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INDOXACARB

Volume 3 – B.6 (AS)

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Co-Rapporteur Member State: Spain

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When	What
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B.6. TOXICOLOGY AND METABOLISM DATA

DPX-KN128 is the insecticidally active, S-enantiomer of a compound belonging to the novel oxadiazine class of insecticides. The R-enantiomer, IN-KN127, is not insecticidally active. DPX-MP062 (75:25) and DPX-JW062 (50:50) are enantiomer blends of DPX-KN128 and IN-KN127. DPX-MP062 contains the respective isomers in a ratio of approximately 75:25, while DPX-JW062 is a racemic (50:50) mixture. Development of the oxadiazine class of insecticides began with DPX-JW062 (50:50). Processes were subsequently developed that allowed for commercial production of DPX-MP062 (75:25), whose enhanced ratio of the insecticidally active enantiomer allowed for lower use rates of the end-use product and thus lower environmental and dietary exposures. Process breakthroughs in 2005 allowed for the commercial production of >99% indoxacarb (DPX-KN128) technical containing $\leq 1\%$ IN-KN127. Today, DPX-KN128 is the primary technical material used as basis for formulation of end-user products. Although DPX-MP062 technical (75:25) was considered the reference material used as basis for the EU approval of the indoxacarb in 2006, DPX-KN128 (99:1) is the active substance intended to be approved in the context of the renewal of indoxacarb.

The concentration of the insecticide active isomer DPX-KN128 and the insecticide inactive isomer IN-KN127 are shown for the 3 forms of technical active materials in the table below:

Test Substance	Percent DPX-KN128	Percent IN-KN127
DPX-JW062	50	50
DPX-MP062	75	25
DPX-KN128	>99	<1

For better clarity, the compounds will be called DPX-JW062 (50:50), DPX-MP062 (75:25) and DPX-KN128 (99:1) in the RAR. It is to be noted that IN-KN127 was written as DPX-KN127 in some toxicity study reports; in the RAR, this denomination was kept in the summaries of these studies.

A full battery of toxicity and metabolism studies was conducted with DPX-JW062 (50:50). Metabolism, acute toxicity, genotoxicity, rat developmental toxicity, neurotoxicity, and targeted repeated dose toxicity studies were conducted with DPX-MP062 (75:25) to demonstrate equivalence with DPX-JW062 (50:50).

Targeted toxicity studies in the most sensitive species and gender were conducted with DPX-KN128 (99:1) to demonstrate equivalence to DPX-JW062 (50:50) and DPX-MP062 (75:25). Since DPX-KN128 was present at 50-75% in the toxicity studies conducted with DPX-JW062 and DPX-MP062, these studies can be used to support the toxicity database for indoxacarb and are relied on where specific studies with DPX-KN128 technical have not been conducted. Additionally, as shown from the results of the three 90-day toxicity studies performed either on DPX-JW062, DPX-MP062 or DPX-KN128, the toxicity of the three compounds seem to be quite similar and no further toxicity testing was considered needed.

The table below illustrates the studies performed on the different technical active materials tested.

Table B.6-1
Indoxacarb studies

Study Type	DPX-JW062 (50:50)	DPX-MP062 (75:25)	DPX-KN128 (99:1)
Rat metabolism	X	X	-
Acute oral/dermal LD ₅₀	-	X	X
Acute inhalation LC ₅₀	X	-	-
Skin sensitization, skin/eye irritation	-	X	X
Phototoxicity	-	-	X
Genotoxicity battery	-	X	X
28-day oral (rats/mice)	X	-	-
28-day dermal	-	X	-
90-day oral (rats)	X	X	X
90-day oral (mice)	X	-	-
90-day oral (dogs)	X	-	-
1-year oral (dogs)	X	-	-
2-year oral (rats)	X	-	-
18-month oral (mice)	X	-	-
Multigeneration reproduction	X	-	-
Rabbit developmental	X	-	-
Rat developmental	X	X	X
Acute oral neurotoxicity (rats)	-	X	-
Subchronic oral neurotoxicity (rats)	-	X	-
Rat developmental neurotoxicity	-	-	X
Immunotoxicity	-	-	X

In this Renewal Assessment Report, new summaries were provided by the applicant for the studies already included in the original DAR. Several studies have also been revised since the first review of the active substance. The reasons for revisions have been included at the beginning of each study summary.

Test substance specification can be determined from the test substance code which is a research and development code number given to a specific batch of produced material (either technical or formulated). The approximate composition of the material(s) used in the various tests is given in the following tables.

Table B.6-2
Active substance specification

Test substance code	Description	Purity (%) ^a	Ratio of Isomers
DPX-JW062-34	DPX-JW062 Technical	94.7	50% DPX-KN128 50% IN-KN127
DPX-JW062-69	DPX-KN128 technical	90.95	90.4% DPX-KN128 0.55% IN-KN127
DPX-JW062-106	DPX-JW062 technical	95	47% DPX-KN128 47% IN-KN127
DPX-JW062-112	DPX-JW062 technical	94.7	50% DPX-KN128 50% IN-KN127
DPX-KN128-31	DPX-KN128 technical	99.7	99.7% DPX-KN128 IN-KN127 not detected
DPX-KN128-098	DPX-KN128 technical	95.5	95.5% DPX-KN128 <0.01% IN-KN127
DPX-KN128-215	DPX-KN128 technical	98.4	98.4% DPX-KN128 IN-KN127 not detected
DPX-KN128-424	DPX-KN128 technical	99.06	99.06% DPX-KN128 <0.1% IN-KN127
DPX-MP062-51	DPX-MP062 technical	94.5	75% DPX-KN128 25% IN-KN127
DPX-MP062-51A	DPX-MP062 technical	94.5	75% DPX-KN128 25% IN-KN127
DPX-MP062-51B	DPX-MP062 technical	94.5	75% DPX-KN128 25% IN-KN127
DPX-MP062-216	DPX-MP062 technical	95.8	72.3% DPX-KN128 23.5% IN-KN127
DPX-MP062 21793-02	DPX-MP062 technical	94.5	75% DPX-KN128 25% IN-KN127

^a Purity refers to the weight percent of the sum of DPX-KN128 + IN-KN127.

Table B.6-3
Metabolite specification

Test substance code	Description	Purity (%)
JT333-1	IN-JT333 technical metabolite	>95%
JT333-20	IN-JT333 technical metabolite	98.7%
KG433-3	IN-KG433 technical metabolite	98%

Classification and labelling:

A harmonised classification and labelling for indoxacarb was adopted by the ECHA Committee for Risk Assessment (RAC) in June 2011. The resulting classification is available in Commission Regulation (EU) No 944/2013 (5th adaptation to technical and scientific progress of Regulation (EC) No 1272/2008) and the classification for human health is the following:

Acute Tox 3 H301

Acute Tox 4 H332

STOT RE 1 H372 (blood, nervous system, heart)

Skin Sens 1B H317

This classification was considered relevant for indoxacarb DPX-KN128 (99:1) (CAS: 173584-44-6) and for the enantiomeric reaction mass 75:25 S:R (CAS: 144171-61-9).

The toxicological studies available in the CLH report are the same as those evaluated in the first DAR of indoxacarb. Therefore, the majority of studies performed on DPX-KN128 (99:1) and submitted for the purpose of this renewal were not included in the CLH report and were not assessed by the ECHA RAC. Nevertheless, the RMS considers that the newly submitted studies do not change the classification adopted by ECHA RAC.

B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

B.6.1.1. Absorption, distribution, metabolism and excretion by oral route

ADME studies were carried out in rats using DPX-JW062 (trifluoromethoxy- and indanone-labels) and DPX-MP062 (75:25) (indanone-label).

Absorption

Upon low dose (5 mg/kg bw) oral administration of DPX-JW062 (50:50) absorption was rather slow and at least 64% of the administered dose for both sexes (trifluoromethoxyphenyl-label). This is based on the sum of urinary excretion and radioactivity levels in tissues by 168 h after administration.

In a separate experiment, absorption defined as the sum of the 48-h urinary and biliary excretion resulted in absorption values of at least 62% for males and at least 44% for females after administration of DPX-JW062 (50:50). However, because of the slow excretion (used to define absorption), the 48-hr observation is considered less relevant. Furthermore, carcass residues were not measured in the bile duct cannulated rats.

Saturation of absorption (less than 11%) occurred at high dose administration (150 mg/kg).

The results of the study with indanone-radiolabelled DPX-MP062 (75:25) (5 mg/kg bw, single dose only) were almost comparable to those obtained from rats dosed with indanone-radiolabelled DPX-JW062 (50:50). One apparent difference was the higher absorption in females of DPX-MP062 (75:25) (58%) compared with DPX-JW062 (50:50) (45%) based on sum of urinary excretion and tissue levels at 168 h in the rats that were administered indanone-labelled test substances.

Absorption was not measured with trifluoromethoxyphenyl-radiolabelled DPX-MP062 (75:25). Since with DPX-JW062 (50:50), absorption with trifluoromethoxyphenyl-labelled test substance was higher than with indanone-labelled test substance, it cannot be excluded that actual absorption of DPX-MP062 (75:25) was higher than 58%. However, since this was not studied, the minimal absorption of DPX-MP062 (75:25) was concluded to be 58% (worst-case assumption) and overall, the agreed oral absorption used in the context of the first inclusion of indoxacarb was 60%.

Repeated low dose administration of trifluoromethoxyphenyl-labelled DPX-JW062 (50:50) to female rats did not appear to influence the absorption observed upon a single dose administration, based on comparison of urinary excretion levels.

Excretion

Excretion of radiolabel was rather slow. By seven days after a single dose (5 mg/kg) of indanone-radiolabelled DPX-JW062 (50:50), excretion of the radiolabel in urine was 37 and 41% for females and males, respectively and in faeces 44% both for females and males. For the trifluoromethoxyphenyl-labelled DPX-JW062 (50:50), these values were somewhat different, due to cleavage of the main structure of the parent compound during biotransformation, i.e. in urine 47 and 55% and in faeces 27 and 30% for females and males, respectively.

Excretion of radiolabel in rats that were administered DPX-MP062 (75:25) (indanone-labelled) was 45 and 35% in urine and 33 and 47% via faeces in female and male rats, respectively. This reflects the higher absorption in females for DPX-MP062 (75:25) compared to DPX-JW062 (50:50).

Distribution

By 168 h after a single oral dose administration of indanone-labelled DPX-JW062 (50:50) the total tissue content of radiolabel was 7.8 and 3.4% for females and males, respectively. Upon administration of the trifluoromethoxyphenyl-labelled DPX-JW062 (50:50), these values were higher, i.e. 17 and 10%, respectively.

By 168 h after a single oral dose administration of DPX-MP062 (75:25) (indanone labelled) the tissue levels were 13 and 4.4% for females and males, respectively. This indicates sex-specific distribution, especially for DPX-MP062 (75:25).

Most of the radiolabel of administered DPX-JW062 (50:50) and DPX-MP062 (75:25) was distributed to fat and blood. Importantly, in comparison with administration of radiolabelled DPX-JW062 (50:50), a greater amount of the radiolabelled DPX-MP062 dose appeared to be retained by tissues, particularly fat. Furthermore, for both test substances, the decline of radiolabel was significantly lower in fat compared to plasma. Remarkably, the radioactivity levels in red blood cells upon administration of [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 but

not [indanone-1-¹⁴C]DPX-JW062 (50:50) were much higher than the levels in plasma apparently due to retention in red blood cells of radioactivity associated with the trifluoromethoxyphenyl portion of the molecule. The latter hypothesis was supported by an additional RBC distribution study with radiolabelled DPX-JW062 (50:50), in which the trifluoromethoxyaniline metabolite P0036 was shown to be exclusively associated with RBCs.

The retention and elimination of the metabolite IN-JT333 from fat and the binding of some component to red blood cells appeared to be the overall rate determining processes for elimination of radioactivity from the body.

Upon repeated dose administration of trifluoromethoxyphenyl labelled DPX-JW062 (50:50) (5 mg/kg) to rats (studied only in female rats), an increase in tissue radioactivity levels was observed for up to 8 days. The relative amount of radiolabel retained in tissues of rats was smaller (8.4% of the total administered radiolabel) than after a single dose administration (17%). In addition, in fat and red blood cells the ratio of the level of radiolabel seven days after the last of multiple dose administration compared to seven days after a single dose, amounted up to a factor 10 and 100, respectively.

Metabolism

DPX-JW062 (50:50) and DPX-MP062 (75:25) were extensively metabolised, based on very low excretion of parent compound in bile (determined for DPX-JW062 (50:50) only) together with extensive excretion of metabolised dose in urine and faeces. Both for DPX-JW062 (50:50) and DPX-MP062 (75:25) some parent compound remained unabsorbed and was excreted in faeces. In urine, no parent compound was observed.

Biotransformation started with either enzymatic removal of the methoxycarbonyl group leading to JT333 (KN125/KN124) or hydroxylation at the benzylic position in the indanone moiety leading to 5-HO-JW062. Both metabolites were found in fat and faeces. For the first reaction, gender specificity and substrate stereospecificity was observed as in females the formation of IN-JT333 was the most important biotransformation step. Furthermore, the S-isomer (KN128) of DPX-JW062 (50:50) was the preferred substrate in this reaction, leading to an increased formation of IN-KN125 over IN-KN124. In male rats, formation of 5-HO-JW062 (sum of two enantiomers) was more important than the formation of IN-JT333 (sum of two enantiomers). Further steps in the extensive biotransformation are indicated in the proposed metabolic pathway as presented in the following section.

In vitro and *in vivo* rat microsomal metabolism

Microsomes from both sexes showed a decline in the concentration of DPX-JW062 (50:50). The rate of decline in male rat microsomal suspensions ($t_{1/2}$ = 25.5 min) was faster than in female suspensions ($t_{1/2}$ = 67.6 min). The corresponding area-under-the-curve (AUC) (nmol × min/ml) for male rat microsomes was 137.8 compared with 500.6 for female rat liver microsomes. These values were used to estimate intrinsic hepatic clearance. The range of estimates for intrinsic hepatic clearance (mL/min/kg body weight) of DPX-JW062 (50:50) in the male rat (26.2 to 35.0 mL/min/kg body weight) were approximately 2.2 to 3.0 times faster than in female rats (11.6 to 11.9 mL/min/kg body weight).

Overall

The toxicokinetics and overall metabolism of DPX-MP062 (indanone radiolabelled) was qualitatively similar to that observed for the corresponding indanone radiolabelled DPX-JW062. However, quantitative differences occurred, especially for total radioactivity observed in fat (greater for DPX-MP062 (75:25)). Furthermore, based on the available data, DPX-MP062 (75:25) seems to be more absorbed in female rats than DPX-JW062 (50:50). Important gender- and stereospecific biotransformation was observed which was more pronounced for DPX-MP062 (75:25) as it contains an enantiomeric excess of the S-enantiomer. It appeared that the S-isomer KN128 is preferred as a substrate for the enzymatic removal of the N-carboxymethyl group in the parent compound leading to IN-JT333, especially in female rats. In male rats an increased (relative to females) formation of 5-HO-JW062 was observed.

DPX-KN128 (99:1)

No ADME study was available with the pure S-enantiomer DPX-KN128. ADME profiles of the racemic DPX-JW062 (50:50) and the enantiomer blend DPX-MP062 (75:25) containing DPX-KN128 and IN-KN127 in a ratio of approximately 75:25 were available and were qualitatively similar. Nevertheless, compared to DPX-JW062 (50:50), administration of DPX-MP062 (75:25) resulted in a higher absorption in female rats and a greater retention of radioactivity in tissues, particularly in fat. DPX-KN128 was shown to be the preferred substrate for the enzymatic removal of the N-carboxymethyl group in the parent compound, leading to the formation of the metabolite IN-JT333.

It can thus be expected that administration of pure DPX-KN128 (99:1) to rats would increase the differences observed between the racemic DPX-JW062 (50:50) and the enantiomeric mixture 75:25 DPX-MP062. Based on these data, it is considered appropriate to use the oral absorption set for DPX-MP062 in the context of the first inclusion of indoxacarb (75:25), i.e. 60%.

According to co-RMS (ES): *“Available data only allow to establish absorption at 60% as worst case assumption. After 168 h absorption in females, based on excretion in urine and tissues is higher for DPX-MP062 (58%) than for DPX-JW062 (45%). A metabolism study with DPX-KN128 would be therefore very convenient.”*

Previous evaluation:	In DAR (2000)
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Report: [REDACTED] (1997b); ¹⁴C-DPX-JW062 (a racemic mixture of DPX-KN128 and IN-KN127): Metabolism in the rat

DuPont Report No.: HLR 283-96

Guidelines: 59 NohSan No.4200 (1985), Directive 87/302/EEC Part B (1987), USEPA 85-1 (1982)

Deviations: 3 rats per sex instead of 4 to evaluate biliary elimination and tissue distribution

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 283-96

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Radiolabeled test material: ¹⁴C-DPX-JW062 technical
 Lot/Batch #: [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062; lot numbers: HOTC 423, HOTC 429, HOTC 456
 [indanone-1-¹⁴C]DPX-JW062; lot numbers: HOTC 421, HOTC 428, HOTC 451
 Purity: HOTC 423: 99.8%; HOTC 429: 99.0%; HOTC 456: 98.4%; HOTC 421: 98.6%; HOTC 428: 98.2%; HOTC 451: 98.1%
 Specific activity: [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062, HOTC 423: 54.0 µCi/mg; HOTC 429: 52.8 µCi/mg; HOTC 456: 51.7 µCi/mg; [indanone-1-¹⁴C]DPX-JW062 - HOTC 421: 55.5 µCi/mg; HOTC 428: 45.1 µCi/mg; HOTC 451: 52.0 µCi/mg
 Description: Solid
 Stability of test compound: The test materials were stable for at least 1–2 months at -20°C
2. Vehicle and/or positive control: The vehicle used was PEG-400
3. Test animals
 Species: Rat
 Strain: CrI:CD[®](SD)BR
 Age at dosing: 7 to 12 weeks of age
 Weight at dosing: 179–267 g (males); 166–242 g (females)
 Source: [REDACTED]
 Acclimation period: Minimum of 5 days followed by 24 hours acclimation to individual metabolism cages
 Diet: Purina[®] Certified Rodent Chow[®] #5002 or Rodent Pellet Certified Formula A/1 Chow, P.J. Noyes Company, Inc. ad libitum except food was withheld from rats overnight before dosing with radiolabelled substance.
 Water: Tap water, ad libitum
- Housing: glass individual metabolism cages
 Husbandry: Husbandry conditions were in accordance with the USPHS-NIH publication Guide to the Care and Use of Laboratory Animals.
4. Environmental conditions
 Temperature: 23 ± 2°C
 Humidity: 50 ± 10%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-Study initiated/completed
 03-March-1994 to 31-July-1997
2. Main study
 Metabolism studies with DPX-JW062 were conducted in male and female CrI:CD[®](SD)BR rats using two ¹⁴C-radiolabels, [indanone-1-¹⁴C]DPX-JW062 and [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062, and two dose levels: 5 and 150 mg/kg. Each dose solution contained enough ¹⁴C-radiolabel to deliver approximately 15 to 40 µCi per subject by oral gavage using polyethylene glycol (PEG-400) as the dose vehicle. End points evaluated included the pharmacokinetic behaviour of radioactive residues in plasma and red blood cells, the disposition and material balance of total radioactive residues among tissues and excreta, the percentage and concentration of residues in tissues at selected times after dosing (e.g., t_{C_{max}}, t_{C_{max}/2}, and terminal sacrifice times), the elimination of radioactive residues in bile, and identification of metabolites.

In addition, a multiple dose experiment was conducted to assess the bioaccumulation of ^{14}C -DPX-JW062 in female rats. The dosing regimen consisted of oral dosing with 5 mg/kg/day of [trifluoromethoxyphenyl(U)- ^{14}C]DPX-JW062 for 14 days with intermediate sacrifices and followed by a 21-day depuration period.

II. RESULTS AND DISCUSSION

A. MAIN STUDY

Pharmacokinetics:

The results of the pharmacokinetic experiments demonstrated that absorption occurred rather slowly following oral gavage at the low dose (5 mg/kg), and that absorption was saturated at the high dose (150 mg/kg). Indeed, despite a 30-fold increase in dose, the C_{max} values were increased only 3- to 5-fold. Absorption plateaued between 6 and 24 hr after dosing. Elimination half-lives for total radioactive residues in plasma were 35-188 hr and depended on the radiolabel given and the sex of the animal. Half-lives were not different among groups of animals given the same radiolabel at the 5 or 150 mg/kg dose levels. However, the half-lives were clearly shorter in male rats compared with female rats. The half-life was prolonged in red blood cells of rats dosed with [trifluoromethoxyphenyl(U)- ^{14}C]DPX JW062 compared to rats dosed with [indanone-1- ^{14}C]DPX-JW062 and also with levels in plasma. This is likely due to retention in red blood cells of radioactivity associated with the trifluoromethoxyphenyl portion of the molecule. This radioactivity was determined to be associated exclusively with the arylamine metabolite IN-P0036 (see Anderson et al. (1999)).

Table B.6.1.1-1
Mean pharmacokinetic parameters for DPX-JW062 in plasma and red blood cells of rats

Parameter	Plasma ^a		Red Blood Cells	
	Male	Female	Male	Female
Group 1A: 5 mg/kg - [indanone-1-¹⁴C]DPX-JW062				
Elimination half-life (t _{1/2} , hr)	35 ± 1 ^b	52 ± 10	97 ± 25	68 ± 7
Area-under-the-curve (AUC, µg/g•hr)	80 ± 27	135 ± 17	82 ± 17	94 ± 13
Peak time (tC _{max} , hr)	<u>6.8</u> ± 3.9	<u>5.3</u> ± 3.4	6.8 ± 2.7	3.3 ± 3.2
Half peak time (tC _{max/2} , hr)	<u>24</u> ± 4	<u>24</u> ± 3	38 ± 11	32 ± 9
Peak concentration (C _{max} , µg/g)	2.4 ± 0.8	3.0 ± 0.3	1.1 ± 0.4	1.5 ± 0.3
Half peak concentration (C _{max/2} , µg/g)	1.2 ± 0.4	1.5 ± 0.2	0.6 ± 0.2	0.7 ± 0.1
Group 1B: 150 mg/kg - [indanone-1-¹⁴C]DPX-JW062				
Elimination half-life (t _{1/2} , hr)	45 ± 2	59 ± 5	84 ± 26	75 ± 6
Area-under-the-curve (AUC, µg/g•hr)	536 ± 295	785 ± 111	437 ± 133	545 ± 53
Peak time (tC _{max} , hr)	8 ± 4	<u>19</u> ± 9	4.0 ± 3.5	9 ± 13
Half peak time (tC _{max/2} , hr)	<u>39</u> ± 8	<u>64</u> ± 13	57 ± 6	88 ± 13
Peak concentration (C _{max} , µg/g)	9.2 ± 3.5	9.3 ± 1.3	4.7 ± 1.4	4.7 ± 0.9
Half peak concentration (C _{max/2} , µg/g)	4.6 ± 1.7	4.6 ± 0.7	2.4 ± 0.7	2.4 ± 0.4
Group 1C: 5 mg/kg - [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062				
Elimination half-life (t _{1/2} , hr)	92 ± 43	114 ± 20	112 ± 6	119 ± 23
Area-under-the-curve (AUC, µg/g•hr)	28 ± 10	55 ± 18	1519 ± 337	2627 ± 327
Peak time (tC _{max} , hr)	<u>8</u> ± 0	<u>8</u> ± 0	24 ± 0	24 ± 0
Half peak time (tC _{max/2} , hr)	<u>16</u> ± 3	<u>19</u> ± 4	140 ± 3	150 ± 26
Peak concentration (C _{max} , µg/g)	0.6 ± 0.1	0.8 ± 0.2	8.4 ± 1.4	13.9 ± 3.6
Half peak concentration (C _{max/2} , µg/g)	0.3 ± 0.1	0.4 ± 0.1	4.2 ± 0.7	7.0 ± 1.8
Group 1D: 150 mg/kg - [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062				
Elimination half-life (t _{1/2} , hr)	96 ± 49	188 ± 42	95 ± 17	138 ± 28
Area-under-the-curve (AUC, µg/g•hr)	241 ± 102	597 ± 343	7656 ± 1620	16648 ± 2296
Peak time (tC _{max} , hr)	<u>3.3</u> ± 1.5	<u>27</u> ± 39	40 ± 14	72 ± 24
Half peak time (tC _{max/2} , hr)	<u>22</u> ± 2	<u>118</u> ± 136	168 ± 5	225 ± 17
Peak concentration (C _{max} , µg/g)	2.7 ± 0.4	2.6 ± 0.3	39 ± 10	63 ± 17
Half peak concentration (C _{max/2} , µg/g)	1.4 ± 0.2	1.3 ± 0.1	19 ± 5	31 ± 8

^a Underlined values were used to set tC_{max} and tC_{max/2} for the tissue residue experiments

^b Data presented as mean ± SD

Disposition and Material Balance

The results of disposition and material balance studies are summarised in the following table. The percentage of the administered dose recovered from all groups by 7 days after dosing was 93% or greater. The distribution of radioactivity for rats dosed with a single 5 mg/kg oral dose of [indanone-1-¹⁴C]DPX JW062 was 37–41% in urine, 44% in faeces, and 3.4–7.8% in tissues. For rats dosed with a single 5 mg/kg oral dose of [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062, the distribution of radioactivity was 47–55% in urine, 27–30% in faeces, and 10–17% in tissues. Most of the dose of either label was excreted by 72–96 hr after dosing. Female rats appeared to retain slightly more residues in tissues than male rats. For rats dosed at the 150 mg/kg level with either radiolabel, the radioactivity was mostly eliminated in faeces (65–78%) compared with elimination in urine (13–20%) and retention in tissues (1.4–4.4%). For female rats dosed with 5 mg/kg/day of [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 for 14 days, the distribution was 56% in the urine, 25% in the faeces, and 8.4% in tissues. Therefore, no important difference was observed between

the single low dose and the multiple low dose groups in females dosed with [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062, except that total tissue radioactivity levels were only 8.4% of the total multiple administered dose compared to 17% after a single dose.

For rats that were administered 5 mg/kg [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062, the sum of urinary excretion and tissue levels by 168 h after administration was 64% and 65% of the administered dose for females and males respectively. The minimum percentage of absorption was therefore concluded to be 64% for both sexes.

Table B.6.1.1-2
Mean disposition and material balance of DPX-JW062

Dose (mg/kg)	Label	Sex	Percent of dose (Mean ± SD)			
			Urine	Faeces	Tissues	Recovered ^a
5	IND	M	41 ± 6 ^b	44 ± 2	3.4 ± 1.0	95 ± 2
		F	37 ± 9	44 ± 9	7.8 ± 1.6	94 ± 2
	TMP	M	55 ± 7	27 ± 3	10.3 ± 2.2	97 ± 3
		F	47 ± 14	30 ± 11	16.6 ± 5.0	98 ± 2
	TMP ^c	F	56 ± 8	25 ± 2	8.4 ± 4.2	96 ± 3
150	IND	M	14 ± 5	78 ± 8	1.4 ± 0.6	98 ± 2
		F	13 ± 4	75 ± 7	3.1 ± 1.9	97 ± 4
	TMP	M	20 ± 7	65 ± 2	3.6 ± 1.7	93 ± 5
		F	14 ± 2	71 ± 6	4.4 ± 1.6	95 ± 2

TMP = [trifluoromethoxyphenyl(U)-¹⁴C], IND = [indanone-1-¹⁴C], M = male, F = female

^a Percent recovered includes cage wash and feed residue which accounts for up to 6% of the dose

^b Data presented as mean ± SD, n = 5

^c Values are for three female rats given 5 mg/kg/day for 14 days

Tissue Residues

In tissues, fat contained the highest tissue ¹⁴C-residue concentration at all sampling times, which appeared to decline less rapidly than ¹⁴C-residue in plasma and other tissues between 24 and 168 hr after dosing. This slow elimination from the fat was more pronounced in females compared to males and female rats retained more residues than males. For example, the fat-to-plasma ratio at 168 hr after dosing with 5 mg/kg [indanone-1-¹⁴C]DPX-JW062 was greater in female rats (29:1) compared to male rats (11:1). Tissues other than fat had tissue-to-plasma ratios ranging from 0.4 to 5.1:1 in female rats and 0.3 to 3.3:1 in male rats at 168 hr after dosing. For animals of the same sex, the concentrations and tissue-to-plasma ratios of [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 and [indanone-1-¹⁴C]DPX-JW062 in fat (and many of the other tissues) were similar. One important difference, however, was the greater association of ¹⁴C-residues with red blood cells of rats dosed with the trifluoromethoxyphenyl label. For rats dosed with 150 mg/kg [indanone-1-¹⁴C]DPX-JW062, the 30-fold increase in dose did not produce a proportional increase in ¹⁴C-tissue residues. Consequently, the tissue-to-plasma ratios were similar across dose levels.

Biliary Elimination

The elimination of ¹⁴C-DPX-JW062 equivalents in bile was quantified for up to 48 hr after dosing and accounted for 17-23% of the dose for bile duct cannulated rats dosed at the 5 mg/kg level with either radiolabel. Essentially no intact DPX-JW062 was detected in bile, and no single metabolite in bile was greater than 4% of the administered dose.

This separate low dose experiment in which biliary and urinary excretions were studied in the same animals up to 48 h after dose administration revealed that for both type of radiolabel, at least 62-66% and 44-46% of the radiolabel was absorbed in males and females respectively (taking into account bile and urinary excretions; percent of dose excreted in carcass and tissue was not measured in this part of the study).

Metabolites

Fat. IN-JT333 was a major metabolite found in the fat and accounted for approximately 1-5% of the dose at terminal sacrifice (7 days after dosing). Chiral HPLC analysis showed that the IN-JT333 in fat was no longer racemic such that the more biologically active enantiomer of IN-JT333 (IN-KN125) was found at a higher concentration compared with the less active enantiomer (IN-KN124). For example, the average residue concentration in fat for the five female rats dosed with 5 mg/kg [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 was comprised of 2.1 ± 0.7 ppm IN-KN125 and 1.0 ± 0.3 ppm IN-KN124. In a single male rat with the same treatment, this radioactivity was comprised of 0.8 ppm IN-KN125 and 0.3 ppm IN-KN124. The slightly greater concentration of IN-KN125 compared with IN-KN124 suggests that the disposition of IN-JT333 involves stereo-selective metabolism and/or distribution. The higher concentration of both isomers in females compared to males indicates a gender-specific biotransformation.

Faeces. A much higher percentage of parent compound was excreted in faeces of rats dosed with 150 mg/kg (68-79% of the administered dose) compared with rats dosed with 5 mg/kg (6-20%). At the 5 mg/kg dose level, the excretion of parent compound in faeces was about 3 times greater for female rats (19-20%) than male rats (6%). The metabolites in the faeces of rats dosed at the 5 mg/kg level were intact compounds (containing both radiolabels). The faecal metabolites of rats dosed with a single oral dose of either radiolabel at the 5 mg/kg level were confirmed to be IN-JT333 (0.4-2.2%) and 5-HO-JW062 in two stereoisomeric forms (3.5-12.7%). Clear gender specific differences were observed in the quantities of IN-JT333 and 5-HO-JW062 found in faeces. Using the trifluoromethoxyphenyl-label, IN-JT333 accounted for $1.4 \pm 0.6\%$ and $0.4 \pm 0.2\%$ of the administered dose in female and male rats, respectively and 5-HO-JW062 accounted for 1.0-2.5% and 6.0-6.7% of the administered dose in females and males, respectively. For 5-HO-JW062, also in faeces, one enantiomer (undefined with respect to R-or S-configuration) was found at higher percentages than the other one, where the ratio between the two enantiomers was 2.5 and 1.1 for females and males, respectively. The sum of IN-JT333 and 5-HO-JW062 in faeces accounted for a total of 5-13% of the administered dose. These results support the existence of a gender-specific and stereo-selective biotransformation of DPX-JW062.

Urine. Metabolites in urine were cleaved products (only containing one of the radiolabels, either indanone or trifluoromethoxyphenyl portions of the parent molecule). The major urinary metabolite identified from rats dosed with [indanone-1-¹⁴C]DPX-JW062 was IN-MU716 (approx. 12% of administered dose in both sexes). Eight other minor indanone-derived metabolites were identified in urine (MX829 sulfate, HO-JU874, IN-MA576, MA576-conjugate, IN-MX829, IN-JU874, ML440-conjugate and MX828-sulfate) whereas five minor metabolites remained unidentified. These were all individually <5% of the dose but collectively indicate extensive metabolism of the indanone group. Major urinary metabolites from rats dosed with [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 included IN-MG195 (14-17% of the administered dose) and IN-MC218 (21-24% of the administered dose). Six other minor metabolites derived from the trifluoromethoxyphenyl group were identified in urine (IN-P0036, a P0036-derivative or conjugate, IN-MZ369, IN-MY795 and HO-KB687-sulfate/glucuronide conjugates) whereas three metabolites remained unidentified (all <4% of the administered dose).

Multiple Dose (Bioaccumulation) Experiment

In female rats given multiple doses of [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062, the relative amount of radiolabel retained in tissues was smaller (8.4% of the total administered radiolabel) than after a single dose administration (17%). However, total radioactive residues in the blood and tissues reached a plateau after 8–14 days of dosing. Compared to the single dose, radioactivity levels in plasma, brain, liver, fat and red blood cells were 1, 2, 3, 13 and 100-fold higher in the multiple low dose group. After the last dose, the decline of total radioactivity in tissues exhibited two phases. In the first phase that occurred between approximately 1 and 7 days after the last dose, the concentration of ¹⁴C-residues in plasma and fat declined to approximately 21 and 18% of the concentrations measured at 1 day after the last dose. For example, the concentration in plasma had declined from 3.7 to 0.78 µg equiv/g in the first 7 days after the last dose. In the second phase, terminal elimination occurred from approximately 7 days after the last dose to the end of the experiment. The tissue concentration data for the elimination phase was used to calculate half-lives. The elimination half-lives for total radioactive residues in tissues were between 7.4 days (for plasma) and 17.7 days (for fat). Similar metabolic profiles in urine and faeces were observed in female rats dosed for either one day or daily for 14 days with [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062.

III. CONCLUSION

DPX-JW062 was readily absorbed at the low dose (5 mg/kg), but saturation of absorption occurred at the high dose (150 mg/kg) following dosing by oral gavage. Commensurate with absorption was extensive metabolism, based on very low excretion of parent compound in bile and extensive excretion of metabolised dose in urine and faeces. Some parent compound remained unabsorbed and was excreted in faeces. Subsequent to absorption, no parent compound was excreted in urine. The elimination half-life of [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 was prolonged due to the retention of radioactivity in the red blood cells associated with the trifluoromethoxyphenyl portion of the molecule. The retention and elimination of the metabolite IN-JT333 from fat appeared to be the overall rate determining process for elimination of radioactive residues from the body.

Previous evaluation:	In DAR (2000)
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Report: [REDACTED] (1997a); ¹⁴C-DPX-MP062 (a 3:1 mixture of DPX-KN128 and IN-KN127): Metabolism in the rat

DuPont Report No.: HL-1997-00439

Guidelines: 87/302/EEC, USEPA 85-1,59 Nohsan No. 4200

Deviations: Deviations include a partial study design involving pharmacokinetics and material balance including rate and extent of excretion and tissue distribution of ¹⁴C-residues. Biliary elimination and earlier tissue distribution times (T_{max} and T_{max}/2) were not evaluated.

Testing Facility: [REDACTED]

Testing Facility Report No.: HL-1997-00439

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Radiolabelled test material: [Indanone-1-¹⁴C] DPX-MP062
 Lot/Batch #: [Indanone-1-¹⁴C] DPX-MP062: HOTC 481
 Purity: [Indanone-1-¹⁴C] DPX-MP062: 96%
 Specific activity: [Indanone-1-¹⁴C] DPX-MP062: 31.4 µCi/mg
 Description: White solid
 Stability of test compound: Not stated in the report.
2. Vehicle and/or positive control: The vehicle used was PEG-400
3. Test animals
 Species: Rat
 Strain: CrI:CD®(SD) BR
 Age at dosing: 7–12 weeks
 Weight at dosing: 187–202 g (males); 170–182 g (females)
 Source: [REDACTED]
 Acclimation period: At least 5 days
 Diet: Purina® Certified Rodent Chow® #5002, or Rodent Pellet Certified Formula A/1 Chow, P.J. Noyes Company, Inc. ad libitum. Food was withheld from rats overnight before dosing with radiolabelled test substance.
 Water: Tap water, ad libitum
 Housing: glass individual metabolism cages
 Husbandry: Husbandry conditions were in accordance with the USPHS-NIH publication Guide to the Care and Use of Laboratory Animals.
4. Environmental conditions
 Temperature: 23 ± 2°C
 Humidity: 50 ± 10%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 29-March-1994 to 31-July-1997
2. Main study
 Metabolism studies with DPX-MP062 were conducted in male and female CrI:CD®(SD)BR rats using a ¹⁴C-radiolabel [indanone-1-¹⁴C]DPX-MP062 at a dose level of 5 mg/kg. The dose solution contained enough ¹⁴C-radiolabel to deliver approximately 30 µCi per subject by oral gavage using polyethylene glycol (PEG-400) as the dose vehicle. End points evaluated included the pharmacokinetic behaviour of radioactive residues in plasma and red blood cells, the disposition and material balance of total radioactive residues among tissues and excreta, and the percentage and concentration of residues in tissues at terminal sacrifice (168 hours after dosing). The results were compared to data from experiments describing the disposition and metabolism of [indanone-1-¹⁴C]DPX-JW062 (see HLR 283-96).

II. RESULTS AND DISCUSSION

A. MAIN STUDY

Pharmacokinetics

The mean peak times ($t_{C_{max}}$) for ^{14}C -residues of DPX-MP062 in plasma were similar for male (8 hr) and female (7 hr) rats. The terminal elimination half-life of [indanone-1- ^{14}C]DPX-MP062 in plasma of male rats was 39 ± 1 hr, and the plasma half-life was 49 ± 10 hr in female rats, suggesting that the elimination of ^{14}C -residues was slightly longer and more variable in female rats than male rats. The retention of [indanone-1- ^{14}C]DPX-MP062 equivalents was greater in red blood cells compared with plasma. For example, the half-life of ^{14}C -residues in red blood cells of males was 91 hr compared with 39 hr in plasma. Comparison of the mean pharmacokinetic parameters indicates no difference between DPX-MP062 and DPX-JW062.

Table B.6.1.1-3
Mean pharmacokinetic parameters for DPX-MP062 or DPX-JW062
in plasma and red blood cells of rats

Parameter	Plasma		Red Blood Cells	
	Male	Female	Male	Female
DPX-MP062: Group 8U (5 mg/kg - [indanone-1-^{14}C] radiolabel)				
Elimination half-life ($t_{1/2}$, hr)	39 ± 1^a	49 ± 10	91 ± 16	74 ± 4
Area-under-the-curve (AUC, $\mu g/g \cdot hr$)	83 ± 9	117 ± 11	80 ± 11	91 ± 22
Peak time ($t_{C_{max}}$, hr)	8.0 ± 3.5	7.3 ± 1.2	8.7 ± 3.1	6.0 ± 3.5
Half peak time ($t_{C_{max}/2}$, hr)	25 ± 4	27 ± 12	35 ± 1	33 ± 8
Peak concentration (C_{max} , $\mu g/g$)	2.3 ± 0.6	2.9 ± 1.1	1.1 ± 0.1	1.4 ± 0.5
Half peak concentration ($C_{max/2}$, $\mu g/g$)	1.2 ± 0.3	1.4 ± 0.6	0.6 ± 0.1	0.7 ± 0.3
DPX-JW062: Group 1A (5 mg/kg - [indanone-1-^{14}C] radiolabel)				
Elimination half-life ($t_{1/2}$, hr)	35 ± 1	52 ± 10	97 ± 25	68 ± 7
Area-under-the-curve (AUC, $\mu g/g \cdot hr$)	80 ± 27	135 ± 17	82 ± 17	94 ± 13
Peak time ($t_{C_{max}}$, hr)	6.8 ± 3.9	5.3 ± 3.4	6.8 ± 2.7	3.3 ± 3.2
Half peak time ($t_{C_{max}/2}$, hr)	24 ± 4	24 ± 3	38 ± 11	32 ± 9
Peak concentration (C_{max} , $\mu g/g$)	2.4 ± 0.8	3.0 ± 0.3	1.1 ± 0.4	1.5 ± 0.3
Half peak concentration ($C_{max/2}$, $\mu g/g$)	1.2 ± 0.4	1.5 ± 0.2	0.6 ± 0.2	0.7 ± 0.1

^a Data presented as mean \pm SD

Absorption

Consistent with the similarities in pharmacokinetic parameters, the oral absorption of DPX-MP062 and DPX-JW062 were also quite equivalent. For DPX-JW062, oral absorption was determined based on total radioactive residues in urine and bile (residues in tissues/carcasses not measured in these animals) or in urine and tissues. A bile elimination study was not conducted with DPX-MP062. Therefore, absorption values for DPX-MP062 were determined based on residues in urine and tissue. Based on these comparisons, the oral absorption of DPX-MP062 and DPX-JW062 were almost comparable. One apparent difference was the higher absorption in females of labelled DPX MP062 (58%) compared with DPX JW062 (45%) based on sum of urinary excretion and tissue levels at 168 h in the rats that were administered indanone-labelled test substances. Since with DPX-JW062, absorption with trifluoromethoxyphenyl-labelled test substance was higher than with indanone-labelled test substance, it cannot be excluded that actual absorption of DPX-MP062 was higher than 58%. However, since this was not studied, the minimal absorption of DPX-MP062 was concluded to be 58% (worst-case assumption).

Disposition and material balance

The disposition and material balance for DPX-MP062 and DPX-JW062 are summarised in Table B.6.1.1-4. Rats dosed with 5 mg/kg [indanone-1-¹⁴C]DPX-MP062 eliminated 35–45% of the dose in urine and 33–47% of the dose in faeces. The percent of dose eliminated in urine of male rats ($35 \pm 5\%$) compared with female rats ($45 \pm 6\%$) appeared to be balanced by a slightly greater elimination in faeces of male rats ($47 \pm 4\%$) compared with female rats ($33 \pm 4\%$). For both sexes, the majority of the dose was excreted within 72 to 96 hr after single oral dosing. The rate and extent to which total ¹⁴C-residues are eliminated in urine and faeces appear to be similar for DPX-MP062 and DPX-JW062, except that absorption in females, based on excretion in urine and tissues (168h) of the indanone label specifically is higher for DPX-MP062 (58%) than for DPX-JW062 (45%). The percent of the DPX-MP062 dose retained in tissues at 168 hr after dosing was $12.9 \pm 4.8\%$ for female rats and $4.4 \pm 0.8\%$ for male rats. This greater retention by female rats compared with males was also observed after dosing with DPX-JW062 ($7.8\% \pm 1.6\%$ in female rats and $3.4\% \pm 1.0\%$ in male rats).

Table B.6.1.1-4
Mean disposition and material balance of DPX-MP062 or DPX-JW062

Dose (mg/kg)	Label	Sex	Percent of dose (Mean \pm SD)			
			Urine	Faeces	Tissues	Recovered ^a
5	IND-DPX-MP062	M	35 ± 5^b	47 ± 4	4.4 ± 0.8	90 ± 3
		F	45 ± 6	33 ± 4	12.9 ± 4.8	95 ± 2
	IND-DPX-JW062	M	41 ± 6	44 ± 2	3.4 ± 1.0	95 ± 2
		F	37 ± 9	44 ± 9	7.8 ± 1.6	94 ± 2

IND = [indanone-1-¹⁴C], M = male, F = female

^a Percent recovered includes cage wash and feed residue which accounts for up to 4% of the dose

^b Data presented as mean \pm SD

Tissue residues

As noted above, the percent dose of the DPX-MP062 retained in tissues at 168 hr after dosing was 2.9-times greater for female rats ($12.9 \pm 4.8\%$) compared with male rats ($4.4 \pm 0.8\%$). For rats dosed with DPX-JW062, female rats also retained more in tissues ($7.8 \pm 1.6\%$) than male rats ($3.4 \pm 1.0\%$). These data suggest that the retention of radioactive residues is 2-3 times greater in female rats compared with male rats. The data also suggest that rats dosed with DPX-MP062 retain slightly more radioactivity in tissues than rats dosed with DPX-JW062. The greater retention of radioactivity in tissues by both female and male rats dosed with DPX-MP062, compared with DPX-JW062, appears to be explained almost entirely by retention of residues in fat. For rats dosed with DPX-MP062, the percent of dose retained in the fat was $2.6 \pm 0.7\%$ and $8.8 \pm 3.8\%$ in males and females, respectively (thus, 3.4-times less for males compared to females). For rats dosed with DPX-JW062, male rats retained $1.8 \pm 0.7\%$ of the dose in fat compared with $4.7 \pm 1.2\%$ in female rats (2.7 times less for males compared to females).

Metabolites and Metabolic Pathway

Fat. IN-JT333, a metabolite found in the fat, accounted for an average of 3-9% of the DPX-MP062 dose at terminal sacrifice (7 days after dosing), i.e. approximately 95% of the total radioactivity in fat (no parent compound was observed in the fat). Rats dosed with DPX-JW062 had an average of 1-5% of the dose retained in fat as IN-JT333. Chiral HPLC analysis showed that the insecticidally active enantiomer of IN-JT333 (IN-KN125) was present at a higher concentration compared with the less active enantiomer (IN-KN124). For example, the average residue concentration in female rats dosed with [indanone-1-¹⁴C]DPX-MP062 was comprised of 5.4 ± 2.5 ppm IN-KN125 and 0.8 ± 0.4 ppm IN-KN124. For female rats dosed with [indanone-1-¹⁴C]DPX-JW062, the average residue concentration was comprised of 2.1 ± 0.7 ppm IN-KN125 and 1.0 ± 0.3 ppm IN-KN124. The higher concentration of IN-KN125 in fat (5.4 ppm) of DPX-MP062 animals compared with DPX-JW062 animals (2.1 ppm) is consistent with the greater proportion of parent DPX-KN128 in the test substance. The higher concentration of IN-KN125 than

IN-KN124 in fat of rats dosed with either DPX-MP062 or DPX-JW062 suggests that the disposition of IN-JT333 involves stereo-selective metabolism and/or distribution.

Faeces. Based on analysis of faecal extracts, 1.4-1.8% of the parent DPX-MP062 was excreted in faeces. In rats dosed with DPX-JW062, the excretion in faeces was 6 and 19% of the dose in males and females, respectively. These data suggest that DPX-MP062 was more readily absorbed than DPX-JW062. The most prominent metabolites in the faeces of rats dosed at the 5 mg/kg level of either DPX-MP062 or DPX-JW062 appeared to be intact (containing both radiolabels), hydroxylated products of the parent compound. The hydroxylated parent compounds (two enantiomers of 5-HO-JW062) were more prominent in faeces from males than from females (more than two-fold higher concentration for the sum of the 2 enantiomers)

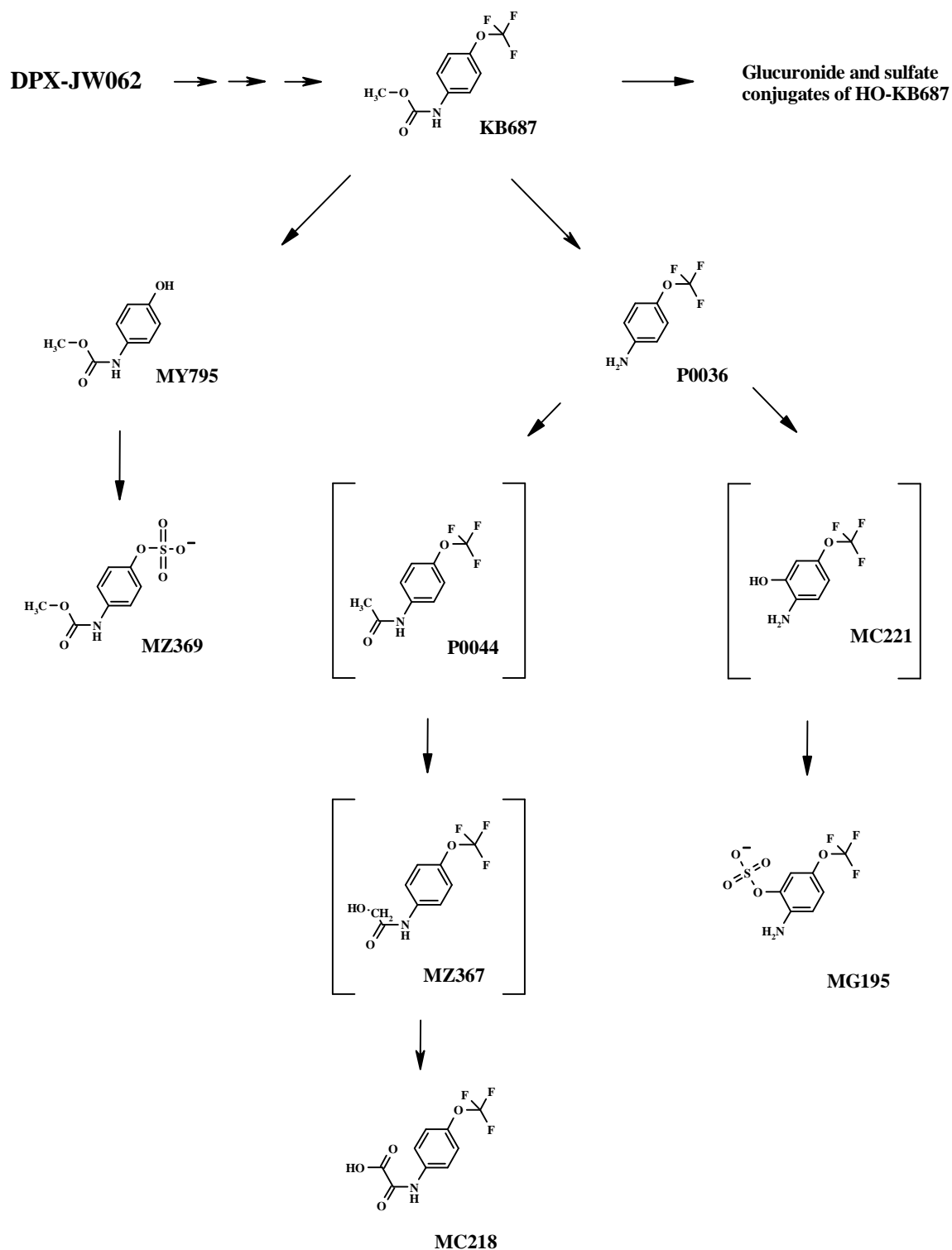
Urine. For DPX-MP062, metabolites in urine were cleaved products (consistent with the metabolism of DPX-JW062). Major urinary metabolites (>5% of the dose) identified from rats dosed with [indanone-1-¹⁴C]DPX-MP062 include IN-MU716 and MX829-sulfate. Seven other minor urinary metabolites (<5% of the dose) included IN-JU874, IN-MA576, IN-MX829, HO-JU874, MA576-conjugate, ML440-conjugate, and MX828-sulfate. These are all individually <5% of the dose, but collectively indicate extensive metabolism of the [indanone-1-¹⁴C]DPX-MP062. The levels of these metabolites are consistent with those identified in the urine of rats given 5 mg/kg [indanone-1-¹⁴C]DPX-JW062. Major urinary metabolites from rats dosed with [trifluoromethoxy-phenyl(U)-¹⁴C]DPX-JW062 include IN-MG195 and IN-MC218.

Chemical reaction scheme for the synthesis of MX829 and its conjugates:

- DPX-MP062 is converted to JT333, 5-HO-JW062, or KG433.
- JT333 is converted to 5-HO-JT333, which then leads to HO-JU874.
- 5-HO-JW062 is converted to JU874.
- KG433 is converted to JU874 or ML440 and conjugate.
- JU874 is converted to MA576 and conjugate, which then leads to MX828.
- MX828 is converted to MX829.
- MX828-sulfate is converted to MX829-sulfate.
- DPX-MP062 is converted to KB687, which leads to Continue.
- MS211 is converted to MU716.

[] Proposed intermediates

Figure B.6.1.1-1
Proposed metabolic pathway of DPX-MP062 in the rat (continued)



III. CONCLUSION

DPX-MP062 was readily absorbed following oral dosing. Commensurate with absorption was extensive metabolism and excretion of metabolised dose in urine and faeces. Some parent compound was excreted in

faeces but not in urine. The results of the study with DPX-MP062 were similar to those obtained from rats dosed with racemic DPX-JW062. A slightly greater amount of the DPX-MP062 dose appeared to be retained by tissues, particularly fat. Other than the differences for total radioactive residues observed in fat and faeces, the pharmacokinetics and overall metabolism of indanone-1-¹⁴C-radiolabel DPX-MP062 was qualitatively very similar to that observed for the corresponding indanone-1-¹⁴C-radiolabel for DPX-JW062.

Previous evaluation:	Submitted for the purpose of renewal
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Report: CA 5.1.1/01 [REDACTED] (2000); ¹⁴C-DPX-JW062 (a racemix mixture of DPX-KN128 and IN-KN127): Metabolism in the rat

DuPont Report No.: HLR 283-96, Supplement No. 1

Guidelines: Directive 87/302/EEC Part B (1987), 59 NohSan No. 4200; **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 283-96, Supplement No. 1

GLP: No

In this supplement to the study HLR 283-96 provided in the original monograph, no additional experiments were performed. The information presented is a further evaluation of data collected concurrently with the *in vitro* microsomal metabolism data originally summarised and reported in Appendix D of DuPont HLR 283-96. These data were not generated in compliance with GLP.

I. MATERIALS AND METHODS

A. MATERIALS

1. Radiolabeled test material: ¹⁴C DPX-JW062
Lot/Batch #: [Indanone-1-¹⁴C]DPX-JW062: HOTC: 428
Radiochemical purity: 98.2%
Description: White solid
CAS#: 144171-61-9
Specific activity: 45.1 µCi/mg
Stability of test compound: The test material was stable for at least 7 days at room temperature.
2. Vehicle and/or positive control:

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
29-March-1994 to 08-February-2000

2. Microsomal analysis

Rat liver microsomes were derived from male and female CrI:CD®(SD)BR rats. For the calculations presented below, only data collected from incubations involving 5 µM [indanone-1-¹⁴C]DPX-JW062 were used based on the requirement for this approach that the analysis be done at a low and presumably non-saturating substrate concentration (Obach *et al.*, 1997; Carlile *et al.*, 1998). The incubations with [indanone-1-¹⁴C]DPX-JW062 were prepared in single vials, started by the addition of NADPH cofactor, and quenched at either 10, 20, or 40 minutes with cold acetonitrile. An incubation of DPX-JW062 and male rat liver microsomes with no added cofactor (NADPH, control) showed no degradation of the parent compound.

3. Description of analytical procedures

The mixtures were centrifuged and the supernatant was concentrated using C18 solid phase extraction (SPE). High performance liquid chromatography (HPLC) analyses were conducted using a Hewlett-Packard HP 1090 Series HPLC with a photodiode detector. Radioactive peaks were detected using a Ramona 92 or Ramona 2000 Flow detector using glass bead solid detector cells (Raytest USA). Metabolites were identified by comparison of retention times with authentic standards, and, when isolated, by LC/MS.

The percent peak areas for DPX-JW062 were used to calculate the decline in concentration as a function of time. Intrinsic clearance by liver microsomes (Cl_{int}) was calculated by two methods published in the literature using either the half-life or AUC of the *in vitro* substrate concentration time course data. The half-life (t_{1/2}) and AUC values were obtained using the commercially available pharmacokinetics analysis software WinNonlin™ Professional (Version 3.0, Pharsight Inc., Mountain View, California).

II. RESULTS AND DISCUSSION

A. MICROSOMAL ANALYSIS

Pharmacokinetics

Microsomes from both sexes showed a decline in the concentration of DPX-JW062. The rate of decline in male rat microsomal suspensions (t_{1/2} = 25.5 min) was faster than in female suspensions (t_{1/2} = 67.6 min). The corresponding AUC (nmol × min/mL) for male rat microsomes was 137.8 compared with 500.6 for female rat liver microsomes (Table 6.1.1-5). These values were used to estimate intrinsic hepatic clearance. The range of estimates for intrinsic hepatic clearance (mL/min/kg body weight) of DPX-JW062 in the male rat (26.2 to 35.0 mL/min/kg body weight) were approximately 2.2 to 3.0 times faster than in female rats (11.6 to 11.9 mL/min/kg body weight; Table 6.1.1-5).

Table B.6.1.1-5
Intrinsic hepatic clearance of DPX-JW062 for male and female rats

Parameter	Male	Female
Initial substrate (nmol)	20	20
Liver/body weight (g/kg bw)	37.7	36.6
Reaction protein concentration (mg/mL)	1.76	1.42
Reaction volume (mL)	4.0	4.0
Liver protein (mg/g liver) ^a	45	45
Half-life, t _{1/2} (min) ^b	25.5	67.6
Area-under-the-curve (nmol × min/mL) ^b	137.8	500.6
Intrinsic clearance (mL/min/mg protein)	0.01545 ^c	0.00722 ^c
	0.02062 ^d	0.00703 ^d
Intrinsic hepatic clearance (mL/min/kg bw)	26.2 ^c	11.9 ^c
	35.0 ^d	11.6 ^d

^a Houston, 1994

^b From WinNonlin™ program

^c Calculation according to Obach *et al.*, 1997

^d Calculation according to Carlile *et al.*, 1998

Metabolism

The profile of metabolites over the 40-minute incubation period showed a clear difference between sexes. The regioisomeric hydroxylation of DPX-JW062 (A3 and A4) was the primary *in vitro* pathway in male microsomal incubations. In female microsomal incubations, A3 and A4 were formed to a much lesser extent. The metabolite IN-JT333 was quantifiable in female microsomes, but for the most part was formed in only trace amounts by the male microsomes, and it was not quantifiable in this experiment. IN-KG433

(A2), another minor *in vitro* microsomal metabolite of DPX-JW062, was not quantifiable in this experiment. The proposed metabolic pathway of DPX-JW062 in rat liver microsomes is shown in Figure B.6.1.1-6.

Table B.6.1.1-6
Summary of DPX-JW062 time course and metabolite profile from liver microsomal incubations with 5 μ M [Indanone-1- 14 C]DPX-JW062

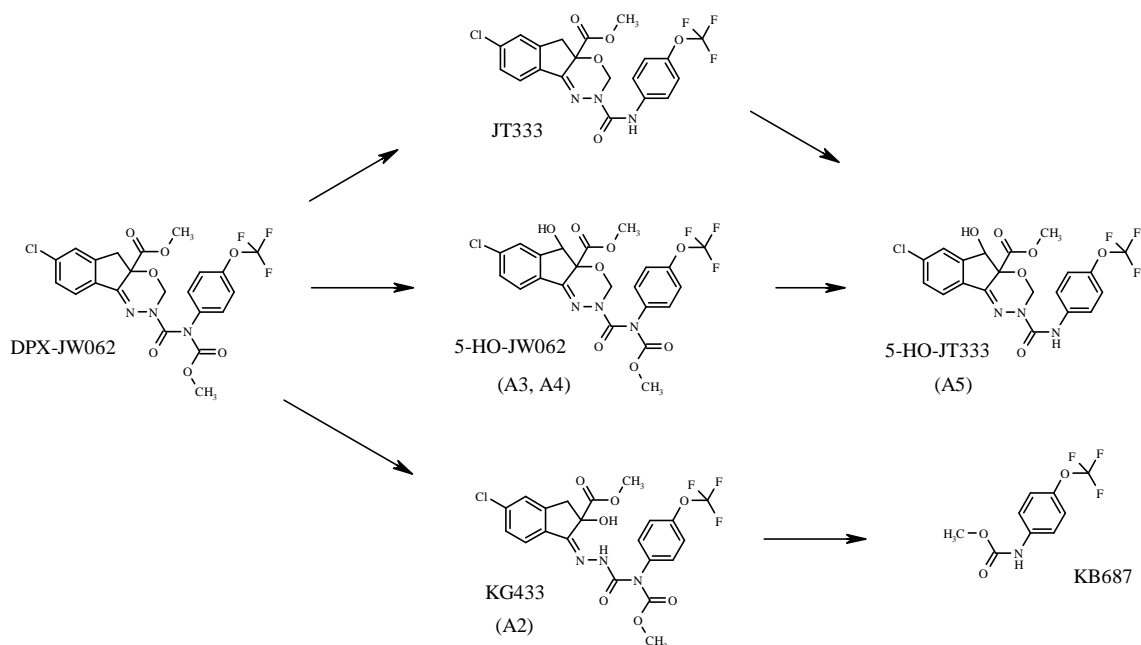
Sex	Incubation time (min)	DPX-JW062 concentration (μ M) ^a	Analyte and radiochromatogram peak area percent			
			DPX-JW062	5-HO-DPX-JW062, A3	5-HO-DPX-JW062, A4	IN-JT333
Male	0	5.00 ^b	100	— ^c	—	—
	10	1.95	39.0	4.3	44.7	—
	20	2.19	43.9	11.5	40.1	—
	40	1.39	27.7	11.7	53.5	—
Female	0	5.00 ^b	100	—	—	—
	10	4.77	95.4	—	—	4.6
	20	4.18	83.7	—	—	10.1
	40	3.37	67.3	—	4.9	10.2

^a Concentrations used for clearance calculations based on proportion of DPX-JW062 peak area measured in microsomal suspension.

^b Initial concentration equivalent to 100% peak area obtained from male microsomal control incubated without NADPH for 20 minutes.

^c Not detected or quantifiable based on 3 \times radiochemical background.

Figure B.6.1.1-2
Proposed *in vitro* metabolic pathway of DPX-JW062 rat liver microsomes



III. CONCLUSION

The *in vitro* metabolism experiments help define the pathway for the initial metabolism of DPX-JW062, and the *in vivo* experiments indicate that these initial metabolites undergo further extensive biotransformation and excretion. In the *in vitro* experiment, liver microsomes from male rats produced predominately hydroxylated DPX-JW062 products whereas microsomes from female rats formed mainly IN-JT333. IN-KG433 is another direct product of DPX-JW062 microsomal metabolism that was identified previously in HLR 283-96 study, but it was not produced in quantifiable amounts in the chromatograms used for this supplement. All three reactions most likely occur in the livers of male and female rats, and they are assumed to be the components of the DPX-JW062 clearance measured in this *in vitro* experiment. The results suggest that the rate of hydroxylation contributes to an estimate of DPX-JW062 clearance that is about twice as fast in male rats compared with female rats. Based on the principles of *in vitro* metabolism, it can be surmised that the products formed in the liver would appear in the systemic circulation and undergo further distribution, metabolism, and/or direct elimination. The greater amount of IN-JT333 formed by the female rat would explain the greater burden of total ¹⁴C-residues in the plasma and fat of this sex. Subsequent redistribution of IN-JT333 from the fat would contribute to the longer half-life of total ¹⁴C-residues in the plasma of female rats compared with the male rats. Furthermore, the sex difference observed in the *in vitro* metabolism experiments, specifically the greater production of IN-JT333 by female rats, is consistent with the profile of metabolites excreted in the faeces following oral gavage dosing. These factors are likely responsible for the greater responsiveness of female rats to the high-dose toxicity of DPX-JW062.

Comparative *in vitro* metabolism study:

A comparative *in vitro* metabolism study using human and animal materials is required according to Regulation (EU) No 283/2013. No study was provided by the applicant. The following justification was submitted:

*“This study was not conducted since an established testing guideline is currently not available. According to SANCO/10181/2013 –rev. 2.1 13 May 2013, Section 4, in cases where “...agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as not test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02.” In addition, as described in CA 5.8.1, study report DuPont 12062, Revision 1, the hemolytic potential of the metabolite IN-MT713 was evaluated in rats, dogs, and humans. In this study, rats were determined to be the most sensitive species for the most sensitive endpoint, hemolysis. Therefore, since guidelines for an *in vitro* comparative metabolism study are not yet available, and since an existing study (DuPont 12062) provides data regarding the critical endpoint in the most sensitive species, an *in vitro* comparative metabolism study has not been conducted at this time.”*

The RMS is of the opinion that the waiving cannot be accepted based on the argument that no test methods or guidance documents are available. Indeed, these types of studies are widely used for certain chemicals (e.g. pharmaceuticals) and protocols of such studies are publicly available.

It is considered that a comparative *in vitro* metabolism study should have been performed for indoxacarb with at least rat, dog and human materials. The aim of this study would be to determine the relevance of the toxicological animal data for risk assessment.

B.6.1.2. Absorption, distribution, metabolism and excretion by other routes

No study was submitted.

B.6.2. ACUTE TOXICITY

Acute toxicity studies, except by inhalation route, were conducted with DPX-KN128 (99:1) and the results are presented below. Acute toxicity studies were also performed with DPX-MP062 (enantiomeric reaction mass 75:25 S:R) or DPX-JW062 (racemic mixture).

Table B.6.2-1
Summary of acute toxicity data for indoxacarb (DPX-KN128, DPX-MP062 and DPX-JW062)

Acute Endpoint	DPX-KN128 (99:1)	DPX-MP062 (75:25)	DPX-JW062 (50:50)
Oral LD ₅₀ (mg/kg bw) Rat	179 (F) ████ 1997 - HLO-1997-00055	268 (F) ████ 1996a - HLR 910-96	-
Dermal LD ₅₀ (mg/kg bw) Rat	>5000 ████ 2003 - DuPont-13019*	>5000 ████ 1996b - HLR 798-96	-
Inhalation LC ₅₀ (mg/L/4 hours) Rat	-	-	4.2 (F) ████ 1995 - HLR 70-95
Skin irritation Rabbit	Not irritating ████ 2003 - DuPont-13164*	Not irritating ████ 1997a - HLR 589-96	-
Eye irritation Rabbit	Not irritating ████ 2003 - DuPont-13020*	Not irritating ████ 1997b - HLR 588-96	-
Skin Sensitization Guinea pig Maximisation test	Sensitizer ████ 2003 - DuPont-13018*	Sensitizer ████ 1996 - HLO 388-96	-
Buehler test (3- induction)	Not sensitizer ████ 2006 - DuPont-18915*	-	-
Phototoxicity Mouse fibroblast cell line Balb/3T3	Not phototoxic Markell, 2015 - DuPont-43522*	-	-

* Studies newly submitted

Acute oral toxicity study with DPX-KN128 (99:1) resulted in a LD₅₀ of 179 mg/kg bw for female rats and 843 mg/kg bw for male rats. The difference in susceptibility between sexes can be explained by the ADME studies where it is shown that the formation of the metabolite IN-JT333 (LD₅₀ of 39 mg/kg bw in females, see B.6.8.1) was the major route of metabolism of DPX-JW062 (50:50) in females, whereas in males, 5-HO-JW062 was the most important metabolite. DPX-KN128 (99:1) is therefore classified as Acute Tox. Cat. 3 H301 ("Toxic if swallowed").

DPX-KN128 (99:1) is not acutely toxic via the dermal route of exposure.

No acute toxicity study by inhalation was conducted with DPX-KN128 (99:1). Acute toxicity study by inhalation was available for DPX-JW062 (racemic mixture). The results of the study with DPX-JW062 (50:50) showed that the LC₅₀ is greater than 5.4 mg/L in males and equal to 4.2 mg/L in females. The absence of data on DPX-KN128 (99:1) was discussed during the ECHA Committee for Risk Assessment in 2011. In the CLH report, an acute inhalation study with a DPX-MP062 manufacturing used product (MUP) is available and showed a LC₅₀ above 5.5 mg/L. Nevertheless, this MUP contains only 70% DPX-MP062 (75:25) equivalent to approximately 52% DPX-KN128 (99:1) on an amorphous silicon dioxide carrier. Given the results of the acute oral toxicity studies performed on DPX-KN128 (99:1) and DPX-MP062 (75:25) showing LD₅₀ of the same order of magnitude for both compounds (the acute toxicity of the S-enantiomer (DPX-KN128) might be only slightly higher compared to the R-enantiomer), the results with the racemic mixture are considered also relevant for DPX-KN128 (99:1) (the LC₅₀ of DPX-KN128 (99:1) is not expected to be < 1 mg/L given that the LC₅₀ of the racemic mixture is 4.2 mg/L). Therefore, classification as Acute Tox. Cat. 4 H332 ("Harmful if inhaled") was considered appropriate (range 1-5 mg/L).

DPX-KN128 (99:1) was not irritating to skin and to the eyes.

DPX-KN128 (99:1) was found to be a skin sensitizer in a Magnusson and Kligman test but not in a Buehler test. Nevertheless, this Buehler test, using only 3 topical inductions, is not considered sensitive enough to detect skin sensitizers. Therefore, DPX-KN128 (99:1) is classified as Skin Sens. Cat. 1B H317 ("May cause an allergic skin reaction") according to Regulation (EC) No 1272/2008 and its 2nd ATP (category 1B assigned when ≥30% of animals responded at >1% induction dose).

As a conclusion, and in accordance to RAC opinion proposing harmonised classification and labelling of indoxacarb (2011) and Regulation (EU) No 944/2013 (5th ATP of Regulation (EC) No 1272/2008), DPX-KN128 (99:1) should be classified Acute Tox 3 H301, Acute Tox 4 H332 and Skin Sens 1B H317. It is noted that acute toxicity studies performed on DPX-KN128 (99:1) were not available in the CLH report (except acute oral

toxicity study). The RAC used the read-across approach to conclude. Nevertheless, in view of the results obtained in these newly submitted studies, the classification adopted by the RAC is confirmed.

DPX-KN128 (99:1) did not show phototoxic potential in the *in vitro* 3T3 NRU phototoxicity test.

B.6.2.1. Oral

Previous evaluation:	In DAR (2000)
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CA 5.2.1/01

Report: [REDACTED] (1997); Acute oral toxicity study with DPX-KN128 technical in male and female rats

DuPont Report No.: HLO-1997-00055

Guidelines: 92/69 ANNEX V, 401, 59 NOHSAN NO. 4200, 81-1; **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: WIL-189093

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb (DPX-KN128) technical
 Lot/Batch #: KN128-31
 Purity: 99.7%
 Description: Off-white crystalline solid
 CAS #: 173584-44-6
 Stability of test compound: In the absence of visible evidence to the contrary, the test substance was assumed to be stable under the conditions of administration.
2. Vehicle: Corn oil
3. Test animals
 Species: Rat
 Strain: Crl:CD[®](SD)BR
 Age at dosing: 51–58 days (males); 72–79 days (females)
 Weight at dosing: 209–263 g for males; 206–227 g for females
 Source: [REDACTED]
 Acclimation period: At least 7 days
 Diet: PMI Feeds, Inc.[®], Certified Rodent LabDiet[®] (#5002), *ad libitum* except when fasted
 Water: Tap water, *ad libitum*
 Housing: Animals were housed singly in stainless steel, wire-mesh-bottom cages suspended above cage boards. Enrichment (e.g., nylabone or nestlet) was placed in each cage.
4. Environmental conditions
 Temperature: 71.6–73.0°F
 Humidity: 34.1–48.4%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

20-December-1996 to 12-February-1997

2. Animal assignment and treatment

Doses of 400, 640, 1000, and 1953 mg/kg body weight for males and 123, 192, 300, and 400 mg/kg body weight for females were selected for this study. Following an overnight fast (approximately 18–20 hours), five male and five female rats per dose group were given a single dose of indoxacarb by gastric intubation. The test substance was administered in corn oil at a volume of 10 mL/kg bw. Animals were observed for mortality and signs of illness, injury, or abnormal behaviour at approximately 1, 3, and 4 hours after dosing and twice daily thereafter for 14 days. The animals were observed for clinical signs at approximately 1, 3, and 4 hours after dosing and once each day thereafter. Animals were weighed on test Days -1, 0, 1, 3, 7, and 14. On test Day 14, surviving animals were euthanized, and all animals were necropsied to detect grossly observable evidence of organ or tissue damage.

3. Statistics

LD₅₀ values and slopes (with 95% confidence limits) were estimated or calculated by the method of Litchfield and Wilcoxon (Probit analysis).

II. RESULTS AND DISCUSSION

A. MORTALITY

While most of the deaths occurred during the first week of the study, there was a tendency for them to be somewhat delayed. Three rats died as late as day 8 and over 80% (19/23) of the deaths occurred on study days 4–8. There were 1/5, 4/5, 2/5, and 3/5 deaths for males in the 400, 640, 1000, and 1953 mg/kg dose groups, respectively. There were 1/5, 3/5, 4/5, and 5/5 deaths for female rats dosed in the 123, 192, 300, and 400 mg/kg dose groups, respectively. Details are provided in the following table.

Table B.6.2.1-1
Acute oral toxicity of indoxacarb: Doses, mortality/animals treated, oral LD₅₀

Dose (mg/kg bw)	Males ^a	Females ^a
123	NA ^b	1/5
192	NA	3/5
300	NA	4/5
400	1/5	5/5
640	4/5	NA
1000	2/5	NA
1953	3/5	NA
Calculated oral LD₅₀:	843 mg/kg bw*	179 mg/kg bw 95% confidence limits: 121-265 mg/kg bw

^a Number of animals which died/number of animals in dose group

^b NA = not applicable

* there was no linear trend or dose-response and confidence limits were not defined

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity most often observed in male and female rats included various external matting/material/staining. Other clinical signs of toxicity observed in male and/or female rats included abnormal excretion (37/40), hypoactivity (28/40), ataxia (27/40), impaired righting reflex (13/40), tremors (5/40), hypothermia (body cool to touch) (4/40), laboured respiration (2/40), clear ocular discharge (1/40), frothy discharge around mouth (1/40), hair loss (1/40), and mechanical trauma to the right forepaw and

distal tail (1/40). With the exception of five rats, all surviving animals appeared normal by Day 14 or earlier.

C. BODY WEIGHT

Losses (0.5–11%) in body weights were noted in surviving animals in all dose groups at various intervals during the first week of the study. All surviving animals that lost weight between Days 0 and 7 gained weight during the second week and surpassed their initial (Day 0) body weights.

D. NECROPSY AND GROSS PATHOLOGY

Gastrointestinal abnormalities were noted for nine rats that were found dead. There were single occurrences of small seminal vesicles and testes and clear fluid uterine contents. Twenty animals that died had various external findings including three rats had dark red area(s) on the lungs and single animals each had various matting (yellow, red) and hair loss on the urogenital area. There were no other gross necropsy findings for animals that died during the study. Pulmonary and external findings were noted for three surviving rats each. There were no other findings at the scheduled necropsy.

III. CONCLUSION

The acute oral LD₅₀ for indoxacarb in fasted rats was 843 and 179 mg/kg of body weight for males and females, respectively.

In accordance with Regulation (EC) No. 1272/2008, indoxacarb is classified in Acute Toxicity Category 3 (H301).

Previous evaluation:	In DAR (2000)
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Report: [REDACTED] (1996a): Acute Oral Toxicity Study with DPX-MP062 Technical (Approximately 75% DPX-KN128 and 25% IN-KN127) in Male and Female Rats. DuPont Report No. HLR 910-96. Published: No.

Guidelines: EEC B.1; OECD 401; US EPA 81-1; MAFF Japan 1985. Deviations: The dosing preparations used in this study were not analysed for stability, homogeneity, or accuracy of concentration. The procedures used by trained staff to prepare the dosing preparations ensured that this did not affect the validity of the study.

GLP: Yes. Certified Laboratory: No (laboratories in the USA are not certified by any governmental agency but are subject to regular GLP inspections from the US EPA).

Materials and methods:

DPX-MP062 technical (purity 94.5%), in corn oil (500 mg/ml), was administered by single gavage to fasted male and female CrI:CD[®](SD)BR rats at concentrations of 1000, 3000, or 5000 mg/kg. In addition, groups of 10 female rats each were dosed at 100 or 250 mg/kg. Surviving animals were observed for clinical signs of toxicity and mortality for up to 24 days after dosing.

Findings:

The results of the oral LD₅₀ study in rats with DPX-MP062 are summarised in the following table.

Table B.6.2.1-2
Acute oral toxicity of DPX-MP062 in Rats: clinical signs and mortality

Dose (mg/kg bw)	Toxicological results ^a	Duration of signs	Time of death	LD ₅₀ (14-24 days)
Male rats				
1000	0/3/5	8 - 15 d	-	
3000	5/5/5	2 - 11 d	6 - 12 d	1730 mg/kg bw
5000	5/5/5	1 - 10 d	4 - 11 d	
Female rats				
100	0/3/10	4 - 15 d	-	
250	7/9/10	1 - 22 d	10 - 21 d	
1000	4/5/5	2 - 17 d	9 - 18 d	268 mg/kg bw
3000	5/5/5	2 - 11 d	4 - 12 d	
5000	5/5/5	1 - 11 d	2 - 12 d	

^a Number of animals which died/number of animals with clinical signs/number of animals used

Clinical signs: Clinical signs of toxicity most often observed in male and female rats included ataxia, ruffled fur, hunched over posture, and stained perineum. Other clinical signs of toxicity observed in male and/or female rats included general spasms, pallor, ocular discharge, immobile, lethargy, piloerection, wet perineum, tremors, salivation, head or facial stains, weakness, leaning to the right, high carriage, splayed rear legs, convulsions, gasping, moribundity, and wet face. Except for rats dosed at 3000 and 5000 mg/kg, these signs tended to occur after day 5 of the recovery period.

Gross necropsy: No test substance-related gross lesions were observed at necropsy.

Conclusion: The oral LD₅₀ for DPX-MP062 in rats was 1730 and 268 mg/kg body weight for males and females, respectively.

B.6.2.2. Dermal

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.2.2/01

Report: [REDACTED] (2003); Indoxacarb (DPX-KN128) technical: Acute dermal toxicity study in rats

DuPont Report No.: DuPont-13019

Guidelines: 59 NohSan No. 4200 (1985), EEC Method B.3. (1992), OPPTS 870.1200 (1998), OECD 402 (1987) **Deviations:** Clinical observations were not done on test days 6-9 and 12 and the day 7 body weights were not taken.

Testing Facility: [REDACTED]

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb (DPX-KN128) technical
Lot/Batch #: KN128-098
Purity: 95.47%
Description: White solid
CAS#: 173584-44-6
Stability of test compound: Not determined. Test sample was applied promptly to the skin after moistening with vehicle.
2. Vehicle and/or positive control: Deionised water
3. Test animals
Species: Rat
Strain: CrI:CD[®](SD)IGS BR
Age at dosing: Approximately 7 weeks old (males)
Approximately 11 weeks old (females)
Weight at dosing: 271.2–294.7 g for males; 223.4–244.5 g for females
Source: XXXXXXXXXX
Acclimation period: 6 days
Diet: PMI[®] Nutrition International, LLC Certified Rodent LabDiet[®] 5002, *ad libitum*
Water: Tap water, *ad libitum*
Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
Temperature: 18–26°C
Humidity: 30–70%
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
25-June-2003 to 04-September-2003
2. Animal assignment and treatment
Approximately 24 hours before dosing, the fur of each animal was closely shaved to expose the back from the scapular to the lumbar region (approximately 10% of each animal's body surface area). A single dose of indoxacarb, moistened with approximately 2 mL of deionised water was applied to the intact skin (approximately 37 square centimetres) of 5 males and 5 females per dose group. The application site was covered with a gauze dressing, stretch gauze bandage, and self-adhesive bandage. After 24 hours, excess test substance was washed from the dorsal skin of each animal with warm water and the skin was dried with a paper towel. Animals were observed for mortality and signs of illness, injury, or abnormal behaviour daily. The animals were observed for clinical signs on test Days 0-5, 10-11, and 13-14. Observations for dermal irritation were made on Test Days 1, 2, 5, 13, and 14. Dermal effects were scored according to the Draize Scale. The animals were weighed on Test Days 0, 1, and 14. On Test Day 14, surviving animals were euthanised and all animals were necropsied to detect grossly observable evidence of organ or tissue damage or dysfunction.
3. Statistics
The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities occurred. Details are provided below.

Table B.6.2.2-1
Acute dermal toxicity of indoxacarb: Doses, mortality/animals treated, dermal LD₅₀

Dose (mg/kg bw)	Males ^a	Females ^a	Combined ^a
5000	0/5	0/5	0/10
Dermal LD₅₀:	>5000 mg/kg bw	>5000 mg/kg bw	>5000 mg/kg bw

^a Number of animals which died/number of animals in dose group

B. CLINICAL OBSERVATIONS

Ocular discharge was observed in eight rats, nasal discharge was observed in one rat, and staining was observed in four rats. One rat exhibited hair loss.

C. BODY WEIGHT

Body weight loss of approximately 5-9% of initial weight occurred in the rats the day after dosing. All rats gained weight by study termination (14 days after dosing).

D. NECROPSY AND GROSS PATHOLOGY

No gross lesions were present in the rats at necropsy.

III. CONCLUSION

The dermal LD₅₀ for indoxacarb was greater than 5000 mg/kg body weight for both male and female rats. In accordance with the provisions of Regulation (EC) No. 1272/2008, classification of indoxacarb by the dermal route is not required.

Previous evaluation:	In DAR (2000)
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Report: [REDACTED] (1996b): Acute Dermal Toxicity Study with DPX-MP062 Technical (Approximately 75% DPX-KN128 and 25% IN-KN127) in Rats. DuPont Report No. HLR 798-96. Published: No.

Guidelines: EEC B.3; OECD 402; US EPA 81-2; MAFF Japan 1985. Deviations: None.

GLP: Yes. Certified Laboratory: No (laboratories in the USA are not certified by any governmental agency but are subject to regular GLP inspections from the US EPA).

Materials and methods:

A single 5000 mg/kg body weight dose of DPX-MP062 technical (purity 94.5%), moistened with approximately 0.3 ml of deionized water, was applied to the shaved, intact skin of 5 male and 5 female CrI:CD[®](SD)BR rats. The application site was occluded for 24 hours, after which, the test substance was removed. The rats were observed for clinical signs and mortality for 14 days following application. Rats were necropsied and examined for gross pathological changes at the end of the 14-day recovery period.

Findings:

The results of the dermal LD₅₀ study in rats with DPX-MP062 are summarised in the following table.

Table B.6.2.2-2
Acute dermal toxicity of DPX-MP062 in rats: Clinical signs and mortality

Dose (mg/kg bw)	Toxicological results ^a	Duration of signs	Time of death	LD ₅₀ (14 days)
Male rats				
5000	0/0/5	-	-	>5000 mg/kg bw
Female rats				
5000	0/0/5	-	-	>5000 mg/kg bw

^a Number of animals which died/number of animals with clinical signs (without considering local findings)/number of animals tested

Clinical signs: No deaths and no clinical signs of toxicity were observed during the study. Two male and 2 female rats exhibited a red ocular discharge 1 day after application. However, this was attributed to stress associated with body wrappings and collars, which were on the rats for approximately 24 hours.

Local findings: The rats exhibited no dermal irritation throughout the study.

Gross necropsy: No gross lesions were detected.

Conclusion: The dermal LD₅₀ for DPX-MP062 in rats was greater than 5000 mg/kg body weight.

B.6.2.3. Inhalation

Previous evaluation:	In DAR (2000)
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CA 5.2.3

Report: [REDACTED] (1995); Inhalation median lethal concentration (LC₅₀) study with DPX-JW062-112 in rats

DuPont Report No.: HLR 70-95

Guidelines: EEC 92/69 Method B2, 59 Nohsan No. 4200, USEPA 81-3, OECD 403 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 70-95

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:
Lot/Batch #: DPX-JW062
Purity: JW062-112
Description: 94.76%
CAS #: Off-white solid (powder)
Stability of test compound: 144171-61-9
Stable in dosing vehicle (air) based on analysis of active ingredient.
2. Vehicle: DPX-JW062-112 was suspended in air
3. Test animals
Species: Rat
Strain: Crl:CD®BR
Age at dosing: Approximately 7 to 8 weeks old
Weight at exposure: 260–299 g for males; 168–230 g for females
Source: XXXXXXXXXX
Acclimation exposure: 6 days
Diet: Purina Certified Rodent Chow® #5002, *ad libitum*,
Water: Tap water, *ad libitum*
Housing: During the test period, rats were housed either singly or in pairs (sexes separate) in 8" × 14" × 8" suspended, stainless steel, wire-mesh cages
4. Environmental conditions
Temperature: 23 ± 2°C
Humidity: 50 ± 10%
Air changes: Not recorded
Photoperiod: 12-hour light/12-hour dark cycle

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
29 November 1994 to 03 February 1995
2. Animal assignment and treatment
Two groups of five male and five female Crl:CD®BR rats each and three groups of ten female Crl:CD®BR rats each were exposed (nose-only) to atmospheres of DPX-JW062 for single, four-hour exposure periods. Male and female rats were exposed to chamber concentrations of 3.3 or 5.4 mg/L DPX-JW062, and female rats only were exposed to chamber concentrations of 0.45, 2.3, or 4.0 mg/L DPX-JW062. Test atmospheres were generated by suspension of DPX-JW062 particulate in air. Gravimetric analysis was used to measure dust concentration, and filters were analysed to verify the percentage of active ingredient. During a 14-day recovery period, rats were weighed and observed for clinical signs of toxicity. All rats found dead and all rats that survived until the end of the recovery period underwent gross pathological examination.
3. Generation of the test atmosphere/chamber description
Atmospheres of DPX-JW062 were generated by suspending the test substance in air with a Fluid Energy Processing Model 00 Jet-0-Mizer Jetmill. The test substance was metered into the jetmill with a K-Tron Model T-20 Twin Screw Volumetric Feeder controlled by a K-Tron Model PCM23401A Speed Controller. Nitrogen was introduced into the bin feeder to prevent moisture absorption by the test substance. Filtered, high-pressure air introduced into the jetmill carried the resulting atmosphere through a 2-L glass cyclone elutriator and into the 34-L glass exposure chamber. A glass baffle was positioned immediately inside the exposure chamber to promote uniform distribution of the test substance. The chamber concentration of DPX-JW062 was controlled by varying the feed rate of test substance to the jetmill. Each chamber atmosphere was exhausted through a high-capacity dust filter and an MSA particulate filter prior to discharge into the fume hood.

The atmospheric concentration of DPX-JW062 was determined by gravimetric analysis at approximately 30-minute intervals during the exposure. Known volumes of chamber atmospheres were drawn from the reference sampling port through a 25 mm filter cassette that contained a pre-weighed Gelman glass fiber (Type A/E) filter. The filters were weighed on a Cahn Model C-30 Microbalance. The atmospheric concentration of DPX-JW062 was calculated from the difference in the pre- and post-sampling filter weights divided by the volume of chamber atmosphere sampled.

Table B.6.2.3-1
Acute inhalation toxicity of DPX-JW062: Exposure atmosphere characteristics

Parameter	Value
Flow rate	38 L/min
Nominal concentration(s) ^a	Gravimetric concentration(s) ^b
0.45 mg/L	1.7 ± 2.4 mg/L
2.3 mg/L	2.1 ± 2.1 mg/L
3.3 mg/L	2.4 ± 2.2 mg/L
4.0 mg/L	2.2 ± 2.2 mg/L
5.4 mg/L	3.2 ± 2.3 mg/L
Particle size MMAD ^c /GSD ^d	2.3 µm/2.2
Particles <1 µm (% w/w)	8–22%
Particles <3 µm (% w/w)	47–77%
Particles <10 µm (% w/w)	91–99%

^a Theoretical atmospheric concentration calculated when the total amount of test substance delivered to the chamber is divided by the total airflow for the exposure.

^b Mean(s) ± SD were gravimetrically determined from chamber samples.

^c MMAD = mass median aerodynamic diameter

^d GSD = geometric standard deviation

4. Statistics

The survival data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

Exposure to 5.4 or 3.3 mg/L DPX-JW062 produced 60% (3/5) and 40% (2/5) mortality in female rats, respectively, and no mortality in male rats (0/5 and 0/5 respectively). A sex-related difference was apparent which indicated female rats were more sensitive to DPX-JW062, and subsequent exposures were conducted with female rats only. Female rats were exposed to concentrations of 4.0, 2.3, or 0.45 mg/L DPX-JW062. Mortality was 40% (4/10), 40% (4/10), and 0% (0/10) for the respective groups of female rats. All rat deaths occurred 6-14 days following exposure to the test substance. No additional exposures were conducted.

B. CLINICAL OBSERVATIONS

During exposures, rats did not exhibit any clinical signs of toxicity. When rats were removed from the restrainers immediately following the exposure, clinical signs included compound-stained facial fur, nasal/ocular discharge, and wet/stained perineum or underbody. Clinical signs in female rats were observed until the end of the recovery period and included hair loss, nasal/ocular discharge, hunched posture, abnormal gait/mobility, lethargy, convulsions, moribundity, weakness, stained fur (face, back, and hind quarters), stained/wet perineum or underbody, head tilt, high/low carriage, and abnormal biting behaviour. Clinical signs observed in male rats were observed until the end of the recovery period and included nasal/ocular discharge, stained/wet perineum, and stained fur (back).

C. BODY WEIGHT

Surviving female rats exhibited slight to severe weight loss up to ten days following exposure to 5.4, 4.0, 3.3, or 2.3 mg/L DPX-JW062; body weight losses ranged from 0.1 to 27 grams (0.1 to 12% of previous body weight). Slight to moderate weight loss was observed in surviving female rats up to two days following exposure to 0.45 mg/L DPX-JW062; body weight losses ranged from 0.7 to 17 grams (0.4 to 10% of previous body weight). Male rats exhibited slight to severe weight loss up to two days following exposure to 5.4 or 3.3 mg/L DPX-JW062; body weight losses ranged from 2.9 to 22 grams (1.0 to 8.4% of previous body weight). Although some surviving rats had transient body-weight loss during the remainder of the recovery period, all surviving rats experienced an overall weight gain by the end of the 14-day recovery period.

D. NECROPSY AND GROSS PATHOLOGY

All exposed rats were subjected to a gross pathological examination following their death or 14 days after exposure. No abnormalities or compound-related gross lesions were detected at necropsy. No target organ was identified in any rats exposed to DPX-JW062.

III. CONCLUSION

The inhalation LC₅₀ for DPX-JW062 was greater than 5.4 mg/L for male rats; the LC₅₀ for female rats was 4.2 mg/L.

On the basis of the LC₅₀ obtained for female rats in this study, and according to Regulation (EC) No 1272/2008, DPX-JW062 is classified as Acute Tox 4 H332.

B.6.2.4. Skin irritation

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.2.4/01

Report: [REDACTED] (2003); Indoxacarb (DPX-KN128) technical: Acute dermal irritation study in rabbits

DuPont Report No.: DuPont-13164

Guidelines: EEC Method B.4. (1992), OPPTS 870.2500 (1998), OECD 404 (1992) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: DuPont-13164

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-------------------------------------|---|
| 1. Test material: | Indoxacarb technical |
| Lot/Batch #: | KN128-098 |
| Purity: | 95.47% |
| Description: | White solid |
| CAS #: | 173584-44-6 |
| Stability of test compound: | Not determined. Test sample was applied promptly to the skin after moistening with vehicle. |
| 2. Vehicle and/or positive control: | Deionised water |
| 3. Test animals | |
| Species: | Rabbit |
| Strain: | New Zealand White |
| Age at dosing: | Young adult |
| Weight at dosing: | 2682–2898 g for males |
| Source: | |
| Acclimation period: | 6 days |
| Diet: | 125 g daily of PMI [®] Nutrition International, LLC Certified Rodent LabDiet [®] 5322 |
| Water: | Tap water, <i>ad libitum</i> |
| Housing: | Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards. |
| 4. Environmental conditions | |
| Temperature: | 16–22°C |
| Humidity: | 30–70% |
| Air changes: | Not recorded |
| Photoperiod: | Alternating 12-hour light and dark cycles |

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
11-June-2003 to 11-August-2003
2. Animal assignment and treatment
Indoxacarb was applied as a single 0.5 g dermal dose to the shaved intact skin of 3 male young adult New Zealand White rabbits. One rabbit was initially tested. The remaining two rabbits were treated if there was no severe irritant response in the first animal. The test substance, moistened with approximately 0.6 mL of deionised water was applied to a 6 cm² area of skin. The application area was covered with a 1-inch, 2-ply gauze square that was held in place with non-irritating tape and covered with porous tape for a semi-occlusive dressing. The rabbits were exposed to the test substance for 4 hours after which the test substance was removed. Test sites were evaluated using the Draize (1959) criteria as described in the report, for signs of dermal irritation 1, 24, 48, and 69/72 hours after test substance removal. The rabbit treated initially was also evaluated immediately after test substance removal.

II. RESULTS AND DISCUSSION

No oedema or erythema was observed. There were no test substance-related body weight effects or clinical signs noted.

Table B.6.2.4-1
Individual dermal irritation scores according to Draize (1959)

Time	Erythema			Oedema		
	36107 ^a	36127 ^a	36128 ^a	36107 ^a	36127 ^a	36128 ^a
1 h	0	0	0	0	0	0
24 h	0	0	0	0	0	0
48 h	0	0	0	0	0	0
69/72 h	0	0	0	0	0	0

^a Animal number

Table B.6.2.4-2
Mean individual dermal irritation scores according to Draize (1959)

Animal number	Erythema ^a	Oedema ^a
36107	0	0
36127	0	0
36128	0	0

^a Mean of 24 h, 48 h, and 72 h readings

III. CONCLUSION

Based on the mean degree of skin reaction observed at 24 to 72 hours, and according to the provisions of Regulation (EC) No. 1272/2008, classification of indoxacarb is not required.

Previous evaluation:	In DAR (2000)
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Report: [REDACTED] (1997a): Primary Dermal Irritation Study with DPX-MP062 Technical (Approximately 75% DPX-KN128 and 25% IN-KN127) in Rabbits. DuPont Report No. HLR 589-96. Published: No.

Guidelines: EEC B.4; OECD 404; US EPA 81-5; MAFF Japan 1985. Deviations: None.

GLP: Yes. Certified Laboratory: No (laboratories in the USA are not certified by any governmental agency but are subject to regular GLP inspections from the US EPA).

Materials and methods:

DPX-MP062 technical (purity 94.5%) was applied as a single 0.5 g dermal dose to 6 male New Zealand White rabbits. The test substance was applied to a 6 cm² clipped area of skin and held in place for 4 hours with a semi-occlusive dressing. Test sites were evaluated for signs of dermal irritation for up to 72 hours after exposure.

Findings:

The results of the dermal irritation study in rabbits with DPX-MP062 are summarised in the following table.

Table B.6.2.4-3
Dermal irritation of DPX-MP062 in Rabbits: Individual dermal responses

Animal No			30966	30967	30972	30974	30975	30976	Mean
Body weight (g)			2492	2410	2359	2530	2612	2654	Scores
	1h	E	0	0	0	0	0	0	

Draize ^b grade after		O	0	0	0	0	0	0	0
	24h	E	0	0	0	0	0	0	0
		O	0	0	0	0	0	0	0
	48h	E	0	0	0	0	0	0	0
		O	0	0	0	0	0	0	0
	72h	E	0	0	0	0	0	0	0
		O	0	0	0	0	0	0	0
Overall all average of means								E	0
								O	0

E = erythema and eschar formation; O = oedema formation

^a For each lesion, calculated as mean score at (24 hours + 48 hours + 72 hours)/3

^b Draize, 1959

Conclusion: Following an exposure period of 4 hours, DPX-MP062 was not irritating to the skin.

B.6.2.5. Eye irritation

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.2.5/01

Report: [REDACTED] (2003); Indoxacarb (DPX-KN128) technical: Acute eye irritation study in rabbits

DuPont Report No.: DuPont-13020

Guidelines: EEC Method B.5. (1992), OECD 405 (2002), OPPTS 870.2400 (1996) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: DuPont-13020

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-------------------------------------|--|
| 1. Test material: | Indoxacarb technical |
| Lot/Batch #: | KN128-098 |
| Purity: | 95.47% |
| Description: | White solid |
| CAS #: | 173584-44-6 |
| Stability of test compound: | Not determined. Test sample was applied promptly to the skin after moistening with vehicle |
| 2. Vehicle and/or positive control: | Test material dosed as received (grounded to obtain a fine powder) |
| 3. Test animals | |
| Species: | Rabbit |
| Strain: | New Zealand White |
| Age at dosing: | Young adult |
| Weight at dosing: | 2914–3172 g for males |
| Source: | |
| Acclimation period: | 6 days |
| Diet: | PMI [®] Nutrition International, LLC Certified Rabbit LabDiet [®] (#5322), approximately 125 g per day |
| Water: | Tap water, <i>ad libitum</i> |
| Housing: | Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards. |
| 4. Environmental conditions | |
| Temperature: | 16–22°C |
| Humidity: | 30–70% |
| Air changes: | Not recorded |
| Photoperiod: | Alternating 12-hour light and dark cycles |

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
25-June-2003 to 04-September-2003
2. Animal assignment and treatment
A single dose of 44 or 45 mg (equivalent to 0.1 mL) of indoxacarb was administered into the lower conjunctival sac of the right eye of 3 male young adult New Zealand White rabbits. One rabbit was initially treated. The remaining 2 rabbits were treated if there was no severe irritant response in the first animal. The eyes were not rinsed after introduction of the test substance. The conjunctiva, iris, and cornea of each treated eye were evaluated for evidence of irritation approximately 1, 24, 48, and 72 hours following administration of the test substance using the Draize scale which is described in the report.

II. RESULTS AND DISCUSSION

Conjunctival redness (score of 1) in 2 rabbits was observed only at the 1 hour observation. No irritation was observed. There were no test substance-related body weight effects or clinical signs noted.

Table B.6.2.5-1
Individual eye irritation scores according to Draize (1959)

Cornea			
Animal no.	36121	36108	36116
1 hour	0	0	0
24 hours	0	0	0
48 hours	0	0	0
72 hours	0	0	0
Iris			
Animal no.	36121	36108	36116
1 hour	0	0	0
24 hours	0	0	0
48 hours	0	0	0
72 hours	0	0	0
Conjunctiva-redness			
Animal no.	36121	36108	36116
1 hour	1	1	0
24 hours	0	0	0
48 hours	0	0	0
72 hours	0	0	0
Conjunctiva-chemosis			
Animal no.	36121	36108	36116
1 hour	0	0	0
24 hours	0	0	0
48 hours	0	0	0
72 hours	0	0	0

Table B.6.2.5-2
Mean individual eye irritation scores according to Draize (1959)

Animal number	Corneal opacity ^a	Iritis ^a	Conjunctival redness ^a	Conjunctival chemosis ^a
36121	0.00	0.00	0.00	0.00
36108	0.00	0.00	0.00	0.00
36116	0.00	0.00	0.00	0.00

^a Mean of 24 h, 48 h, and 72 h readings.

III. CONCLUSION

Based on the mean degree of eye irritation observed at 24 to 72 hours, and according to the provisions of Regulation (EC) No. 1272/2008, classification of indoxacarb is not required.

Previous evaluation:	In DAR (2000)
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Report: [REDACTED] (1997b): Primary Eye Irritation Study with DPX-MP062 Technical (Approximately 75% DPX-KN128 and 25% IN-KN127) in Rabbits. DuPont Report No. HLR 588-96. Published: No.

Guidelines: EEC B.5; OECD 405; US EPA 81-4; MAFF Japan 1985. Deviations: None.

GLP: Yes. Certified Laboratory: No (laboratories in the USA are not certified by any governmental agency but are subject to regular GLP inspections from the US EPA).

Materials and methods:

A single dose of 48.0 mg of DPX-MP062 technical (purity 94.5%) was administered into the lower conjunctival sac of the right eye of 6 male New Zealand White rabbits. The eyes were not rinsed after introduction of the test substance. The conjunctiva, iris, and cornea of each treated eye were evaluated for evidence of irritation 1, 24, 48, and 72 hours following administration of the test substance.

Findings:

The results of the ocular irritation study in rabbits with DPX-MP062 are summarised in the following table.

Table B.6.2.5-3
Ocular irritation of DPX-MP062 in Rabbits: Individual ocular responses

Animal No	Tissue	Signs	DRAIZE ^a grades				Comment
			1h	24h	48h	72	
30988	Cornea	Opacity	2	0	0	0	
		Area	4	0	0	0	
	Iris	Iritis	1	0	0	0	
		Chemosis	2	1	1	0	
		Redness	2	2	1	0	
		Discharge	2	0	0	0	
30989	Cornea	Opacity	1	0	0	0	
		Area	4	0	0	0	
	Iris	Iritis	1	0	0	0	
		Chemosis	2	1	0	0	
		Redness	2	1	0	0	
		Discharge	2	0	0	0	
30990	Cornea	Opacity	1	0	0	0	
		Area	3	0	0	0	
	Iris	Iritis	1	0	0	0	
		Chemosis	2	0	0	0	
		Redness	2	1	0	0	
		Discharge	2	0	0	0	
30991	Cornea	Opacity	1	0	0	0	
		Area	2	0	0	0	
	Iris	Iritis	1	0	0	0	
		Chemosis	2	1	0	0	
		Redness	2	1	0	0	
		Discharge	2	0	0	0	
30992	Cornea	Opacity	1	2	0	0	
		Area	4	4	0	0	
	Iris	Iritis	0	1	0	0	
		Chemosis	2	2	1	0	
		Redness	2	3	2	0	
		Discharge	0	0	0	0	
30993	Cornea	Opacity	1	0	0	0	
		Area	4	0	0	0	
	Iris	Iritis	1	0	0	0	
		Chemosis	2	1	0	0	
		Redness	2	1	1	0	
		Discharge	1	0	0	0	
Mean Scores	Cornea	Opacity		0.33	0	0	Overall average of means^b
	Iris	Iritis		0.17	0	0	
	Conjunctiva	Chemosis		1.0	0.33	0	
		Redness		1.5	0.67	0	

^a Draize, 1959

^b For each lesion, calculated as mean score at (24 hours + 48 hours + 72 hours)/3

Clinical signs: No adverse clinical signs of toxicity were observed and no deaths occurred.

Ocular effects: Blistering of the conjunctiva was observed in all rabbits by 1-hour post-treatment. Corneal opacity, iritis, conjunctival redness, conjunctival chemosis, and discharge were observed during the study. Ocular irritation had cleared in all animals by 72 hours post-treatment.

Conclusions: DPX-MP062 produced no significant ocular lesions according to criteria defined in Regulation (EC) No 1272/2008 and is thus not considered to be irritating to the eye.

B.6.2.6. Skin sensitization

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.2.6/01

Report: [REDACTED] (2003); Indoxacarb (DPX-KN128) technical: Dermal sensitization - Magnusson-Kligman maximization method

DuPont Report No.: DuPont-13018

Guidelines: OPPTS 870.2600 (1998) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: 13984

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
 Lot/Batch #: KN128-098
 Purity: 95.47%
 Description: White solid
 CAS #: 173584-44-6
 Stability of test compound: Not determined.
2. Vehicle and/or positive control: Vehicle: mineral oil
3. Test animals
 Species: Guinea pig
 Strain: Hartley albino
 Age at dosing: Young adult
 Weight at dosing: 360–433 g for males
 Source: [REDACTED]
 Acclimation period: 11 days
 Diet: Pelleted Purina Guinea Pig Chow (#5025), *ad libitum*
 Water: Tap water, *ad libitum*
 Housing: Animals were group housed in stainless steel cages with wire-mesh floors suspended above cage boards.
4. Environmental conditions
 Temperature: 18–23°C
 Humidity: 39–70%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
28-July-2003 to 29-August-2003

2. Animal assignment and treatment

Range finding study: Preliminary irritation testing was performed on 12 male animals to determine appropriate concentrations of the test substance that could be used for both intradermal and topical induction as well as topical challenge. The concentration selected for the intradermal induction was 5% w/w mixture in mineral oil, and the concentration selected for topical induction was 60% in mineral oil. The highest non-irritating concentration selected for the challenge phase was 45% w/w mixture in mineral oil.

Main study: Based on the results of the preliminary irritation testing, 20 male animals were intradermally induced on Day 1 with pairs of injections of the test substance (5% w/w mixture in mineral oil), test substance combined with Freund's Complete adjuvant (5% w/w mixture of test substance in adjuvant), and adjuvant alone (50% v/v adjuvant in distilled water). A test vehicle control group (10 animals) received injections of mineral oil, mineral oil combined with Complete Freund's adjuvant, and adjuvant alone. Approximately one week later, animals were topically induced with 0.5 g of a 60% w/w mixture of the test substance in mineral oil. Animals were challenged on Test Day 22 with 0.5 g of a 45% w/w mixture of the test substance in mineral oil and 0.5 g of a 14.85% w/w mixture of the test substance in mineral oil on two separate test sites. The vehicle control group was also treated with the test substance, and test mineral oil at challenge. Approximately 24 and 48 hours after the challenge phase, the test sites were evaluated for signs of elicited sensitisation.

II. RESULTS AND DISCUSSION

One test animal was found dead on day 11 of the study. Toxic signs noted prior to death included hypoactivity, labored breathing and a prone posture. Gross necropsy revealed extreme red oral discharge. All other test animals survived and gained body weight during the study.

Table summarising the incidence of the sensitisation response noted after challenge is found below.

Under the conditions of this study, indoxacarb was considered to be a sensitiser.

Appropriate historical control data using α -hexylcinnamaldehyde demonstrated a positive response.

Table B.6.2.6-1
Maximisation test with Indoxacarb: Dermal response 24 and 48 hours after challenge

Hours	Test substance group				Control group	
	45%		14.85%		Mineral Oil	
	24	48	24	48	24	48
Number of animals with a score of 1 or greater*	11/19 ^a	9/19	7/19	6/19	0/19	0/19

^a Number of animals with positive dermal score/number of animals in dose group

* According to the scoring system, the score of 0.5 (very faint erythema) is not considered a positive reaction.

III. CONCLUSION

Indoxacarb does possess skin sensitising potential under the conditions of the maximisation test. In accordance with Regulation (EC) No. 1272/2008, indoxacarb is classified in Category 1 for skin sensitization.

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.2.6/02

Report: [REDACTED] (2006); Indoxacarb (DPX-KN128) technical: Dermal sensitization test - Buehler method

DuPont Report No.: DuPont-18915

Guidelines: OECD 406 (1992), OPPTS 870.2600 (1998) Deviations: None

Testing Facility: [REDACTED]

Testing Facility Report No.: 19265

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
 Lot/Batch #: KN128-098
 Purity: 96.17%
 Description: Off-white solid
 CAS #: 173584-44-6
 Stability of test compound: The test substance was expected to be stable for the duration of testing.
2. Vehicle and/or positive control: Mineral oil
3. Test animals
 Species: Guinea pig
 Strain: Hartley albino
 Age at dosing: Young adult
 Weight at dosing: 313–385 g
 Source: [REDACTED]
 Acclimation period: 5–37 days
 Diet: Pelleted Purina Guinea Pig Chow (#5025), *ad libitum*
 Water: Filtered tap water, *ad libitum*
 Housing: Animals were group housed in stainless steel cages with wire-mesh floors or plastic perforated bottom caging suspended above cage boards.
4. Environmental conditions
 Temperature: 19–22°C
 Humidity: 25–70%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 06-March-2006 to 06-April-2006
2. Animal treatment
Range finding study: Preliminary irritation testing was performed on 2 groups of four animals to determine appropriate concentrations of the test substance for topical induction and challenge.

From these results, the HNIC (the highest concentration that produced responses in four guinea pigs no more severe than two scores of 0.5 and two scores of zero) was established and used for the challenge. The HNIC selected for the challenge phase was a 49% w/w mixture of test substance in mineral oil. The 65% w/w mixture of test substance in mineral oil produced very faint irritation in three of four of the animals and was used for the induction phase.

Main study: Twenty animals (20 male) were topically induced with a 65% w/w mixture of test substance in mineral oil. This procedure was performed once a week for 3 weeks (three 6-hour applications). Twenty-seven days after the first induction application, animals received a challenge concentration of a 49% w/w mixture of test substance in mineral oil on the clipped, naive, right flank. Approximately 24 and 48 hours after induction treatments, the test sites were evaluated for dermal irritation. Approximately 24 and 48 hours after the challenge treatment, the test sites were evaluated for signs of elicited sensitisation. Very faint redness (usually non-confluent, score of 0.5) was not considered a positive dermal reaction. Scores of 1 (faint redness, usually confluent) or greater were required to be indicative of sensitisation. The same procedures were performed on a contemporaneous vehicle control group, except that the test substance was replaced by the vehicle, mineral oil, during induction. Vehicle control animals were challenged with both indoxacarb and mineral oil. No contemporaneous positive control was evaluated; however, HCA is periodically tested in order to document the effect of a known sensitizer in this test system.

II. RESULTS AND DISCUSSION

Very faint erythema (0.5) was noted for most test sites at various intervals throughout the induction phase. For the animals challenged with a 49% w/w mixture of test substance in mineral oil, very faint erythema (0.5) was noted for eight of twenty test sites 24 hours after challenge. Irritation persisted at two of these sites through 48 hours. No responses were noted in the vehicle control animals. Appropriate historical control data using HCA demonstrated a positive response. No test substance-related clinical signs of toxicity were observed. There were no test substance-related body weight effects noted.

Table B.6.2.6-2
Buehler test with indoxacarb: Dermal response to challenge

Group	24 hours	48 hours
Test group	0/20 ^a	0/20
Test group vehicle control (right front flank challenged with 49% w/w mixture of test substance in mineral oil)	0/10	0/10
Test group vehicle control (right rear flank challenged with 100% mineral oil)	0/10	0/10

^a Number of animals with positive dermal response/number of animals in dose group

III. CONCLUSION

Under conditions of the Buehler method, indoxacarb did not produce a dermal sensitisation response in guinea pigs. According to the provisions of Regulation (EC) No. 1272/2008 classification of indoxacarb for dermal sensitisation is not required.

Previous evaluation:	In DAR (2000)
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Report: [REDACTED] (1996): Guinea Pig Dermal Sensitization - Magnusson-Kligman Maximization Test with DPX-MP062 Technical (Approximately 75% DPX-KN128, 25% IN-KN127). DuPont Report No. HLO 388-96. Published: No.

Guidelines: EEC B.6; OECD 406; US EPA 81-6 and 40 CFR 798; MAFF Japan 1985. Deviations: Treatment solutions were not analysed for concentration, uniformity or stability of the test and control articles. The procedures used by trained personnel to prepare the treatment solutions insured that this did not affect the validity of the study.

GLP: Yes. Certified Laboratory: No (laboratories in the USA are not certified by any governmental agency but are subject to regular GLP inspections from the US EPA).

Materials and methods:

The dermal sensitisation potential of DPX-MP062 (purity 94.5%) was evaluated by the Magnusson-Kligman Maximisation method in male Hartley albino guinea pigs. Twenty animals were intradermally induced on day 1 with 0.1 ml of a 5% w/v dilution of DPX-MP062 technical in propylene glycol emulsified with Freund's complete adjuvant. On test day 8, animals were topically induced with 0.5 g of the test substance moistened with 0.5 ml of propylene glycol. Animals were challenged on test day 22 with 0.5 g of the test substance moistened in 0.5 ml propylene glycol (considered 100%) and 0.5 ml of a 33% w/v concentration of the test substance in propylene glycol on two separate test sites. The same procedures were carried out on a contemporaneous control group except that for the induction phases the test article was replaced by propylene glycol (vehicle control). In addition, 1-chloro-2,4-dinitrobenzene (DNCB - positive control) and ethanol/acetone (positive control vehicle) groups were also evaluated.

Findings:

The results of the dermal sensitisation study in guinea pigs with DPX-MP062 are summarised in the following table.

Table B.6.2.6-3
Dermal sensitisation of DPX-MP062 in Guinea Pigs: Dermal response to sensitisation challenge

Skin reddening following challenge: Vehicle control group							Skin reddening following challenge: Test substance group						
Animal No	Test substance patch (100%) ^a		Test substance patch (33%)		Control patch		Animal No	Test substance patch (100%)		Test substance patch (33%)		Control patch	
	24h	48h	24h	48h	24h	48h		24h	48h	24h	48h	24h	48h
21	0	0	0	0	0	0	1	2	2	1	1	0	0
22	0	0	0	0	0	0	2	2	3	1	1	0	0
23	0	0	0	0	0	0	3	0.5	0	0	0	0	0
24	0	0	0	0	0	0	4	2	2	0.5	0	0	0
25	0	0	0	0	0	0	5	1	1	1	0.5	0	0
26	0	0	0	0	0	0	6	1	1	0.5	1	0	0
27	0	0	0	0	0	0	7	0	0	0	0	0	0
28	0	0	0	0	0	0	8	2	1	1	0.5	0	0
29	0	0	0	0	0	0	9	1	2	0.5	1	0	0
30	0.5 ^b	0	0	0	0	0	10	1	1	1	0	0	0
31	0	0	0	0	0	0	11	2	1	1	1	0	0
32	0.5	0	0	0	0	0	12	0.5	0.5	0	0	0	0
33	0	0	0	0	0	0	13	1	0.5	0.5	0.5	0	0
34	0	0	0	0	0	0	14	0.5	0	0.5	0	0	0
35	0	0	0	0	0	0	15	0.5	0	0	0	0	0
36	0	0	0	0	0	0	16	2	1	2	2	0	0
37	0	0	0	0	0	0	17	0.5	1	0.5	1	0	0
38	0	0	0	0	0	0	18	1	1	0.5	0	0	0
39	0	0	0	0	0	0	19	0.5	0.5	0	0	0	0
40	0	0	0	0	0	0	20	1	1	0.5	0	0	0

^a 0.5 g of test substance moistened with 0.5 ml of propylene glycol was considered 100%.

^b Scores of 0.5 (barely perceptible redness) were not considered a positive reaction.

The percentage of sensitisation at 24 and/or 48 hours for the test article animals challenged with 0.5 g of test article moistened with 0.5 ml of propylene glycol was 70% (14/20) and this percentage was 50% (10/20) in animals challenged with a 33% concentration of the test substance. No redness to moderate redness was observed at 24 hours and no redness to intense redness with or without swelling was observed at 48 hours. A dermal sensitisation response was noted in 100% of the 0.01% DNCB (positive control) animals. No responses were noted in the vehicle or positive control vehicle animals.

Conclusions: DPX-MP062 has skin sensitising potential under the conditions of the Maximisation test.

B.6.2.7. Phototoxicity

Previous evaluation:	Submitted for the purpose of renewal
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Report: Markell, L.K. (2015) Indoxacarb (DPX-KN128) technical: *In vitro* 3T3 NRU phototoxicity test

DuPont Report No.: DuPont-43522

Guidelines: OECD Guidelines for the Testing of Chemicals Section 4 (Part 432) (2004)

Deviations: the study design complied with the above mentioned test guideline except as noted below:

- The OECD guideline indicates that the highest non-cytotoxic UVA dose should be used. Therefore, a sensitivity test was performed to ensure that the UVA dose chosen produced less than 20% cytotoxicity. In this study, the dose of UVA to be used was also determined based on the ability to classify the positive control chemical, chlorpromazine (CPZ), correctly. The guideline outlines test acceptance criteria for CPZ in the presence (IC₅₀ = 0.1 to 2.0 µg/mL) and absence (IC₅₀ = 7.0 to 90.0 µg/mL) of UVA irradiation. This measure served as an additional control that the test system was functioning as expected.

- In this study, the cells were treated with test substance prepared in Hank's Buffered Salt Solution (HBSS) and incubated for approximately 1 hour 45 minutes. During this time, the cells in culture tended to detach from the cell culture plates due to sub-optimal growth conditions. Therefore, the 96-well plates were only washed once with HBSS following the treatment with test substance. This exception did not affect the objectives or the validity of the study because this protocol change has been tested repeatedly during proficiency chemical testing and correct classification was still attained.

- In this study, the Neutral Red was prepared in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% newborn calf serum at a concentration of 40 µg/mL. The OECD guideline specifies that Neutral Red will be prepared in media without serum at a concentration of 50 µg/mL. Newborn calf serum (5%) was used due to cell detachment that was observed when media did not include any serum. Neutral Red (40 µg/mL) was used due to Neutral Red crystal formation that was observed at the higher concentration. This exception does not affect the objectives or the validity of the study because this protocol change has been tested repeatedly during proficiency chemical testing and correct classification was still attained.

Testing Facility: DuPont Haskell Laboratory, Newark, Delaware, USA

Testing Facility Report No.: DuPont-43522

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
Lot/Batch #: KN128-424
Purity: 99.06%
Description: Solid, crystalline
CAS #: 173584-44-6
Stability of test compound: The test substance was assumed to be stable throughout the exposure phase of the study; no evidence of instability was observed.
2. Solvent used: Acetone
Control materials
Negative (solvent) control/final concentration: 1% Acetone
Positive control: Chlorpromazine (CPZ), diluted in 1 % DMSO to 0.032, 0.1, 0.32, 1, 3.2, 10, 31.6, and 100 µg/mL

3. Test system

A permanent mouse fibroblast cell line, Balb/3T3, clone A31, was obtained from American Type Culture Collection (ATCC®) (Manassas, Virginia, U.S.A., catalogue number CCL-163). The cells were stored frozen in liquid nitrogen. Balb/3T3 cells were started from frozen batches and were cultured for at least one passage prior to their use in an assay. Cells were checked regularly for the absence of mycoplasma contamination.

The cells were grown in media containing DMEM media base with 10% newborn bovine serum. Cells were incubated at approximately 37°C in an atmosphere of approximately 5% CO₂. Passage #72 was used for run 1, and passage #72 was used for run 2.

The current EU Data requirements in Reg. 283/2013 request submission of an in vitro 3T3 NRU phototoxicity testing in case the active substance absorbs electromagnetic radiation in the range 290-700 nm with a molar absorption coefficient > 10 L mol⁻¹ cm⁻¹. However, the direct irradiation of the cells between 290 and ~315 nm results in cytotoxicity driven by the irradiation alone. This is also clearly described in the respective OECD test guideline 432: “... *Light of the UVA and visible regions is usually associated with phototoxic reactions in vivo, whereas generally UVB is of less relevance but is highly cytotoxic; the cytotoxicity increases 1000-fold as the wavelength goes from 313 to 280 nm...*”. As the scope of the study is to compare cytotoxicity of the test item in presence and absence of light exposure, it is not compatible with the scope of the assay to induce cytotoxicity by the light exposure itself. In addition, the wavelengths and doses employed should not be unduly deleterious to the test system. Therefore the SOL-500 was used with irradiance in the UV/visible range of 295-780 nm. The H1 filter allows for a light source almost devoid of UVB with a filtering transmission range from 315 nm. According to the ultraviolet/visible spectra of indoxacarb (see Volume 3CA B2), the pH <2 samples showed absorbance maxima at approximately 203, 230, 285, and 311 nm. The pH 7 and pH >10 samples showed absorbance maxima at 235, 285, and approximately 311 nm. At these wavelengths, molar absorption coefficient ranged approximately from 10 to 20 L mol⁻¹ cm⁻¹. UV/VIS spectra showed absorbance until approx. 340 nm. Although the absorbance pics were observed below 315 nm, it cannot be excluded that molar absorption coefficient is > 10 L mol⁻¹ cm⁻¹ at wavelength greater than 315 nm. Therefore, this test is considered adequate.

4. Dosimetry

The intensity of light (irradiance) was regularly checked before each phototoxicity test using a Honle Basic UV Meter and UVA sensor with spectrum detection (330–400 nm); maximum intensity 5 W/cm². The intensity was measured through the same type of 96-well plate lid as was used in the assay. The UV-meter was calibrated to zero by exposure to no UVA irradiation (covering sensor).

A dose of 10 J/cm² (as measured in the UVA range) was determined to be non-cytotoxic to Balb/3T3 cells and sufficiently potent to excite chemicals to elicit phototoxic reactions. The exposure time was calculated in the following way:

$$t \text{ (min)} = \frac{\text{irradiation dose (J/cm}^2\text{)} \times 1000}{\text{irradiance (mW/cm}^2\text{)} \times 60} \quad (1\text{J} = 1\text{ Wsec})$$

UVA irradiance during run 1 was 3.63 mW/cm², and cells were irradiated for 45 minutes and 54.6 seconds. The UVA irradiance during run 2 was 4.98 mW/cm², and cells were irradiated for 33 minutes and 27.6 seconds. The light source was approximately 76 cm from the test plates during run 1 and approximately 63 cm from the test plates during run 2.

The UVA sensitivity of the cells was checked approximately every fifth passage for sensitivity to the light source by assessing their viability following exposure to increasing doses of irradiation. Several doses of irradiation were used in this assessment. Cells were seeded at the density used in the *in vitro* 3T3 NRU phototoxicity test and irradiated the next day. Cell viability was then determined 1 day later using Neutral Red uptake. It was demonstrated that the UVA dose of 10 J/cm² was sufficient to classify the positive control chemical correctly while producing less than 20% cytotoxicity compared to the non-irradiated solvent control.

5. Culture preparation

Cell culture maintenance and stock dosing media contained DMEM media base (with 10% calf bovine serum). Cells from frozen stock cultures were seeded in culture medium at an appropriate density and subcultured at least once before they were used in the *in vitro* 3T3 NRU phototoxicity test.

Cells from frozen stock cultures were seeded in culture medium at an appropriate density and subcultured at least once before they were used in the *in vitro* 3T3 NRU phototoxicity test.

Cells used for the phototoxicity test were seeded in culture medium at 1×10^4 cells per well, so that cultures did not reach over-confluence by the end of the test, *i.e.*, when cell viability was determined approximately 48 hours after seeding of the cells.

For the test substance or positive control, cells were seeded identically in two separate 96-well plates, which were then taken concurrently through the entire test procedure under identical culture conditions except for the time period where one of the plates was irradiated (+Irr), and the other one was kept in the dark (-Irr).

6. Test compound preparation

Test substances were prepared fresh immediately prior to use.

Solubility testing was conducted prior to study initiation with the solvents DMSO, ethanol, acetonitrile and acetone, up to 1000 µg/mL. Acetone was determined to yield the highest dosing concentration.

The test substance was dissolved in acetone. Solvent was present at a constant volume, and testing doses contained 1% solvent in HBSS in all cultures, *i.e.*, in the solvent controls as well as in all concentrations of the test substance, and were non-cytotoxic at that concentration. Test substance concentrations were selected to avoid precipitation or cloudy solutions. To avoid toxicity induced by improper culture conditions or by highly acidic or alkaline chemicals, the pH of the cell cultures with added test chemical was in the range 6.5–7.8. Precipitate of KN128 was observed microscopically in a cell culture plate when prepared at dosing concentrations higher than 10 µg/mL, at 1% v/v acetone in Hank's Balanced Salt Solution (HBSS). The test substance was soluble in acetone, with no precipitate observed when solubilized in 1% acetone in HBSS up to 10 µg/mL. The test substance also had an appropriate pH at 10 µg/mL; therefore, 10 µg/mL was selected as the upper limit concentration for this assay. It was determined that the test substance was less soluble with the solvents DMSO and ethanol.

Vortex mixing and warming were used to aid solubilisation.

The ranges of concentrations of a test substance in the presence (+Irr) and in the absence (-Irr) of light were adequately determined in dose range-finding experiments. The test substance was tested at the following concentrations: 0.0032, 0.01, 0.032, 0.1, 0.32, 1.0, 3.2, and 10 µg/mL solubilised in 1% solvent in HBSS during definitive experiments (run 1 and run 2). Nevertheless, the analysis of dosing solutions showed that the test substance was between approximately 50% and 70% of targeted concentrations.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

18-December-2014 to 23-December-2014

2. Day 1

A 100-µL volume of culture medium (DMEM + 10% Newborn Calf Serum) was dispensed into the peripheral wells of a 96-well tissue culture microtiter plate (= blanks). In the remaining wells, 100 µL of a cell suspension of 1×10^5 cells/mL in culture medium ($= 1 \times 10^4$ cells/well) was dispensed. Two plates were prepared for each individual test substance concentration and for the positive control. Plates were labelled with the Work Request and Service Code for each assay, and a well map was included in the study records to identify the contents of each well.

The cells were incubated for 24 ± 2 hours at approximately 37°C and approximately 5% CO₂ in a humidified incubator. This incubation period allowed for cell recovery, adherence, and exponential growth.

3. Day 2

From the two plates prepared for each series of test substance concentrations and the controls, one was selected for the determination of cytotoxicity (-Irr, the control plate), and one (the UVA treatment plate) for the determination of photocytotoxicity (+Irr).

Prior to dosing, a mastermix of each concentration of test substance or positive control stock solution was prepared by adding 40 µL of the stock solution to 3.960 mL of HBSS with Ca²⁺/Mg²⁺. Also, a mastermix containing 40 µL of the solvent and 3.960 mL of HBSS was prepared. This solution was used to dose the solvent control wells. The final solvent concentration in all dosing solutions was 1.0%.

After a 24 ± 2 -hour pre-incubation of plated cells, the plates were removed from the incubator and checked for attachment and morphology prior to dosing. The cell media was removed, dosing buffer (100 µL/well of the appropriate mastermix) was added, and the plates were returned to the incubator (37°C, 5% CO₂) and incubated for approximately 1 hour.

To perform the +Irr exposure, the cells were irradiated at room temperature for approximately 30-50 minutes through the lid of the 96-well plate. Average temperatures recorded during the UVA light exposure range from 37–42°C. The non-irradiated plates (-Irr) were kept at room temperature in the dark for an incubation equivalent to the UVA light exposure time.

Test solution was decanted and carefully washed once with 150 µL of HBSS with Ca²⁺/Mg²⁺. The buffer was replaced with culture medium and incubated overnight (18–22 hours) at approximately 37°C and approximately 5% CO₂ in a humidified incubator.

A 40-µg/mL preparation of Neutral Red (NR) (3-amino-7-dimethylamino-2-methylphenazine hydrochloride, CAS number 553-24-2) in DMEM, 5% Newborn Calf Serum was made and incubated at approximately 37°C overnight for use on Day 3.

4. Day 3

Microscopic evaluation

Cells were examined for growth, morphology, and integrity of the monolayer using a phase contrast microscope. Changes in cell morphology and effects on cell growth were recorded.

Neutral Red uptake test

Media was removed, and the cells were washed with 150 µL of HBSS. The washing solution was removed. A 100-µL volume of 40 µg/mL NR in DMEM, 5% Newborn Calf Serum was added to each well and incubated at approximately 37°C and approximately 5% CO₂ in a humidified incubator for 3 hours ± 30 minutes.

After incubation, the NR medium was removed, and the cells washed with 150 µL of HBSS with Ca²⁺/Mg²⁺. HBSS was decanted and the excess removed by blotting.

A 150-µL volume of NR desorb solution (freshly prepared 49 parts water, 50 parts ethanol, and 1 part acetic acid) was added.

The microtiter plate was shaken gently on a microtiter plate shaker for approximately 10-15 minutes until NR had been extracted from the cells and had formed a homogeneous solution.

The optical density of the NR extract was measured at 540 nm in a spectrophotometer, using blanks as a reference. The data were saved in an appropriate electronic file format for subsequent analysis.

5. Data analysis

Absorbance values for both vehicle controls (-Irr and +Irr) were determined, and an average was calculated for each. Percent viability values were determined by dividing the absorbance of the treatment group by the mean absorbance of the vehicle control group and multiplying by 100. The average % control was determined for each concentration, along with standard deviation. These averages were graphed using a bar graph with the standard deviation represented by the error bars.

To enable evaluation of the data, a PIF or MPE was calculated. Phototox prediction software Phototox Version 2.0, ZEBET was used to determine both the PIF and MPE. The calculations performed by the software are described below.

For the calculation of the measures of photocytotoxicity, the set of discrete dose-response values was approximated by an appropriate continuous dose-response curve (model). Fitting of the curve to the data was commonly performed by a non-linear regression method. To assess the influence of data variability on the fitted curve, a bootstrap procedure is recommended.

A PIF was calculated using the following formula:

$$\text{PIF} = \frac{\text{IC}_{50}(-\text{Irr})}{\text{IC}_{50}(+\text{Irr})}$$

An IC₅₀ in the presence or absence of light could not be calculated; therefore, a PIF was not determined for the test substance.

The MPE was based on comparison of the complete concentration response curves. It was defined as the weighted average across a representative set of photo effect values.

$$\text{MPE} = \frac{\sum_{i=1}^n w_i \text{PE}_{c_i}}{\sum_{i=1}^n w_i}$$

The photo effect (PEc) at any concentration (C) was defined as the product of the response effect (REc) and the dose effect (DEc), *i.e.*, $PEc = REc \times DEc$. The REc was the difference between the responses observed in the absence and presence of light, *i.e.*, $REc = Rc(-Irr) - Rc(+Irr)$. The dose-effect was given by:

$$DEc = \frac{C/C^* - 1}{C/C^* + 1}$$

where C^* represents the equivalence concentration, *i.e.*, the concentration at which the +Irr response equals the -Irr response at concentration C. If C^* could not be determined because the response values of the +Irr curve were systematically higher or lower than $Rc(-Irr)$, the dose effect was set to 1. The weighting factors, w_i , were given by the highest response value, *i.e.*, $w_i = \text{MAX} \{Ri(+Irr), Ri(-Irr)\}$. The concentration grid C_i was chosen such that the same number of points falls into each of the concentration intervals defined by the concentration values used in the experiment. The calculation of MPE was restricted to the maximum concentration value at which at least one of the two curves still exhibited a response value of at least 10%. If this maximum concentration was higher than the highest concentration used in the +Irr experiment, the residual part of the +Irr curve was set to the response value "0." Depending on whether the MPE value was larger than a properly chosen cut-off value ($MPEc = 0.15$) or not, the test substance was classified as phototoxic.

Interpretation of results:

PIF	MPE	Prediction
<2	<0.1	No phototoxicity
>2 and >5	>0.1 and <0.15	Probable phototoxicity
>5	>0.15	Phototoxicity

6. Evaluation criteria

The test data should allow a meaningful analysis of the concentration-response obtained in the presence and in the absence of irradiation, and if possible the concentration of test chemical by which cell viability was reduced to 50% (IC_{50}). If cytotoxicity was found, both the concentration range and the intercept of individual concentrations were set in a way to allow the fit of a curve to the experimental data.

For both clearly positive and clearly negative results, the primary experiment, supported by one or more preliminary dose range-finding experiment(s), was sufficient.

Equivocal, borderline, or unclear results should be clarified by further testing. In such cases, modification of experimental conditions should be considered. Experimental conditions that might be modified include the concentration range or spacing, the pre-incubation time, and the irradiation-exposure time. A shorter exposure time may be appropriate for water-unstable chemicals.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Dose samples containing indoxacarb at the concentrations of 0.0032, 0.010, 0.032, 0.10, 0.32, 1.0, 3.2, and 10 µg/mL prepared on December 18 and December 22, 2014 were submitted for concentration verification and room temperature stability analyses. Three different concentrations of each batch of samples were analyzed: 0.10, 1.0, and 10 µg/mL. Due to detection limits, the lowest concentrations were not analyzed. The analysis results show that the test substance was between approximately 50% and 70% of targeted concentrations and outside the acceptable ranges (see Volume 3B5). The concentrations of the test

substance in the vehicle remained consistent for the duration of the experiment. Indoxacarb was assumed to be stable throughout the exposure phase of the study; no evidence of instability was observed. Indoxacarb was not found in the 0 mg/mL samples.

B. ASSAY PERFORMANCE

MPE and PIF values of CPZ in the presence and absence of UVA irradiation were within the acceptable ranges as specified by the test guideline. The IC_{50} value for run 2 of CPZ was slightly outside the guideline range ($IC_{50} = 2.07 \mu\text{g/mL}$ measured vs. guideline IC_{50} range = 0.1 to 2.0 $\mu\text{g/mL}$). However, this value was similar to the historical range for the positive control.

For the runs performed with the positive control CPZ and the test substance, the irradiated vehicle control showed a viability of more than 80% when compared with the non-irradiated vehicle control for both experimental runs.

Absorbance of the vehicle control was assessed to demonstrate that the cells seeded in well had grown with a normal doubling time during the 2 days of the assay. The absolute optical densities of the Neutral Red extracted from various irradiated and non-irradiated plates containing solvent controls were slightly below the acceptance criteria. However, these values were within the historical range of assay performance for both irradiated and non-irradiated plates.

C. BALB/3T3 SENSITIVITY TO UVA IRRADIATION

The Balb/3T3 cells were treated with increasing intensities of UVA irradiation, and viability was compared to non-irradiated cells. 10 J/cm², the dose used for both CPZ and indoxacarb treatment, showed a viability of more than 80% meeting the guideline quality criteria.

D. PHOTOTOXICITY

No phototoxic effect was observed with the test compound following UVA irradiation. Under the conditions of this study, the MPE for test compound was determined to be -0.024 and 0.035 for runs 1 and 2, respectively, and the PIF of *1 was identified, indicating that indoxacarb was not phototoxic. A major limitation of the PIF prediction model is that if IC_{50} values cannot be determined, as found in this case for the test compound, a PIF cannot be calculated. To overcome this limitation, a novel measure to assess the phototoxic potential of chemicals, the MPE, was used. At the concentrations of indoxacarb tested, there was no inherent cytotoxicity or phototoxicity to Balb/3T3 cells.

As expected, the positive control, CPZ, showed a substantial increase in toxicity following UVA irradiation compared to un-irradiated CPZ (Table). The positive control was within acceptable ranges based upon OECD guidelines (run 1) and on historical ranges (run 2) and provided confidence in the test system.

Controls: The positive and solvent controls fulfilled the requirements for a valid test.

Table B.6.2.7-1
Summary of PIF and MPE results

	Run	PIF ^a	MPE ^b	IC ₅₀ -Irr ^c (µg/mL)	IC ₅₀ +Irr ^d (µg/mL)
CPZ ^e	1	37.180	0.447	33.243	0.898
	2	24.727	0.376	51.244	2.078
Indoxacarb	1	*1	-0.024	— ^f	— ^f
	2	*1	0.035	— ^f	— ^f

^a Photo-irritation-factor

^b Mean photo effect

^c Non-irradiated cells

^d Irradiated cells

^e Chlorpromazine

^f Both IC₅₀ (-Irr) and IC₅₀ (+Irr) cannot be calculated for indoxacarb because no cytotoxicity was demonstrated up to the highest concentration tested (10 µg/mL), which indicates no cytotoxic potential. In this case, the PIF - *1.

Table B.6.2.7-2
Cell viability after exposure to 10 J/cm²

	Run	Percent control ^a	SEM
Indoxacarb -Irr ^b	1	100	0.0116
Indoxacarb +Irr ^c	1	88.5	0.0109
Indoxacarb -Irr	2	100	0.0129
Indoxacarb +Irr	2	96.3	0.0138
CPZ ^d -Irr	1	100	0.0275
CPZ +Irr	1	116.8	0.0245
CPZ -Irr	2	100	0.0100
CPZ +Irr	2	94.4	0.0128

^a Percent viability was calculated by comparing the +Irr solvent control average absorbance (OD₅₄₀ NRU) to the -Irr solvent control average absorbance (OD₅₄₀ NRU).

^b Non-irradiated cells

^c Irradiated cells

^d Chlorpromazine

III. CONCLUSION

Under the conditions of the study, IC₅₀ values and a PIF could not be calculated. The MPE for test compound was determined to be -0.024 and 0.035 for runs 1 and 2, respectively. This result suggests that following UVA irradiation, indoxacarb was not phototoxic when tested at targeted concentrations up to 10 µg/mL (measured concentrations of 50 to 70% of the targeted concentrations).

B.6.3. SHORT-TERM TOXICITY

The sub-chronic toxicity of DPX-KN128 (coded DPX-JW062-69 in the study report and containing 91.2% of the S-enantiomer), DPX-MP062 (75:25) and DPX-JW062 (50:50) has been evaluated in 90-day feeding studies in rats. DPX-JW062 (50:50) has also been evaluated in 90-day feeding study in mice and 90-day and 1-year feeding study in dogs. A 28-day dermal toxicity study was also performed on DPX-MP062 (75:25). All these studies were already assessed in the first DAR (2000) or Addendum to DAR (2001).

Table B.6.3-1
Summary of short-term toxicity studies for indoxacarb (DPX-KN128 (99:1), DPX-MP062 (75:25) and DPX-JW062 (50:50))

Type of study and Test Substance	Dose range tested	NOAEL		LOAEL		Target organ(s) and effects	Reference
		ppm	mg/kg/d	ppm	mg/kg/d		
Oral (Feeding), 90-day Rat DPX-JW062-69 [91.2% DPX-KN128 and 0.3% DPX-KN127]	M: 0, 8, 20, 50, 100, 200 ppm – 0, 0.56, 1.4, 3.2, 6.6, 14 mg/kg bw/d F: 0, 3, 8, 20, 50, 100 ppm – 0, 0.25, 0.68, 1.7, 4.1, 8.5 mg/kg bw/d	M: 20 F: 8	M: 1.4 F: 0.68	M: 50 F: 20	M: 3.2 F: 1.7	At LOAELs: haemolytic effects on circulating RBC (<i>up to -6% and -9% decreases in M and F respectively, Met-Hb in M</i>) and histopathological spleen effects. At higher doses: decreased body weights, body weight gains, food consumption, increased MCV and/or reticulocytes, methaemoglobinemia, histopathological findings in the liver and bone marrow.	1997a HLR 301-94, Revision No. 2
Oral (Feeding), 90-day - rat DPX-MP062 (75:25)	M: 0, 10, 50, 100, 200 ppm – 0, 0.62, 3.09, 6.01, 15.0 mg/kg bw/d F: 0, 10, 25, 50, 100 ppm – 0, 0.76, 2.13, 3.78, 8.94 mg/kg bw/d	M: 10 F: <10	M: 0.62 F: <0.76	M: 50 F: 10	M: 3.09 F: 0.76	At LOAELs: haemolytic effects on circulating RBC (<i>up to -6% and -7% decreases in M and F respectively</i>) and histopathological spleen effects At higher doses: decreased body weights, body weight gains, food consumption, increased MCV, decreased total protein and globulin concentrations (M), histopathological findings in the liver, kidney, thymus and bone marrow. At 100 ppm only, in F: mortality	1997 HL-1997-00056, Revision No. 1
Oral (Feeding), 90-day - Rat DPX-JW062 (50:50)	M: 0, 30, 60, 125, 250 ppm – 0, 1.9, 3.9, 8.0, 16 mg/kg bw/d F: 0, 15, 30, 60, 125 ppm – 0, 0.99, 2.3, 4.6, 9.5 mg/kg bw/d	M: <30 F: 15	M: <1.9 F: 0.99	M: 30 F: 30	M: 1.9 F: 2.3	At LOAELs: haemolytic effects on circulating RBC (<i>up to -7% and -9% decreases in M and F respectively</i>) and histopathological spleen effects At higher doses: decreased body weights, body weight gains, food consumption, increased MCV and reticulocytes, histopathological findings in the liver and bone marrow	1997b HLR 751-93, Revision No. 2
Oral (Feeding), 90-day – with reversibility Rat DPX-JW062 (50:50)	M: 0, 30, 60, 125, 250 ppm – 0, 1.8, 3.7, 7.5, and 15 mg/kg bw/day F: 0, 15, 30, 60, 125 ppm – 0, 1.2, 2.5, 4.9, and 12 mg/kg bw/day	Not applicable*		Not applicable*		Haematological effects (decreased RBC, Hb, Ht, and increased MCV) were reversible for both males and females within the 21-day recovery period. <i>*The study did not include ophthalmology, clinical chemistry, urinalysis, organ weighing, and histopathology and was designed to assess the reversibility of haematological effects.</i>	1998 HL-1998-01200

Oral (Feeding), 90-day - Mouse DPX- JW062 (50:50)	0, 35, 75, 150, 10/300 ppm M: 0, 5.5, 12, 23, 1.7/44 mg/kg bw/d F: 0, 7.0, 16, 30, 2.1/51	M: 35 F: 35	M: 5.5 F: 7.0	M: 75 F: 75	M: 12 F: 16	At LOAELs: slight haemolytic effects on circulating RBC, Heinz bodies, increased reticulocytes and histopathological spleen effects At higher doses: decreased body weights, body weight gains, food consumption, histopathological findings in the liver, clinical signs indicative of neurotoxicity	1997 HLR 750- 93, Revision No. 1
Oral (Feeding) 90-day Dog DPX- JW062 (50:50)	0, 40, 80, 160, 640 ppm M: 0, 1, 2, 5, 18 mg/kg bw/d F: 0, 1, 3, 5, 17 mg/kg bw/d	M: <40 F: <40	M: <1 F: <1	M: 40 F: 40	M: 1 F: 1	At LOAELs: haemolytic effects on circulating RBC (<i>up to -14% and -13% decreases in M and F respectively, increased MCV</i>) and histopathological effects in spleen, liver, kidney, bone marrow At higher doses: Heinz bodies, increased bilirubin levels, increased phosphatase alkaline and liver weights in F	1997a HLO 494- 95, Revision No. 3
Oral (Feeding) 1-year dog study DPX- JW062 (50:50)	0, 40, 80, 640, 1280 ppm M: 0, 1.1, 2.3, 17.5, 33.6 mg/kg bw/d F: 0, 1.3, 2.4, 18.9, 36.1 mg/kg bw/d	M: <40 F: <40	M: <1.1 F: <1.3	M: 40 F: 40	M: 1.1 F: 1.3	At LOAELs: haemolytic effects on circulating RBC (<i>up to -14% and -3% decreases in M and F respectively, increased MCV and/or reticulocytes</i>) and histopathological effects in spleen, liver, kidney, bone marrow, increased plasma bilirubin levels, bilirubinuria At higher doses: Heinz bodies, increased phosphatase alkaline and liver weights	1997b HLO 885- 96, Revision No. 1
Dermal, 28-day - Rat DPX- MP062 (75:25)	0, 50, 500, 1000, 2000 mg/kg bw/d	–	M: <50 F: <50	–	M: 50 F: 50	At LOAELs: slight haemolytic effects and histopathological spleen effects At higher doses: decreased body weights, body weight gains, food consumption, increased MCV and/or reticulocytes, methaemoglobinemia, increased spleen weights	1999 DuPont- 2813

Sub-chronic studies in rats (90-day feeding studies in rats) demonstrate that the insecticides DPX-KN128 (99:1), as well as DPX-MP062 and DPX-JW062 (containing the isomers DPX-KN128 and IN-KN127 in ratios of approximately 75:25 and 50:50, respectively) have similar toxicities at their low-effect levels. The LOAELs in rats were based on haemolytic effects. At the LOAELs, the haemolytic effects typically consisted of decreases in number of erythrocytes, haemoglobin concentration, and haematocrit, accompanied by secondary changes in the spleen such as increased haemosiderin pigments. At higher dose levels, histopathological secondary changes were also reported in liver, kidneys and bone marrow, and clinical chemistry findings were also noted.

Increased reticulocyte counts and MCV observed at highest dose levels are indicative of a regenerative anaemia. Moreover, in the only 90-day study in which Met-Hb was measured (study conducted on DPX-KN128), its level was found to be increased. It is to be noted that Heinz bodies were not measured in the 90-day rat studies. Regenerative responses were evident in peripheral blood (increased MCV, reticulocyte counts) and also in spleen (increased extramedullary haemopoiesis) and bone marrow (mixed cell hyperplasia). Consistent with the regenerative nature of these effects, the 90-day reversibility feeding study in rats with DPX-JW062 (50:50) demonstrated that haematologic effects (decreased RBC, Hb, Ht and increased MCV) were reversible within 21 days (the earliest recovery interval assessed). Nevertheless, the study design did not allow demonstrating the reversibility of histopathological findings.

Statistical modelling of the haematologic effects in subchronic studies with DPX-MP062 (75:25), DPX-JW062 (50:50), and DPX-KN128 (99:1) demonstrated that the magnitude of the haematologic effects were similar irrespective of the isomer blend (Green, 1999/DuPont-2780, see B.6.8.2).

The other effects commonly observed in rats administered DPX-KN128 (99:1), DPX-MP062 (75:25) or DPX-JW062 (50:50) were decreased body weight gains, body weights and food consumption.

Mortality was also observed in one of the three 90-day rat studies. Five out of ten females administered 100 ppm of DPX-MP062 (75:25) (equivalent to 8.94 mg/kg bw/d) died during the course of the study (between test days 8 and 19). The decedent rats had atrophy of the spleen, thymus and/or bone marrow at necropsy, and haemoglobin pigment was found in the renal tubule cells and/or lumens. Therefore, it could not be ruled out that these deaths are related to haematotoxicity. It should be noted that mortality was also reported in other studies: in the 28-day studies at the dose levels of 23.5 mg/kg bw/d in 2/5 female rats and at 34 and 35.3 mg/kg bw/d in 1/10 male and 1/10 female mice respectively; in the subchronic oral neurotoxicity study at the dose level of 6.09 mg/kg bw/d in 3/12 female rats on test days 9-12; in the developmental neurotoxicity study at the dose level of 3 mg/kg bw/d in 3/25 female rats showing also clinical signs of neurotoxicity, on gestation day 19 to lactation day 3.

Overall, females appeared to be more sensitive to the effects of indoxacarb than males. This is in agreement with ADME data which showed gender differences in absorption and biotransformation of indoxacarb (see B.6.1).

Sub-chronic feeding studies have also been conducted in mice with DPX-JW062 (50:50). Effects seen were qualitatively similar to those observed in rats: haemolytic effects accompanied by secondary histopathological spleen effects were observed at the LOAEL. Mice were less sensitive to the subchronic toxicity of DPX-JW062 (50:50) relative to the rat and dog. Body weight and nutritional effects, as well as clinical signs suggestive of neurotoxicity (see B.6.7), were also reported at higher dose levels.

Repeated dose toxicity studies were conducted with DPX-JW062 (50:50) in dogs. The LOAELs in the 90-day and 1-year dog studies with DPX-JW062 (50:50) were also based on haemolytic anaemia: decreases in number of erythrocytes, haemoglobin concentration, and haematocrit, accompanied by secondary changes in the spleen, liver, kidney and bone marrow. The pattern of changes in the mean values of the erythrocyte indices (MCV, MCH, and MCHC), platelet counts, and reticulocyte counts indicated that the anaemia was regenerative. At higher dose levels, increased numbers of Heinz bodies (parameters only measured in the dog studies) indicated that oxidative denaturation of haemoglobin may have been the cause of haemolysis. No NOAELs can be determined in these dog studies.

A sub-chronic dermal toxicity study was conducted in rats with DPX-MP062 (75:25). Similar effects than those observed after oral administration were observed from the low dose level of 50 mg/kg bw/d on haematology endpoints (decreased red blood cell parameters and secondary changes in the spleen in the majority of the animals). No unique additional target organs were detected. Based on the lower systemic toxicity from dermal exposure and the similarity of the systemic response compared to the oral route of exposure, sub-chronic dermal toxicity studies were not conducted with DPX-JW062 (50:50) or DPX-KN128 (99:1).

Based on the mortality observed in females in the 90-day rat study with DPX-MP062 (75:25), supported by the mortality data of the 28-day rat and mouse studies with DPX-JW062 (50:50), and based on the haemolytic anemia observed in the subchronic studies with DPX-KN128 (99:1), DPX-MP062 (75:25) and DPX-JW062 (50:50) and the dose levels at which these effects occurred, a classification STOT-RE Category 1 was adopted by the ECHA Committee for Risk Assessment (2011).

Interpretation of haematological effects:

During the peer review process for inclusion of indoxacarb in Annex I of Directive 91/414/EEC, the critical effects to take into account for the setting of the NOAELs were deeply discussed between Member States during the years 2000-2005. Based on the same toxicological database, the JMPR (2005) came to different conclusions than those taken at EU level. In view of these discrepancies, the Commission requested the RMS NL to write an addendum to re-evaluate the derivation of the ADI. This post-approval addendum was available in 2007.

As described in this post-approval addendum prepared by NL, the evaluation of the haematological effects in the EU and by the JMPR was as follows:

- EU: *“In the evaluation of the effects on circulating red blood cells in the individual studies, a slight but significant decrease in one of the parameters of circulating erythrocyte numbers (RBC count, Hb, Ht) was considered as adverse. It is however recognized that a very slight but significant deviation in one circulating red blood cell parameter, in the absence of other effects (increased haemosiderin pigment in liver, kidneys, spleen and bone marrow, presence of Heinz bodies and met-Hb, increased serum bilirubin values, absence of regenerative responses (increased MCV and reticulocyte counts, extramedullary haemopoiesis and mixed cell hyperplasia in bone marrow)), is likely to be fortuitous. Furthermore, dose-dependency naturally plays an important role in assessing the adversity of the effects. Unfortunately, in the available studies the existence of a dose-response relationship was obscured by the small differences in the dose levels.*

Keeping this in mind and comparing all available data with DPX-MP062 (enriched isomer mixture) and DPX-JW062 (racemic mixture), it is concluded that the overall NOAEL for DPX-MP062 for short- and long-term exposure would be 10 ppm for male and female rats, equivalent to 0.6 mg/kg bw. This ‘overall NOAEL’ for DPX-MP062 of 0.6 mg/kg bw is used to derive the AOEL and ADI.”

- JMPR: *“The 2005 JMPR considered the establishment of an ADI and ARfD for indoxacarb. The Joint Meeting concluded that the mild haemolysis observed in studies in rats and dogs given repeated doses was characterized by a reduced erythrocyte count, erythrocyte volume fraction, haemoglobin concentration, and a secondary physiological response involving increased haemopoiesis and deposition of haemosiderin in the spleen and liver. While the reductions in erythrocyte numbers through oxidative damage of haemoglobin occurred with a rather shallow dose-response curve, they achieved statistical significance relative to concurrent controls. However, the JMPR considered that these small changes in circulating erythrocyte mass in the absence of a concomitant increase in haematopoiesis were of no toxicological importance. As a consequence, the ADI of 0–0.01 mg/kg bw per day was based on a NOAEL of 1.1 mg/kg bw per day for erythrocyte damage, Heinz body formation and the secondary increase in haematopoiesis in the spleen and liver in a 1-year dietary study in dogs and using a 100-fold safety factor. This NOAEL was supported by a similar value (1.3 mg/kg bw per day) in a two-generation study of reproduction in rats in which reduced body-weight gain and food consumption in dams was observed. At higher doses, the pups had reduced bodyweight gain during lactation.”*

Therefore, the RMS NL re-evaluated some of the critical studies in the post-approval addendum:

“Different opinions are found among scientists whether anaemia itself is a critical effect or that the adaptation to the anaemia (higher MCV of red blood cells, increase of haematopoiesis, appearance of Heinz bodies) is the critical effect.

In the EU-DAR, a statistically significant and dose-dependent decrease in one or more indicators of circulating erythrocyte mass was considered to be an adverse effect (also when the decrease was <10%), and the simultaneous occurrence of a regenerative response was not considered a prerequisite for this judgement.

The JMPR considered that small changes in circulating erythrocyte mass in the absence of a concomitant increase in haematopoiesis were of no toxicological importance.

In the current re-evaluation of indoxacarb, the RMS agrees with the JMPR that the evaluation in the EU-DAR has been conservative and that a mild anaemia alone, without a regenerative response, should not be considered as adverse. In this addendum, the RMS used the following criteria to determine whether the haematological effects are adverse or not:

- *a statistical significant decrease in RBC count, Hb or Ht, higher than 10%, is considered adverse*
- *a statistical significant decrease in RBC count, Hb or Ht, in combination with a regenerative response (higher MCV of red blood cells, higher reticulocyte count, increase of haematopoiesis, appearance of Heinz bodies, increased pigment) is considered adverse.”*

In the sake of transparency, the RMS FR has included at the end of each study:

- the conclusions from the original DAR,
- the conclusions from the post-annex I addendum (if available) which take into account the JMPR evaluation,
- the conclusions proposed by the applicant in the renewal dossier,
- the conclusions proposed by the RMS FR in the context of the renewal of the active substance.

The RMS FR is in general in agreement with the assessment proposed by the RMS NL in the post-approval addendum. To determine the LOAELs of each study, the following criteria were considered adverse:

- a decrease in red blood cell count, haemoglobin concentration and/or haematocrit higher than 10%. As highlighted in the JMPR Guidance Document for WHO monographers and reviewers (2015), a decrease in haemoglobin value by more than about 10% is a starting point to judge anaemia.

- a decrease in red blood cell count, haemoglobin concentration and/or haematocrit around 10% but associated with at least one of the following effect:

- increased mean corpuscular volume greater than 5% (according to JMPR Guidance Document 2015)
- increased reticulocyte count
- increased level of methaemoglobin greater than 5% in dogs and 1.5% in rats (according to JMPR Guidance Document 2015). This parameter was nevertheless only measured in the 90-day rat study conducted on DPX-KN128 (99:1).
- increased haemosiderin deposits in the spleen, liver, kidney and/or bone marrow compared to the control group. Indeed, according to Muller et al. (2006)¹, “*Since a low extent of haemosiderosis is a normal age-related lesion that may show some degree of interindividual variation, only clear increases in haemosiderin deposition compared to the internal control group should be considered as treatment-related effects*”.
- increased haematopoiesis
- appearance of Heinz bodies. This parameter was nevertheless only measured in the 90-day and 1-year dog studies.

The applicant proposal for haematologic effects assessment is as follows:

“As with other target organ toxicities, a number of parameters were used to assess the adversity of the haematologic effects produced following exposure to Indoxacarb. In all studies, no-observed-adverse-effect levels (NOAELs) for haematology were based on the presence of most or all of the following observations:

- *Changes in group means for RBC mass parameters were very slight (generally decreased <10% relative to controls).*
- *RBC mass parameters for individual animals were generally within or only slightly outside reference values.*
- *Effects on the RBC, if present, were usually not of sufficient magnitude to provoke a reticulocytosis (regenerative response).*
- *Evidence of oxidant effects on red cells was miniscule or absent. For example at the NOAEL, Heinz body counts were <0.2% in dogs in the subchronic and chronic feeding studies.*

The factors noted above are consistent with recommendations for assessing adversity as given in the OECD’s Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies (OECD, 2000). In this guidance document, an adverse response is defined as “any treatment-related response that results in change in the morphology, physiology, growth development or life span of an organism, which results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other environmental influences”.

Thus, in determining the adversity of a given effect, consideration is given to

- 1) *The functional consequences to the target organ*
- 2) *The ability of the target organ to compensate for additional stress, and*
- 3) *The presence or absence of increased susceptibility to further insult*

Functional Consequences

The function of the red blood cell is oxygen delivery to the tissues. One indicator of altered tissue oxygen homeostasis is a rightward shift in the oxygen-hemoglobin dissociation curve. In humans, this shift occurs when hemoglobin values are decreased by about 30-40% of normal (Stehling and Simon, 1994). Decreases in hemoglobin concentration of this magnitude did not occur in any of the species tested following exposure to Indoxacarb, even at the highest doses tested. As noted above, one of several factors considered in assessing the significance of indoxacarb-induced hematological effects was whether red cell mass parameters were decreased by more than 10% relative to study controls. Based on a functional consideration of the erythron, this value represents a conservative criterion for adversity.

Compensatory ability.

Indoxacarb-induced haematologic effects are the result of a mild increase in the normal physiological process of red cell turnover. The compound produces no effect on the compensatory (regenerative) capacity of the bone marrow, even at high doses. Thus, indoxacarb-induced hematological effects are regenerative during continuous dosing (at doses high enough to provoke a regenerative response) and are rapidly and completely reversible following cessation of dosing (DuPont Report HL-1998-01200 Point CA 5.3.2 of this document).

¹ Muller A. et al., Hazard classification of chemicals inducing haemolytic anaemia: an EU regulatory perspective. Regulatory toxicology and Pharmacology 45 (2006) 229-241

Susceptibility to further insult.

A decrease in red cell mass, if of sufficient magnitude, could deplete red cell reserve resulting in increased susceptibility to additional stress to the erythron. However, at the NOAELs for indoxacarb induced hematology effects, parameters of red cell mass for individual animals were generally within, or only slightly outside, laboratory reference ranges for normal animals. Therefore, at the NOAELs for haematologic effects, no significant increase in susceptibility to additional hematological stress is expected.

In most of the subchronic and chronic studies with DPX-KN128, DPX-MP062, or DPX-JW062, some evidence of slight and/or transient increases in red cell turnover was present at doses that were not considered to be biologically adverse. At these lower doses, differences in group means for the parameters of red cell mass were small (usually less than 10% relative to controls), and clinical anaemia was generally absent based on assessment of red cell mass parameters in individual animals. Thus, any red cell effects that may have been present at these lower doses were fully compensated. Compensated haemolytic state occurs when some evidence of increased red cell turnover is present but without adverse changes such as anaemia or alterations in tissue oxygenation (Dornfest et al., 1983; Smith and Their, 1985). The presence of secondary microscopic changes such as slight increases in iron storage, extramedullary haemopoiesis of the spleen, and increased erythrocytic Heinz bodies also occurred at doses not considered to be adverse-effects levels. These are normal, physiologic responses to increased red cell turnover (Glaister, 1986; Lomax et al., 1990) and were not associated with primary toxicity to the spleen, even following chronic dietary exposure in rats. Since the spleen is particularly efficient at removing red cells, even those with functionally inconsequential changes (Cooper and Shattil, 1971), such microscopic changes may be present even if red cell effects are fully compensated. Heinz bodies represent oxidatively denatured haemoglobin and are common findings in oxidant-induced haemolytic anaemia (Duncan et al., 1994). Thus, while these secondary microscopic changes were useful biological indicators of exposure to a red cell oxidant, they were not considered to be representative of primary target organ toxicity or to be biologically adverse. Rather, biological adversity was determined based on changes in the primary target tissue, the red blood cell.

The changes in haematologic parameters following exposure to DPX-JW062 were qualitatively similar across all species evaluated, and were consistent with a mild regenerative anaemia whose underlying mechanism was accelerated red cell turnover (haemolysis). The minimal nature of the hematology responses in rats and dogs is underscored by a statistical study in which indoxacarb-induced hematology changes were evaluated relative to historical control data (DuPont-2780, Point CA 5.8.2 and DuPont-6122, Point CA 5.8.2). Specifically, for the one-year dog study (Point CA5.3.2) with DPX-JW062, and the 90-day study in rats with DPX-MP062 (Point CA 5.3.2), effects on red cell mass parameters (RBC, HB, HT) relative to study controls were statistically analyzed using variances derived from the respective historical control populations. In dogs, there were no statistically significant decreases in red cell mass parameters at the low dose (40 ppm) at any time point in either males or females. Similarly, in rats, there were no statistically significant decreases at 10 ppm in males or females. Also, with the exception of inconsistent decreases in haemoglobin, no significant decreases were present at 50 and 25 ppm for male and female rats, respectively. Under the conditions of this analysis, the lack of a statistically significant difference between these treated groups and their respective control indicates that any differences observed are no greater than would be expected had both groups (i.e., both treated and control) been randomly drawn from a normal (control) population. These results support the conclusion that any haematologic effects occurring at the low dose in rats and dogs were not biologically significant since differences between treated and controls were no different than differences which may be seen among historical controls."

B.6.3.1. Oral 28-day study

The 28-day oral toxicity studies available with the racemic mixture DPX-JW062 were submitted for the first inclusion of the active substance. According to the applicant, these studies were not relied upon for this renewal. Indeed, deviations from the OECD guidelines were highlighted (deviations include absence of haematology and clinical biochemistry endpoints, endocrine endpoints, and absence of histopathological evaluations on tissues). These studies were used to select dietary concentrations for the 90-day studies and were not intended to determine NOAELs.

Nevertheless, summaries of these 28-day rat and mouse studies, as provided in the original DAR, are copied below for information.

Previous evaluation:	In DAR (2000)
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reference/notifier	: [REDACTED] 1993b	exposure	: 28 days, diet
type of study	: 28-day oral toxicity study, range finding	doses	: 0, 8/400 ¹ , 12, 29, 59, 118, and 235 mg/kg food ²
year of execution	: 1993	vehicle	: acetone ³
test substance	: DPX-JW062-34, lot no. not indicated, purity 94.7%, tan solid	GLP	: no statement
route	: oral	guideline	: range finding
species	: rat, strain CrI:CD®BR, both sexes	acceptability	: supplementary
group size	: 5/sex/dose	NOAEL	: not applicable

¹ Animals were exposed to 8 mg/kg food from day 1-17 and to 400 mg/kg food for the remainder of the study.

² Mean intakes of test substance were equal to 0, 0.713/23.4, 1.02, 2.47, 5.89, 8.85, and 20.6 mg/kg bw/day for males and 0, 0.738/14.0, 1.08, 2.61, 4.72, 9.29, and 23.5 mg/kg bw/day for females, respectively.

³ The test substance was dissolved in acetone prior to mixing with the diet.

Study design

The study was not performed in accordance with OECD guideline 407, because no haematology, clinical chemistry, and microscopy was conducted. Moreover, only a limited number of organs (liver, kidneys, testes, and brain) were weighed.

Results

The results are summarized below.

Table B.6.3.1-1. Summary of results

Dose (mg/kg food)	0	12	29	59	118	235	8/400	dr
	m f	m f	m f	m f	m f	m f	m f	
Mortality						2	3	
Clinical signs¹						++ ++	++	
Body weight				d	dc	dc dc	d dc	f
Food consumption				dc ₂	dc	dc ₂	d dc	
Haematology	not performed							
Clinical chemistry	not performed							
Organ weights								
liver						d ^a	dc ^a	
kidneys				dc	dc	dc	dc ^a	f
brain					ic ^r	ic ^r	ic ^r	f
Pathology								
<u>macroscopy</u>	no treatment-related findings							
<u>microscopy</u>	not performed							

¹ clinical signs included ruffled fur in 235 mg/kg food males, signs of general ill health (e.g. pallor and ruffled fur) in 235 mg/kg food females, and abnormal gaits, dehydration, and signs of general ill health in 400 mg/kg food females.

² statistical significance was only reached for food consumption from day 0 to 17, and not from day 0 to 27.

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/c decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

+ present in one/a few animals

++ present in most/all animals

Acceptability

The study was intended as a range finding study. Haematology, clinical chemistry, and microscopy were not performed. Furthermore, only a few organ weights were determined. Results of stability and homogeneity analyses of diet samples were not included in the study report. Therefore, the study was considered of limited value for the overall toxicological evaluation of the test substance and should be considered as supplementary.

Conclusions

Because critical endpoints were not examined, only a provisional NOAEL can be established. Based on effects on body weight in female rats treated with 59 mg/kg food, the provisional NOAEL for the test substance was found to be 29 mg/kg food, equal to 2.5 mg/kg bw/day.

Previous evaluation:		In DAR (2000)	
reference/notifier	: [REDACTED] 1993a	exposure	: 28 days, diet
type of study	: 28-day oral toxicity study, range finding	doses	: 0, 12, 29/400 ¹ , 59, 118, 235, 1225, and 2450 mg/kg food ²
year of execution	: 1993	vehicle	: acetone ³
test substance	: DPX-JW062-34, lot no. not indicated, purity 94.7%, tan solid	GLP	: no statement
route	: oral	guideline	: range finding
species	: mouse, strain Crl:CD [®] -1(ICR)BR, both sexes	acceptability	: supplementary
group size	: 10/sex/dose	NOAEL	: not applicable

¹ Animals were exposed to 29 mg/kg food from day 1-7 and to 400 mg/kg food for the remainder of the study.

² Due to considerable mortality and severe animal distress, all animals from the 1225 and 2450 mg/kg food groups were sacrificed after 7 days on study. For the remaining dose groups, mean intakes of test substance were equal to 0, 2.06, 5.23/60.3, 10.8, 17.9, and 34.0 mg/kg bw/day for males and 0, 2.52, 6.83/56.0, 11.8, 21.5, and 35.3 mg/kg bw/day for females, respectively.

³ The test substance was dissolved in acetone prior to mixing with the diet.

Study design

The study was not performed in accordance with OECD guideline 407, because no haematology, clinical chemistry, and microscopy was conducted. Moreover, only a limited number of organs (liver, kidneys, testes, and brain) were weighed.

Results

The results are summarized below.

Table B.6.3.1-2. Summary of results

Dose (mg/kg bw)	0		12		59		118		235		29/400		dr
	m	f	m	f	m	f	m	f	m	f	m	f	
Mortality									1	1	1		
Clinical signs¹							+	+	++	++	++	++	mf
Body weight							dc		dc	dc	dc	dc	mf
Food consumption							dc		dc	dc	dc	dc	f
Haematology	not performed												
Clinical chemistry	not performed												
Organ weights													

Dose (mg/kg bw)	0		12		59		118		235		29/400		dr
	m	f	m	f	m	f	m	f	m	f	m	f	
liver									ic ^r		dc ^a	dc ^a	f
kidneys					ic ^r					dc ^a	dc ^a	dc ^a	
testes											ic ^r		
brain							ic ^r		ic ^r	ic ^r	ic ^r	ic ^r	
Pathology													
<u>macroscopy</u>	no treatment-related findings												
<u>microscopy</u>	not performed												

1 animals exhibited clinical signs suggestive of neurotoxicity (e.g. abnormal gait, head tilt, tremors)

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/c decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

+ present in one/a few animals

++ present in most/all animals

Acceptability

The study was intended as a range finding study. Haematology, clinical chemistry, and microscopy were not performed. Furthermore, only a few organ weights were determined. Results of stability and homogeneity analyses of diet samples were not included in the study report. Therefore, the study was considered of limited value for the overall toxicological evaluation of the test substance and should be considered as supplementary.

Conclusions

Because critical endpoints were not examined, only a provisional NOAEL can be established. Although the increase in relative weight of the kidneys observed in 59 mg/kg food males was statistically significant, it was not considered biologically relevant because of the lack of a dose-response relationship. Therefore, based on effects on body weight in male mice treated with 118 mg/kg food, the provisional NOAEL for the test substance was found to be 59 mg/kg food, equal to 10.8 mg/kg bw/day.

B.6.3.2. Oral 90-day study

Oral 90-day toxicity in the rat

Previous evaluation:	HLR 301-94 Revision No.1 : In DAR (2000) HLR 301-94 Revision No.2 : Submitted for the purpose of renewal (see reasons for revision below)
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CA 5.3.2/02

Report: [REDACTED] (1997a); Subchronic oral toxicity: 90-Day study with DPX-JW062-69 (99.7% DPX-KN128) feeding study in rats

DuPont Report No.: HLR 301-94, Revision No. 2

Guidelines: OECD 408 (1987), U.S. EPA 82-1, Nohsan No. 4200, 87/302/EEC Part B

Deviations: weekly detailed clinical observations were not evaluated in an open field arena, body weight at the initiation of the study exceeded 20% of the mean for males, and histopathological evaluation of Peyer's patches in the intestines was not specifically noted in the report since they are routinely collected and evaluated as part of the intestines. However, reconducting the study is unlikely to yield a significantly different result since detailed observations in an open field arena were conducted in the 90-day neurotoxicity study (HLR 1116-96, Revision No. 1). In addition, the exceedance of the 20% of mean body weight range at study start did not affect interpretation of the test results. The absence of histopathological evaluation of Peyer's patches is unlikely to alter the interpretation of the study since no effects on the immune system were observed in other immune

system tissues evaluated in this study or in a 28-day immunotoxicity study in mice (DuPont-29280) with indoxacarb.

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 301-94, Revision No. 2

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

Reason for Revision 2:

1. Clarification of nomenclature regarding the distinction between the insecticidally active enantiomer DPX-KN128, the insecticidally inactive enantiomer, and the racemic mixture of enantiomers.
2. Mean daily intake values were added for the corresponding NOAEL values.
3. Discussions of hematology and microscopic findings were updated following re-evaluation.
4. Statements regarding results from other 90-day studies with the racemic forms of Indoxacarb were revised due to re-assessment of the hematology and microscopic findings in these studies.
5. Addition of literature citations regarding the re-assessment of hematology and microscopic findings.
6. Addition of historical control data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

DPX-JW062 technical
Lot/Batch #: JW062-69
Purity: 91.5% DPX-JW062; 99.7% of the chemical purity is DPX-KN128
(91.2% DPX-KN128 and 0.3% DPX-KN127, by analysis)
Description: Pale yellow amorphous glass
CAS #: DPX-JW062: 144171-61-9
DPX-KN128: 173584-44-6
Stability of test compound: Analyses confirmed that test material was stable in feed for at least 14 days at room temperature, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.
2. Vehicle and/or negative control: Untreated diet. The test substance was dissolved in acetone and then the resulting solution mixed with feed. Control feed was mixed with acetone alone
3. Test animals

Species: Rat
Strain: CrI:CD [®] BR
Age at initial dosing: Approximately 42 days old
Weight at initial dosing: 154.6–251.2 g for males; 124.6–178.5 g for females
Source: XX
Acclimation period: 20 days
Diet: Irradiated Purina Certified Rodent Chow [®] (#5002), <i>ad libitum</i> . During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water: Tap water, <i>ad libitum</i>
Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions

Temperature: 21–25°C
Humidity: 40–60%
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

17-January-1994 to 19-April-1994
2. Animal assignment and treatment

Six groups of 10 animals/sex/concentration were administered concentrations of DPX-JW062 in feed daily for 90 days. Males received 0, 8, 20, 50, 100, and 200 ppm and females received 0, 3, 8, 20, 50, and 100 ppm. Dosages were based on a 28-day range-finding study and a previous 90-day feeding study, both conducted with DPX-JW062. It was assumed that these doses would demonstrate at least minimal toxicity as well as a clear NOAEL. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet.

Table B.6.3.2-1
Study design: 90-day feeding study in rats

Males				Females			
Group no.	No./ group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw	Group no.	No./ group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw
I	10	0 (control)	0 (control)	II	10	0 (control)	0 (control)
III	10	8	0.56	IV	10	3	0.25
V	10	20	1.4	VI	10	8	0.68
VII	10	50	3.2	VIII	10	20	1.7
IX	10	100	6.6	X	10	50	4.1
XI	10	200	14	XII	10	100	8.5

^a Weight/weight concentration of active ingredient in test substance (adjusted for 91.5% purity)

3. Diet preparation and analysis

The test substance (dissolved in acetone) was added to the rodent diet and thoroughly mixed for 3 minutes. Control diets were mixed for the same period of time. All diets were prepared weekly and refrigerated until used. The stability, homogeneity, and concentration of DPX-JW062 in the dietary mixtures were checked by analysis using HPLC near the beginning and end of study and approximately 7 months following the in-life phase of the study. The test substance was at target concentrations (83.7–102%), homogeneous (83.7–102%) throughout the feed and was stable (82.9–109% of nominal) for up to 14 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

Body weights, body weight gains, food consumption, food efficiency, organ weights, and clinical pathology measurements were analysed by a one-way analysis of variance. Pairwise comparisons between test and control groups were made with the Dunnett's test. Survival among groups on the final weigh day for all groups of rats was compared with the Cochran-Armitage test for trend. Increases in the incidences of clinical observations were evaluated by the Cochran-Armitage test for trend.

Increases in the incidences of all microscopic observations in tissues and organs were statistically evaluated. The Cochran-Armitage trend test was applied to all microscopic observations in tissues or organs where all of these tissues or organs (subject to availability) had been evaluated in all groups. In addition, the Cochran-Armitage trend test was also applied to decreased incidences of progressive rodent nephropathy in the kidneys. When all tissues or organs were not microscopically evaluated in all groups, the Fisher's exact test was applied to compare the control and high-dose group lesion incidences.

The Bartlett's test for homogeneity of variances was performed on the organ weight and clinical pathology data and, if significant, was followed by nonparametric procedures. The Kruskal-Wallis test for equal medians and the Mann-Whitney U test or the Dunn's multiple comparison test (nonparametric procedures) were performed.

Except for the Bartlett's test ($p < 0.005$), all significance was judged at $p < 0.05$.

C. METHODS

1. Observations

Animals were observed at least once daily for mortality and morbidity and for signs of abnormal behaviour and appearance. On days when they were weighed, each animal was individually handled, examined for abnormal behaviour and appearance.

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded for each animal over the weighing interval by weighing the feeder at the beginning and end of the interval and subtracting the final weight and the amount of spillage from the feeder from the initial. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology, clinical chemistry, and urinalysis)

Blood and urine samples were collected from all animals approximately 45 and 90 days after initiation of the study. Animals were fasted approximately 16 hours prior to sample collection. Evaluation of haematology, clinical chemistry, and urinalysis parameters were performed for all animals. Bone marrow smears were prepared at the final sacrifice from all main study animals, but experimental findings did not warrant analysis and samples were discarded.

6. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in the following table. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (200 ppm for males; 100 ppm for females) and from the control (0 ppm) and from an animal (200 ppm male) sacrificed *in extremis* prior to the

scheduled sacrifice were processed to slides and evaluated microscopically. Gross lesions and suspected target tissues (liver, kidneys, lungs, spleen, bone marrow), were processed to slides and examined microscopically for all animals. Sternal bone, sternal bone marrow, femoral bone, and femoral bone marrow from all rats in all dose groups were evaluated histomorphologically.

Table B.6.3.2-2
90-Day feeding study in rats: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Brain	X	X
Spleen		X
Heart		X
Liver	X	X
Kidneys	X	X
Oesophagus		X
Adrenal glands	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Skin		X
Trachea		X
Thymus		X
Mesenteric lymph node		X
Mandibular lymph node		X
Bone marrow		X
Thyroid gland		X
Parathyroid glands		X
Eyes		X
Exorbital lacrimal glands		X
Harderian glands		X
Testes	X	X
Epididymides		X
Prostate		X
Seminal vesicles		X
Ovaries		X
Uterus		X
Mammary glands (females)		X
Vagina		X
Stomach		X
Pituitary		X
Lungs		X
Spinal cord		X
Sciatic nerve		X
Skeletal muscle		X
Femur/knee joint		X
Sternum		X
Aorta		X
Urinary bladder		X
Gross observations		X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No test-substance related clinical signs of toxicity were observed for any dietary concentration in either males or females.

2. Mortality

Test substance-related mortality did not occur during the course of this study. One 200 ppm male rat was sacrificed in extremis on test Day 87; there were no gross or microscopic findings to suggest that the moribund condition was attributable to compound administration.

B. BODY WEIGHT AND BODY WEIGHT GAIN

At test Day 91 body weights were 9.7 and 29% lower than controls in the 100 ppm and 200 ppm males, respectively. At test Day 91 body weights were 15 and 19% lower than controls in the 50 ppm and 100 ppm females, respectively. The overall body weight gains were 16 and 45% lower than controls in the 100 ppm and 200 ppm males, respectively. The overall body weight gains were 32 and 41% lower than controls in the 50 ppm and 100 ppm females, respectively.

Table B.6.3.2-3
90-Day feeding study in rats: Body weights (g)

Day	0 ppm (control)	8 ppm	20 ppm	50 ppm	100 ppm	200 ppm
Males:						
Day 0	193.1	193.7	193.9	196.6	196.5	193.3
Day 7	255.1	253.7	254.7	255.4	242.5	197.7 ^a
Day 14	313.6	309.8	308.8	319.0	298.3	235.9 ^a
Day 21	360.5	358.7	356.3	366.1	339.5	276.6 ^a
Day 28	405.2	402.0	392.5	396.2	382.0	297.5 ^a
Day 35	438.3	440.0	421.8	443.0	403.6	333.9 ^a
Day 42	467.6	463.4	447.1	463.0	424.7	347.6 ^a
Day 49 ^b	480.9	478.9	462.8	480.8	436.9	349.4 ^a
Day 56	502.9	503.8	489.5	499.2	457.4	357.6 ^a
Day 63	531.0	524.2	509.9	522.8	478.7	377.0 ^a
Day 70	537.5	536.8	526.4	541.9	494.9	393.4 ^a
Day 77	554.6	549.5	538.8	557.0	505.1	397.2 ^a
Day 84	569.5	566.4	554.6	572.4	518.5	400.2 ^a
Day 91	555.9	549.5	541.8	557.3	502.2 (-9.7%)	393.2 ^a (-29%)

^a Significantly different from control by the one-way analysis of variance and Dunnett's test criteria, $p < 0.05$.

^b Rats were fasted approximately 20 hours prior to clinical evaluation (test day 45).

Table B.6.3.2-4
90-Day feeding study in rats: Body weights (g)

Day	0 ppm (control)	3 ppm	8 ppm	20 ppm	50 ppm	100 ppm
Females:						
Day 0	152.3	155.9	152.0	154.0	154.0	153.6
Day 7	176.3	181.1	176.4	176.7	166.4	144.2 ^a
Day 14	198.8	206.4	199.1	197.7	198.1	167.7 ^a
Day 21	217.1	228.2	216.7	216.6	203.8	182.4 ^a
Day 28	232.7	242.0	230.2	230.5	190.6 ^a	205.4 ^a
Day 35	247.3	259.1	243.5	245.1	225.3 ^a	197.8 ^a
Day 42	256.9	269.3	254.3	254.9	227.7 ^a	210.1 ^a
Day 49 ^b	258.8	269.8	257.4	255.9	232.1 ^a	207.9 ^a
Day 56	265.9	278.5	265.5	264.4	240.7 ^a	219.5 ^a
Day 63	277.8	289.6	273.2	270.1	240.4 ^a	228.4 ^a
Day 70	282.7	293.0	277.6	277.8	248.9 ^a	230.7 ^a
Day 77	284.6	296.5	281.6	285.6	250.4 ^a	235.6 ^a
Day 84	292.0	306.3	289.1	296.1	252.1 ^a	238.4 ^a
Day 91	293.6	305.4	289.8	281.3	250.1 ^a (-15%)	236.5 ^a (-19%)

^a Significantly different from control by the one-way analysis of variance and Dunnett's test criteria, $p < 0.05$.

^b Rats were fasted approximately 20 hours prior to clinical evaluation (test day 46).

Table B.6.3.2-5
90-Day feeding study in rats: Body weight gain (g)

Parameter	0 ppm	3 ppm	8 ppm	20 ppm	50 ppm	100 ppm	200 ppm
Males:							
Body weight gain, Day 0-7	62.1	na ^c	60.0	60.8	58.8	46.0 ^d	4.5 ^d
Body weight gain, Day 7-14	58.4	na	56.1	54.1	63.5	55.9	38.2 ^d
Body weight gain, Day 14-21	46.9	na	48.9	47.5	47.1	41.1	40.7
Body weight gain, Day 21-28	44.8	na	43.3	36.3	30.1 ^d	42.6	20.9 ^d
Body weight gain, Day 28-35	33.0	na	38.0	29.2	46.8	21.6	36.4
Body weight gain, Day 35-42	29.3	na	23.3	25.4	20.0	21.1	13.7
Body weight gain, Day 42-49 ^a	13.3	na	15.5	15.6	17.8	12.2	1.8
Body weight gain, Day 49-56	22.1	na	24.9	26.7	18.4	20.5	8.2 ^d
Body weight gain, Day 56-63	28.1	na	20.4	20.4	23.6	21.3	19.4
Body weight gain, Day 63-70	6.5	na	12.6	16.5	19.1	16.2	16.4
Body weight gain, Day 70-77	17.0	na	12.7	12.3	15.1	10.3	3.8 ^d
Body weight gain, Day 77-84	14.9	na	16.9	15.8	15.4	13.4	3.0
Body weight gain, Day 84-91	-13.6	na	-16.8	-12.8	-15.1	-16.4	-22.2
Overall body weight gain, Day 0 - 91	362.9	na	355.8	347.9	360.6	305.7 (-16%)	199.9 ^d (-45%)
Females:							
Body weight gain, Day 0-7	24.1	25.2	24.5	22.7	12.4 ^d	-9.4 ^d	na
Body weight gain, Day 7-14	22.5	25.3	22.6	21.0	31.6 ^d	23.5	na
Body weight gain, Day 14-21	18.3	21.7	17.7	18.9	5.7 ^d	14.8	na
Body weight gain, Day 21-28	15.6	13.8	13.4	13.9	-13.2 ^d	23.0 ^d	na
Body weight gain, Day 28-35	14.6	17.1	13.4	14.5	34.8 ^d	-7.6 ^d	na

Body weight gain, Day 35-42	9.6	10.2	10.8	9.8	2.4	12.3	na
Body weight gain, Day 42-49 ^b	1.9	0.5	3.1	1.0	4.3	-2.2	na
Body weight gain, Day 49-56	7.1	8.8	8.1	8.4	8.6	11.6	na
Body weight gain, Day 56-63	12.0	11.1	7.7	5.7	-0.3 ^d	8.9	na
Body weight gain, Day 63-70	4.9	3.4	4.5	7.7	8.5	2.3	na
Body weight gain, Day 70-77	1.9	3.5	4.0	7.9	1.4	4.9	na
Body weight gain, Day 77-84	7.5	9.8	7.5	10.5	1.8	2.8	na
Body weight gain, Day 84-91	1.6	-0.9	0.7	-14.8	-2.0	-1.9	na
Overall body weight gain, Day 0 - 91	141.3	149.5	137.8	127.3	96.1 ^d (-32%)	82.8 ^d (-41%)	na

^a Rats were fasted approximately 20 hours prior to clinical evaluation (test day 45).

^b Rats were fasted approximately 20 hours prior to clinical evaluation (test day 46).

^c na = not applicable; dosage not administered to that sex.

^d Significantly different from control by the one-way analysis of variance and Dunnett's test criteria, p <0.05.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Males administered the 100 ppm and 200 ppm diet had lower food consumption compared to controls (9.8 and 21%, respectively). Females administered the 50 ppm and 100 ppm diet had lower food consumption compared to controls (11 and 15% lower, respectively). Test substance-related decreases in food efficiency occurred in males administered 100 ppm and 200 ppm (7.2 and 30% lower, respectively.) Test substance-related decreases in food efficiency occurred in females administered 50 ppm and 100 ppm (24 and 29% lower, respectively).

Table B.6.3.2-6
90-Day feeding study in rats: Body weight gain/food consumption/food efficiency

Parameter	0 ppm	3 ppm	8 ppm	20 ppm	50 ppm	100 ppm	200 ppm
Males:							
Body weight gain, Day 0-91 ^a	362.9	na ^c	355.8	347.9	360.6	305.7 (-16%)	199.9 ^d (-45%)
Food consumption, Day 0-91 ^a	29.6	na	30.2	29.6	28.5	26.7 (-9.8%)	23.5 ^d (-21%)
Food efficiency, Day 0-91 ^b	0.138	na	0.132	0.131	0.142	0.128 (-7.2%)	0.096 ^d (-30%)
Females:							
Body weight gain, Day 0-91 ^a	141.3	149.5	137.8	127.3	96.1 ^d (-32%)	82.8 ^d (-41%)	na
Food consumption, Day 0-91 ^a	20.7	21.4	21.0	20.6	18.4 (-11%)	17.5 ^d (-15%)	na
Food efficiency, Day 0-91 ^b	0.076	0.078	0.073	0.069	0.058 ^d (-24%)	0.054 ^d (-29%)	na

^a In grams

^b Grams weight gain/ grams food consumed

^c na = not applicable; dosage not administered to that sex.

^d Significantly different from control by the one-way analysis of variance and Dunnett's test criteria, p <0.05.

D. OPHTHALMOLOGICAL EXAMINATIONS

No test-substance related ophthalmological observations were observed for any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Haematology

Males and females had decreases in the indicators of erythrocyte mass: red blood cell count, haemoglobin concentration, and haematocrit. These changes were associated with a regenerative response by the haematopoietic system based on increased MCV and/or increased reticulocyte counts at the two highest tested doses in males and females. Slight increases in methaemoglobin were also present in these groups. Methaemoglobin results from oxidation of haemoglobin iron from the ferrous to the ferric state. Taken together, the findings decreased erythrocyte mass, an associated regenerative response, and slight methaemoglobinaemia are consistent with an oxidant-induced haemolytic process. Secondary microscopic findings such as increased iron pigment storage and extramedullary haematopoiesis in the spleen were also consistent with a haemolytic process.

Table B.6.3.2-7
90-Day feeding study in rats: Haematology findings

Parameter	0 ppm	3 ppm	8 ppm	20 ppm	50 ppm	100 ppm	200 ppm
Males (Test Day 45):							
RBC × 10 ⁶ /μL	7.49	na ^d	7.51	7.30 -2.5%	7.31 -2.4%	7.37 -1.6%	6.95 -7.2%
Hb (g/dL)	15.8	na	15.3 -3.2%	15.4 -2.5%	15.2 -3.8%	15.3 -3.2%	14.9 -5.7%
Ht %	40	na	41	40	39 -2.5%	41	39 -2.5%
Reticulocytes × 10 ³ /μL	157	na	208	225	217	198	244
MCV (fl)	54	na	54	55	54	55	57 ^b +5.6%
MCH (pg)	21	na	21	21	21	21	22
MCHC (g/dL)	39	na	38	39	39	38	38
Platelets × 10 ³ /μL	1123	na	1012 ^a	1064	1014	1076	1102
Met-Hb (%)	c	na	c	c	c	c	c
WBC × 10 ³ /μL	19.6	na	18.8	22.5	20.1	22.2	22.3
Neutrophils (WBC × %)	1599	na	1689	3182 ^a	1346	2402 ^a	3605 ^a
Lymphocytes (WBC × %)	16234	na	14595	17357	16781	17829	16747
Males (Test Day 90):							
RBC × 10 ⁶ /μL	8.13	na	8.22	7.80 -4.1%	7.93 -2.5%	7.49 ^b -7.9%	7.33 ^b -9.8%
Hb (g/dL)	16.3	na	15.9 ^a -2.5%	15.5 -4.9%	15.4 ^a -5.5%	15.2 ^a -6.7%	14.8 ^a -9.2%
Ht %	49	na	48 -2%	47 -4.1%	46 ^a -6.1%	45 ^a -8.2%	46 -6.1%
Reticulocytes × 10 ³ /μL	106	na	91	104	155	168	188 ^b
MCV (fl)	60	na	58	61	58	60	62 ^b +3.3%
MCH (pg)	20	na	19	20	19	20	20
MCHC (g/dL)	34	na	34	33	34	34	33
Platelets × 10 ³ /μL	1176	na	979 ^b	1111	1104	1135	1197
Met-Hb (%)	0	na	1 ^a	1 ^a	2 ^a	4 ^a	8 ^a
WBC × 10 ³ /μL	15.6	na	12.8 ^a	16.3	13.7	15.1	18.6
Neutrophils (WBC × %)	1840	na	1362	3138	2096	2852	4209
Lymphocytes (WBC × %)	11322	na	10003	11566	9866	11157	13128

Table B.6.3.2-8
90-Day feeding study in rats: Haematology findings

Parameter	0 ppm	3 ppm	8 ppm	20 ppm	50 ppm	100 ppm
Females (Test Day 45):						
RBC × 10 ⁶ /μL	7.68	7.36 -4.2%	7.10 ^a -7.6%	7.00 ^a -8.9%	6.40 ^a -16.7%	7.05 ^a -8.2%
Hb (g/dL)	16.3	15.8 ^a -3.1%	15.4 ^a -5.5%	14.8 ^a -9.2%	14.1 ^a -13.5%	15.3 ^a -6.1%
Ht %	43	41 -4.7%	40 ^a -7%	39 ^a -9.3%	37 ^a -14%	41 -4.7%
Reticulocytes × 10 ³ /μL	66	85	85	161 ^b	130 ^b	124

MCV (fl)	55	56 +1.8%	56 +1.8%	56 +1.8%	58 ^b +5.5%	58 ^b +5.5%
MCH (pg)	21	21	22	21	22 ^b	22
MCHC (g/dL)	38	39	39	38	38	37 ^a
Platelets × 10 ³ /μL	1097	1006	982 ^a	1006	1084	899
Met-Hb (%)	c	c	c	c	c	c
WBC × 10 ³ /μL	16.0	17.0	15.1	14.5	15.1	20.6 ^b
Neutrophils (WBC × %)	1478	1692	1160	1298	1337	1404
Lymphocytes (WBC × %)	13581	13640	13063	12480	13231	18412 ^b
Females (Test Day 90):						
RBC × 10 ⁶ /μL	8.24	7.74 ^b -6.1%	7.75 ^b -5.9%	7.57 ^b -8.1%	7.09 ^b -14%	6.84 ^b -17%
Hb (g/dL)	16.8	16.2 -3.6%	16.0 ^b -4.8%	15.6 ^b -7.1%	15.0 ^b -10.7%	15.1 ^b -10.1%
Ht %	52	49 ^b -5.8%	49 ^b -5.8%	48 ^b -7.7%	47 ^b -9.6%	46 ^b -11.5%
Reticulocytes × 10 ³ /μL	96	66	62	83	131	96
MCV (fl)	63	63	63	63	66 ^b +4.8%	67 ^b +6.3%
MCH (pg)	20	21	21	21	21	22 ^b
MCHC (g/dL)	33	33	33	33	32	33
Platelets × 10 ³ /μL	964	936	949	1110	1184 ^b	1081
Met-Hb (%)	0	0	1	0	2 ^a	4 ^a
WBC × 10 ³ /μL	9.8	9.9	9.9	10.7	13.1	14.2 ^b
Neutrophils (WBC × %)	785	640	724	1086	1141	783
Lymphocytes (WBC × %)	8346	8678	8639	8973	11348	13006 ^b

^a Significantly different from control by the Mann-Whitney U criteria, p <0.05.

^b Significantly different from control by the Dunnett criteria, p <0.05.

^c Evaluated for Day 90 only

^d na = not applicable (dosage not administered to that sex)

RBC Red blood cells

Hb Haemoglobin

Ht Haematocrit

MCV Mean Corpuscular Volume

MCH Mean Corpuscular Haemoglobin

MCHC Mean Corpuscular Haemoglobin Concentration

Met-Hb Methaemoglobin

WBC White blood cells

2. Clinical chemistry

There were no toxicologically significant changes in clinical chemistry parameters in male or female rats.

3. Urinalysis

There were no adverse changes in urine parameters in male or female rats.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

There were no organ weight effects that were considered directly related to the administration of the test substance. Any organ weight alterations were judged to be secondary effects of toxicologically significant body weight decrements in male rats at 100 and 200 ppm and/or female rats at 50 and 100 ppm.

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy.

Statistically significant test substance-related and microscopic changes were observed in males and in females and consisted of yellow-green pigment in liver Kupffer cell (for the 200 ppm male and 100 ppm female groups), increased amounts of yellow-brown pigment in macrophages of the spleen (males: 50 ppm and above, and females: 20 ppm and above), erythrocytic hyperplasia in the spleen (males: 50 ppm and above, and females: 20 ppm and above), and mixed hyperplasia of bone marrow (females: 50 ppm and above).

Perls iron stain demonstrated the presence of hemosiderin in pigment-containing Kupffer cells of the liver and in pigment contained in macrophages in the spleen of representative male and female high concentration rats.

A decreased incidence of progressive rodent nephropathy in the kidneys was statistically significant in male rats at 200 ppm (1/10) and in female rats at 50 and 100 ppm (0/10 and 0/10, respectively) compared to male control (9/10) and female control (2/10) rats. This decreased incidence was most likely due to self-induced caloric and protein restriction as evidenced by significant decreases in food consumption and food efficiency by rats in these groups and was judged to be a compound-related effect secondary to nutritional alterations but was not considered toxicologically important.

Table B.6.3.2-9
90-Day feeding study in rats: Incidences of microscopic effects

Test substance concentration	0 ppm	3 ppm	8 ppm	20 ppm	50 ppm	100 ppm	200 ppm
Number of rats/group:	10	10	10	10	10	10	10
Males:							
Liver							
Pigment, increased Kupffer cell	0 ^a	na ^b	0	0	0	1	8 ^c
Spleen							
Hyperplasia, erythrocytic	0	na	0	1	8 ^c	9 ^c	9 ^c
Pigment, increased (macrophages)	0	na	1	2	6 ^c	10 ^c	10 ^c
Bone marrow							
Hyperplasia, mixed	3	na	5	3	4	6	5
Females:							
Liver							
Pigment, increased Kupffer cell	0	0	0	0	1	6 ^c	na
Spleen							
Hyperplasia, erythrocytic	0	0	2	2	5 ^c	8 ^c	na
Pigment, increased (macrophages)	0	1	2	10 ^c	10 ^c	10 ^c	na
Bone marrow							
Hyperplasia, mixed	0	1	1	1	5 ^c	7 ^c	na

^a Number of organs with microscopic change.

^b na = not applicable; dosage not administered to that sex.

^c Statistically significant by the Cochran-Armitage trend test criteria, $p < 0.05$.

Conclusions from the original DAR (2000, RMS NL):

The authors considered the haematological changes only relevant when all of the following criteria were met: (1) a change of >10% compared to control values, (2) the presence of the effect both at 45 and 90 days after the start of treatment, and (3) the presence of a regenerative bone marrow response (MCV, reticulocytes). Based on these

considerations they established a NOAEL of 50 mg/kg food for males (based on effects on body weight and food consumption at 100 mg/kg food) and of 20 mg/kg food for females (based on effects on red blood cell parameters at 50 mg/kg food). However, the reviewer considers a statistically significant and dose-dependent change in one or more indicators of circulating erythrocyte mass to be an adverse effect of the treatment because these changes, in combination with the microscopic changes in the liver and spleen, indicate the presence of haemolytic anaemia. A simultaneous regenerative (bone marrow) response is not considered to be a prerequisite for this judgement. It is noted by the reviewer that increased serum chloride levels have been suggested to be associated with anaemia, although sufficient scientific evidence is lacking. Increased pigment levels noted in several organs at microscopic examination as well as increased extramedullary haemopoiesis in the spleen and hyperplasia in bone marrow, are considered to be secondary to the observed effects on circulating red cell parameters. The significantly decreased incidence of progressive rodent nephropathy in the kidneys was judged by the authors to be a compound-related effect secondary to nutritional alterations (restricted dietary caloric and protein intake), but was not considered toxicologically relevant.

Based on these considerations, the NOAEL for semichronic exposure to the test substance was set at <8 mg/kg food for male rats, equal to <0.56 mg/kg bw/d, and at <3 mg/kg food for female rats, equal to <0.25 mg/kg bw/d.

Conclusions from the Post-Annex I Addendum (2007, RMS NL):

Study not reviewed.

Conclusions proposed by the applicant (2015):

The NOAEL for DPX-JW062-69 [91.2% DPX-KN128 and 0.3% DPX-KN127] was 50 ppm (3.2 mg/kg bw/day) for males and 20 ppm (1.7 mg/kg bw/day) for females. This NOAEL was based on the presence of compound-related, toxicologically significant decrements in body weight, body weight gain, food consumption, and food efficiency in males at the LOAEL, 100 ppm, and the presence of compound-related, toxicologically significant decrements in body weight, body weight gain, food consumption, food efficiency, and haemolysis of equivocal significance in females at the LOAEL, 50 ppm.

RMS FR assessment (2016):

Splenic effects in the majority of animals (haemosiderosis and erythrocytic hyperplasia) associated with decreased in red blood cell parameters were considered adverse from the dose levels of 50 ppm in males and 20 ppm in females. Methaemoglobinemia was also observed in males and females from the dose level of 50 ppm. The NOAEL is thus set at 20 ppm (1.4 mg/kg bw/d) for males and 8 ppm (0.68 mg/kg bw/d) for females. At higher dose levels, decreased body weight, body weight gains and food consumption were observed, as well as increased MCV and/or reticulocytes, hemosiderosis in the liver and bone marrow hyperplasia in females.

Previous evaluation:	HL-1997-00056 : In DAR (2000) HL-1997-00056 Revision No.1 : Submitted for the purpose of renewal (see reasons for revisions below)
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CA 5.3.2/01

Report: [REDACTED] (1997); Subchronic oral toxicity: 90-Day study with DPX-MP062 (approximately 75% DPX-KN128, 25% DPX-KN127) feeding study in rats

DuPont Report No.: HL-1997-00056, Revision No. 1

Guidelines: Directive 87/302/EEC Part B (1987), U.S. EPA 82-1 (1982), 59 NohSan No. 4200 (1985), OECD 408 (1987)

Deviations: Ophthalmological findings and gross pathological lesion incidences were not evaluated by statistics. Detailed clinical observations were not evaluated in an open field arena, and histopathological evaluation of Peyer's patches in the intestines was not specifically noted in the report since they are routinely collected and evaluated as part of the intestines. However, reconducting the study is unlikely to yield a significantly different result since detailed observations in an open field arena were conducted in the 90-day neurotoxicity study (HLR 1116-96, Revision No. 1). The absence of histopathological evaluation of Peyer's patches is unlikely to alter the interpretation of the study since no effects on the immune system were observed in other immune system tissues evaluated in this study or in a 28-day immunotoxicity study in mice (DuPont-29280) with indoxacarb (DPX-KN128).

Testing Facility: [REDACTED]

Testing Facility Report No.: HL-1997-00056, Revision No. 1

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

Reason for Revision No. 1:

1. Clarification of nomenclature regarding the distinction between the insecticidally active enantiomer DPX-KN128, the insecticidally inactive enantiomer, and the racemic mixture of enantiomers.
2. The bases for dose level selection was updated
3. Discussions of hematology and microscopic findings were updated following re-evaluation.
4. Addition of literature citations regarding the re-assessment of hematology and microscopic findings.
5. Addition of historical control data

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical. As stated in the study report, the isomers ratio is 79% DPX-KN128, 21% DPX-KN127.
Lot/Batch #: MP062-51A
Purity: 94.5%
Description: Off-white solid
CAS #: None for DPX-MP062
DPX-KN128: 173584-44-6
Stability of test compound: Analyses confirmed that test material was stable in feed for up to 21 days at room temperature or refrigerated, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared every other week.
2. Vehicle and/or negative control: Untreated diet (containing the same amount of acetone used to solubilize the test substance)
3. Test animals
Species: Rat
Strain: CrI:CD[®](SD)BR
Age at initial dosing: Approximately 49 days old
Weight at initial dosing: 171.3–265.7 g for males; 149.0–194.2 g for females
Source: [REDACTED]
Acclimation period: Minimum of 2 weeks
Diet: PMI[®] Feeds, Inc.[™] Irradiated Certified Rodent Diet[™] (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water: Tap water, *ad libitum*
Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
Temperature: 23 ± 1°C
Humidity: 50 ± 10%
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

03-September-1996 to 05-December-1996

2. Animal assignment and treatment

Five groups of 10 animals/sex/concentration were administered concentrations of DPX-MP062 in feed daily for 90 days. Males received 0, 10, 50, 100, and 200 ppm, and females received 0, 10, 25, 50, and 100 ppm. These doses were selected based on test substance-related body weight and haematology effects observed previously with DPX-JW062, a closely related isomeric mixture. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet.

Table B.6.3.2-10
Study design: 90-Day feeding study in rats

Males				Females			
Group no.	No./ group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw	Group no.	No./ group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw
I	10	0 (control)	0 (control)	II	10	0 (control)	0 (control)
III	10	10	0.620	IV	10	10	0.760
V	10	50	3.09	VI	10	25	2.13
VII	10	100	6.01	VIII	10	50	3.78
IX	10	200	15.0	X	10	100	8.94

^a Weight/weight concentration of active substance (total isomers) in DPX-MP062 (adjusted for 94.5% purity)

3. Diet preparation and analysis

The test substance (dissolved in acetone) was added to the rodent diet and thoroughly mixed. Control diets were mixed with acetone under the same conditions as the diets prepared with test substance. All diets were prepared every other week and refrigerated until used. The homogeneity and concentration of DPX-MP062 in the dietary mixtures were checked by analysis using HPLC on Day -7 and Days 49, and 76, respectively. Stability was assessed at 7, 14, and 21 days after preparation in a separate neurotoxicity study with DPX-MP062. The test substance was at target concentrations (88.5% to 99.8% of nominal), homogeneous (C.V. = 2.2% to 8.6%) throughout the feed, and was stable (89.5% to 97.5% of nominal) for up to 21 days at room temperature or refrigerated. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

Body weights, body weight gains, food consumption, food efficiency, clinical pathology measurements, and organ weights were analysed by a one-way analysis of variance (ANOVA). Pairwise comparisons between test and control groups were made with the Dunnett's test. The Bartlett's test for homogeneity of variances was performed on the clinical pathology and organ weight data and, if significant, the following nonparametric procedures were performed: the Kruskal-Wallis test for equal medians and the Mann-Whitney U test (clinical pathology) or Dunn's multiple comparison test (organ weight data). Within each sex, increased incidences of clinical observations among groups and survival on the final weigh and food consumption day were compared using the Cochran-Armitage trend test. Except for Bartlett's test ($p < 0.005$), significance was judged at $p < 0.05$.

C. METHODS

1. Observations

Animals were observed at least once daily for mortality and morbidity and for signs of abnormal behaviour and appearance. On days when they were weighed, each animal was individually handled, examined for abnormal behaviour and appearance, and subjected to detailed clinical observations.

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology, clinical chemistry, and urinalysis)

Blood and urine samples were collected from all animals approximately 50 and 90 days after initiation of the study. Animals were fasted approximately 16 hours prior to sample collection. At sacrifice blood, bone marrow, and urine were collected. Evaluation of haematology, clinical chemistry, and urinalysis parameters were performed for all animals. Bone marrow smears were prepared at the final sacrifice from all main study animals, but experimental findings did not warrant analysis and samples were discarded.

6. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in the following table. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (200 ppm for males; 100 ppm for females) and control (0 ppm) and from animals that died or were sacrificed prior to the scheduled sacrifice were processed to slides and evaluated microscopically. Gross lesions and suspected target tissues (liver, kidney, lungs, spleen, and bone marrow [sternum]), as determined by examination of the control and high dose animals, were processed to slides and examined microscopically for all animals.

Table B.6.3.2-11
90-Day feeding study in rats: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted ^a
Brain	X	X
Spleen		X
Heart		X
Liver	X	X
Kidneys	X	X
Oesophagus		X
Adrenal glands	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Skin		X
Trachea		X
Nose		X ^a
Thymus		X
Mesenteric lymph node		X
Mandibular lymph node		X
Bone marrow		X
Thyroid gland		X
Parathyroid glands		X
Harderian glands		X
Exorbital lacrimal glands		X
Eyes		X
Testes	X	X
Epididymides		X
Prostate		X
Seminal vesicles		X
Ovaries		X
Uterus		X
Vagina		X
Mammary glands (females)		X
Stomach		X
Pituitary gland		X
Lungs		X
Spinal cord		X
Sciatic nerve		X
Skeletal muscle		X
Femur (including joint)		X
Sternum		X
Aorta		X
Urinary bladder		X
Gross lesions		X

^a Evaluated microscopically only if a gross lesion was present

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

Test substance-related clinical signs of toxicity (weakness and ataxia) were observed in the 100 ppm females over the first 3 weeks of exposure. Tremors (1/10) and hyperreactivity (2/10) were also observed in this group: it is unclear whether these signs were test substance-related because the incidences were low, the increases were not statistically significant, and hyperreactivity was also observed at lower concentrations, in a non-dose-related pattern. No test substance-related clinical signs of toxicity were observed for any dietary concentration in males or for any dietary concentration at or below 50 ppm in females.

2. Mortality

One control female was found dead. She died of a complex of urinary calculi, inflammation and obstruction. Test substance-related mortality (five of 10) occurred in 100 ppm females. They were found dead or sacrificed in extremis during the second or third week of dietary exposure (test days 8, 9, 10, 13 and 19). These rats had all lost weight prior to death and some exhibited weakness and/or ataxia. These rats had atrophy of spleen, thymus, and/or bone marrow at necropsy, and haemoglobin pigment was found in the renal tubule cells and/or lumens. Test substance-related mortality in male rats did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

At test Day 90 body weights of 200 ppm males were 72% of control. The overall body weight gains for 200 ppm males were 54% of control. Body weight gains of 100 ppm males were also decreased compared to control group, although no statistically significant decrease in mean final body weight was observed in this group. Mean final body weight was 83% and 85% of control for the 100 and 50 ppm females, respectively at test Day 90. The overall body weight gains were 58% and 62% of control for the 100 ppm and 50 ppm females, respectively.

There were no test substance-related effects on body weights or body weight gains in males at or below 50 ppm or in females at or below 25 ppm. Summaries of body weights and body weight gains are provided in the tables below. Sporadic statistical differences in weight gain were observed at other time intervals, however they were not consistent with mean body weights, and therefore were not considered to be adverse.

Table B.6.3.2-12
90-Day feeding study in rats: Body weights (g)

Day	0 ppm (males and females)	10 ppm (males and females)	50 ppm (males) 25 ppm (females)	100 ppm (males) 50 ppm (females)	200 ppm (males) 100 ppm (females)
Males:					
Day 0	236.5	238.8	229.5	236.5	225.7
Day 90	544.9	535.7 -2%	530.4 -3%	499.4 -8%	393.2 ^a -28%
Females:					
Day 0	170.6	174.9	170.0	171.8	172.9
Day 90	282.8	296.5 -5%	267.1 -6%	240.4 ^a -15%	233.4 ^a -17%

^a Significantly different from control by the ANOVA and Dunnett's tests criteria, $p < 0.05$.

Table B.6.3.2-13
90-Day feeding study in rats: Body weight gain

Parameter	0 ppm (males and females)	10 ppm (males and females)	50 ppm (males) 25 ppm (females)	100 ppm (males) 50 ppm (females)	200 ppm (males) 100 ppm (females)
Males:					
Body weight gain, Day 0–13 (g)	93.3	88.0	90.8	76.4	28.2 ^a
Body weight gain, Day 0–41 (g)	216.0	203.9	211.2	178.3 ^a	82.3 ^a
Overall body weight gain, Day 0–90 (g)	308.5	297.0	290.5	262.9	167.4 ^a
Females:					
Body weight gain, Day 0–13 (g)	32.6	31.7	26.6	11.3 ^a	-30.9 ^a
Body weight gain, Day 0–41 (g)	77.3	79.4	68.3	48.3 ^a	35.3 ^a
Overall body weight gain, Day 0–90 (g)	111.2	121.6	97.1	68.6 ^a	64.5 ^a

^a Significantly different from control by the ANOVA and Dunnett's tests criteria, p <0.05.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Males administered the 200 ppm diet had lower food consumption compared to controls (85% of the control value). There were no test substance-related effects on overall food consumption in female rats at any concentration. Test substance-related decreases in food efficiency occurred in males administered 200 ppm DPX-MP062 (65% of the control value). Test substance-related decreases in food efficiency occurred in females administered 50 ppm or 100 ppm DPX-MP062 (75 % and 71% of the control values, respectively). There were no test substance-related effects on food consumption or food efficiency in male rats at 100 ppm or below. Transient, sporadic statistical differences were observed at other time points, however, overall food consumption was not affected, and therefore these differences were not considered to be adverse. Body weight gain, food consumption, and food efficiency data are summarised in the following table.

Table B.6.3.2-14
90-Day feeding study in rats: Body weight gain/food consumption/food efficiency

Parameter	0 ppm (males and females)	10 ppm (males and females)	50 ppm (males) 25 ppm (females)	100 ppm (males) 50 ppm (females)	200 ppm (males) 100 ppm (females)
Males:					
Body weight gain, Day 0–90 (g)	308.5	297.0	290.5	262.9	167.4 ^a
Food consumption, Day 0–90 (g)	26.7	26.5	26.7	24.4	22.8 ^a
Food efficiency, Day 0–90 (g wt. gain/g food consumed)	0.130	0.125	0.122	0.121	0.085 ^a
Females:					
Body weight gain, Day 0–90 (g)	111.2	121.6	97.1	68.6 ^a	64.5 ^a
Food consumption, Day 0–90 (g)	19.9	19.1	19.9	16.3	17.9
Food efficiency, Day 0–90 (g wt. gain/g food consumed)	0.063	0.071	0.055	0.047 ^a	0.045 ^a

^a Significantly different from control by the ANOVA and Dunnett's tests criteria, p <0.05.

D. OPHTHALMOLOGICAL EXAMINATIONS

No ophthalmological observations were observed for any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Haematology

Haemolytic changes were present in male rats from the dose level of 50 ppm and in female rats at and above 10 ppm. Erythrocytic changes were characterised by dose-related decreases in the indicators of erythrocyte mass: erythrocyte count (RBC), haemoglobin concentration (Hb), and haematocrit (Ht). On a group mean basis, these parameters were mildly decreased (in some instances by 10% or more, relative to controls) at both sampling times in both sexes. Increased mean cell volume (MCV) observed in the highest tested groups was suggestive of a normal bone marrow regenerative response. It should however be noted that no significant increased of reticulocytes count was noted. The haemolytic nature of the anaemia was confirmed by secondary microscopic findings such as increased pigment storage in macrophages of the liver and spleen, and increased haemopoiesis in the spleen.

Table B.6.3.2-15
90-Day feeding study in rats: Haematology findings

Parameter	0 ppm (males and females)	10 ppm (males and females)	50 ppm (males) 25 ppm (females)	100 ppm (males) 50 ppm (females)	200 ppm (males) 100 ppm (females)
Males (Test Day 45):					
RBC ^a ($\times 10^6/\mu\text{L}$) (SD ^b)	7.99 (0.28)	8.31 (0.28) +4%	8.26 (0.32) +3%	7.57 (0.39) -5%	7.27 (0.73)^c -9%
Hb ^d (g/dL) (SD)	16.2 (0.8)	16.1 (0.5) -1%	15.7 (0.5) -3%	15.3 (0.6)^c -6%	15.1 (1.1)^c -7%
Ht ^e (%) (SD)	47 (3)	48 (2) +2%	47 (2) 0%	45 (2) -4%	45 (4) -4%
MCV ^f (fl) (SD)	59 (2)	58 (2)	57 (2)	60 (3)	62 (3)^c
Males (Test Day 90):					
RBC ($\times 10^6/\mu\text{L}$) (SD)	8.54 (0.28)	8.78 (0.29) +3%	8.29 (0.39) -3%	7.78 (0.39)^c -9%	7.19 (0.45)^c -16%
Hb (g/dL) (SD)	16.1 (0.5)	16.2 (0.5) +1%	15.1 (0.5)^c -6%	14.8 (0.5)^c -8%	14.4 (0.5)^c -11%
Ht (%) (SD)	47 (1)	48 (2) +2%	45 (2) -4%	44 (2)^c -6%	43 (2)^c -9%
MCV (fl) (SD)	55 (2)	55 (2)	55 (1)	57 (2)	60 (2)^c
Females (Test Day 45):					
RBC ($\times 10^6/\mu\text{L}$) (SD)	8.12 (0.50)	7.73 (0.28) -5%	7.60 (0.33)^c -6%	7.61 (0.35)^c -6%	6.68 (0.22)^c -18%
Hb (g/dL) (SD)	16.3 (0.5)	15.6 (0.5)^c -4%	15.4 (0.3)^c -6%	15.5 (0.6)^c -5%	14.4 (0.2)^c -12%
Ht (%) (SD)	50 (3)	47 (2)^c -6%	47 (1)^c -6%	48 (2)^c -4%	43 (0)^c -14%
MCV (fl) (SD)	62 (2)	61 (2)	62 (2)	63 (2)	64 (3)
Females (Test Day 90):					
RBC ($\times 10^6/\mu\text{L}$) (SD)	8.19 (0.50)	7.64 (0.62) -7%	7.71 (0.22) -6%	7.36 (0.53)^c -10%	7.00 (0.21)^c -15%
Hb (g/dL) (SD)	16.0 (1.0)	15.4 (0.7) -4%	15.1 (0.3)^c -6%	14.9 (0.7)^c -7%	14.7 (0.3)^c -8%
Ht (%) (SD)	49 (3)	46 (3)^c -6%	46 (1)^c -6%	45 (2)^c -8%	45 (1)^c -8%
MCV (fl) (SD)	60 (1)	60 (2)	60 (1)	61 (2)	65 (1)^c

^a RBC = red blood cells

^b SD = standard deviation

^c Significantly different from control by the Dunnett criteria, $p < 0.05$.

^d Hb = haemoglobin

^e Ht = haematocrit

^f MCV = mean corpuscular volume

2. Clinical chemistry

Male rats exposed to 200 ppm of DPX-MP062 exhibited mildly decreased total protein and globulin concentrations at the 45- and 90-day evaluations. These changes may have been due to decreased globulin synthesis, and they were considered to be biologically adverse. There were no statistically significant or biologically significant changes in clinical chemistry parameters in female rats.

Table B.6.3.2-16
90-Day feeding study in rats: Clinical chemistry evaluation

Parameter	0 ppm	10 ppm	50 ppm	100 ppm	200 ppm
Males (Test Day 45):					
Total protein (g/dL)	6.6	6.6	6.6	6.4	6.1 ^a
Globulin (g/dL)	2.0	2.0	2.0	1.9	1.6 ^a
Males (Test Day 90):					
Total protein (g/dL)	7.1	7.0	7.3	7.2	6.6 ^a
Globulin (g/dL)	2.3	2.2	2.3	2.3	1.9 ^a

^a Significantly different from control by Dunnett criteria, $p < 0.05$.

3. Urinalysis

There were no adverse changes in urine parameters in male or female rats.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

Organ weight data are shown in Table . Mean absolute and relative (to brain weight) liver and kidney weights were significantly decreased and mean relative (to body weight) adrenal glands, testes, and brain weights were significantly increased in 200 ppm males. Mean relative (to body weight) brain weights were significantly increased in 100 ppm males. Mean relative (to body weight) liver and brain weights were significantly increased in 100 ppm females. Mean relative (to body weight) brain weight was statistically increased, and mean absolute and relative (to brain weight) adrenal weights were statistically decreased in 50 ppm females. These weight changes were considered to be the result of lower final body weights in test substance-exposed rats and were not indicative of target organ effects. No test substance-related changes in mean organ weights or organ weights relative to final body weight or brain weight were apparent at dietary concentrations below 100 ppm for male rats or below 50 ppm for female rats.

Table B.6.3.2-17
90-Day feeding study in rats: Organ weights

Parameter	0 ppm (males and females)	10 ppm (males and females)	50 ppm (males) 25 ppm (females)	100 ppm (males) 50 ppm (females)	200 ppm (males) 100 ppm (females)
Males:					
Absolute liver weight (g)	16.345	15.892	16.046	14.976	11.614 ^b
Relative liver weight ^a	3.114	3.058	3.134	3.157	3.093
Liver to brain weight ratio	7.779	7.394	7.356	6.926	5.670 ^b
Absolute kidney weight (g)	4.046	3.940	3.919	3.805	2.993 ^b
Relative kidney weight ^a	0.774	0.761	0.768	0.802	0.801
Kidney to brain weight ratio	1.924	1.831	1.796	1.759	1.461 ^b
Absolute adrenal gland weight (g)	0.063	0.061	0.065	0.063	0.057
Relative adrenal gland weight ^a	0.012	0.012	0.013	0.013	0.015 ^b
Adrenal gland to brain weight ratio	0.030	0.029	0.030	0.029	0.028
Absolute testes weight (g)	3.199	3.211	3.429	3.186	3.077
Relative testes weight ^a	0.615	0.624	0.679	0.673	0.829 ^b
Testes to brain weight ratio	1.524	1.494	1.564	1.471	1.509
Absolute brain weight (g)	2.104	2.149	2.184	2.163	2.050
Relative brain weight ^a	0.404	0.418	0.431	0.458 ^b	0.554 ^b
Females:					
Absolute liver weight (g)	8.025	8.766	7.535	7.308	7.323
Relative liver weight ^a	2.997	3.104	2.978	3.228	3.365 ^b
Liver to brain weight ratio	4.063	4.567	3.877	3.770	3.896
Absolute kidney weight (g)	2.068	2.234	2.019	2.004	1.812
Relative kidney weight ^a	0.774	0.799	0.802	0.885	0.832
Kidney to brain weight ratio	1.047	1.174	1.039	1.034	0.964
Absolute adrenal gland weight (g)	0.078	0.072	0.073	0.065 ^b	0.070
Relative adrenal gland weight ^a	0.029	0.026	0.029	0.029	0.032
Adrenal gland to brain weight ratio	0.039	0.037	0.037	0.034 ^b	0.037
Absolute brain weight (g)	1.972	1.921	1.944	1.941	1.879
Relative brain weight ^a	0.738	0.685	0.773	0.861 ^b	0.864 ^b

^a Relative weight is defined as percent of body weight.

^b Significantly different from control by the Dunnett's Test criteria, $p < 0.05$.

2. Gross pathology and histopathology

The only test substance-related gross finding at necropsy was the thin body status of 100 ppm females found dead or sacrificed *in extremis*.

Minimal to mild secondary test substance-related microscopic findings were present in liver, kidney, and spleen of males and females at several exposure levels, as summarised in the table below. It is to be noted that statistical analyses were not conducted for histopathological part of this study.

The brown pigment in macrophages of liver and spleen of males and females was positive for iron by the Perls' stain and was indicative of hemosiderin, suggesting an hemolysis context.

Compound-related histopathologic lesions were observed in the 100 ppm female rats which were found dead or sacrificed *in extremis* during the first few weeks of the study. Atrophy of the spleen and bone marrow was observed in these 5 females, atrophy of thymus was observed in 4 of them. These effects were due to loss of lymphoid and haemopoietic cells. This tissue atrophy was most likely due to a combination of weight loss, nutritional imbalance, and stress. Haemoglobin

pigment was also observed in renal tubule cells and/or lumens of these early-death rats but was not observed in rats in this group which were sacrificed by design. The presence of haemoglobin in the kidneys suggests that these rats may have undergone lysis of erythrocytes in the blood stream. This is in contrast to the other rats in which hemosiderin pigment was observed in macrophages, suggesting phagocytosis of erythrocytes by hepatic and splenic macrophages. Other histopathological observations in this study were consistent with normal background lesions of rats of this age and strain.

Table B.6.3.2-18
90-Day feeding study in rats: Incidences of microscopic effects

DPX-MP062 (ppm)	0 ppm (males and females)	10 ppm (males and females)	50 ppm (males) 25 ppm (females)	100 ppm (males) 50 ppm (females)	200 ppm (males) 100 ppm (females)
Number of rats/group:	10	10	10	10	10
Males:					
Liver					
Pigment, increased	0 ^a	0	0	0	1
Spleen					
Pigment, increased	0	0	2	6	10
Haemopoiesis, increased	0	0	7	6	9
Females:					
Liver					
Pigment, increased	0	0	0	3	10
Kidney					
Haemoglobin pigment	0	0	0	0	5 ^b
Spleen					
Atrophy	1	0	0	1	5 ^b
Pigment, increased	1	6	10	9	5 ^c
Haemopoiesis, increased	0	4	4	6	2 ^c
Thymus					
Atrophy	1	0	0	0	4 ^b
Bone marrow					
Atrophy	0	0	0	0	5 ^b

^a Number of organs with microscopic change.

^b Findings observed in the found dead/sacrificed female rats (n=5)

^c Findings observed in the surviving female rats (n=5)

Conclusions from the original DAR (2000, RMS NL):

The authors considered the significant decreases noted in some indicators of circulating erythrocyte mass in ≤100 mg/kg treated males and ≤50 mg/kg treated females biologically irrelevant, because the magnitude of the changes was <10% compared to controls and no regenerative bone marrow response (suggested by increased MCV values) was absent. As a consequence, the authors established a NOAEL of 100 mg/kg food for males (based on effects on body weight, food consumption, haematology, and clinical chemistry at the top dose level) and of 25 mg/kg food for females (based on effects on body weight and food consumption at the next higher

dose level). However, the reviewer considers statistically significant and dose-dependent decreases in one or more indicators of circulating erythrocyte mass to be an adverse effect of the test substance because these changes, in combination with the microscopic changes in the liver, spleen and kidneys, may indicate the presence of haemolytic anaemia. The increased serum chloride levels noted both in males and females were not considered to reflect the occurrence of hyperchloremia by the authors, because no other parameters indicative of such a disease state were affected. The reviewer notes that increased serum chloride levels have been suggested to be associated with anaemia, although sufficient scientific evidence is lacking. The microscopic changes noted in liver, spleen, and kidneys were concluded to be secondary to the effects on circulating red cells. Based on the effects on circulating red blood cell parameters noted in males at ≥ 50 mg/kg food and in females at ≥ 10 mg/kg food, the NOAEL for semichronic oral toxicity in rats was set at 10 mg/kg food (equal to 0.62 mg/kg bw/day) for males and at <10 mg/kg food (equal to <0.76 mg/kg bw/day) for females.

Conclusions from the Post-Annex I Addendum (2007, RMS NL):

The reviewer considers the increased extramed. haemopoiesis in the spleen in females treated with 10 mg/kg food as a regenerative response, which should be considered as adverse. It should be noted though, that at this dose level the MCV values are not increased. The increased serum chloride levels noted both in males and females were not considered to reflect the occurrence of hyperchloremia by the authors, because no other parameters indicative of such a disease state were affected.

The microscopic changes noted in liver, spleen, and kidneys were concluded to be secondary to the effects on circulating red cells. Based on the effects on circulating red blood cell parameters in combination with increased extramed. haemopoiesis in the spleen noted in females at ≥ 10 mg/kg food, the NOAEL for semichronic oral toxicity in rats was set at **<10 mg/kg food** (equal to <0.76 mg/kg bw/day) for females.

The JMPR concluded in their 2005 evaluation: On the basis of minimal effects of haemolysis at lower doses that were not considered to be adverse, the NOAEL for reduced body-weight gain and haematological changes was 25 ppm in females (2.1 mg/kg bw per day) and 50 ppm (3.1 mg/kg bw per day) in males (MacKenzie, 1997).

Conclusions proposed by the applicant (2015):

The NOAEL was 100 ppm (6.01 mg/kg bw/day) for males and 25 ppm (2.13 mg/kg bw/day) for females. This NOAEL was based on decreased body weight and nutritional parameters, mild haemolytic anaemia, and decreased total protein and globulin concentration in males at the LOAEL, 200 ppm, and decreased body weight parameters and food efficiency in females at the LOAEL, 50 ppm.

RMS FR assessment (2016):

Increased extramedullary haematopoiesis and pigments were observed in the spleen at the dose levels of 50 ppm in males and 10 ppm in females. Although at these dose levels, effects on red blood cell parameters were quite minimal (around -4/-7% compared to controls) and MCV was not reduced, it is considered that, according to the conclusions reached during the first EU review of indoxacarb, these histopathological effects occurring in the majority of animals should be considered adverse and the NOAEL is set at <10 ppm (<0.76 mg/kg bw/d) for females and equal to 10 ppm (0.62 mg/kg bw/d) for males. Effects observed at the highest dose levels were decreased body weights, body weight gains and food consumption, increased MCV, decreased total protein and globulin concentrations in males, as well as histopathological effects in liver, kidney, thymus, spleen and bone marrow. Mortality was seen in 5 out of 10 females at the highest dose level of 100 ppm (8.94 mg/kg bw/d).

Previous evaluation:	HLR 751-93 Revision No.1 : In DAR (2000) HLR 751-93 Revision No.2 : Submitted for the purpose of renewal (see reasons for revisions below)
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CA 5.3.2/03

Report: [REDACTED] (1997b); Subchronic oral toxicity: 90-Day study with DPX-JW062-34 (50% DPX-KN128, 50% DPX-KN127) feeding study in rats

DuPont Report No.: HLR 751-93, Revision No. 2

Guidelines: OECD 408 (1987), U.S. EPA 82-1, Nohsan No. 4200, 87/302/EEC Part B

Deviations: Detailed clinical observations were not evaluated in an open field arena, body weight at the initiation of the study exceeded 20% of the mean for males, and histopathological evaluation of Peyer's patches in the intestines was not specifically noted in the report since they are routinely collected and evaluated as part of the intestines. However, reconducting the study is unlikely to yield a significantly different result since detailed observations in an open field arena were conducted in the 90-day neurotoxicity study (HLR 1116-96, Revision No. 1). In addition, the exceedance of the 20% of mean body weight range at study start did not affect interpretation of the test results. The absence of histopathological evaluation of Peyer's patches is unlikely to alter the interpretation of the study since no effects on the immune system were observed in other immune system tissues evaluated in this study or in a 28-day immunotoxicity study in mice (DuPont 29280) with indoxacarb (DPX-KN128).

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 751-93, Revision No. 2

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

Reason for Revision No. 2:

1. Clarification of nomenclature regarding the distinction between the insecticidally active enantiomer DPX-KN128, the insecticidally inactive enantiomer, and the racemic mixture of enantiomers.
2. Discussions of hematology and microscopic findings were updated following re-evaluation.
3. The NOAEL of 125 ppm for males was changed to 60 ppm.
4. Addition of literature citations regarding the re-assessment of hematology and microscopic findings.
5. Addition of historical control data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062 technical
 Lot/Batch #: JW062-34
 Purity: 94.7%
 Description: Tan solid
 CAS #: 144171-61-9
 Stability of test compound: Analyses confirmed that test material was stable in feed for at least 7 days at room temperature, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared at biweekly intervals until week 8 and weekly for the remainder of the study.
2. Vehicle and/or negative control: The test substance was dissolved in acetone and then the resulting solution mixed with feed. Control feed was mixed with acetone alone.
3. Test animals
 Species: Rat
 Strain: CrI:CD[®]BR
 Age at initial dosing: Approximately 49 days old
 Weight at initial dosing: 189.1–256.2 g for males; 143.2–186.3 g for females
 Source: XX
 Acclimation period: 14 days
 Diet: Irradiated Purina Certified Rodent Chow[®] (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
 Water: Tap water, *ad libitum*
 Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
 Temperature: 21–25°C
 Humidity: 40–60%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed
 15-June-1993 to 15-September-1993
2. Animal assignment and treatment
 Five groups of 10 animals/sex/concentration were administered concentrations of DPX-JW062 in feed daily for 90 days. Males received 0, 30, 60, 125, and 250 ppm and females received 0, 15, 30, 60, and 125 ppm. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet.

Table B.6.3.2-19
Study design: 90-day feeding study in rats

Males				Females			
Group no.	No./ group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw	Group no.	No./ group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw
I	10	0 (control)	0 (control)	II	10	0 (control)	0 (control)
III	10	30	1.9	IV	10	15	0.99
V	10	60	3.9	VI	10	30	2.3
VII	10	125	8.0	VIII	10	60	4.6
IX	10	250	16	X	10	125	9.5

^a Weight/weight concentration of active ingredient in DPX-JW062 (adjusted for 94.7% purity).

3. Diet preparation and analysis

The test substance dissolved in acetone was added to the rodent diet and thoroughly mixed for 3 minutes until test Day 13. During preparation of the 60 ppm diet on test Day 13, it was apparent that all of the acetone did not evaporate when the diet was mixed for 3 minutes and therefore the diet mixing procedure was changed. The 125 ppm and 250 ppm diets prepared on test Day 13 and all diets prepared thereafter were mixed for 3 minutes, and then were manually stirred with a scoop before mixing for an additional minute. Control diets were mixed for the same period of time. All diets were prepared biweekly until test week 8 and weekly for the remainder of the study and refrigerated until used. The stability, homogeneity, and concentration of DPX-JW062 in the dietary mixtures were checked by analysis using HPLC at beginning, in the middle and near the end of the study. Due to the change in diet mixing procedures on test Day 13, additional samples were collected to verify the stability and homogeneity of distribution of DPX-JW062 in the diets. Because the results of the analysis of the 15 ppm diets prepared on test Day -1 suggested that DPX-JW062 may not have been homogeneously distributed throughout the diet and may not have been present at the targeted concentration, a sample of 15 ppm diet prepared on test Day 27 was collected and then refrigerated for 5 days. This sample was used to verify the concentration of DPX-JW062 in the 15 ppm diet. The test substance was at target concentrations $\pm 14\%$ (except for the 15 ppm diet during the first 4 weeks; see below), homogeneous (80.3–108%, based on the four-minute mixing procedure begun on test Day 13) throughout the feed and was stable (81.3–104%) for up to 14 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

Results of the analysis of samples from the 15 ppm diet prepared on test Day -1 suggested that the active substance may not have been homogeneously distributed throughout the diet and may not have been present at the targeted concentration of 15 ppm. Measured concentrations of active substance in samples taken from the top, middle, and bottom regions of the mixer ranged from 6.94 to 13.3 ppm with a coefficient of variation of 19. These data were obtained from a total of 21 extractions (7 estimates of active substance in the diet were obtained from the top, middle, and bottom regions of the mixer). Since the study records indicate that the appropriate amounts of test substance and chow were present in the mixer, these data suggest that either the test substance was not completely dissolved or suspended in the vehicle (*i.e.*, acetone), and/or the diet was not thoroughly mixed. After modification of the mixing procedure, a sample of 15 ppm diet prepared on test Day 27 was collected and refrigerated for 5 days. Measured concentration of active substance in this sample was 14.7 ppm, indicating that DPX-JW062 was present at the targeted concentration.

4. Statistics

Body weights, body weight gains, food consumption, food efficiency, organ weights, and clinical pathology measurements were analysed by a one-way analysis of variance. Pairwise comparisons between test and control groups were made with the Dunnett's test. Survival among groups on the final weigh day for all groups of rats was compared with the Cochran-Armitage trend test. Increases in the incidences of clinical observations were evaluated by the Cochran-Armitage trend test.

The Bartlett's test for homogeneity of variances was performed on organ weight data and clinical pathology data and, if significant, was followed by nonparametric procedures.

The incidences of microscopic observations were analysed with the Cochran-Armitage trend test. When the trend test was positive for the high and intermediate-concentration groups, the incidence observed in the lowest concentration group was compared to its respective control group observation incidence using the Fisher's exact test.

Except for the Bartlett's test ($p < 0.005$), all significance was judged at $p < 0.05$.

C. METHODS

1. Observations

Animals were observed at least once daily for mortality and morbidity and for signs of abnormal behaviour and appearance. On days when they were weighed, each animal was individually handled, examined for abnormal behaviour and appearance.

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded for each animal over the weighing interval by weighing the feeder at the beginning and end of the interval and subtracting the final weight and the amount of spillage from the feeder from the initial weight. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology, clinical chemistry, and urinalysis)

Blood and urine samples were collected from all animals approximately 48 and 91 days after initiation of the study. Animals were fasted approximately 16 hours prior to sample collection. Evaluation of haematology, clinical chemistry, and urinalysis parameters were performed for all animals. Bone marrow smears were prepared at the final sacrifice from all main study animals, but experimental findings did not warrant analysis and samples were discarded.

6. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in the following table. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (250 ppm for males; 125 ppm for females) and control (0 ppm) and from the one animal that died prior to the scheduled sacrifice were processed to slides and evaluated microscopically. Gross lesions and suspected target tissues (liver, kidney, lungs, spleen, and bone marrow), which were judged to be potentially relevant to an assessment of the toxicity of the compound, were processed to slides and examined microscopically for all animals.

Table B.6.3.2-20
90-Day feeding study in rats: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted ^a
Brain	X	X
Spleen		X
Heart		X
Liver	X	X
Kidneys	X	X
Oesophagus		X
Adrenal glands	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Skin		X
Trachea		X
Thymus		X
Mesenteric lymph node		X
Mandibular lymph node		X
Bone marrow		X
Thyroid gland		X
Parathyroid glands		X
Eyes		X
Exorbital lacrimal glands		X
Harderian glands		X
Testes	X	X
Epididymides		X
Prostate		X
Seminal vesicles		X
Ovaries		X
Uterus		X
Mammary glands (females)		X
Vagina		X
Stomach		X
Pituitary		X
Lungs		X
Spinal cord		X
Sciatic nerve		X
Skeletal muscle		X
Femur/knee joint		X
Sternum		X
Aorta		X
Urinary bladder		X
Gross observations		X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No test-substance related clinical signs of toxicity were observed for any dietary concentration in either males or females.

Statistically significant increased incidence of corneal opacity was observed in 2 male rats at 250 ppm. This clinical sign was not judged to be toxicologically important (see D. Ophthalmological examinations).

2. Mortality

One female in the 125 ppm group was found dead on test Day 26. There were no gross or microscopic pathological findings that indicated the death was test substance related.

B. BODY WEIGHT AND BODY WEIGHT GAIN

At test Day 90 body weights were 8.7 and 16% lower than controls in the 250 ppm males and in the 125 ppm females, respectively. The overall body weight gains were 16 and 35% lower than controls in the 250 ppm males and in the 125 ppm females, respectively.

Table B.6.3.2-21
90-Day feeding study in rats: Body weights (g)

Day	0 ppm	15 ppm	30 ppm	60 ppm	125 ppm	250 ppm
Males:						
Day 0 (SD ^a)	210.4 (15.7)	na ^d	214.9 (14.2)	218.7 (15.5)	216.0 (13.9)	217.4 (17.7)
Day 7 (SD)	267.6 (18.9)	na	272.6 (16.8)	264.2 (15.8)	262.9 (16.0)	248.6 (28.0)
Day 14 (SD)	317.3 (21.0)	na	326.4 (21.6)	321.9 (25.0)	312.3 (19.3)	294.1 (31.3)
Day 21 (SD)	360.9 (24.8)	na	373.9 (25.0)	372.2 (32.3)	351.7 (22.3)	323.7 (46.7) ^e
Day 28 (SD)	396.6 (26.8)	na	411.4 (29.4)	410.2 (37.3)	385.7 (21.1)	361.6 (41.3)
Day 35 (SD)	421.8 (25.5)	na	436.7 (28.9)	438.2 (40.2)	407.1 (21.8)	388.1 (43.1)
Day 42 (SD)	445.9 (29.9)	na	463.5 (33.1)	462.0 (40.2)	430.4 (23.5)	405.9 (44.0) ^e
Day 49 ^b (SD)	461.3 (30.5)	na	475.3 (35.1)	475.9 (43.9)	440.1 (29.1)	407.6 (47.8) ^e
Day 56 (SD)	482.9 (32.3)	na	500.5 (34.4)	503.3 (45.5)	467.9 (27.6)	435.4 (48.4) ^e
Day 62 (SD)	498.6 (33.9)	na	520.4 (39.8)	520.6 (49.1)	485.4 (29.4)	455.5 (51.6)
Day 69 (SD)	517.8 (37.8)	na	537.8 (37.9)	538.5 (51.5)	506.0 (27.4)	476.0 (54.0)
Day 76 (SD)	533.7 (36.7)	na	554.0 (46.0)	555.1 (52.7)	520.9 (29.9)	486.7 (54.3)
Day 84 (SD)	544.6 (39.6)	na	558.8 (51.2)	565.7 (54.8)	531.6 (30.0)	497.6 (54.4)
Day 90 (SD)	552.5 (38.9)	na	564.0 (62.6)	572.6 (55.7)	542.7 (35.8)	504.7 (56.1)
Females:						
Day 0 (SD)	164.3 (8.7)	168.8 (13.2)	164.2 (8.7)	167.0 (8.9)	165.1 (9.7)	na
Day 7 (SD)	191.8 (15.0)	195.3 (16.6)	185.8 (9.4)	187.3 (13.9)	157.5 (19.5) ^e	na
Day 14 (SD)	214.1 (18.8)	217.7 (20.2)	209.1 (9.7)	207.4 (16.8)	183.7 (15.9) ^e	na
Day 21 (SD)	234.1 (27.7)	237.7 (22.6)	226.9 (12.5)	224.5 (19.6)	190.2 (21.0) ^e	na
Day 28 (SD)	247.1 (25.1)	253.9 (26.6)	242.9 (12.9)	236.2 (17.6)	215.5 (17.3) ^e	na
Day 35 (SD)	256.6 (26.5)	266.5 (30.2)	253.3 (12.6)	250.0 (20.1)	216.8 (22.2) ^e	na
Day 42 (SD)	265.4 (28.1)	280.5 (32.5)	262.4 (15.2)	259.4 (24.4)	228.2 (20.1) ^e	na
Day 49 ^c (SD)	252.3 (26.8)	262.5 (30.6)	247.0 (17.3)	243.8 (23.4)	212.3 (19.4) ^e	na
Day 56 (SD)	276.5 (30.7)	290.2 (35.1)	275.7 (16.4)	270.3 (24.6)	241.5 (19.8) ^e	na
Day 62 (SD)	282.5 (33.3)	299.2 (40.4)	280.0 (20.9)	274.9 (29.7)	239.5 (25.4) ^e	na
Day 69 (SD)	287.2 (36.5)	303.0 (38.8)	281.8 (21.6)	282.3 (32.4)	245.4 (22.5) ^e	na
Day 76 (SD)	297.1 (38.8)	310.0 (38.1)	288.1 (22.7)	287.1 (31.4)	249.0 (25.2) ^e	na
Day 84 (SD)	303.5 (47.0)	315.9 (39.1)	297.2 (19.3)	290.0 (29.9)	256.4 (25.6) ^e	na
Day 90 (SD)	304.1 (50.1)	316.3 (41.3)	295.6 (24.7)	296.9 (28.6)	255.4 (28.9) ^e	na

^a SD = standard deviation.

^b Rats were fasted approximately 16 hours prior to clinical evaluation (test Day 48).

^c Rats were fasted approximately 16 hours prior to clinical evaluation (test Day 49).

^d na = not applicable; dosage not administered to that sex

^e Significantly different from control by the one-way analysis of variance and Dunnett's test criteria, $p < 0.05$.

Table B.6.3.2-22
90-Day feeding study in rats: Body weight gain

Parameter	0 ppm	15 ppm	30 ppm	60 ppm	125 ppm	250 ppm
Males:						
Day 0-7 (SD ^a)	57.2 (4.6)	na ^d	57.8 (6.5)	45.4 (15.7)	46.9 (5.7)	31.2 (14.6) ^e
Day 7-14 (SD)	49.7 (4.6)	na	53.8 (7.5)	57.8 (17.2)	49.5 (5.2)	45.5 (6.5)
Day 14-21 (SD)	43.6 (4.6)	na	47.5 (5.2)	50.3 (8.3)	39.3 (5.1)	29.6 (24.3) ^e
Day 21-28 (SD)	35.8 (4.9)	na	37.5 (5.7)	38.0 (6.0)	34.0 (3.8)	38.0 (16.2)
Day 28-35 (SD)	25.2 (6.0)	na	25.3 (3.9)	28.0 (5.6)	21.4 (8.4)	26.5 (6.1)
Day 35-42 (SD)	24.1 (6.6)	na	26.7 (7.4)	23.8 (5.6)	23.3 (10.1)	17.8 (5.8)
Day 42-49 ^b (SD)	15.4 (6.8)	na	11.8 (6.8)	14.0 (8.5)	9.7 (8.1)	1.7 (9.9) ^e
Day 49-56 (SD)	21.6 (7.9)	na	25.2 (7.1)	27.3 (8.7)	27.8 (5.4)	27.8 (6.6)
Day 56-62 (SD)	15.7 (4.3)	na	19.9 (7.8)	17.4 (6.5)	17.5 (3.8)	20.1 (7.2)
Day 62-69 (SD)	19.2 (5.1)	na	17.5 (8.6)	17.8 (6.9)	20.6 (4.5)	20.5 (5.9)
Day 69-76 (SD)	15.8 (6.5)	na	16.2 (10.7)	16.7 (7.6)	14.9 (5.0)	10.8 (5.3)
Day 76-84 (SD)	10.9 (5.2)	na	4.8 (14.2)	10.5 (6.5)	10.7 (7.2)	10.9 (7.2)
Day 84-90 (SD)	7.9 (7.0)	na	5.2 (24.4)	6.9 (10.3)	11.1 (9.5)	7.0 (6.0)
Overall body weight gain, Day 0-90 (SD)	342.1 (33.3)	na	349.2 (56.5)	353.9 (45.0)	326.7 (29.1)	287.3 (41.0) ^e
Females:						
Day 0-7 (SD)	27.5 (9.0)	26.5 (6.4)	21.6 (4.1)	20.3 (5.8)	-7.6 (19.1) ^e	na
Day 7-14 (SD)	22.4 (7.2)	22.4 (5.6)	23.3 (4.4)	20.0 (5.5)	26.2 (9.2)	na
Day 14-21 (SD)	20.0 (11.1)	19.9 (5.9)	17.9 (3.4)	17.1 (5.7)	6.4 (9.8) ^e	na
Day 21-28 (SD)	13.0 (8.7)	16.2 (7.7)	15.9 (5.6)	11.6 (7.6)	22.9 (7.7) ^e	na
Day 28-35 (SD)	9.5 (5.5)	12.6 (7.8)	10.4 (5.8)	13.9 (5.1)	1.3 (8.6) ^e	na
Day 35-42 (SD)	8.9 (5.1)	14.0 (5.0)	9.1 (4.8)	9.0 (6.0)	11.4 (9.6)	na
Day 42-49 ^c (SD)	-13.1 (3.5)	-18.0 (5.1)	-15.4 (4.9)	-15.6 (7.0)	-15.9 (6.6)	na
Day 49-56 (SD)	24.2 (6.0)	27.7 (6.6)	28.7 (4.3)	26.4 (5.3)	29.2 (6.8)	na
Day 56-62 (SD)	6.0 (5.5)	9.0 (12.6)	4.3 (9.4)	4.7 (8.7)	-2.0 (6.8)	na
Day 62-69 (SD)	4.6 (5.8)	3.8 (9.3)	1.9 (5.9)	7.4 (7.5)	5.8 (7.4)	na
Day 69-76 (SD)	9.9 (5.8)	7.1 (7.9)	6.3 (9.0)	4.8 (9.5)	3.6 (10.8)	na
Day 76-84 (SD)	6.5 (10.6)	5.9 (6.0)	9.0 (7.1)	2.9 (4.7)	7.4 (6.5)	na
Day 84-90 (SD)	0.5 (6.1)	0.3 (6.9)	-1.6 (9.3)	6.9 (6.8)	-1.0 (10.1)	na
Overall body weight gain, Day 0-90 (SD)	139.8 (45.0)	147.4 (29.8)	131.4 (19.7)	129.9 (21.6)	91.0 (25.2) ^e	na

^a SD = standard deviation.

^b Rats were fasted approximately 16 hours prior to clinical evaluation (test Day 48).

^c Rats were fasted approximately 16 hours prior to clinical evaluation (test Day 49).

^d na = not applicable; dosage not administered to that sex

^e Significantly different from control by the one-way analysis of variance and Dunnett's test criteria, p <0.05.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Males and females administered the 250 ppm and 125 ppm diet, respectively, had lower food consumption compared to controls (8.7% lower for males and 19% lower for females). Test substance-related decreases in food efficiency occurred in males and females administered 250 ppm and 125 ppm DPX-JW062 (7.9% lower in males and 20% lower in females).

Table B.6.3.2-23
90-Day feeding study in rats: Body weight gain/food consumption/food efficiency

Parameter	0 ppm	15 ppm	30 ppm	60 ppm	125 ppm	250 ppm
Males:						
Body weight gain, Day 0-90 (SD ^a)	342.1 (33.3)	na ^b	349.2 (56.5)	353.9 (45.0)	326.7 (29.1)	287.3 (41.0) ^c
Food consumption, Day 0-90 (SD)	27.6 (1.5)	na	28.1 (2.1)	28.6 (2.3)	26.6 (1.8)	25.2 (2.2) ^c
Food efficiency, Day 0-90 (SD)	0.139 (0.008)	na	0.139 (0.016)	0.139 (0.011)	0.138 (0.011)	0.128 (0.013)
Females:						
Body weight gain, Day 0-90 (SD)	139.8 (45.0)	147.4 (29.8)	131.4 (19.7)	129.9 (21.6)	91.0 (25.2) ^c	na
Food consumption, Day 0-90 (SD)	20.5 (2.2)	20.2 (2.2)	19.4 (1.7)	19.2 (1.7)	16.6 (1.9) ^c	na
Food efficiency, Day 0-90 (SD)	0.076 (0.017)	0.082 (0.010)	0.076 (0.008)	0.076 (0.007)	0.061 (0.013) ^c	na

^a SD = standard deviation.

^b na = not applicable; dosage not administered to that sex

^c Significantly different from control by the one-way analysis of variance and Dunnett's test criteria, $p < 0.05$.

D. OPHTHALMOLOGICAL EXAMINATIONS

No test-substance related ophthalmological observations were observed for any dietary concentration in either males or females. During the final ophthalmological evaluation, a left-eye shrunken globe was observed in 1 female rat at 60 ppm, and left-eye corneal opacities were observed in 2 male rats at 250 ppm. The ocular lesions noted at this examination were correlated with corneal opacities and/or other ocular findings reported through clinical observations of the same rats in the latter half of the study. Given that blood was drawn from rats by orbital sinus puncture of the left eye for the 45-day clinical pathology evaluation, it is likely that the ocular findings noted during the final ophthalmological examination (test day 86) were due to this procedure and were not related to the dietary administration of DPX-JW062.

E. CLINICAL PATHOLOGY

1. Haematology

Compound- and dose-related haemolytic anaemia was observed in males and females. The mean values for the indicators of erythrocyte mass (red blood cell count, haematocrit, and/or haemoglobin concentration) were decreased at the 45- and 90-day sampling times for all treated groups, reaching statistical significance in all male groups at day 45, in the 60, 125 and 250 ppm male groups at day 90, in the 60 and 125 ppm female groups at day 45 and in the 30, 60 and 125 ppm female groups at day 90. Reticulocytes and MCV counts were statistically significantly increased at the highest tested doses (250 ppm for males, 60 and 125 ppm for females).

Statistically significant leucocytes findings in male rats and female rats were considered not to be compound related as they were small in magnitude and within the range of normal biological variation, and comparable changes did not occur at both sampling times.

Table B.6.3.2-24
90-Day feeding study in rats: Haematology findings

Parameter	0 ppm	30 ppm	60 ppm	125 ppm	250 ppm
Males (Test Day 45):					
RBC × 10 ⁶ /μL	7.95	7.43 ^a -6.5%	7.46 ^a -6.2%	7.18 ^a -9.7%	6.76 ^a -15%
Hb (g/dL)	16.2	15.1 ^a -6.8%	14.9 ^a -8%	14.9 ^a -8%	14.5 ^a -6.2%
Ht %	44	41 ^a -6.8%	40 ^a -9.1%	41 ^a -6.8%	40 ^a -9.1%
Reticulocytes × 10 ³ /μL	45	56	67	73	124 ^a
MCV (fl)	55	55	54	57 +3.6%	59 ^a +7.3%
MCH (pg)	20	21	20	21	22
MCHC (g/dL)	38	37	37	36	37
Platelets × 10 ³ /μL	1112	1160	1039	1107	1129
WBC × 10 ³ /μL	15.1	17.0	16.8	17.9	20.3
Neutrophils (WBC × %)	1483	2784	2022	2185	1824
Lymphocytes (WBC × %)	12823	13172	13814	14973	17580 ^a
Band neutrophils (WBC × %)	0	0	0	0	0
Atypical lymphocytes (WBC × %)	12	14	13	17	65
Monocytes (WBC × %)	796	925	807	588	773
Eosinophils (WBC × %)	26	106	125 ^b	169	39
Basophils (WBC × %)	0	0	0	0	0
Males (Test Day 90):					
RBC × 10 ⁶ /μL (SD)	8.49	8.36 -1.2%	8.10 -4.6%	7.47 ^a -12%	7.10 ^a -16.4%
Hb (g/dL) (SD)	16.3	15.6 -4.3%	15.0 ^a -8%	14.8 ^a -9.2%	14.5 ^a -11%
Ht %	44	43 -2.3%	41 ^a -6.8%	41 ^a -6.8%	40 ^a -9.1%
Reticulocytes × 10 ³ /μL	100	128	121	136	224 ^a
MCV (fl)	52	51	51	55 +5.8%	56 ^a +7.7%
MCH (pg)	19	19	19	20	21 ^a
MCHC (g/dL)	37	37	36	37	36
Platelets × 10 ³ /μL	1094	1178	1107	1069	1028
WBC × 10 ³ /μL	11.2	13.6	12.5	12.6	13.0
Neutrophils (WBC × %)	1114	2185 ^a	1966 ^a	1878 ^a	1449
Lymphocytes (WBC × %)	9021	10541	9831	10098	11108
Band neutrophils (WBC × %)	0	0	0	0	0
Atypical lymphocytes (WBC × %)	0	0	20	32	13
Monocytes (WBC × %)	939	785	547	452 ^a	438 ^a
Eosinophils (WBC × %)	116	120	156	111	36 ^b
Basophils (WBC × %)	0	0	0	0	0

Table B.6.3.2-24
90-Day feeding study in rats: Haematology findings (continued)

Parameter	0 ppm	15 ppm	30 ppm	60 ppm	125 ppm
Females (Test Day 45):					
RBC × 10 ⁶ /μL	7.63	7.57 -0.8%	7.62 -0.1%	7.08 ^a -7.2%	6.32 ^a -17.2%
Hb (g/dL)	15.9	15.7 -1.3%	15.7 -1.3%	15.2) -4.4%	14.1 ^a -11.3%
Ht %	44	44	44	43 -2.3%	39 ^a -11.4%
Reticulocytes × 10 ³ /μL	62	33	49	54	132 ^a
MCV (fl)	58	59	58	60 ^a +3.4%	61 ^a +5.2%
MCH (pg)	21	21	21	22	22 ^a
MCHC (g/dL)	36	35 ^a	36	36	36
Platelets × 10 ³ /μL	1122	1046	1103	1081	1202
WBC × 10 ³ /μL	16.0	16.4	13.8	16.1	18.2
Neutrophils (WBC × %)	1492	1435	1384	1740	1407
Lymphocytes (WBC × %)	13466	14336	11908	13719	16333
Band neutrophils (WBC × %)	0	0	0	0	0
Atypical lymphocytes (WBC × %)	10	11	83 ^b	70	57
Monocytes (WBC × %)	893	567	413	422	338 ^b
Eosinophils (WBC × %)	98	83	53	99	31
Basophils (WBC × %)	0	0	0	0	0
Females (Test Day 90):					
RBC × 10 ⁶ /μL	8.12	7.83 -3.6%	7.53 ^a -7.3%	7.27 ^a -10.5%	6.89 ^a -15%
Hb (g/dL)	16.3	15.6 -4.3%	14.9 ^a -8.6%	15.1 ^a -8.9%	14.5 ^a -11%
Ht %	44	44	42 -4.5%	43 -2.3%	41 ^a -6.8%
Reticulocytes × 10 ³ /μL	54	66	106	165 ^a	147 ^a
MCV (fl)	55	56 +2%	56 +2%	59 ^a +7.2%	60 ^a +9%
MCH (pg)	20	20	20	21	21
MCHC (g/dL)	37	36	35 ^a	36 ^a	35 ^a
Platelets × 10 ³ /μL	1055	1016	1035	1083	1101
WBC × 10 ³ /μL	8.6	9.9	9.1	8.7	9.1
Neutrophils (WBC × %)	434	531	622	476	506
Lymphocytes (WBC × %)	7923	9042	8079	7929	8391
Band neutrophils (WBC × %)	0	0	0	0	0
Atypical lymphocytes (WBC × %)	0	98	100	140	69
Monocytes (WBC × %)	283	200	254	160	156
Eosinophils (WBC × %)	0	20	36	35	0
Basophils (WBC × %)	0	0	0	0	0

^a	Significantly different from control by the Dunnett criteria, $p < 0.05$.
^b	Significantly different from control by the Mann-Whitney U criteria, $p < 0.05$.
RBC	Red blood cells
Hb	Haemoglobin
Ht	Haematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
WBC	White blood cells

2. Clinical chemistry

Female rats in the 125 ppm group had statistically significantly decreased serum total protein and globulin concentrations at the 45- and 90-day sampling times. These changes were consistent and considered to be compound related. Decreased globulin synthesis was a likely cause of these changes; thus, they were considered to be toxicologically important. Similar effects were not observed in male rats.

Table B.6.3.2-25
90-Day feeding study in rats: Clinical chemistry evaluation

Parameter	0 ppm	15 ppm	30 ppm	60 ppm	125 ppm
Females (Test Day 45):					
Total protein (g/dL)	7.3	6.8 ^a	7.0	6.6 ^a	6.4 ^a
Globulin concentrations (g/dL)	2.1	1.8 ^a	2.0	1.8 ^a	1.6 ^a
Females (Test Day 90):					
Total protein (g/dL)	7.8	7.4	7.7	7.5	7.0 ^a
Globulin concentrations (g/dL)	2.2	2.0 ^a	2.1	2.1	1.9 ^a

^a Significantly different from control by Dunnett criteria, $p < 0.05$.

3. Urinalysis

Compound-related changes in urinalysis parameters did not occur in male or female rats during the conduct of this study.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

No test substance-related changes in mean organ weights or organ weights relative to final body weight were apparent at any dietary concentration.

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy. Microscopic changes secondary to compound-induced haemolysis were observed in the liver, spleen, and/or bone marrow of male and female rats in the 30 ppm groups and above. These changes were characterized by yellow-green pigment (hemosiderin) in liver Kupffer cells, increased amounts of yellow-brown pigment (hemosiderin) in macrophages of the spleen, erythrocytic hyperplasia in the spleen, and mixed hyperplasia of bone marrow. They represent a normal secondary physiological response to test substance induced haemolysis.

Table B.6.3.2-26
90-Day feeding study in rats: Incidences of microscopic effects

DPX-JW062	0 ppm	15 ppm	30 ppm	60 ppm	125 ppm	250 ppm
Number of rats/group:	10	10	10	10	10	10
Males:						
Liver						
Pigment, increased Kupffer cell	0 ^a	na ^b	0	0	0	4 ^c
Spleen						
Hyperplasia, erythrocytic	0	na	0	4 ^c	10 ^c	10 ^c
Pigment, increased (macrophages)	2	na	7 ^c	10 ^c	10 ^c	10 ^c
Bone marrow						
Hyperplasia, mixed	0	na	1	2	3	4 ^c
Females:						
Liver						
Pigment, increased Kupffer cell	0	0	1	2	7 ^c	na
Spleen						
Hyperplasia, erythrocytic	0	0	1	3 ^c	4 ^c	na
Pigment, increased (macrophages)	1	1	10 ^c	10 ^c	10 ^c	na
Bone marrow						
Hyperplasia, mixed	1	1	1	1	5 ^c	na

^a Number of organs with microscopic change.

^b na = not applicable; dosage not administered to that sex

^c Statistically significant by the Cochran-Armitage trend test criteria, p <0.05.

Conclusions from the original DAR (2000, RMS NL):

The authors established a NOAEL of 125 and 60 mg/kg food for males and females, respectively, based on effects on body weight, food consumption, and red blood cell parameters at the top dose level. With regard to the haematological changes the authors concluded that they were only toxicologically relevant in the highest dose groups because indicators of circulating erythrocyte mass were generally decreased more than 10% and reticulocyte counts were increased, the latter observation pointing to a regenerative bone marrow response. The reviewer does however not concur with this conclusion, as a statistically significant and dose-dependent decrease in one or more indicators of circulating erythrocyte mass is considered to be an adverse effect. The simultaneous occurrence of a regenerative (bone marrow) response is not considered a prerequisite for this judgement.

Haematological changes were indicative of a haemolytic effect of the test substance or a metabolite. The statistically significant increase in serum chloride levels (suggested to be associated with anaemia) is in line with this observation. Microscopic changes secondary to a compound-induced effects on circulating red blood cells were observed in the liver, spleen, and/or bone marrow of male and female rats.

Therefore, based on (primary and secondary) haematological changes noted in 30 mg/kg food males as well as females, the NOAEL is set at <30 mg/kg food for males (equal to <1.9 mg/kg bw/day), and 15 mg/kg food for females (equal to 0.99 mg/kg bw/day).

Conclusions from the Post-Annex I Addendum (2007, RMS NL):

The authors established a NOAEL of 125 and 60 mg/kg food for males and females, respectively, based on effects on body weight, food consumption, and red blood cell parameters at the top dose level. With regard to the haematological changes the authors concluded that they were only toxicologically relevant in the highest dose groups because indicators of circulating erythrocyte mass were generally decreased more than 10% and reticulocyte counts were increased, the latter observation pointing to a regenerative bone marrow response. The reviewer does however not concur with this conclusion, as a statistically significant and dose-dependent decrease in one or more indicators of circulating erythrocyte mass is observed in combination with increased pigment in the macrophages in the spleen, indicating breakdown of erythrocytes (in 30 mg/kg food males and females).

Haematological changes were indicative of a haemolytic effect of the test substance or a metabolite. Microscopic changes secondary to a compound-induced effects on circulating red blood cells were observed in the liver, spleen, and/or bone marrow of male and female rats.

Therefore, based on (primary and secondary) haematological changes noted in 30 mg/kg food males as well as females, the NOAEL is set at <30 mg/kg food for males (equal to <1.9 mg/kg bw/day), and 15 mg/kg food for females (equal to 0.99 mg/kg bw/day).

The JMPR concluded in their 2005 evaluation: As the effects at 30 ppm in females and 60 ppm in males were considered not to be adverse, the NOAEL was 30 ppm in females (equal to 2.3 mg/kg bw per day) and 60 ppm in males (equal to 3.9 mg/kg bw per day) on the basis of increased reticulocyte counts in males and microscopic evidence of mild haemolysis in both sexes at higher doses (=2.3 mg/kg bw per day).

Conclusions proposed by the applicant (2015):

The NOAEL was 60 ppm (3.9 mg/kg bw/day) for males and 60 ppm (4.6 mg/kg bw/day) for females. This NOAEL was based on toxicologically significant haemolytic anaemia in males at the LOAEL, 125 ppm, and on toxicologically significant decrements in body weight, body weight gain, food consumption, and/or food efficiency in males at 250 ppm and on toxicologically significant decrements in body weight, body weight gain, food consumption, and/or food efficiency, and toxicologically significant haemolytic anaemia in females at the LOAEL, 125 ppm.

RMS FR assessment (2016):

The NOAEL is set at <30 ppm (<1.9 mg/kg bw/d) in males and 15 ppm (0.99 mg/kg bw/d) in females. Indeed, at the dose level of 30 ppm, histopathological effects indicative of haemolytic anemia (particularly haemosiderin in the spleen) were observed in males and females. At the LOAEL of 30 ppm, decrease in red blood cell parameters were about -7% at Day 45 and slight at Day 90 for males, and slight at Day 45 and about -5/-9% at Day 90 for females. At the highest dose levels, increased MCV and reticulocytes were measured as well as haemosiderin in the liver and erythrocytis hyperplasia in the bone marrow and the spleen. Decreased body weights, body weight gains and food consumption were also noted.

Previous evaluation:	In DAR (2000)
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CA 5.3.2

Report: [REDACTED] (1998); DPX-JW062 technical: Subchronic toxicity; 90-day feeding study in rats

DuPont Report No.: HL-1998-01200

Guidelines: Directive 87/302/EEC Part B (1987), USEPA 82-1 (1982), 59 NohSan No. 4200 (1985), OECD 408 (1981)

Deviations: The study was only partly performed in accordance with OECD guideline 408 as it was designed to evaluate the reversibility of haematological effects as observed in a previous 90-day oral study in rats with the same test compound and concentrations. The study did not include ophthalmoscopy, clinical chemistry, urinalysis, organ weighing, and histopathology

Testing Facility: [REDACTED]

Testing Facility Report No.: HL-1998-01200

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062 technical
 Lot/Batch #: JW062-106
 Purity: 95.03%
 Description: Not stated
 CAS #: 144171-61-9
 Stability of test compound: Analyses confirmed that test material was stable in feed under the conditions of the study, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.
2. Vehicle and/or negative control: Substance was dissolved in acetone prior to mixing in the diet.
3. Test animals
 Species: Rat
 Strain: Crl:CD[®](SD) BR
 Age at initial dosing: Approximately 42 days old
 Weight at initial dosing: 161.0–225.9 g for males; 124.9–175.4 g for females
 Source: [REDACTED]
 Acclimation period: 23 days
 Diet: PMI[®] Nutrition International, Inc., Irradiated Certified Rodent LabDiet[®] (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
 Water: Tap water, *ad libitum*
 Housing: Animals were housed three per cage, sexes separate, in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
 Temperature: 23 ± 1°C
 Humidity: 50 ± 10%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed
 05-January-1998 to 15-May-1998
2. Methods
 This 90-day feeding study was conducted to confirm the reversibility of haematologic effects observed in previous studies. DPX-JW062 was administered in the diet to male and female Crl:CD[®](SD)BR rats (10 rats/sex/concentration). Male rats were administered concentrations of 0, 30, 60, 125, or 250 ppm. Females were administered concentrations of 0, 15, 30, 60, or 125 ppm. The mean daily intakes for male rats were 0, 1.8, 3.7, 7.5, and 15 mg/kg bw/day. The mean daily intakes for female rats were 0, 1.2, 2.5, 4.9, and 12 mg/kg bw/day. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, haematology, and gross pathology. Since haematologic effects were reversible following a 21-day recovery period, all rats were sacrificed and necropsied on recovery Days 31 and 32 for males and females, respectively.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity
 Test substance-related mortality or clinical signs of toxicity did not occur in this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight changes in these groups correlated with test substance-related decrements in food consumption or food efficiency. During the recovery period, rats in these groups had significantly greater mean body weight gain and food efficiency, compared to control, resulting in mean body weights that were similar to controls by the end of the recovery period.

Table B.6.3.2-27
90-Day feeding study in rats: Body weight/body weight gain/food consumption/food efficiency

Male rats		0 ppm	30 ppm	60 ppm	125 ppm	250 ppm
Body weight (g)	Day 98	499.7	500.1	491.0	459.1	443.1 ^b
	Day 126 ^a	528.2	534.9	525.8	501.6	495.9
Body weight gain (g)	Day 0-98	301.0	306.3	292.8	261.1	247.2 ^b
	Day 98-126	28.6	34.8	34.8	42.5 ^b	52.8 ^b
Food consumption (g)	Day 0-98	25.0	24.3	24.4	22.4 ^b	21.6 ^b
	Day 98-126	24.4	24.6	24.7	24.3	24.6
Food efficiency (g wt/g fd consumed)	Day 0-98	0.124	0.129	0.122	0.119	0.117
	Day 98-126	0.042	0.050	0.050	0.062 ^b	0.076 ^b
Female rats		0 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Body weight (g)	Day 98	283.4	280.3	266.0	254.0	239.4 ^b
	Day 126 ^a	295.5	296.9	289.7	281.6	272.7
Body weight gain (g)	Day 0-98	135.4	129.3	120.2	107.6 ^b	92.3 ^b
	Day 98-126	12.1	16.5	23.7	27.5 ^b	33.3 ^b
Food consumption (g)	Day 0-98	20.7	19.5	18.4	18.0	18.9
	Day 98-126	20.4	19.3	20.1	21.2	22.3
Food efficiency (g wt/g fd consumed)	Day 0-98	0.068	0.068	0.067	0.061	0.053 ^b
	Day 98-126	0.021	0.030	0.042 ^b	0.046 ^b	0.053 ^b

^a Day 98-126 was a recovery period

^b Significantly different from control by the Dunnett's test, $p < 0.05$

C. CLINICAL PATHOLOGY

1. Haematology

Mild haemolytic anaemia occurred in male and female groups. At both the 45- and 90-day sampling times, the group means for the indicators of circulating erythrocyte mass (RBC, Hb, Ht) were decreased compared to controls. After the 21-day recovery period, there were no changes in the indicators of circulating erythrocyte mass. Thus, there was complete recovery from the test substance-related haemolytic anaemia in the male and female groups.

Table B.6.3.2-28
90-Day reversibility feeding study in rats: Haematological evaluation

Male rats	0 ppm	30 ppm	60 ppm	125 ppm	250 ppm
RBC (% Control) Day 90:	8.56	8.27	8.09	7.89 ^a	7.09 ^a
		-3%	-5%	-8%	-17%
21-Day Recovery:	8.52	8.45	8.54	8.64	8.26
Hb (% Control) Day 90:	16.8	15.8 ^a	15.6 ^a	15.7 ^a	14.7 ^a
		-6%	-7%	-7%	-12%
21-Day Recovery:	16.7	16.3	16.5	16.7	16.5
HCT (%) Day 90:	50	48	47 ^a	47 ^a	44 ^a
		-4%	-6%	-6%	-12%
21-Day Recovery:	50	49	50	50	50
MCV (fl) Day 90:	59	58	59	59	62 ^a
21-Day Recovery:	59	58	58	59	60
MCH (pg) Day 90:	20	19	20	20	21 ^a
21-Day Recovery:	20	19	19	19	20
MCHC (g/dL) Day 90:	33	33	33	34	34
21-Day Recovery:	33	33	33	33	33
Female rats	0 ppm	15 ppm	30 ppm	60 ppm	125 ppm
RBC (% Control) Day 90:	8.13	7.90	7.49 ^a	7.29 ^a	6.95 ^a
		-3%	-8%	-10%	-15%
21-Day Recovery:	8.02	8.07	7.95	8.25	7.62
Hb (% Control) Day 90:	16.5	15.9	15.3 ^a	14.9 ^a	15.2 ^a
		-4%	-7%	-10%	-8%
21-Day Recovery:	16.4	16.5	16.2	16.7	16.1
HCT (%) Day 90:	51	49	47 ^a	47 ^a	46 ^a
		-4%	-8%	-8%	-10%
21-Day Recovery:	51	50	50	52	49
MCV (fl) Day 90:	62	62	63	64	66 ^a
21-Day Recovery:	63	62	63	63	65
MCH (pg) Day 90:	20	20	21	21	22 ^a
21-Day Recovery:	21	20	21	20	21
MCHC (g/dL) Day 90:	33	32	33	32	33
21-Day Recovery:	33	33	33	32	33

^a Significantly different from control by Dunnett's, $p < 0.05$

RBC = Red blood cells, Hb = Haemoglobin, HCT = Haematocrit, MCV = Mean Corpuscular Volume

MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration

G. SACRIFICE AND PATHOLOGY

1. Gross pathology

No test substance-related gross lesions were observed at necropsy.

Conclusions from the original DAR (2000, RMS NL):

The authors established a NOAEL of 125 mg/kg food for males (based on effects on body weight, food consumption, and haematology at the top dose level) and of 60 mg/kg food for females (based on effects on body weight and haematology at the top dose level). However, the reviewer considers statistically significant and dose-dependent decreases in one or more indicators of circulating erythrocyte mass to be an adverse effect of the test substance because these changes may indicate the presence of haemolytic anaemia. Based on the effects on circulating red blood cell parameters noted in males and in females at all dose levels, the NOAEL for semichronic oral toxicity in rats was set at <30 mg/kg food (equal to <1.83 mg/kg bw/day) for males and at <15 mg/kg food (equal to <1.23 mg/kg bw/day) for females. All effects on circulating red blood cell parameters were reversible within the 21-day recovery period.

Conclusions proposed by the applicant (2015):

The NOAEL in the 90-day reversibility feeding study in rats was 125 ppm (7.5 mg/kg/day) for male rats and 60 ppm (4.9 mg/kg/day) for female rats. These NOAELs are based on body weight and haemolytic effects at higher concentrations. The haemolytic and body weight effects were reversible within 21 days in both male and female rats.

RMS FR assessment (2016):

This study is considered as supplementary and is not intended to set NOAELs given that only limited parameters were investigated. In this study, haematological effects (decreased RBC, Hb and Ht, increased MCV) were shown to be reversible within the 21-day recovery period.

Oral 90-day toxicity in the mouse

Previous evaluation:	In DAR (2000)
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CA 5.3.2

Report: [REDACTED] (1997); Subchronic oral toxicity: 90-Day study with DPX-JW062-34 (50% DPX-KN128, 50% DPX-KN127) feeding study in mice

DuPont Report No.: HLR 750-93, Revision No. 1

Guidelines: Directive 87/302/EEC Part B (1987), USEPA 82-1 (1982), 59 NohSan No. 4200 (1985), OECD 408 (1981)

Deviations: The body weight variation of the female animals exceeded $\pm 20\%$ of the mean at the commencement of the test. However, animals were randomly assigned to study groups and no statistically significant differences among group body weight means were present. Therefore, this deviation from the OECD guideline is considered not to affect the validity of the study. Clinical chemistry was only performed to a very limited extent as only plasma protein levels were determined.

Weekly assessment of detailed clinical observations in an open field arena was not conducted. Clinical biochemistry endpoints were not measured due to small sample size available from this species (mice). Full biochemistry was conducted in the 90-day rat studies. Histopathological evaluation of Peyer's patches in the intestines was not specifically noted in the report since they are routinely collected and evaluated as part of the intestines. However, reconducting the study is unlikely to yield a significantly different result since detailed observations in an open field arena were conducted in the 90-day neurotoxicity study (HLR-1116-96, Revision No. 1). In addition, the absence of the 20% of mean body weight range at study start did not affect interpretation of the test results. The absence of histopathological evaluation of Peyer's patches is unlikely to alter the interpretation of the study since no effects on the immune system were observed in other immune system tissues evaluated in this study or in a 28-day immunotoxicity study in mice (DuPont-29280) with indoxacarb.

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 750-93, Revision No. 1

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062 technical
Lot/Batch #: JW062-34
Purity: 94.7%
Description: Tan solid
CAS #: 144171-61-9
Stability of test compound: Analyses confirmed that test material was stable in feed for at least 14 days at room temperature, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared every other week and refrigerated until used.
2. Vehicle and/or negative control: Untreated diet with acetone
3. Test animals
Species: Mouse
Strain: Crl:CD-1[®](ICR)BR
Age at initial dosing: Approximately 51 days old
Weight at initial dosing: 28.7–35.9 g for males; 19.7–28.6 g for females
Source: XXXXXXXXXX
Acclimation period: 22 days
Diet: Purina Certified Rodent Chow[®] #5002, *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water: Tap water, *ad libitum*
Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
Temperature: 23 ± 2°C
Humidity: 50 ± 10%
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed
16-June-1993 to 17-September-1993
2. Animal assignment and treatment
Five groups of 10 animals/sex/concentration were administered concentrations of DPX-JW062 in feed daily for 90 days. From test Days 0 through 42, the animals received 0, 10, 35, 75, and 150 ppm; from test Days 42 through 91, the animals received 0, 35, 75, 150, and 300 ppm. The 10 ppm concentration was increased to 300 ppm from test Day 42 through the end of the study since data obtained during the first 6 weeks suggested that continued feeding at 150 ppm might not result in adequate toxicity. Dosing levels were based on a previous 28-day diet toxicity study in mice. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet.

Table B.6.3.2-29
Study design: 90-Day study in mice

Males				Females			
Group no.	No./ group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw	Group no.	No./ group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw
I	10	0 (control) ^b	0 (control)	II	10	0 (control) ^c	0 (control)
III	10	10 ^b	1.7	IV	10	10 ^b	2.1
V	10	35 ^b	5.5	VI	10	35 ^c	7.0
VII	10	75 ^b	12	VIII	10	75 ^c	16
IX	10	150 ^b	23	X	10	150 ^c	30
III	10	300 ^c	44	IV	10	300 ^c	51

^a Weight/weight concentration of test substance in DPX-JW062-34 (adjusted for 94.7% purity).

^b Test Day 0 through test Day 42 (morning).

^c Test Day 42 (afternoon) through test Day 91.

3. Diet preparation and analysis

The test substance (dissolved in acetone) was added to the rodent diet and thoroughly mixed for 3 minutes. Control diets were mixed for the same period of time. All diets were prepared every other week and refrigerated until used. The stability, homogeneity and concentration of DPX-JW062 in the dietary mixtures were checked by analysis using HPLC at beginning, middle, and end of study. The test substance was at target concentrations, homogeneous (88.1% to 106%) throughout the feed, and was stable (91.7% to 107%) for up to 14 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

Body weights, body weight gains, food consumption, food efficiency, organ weights, and haematology measurements were analysed by a one-way analysis of variance. Pairwise comparisons between test and control groups were made with the Dunnett's test. Survival among groups on the final food consumption day was compared for all groups of mice with the Cochran-Armitage test for trend. Increases in the incidences of clinical observations were evaluated by the Cochran-Armitage test for trend. Incidence of Heinz body formation in erythrocytes was evaluated by the Fisher's exact test.

The Bartlett's test for homogeneity of variances was performed on the organ weight and clinical laboratory data and, if significant, was followed by the Kruskal Wallis test and Mann-Whitney U test.

The incidences of microscopic findings for tissues which were examined at all dose levels (liver, gallbladder, kidneys, lungs, and spleen) were analysed by the Cochran-Armitage test for trend. When the trend test was positive for the high- and intermediate-concentration groups, the incidence observed in the low-concentration group was compared to its respective control group incidence using the Fisher's exact test. The Fisher's exact test was also used to compare the control and high concentration incidences of microscopic findings for all other tissues.

Except for the Bartlett's test ($p < 0.005$), all significance was judged at $p < 0.05$.

C. METHODS

1. Observations

Animals were observed at least once daily for mortality and morbidity and for signs of abnormal behaviour and appearance. On days when they were weighed, each animal was individually handled, examined for abnormal behaviour and appearance.

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency and daily intake

Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by focal illumination and indirect ophthalmoscopy prior to study start. All surviving animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology)

Blood samples were collected from all animals approximately 49–50 and 92–93 days after initiation of the study. At sacrifice, blood and bone marrow were collected. Evaluation of haematology were performed for all animals. Bone marrow smears were prepared at the final sacrifice from all main study animals, but experimental findings did not warrant analysis.

6. Sacrifice and pathology

At termination, animals were sacrificed by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in the following table. Organ weight/final body weight and organ weight/brain weight ratios were calculated. Tissues collected from animals receiving the highest dose (300 ppm) and control (0 ppm) and from animals that died or were sacrifice prior to scheduled sacrifice were processed to slides and evaluated microscopically. Gross lesions and suspected target tissues (liver, gallbladder, kidney, lung, and spleen,), as determined by examination of the control and high dose animals, were processed to slides and examined microscopically for all animals.

Table B.6.3.2-30
90-Day feeding study in mice: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Brain	X	X
Spleen		X
Heart		X
Liver	X	X
Kidneys	X	X
Oesophagus		X
Adrenal glands	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Gallbladder		X
Skin		X
Trachea		X
Thymus		X
Mesenteric lymph node		X
Mandibular lymph node		X
Bone marrow (sternum and femur)		X
Thyroid gland		X
Parathyroid glands		X
Eyes		X
Exorbital lacrimal glands		X
Harderian glands		X
Testes	X	X
Epididymides		X
Prostate		X
Seminal vesicles		X
Ovaries		X
Uterus		X
Vagina		X
Mammary glands (females)		X
Stomach		X
Pituitary		X
Lungs		X
Spinal cord		X
Sciatic nerve		X
Skeletal muscle		X
Femur/knee joint		X
Sternum		X
Aorta		X
Urinary bladder		X
Gross observations		X

II. RESULTS AND DISCUSSION

A. FINDINGS

Findings from the 90-day feeding study with DPX-JW062 in mice are summarised in the following table.

Clinical observations suggestive of neurotoxicity (*i.e.*, mice observed leaning to one side and/or with abnormal gait or mobility) were exhibited by male mice at 300 ppm and by female mice at 150 and 300 ppm. Toxicologically significant decreases in mean body weight and mean body weight gain relative to controls were present in male mice at 300 ppm and in female mice at 150 and 300 ppm. Body weight effects were correlative to toxicologically significant decrements in mean daily food consumption and mean food efficiency in these groups relative to controls.

At the 90-day sampling time for haematology, blood smears from mice in the 150 and 300 ppm groups had increased incidences of erythrocytic Heinz bodies and the haematologic data indicated that mild haemolysis had occurred. These groups of mice also had increased reticulocyte counts, and microscopically increased pigment (liver and spleen) and haemopoiesis of the spleen were present in several dosed groups. Circulating erythrocyte mass parameters (RBC count, haemoglobin concentration, and haematocrit) were not significantly decreased.

Table B.6.3.2-31
Summary of results of the 90-day feeding study in mice with DPX-JW062

Dosage	Male						
	Dietary conc.:	0	10 ^a	35	75	150	300 ^a ppm
	Mean daily intake:	0	1.7	5.5	12	23	44 mg/kg/day
	Female						
	Dietary conc.:	0	10 ^a	35	75	150	300 ^a ppm
	Mean daily intake:	0	2.1	7.0	16	30	51 mg/kg/day
Analytical	The test substance was distributed homogeneously and was stable in the diet. Measured concentrations of DPX-JW062 were from 86.3 to 95.7% and 93.3 to 96.7% for samples taken on Test Days 41 and 83, respectively.						
	Dose Group (ppm)	Results					
General observations	300 m ^c , ≥150 f ^c	Abnormal gait, leaning to one side					
Mortality	300 m	One death on Day 85					
Body weight	≥150 f	Decreased (8–9%)					
	300 m	Decreased (13%)					
Body weight gain	150 f	Decreased (45%)					
	300 m, f	Body weight loss relative to body weight on Day 42					
Food consumption	150 f	Decreased (12%)					
	300 m, f	Decreased (m-18%; f-30%)					
Food efficiency	150 f	Decreased (38%)					
	300 m, f	Decreased (negative food efficiency)					
Ophthalmology	nad ^b						
Haematology	≥75	No important changes in red blood cell parameters Some evidence of slight haemolysis: slight decreases in indicators of circulating erythrocyte mass and Heinz bodies reticulocytes increased					
Gross pathology	300 f	Small spleens (lymphoid depletion; likely stress-related)					
Organ weights	Nad except for changes attributable to body weight effects						
Histopathology	Effects secondary to slight red blood cell changes:						
	≥75 m, f	Haemosiderin in spleen increased					
	≥150 m, f	Haemopoiesis in spleen increased					
	300 m, f	Haemosiderin in liver increased					

^a The dietary concentration in the 10 ppm group was increased to 300 ppm on Test Day 42.

^b No abnormalities detected that were considered test substance-related and/or biologically adverse

^c m = male; f = female

All increases or decreases are relative to controls unless otherwise noted.

Table B.6.3.2-32: Summary of histopathological findings of the 90-day feeding study in mice with DPX-JW062

Dose (mg/kg food)	0		35		75		150		10/300	
	m	f	m	f	m	f	m	f	m	f
-increased pigment (Kupffer cells), liver	0	0	0	0	0	0	0	0	10 (ic)	5 (ic)
-focal alveolar inflammation, lungs	0	0	0	0	0	0	0	0	2 (ic)	0
-increased extramed. haemopoiesis, spleen	0	1	0	0	0	1	7 (ic)	4 (ic)	7 (ic)	6 (ic)
-lymphoid depletion, spleen	1	1	1	0	0	0	0	1	1	4 (ic)
-increased pigment macrophages, spleen	0	2	0	2	4 (ic)	8 (ic)	9 (ic)	9 (ic)	7 (ic)	9 (ic)

ic statistically significantly increased compared to the controls

Conclusions from the original DAR (2000, RMS NL):

A mild, compound-related Heinz body haemolytic effect occurred in ≥ 75 mg/kg food exposed animals. Since it is known that erythrocytic Heinz bodies interfere with spectrophotometric determination of Hb, MCH and MCHC, changes in these parameters are difficult to interpret. Other parameters of circulating erythrocyte mass (RBC count, Ht) were not significantly affected, although microscopically increased haemosiderin pigments were noted in the spleen in 75 mg/kg food and higher dose groups. A dose-related bone marrow regenerative response (increased reticulocyte counts) was observed in males at ≥ 75 mg/kg food. The authors did not consider the erythrocyte effects to be adverse and established a NOAEL of 150 mg/kg food for males and of 75 mg/kg food for females (both based on clinical signs, decreased body weights and food consumption). However, the reviewer does not concur with this conclusion and considers the effects noted at 75 mg/kg food and higher an adverse effect of the treatment. Based on these considerations, the NOAEL for semichronic exposure of male and female mice was established at 35 mg/kg food, equal to 5.5 mg/kg bw/day.

Conclusions proposed by the applicant (2015):

The no-observed-adverse-effect level (NOAEL) was 150 ppm for males and 75 ppm for females, equivalent to 23 and 16 mg/kg/day, respectively. These NOAELs were based on decreases in body weight, body weight gain, food consumption, and/or food efficiency relative to controls, and on clinical signs suggestive of neurotoxicity at 300 ppm in male mice and at 150 and 300 ppm in female mice.

RMS FR assessment (2016):

The conclusions from the original DAR are supported: the NOAEL of this study is set at 35 ppm (equivalent to 5.5 mg/kg bw/d in males and 7.0 mg/kg bw/d in females), based on findings related to haemolytic anemia observed at the LOAEL of 75 ppm (equivalent to 12 mg/kg bw/d in males and 16 mg/kg bw/d in females).

Oral toxicity in the dog

Previous evaluation:	In Addendum to DAR (2001)
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CA 5.3.2/06

Report: [REDACTED] (1995); Six-week palatability and toxicity study with DPX-JW062-106 feeding study in dogs

DuPont Report No.: HLO 162-95

Guidelines: None

Testing Facility: [REDACTED]

Testing Facility Report No.: WIL-189015

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

A. MATERIALS

- ## B. STUDY DESIGN

- ## 2. Animal assignment and treatment

Two groups of 2 animals/sex/concentration were administered concentrations of DPX-JW062 in feed daily for 6 weeks. Males and females received 80, and 160 ppm concentrations at the initiation of the study. In attempts to obtain a maximum tolerated dose, the 80 ppm concentration was increased to 320 ppm concentration at the third week of dosing (Week 2) for both males and females and to 640 ppm concentration at the fifth week of dosing (Week 4) for females, while the 160 ppm concentration was increased to 480 ppm concentration at the third week of dosing (Week 2) for females and at the fifth week of dosing (Week 4) for males. The route was oral because that is a possible route of exposure in humans. At the discretion of the study director, dogs judged to be suitable for testing, based on the pretest data, were assigned randomly to study groups using a computer-generated program. A control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table B.6.3.2-33
Study design: 6 week feeding study in dogs

Group no.	Weeks	No. females/ group	No. males/ group	Conc. in diet (ppm) ^a	Mean daily intakes/ males (mg/kg bw)	Mean daily intakes/ females (mg/kg bw)
1	1-6	2	2	0 (control)	0 (control)	0 (control)
2	0-2	2	2	80	3	2
2	2-3	2	2	320	11	10
2	3-4	2	2	320	11	8
2	4-5	2	2	320 males 640 females	11	17
2	5-6	2	2	320 males 640 females	10	11
3	0-1	2	2	160	5	6
3	1-2	2	2	160	5	5
3	2-3	2	2	160 males 480 females	6	17
3	3-4	2	2	160 males 480 females	6	18
3	4-6	2	2	480	16	17

^a Weight/weight concentration of test substance

3. Diet preparation and analysis

The test substance (dissolved in acetone) was added to a small amount of diet and thoroughly mixed in a Hobart mixer for 10 minutes. The premix was transferred to a twin-shell mixer with intensifier bar and mixed for 15 minutes with enough feed to achieve a total batch size of homogenous diet. Control diets were mixed for the same period of time. All diets were prepared weekly and refrigerated until used.

4. Statistics

All analyses were conducted using two-tailed tests for minimum significance levels of 1% and 5% comparing the treated group to the vehicle control group by sex. All means were presented with standard deviations (S.D.) and the numbers of sampling units (N) used to calculate the means. All statistical tests were performed by a Digital[®] MicroVAX[®] 3400 computer with appropriate programming. Analysis of body weights, body weight changes, food consumption, and clinical pathology values were analysed by a one-way analysis of variance, followed by Dunnett's test.

C. METHODS

1. Observations

Animals were observed twice a day for mortality, morbidity, and the availability of food and water. All animals were observed at the time of feeding and approximately one hour following feeding for overt signs of toxicity. Detailed physical examinations were conducted on all animals weekly.

2. Body weights

All animals were weighed twice weekly.

3. Food consumption, food efficiency and daily intake

Food consumption was recorded daily for each animal and reported as a daily average over the weekly weighing interval. Food efficiency and daily intake of the test article were calculated from food consumption and body weight gain data.

4. Ophthalmological examinations

All animals were examined with an indirect ophthalmoscope and/or a hand-held slit lamp prior to study start. All animals were examined again during study Week five.

5. Clinical pathology (haematology)

Blood samples were collected from all animals at pretest (1 week apart), and during study Weeks 1, 3, and 5. Animals were fasted (length of time not stated in the report) prior to sample collection. Evaluations of haematology parameters were performed for all animals.

6. Sacrifice and pathology

All dogs were euthanised by anaesthesia with sodium pentobarbital and exsanguination. Gross examinations were performed on all animals. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the thoracic, abdominal and pelvic cavities including contents. At the time of necropsy, gross lesions suspected of being related to treatment were collected and placed in 10% neutral buffered formalin. Additionally, the eyes from all animals were placed in Bouin's fixative for approximately three hours and then placed in 70% ethanol. Eyes from all dogs were trimmed, according to [REDACTED] procedure, routinely processed, embedded in paraffin, sectioned at approximately 5 micrometers, stained with hematoxylin and eosin (H&E) and examined with a light microscope at [REDACTED].

Table B.6.3.2-34
6-week feeding study in dogs: Organs/tissues collected for pathological examination

Organ	Microscopic/histopathologic evaluation conducted
Eyes	X
Gross observations	X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related clinical signs of toxicity were observed at any dose level/dietary concentration in either males or females.

2. Mortality

Instances of mortality did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Mean body weight losses were observed from week 4 in animals dosed at 640 ppm concentration (only females were dosed at this level), resulting in a statistically significant reduction in mean body weights throughout dosing. Mean body weight for this group was 15.1% lower than controls at the terminal sacrifice.

The only statistically significant difference in male animals was a decreased body weight gain in animals dosed at 80 ppm concentration (Group 2); however, a significant decrease was not seen at higher concentrations, and the Group 2 male body weights always stayed within 4.8% of the control. Thus, the decreased mean body weight gain for this group was not considered to be test substance related.

Table B.6.3.2-35
6-week dietary study in dogs: Body weights (g)

Week	0 ppm	80 ppm (weeks 0-2) 320 ppm (M Weeks 2-6, F Weeks 2-4) 640 ppm (F Weeks 4-6)	160 ppm (M Weeks 0-4, F Weeks 0-2) 480 ppm (M Weeks 4-6, F Weeks 2-6)
Males:			
Week 0	10141	10234	9931
Week 0 ^a	10490	10669	10235
Week 1	10486	10575	10365
Week 1 ^a	10783	10687	10535
Week 2	11013	10908	10769
Week 2 ^a	11194	11062	11033
Week 3	11422	11350	11123
Week 3 ^a	11571	11165	11220
Week 4	11717	11418	11497
Week 4 ^a	11788	11562	11536
Week 5	11761	11628	11829
Week 5 ^a	12059	11810	11982
Week 6	12333	11747	12351
Females:			
Week 0	8401	9030	8960
Week 0 ^a	8719	8939	9102
Week 1	8688	8892	9197
Week 1 ^a	8812	8604	9346
Week 2	8954	8772	9436
Week 2 ^a	9184	9022	9695
Week 3	9274	9098	9615
Week 3 ^a	9412	9061	9812
Week 4	9540	9105	10068
Week 4 ^a	9780	9097	10177
Week 5	9864	8998 ^b	10319
Week 5 ^a	10080	8811 ^b	10511
Week 6	10234	8685 ^b	10582

^a Second weekly body weight.

^b Significantly different from control by the Dunnett's Test, p <0.05.

Table B.6.3.2-36
6-week dietary study in dogs: Body weight gain (g)

Parameter	0 ppm	80 ppm (Weeks 0-2) 320 ppm (M Weeks 2-6, F Weeks 2-4) 640 ppm (F Weeks 4-6)	160 ppm (M Weeks 0-4, F Weeks 0-2) 480 ppm (M Weeks 4-6, F Weeks 2-6)
Males:			
Body weight gain, Week 0–0 ^a	350	435	304
Body weight gain, Week 0 ^a –1	-5	-94	130
Body weight gain, Week 1–1 ^a	298	112 ^b	170
Body weight gain, Week 1 ^a –2	230	221	235
Body weight gain, Week 2–2 ^a	181	154	264
Body weight gain, Week 2 ^a –3	228	289	91
Body weight gain, Week 3–3 ^a	150	-186	97
Body weight gain, Week 3 ^a –4	146	254	277
Body weight gain, Week 4–4 ^a	72	144	39
Body weight gain, Week 4 ^a –5	-27	66	293
Body weight gain, Week 5–5 ^a	298	182	153
Body weight gain, Week 5 ^a –6	274	-63	369
Overall body weight gain, Week 0–6	2192	1513	2420
Females:			
Body weight gain, Week 0–0 ^a	319	-91	142
Body weight gain, Week 0 ^a –1	-32	-48	95
Body weight gain, Week 1–1 ^a	124	-288	150
Body weight gain, Week 1 ^a –2	142	168	90
Body weight gain, Week 2–2 ^a	231	250	259
Body weight gain, Week 2 ^a –3	90	77	-81
Body weight gain, Week 3–3 ^a	138	-37	197
Body weight gain, Week 3 ^a –4	129	44	257
Body weight gain, Week 4–4 ^a	240	-8 ^b	109
Body weight gain, Week 4 ^a –5	84	-100	142
Body weight gain, Week 5–5 ^a	216	-187 ^c	193
Body weight gain, Week 5 ^a –6	155	-126	71
Overall body weight gain, Week 0–6	1834	-345 ^b	1623

^a Second weekly body weight.

^b Significantly different from control by the Dunnett's test, $p < 0.05$.

^c Significantly different from control by the Dunnett's test, $p < 0.01$.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

There was a test substance-related decrease in food consumption in Group 2 females during the week 5-6 interval, when these animals were receiving the 640 ppm concentration dose. Food efficiency was also decreased for Group 2 females while they were being dosed at the 640 ppm concentration dose.

Table B.6.3.2-37
6-Month dietary study in dogs: food consumption (grams/animal/day)/food efficiency (bw gained/% feed consumed)

Parameter	0 ppm	80 ppm (Weeks 0-2) 320 ppm (M Weeks 2-6, F Weeks 2-4) 640 ppm (F Weeks 4-6)	160 ppm (M Weeks 0-4, F Weeks 0-2) 480 ppm (M Weeks 4-6, F Weeks 2-6)
Males:			
Food Consumption, Week 0–1	344.0	344.0	298.0
Food Consumption, Week 1–2	370.0	362.0	310.0
Food Consumption, Week 2–3	395.0	383.0	358.0
Food Consumption, Week 3–4	392.0	368.0	363.0
Food Consumption, Week 4–5	398.0	394.0	382.0
Food Consumption, Week 5–6	399.0	388.0	380.0
Food Efficiency, Week 0–1	13.89	14.11	20.83
Food Efficiency, Week 1–2	20.54	13.11	18.17
Food Efficiency, Week 2–3	14.79	16.46	14.28
Food Efficiency, Week 3–4	10.82	2.58	15.10
Food Efficiency, Week 4–5	1.61	7.63	12.42
Food Efficiency, Week 5–6	20.49	3.90	19.39
Females:			
Food Consumption, Week 0–1	280.0	213.0	326.0
Food Consumption, Week 1–2	311.0	184.0	320.0
Food Consumption, Week 2–3	360.0	279.0	338.0
Food Consumption, Week 3–4	329.0	208.0	363.0
Food Consumption, Week 4–5	360.0	231.0	363.0
Food Consumption, Week 5–6	358.0	147.0 ^b	366.0
Food Efficiency, Week 0–1	14.68	-36.54	10.46
Food Efficiency, Week 1–2	12.29	-68.56	10.10
Food Efficiency, Week 2–3	12.57	17.79	7.97
Food Efficiency, Week 3–4	10.98	1.10	16.76
Food Efficiency, Week 4–5	12.84	-7.61 ^a	9.95
Food Efficiency, Week 5–6	15.03	-29.32 ^b	9.62

^a Significantly different from control by the Dunnett's Test, $p < 0.01$.

^b Significantly different from control by the Dunnett's Test, $p < 0.05$.

D. OPHTHALMOLOGICAL EXAMINATIONS

No test substance-related ophthalmological observations were observed for any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Haematology

Numerous test substance-related changes in haematology parameters were apparent at dose concentrations of 160 ppm and higher. Red blood cell, haemoglobin and haematocrit means were decreased at the study Week 1 evaluation in the Group 3 (160 ppm) males and females and at the study Week 3 and 5 evaluations in all animals treated with DPX-JW062 (dose concentrations of 160, 320, 480, and 640 ppm). The lower red blood cell parameters were associated with increases in MCV values and decreases in MCHC values. When compared to the control group, the differences in these parameters were often statistically significant ($p < 0.05$ or $p < 0.01$) in all treated groups. Mean platelet counts were generally increased at the study Week 3 and 5 evaluations in all treated animals (dose

concentrations of 160, 320, 480, and 640 ppm). The differences in platelet means were statistically significant at the study week 3 evaluation in the Group 3 (160 ppm) males ($p < 0.05$) and at the study Week 5 evaluation in the Group 2 and 3 (640 and 480 ppm, respectively) females ($p < 0.05$) and the Group 2 and 3 (160 and 480 ppm, respectively) males ($p < 0.01$). No other toxicologically significant differences in haematology parameters were observed in any of the groups treated with DPX-JW062.

Table B.6.3.2-38 6-Week dietary study in dogs: Haematology findings

Parameter	0 ppm	80 ppm (Weeks 0-2) 320 ppm (M Weeks 2-6, F Weeks 2-4) 640 ppm (F Weeks 4-6)	160 ppm (M Weeks 0-4, F Weeks 0-2) 480 ppm (M Weeks 4-6, F Weeks 2-6)
Males (Test Week 1):			
RBC ^a	6.21	5.79	5.09
Hb ^b	14.4	12.9	11.9
Hematocrit (%)	43.7	40.4	37.7
MCV ^c (fl)	70.4	69.9	74.1
MCH ^d (pg)	23.2	22.2	23.3
MCHC ^e (g/dL)	32.9	31.9	31.5
WBC ^f × 10 ³ /μL	14.3	16.1	15.4
Platelets × 10 ³ /μL	387	508	517
Males (Test Week 3):			
RBC	6.20	4.54	4.78
Hb	14.6	10.7 ^g	11.7
Hematocrit (%)	43.3	34.3	37.1
MCV (fl)	70.0	75.5 ^g	77.5 ^g
MCH (pg)	23.7	23.5	24.5
MCHC (g/dL)	33.9	31.1	31.6
WBC × 10 ³ /μL	14.8	14.2	18.3
Platelets × 10 ³ /μL	359	565	640 ^g
Males (Test Week 5):			
RBC	6.29	4.89	5.10
Hb	14.9	11.4 ^g	12.0 ^g
Hematocrit (%)	44.1	37.2	38.6
MCV (fl)	70.3	76.1	75.8
MCH (pg)	23.7	23.4	23.5
MCHC (g/dL)	33.7	30.7 ^h	31.0 ^h
WBC × 10 ³ /μL	13.9	11.4	14.2
Platelets × 10 ³ /μL	355	552 ^h	622 ^h
Females (Test Week 1):			
RBC	6.27	6.99	6.01
Hb	14.5	15.3	13.6
Hematocrit (%)	44.2	48.6	43.3
MCV (fl)	70.6	69.6	72.1
MCH (pg)	23.2	21.9	22.6
MCHC (g/dL)	32.8	31.4 ^g	31.3 ^g
WBC × 10 ³ /μL	13.6	8.7	19.3
Platelets × 10 ³ /μL	428	476	527
Females (Test Week 3):			
RBC	6.38	5.82	4.72
Hb	15.3	13.2	11.6
Hematocrit (%)	44.9	42.9	36.6
MCV (fl)	70.4	73.7	77.7 ^g
MCH (pg)	24.0	22.7	24.6
MCHC (g/dL)	34.0	30.8 ^h	31.6 ^g
WBC × 10 ³ /μL	11.4	11.5	17.3
Platelets × 10 ³ /μL	408	632	697

Table B.6.3.2-38 6-Week dietary study in dogs: Haematology findings (continued)

Parameter	0 ppm	80 ppm (Weeks 0-2) 320 ppm (M Weeks 2-6, F Weeks 2-4) 640 ppm (F Weeks 4-6)	160 ppm (M Weeks 0-4, F Weeks 0-2) 480 ppm (M Weeks 4-6, F Weeks 2-6)
Females (Test Week 5):			
RBC	5.90	5.70	5.53
Hb	14.2	13.6	12.9
Hematocrit (%)	42.2	43.7	41.9
MCV (fl)	71.5	76.6 ^g	75.7 ^g
MCH (pg)	24.0	23.8	23.4
MCHC (g/dL)	33.6	31.0 ^h	30.9 ^h
WBC × 10 ³ /μL	11.6	8.9	17.8
Platelets × 10 ³ /μL	404	685 ^g	661 ^g

^a

Red blood cells

^b

Haemoglobin

^c

Mean Corpuscular Volume

^d

Mean Corpuscular Haemoglobin

^e

Mean Corpuscular Haemoglobin Concentration.

^f

White blood cells.

^g

Significantly different from control by the Dunnett criteria, p <0.05.

^h

Significantly different from control by the Dunnett criteria, p <0.01.

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy. There were no test substance-related microscopic findings in the eyes from any dog. All histopathological observations in this study were consistent with normal background lesions of dogs of this age and breed.

III. CONCLUSION

The applicant proposed a NOAEL of 80 ppm (3 mg/kg bw/day) for males and 80 ppm (2 mg/kg bw/day) for females. This NOAEL was based on decreased food efficiency, food consumption, body weights, and body weight gains in females and on haematology parameters (red cell count, haemoglobin, haematocrit, and MCHC) in males and females at the LOAEL of 160 ppm.

RMS comment: It should be noted that this type of study design, with increasing dose levels throughout the study, is not primarily intended to set NOAEL but to determine dose levels for the subchronic toxicity study.

Previous evaluation:	HLR 494-95 : In DAR (2000) HLR 494-95 Revision No.3 : Submitted for the purpose of renewal (see reasons for revisions below)
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CA 5.3.2/04

Report: [REDACTED] (1997a); Subchronic oral toxicity: 90-Day study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) feeding study in dogs

DuPont Report No.: HLO 494-95, Revision No. 3

Guidelines: U.S. EPA 82-1, 59 Nohsan No. 4200, OECD 409, Directive 87/302/EEC Part B

Deviations: Histopathological evaluation of Peyer's patches in the intestines was not specifically noted in the report since they are routinely collected and evaluated as part of the intestines. However, reconducting the study is unlikely to yield a significantly different result. The absence of histopathological evaluation of Peyer's patches is unlikely to alter the interpretation of the study since no effects on the immune system were observed in other immune system tissues evaluated in this study or in a 28-day immunotoxicity study in mice (DuPont-29280) with indoxacarb (DPX-KN128).

Testing Facility: [REDACTED]

Testing Facility Report No.: WIL-189016

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

Reason for Revision Nos. 1-3:

1. Signature of the study director was not necessary on the histopathology report.
2. Correction of the number of dogs evaluated in the histopathology summary.
3. Study title was changed to add the concentration of the enantiomers DPX-KN128 and DPX-KN127.
4. Discussions of hematology, clinical chemistry, and microscopic findings were updated following re-evaluation.
5. The NOAEL conclusion was changed to 80 ppm for males and 160 ppm for females.
6. Addition of literature citations regarding the re-assessment of hematology and microscopic findings.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062 technical
 Lot/Batch #: JW062-106
 Purity: 95.03%
 Description: Off-white solid
 CAS #: 144171-61-9
 Stability of test compound: Analyses confirmed that test material was stable in feed for at least 14 days refrigerated or at room temperature, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.
2. Vehicle and/or negative control: Untreated diet mixed with acetone
3. Test animals
 Species: Dog
 Strain: Beagle
 Age at initial dosing: Approximately 6–7 months old
 Weight at week 0: 7.9–13.4 kg for males; 7.3–10.0 kg for females
 Source: XXXXXXXXXX
 Acclimation period: Minimum 14 days
 Diet: Purina® Certified Canine Chow® #5007, offered for approx. one hour per day. During the test period, test substance was incorporated into the feed of all animals except negative controls. Additional nutritional support was provided for one dog in the 640 ppm group due to poor food consumption.
 Water: Tap water, *ad libitum*
 Housing: Animals were housed individually in stainless steel cages.
4. Environmental conditions
 Temperature: 20.6–26.3°C
 Humidity: 30.9–57.0%
 Air changes: 10–15 changes/hour
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed
 16-February-1995 to 19-May-1995

2. Animal assignment and treatment

In a previous 6-week palatability study in male and female dogs, weight gain, food consumption, and food efficiency were decreased in females administered diet containing 640 ppm. In addition, decreased red blood cell parameters and increased MCV and platelet counts were observed in males and females administered 160 ppm and above. The NOAEL in the 6-week palatability study was considered to be 80 ppm. Based on the results of the 6-week palatability study, the dietary concentrations of 40, 80, 160, and 640 ppm were selected for the 90-day study. Five groups of four animals/sex/concentration were administered concentrations of DPX-JW062 in feed daily for 90 days. Males and females received 40, 80, 160, and 640 ppm. Animals were assigned to dose groups by a standard block randomisation procedure using body weights as the block. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide for the Care and Use of Laboratory Animals* (USPHS-NIH Publication No. 86-23).

Table B.6.3.2-39
Study design: 90-Day feeding study in dogs

Group no.	No. females/ group	No. males/ group	Conc. in diet (ppm) ^a	Mean daily intakes/ males (mg/kg bw)	Mean daily intakes/ females (mg/kg bw)
1	4	4	0 (control)	0 (control)	0 (control)
2	4	4	40	1	1
3	4	4	80	2	3
4	4	4	160	5	5
5	4	4	640	18	17

^a Weight/weight concentration of test substance

3. Diet preparation and analysis

The test substance (dissolved in acetone) was added to the diet and thoroughly mixed for 25 minutes. Control diets were mixed for the same period of time. All diets were prepared weekly and under ambient conditions until used. The stability, homogeneity, and concentration of DPX-JW062 in the dietary mixtures were checked by analysis using HPLC at the beginning of the study (stability and homogeneity) or on Days 45 and 90 (concentration). The test substance was at target concentrations, homogeneous throughout the feed, and stable for up to 14 days refrigerated or at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

All analyses were conducted using two-tailed tests for significance levels of 5% and 1% comparing the treatment groups to the vehicle control group by sex. All statistical tests were performed by a Digital® MicroVAX 3400 computer with appropriate programming. Body weights, body weight changes, food consumption, clinical laboratory values, and absolute and relative organ weights were analysed by a one-way analysis of variance followed by Dunnett's Test. Clinical laboratory values for cell types that occur at a low incidence (*i.e.*, monocytes, eosinophils, basophils, and unsegmented neutrophils) were not subjected to statistical analysis.

C. METHODS

1. Observations

Animals were observed twice a day for mortality, morbidity, and overt signs of toxicity. Detailed examinations for clinical signs of toxicity were conducted weekly.

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded daily for each animal and the mean group food consumption was calculated over the weekly weighing interval. Food efficiency and daily intake of the test article were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by indirect ophthalmoscopy and/or a hand-held slit lamp prior to study start. All animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology, clinical chemistry, and urinalysis)

Blood and urine samples were collected from all animals once during pretest (Week 1), and during study Weeks 3, 8, and 12. Blood samples were collected prior to the daily administration of the test

diets. Evaluation of haematology, clinical chemistry, and urinalysis parameters were performed for all animals. Bone marrow smears were prepared at the final sacrifice form all main study animals.

It is to be noted that Heinz body counts were added to the parameters to be evaluated. Indeed, during the course of enumerating the reticulocyte counts, Heinz bodies were noted for various animals at certain time points.

6. Sacrifice and pathology

All dogs were euthanised by anaesthesia with sodium pentobarbital and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in the table below. Organ weight/final body weight ratios were calculated. Tissues collected from animals in all dose groups were processed to slides and evaluated microscopically.

Table B.6.3.2-40
90-Day feeding study in dogs: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Brain	X	X
Spleen		X
Heart		X
Liver	X	X
Kidneys	X	X
Oesophagus		X
Adrenal glands	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Gall bladder		X
Skin		X
Trachea		X
Thymus		X
Mesenteric lymph node		X
Suprathyroid lymph node		X
Bone marrow		X
Thyroid gland (with parathyroids)	X	X
Eyes (with optic nerve)		X
Testes	X	X
Epididymides	X	X
Prostate		X
Ovaries (without oviducts)	X	X
Uterus (with vagina)		X
Mammary glands (females)		X
Stomach		X
Pituitary		X
Lungs (including bronchi)		X
Spinal cord		X
Sciatic nerve		X
Skeletal muscle		X
Femur/knee joint		X
Rib		X
Aorta		X
Urinary bladder		X
Gross lesions		X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No test-substance related clinical signs of toxicity were observed for any dietary concentration in either males or females.

2. Mortality

No mortality occurred during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Mean body weights in the 640 ppm group females were lower than the control group values from Week 3 through the remainder of the study (Week 13). None of these differences were statistically significant. The mean body weight losses and/or reduced body weight gains in the 640 ppm group females were due to one female (no. 3014), which began experiencing an obvious reduction in food consumption and body weight gain during the second week of the study. In an attempts to stimulate appetite, the dietary admix of this female was supplemented with Alpo® or bouillon near the end of study week 7 to 8 through the end of study week 9 to 10). When the values from female no. 3014 are excluded from the 640 ppm group data, the means for this group are similar to the control group. In light of the definitive test substance-related effect on the haematologic parameters in this dog, one cannot exclude the possibility that the reduced food consumption and weight gain at the 640 ppm concentration were test substance-related effects. There were no test substance-related effects on body weights or body weight gains in any of the other groups.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Mean food consumption was often reduced during the first eight weeks of administration of DPX-JW062 in the 640 ppm group females. None of these differences were statistically significant; the differences were primarily due to female no. 3014, which began experiencing an obvious reduction in food consumption and body weight gains during the second week of the study. The other dogs in the 640 ppm group continued to eat well, accounting for the small difference (and lack of statistical significance) between the control and 640 ppm group female means. Food efficiency in the 640 ppm group females was generally below the control group values except during the period that female no. 3014 had the diet supplemented with Alpo® or bouillon. Negative food efficiency values were observed in this group for those weeks in which female no. 3014 experienced body weight losses. These decreases in food efficiency were never statistically significant. There were no test substance-related effects on food consumption or food efficiency in any other groups.

D. OPHTHALMOLOGICAL EXAMINATIONS

No test-substance related ophthalmological observations were observed for any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Haematology

Decreases in the indicators of erythrocyte mass (red blood cell count, haemoglobin concentration, and haematocrit) were present in males and females at the Week 3, 8, and/or 12 evaluations. The pattern of changes in the mean values of the erythrocyte indices (mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH], and mean corpuscular haemoglobin concentration [MCHC]) in these groups was suggestive of haemolysis with a regenerative response (*i.e.*, MCV and MCH were generally increased and MCHC was decreased). Mean absolute reticulocyte counts were minimally increased at the highest dose levels, indicating that a bone marrow regenerative response had occurred. An increased number of Heinz bodies in the 80, 160 and 640 ppm group males and females indicated that oxidative denaturation of haemoglobin may have been the cause of haemolysis.

Table B.6.3.2-41
90-Day feeding study in dogs: Haematology findings

Parameter	0 ppm	40 ppm	80 ppm	160 ppm	640 ppm
Males (Test Week 3):					
RBC ^a (% control)	100	86 ^b	87	80 ^c	81 ^c
Hb ^d (% control)	100	88	91	84 ^c	83 ^c
Ht ^e (% control)	100	92	93	90	92
MCV ^f (µm ³)	68.6	73.8 ^b +7.6%	73.5 ^b +7.1%	76.7 ^c +11.8%	78.2 ^c +14%
MCH ^g (pg)	23.2	24.0	24.4	24.2	23.7
MCHC ^h (g/dL)	33.8	32.5	33.1	31.5 ^b	30.4 ^c
Reticulocytes (%)	0.3	1.0	1.2	2.0 ^b	1.7
Heinz bodies (%)	0.0	0.0	0.0	0.2	0.3 ^b
Males (Test Week 8):					
RBC (% control)	100	103	99	88	85
Hb (% control)	100	106	101	93	90
Ht (% control)	100	110	103	96	95
MCV (µm ³)	68.2	73.1 ^b +7.2%	71.0 +4.1%	74.3 ^c +8.9%	76.6 ^c +12.3%
MCH (pg)	23.1	23.7	23.5	24.1	24.3 ^b
MCHC (g/dL)	33.8	32.3 ^b	33.1	32.4	31.8 ^c
Reticulocytes (%)	0.2	0.9	0.3	1.2	1.7 ^b
Heinz bodies (%)	0.0	0.0	0.1	0.3 ^b	0.4 ^c
Males (Test Week 12):					
RBC (% control)	100	93	94	81 ^c	81 ^c
Hb (% control)	100	93	95	84 ^b	84 ^b
Ht (% control)	100	99	99	87	90
MCV (µm ³)	69.4	73.6 +6.1%	73.0 +5.2%	74.3 ^b +7.1%	76.6 ^c +10.4%
MCH (pg)	22.9	22.8	23.0	23.6	23.5
MCHC (g/dL)	32.9	31.0	31.5	31.8	30.7
Reticulocytes (%)	0.2	0.8	0.3	1.1	2.4 ^c
Heinz bodies (%)	0.0	0.0	0.1	0.1	0.1
Females (Test Week 3):					
RBC (% control)	100	87	84	85	83
Hb (% control)	100	92	89	91	87
Ht (% control)	100	92	91	95	97
MCV (µm ³)	68.1	71.6 +5.1%	73.3 ^c +7.6%	75.8 ^c +11.3%	79.2 ^c +16.3%
MCH (pg)	23.3	24.5	24.7	24.7	24.5
MCHC (g/dL)	34.2	34.2	33.7	32.6	31.0
Reticulocytes (%)	0.2	0.5	0.8	1.4 ^b	1.5 ^b
Heinz bodies (%)	0.0	0.0	0.0	0.2 ^b	0.1
Females (Test Week 8):					
RBC (% control)	100	95	96	93	76 ^c
Hb (% control)	100	95	92	93	78 ^c
Ht (% control)	100	99	102	100	87
MCV (µm ³)	67.7	70.6 +4.3%	71.5 ^c +5.6%	73.2 ^c +8.1%	77.3 ^c +14.2%
MCH (pg)	23.8	23.9	22.9	23.8	24.6

MCHC (g/dL)	35.2	33.8	32.0 ^c	32.5 ^c	31.8 ^c
Reticulocytes (%)	0.3	0.2	0.4	1.3	2.3 ^b
Heinz bodies (%)	0.0	0.0	0.1	0.4 ^b	0.4 ^b

Table B.6.3.2-41
90-Day feeding study in dogs: Haematology findings (continued)

Parameter	0 ppm	40 ppm	80 ppm	160 ppm	640 ppm
Females (Test Week 12):					
RBC (% control)	100	91	88	91	79 ^c
Hb (% control)	100	93	91	97	78 ^b
Ht (% control)	100	94	93	99	90
MCV (µm ³)	69.0	71.4 +3.5%	72.7 ^b +5.4%	75.0 ^c +8.7%	78.0 ^c +13%
MCH (pg)	22.3	23.0	23.1	23.6	22.0
MCHC (g/dL)	32.4	32.2	31.7	31.5	28.2 ^c
Reticulocytes (%)	0.1	0.4	1.1	1.7	2.3 ^c
Heinz bodies (%)	0.0	0.0	0.2	0.5	0.4

^a RBC = Red blood cells

^b Significantly different from control by the Dunnett's test criteria, p <0.05.

^c Significantly different from control by the Dunnett's test criteria, p <0.01.

^d Hb = Haemoglobin

^e Ht = Haematocrit

^f MCV = Mean corpuscular volume

^g MCH = Mean corpuscular haemoglobin

^h MCHC = Mean corpuscular haemoglobin concentration

2. Clinical chemistry

Male and female dogs exposed to 160 ppm or 640 ppm DPX-JW062 exhibited minimally increased mean bilirubin concentrations at all evaluations. The increases in bilirubin concentration were most likely secondary to haemolysis.

Mean alkaline phosphatase was increased in 160 and 640 ppm females during test weeks 3, 8, and 12. Although the increases were statistically significant in 160 and 640 ppm females during weeks 8 and 12, the increase was not clearly dose-related at week 12. Laboratory historical control data reported in the study report give a mean of 97 U/L (range 59.6-134.4) for females. Nevertheless, historical control data seems to correspond to Day 0 values although it is known that these values decreased with time in dogs. These HCD are thus not relevant for comparison with values of the treated groups at WK3, 8 and 12. Given the magnitude of the increase and the associated increased liver weight, it cannot be excluded that this effect is treatment-related.

Similar changes were not observed in males. Therefore, these changes were not considered to be test substance related.

Table B.6.3.2-41
90-Day feeding study in dogs: Haematology findings

	Males					Females				
Parameter	0	40	80	160	640	0	40	80	160	640
Bilirubin (mg/dL)										
WK -1	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.2
WK 3	0.1	0.2*	0.2	0.3**	0.2*	0.2	0.2	0.3	0.3	0.3
WK 8	0.1	0.2	0.2	0.3**	0.4**	0.2	0.2	0.3*	0.4**	0.4**
WK 12	0.2	0.3	0.3	0.3*	0.4**	0.2	0.2	0.2	0.3	0.4**
Alkaline phosphatase (U/L)										
WK -1	98	115	92	105	97	99	98	85	113	114
WK 3	102	114	98	118	109	91	102	88	143	146
WK 8	94	100	91	105	90	76	98	85	134	136*
WK 12	98	87	84	94	81	69	85	80	134*	130*

* Significantly different from control by the Dunnett's test criteria, $p < 0.05$.

** Significantly different from control by the Dunnett's test criteria, $p < 0.01$.

3. Urinalysis

There were no adverse changes in urine parameters in male or female dogs.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

No test substance-related changes in mean organ weights or organ weights relative to final body weight were apparent at any dietary concentration. Absolute liver weight was slightly increased in 160 and 640 ppm females (not statistically significant); and relative liver weight was significantly increased in 640 ppm females. However, no test substance-related liver lesions were observed upon microscopic examination. Nevertheless, given the increase in alkaline phosphatase levels observed in these groups, it cannot be excluded that these effects are treatment-related. No effects were observed on liver weights or liver histopathology in male dogs at any dietary concentration.

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy. At the microscopic examination, test substance-related extramedullary haematopoiesis of the spleen and erythrocyte hyperplasia of the bone marrow were present at 80 ppm and above in male dogs and at 40 ppm and above in female dogs. Yellow brown pigment (interpreted as hemosiderin) was slightly increased in one or more of the following organs from males and females at 40 ppm and above: liver, kidneys, spleen, and bone marrow. The erythrocytic hyperplasia observed in spleen and bone marrow and hemosiderin accumulation in various tissues are typical secondary physiologic responses to a test substance-induced haemolytic effect. All other histopathological observations in this study were consistent with normal background lesions of dogs of this age and breed.

Table B.6.3.2-43
90-Day feeding study in dogs: Incidences of microscopic effects

DPX-JW062 (ppm)	0	40	80	160	640
Number of dogs/group:	4	4	4	4	4
Males:					
Liver					
Pigment, increased, Kupffer cell	0 ^a	1	4	4	4
Kidneys					
Pigment, increased, tubular epithelium	0	1	2	3	4
Spleen					
Pigment, increased, macrophages	0	4	4	4	4
Haematopoiesis, increased, extramedullary	0	0	2	4	4
Bone Marrow					
Pigment, increased, macrophages	0	0	2	4	4
Hyperplasia, erythrocytic	0	0	3	4	4
Females:					
Liver					
Pigment, increased, Kupffer cell	0	1	4	4	4
Kidneys					
Pigment, increased, tubular epithelium	0	0	1	3	3
Spleen					
Pigment, increased, macrophages	0	2	4	3	4
Haematopoiesis, increased, extramedullary	0	3	1	3	3
Bone Marrow					
Pigment, increased, macrophages	0	3	4	4	4
Hyperplasia, erythrocytic	0	4	4	4	4

^a Number of organs with microscopic change.

Conclusions from the original DAR (2000, RMS NL):

Based on body weight effects in the 640 mg/kg food treated females and changes indicative of the occurrence of mild anaemia in ≥ 160 mg/kg food males and 640 mg/kg food females, the authors concluded the NOAEL of the test substance for 90-days oral exposure to be 80 mg/kg food for male dogs and 160 mg/kg food for female dogs. Several changes, such as increased serum bilirubin levels and the occurrence of haemosiderin pigment in liver, spleen, kidneys, and bone marrow, were only considered secondary to the effects on circulating red blood cells and were not used to support the occurrence of effects at lower dose levels. Since it is known that erythrocytic Heinz bodies interfere with spectrophotometric determination of Hb, MCH and MCHC, changes in these parameters are difficult to interpret. Therefore, the increase in haemosiderin pigment together with the regenerative responses in bone marrow and spleen observed in 40 mg/kg food animals are considered indicative of the occurrence of effects on red blood cells, i.e. an adverse effect of the test substance at this dose level. Therefore, the NOAEL for 90-days exposure to dogs is set at <40 mg/kg food, equal to <1 mg/kg bw/d, for both males and females.

Conclusions from the Post-Annex I Addendum (2007, RMS NL):

Based on body weight effects in the 640 mg/kg food treated females and changes indicative of the occurrence of mild anaemia in ≥ 160 mg/kg food males and 640 mg/kg food females, the authors concluded the NOAEL of the test substance for 90-days oral exposure to be 80 mg/kg food for male dogs and 160 mg/kg food for female dogs. Several changes, such as increased serum bilirubin levels and the occurrence of haemosiderin pigment in liver, spleen, kidneys, and bone marrow, were only considered secondary to the effects on circulating red blood cells and were not used to support the occurrence of effects at lower dose levels. Since it is known that erythrocytic Heinz bodies interfere with spectrophotometric determination of Hb, MCH and MCHC, changes in these

parameters are difficult to interpret. Therefore, the increase in haemosiderin pigment together with the regenerative responses in bone marrow and spleen observed in 40 mg/kg food animals are considered indicative of the occurrence of effects on red blood cells, i.e. an adverse effect of the test substance at this dose level. Therefore, the NOAEL for 90-days exposure to dogs is set at <40 mg/kg food, equal to <1 mg/kg bw/d, for both males and females.

The JMPR concluded in their 2005 evaluation: Microscopically, haemosiderin was present in the spleen and liver of male and female dogs at 40 ppm and above. The changes in erythrocyte parameters in association with the presence of pigment in the kidneys and bone marrow, and mild erythrocytic hyperplasia of the spleen and bone marrow present in females at 160 ppm and males at 640 ppm were considered to be adverse findings. On the basis of mild haemolysis observed in both sexes, the NOAEL in females was 80 ppm (3 mg/kg bw per day) and 160 ppm (5 mg/kg bw per day) in males.

Conclusions proposed by the applicant (2015):

The NOAEL was 80 ppm (2 mg/kg bw/day) for males and 160 ppm (5 mg/kg bw/day) for females. This NOAEL was based on mild anaemia in males at the LOAEL, 160 ppm and mild anaemia and decreased food efficiency, body weight gains, and body weights in females at the LOAEL, 640 ppm.

RMS FR assessment (2016):

At the lowest dose level of 40 ppm, decreased red cell mass parameters of up to -14% and -13% in males and females respectively and increased MCV were observed, associated with histopathological findings indicative of haemolytic anemia in the spleen, liver, kidney and bone marrow (hemosiderosis in males and females, bone marrow hyperplasia and extramedullary haematopoiesis in the spleen in females). The NOAEL of this 90-d dog study is therefore set at <40 ppm (1 mg/kg bw/d). Heinz bodies were observed at higher dose levels in this study.

Previous evaluation:	HLR 885-96 : In DAR (2000) HLR 885-96 Revision No.1 : Submitted for the purpose of renewal (see reasons for revisions below)
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CA 5.3.2/05

Report: [REDACTED] (1997b); Chronic toxicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) one year feeding study in dogs

DuPont Report No.: HLO 885-96, Revision No. 1

Guidelines: OECD 452, 59 Nohsan N0. 4200, U.S. EPA 83-1, Directive 87/302/EEC Part B

Deviations: Histopathological evaluation of Peyer's patches in the intestines was not specifically noted in the report since they are routinely collected and evaluated as part of the intestines. However, reconducting the study is unlikely to yield a significantly different result. The absence of histopathological evaluation of Peyer's patches is unlikely to alter the interpretation of the study since no effects on the immune system were observed in other immune system tissues evaluated in this study or in a 28-day immunotoxicity study in mice (DuPont 29280) with indoxacarb (DPX-KN128).

Testing Facility: [REDACTED]

Testing Facility Report No.: WIL-189019

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

Reason for Revision No. 1:

1. Discussions of hematology and microscopic findings were updated following re-evaluation.

2. Addition of literature citations regarding the re-assessment of hematology and microscopic findings.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

DPX-JW062 technical
Lot/Batch #: JW062-106
Purity: 95.03%
Description: Off-white powder
CAS #: 144171-61-9
Stability of test compound: Analyses confirmed that test material was stable in feed for at least 7 or 14 days at room temperature, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.
2. Vehicle and/or negative control: Untreated diet with acetone
3. Test animals

Species: Dog
Strain: Beagle
Age at initial dosing: Approximately 25 weeks old
Weight at initial dosing: 7.8–10.1 kg for males; 6.3–8.6 kg for females
Source: [REDACTED]
Acclimation period: 20 days
Diet: PMI® Feeds, Inc., Certified Canine LabDiet® #5007, offered for one hour per day. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water: Tap water, <i>ad libitum</i>
Housing: Animals were housed individually in stainless steel cages.
4. Environmental conditions

Temperature: 21.2–24.7°C
Humidity: 28.6–69.9%
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

07-September-1995 to 06-September-1996
2. Animal assignment and treatment

Five groups of five animals/sex/concentration were administered concentrations of DPX-JW062 in feed daily for 52 weeks. Males and females received 0, 40, 80, 640, and 1280 ppm. Dietary concentrations were selected based on the results of the 90-day dog study (HLO-494-95, Revision No. 3 summarised in this document). The selected route of administration was oral (dietary) since this is a possible route of human exposure. Animals were assigned to dose groups by a standard block randomisation procedure using body weights as the block. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Table B.6.3.2-44
Study design: 1-Year feeding study in dogs

Group no.	No. females/ group	No. males/ group	Conc. in diet (ppm) ^a	Mean daily intakes/ males (mg/kg bw)	Mean daily intakes/ females (mg/kg bw)
1	5	5	0 (control)	0 (control)	0 (control)
2	5	5	40	1.1	1.3
3	5	5	80	2.3	2.4
4	5	5	640	17.5	18.9
5	5	5	1280	33.6	36.1

^a Weight/weight concentration of test substance

3. Diet preparation and analysis

The test substance dissolved in acetone was added to a small amount of feed and thoroughly premixed for 10 minutes, followed by 15 minutes of mixing with enough feed to achieve a total batch size of homogenous diet. Control diets were mixed for the same period of time. All diets were prepared weekly and stored at room temperature until used. The homogeneity of DPX-JW062 was checked by analysis using HPLC from samples taken at the beginning of the study and at Months 3 and 8. Concentrations of DPX-JW062 in the dietary mixtures were checked by analysis using HPLC at Months 1, 3, 6, 9, and 12. The stability of test substance in the dietary mixtures for 7 or 14 days at room temperature or 14 days refrigerated was checked by analysis using HPLC at the beginning of the study. The test substance was at target concentrations (87.3–96.9% of nominal, 91.8–101.6% of nominal, 92.0–95.5% of nominal, 93.0–96.3% of nominal, and from 93.3 to 96.1% of nominal for preparation dates month 1, month 3, month 6, month 9, and month 12, respectively), homogeneous (C.V. = 0.5–7.8%) throughout the feed, and was stable (88.3–96.4% of nominal) for 7 or 14 days at room temperature or 14 days refrigerated. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

All analyses were conducted using two-tailed tests for significance levels of 5% and 1% comparing the treatment groups to the vehicle control group by sex. Statistical tests were performed by a Digital® MicroVAX® 3400 computer with appropriate programming. Body weight, body weight change, food consumption, clinical pathology, and absolute and relative organ weight data were subjected to one-way analysis of variance followed by Dunnett's test. Clinical pathology values for leukocytes that occur at low incidence (*i.e.*, monocytes, eosinophils, and basophils) were not subjected to statistical analysis.

C. METHODS

1. Observations

Animals were observed twice a day for mortality, moribundity, and overt signs of toxicity. Detailed examinations for clinical signs of toxicity were conducted weekly.

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency and daily intake

Food consumption was recorded daily for each animal and the mean group food consumption was calculated over the weekly weighing interval. Food efficiency and daily intake of the test article were calculated from food consumption and body weight data.

4. Ophthalmological examinations

All animals were examined by indirect ophthalmoscopy preceded by mydriasis prior to study start. All animals were examined again prior to scheduled sacrifice.

5. Clinical pathology (haematology, clinical chemistry, and urinalysis)

Blood and urine samples were collected from all animals during pretest (Week -1) and Weeks 12, 25, and 51. Collection of blood and urine samples was conducted prior to the feeding period. Evaluation of haematology (including Heinz bodies), clinical chemistry, and urinalysis parameters were performed for all animals. Bone marrow smears were prepared at the final sacrifice from all main study animals.

6. Sacrifice and pathology

All dogs were euthanized by anaesthesia with sodium pentobarbital and exsanguination. Gross examinations were performed on all main study animals. Organs that were weighed are listed in the following table. Organ weight/final body weight and organ weight/brain weight ratios were calculated. All collected tissues were processed to slides and evaluated microscopically.

Table B.6.3.2-45
1-Year feeding study in dogs: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Brain	X	X
Spleen		X
Heart		X
Liver	X	X
Kidneys	X	X
Oesophagus		X
Adrenal glands	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands		X
Pancreas		X
Gall bladder		X
Skin		X
Trachea		X
Thymus		X
Mesenteric lymph node		X
Suprathyroid lymph node		X
Bone marrow		X
Thyroid gland (with parathyroid)	X	X
Eyes with optic nerve		X
Testes	X	X
Epididymides	X	X
Prostate		X
Ovaries (including oviducts) (weighed without oviducts)	X	X
Uterus with vagina		X
Mammary glands (females)		X
Stomach		X
Pituitary		X
Lungs		X
Sciatic nerve		X
Spinal cord		X
Skeletal muscle		X
Aorta		X
Urinary bladder		X
Bone (femur with joint; rib with costochondral junction)		X
Gross observations		X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No test-substance related clinical signs of toxicity were observed for any dietary concentration in either males or females.

2. Mortality

Mortality did not occur during the course of this study

B. BODY WEIGHT AND BODY WEIGHT GAIN

Mean body weights in the 1280 ppm group males generally remained lower than the control group throughout the study. Decreased mean body weight gains were observed in this group during approximately the first three months of the study (statistically significant during study weeks 8-9 and 9-10). One high dose male was responsible for the magnitude of the changes. In the 1280 group females, mean body weights were reduced for approximately the first two months of the study, and were similar to or greater than the control group thereafter. No test substance-related effects were observed in mean body weights in any of the remaining groups.

Table B.6.3.2-46
1-Year feeding study in dogs: Body weights (g)

Day	0 ppm	40 ppm	80 ppm	640 ppm	1280 ppm
Males:					
Week 0	9201	9206	9167	9276	9306
Week 12	12289	12377	11819	12347	10276
Week 24	13278	13361	13210	13518	11026
Week 36	13357	13633	13572	13278	11230
Week 52	13521	13634	13724	13413	12424
Females:					
Week 0	7670	7558	7864	7561	7947
Week 12	9501	9387	9589	9101	9111
Week 24	10352	10117	10396	9827	10363
Week 36	10536	10433	10738	9867	10744
Week 52	10521	10696	11064	10109	10826

Table B.6.3.2-47
1-Year feeding study in dogs: Body weight gain (g)

Parameter	0 ppm	40 ppm	80 ppm	640 ppm	1280 ppm
Males:					
Overall body weight gain, Week 0–52	4320	4428	4557	4137	3118
Females:					
Overall body weight gain, Week 0–52	2851	3138	3200	2548	2879

^a Significantly different from control by the Dunnett's test criteria, $p < 0.05$.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Test substance-related effects on food consumption consisted of decreases in the 1280 ppm group. Food consumption in the 1280 ppm group males was reduced for approximately the first nine months of the study. During the last three months of the study, food consumption was similar or slightly below controls. In the 1280 group females, food consumption was decreased approximately the first two months of the study. The differences from the control group were statistically significant during study weeks 2–3 and 8–9. No test substance-related effects were observed in food consumption in the 40, 80, and 640 ppm groups.

Food efficiency was decreased in the 1280 ppm group males and females during approximately the first eight and two months, respectively. The decreases were of sporadic statistical significance and the magnitude of the decreases was primarily a result of male no. 3378 and female no. 3408. No test substance-related effects on food efficiency were noted in the 40, 80, and 640 ppm groups.

D. OPHTHALMOLOGICAL EXAMINATIONS

No test-substance related ophthalmological observations were observed for any dietary concentration in either males or females.

E. CLINICAL PATHOLOGY

1. Haematology

Males and females displayed test substance-related haemolytic anaemia. Dose related decreases in the indicators of circulating erythrocytic mass (red blood cell count, haemoglobin concentration, and haematocrit) were present in the 80, 640, and 1280 ppm male and female groups and in the 40 ppm male group at all evaluations. Increased Heinz bodies (denatured haemoglobin) suggest that the red cell effects were due to oxidant-induced haemolysis. Other haematologic changes were consistent with a typical regenerative response to haemolytic anaemia. These included increased mean reticulocyte counts, increased mean corpuscular volume, decreased mean corpuscular haemoglobin concentration, erythrocyte morphological changes (increased incidence of Howell-Jolly bodies, polychromasia, hypochromasia), and/or increased mean platelet counts. Based on the presence of a regenerative response, the haematologic effects noted above are expected to be reversible following cessation of exposure to the test substance.

Table B.6.3.2-48
1-Year feeding study in dogs: Haematology findings

Parameter	0 ppm	40 ppm	80 ppm	640 ppm	1280 ppm
Males (Week 51)					
RBC ^a × 10 ⁶ /μL	7.18	6.21 ^c -14%	6.07 ^c -15%	5.34 ^c -25%	5.29 ^c -26%
Hb (g/dL)	16.3	14.6 ^c -10%	14.0 ^c -14%	12.8 ^c -21%	12.4 ^c -24%
Haematocrit (%)	47.5	43.0 ^b -9.5%	42.1 ^c -11.4%	38.2 ^c -19.6%	37.7 ^c -21%
Reticulocytes × 10 ⁶ /μL	0.032	0.017	0.057	0.050	0.079 ^b
MCV (μ ³)	66.3	69.3 +4.5%	69.4 +4.7%	71.7 ^c +8.1%	71.4 ^c +7.7%
MCHC (g/dL)	34.3	34.0	33.3 ^b	33.5	32.9 ^c
Platelet × 10 ³ /μL	293	377	386	541 ^c	482 ^c
Heinz bodies (%)	0.0	0.0	4.4	11.0 ^c	12.4 ^c
Females (Week 51)					
RBC × 10 ⁶ /μL	6.70	6.47 -3.4%	5.66 ^a -15.6%	5.60 ^a -16%	5.01 ^b -25%
Hb (g/dL)	15.5	15.3 -1.2%	13.2 ^b -14.8%	13.3 ^b -14%	12.1 ^b -22%
Haematocrit (%)	45.4	45.3	39.2 ^a -13.7%	39.8 ^a -12%	36.0 ^b -21%
Reticulocytes × 10 ⁶ /μL	0.010	0.039	0.033	0.092 ^b	0.048
MCV (μ ³)	67.8	70.1 +3.3%	69.4 -2.3%	71.1 +4.9%	72.1 ^a +6.3%
MCHC (g/dL)	34.3	33.8	33.7	33.6	33.5 ^a
Platelet × 10 ³ /μL	295	360	530 ^b	502 ^b	501 ^b
Heinz bodies (%)	0.0	0.1	7.2	11.9 ^b	14.8 ^b

^a RBC = Red blood cells

^b Significantly different from control by the Dunnett's Test criteria, p <0.05.

^c Significantly different from control by the Dunnett's Test criteria, p <0.01.

^d Hb = Haemoglobin

^e MCV = Mean corpuscular volume

^f MCHC = Mean corpuscular haemoglobin concentration

2. Clinical chemistry

No primary adverse test substance-related effects were observed on clinical chemistry parameters. Mean total bilirubin concentrations were increased in the 80, 640, and 1280 ppm groups, but these changes were considered secondary to haemolytic anaemia.

Mean alkaline phosphatase was significantly increased in 1280 ppm males at Week 51 and in 640 and/or 1280 ppm females during Weeks 12, 25, and 51. Although a dose-related response was not clearly evident and no corresponding microscopic changes were evident in the liver, it cannot be totally excluded that this effect is treatment-related given the increases in liver weight at these dose levels.

Table B.6.3.2-41
90-Day feeding study in dogs: Haematology findings

	Males					Females				
Parameter	0	40	80	640	1280	0	40	80	640	1280
Bilirubin (mg/dL)										
WK -1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
WK 12	0.1	0.1	0.2**	0.3**	0.3**	0.1	0.3**	0.3**	0.3**	0.3**
WK 25	0.2	0.2	0.2	0.3**	0.3**	0.2	0.2	0.3*	0.3*	0.3**
WK 51	0.2	0.2	0.3**	0.3*	0.3**	0.2	0.3	0.3	0.3	0.3*
Alkaline phosphatase (U/L)										
WK -1	142	136	126	129	130	143	123	108	119	111
WK 12	110	114	134	129	125	112	115	112	152*	148
WK 25	70	67	98	99	96	75	82	78	132**	118*
WK 51	59	51	89	108	118*	68	82	98	132**	114*

* Significantly different from control by the Dunnett's test criteria, p <0.05.

** Significantly different from control by the Dunnett's test criteria, p <0.01

3. Urinalysis

The 1280 ppm group males had an increased incidence of bilirubinuria at each evaluation time points, which may have been secondary to the haemolytic anemia. Other groups also showed bilirubinuria as detailed in the following table.

Table B.6.3.2-50
1-Year feeding study in dogs: Urinalysis - bilirubinuria

Bilirubinuria	0 ppm	40 ppm	80 ppm	640 ppm	1280 ppm
Males:					
WK -1	0	0	0	0	0
WK 12	0	0	0	0	3 (2+)
WK 25	0	0	0	3 (1+, 1+, 2+)	4 (1+, 1+, 2+, 2+)
WK51	0	3 (1+, 1+, 2+)	2 (2+, 3+)	1 (3+)	4 (2+, 2+, 3+, 3+)
Females:					
WK -1	0	0	0	0	0
WK 12	0	0	1 (2+)	0	1 (1+)
WK 25	0	0	0	0	0
WK51	0	0	1 (1+)	1 (1+)	2 (1+, 2+)

Number of animals affected. (total number of animals per group is 5)

In brackets: grade – 1+: trace to slight; 2+: slight to moderate; 3+: moderate to abundant

F. SACRIFICE AND PATHOLOGY

1. Organ weight

Mean liver weights were significantly increased in 640 ppm males, and relative liver weight was significantly increased in 640 and 1280 ppm males. Mean liver weights were significantly increased in 80 and 1280 ppm females; and relative liver weight was increased in 640 and 1280 ppm females. Although the increases were not clearly dose-related, given the changes in alkaline phosphatase levels and the magnitude of the liver weights increases, a test substance related effect cannot be excluded from the dose levels of 640 ppm in males and 80 ppm females.

Table B.6.3.2-51
1-Year feeding study in dogs: Organ weights

Parameter	0 ppm	40 ppm	80 ppm	640 ppm	1280 ppm
Males:					
Absolute liver weight (g)	356.12	372.56 +4.6%	392.37 +10.2%	532.93 ^c +49.7%	423.75 +19%
Relative ^a liver weight	2.626	2.685 +2.2%	2.834 +7.9%	3.902 ^c +48.6%	3.430 ^c +30.6%
Liver to brain weight	431.456	470.048 +8.9%	496.766 +15.1%	655.549 ^c +51.9%	516.656 +19.7%
Females:					
Absolute liver weight (g)	281.67	335.93 +19.3%	363.36 ^b +29.0%	359.80 +27.7%	389.05 ^c +38.1%
Relative ^a liver weight	2.694	3.133 +16.3%	3.277 +21.6%	3.486 ^c +29.4%	3.560 ^c +32.1%
Liver to brain weight	369.070	450.170 +22.0%	466.759 +26.5%	455.386 +23.4%	520.588 ^c +41.1%

^a Relative weight is defined as the organ to body weight ratio.

^b Significantly different from control by the Dunnett's test criteria, $p < 0.05$.

^c Significantly different from control by the Dunnett's test criteria, $p < 0.01$.

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy.

Microscopic changes that were secondary to the test substance-induced haemolytic effect were observed in the liver, spleen, kidneys, and/or bone marrow of all treated groups. These secondary changes were noted in some animals in the 40 ppm group.

Table B.6.3.2-52
1-Year feeding study in dogs: Incidences of microscopic effects

DPX-JW062 (ppm)	0	40	80	640	1280
Number of dogs/group:	5	5	5	5	5
Males:					
Liver					
Pigment, increased, Kupffer cell	0 ^a	2	4	5	5
Kidneys					
Pigment, tubular	0	3	5	4	5
Spleen					
Pigment, increased	0	0	0	3	3
Haematopoiesis, increased, extramedullary	0	0	3	5	5
Bone Marrow					
Pigment, increased	0	2	1	5	5
Hyperplasia, mixed	0	2	3	5	5
Females:					
Liver					
Pigment, increased, Kupffer cell	0	1	5	5	5
Kidneys					
Pigment, tubular	0	0	3	5	4
Spleen					
Pigment, increased	0	0	1	3	5
Haematopoiesis, increased, extramedullary	0	1	5	5	5
Bone Marrow					
Pigment, increased	0	0	4	5	5
Hyperplasia, mixed	0	4	5	5	5

^a Number of organs with microscopic change.

Conclusions from the original DAR (2000, RMS NL):

The authors concluded the NOAEL to be 40 mg/kg food (equivalent to 1.1 mg/kg bw/day), based on the presence of 'mild' haemolytic anaemia at 80 mg/kg food. However, the reviewer considers the statistically significant changes in RBC count, haemoglobin and hematocrit observed in males at 51 weeks after exposure to 40 mg/kg food an adverse effect. The increased plasma bilirubin levels (females), increased pigments in liver, kidney and bone marrow (males), and mixed hyperplasia at this dose level is in line with a haemolytic effect at this dose level. Therefore, the NOAEL for semichronic toxicity (52 weeks) of the test substance in dogs is established at <40 mg/kg food, equal to <1.1 mg/kg bw/day.

Conclusions from the Post-Annex I Addendum (2007, RMS NL):

The authors concluded the NOAEL to be 40 mg/kg food (equivalent to 1.1 mg/kg bw/day), based on the presence of 'mild' haemolytic anaemia at 80 mg/kg food. However, the reviewer considers the statistically significant changes in RBC count, haemoglobin and hematocrit observed in males at 51 weeks after exposure to 40 mg/kg food, in combination with the increased plasma bilirubin levels (females), increased pigments in liver, kidney and bone marrow (males), and mixed hyperplasia at this dose level in line with a haemolytic effect. Therefore, the NOAEL for semichronic toxicity (52 weeks) of the test substance in dogs is established at <40 mg/kg food, equal to <1.1 mg/kg bw/day.

The JMPR concluded in their 2005 evaluation: Haemolytic effects were seen in males at = 40 ppm and females at = 80 ppm. Haematology values were outside historical control values only at = 640 (males) and = 80 ppm (females), although statistical significance was demonstrated (Dunnett test) at lower doses. The effect was greatest at the 12-week sampling time and values increased thereafter, but were still significantly lower than controls at 51 weeks at = 40 ppm (males) and at = 80 ppm (females). There were no other significant serum

Note from the RMS: the JMPR compared haematology values with historical control values. The SCP also took historical values into account in their opinion. These parameters are however very sensitive parameters when compared to the concurrent control, but become less sensitive when compared to historical control values. In the opinion of the RMS, prevalence should be given to the concurrent control values.

The no-observed-adverse-effect-level (NOAEL) for males was 40 ppm (1.1 mg/kg bw/day) based on the presence of haemolytic anaemia in males at the lowest-observed-adverse-effect-level (LOAEL) of 80 ppm (2.3 mg/kg bw/day). The NOAEL for females was 40 ppm (1.3 mg/kg bw/day) based on the presence of haemolytic anaemia in females at the LOAEL of 80 ppm (2.4 mg/kg bw/day).

Considering the effects indicative of haemolytic anemia observed at the lowest tested dose, the NOAEL is set at <40 ppm for males (1.1 mg/kg bw/d) and females (1.3 mg/kg bw/d). Indeed, at this dose level, histopathological effects in the kidney, liver, bone marrow and/or spleen occurred in both sexes and increased plasma bilirubin levels were observed in females and bilirubinuria was observed in males. Red blood cell parameters were decreased in males by up to 14% whereas they are only slightly changed in females, for which MCV and reticulocytes were nevertheless increased. At higher dose levels, Heinz bodies were recorded in both sexes.

Previous evaluation:	In Addendum to DAR (2001)
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Report: [REDACTED] (1999); DPX-MP062 technical: Repeated -dose dermal toxicity 28-day study in rats

Guidelines: OECD 410 (1981),EEC Method B.9. (1992),USEPA 870.3200 (1996), 59 Nohsan N0. 4200
Deviations: None

Testing Facility: [REDACTED]

GLP: Yes

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:
Lot/Batch #: DPX-MP062 technical
Purity: MP062-216
Description: 95.83%
CAS #: White solid
Stability of test compound: 144171-61-9
The test substance was determined to be stable for the length of the study
2. Vehicle and/or positive control: Deionized water
3. Test animals
Species: Rat
Strain: CrI:CD[®](SD)IGS BR
Age at dosing: Approximately 57 days old
Weight at dosing: 246.0-298.8 g for males; 174.9-222.7 g for females
Source: [REDACTED]
Acclimation period: 6 days
Diet: PMI Nutrition International, Inc. Certified Rodent LabDiet[®] (#5002)
Water: Tap water, *ad libitum*
Housing: Rats were housed singly with the sexes separate in stainless steel, wire-mesh cages.
4. Environmental conditions
Temperature: 19 to 25°C
Humidity: 40 to 60%
Air changes: Not included in report
Photoperiod: 12-hour light/dark cycle

B. STUDY DESIGN

1. In-life initiated/completed
05-May-1999 to 03-June-1999
2. Methods
In a previous 28-day dermal study (HLO 747-96) in rats with DPX-MP062, the test substance applied to the skin did not cover 10% of the skin surface area in all dose groups as required by guidelines EEC B.9 and OECD 410. In this repeat 28-day dermal study, DPX-MP062 was moistened with deionised water and applied to the shaved, intact dorsal skin of male and female CrI:CD[®](SD)IGS BR rats (10/sex/dose) for 28 daily (consecutive) applications. The material was applied to the skin to cover approximately 10% of the total body surface area. The rats were exposed to the test substance for 6 hours per day. Exposure doses were 0, 50, 500, 1000, or 2000 mg/kg/day. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, clinical pathology, organ weights, ophthalmology, and gross and microscopic pathology.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity
There were no test substance-related clinical signs of systemic toxicity in any male group. A low, but statistically significant increase in the incidences of cool body and hunched over posture was observed in 2000 mg/kg/day females. These signs may represent a response to systemic exposure to DPX-MP062 or may be due to the stress of the dosing procedure, as they were only observed after the exposure period. Statistically significant increases in the incidence of stains were observed in the inguinal area, the head, the perineum, and/or the underbody in male and female rats exposed to 500, 1000, and/or 2000 mg/kg/day.

2. Mortality

Mortality (3 of 10) occurred in 2000 mg/kg females. One of these deaths was accidental (the result of a fractured nose). The two other female rats in the 2000 mg/kg/day group exhibited body weight loss, stained perineum, hunched over posture, and/or weakness. No apparent cause for either death was found on necropsy. However, these two deaths were considered to be test substance-related.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Mean body weight gain in males exposed to 2000 mg/kg/day was lower than that of control male rats (76% of control; statistically significant). Test substance-related decreases in body weight gain were observed in female rats exposed to 500 mg/kg/day and above of DPX-MP062. Statistically significant reductions in overall mean body weight gain in the 2000, 1000, and 500 mg/kg/day female groups were 53, 53, and 55% of control value, respectively. Mean body weights of female rats in these groups were also lower than controls.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

Males treated with 2000 mg/kg had lower food consumption compared to controls (91% of control). Females treated with 500 mg/kg and above had lower food consumption compared to controls (83-87% of controls). Test substance-related decreases in food efficiency were also observed in these female groups (64-65% of controls).

Table B.6.3.3-1
28-Day dermal study in rats: Body weight/body weight gain/food consumption/food efficiency

	0 mg/kg	50 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg
Males					
Body weight (g), Day 28	379.7	386.4	364.4	367.4	355.3
Body weight gain (g), Day 1-28	108.9	113.3	97.7	96.4	82.8 ^a
Food consumption (g), Day 1-28	27.1	26.9	26.4	25.5	24.7 ^a
Food efficiency (g wt/g food consumed), Day 1-28	0.148	0.156	0.138	0.141	0.124
Females					
Body weight (g), Day 28	258.1	245.6	230.5 ^a	231.5 ^a	230.5 ^a
Body weight gain (g), Day 1-28	64.4	47.4	35.5 ^a	33.9 ^a	34.3 ^a
Food consumption (g), Day 1-28	22.3	20.6	19.4 ^a	18.6 ^a	18.6 ^a
Food efficiency (g wt/g food consumed), Day 1-28	0.105	0.085	0.067 ^a	0.067 ^a	0.068 ^a

^a Significantly different from control by the Dunnett's test, $p < 0.05$.

D. CLINICAL PATHOLOGY

1. Haematology

At 500, 1000, and 2000 mg/kg/day, decreases in RBC mass parameters (haemoglobin, haematocrit, red cell count), and associated changes in red cell indices (MCV, MCH, MCHC) and reticulocyte counts were present in male and female rats. Slight increases in methaemoglobin were also present. RBC mass parameters were decreased by 2 to 10% compared to controls. The decreases in mean and individual animal RBC mass parameters and reticulocytes were similar in the three highest dose groups in both sexes despite the 4-fold range in applied dose. At 50 mg/kg/day, a mild statistically significant increase in reticulocytes was observed in females.

Table B.6.3.3-2
28-Day dermal study in rats: Haematological evaluation

	0 mg/kg	50 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg
Males					
RBC (% Control)	8.12	8.00 -1.5%	7.36 ^a -9.4%	7.56 ^a -6.9%	7.41 ^a -8.7%
Hb (% Control)	15.3	15.0 -2%	14.2 ^a -7.2%	14.4 ^a -5.9%	14.4 ^a -5.9%
HCT (%)	47.7	46.7 -2.1%	44.8 ^a -6.1%	46.6 -2.3%	45.8 -4.0%
Reticulocytes × 10 ³ /μL	187.3	217.0	411.9 ^a	497.9 ^a	472.4 ^a
MCV (fl)	58.8	58.4	60.9	61.8 ^a	61.8 ^a
MCH (pg)	18.8	18.8	19.3	19.2	19.4
MCHC (g/dL)	32.0	32.1	31.6	31.0 ^a	31.4 ^a
Met-Hb (%)	0.5	0.5	0.8 ^b	1.0 ^b	1.1 ^b
Females					
RBC (% Control)	7.80	7.70 -1.3%	7.09 ^a -9.1%	7.15 ^a -8.3%	7.01 ^a -10.1%
Hb (% Control)	15.4	14.9 -3.2%	14.4 ^a -6.5%	14.4 ^a -6.5%	14.2 ^a -7.8%
HCT (%)	47.2	46.5 -1.5%	45.2 -4.2%	45.4 -3.8%	45.4 -3.8%
Reticulocytes × 10 ³ /μL	168.3	301.2 ^a	409.1 ^a	345.9 ^a	416.7 ^a
MCV (fl)	60.7	60.4	63.8 ^a	63.6 ^a	64.8 ^a
MCH (pg)	19.8	19.4	20.3	20.2	20.3
MCHC (g/dL)	32.6	32.2	31.9 ^a	31.7 ^a	33.3 ^a
Met-Hb (%)	0.6	0.6	0.9	1.2 ^b	1.6 ^b

^a Significantly different from control by Dunnett's, p <0.05.

^b Significantly different from control by Dunnett's test, p <0.05.

RBC = Red blood cells, Hb = Haemoglobin, HCT = Haematocrit, MCV = Mean Corpuscular Volume

MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration

Met-Hb = Methaemoglobin

2. Clinical chemistry

There were no biologically significant changes in clinical chemistry parameters.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

There were no primary effects on organ weights. Effects secondary to the haematology changes were noted in the spleen. Increased spleen weight parameters occurred in males at 500 mg/kg/day and above and in 2000 mg/kg/day females.

2. Gross pathology and histopathology

Grossly increased incidences of spleen discoloration were increased in males and females administered 500 mg/kg/day and above. Microscopically, increased extramedullary haematopoiesis was seen in all treated male and female groups. Increased pigmented macrophages (haemosiderin) were also seen in all treated male and female groups, except the 50 mg/kg/day males.

Table B.6.3.3-3: gross pathology and histopathology findings

Dose (mg/kg bw/d)	0		50		500		1000		2000	
	m	f	m	f	m	f	m	f	m	f
<u>Macroscopy</u> Spleen - Spleen discoloration					+	++	++	++	+	++
<u>Microscopy</u> Spleen - increased extramed. haemopoiesis	0	0	6	3	10	9	10	7	10	6
- increased pigment (hemosiderin)	0	1	0	7	10	10	10	10	10	8

+ / ++ present in one or a few animals / present in most or all animals

Conclusions from the original DAR (2000, RMS NL):

The authors of the study established a NOAEL of 50 mg/kg bw/day based on reduced body weight, body weight gain, food consumption, and food efficiency in female rats exposed to 500 mg/kg bw/day. The reviewer considers this dose level to be a LOAEL, based on microscopic effects in the spleen (increased pigment and extramedullary haematopoiesis) and increased reticulocyte counts observed in females. Under the conditions of this study, the NOAEL is considered < 50 mg/kg bw/day.

Conclusions proposed by the applicant (2015):

The NOAEL in the 28-day dermal study in rats was 1000 mg/kg/day for males and 50 mg/kg/day for females. The NOAEL for male rats was based on reduced body weight gain and food consumption in rats exposed to 2000 mg/kg/day. The NOEL in female rats was based on reduced body weight, body weight gain, food consumption, and food efficiency in females exposed to 500 mg/kg/day and above.

RMS FR assessment (2016):

The NOAEL is set at <50 mg/kg bw/d based on histopathological spleen effects and slight indication of haemolytic anemia (slight decrease in red blood cell mass parameters and increased reticulocytes in females) observed at this dose level. At higher doses, reduced body weights, body weight gains and food consumption and increased spleen weights were observed, as well as increased MCV and/or reticulocytes and methaemoglobinemia.

B.6.4. GENOTOXICITY

Genotoxicity studies were conducted with DPX-KN128 (99:1) and DPX-MP062 (75:25). Summaries of these studies are presented below.

Table B.6.4-1
Summary of genotoxicity studies for indoxacarb (DPX-KN128 and DPX-MP062)

Type of study	Test system	Concentration range tested	Result	Reference
<i>In vitro</i> bacterial mutagenicity (Ames)				
DPX-KN128 (99:1)	<i>Salmonella typhimurium</i> and <i>Escherichia coli</i>	0 - 5000 µg/plate (with and without S9)	Negative	Wagner 2004 DuPont-14332*
DPX-MP062 (75:25)	<i>Salmonella typhimurium</i> and <i>Escherichia coli</i>	0 - 5000 µg/plate (with and without S9)	Negative	Mathison 1997 HLR 831-96
<i>In vitro</i> mammalian cell gene mutation (CHO/HGPRT)				
DPX-KN128 (99:1)	CHO cells	0 - 50 µg/mL (with and without S9)	Negative	San and Clarke 2003 DuPont-13023*
DPX-MP062 (75:25)	CHO cells	0 - 250 µg/mL (with and without S9)	Negative	San and Clarke 1997 HLO-1997-00030
<i>In vitro</i> chromosome aberration				
DPX-KN128 (99:1)	Human lymphocytes	0 – 75 µg/mL with S9 0 – 50 µg/mL without S9	Negative	Gudi and Rao 2004 DuPont-13022, Revision No. 1*
DPX-MP062 (75:25)	Human lymphocytes	0 - 1000 µg/mL (with and without S9)	Negative	Gudi and Schadly 1996a HLO 979-96
<i>In vitro</i> unscheduled DNA synthesis				
DPX-MP062 (75:25)	Rat primary hepatocytes	0 - 200 µg/mL	Negative	San and Sly 1997a HLO-1997-00033
<i>In vivo</i> micronucleus				
DPX-KN128 (99:1)	Mouse bone marrow	0, 500, 1000, 2000 mg/kg bw	Negative	2003 DuPont-13021*
DPX-MP062 (75:25)	Mouse bone marrow	0, 3000, 4000 mg/kg bw (M) 0, 1000, 2000 mg/kg bw (F)	Negative	1997 HLR 1046-96

* Studies newly submitted

DPX-KN128 (99:1) did not induce mutations in bacteria or mammalian cells *in vitro*, and did not induce structural chromosomal aberrations or polyploidy in mammalian cells *in vitro* either in the presence or in the absence of metabolic activation. DPX-KN128 (99:1) did not induce an increase in micronucleated polychromatic erythrocytes in bone marrow of mice. The same results are reported for the 75:25 S:R enantiomeric blend DPX-MP062. DPX-MP062 (75:25) also did not cause unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

As a conclusion, the available data indicate that DPX-KN128 (99:1) and DPX-MP062 (75:25) did not show a genotoxic potential.

Photogenotoxicity potential:

No photogenotoxicity assay was provided for indoxacarb.

The following justification was provided by the applicant: “*This study was not conducted since an established testing guideline is currently not available. According to SANCO/10181/2013 –rev. 2.1 13 May 2013, Section 4, in cases where “...agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, waiving of these particular data requirement points is considered acceptable as long as not test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02.” In addition, Indoxacarb was negative in the phototoxicity assay at the limit of solubility (MCA 5.2.7), and was negative in all in vitro and in vivo genotoxicity assays (MCA 5.4.4), and no further testing is warranted based on these results. Further, according to the attached publication by Lynch et al, (2011), the International Workshop of Genotoxicity Testing has concluded that*

photogenotoxicity testing should no longer be recommended as part of the standard photosafety testing strategy.”

The RMS agreed with the applicant. However, according to Regulation (EU) no 283/2013, a photomutagenicity test would not be required for indoxacarb as its Ultraviolet/visible molar extinction/absorption coefficient is less than $1\,000\text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$.

B.6.4.1. *In vitro* studies

Bacterial assay for gene mutation

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.4.1/03

Report: Wagner, V.O., Klug, M.L. (2004); Indoxacarb (DPX-KN128) technical: Bacterial reverse mutation test

DuPont Report No.: DuPont-14332

Guidelines: OPPTS 870.5100, EC 2000/32/EC, OECD 471, JMAFF NohSan 8147 **Deviations:** None

Testing Facility: BioReliance, Rockville, Maryland, USA

Testing Facility Report No.: AA78LT.503.BTL

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any government agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|---|--|
| 1. Test material: | Indoxacarb technical |
| Lot/Batch #: | KN128-098 |
| Purity: | 95.47% |
| Description: | White solid |
| CAS # | 173584-44-6 |
| Stability of test compound: | Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study. |
| Solvent | Dimethyl sulfoxide (DMSO) |
| 2. Control materials | |
| Negative/Solvent control/final concentration: | DMSO at 50 µL/plate |

Positive control, non activation:

Positive control	Dose (µg/plate)	Strain(s)
2-Nitrofluorene	1	TA98
Methyl methanesulfonate	1000	WP2 <i>uvrA</i>
Sodium azide	1	TA100, TA1535
9-Aminoacridine	75	TA 1537

Positive control, activation:

Positive control	Dose (µg/plate)	Strain
2-Aminoanthracene	1	TA100
	1	TA98, TA1535, TA1537
	10	WP2 <i>uvrA</i>

3. Activation: Rat liver S9 from Male Sprague-Dawley rats induced with Aroclor 1254.
 Lot number: Not applicable
 Source: BioReliance, Rockville, Maryland
 Protein content: Not given
- Characterisation: The metabolic activation ability of the S9 was characterised using varying S9 and positive control concentrations. Each bulk preparation of S9 was tested for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100 (data not available to the RMS).
- S9 mix composition
 Sodium phosphate buffer (pH 7.4): 100 mM
 Glucose-6-phosphate: 5 mM
 NADP: 4 mM
 KCl: 33 mM
 MgCl₂: 8 mM
 S9 homogenate: 10% (v/v)
4. Test organisms
Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvrA* were properly maintained and were checked for appropriate genetic markers (rfa mutation, R factor).
5. Test concentrations for plate incorporation assay
Trial 1: 2.5, 7.5, 25, 75, 200, 600, 1800, and 5000 µg indoxacarb/plate in duplicate in the presence and absence of S9 activation.
Trial 2: 75, 200, 600, 1800, and 5000 µg indoxacarb/plate in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 13-February-2004 to 23-February-2004

2. Plate incorporation assay

This study consisted of 2 independent trials: a preliminary toxicity-mutation assay (Trial 1) and a mutagenicity assay (Trial 2). In both trials, 0.5 mL of S9 or Sham mix, 100 μ L of tester strain (cells seeded) and 50 μ L of solvent or test substance dilution were added to 2.0 mL of molten selective top agar at $45 \pm 2^\circ\text{C}$. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test substance aliquot was replaced by a 50 μ L aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at $37 \pm 2^\circ\text{C}$. Plates that were not counted immediately following the incubation period were stored at 2 to 8°C until colony counting could be conducted. All dosing solutions were plated in duplicate in the toxicity-mutation assay, and in triplicate in the mutagenicity assay.

Bacterial background lawns were evaluated for evidence of test substance precipitation and toxicity. Toxicity was scored relative to the concurrent negative control plates and recorded with the mean revertant count for the strain, condition, and concentration. Revertant colonies for a given tester strain and condition were counted by an automated colony counter.

3. Statistics

Data for each tester strain were evaluated independently. For each tester strain, the mean number of revertants and the standard deviation at each concentration in the presence of and absence of exogenous metabolic activation system were calculated. No further statistical analyses were conducted.

4. Evaluation criteria

A test substance was classified as positive when the mean number of revertants in any strain except TA1535 and TA1537, and at any test substance concentration was at least 2 times greater than the mean number of revertants in the concurrent negative control and occurred in a positive dose-response relationship. For strains TA1535 and TA1537 a mean number of revertants of at least 3 times greater than the negative control were needed to be considered a positive response. An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The analytical method is validated (see Volume 3B5). Indoxacarb was present at acceptable concentrations in the dosing solutions (within 10% of nominal concentrations). Indoxacarb was shown to be stable in the dosing solutions under the conditions of the study. Indoxacarb was not found in the 0 mg/mL samples. The positive and solvent controls fulfilled the requirements for a valid test.

B. MUTATION ASSAYS

DMSO was selected as the solvent of choice based on a solubility determination of the test substance and compatibility with the target cells. The test substance was soluble in DMSO at a maximum concentration of 100 mg/mL (corresponding to 5000 μ g/plate). Precipitate was observed beginning at 1800 μ g per plate in both trials. No test substance-related toxicity was observed in either trial as is evidenced by a reduction of the microcolony background lawns and/or by a concentration-related decrease in mean revertants per plate. All positive controls exhibited more than a 3-fold increase in mean revertants over the respective mean of the negative controls. For strains TA100, TA98 and WP2 *uvrA*, no test substance concentration produced a mean 2 times greater than the mean of its respective negative control. For strains TA1535 and TA1537, no test substance concentration produced a mean 3 times greater than the mean of its respective negative control. There was no concentration-related increase in the mean revertants per plate in any strain

Controls: The positive and solvent controls fulfilled the requirements for a valid test.

Table B.6.4.1-1
Summary of average revertants/plate without activation

Compound	Conc. µg/plate	TA100		TA1535		TA1537		TA98		WP2 <i>uvrA</i>	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
DMSO	50 µL	143 ^a	101 ^b	27	16	4	7	21	18	18	16
Indoxacarb	2.5	135	ne	23	ne	8	ne	24	ne	16	ne
	7.5	152	ne	30	ne	6	ne	20	ne	10	ne
	25	143	ne	24	ne	6	ne	18	ne	16	ne
	75	155	106	29	16	11	6	17	19	14	15
	200	154	97	27	16	6	7	21	20	12	17
	600	135	111	23	16	9	9	24	16	16	13
	1800	148	102	25	16	8	7	16	20	12	15
	5000	140	99	28	15	7	6	18	17	13	15
SA	1	656	554	432	444	ne	ne	ne	ne	ne	ne
9-AA	75	ne	ne	ne	ne	498	505	ne	ne	ne	ne
2NF	1	ne	ne	ne	ne	ne	ne	139	192	ne	ne
MMS	1000	ne	ne	ne	ne	ne	ne	ne	ne	132	270

^a Average of 2 replicates per trial

^b Average of 3 replicates per trial

2NF = 2-nitrofluorene; SA = sodium azide; 9-AA = 9-aminoacridine; MMS = methyl methanesulfonate; ne = not evaluated

Table B.6.4.1-2
Summary of average revertants/plate with activation

Compound	Conc. µg/plate	TA100		TA1535		TA1537		TA98		WP2 <i>uvrA</i>	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
DMSO	50 µL	136 ^a	107 ^b	16	13	8	5	32	29	14	14
Indoxacarb	2.5	139	ne	16	ne	7	ne	25	ne	13	ne
	7.5	142	ne	14	ne	6	ne	31	ne	16	ne
	25	136	ne	17	ne	5	ne	38	ne	16	ne
	75	148	129	19	13	5	6	40	24	16	15
	200	148	122	15	13	9	4	33	30	13	13
	600	147	120	15	14	11	6	38	25	14	14
	1800	145	109	22	14	3	6	32	29	15	13
	5000	152	98	13	14	6	6	32	27	11	14
2AA	1	2001	703	173	337	195	172	1157	630	ne	ne
	10	ne	ne	ne	ne	ne	ne	ne	ne	953	504

^a Average of 2 replicates per trial.

^b Average of 3 replicates per trial.

2AA = 2-aminoanthracene; ne = not evaluated

III. CONCLUSION

Indoxacarb was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay.

Previous evaluation:	In DAR (2000)
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Report: Mathison, B.H. (1997): DPX-MP062 (Approximately 75% DPX-KN128, 25% DPX-KN127): Mutagenicity Testing in the *Salmonella typhimurium* and *Escherichia coli* Plate Incorporation Assay. DuPont Report No. HLR 831-96. Published: No.

Guidelines: EEC B.13 and B.14; OECD 471 and 472; US EPA 84-2; MAFF Japan 1985. Deviations: Treatment solutions or suspensions were not analysed for identity, composition, uniformity, or stability of the test and control articles. The procedures used by trained personnel to prepare the treatment solutions were intended to ensure that this did not affect the validity of the study.

GLP: Yes. Certified Laboratory: No (laboratories in the USA are not certified by any governmental agency but are subject to regular GLP inspections from the US EPA).

Materials and methods:

DPX-MP062 (purity 94.5%), was evaluated for mutagenicity in *Salmonella typhimurium* strains TA100, TA1535, TA97a, and TA98 and in *Escherichia coli* strain WP2uvrA(pKM101) with and without an exogenous metabolic activation system (S9). Nominal concentrations of 10, 50, 100, 250, 500, 1000, 2500, and 5000 µg/plate were evaluated in 2 trials (the 10 and 50 µg/plate concentrations were not conducted in the second trial). The test substance was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mg/ml. Positive indicators were 2-aminoanthracene (2AA), 2-nitrofluorene (2NF), sodium azide (NAAZ), ICR-191 Acridine (ICR-191), and methyl methanesulfonate (MMS). A test substance was classified as positive when the average number of revertants in any strain at any test substance concentration was at least 2 times greater than the negative control and occurred in a dose-response relationship.

Findings:

Findings from the mutagenicity test with DPX-MP062 in the *Salmonella typhimurium* and *Escherichia coli* plate incorporation assay are summarised in the following table.

Table B.6.4.1-3
Mutagenicity test with DPX-MP062: Summary of average revertants/plate

Average revertants/plate without activation											
Compound	Conc. µg/plate	TA100		TA1535		TA97		TA98		WP2uvrA	
		Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
DPX-MP062	0	123	175	13	12	96	87	35	29	188	146
	10	129	ne	12	ne	114	ne	31	ne	244	ne
	50	121	ne	9	ne	109	ne	36	ne	237	ne
	100	122	138	13	11	93	78	36	41	259	146
	250	121	163	14	13	110	80	30	32	236	165
	500	136	163	11	17	123	79	41	33	185	178
	1000	133	167	17	12	114	91	37	31	242	178
	2500	131	170	15	12	125	90	33	35	198	170
	5000	130	184	14	15	104	97	36	34	230	184
NAAZ	2	560	1498	450	440	ne	ne	ne	ne	ne	ne
2AA	1	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ICR-191	2	ne	ne	ne	ne	1361	1306	ne	ne	ne	ne
2NF	25	ne	ne	ne	ne	ne	ne	1334	1203	ne	ne
MMS	1000	ne	ne	ne	ne	ne	ne	ne	ne	1531	2290
Average Revertants/Plate with Activation											
DPX-MP062	0	139	153	10	7	113	102	34	36	196	163
	10	114	ne	10	ne	129	ne	29	ne	272	ne
	50	101	ne	9	ne	131	ne	33	ne	249	ne
	100	146	136	10	12	107	97	36	33	277	175
	250	132	151	9	13	135	87	35	32	252	167
	500	120	145	11	15	156	101	37	40	274	157
	1000	139	142	11	14	141	102	32	29	210	161
	2500	129	154	13	12	129	105	33	36	202	162
	5000	129	166	12	18	136	104	39	42	239	164
2AA	1	1340	2015	ne	ne	1231	625	ne	ne	ne	ne
	2	ne	ne	246	392	ne	ne	1907	1876	ne	ne
	250	ne	ne	ne	ne	ne	ne	ne	ne	1537	2039

Note: Average of 3 replicates per trial

2AA = 2-aminoanthracene; 2NF = 2-nitrofluorene; NAAZ = sodium azide; ICR-191= ICR-191 Acridine;

MMS = methyl methanesulfonate; ne = not evaluated

There was no evidence of toxicity by the test compound. The number of revertants at all concentrations of the test substance was similar to controls in studies both with and without activation.

Conclusion: DPX-MP062 was negative for mutagenic activity in the *in vitro* bacterial gene mutation assay.

CHO/HGPRT

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.4.1/02

Report: San, R.H.C., Clarke, J. (2003); Indoxacarb (DPX-KN128) technical: *In vitro* mammalian cell gene mutation test (CHO/HGPRT test)

DuPont Report No.: DuPont-13023

Guidelines: 12 NohSan No. 8147 (2000), EC Directive 2000/32/EC Annex 4E Number L 136, OECD 476 (1998), OPPTS 870.5300 (1998) **Deviations:** None

Testing Facility: BioReliance, Rockville, Maryland, USA

Testing Facility Report No.: AA78LT.782.BTL

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
Lot/Batch #: KN128-098
Purity: 95.47%
Description: White solid
CAS #: 173584-44-6
Stability of test compound: Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study.
2. Solvent: Dimethyl sulfoxide (DMSO)
Control materials
Negative (solvent) control: DMSO, 1 %
Positive, non-activation: Ethyl methanesulfonate (EMS) in DMSO at a final concentration of 0.2 µL/mL
Positive, activation: Benzo(a)pyrene (BaP) in DMSO at a final concentration of 4 µg/mL
3. Activation: Rat liver S9 from male Sprague-Dawley rats induced with Aroclor 1254
Lot number: Not applicable
Source: BioReliance, Rockville, Maryland
Protein content: Not specified
Characterisation: The metabolic activation ability of each bulk of S9 was characterised by its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.
S9 mix composition
Sodium phosphate buffer (pH 8.0): 50 mM
Glucose-6-phosphate: 5 mM
NADP: 4 mM
KCl: 30 mM
MgCl₂: 10 mM
Calcium chloride: 10 mM
S9 homogenate: 10% (v/v)
4. Test cells
Chinese Hamster Ovary cells (CHO-K1) were properly maintained, periodically checked for mycoplasma contamination, and periodically “cleansed” against high spontaneous background. Cells used in the mutation assay were within four subpassages from cleansing in order to assure karyotypic stability.

5. Culture medium

F12FBS5-Hx, Ham's F12 medium without hypoxanthine supplemented with 5% foetal bovine serum (FBS), 100 units penicillin/mL, 100 µg streptomycin/mL and 2 mM L-glutamine/mL.

6. Locus examined

Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) – the selection agent used was 10 µM 6-thioguanine (6TG, 2-amino-6-mercaptopurine).

7. Test compound concentrations used

Preliminary cytotoxicity

Trial 1: 0.5, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg indoxacarb/mL in duplicate in the presence and absence of S9 activation.

Mutagenesis assay

Trial 2: 5.0, 15, 25, 50, 75, and 100 µg indoxacarb/mL in duplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion:

05-August-2003 to 03-October-2003

2. Preliminary cytotoxicity assay

CHO cells were exposed for 5 hours to vehicle alone and nine concentrations of test substance ranging from 0.5 to 5000 µg/mL in both the absence and presence of S9-activation for evaluation of test substance effect on colony-forming efficiency (CE).

3. Mutagenesis assay

CHO cells were exposed for 5 hours to the vehicle alone, appropriate positive controls, and six concentrations of test substance in duplicates in both the absence and presence of S9-activation. After 5 hours, the cells were washed with Ca^{++} and Mg^{++} – free Hanks' balanced salt solution (CMF-HBSS) and cultured for an additional 18-24 hours. At this time, the cells were subcultured to assess cytotoxicity and to initiate the phenotypic expression period.

Cytotoxicity: The replicates from each treatment condition were detached using trypsin and subcultured in triplicate at a density of 100 cells/60 mm dish. After 7-10 days incubation, the colonies were rinsed with HBSS, fixed with methanol, stained with 10% aqueous Giemsa, counted, and cloning efficiency determined.

Phenotypic expression/selection: The replicates from each treatment condition were detached using trypsin and subcultured in duplicates at a density no greater than 10^6 cells/100 mm dish. Subculturing at 2-3 day intervals was employed for the 7-9 day expression period. For selection of the TG-resistant phenotype, the replicates from each treatment condition were trypsinised and replated, in quintuplicate, at a density of 2×10^5 cells/100 mm dish in medium containing 10 µM TG. For cloning efficiency determination at the time of selection, 100 cells/ 60 mm dish were plated in triplicate. After 7-10 days of incubation, the colonies were fixed, stained, and counted for both cloning efficiency and mutant selection.

4. Statistics

The data did not warrant statistical analysis.

5. Evaluation criteria

The test substance was considered to induce a positive response if there was a concentration-related increase in mutant frequencies with at least two consecutive doses showing mutant frequencies of >40 mutants per 10^6 clonable cells. If no culture exhibited a mutant frequency of >40 mutants per 10^6 clonable cells, the test substance was considered negative.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The analytical method is validated (see Volume 3B5). Indoxacarb was present at acceptable concentrations in the dosing solutions (within 7.4% of nominal concentrations). Indoxacarb was shown to be stable in the dosing solutions under the conditions of the study. Indoxacarb was not found in the 0 mg/mL samples.

B. PRELIMINARY CYTOTOXICITY ASSAY

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance was soluble in DMSO at a maximum concentration of 500 mg/mL. There was visible precipitate in the treatment medium at test substance concentrations ≥ 150 $\mu\text{g/mL}$. No visible precipitate was observed at concentrations ≤ 50 $\mu\text{g/mL}$ in the beginning and at the end of the treatment period. Cloning efficiency at 5000 $\mu\text{g/mL}$ was 43% without activation and 54% with activation. Based on the results of the toxicity test, the doses chosen for the mutagenesis assay ranged from 15-200 $\mu\text{g/mL}$ for both the non-activated and S9-activated cultures.

C. MUTAGENESIS ASSAY

The first trial of the assay was terminated due to contamination (data recorded but not reported). The assay was repeated using the concentrations of 5.0, 15, 25, 50, 75 and 100 $\mu\text{g/mL}$. In both the non-activated and S9-activated systems, cultures treated with concentrations of 5.0, 15, 25 and 50 $\mu\text{g/mL}$ were cloned for concurrent cytotoxicity. There was visible precipitate in the treatment medium at test substance concentrations ≥ 50 $\mu\text{g/mL}$. No visible precipitate was observed at concentrations ≤ 25 $\mu\text{g/mL}$ in the beginning and at the end of the treatment period. Cultures treated with the test substance at ≥ 75 $\mu\text{g/mL}$ were terminated before cloning due to precipitation. Relative cloning efficiency was 96% and 69% at 50 $\mu\text{g/mL}$, the highest dose tested, in the non-activated and S9-activated systems respectively. None of the treated cultures exhibited mutant frequencies of greater than 40 mutants per 10^6 clonable cells. The non-activated and S9-activated positive controls induced mutant frequencies that were at least three times that of the solvent control and exceeded 40 mutants per 10^6 clonable cells.

Controls: The positive and solvent controls fulfilled the requirements for a valid test.

Table B.6.4.1-4
***In vitro* mammalian gene mutation: Summary of mutagenicity findings**

Compound	Conc. $\mu\text{g/mL}$	Mutation frequency without activation ^a	Mutation frequency with activation ^a
		Trial 2	Trial 2

Indoxacarb	5.0	12.9	2.3
	15	3.4	6.3
	25	<0.7	0.8
	50	<0.8	6.3
DMSO	solvent	1.2	6.4
EMS	0.2 µL/mL	350.8	ne
B(a)P	4.0 µg/mL	ne	284.3

^a Mutants per 1×10^6 surviving cells defined as: total mutant colonies/(number of selection dishes x cloning efficiency $\times 2 \times 10^5$ cells) $\times 10^6$

EMS = ethyl methanesulfonate

B(a)P = benzo(a)pyrene

ne not evaluated

III. CONCLUSION

Indoxacarb was negative in the non-activated and S9-activated test systems in the CHO/HGPRT mutation assay.

Previous evaluation:	In DAR (2000)
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Report: San, R.H.C., Clarke, J.J. (1997); DPX-MP062 (approximately 75% DPX-KN128, 25% DPX-KN127): *In vitro* mammalian cell gene mutation test with an independent repeat assay

DuPont Report No.: HLO-1997-00030

Guidelines: 87/302/EEC, U.S. EPA 84-2, OECD 476

Deviations: The identity, strength, purity and composition or other characteristics to define the test or control article were not determined by the testing facility. Analyses to determine the uniformity, concentration, or stability of the test or control mixture were not performed by the testing facility. The stability of the test or control article under the test conditions was not determined by the testing facility. The procedure used by trained personnel to prepare the treatment solutions were intended to ensure that these deviations did not affect the validity of the study.

GLP: Yes. Certified Laboratory: No (laboratories in the USA are not certified by any governmental agency but are subject to regular GLP inspections from the US EPA).

Materials and methods:

DPX-MP062 technical (purity 94.5%, batch MP062-51A) was tested in the CHO/HGPRT mutation assay in the presence and absence of Aroclor-induced rat liver S9. Both an initial and an independent repeat assay were conducted. Following a preliminary toxicity assay, duplicate flasks of exponentially growing CHO-K₁-BH₄ cells were exposed for 5 hours at $37 \pm 1^\circ\text{C}$ to DPX-MP062 at concentrations of 3.1 to 250 µg/ml. Cells were then independently subcultured for assessment of cytotoxicity (cloning efficiency) and for expression and selection of the TG-resistant phenotype. The test substance was dissolved in DMSO vehicle at a maximum concentration of 100 mg/ml. Ethyl methanesulfonate and benzo(a)pyrene were used as positive controls for the non-activated and activated test systems, respectively. Toxicity was defined as a cloning efficiency of $\leq 50\%$ of the solvent controls. The assay was considered positive when a dose-dependent increase in mutation frequencies occurred with at least 2 consecutive doses having mutation frequencies of greater than 40 mutants per 10^6 clonable cells.

Findings:

The preliminary toxicity assay revealed a visible precipitate at 1000 µg/mL, and a cloudy treatment medium with no visible precipitate at 150, and 500 µg/mL. Concentrations ≤ 50 µg/mL were soluble in treatment medium. Selection of dose levels for the mutagenesis assay was based on the solubility limitations. Substantial toxicity, defined as cloning efficiency $\leq 50\%$ of the solvent control, was not observed at any dose level with or without S9 activation.

Findings from the *in vitro* mammalian cell gene mutation test for with DPX-MP062 are summarised in the following table. There was no evidence of toxicity and no positive responses in any of the assays at any of the concentrations of the test substance evaluated.

Table B.6.4.1-5
***In Vitro* Mammalian Gene Mutation with DPX-MP062: Summary of**
Mutagenicity Findings

Compound	µg/ml	Mutation Frequency without Activation ^a		Mutation Frequency with Activation ^a	
		Third assay ^b	Fourth assay ^b	First assay	Second assay
DPX-MP062	0	0.4	1.3	0.6	2.6
	3.1	2.6	6.5	ne	2.6
	6.3	6.8	9.4	ne	25.5
	12.5	<0.6 ^c	8.3	2.9	15.5
	25	11.3	10.8	13.3	12.3
	50	ne	ne	6.9	ne
	100	4.4 ⁺	6.4 ⁺	4.3	4.7
	250	7.1 ⁺	8.1*	<0.6 ^{c+}	16.9 ⁺
EMS	0.2	301.1	378.5	ne	ne
B(a)P	4.0	ne	ne	158	282.1

a mutants per 1×10^6 surviving cells defined as: total mutant colonies/(number of selection dishes x cloning efficiency x 2×10^5 cells) x 10^6

b The non-activated portions of both the first and second assays were repeated due to low cloning efficiency of solvent controls in the first assay, and loss of mutation plates due to dilution or readjustment errors in the second assay. These repeated assays are referenced in the study report as the third and fourth assays, respectively.

c Calculated on the basis of <1 mutant colony observed in the total number of dishes prepared.

+ Treatment medium cloudy with no visible precipitate

* Visible precipitate observed in treatment medium

ne = not evaluated; EMS = ethyl methanesulfonate; B(a)P = benzo(a)pyrene

Conclusion: DPX-MP062 was negative in the CHO/HGPRT mutation assay.

Chromosomal aberration

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.4.1/01

Report: Gudi, R., Rao, M. (2004); Indoxacarb (DPX-KN128) technical: *In vitro* mammalian chromosome aberration study in human peripheral blood lymphocytes

DuPont Report No.: DuPont-13022, Revision No. 1

Guidelines: 59 NohSan No. 4200 (1985), OPPTS 870.5375 (1998), EC Directive 2000/32/EC Annex 4D-L136, OECD 473 (1998) **Deviations:** None

Testing Facility: BioReliance, Rockville, Maryland, USA

Testing Facility Report No.: AA78LT.341.BTL

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
 Lot/Batch #: KN128-098
 Purity: 95.47%
 Description: White solid
 CAS #: 173584-44-6
 Stability of test compound: Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study.
- Solvent: Dimethyl sulfoxide (DMSO)
2. Control materials
 Negative (Solvent) control/final concentration: DMSO, 1%
 Positive, non-activation: Mitomycin C (MMC) in water at 0.30 and 0.60 µg/mL
 Positive, activation: Cyclophosphamide (CP) in water at 20 and 40 µg/mL
3. Activation: Rat liver S9 from Male Sprague-Dawley rats induced with Aroclor1254
 Lot number: Not applicable
 Source: BioReliance, Rockville, Maryland
 Protein content: Not specified
 Characterisation: The metabolic activation ability of each bulk of S9 was characterised by its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100
- S9 mix composition
 Glucose-6-phosphate: 1 mM
 NADP: 1 mM
 KCl: 6 mM
 MgCl₂: 2 mM
 S9 homogenate: 0.02% (v/v)
4. Test cells
 Human lymphocytes obtained from human venous blood from a normal, healthy donor.
5. Culture medium
 RPMI 1640, supplemented with 15% foetal bovine serum, 1% phytohaemagglutinin (to stimulate lymphocytes to divide), 1% penicillin and streptomycin, and 2 mM L-glutamine.
6. Test compound concentrations used in the chromosome aberration assay
 Non-activated 4-hour exposure group: 3.13, 6.25, 12.5, 25, 50, 75, and 100 µg indoxacarb/mL in duplicate in the absence of S9 activation.
 S9-activated 4-hour exposure group: 3.13, 6.25, 12.5, 25, 50, 75, and 100 µg indoxacarb/mL in duplicate in the presence of S9 activation.
 Non-activated 20-hour exposure group: 1.25, 2.5, 5, 10, 15, 20, and 30 µg indoxacarb/mL in duplicate in the absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
 06-August-2003 to 23-September-2003

2. Preliminary cytotoxicity assay

The toxicity test was performed for the purpose of selecting concentrations for the chromosome aberration assay and consisted of an evaluation of test substance effect on mitotic index (MI, number of dividing cells/500 cells counted). The cells were exposed to solvent alone and to concentrations of the test substance ranging from 0.5 to 5000 µg/mL (10 mM) for 4 hours in both the presence and absence of S9 activation and for 20 hours continuously in the absence of S9 activation.

3. Cytogenetic assay

Cell treatment: Cells were exposed to test compound, solvent, or positive control for 4 h or 20 h (non-activated) or 4 h (activated).

Spindle inhibition: Two hours prior to the scheduled cell harvest at approximately 20 h after treatment initiation, Colcemid® was added to the cell cultures at a final concentration of 0.1 µg/mL.

Cell harvest: Two hours after the addition of Colcemid®, metaphase cells were harvested for both the activated and non-activated studies by centrifugation at 1200 rpm for about 5 minutes. The cell pellet was resuspended in 5 mL of 0.075 M KCl and incubated at $37 \pm 1^\circ\text{C}$ for 20 minutes. At the end of the KCl treatment and immediately prior to centrifuging, the cells were gently mixed and approximately 0.5 mL of fixative (methanol:glacial acetic acid, 3:1 v/v) was added to each tube. The cells were collected by centrifugation, the supernatant aspirated, and the cells were fixed with two washes with approximately 3-5 mL of fixative and stored in fixative overnight or longer at approximately 2-8°C.

Slide preparation: The cells were collected by centrifugation and the supernatant aspirated, leaving 0.1 to 0.3 mL fixative above the cell pellet. An aliquot of the cell suspension was dropped onto a glass slide and allowed to air dry overnight. The dried slides were stained with 5% Giemsa, air dried, and permanently mounted.

Evaluation of metaphase cells: Metaphase cells with 46 centromeres were examined under oil immersion without prior knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase-spreads (100 per replicate treatment condition) were examined for chromatid-type and chromosome-type aberrations. Chromatid type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverised chromosome(s), pulverised cells, and severely damaged cells (≥ 10 aberrations) also were recorded. Chromatid gaps and isochromatid gaps were recorded but not included in the analysis. The mitotic index was recorded as the percentage of cells in mitosis per 500 cells counted. The percent polyploid and endoreduplicated cells were evaluated per 100 cells.

4. Statistics

Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test ($p \leq 0.05$). In the event of a positive Fisher's exact test at any test substance concentration, the Cochran-Armitage test was used to measure dose responsiveness.

5. Evaluation criteria

The test substance was considered to induce a positive response if the percentage of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the concurrent negative control group ($p \leq 0.05$) or if a reproducible and significant increase in the percentage of cells with aberrations occurred at a single dose level relative to concurrent negative controls. However, values that are statistically significant, but do not exceed the range of historical solvent controls, may be judged as not biologically significant. Test substances not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The analytical method is validated (see Volume 3B5). Indoxacarb was present at acceptable concentrations in the dosing solutions (within 9.6% of nominal concentrations). Indoxacarb was shown to be stable in the dosing solutions under the conditions of the study. Indoxacarb was not found in the 0 mg/mL samples.

B. PRELIMINARY CYTOTOXICITY ASSAY

DMSO was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance was soluble in DMSO at a maximum concentration of 500 mg/mL. Visible precipitate was observed in treatment medium at concentrations ≥ 150 $\mu\text{g/mL}$. Toxicity (mitotic inhibition) in excess of 50%, relative to the solvent control, was observed at concentrations ≥ 50 $\mu\text{g/mL}$ in the non-activated and S9 activated 4 h exposure groups and at concentrations ≥ 15 $\mu\text{g/mL}$ in the non-activated 20 h exposure group.

C. CHROMOSOME ABERRATION ASSAY

Non-activated 4 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 50 $\mu\text{g/mL}$, mitotic inhibition was 52%, relative to the solvent control. The percentage of cells with structural and numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control ($p > 0.05$, Fisher's exact test).

Activated 4 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 75 $\mu\text{g/mL}$, mitotic inhibition was 51%, relative to the solvent control. The percentage of cells with structural and numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control ($p > 0.05$, Fisher's exact test).

Non-activated 20 h exposure group: At the highest test concentration evaluated for chromosome aberrations, 20 $\mu\text{g/mL}$, mitotic inhibition was 53%, relative to the solvent control. The percentage of cells with structural and numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control ($p > 0.05$, Fisher's exact test).

Controls: The positive and solvent controls fulfilled the requirements for a valid test.

Table B.6.4.1-6
Summary of chromosome aberration data in Trial 2

Treatment	Conc. $\mu\text{g/mL}$	% of cells with structural aberrations:			% of cells with polyploidy:		
		S9+	S9-		S9+	S9-	
		4 h	4 h	20 h	4 h	4 h	20 h

DMSO	0	0	0	0	0	0	0
Indoxacarb	3.13	0	0	ne	0	0	ne
	5	ne	ne	0	ne	ne	0
	10	ne	ne	0	ne	ne	0
	12.5	0	0	ne	0	0	ne
	20	ne	ne	0.5	ne	ne	0
	50	ne	0	ne	ne	0	ne
	75	0	ne	ne	0	ne	ne
MMC	0.3	ne	ne	14.0 ^a	ne	ne	0
MMC	0.6	ne	8 ^a	ne	ne	0	ne
CP	20	16.0 ^a	ne	ne	0	ne	ne

^a Statistically significant ($p \leq 0.05$; Fisher's exact test)

MMC = mitomycin C; CP = cyclophosphamide; ne = not evaluated

III. CONCLUSIONS

Indoxacarb was negative for structural and numerical chromosome aberrations in the non-activated and S9-activated test systems in the *in vitro* test for clastogenicity in mammalian cells using human peripheral blood lymphocytes.

Previous evaluation:	In DAR (2000)
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Report: Gudi, R.; Schady, E.H. (1996a): DPX-MP062 (Approximately 75% DPX-KN128, 25% DPX-KN127): *In Vitro* Mammalian Cytogenetic Test Using Human Peripheral Lymphocytes. DuPont Report No. HLO 979-96. Published: No.

Guidelines: EEC B.10; OECD 473; US EPA 84-2.

Deviations: The identity, strength, purity and composition or other characteristics to define the test or control article were not determined by the testing facility. Analyses to determine the uniformity, concentration, or stability of the test or control mixture were not performed by the testing facility. The stability of the test or control article under the test conditions was not determined by the testing facility. The procedure used by trained personnel to prepare the treatment solutions were intended to ensure that these deviations did not affect the validity of the study.

GLP: Yes. Certified Laboratory: No (laboratories in the USA are not certified by any governmental agency but are subject to regular GLP inspections from the US EPA).

Materials and methods:

DPX-MP062 technical (purity 94.5%) was tested in the *in vitro* mammalian cytogenetic test using human peripheral blood lymphocytes (HPBL) both in the absence and presence of Aroclor-induced S9 metabolic activation. Based on the results of a preliminary toxicity assay, dose levels in the initial assay were 15.7, 31.3, 62.5, 125, 250, 500, 750, and 1000 µg/ml. Dose levels in an independent repeat assay were 125, 250, 500, 750, and 1000 µg/ml. For both trials, evaluations were necessary only at 250 µg/ml and above. The test substance was dissolved in dimethyl sulfoxide (DMSO) at a maximum concentration of 100 mg/ml. Mitomycin C and cyclophosphamide were used as the positive control for the non-activated and S9 activated studies, respectively. HPBL were treated for 4-hours, and metaphase cells (induced with Colcemid[®]) were harvested about 24 hours (24 and 48 hours in the repeat assay) following the initiation of treatment. Cells were evaluated for structural and numerical chromosome aberrations, as well as for toxicity (mitotic inhibition).

Findings:

Findings from the *in vitro* mammalian cytogenetic test for chromosome aberrations with DPX-MP062 are summarised in the following table.

Table B.6.4.1-7
Mammalian cytogenetic test with DPX-MP062: Summary of chromosome aberration data

Compound	Conc. µg/ml	Initial assay		Repeat Assay					
		% of cells with structural aberrations:		% of cells with structural aberrations:				% of cells with polyploidy:	
		S9-	S9+	S9-		S9+		S9-	S9+
		24 hr	24 hr	24 hr	48 hr	24 hr	48 hr	48 hr	48 hr
Untreated	-	0.0	0.0	0.0	0.0	0.0	2.0	1.0	0.0
DMSO	-	1.5	0.0	0.0	0.0	0.0	0.5	0.5	0.5
DPX-MP062	250.0	1.5	0.5	2.0	0.0	0.5	1.0	0.0	0.0
	500.0	1.5	0.5	1.5	0.0	0.0	1.5	0.5	0.5
	750.0	0.0	0.5	0.5	0.0	1.5	1.0	0.5	1.0
	1000.0	0.0	1.5	0.0	0.5	0.0	0.0	0.0	0.5
MMC	0.25	15.0*	ne	25.0*	6.5*	ne	ne	0.5	ne
CP	25.0	ne	25.5*	ne	ne	14.0*	14.5*	ne	2.5 [#]

MMC = mitomycin C; CP = cyclophosphamide; DMSO = dimethyl sulfoxide

statistically significant (p ≤0.05; Fisher's exact test)

* statistically significant (p ≤0.01; Fisher's exact test)

The positive, untreated, and solvent controls fulfilled the requirements for a valid test. In the initial chromosome aberration assay, toxicity (mitotic inhibition) was approximately 82% and 76% at 1000 µg/ml in the non-activated and S9-activated studies, respectively. In the independent repeat assay, toxicity was approximately 73% and 90% at 1000 µg/ml in the nonactivated studies at the 24- and 48-hour harvests, respectively. In the S9 activated repeat studies, toxicity was approximately 90% and 83% at 1000 µg/ml for the 24- and 48-hour harvests, respectively. No statistically significant increases in structural chromosome aberrations were observed in either trial at any of the concentrations evaluated. In addition no statistically significant increases in polyploidy (evaluated only at the 48-hour harvest time in the repeat assay) were observed.

Conclusion: DPX-MP062 technical was negative for chromosome aberrations in the nonactivated and S9-activated test systems in the *in vitro* mammalian cytogenetics test using human peripheral blood lymphocytes.

In vitro unscheduled DNA synthesis

Previous evaluation:	In DAR (2000)
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CA 5.4.1

Report: San, R.H.C., Sly, J.E. (1997a); DPX-MP062 (approximately 75% DPX-KN128, 25% DPX-KN127): Unscheduled DNA synthesis in mammalian cells *in vitro* with an independent repeat assay

DuPont Report No.: HLO-1997-00033

Guidelines: USEPA 84-4, OECD 482, Directive 87/302/EEC Part B, 59 Nohsan N0. 4200 **Deviations:** None

Testing Facility: Microbiological Associates, Inc., Rockville, Maryland, USA

Testing Facility Report No.: G96CF47.380001

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.5%
 Description: Off-white solid
 CAS #: None for DPX-MP062
 DPX-KN128 (active isomer): 173584-44-6
 Stability of test compound: Under the absence of visible evidence to the contrary, the test substance was assumed to be stable under the conditions of the study.
- Solvent: Dimethyl sulfoxide (DMSO)
2. Control materials: Dimethylbenza(a)anthracene (DMBA)

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 12-December-1996 to 21-April-1997
2. Methods
 DPX-MP062 technical (purity 94.5%) was tested in the Unscheduled DNA Synthesis (UDS) assay using primary cultures of rat hepatocytes. Based on the results of a preliminary toxicity assay, concentrations tested were 1.56, 3.13, 6.3, 12.5, 25, 50, 100, and 200 µg/mL. Evaluations for UDS were conducted at dose levels of 12.5 µg/mL and above. For both the initial and independent repeat assays, the test substance was dissolved in dimethyl sulfoxide (DMSO) at a concentration of approximately 67 mg/mL. Dimethylbenz(a)anthracene (DMBA) and DMSO served as positive and negative controls, respectively. Hepatocyte cultures were exposed to their respective concentrations of the test substance (or positive or negative control), along with 10 µCi 3H-thymidine/mL, for approximately 18-20 hours. Mean net nuclear grain counts and percent of cells in repair (cells with ≥5 net nuclear grains) were obtained for each group from autoradiography slides. Cytotoxicity was assessed based on lactate dehydrogenase (LDH) concentration in the culture medium and by microscopic examination of hepatocyte cultures and of fixed and stained cells.

II. RESULTS AND DISCUSSION

A. UDS ASSAYS

Microscopic examination of hepatocyte cultures at the termination of treatment indicated a low level of toxicity in the repeat (but not the initial) assay at 100 and 200 µg/mL and normal morphology at lower concentrations. However, examination of fixed and stained cells indicated that cells treated with all concentrations of the test substance could be evaluated for UDS. None of the concentrations of the test substance caused a significant increase in the mean net nuclear counts when compared to negative controls.

Table B.6.4.1-8
Summary of UDS assay with DPX-MP062

Treatment	Concentration	Mean net grains/nucleus ^a		% of cells in repair ^b	
		Initial assay	Repeat assay	Initial assay	Repeat assay

DMSO	10 µL/mL	-1.7 (2.1) ^c	-1.6 (2.5)	0	2
DPX-MP062	12.5 µg/mL	-1.8 (2.3)	-1.8 (1.8)	1	0
	25 µg/mL	-1.8 (1.9)	-1.8 (2.1)	0	0
	50 µg/mL	-1.4 (2.5)	-1.4 (3.5)	3	3
	100 µg/mL	-2.0 (1.7)	-1.4 (2.0)	0	2
	200 µg/mL	-1.7 (2.2)	-1.5 (2.2)	2	1
DMBA	10 µg/mL	14.3 (8.3) ^d	17.2 (7.8) ^d	91	93

^a Represents the grand mean of 3 slides per treatment; 50 nuclei per slide were counted

^b Cells in repair = those with 5 or more net nuclear grains

^c Standard deviation in parenthesis

^d Significant based on increase of ≥5 net nuclear grain counts relative to control

DMSO = dimethyl sulfoxide; DMBA = dimethylbenz(a)anthracene

III. CONCLUSION

DPX-MP062 did not cause a significant increase in unscheduled DNA synthesis and was concluded to be negative.

B.6.4.2. *In vivo* studies in somatic cells

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.4.2/01

Report: [REDACTED] (2003); Indoxacarb (DPX-KN128) technical: Mouse bone marrow micronucleus test

DuPont Report No.: DuPont-13021

Guidelines: EC Directive 2000/32/EC Annex 4C-B12 Number L 136, MAFF 59 Nohsan No. 4200 (1985), OECD 474 (1998), OPPTS 870.5395 (1998) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: DuPont-13021

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
 Lot/Batch #: KN128-098
 Purity: 95.47%
 Description: White solid
 CAS #: 173584-44-6
 Stability of test compound: The test substance was prepared homogenously, at the targeted concentrations, and stable when stored in the vehicle at room temperature during the study period.
- Solvent used: Polyethylene glycol (PEG)
2. Control materials
 Negative and solvent: Polyethylene glycol (PEG)
 Positive: Cyclophosphamide at 30 mg/kg
3. Test animals
 Species: Mouse
 Strain: Crl:CD-1[®] (ICR)BR
 Age at dosing: Approximately 7 weeks
 Weight at dosing: 26.032.4 g for males; 21.5-27.8 g for females
 Source: [REDACTED]
 Acclimation period: 6 days
 Diet: PMI[®] Nutrition International, LLC Certified Rodent LabDiet[®] (#5002), *ad libitum*.
 Water: Tap water, *ad libitum*
 Housing: Mice were housed one per cage in wire-mesh cages suspended above cage boards.
4. Environmental conditions
 Temperature: 18-26°C
 Humidity: 30-70%
 Air changes: Not reported
 Photoperiod: Alternating 12-hour light and dark cycles
5. Number of animals per dose
 Dose range-finding study: Three male animals
 Definitive micronucleus assay: Five/sex/dose/sacrifice time (low - and intermediate-dose)
 Seven/sex/sacrifice time (high-dose)
6. Test compound concentrations used
 Dose range-finding study: 2000 mg indoxacarb/kg
 Definitive micronucleus assay: 500, 1000, or 2000 mg indoxacarb/kg

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 29-July-2003 to 26-August-2003
2. Dose range-finding study
 Three male mice were administered 2000 mg indoxacarb/kg bw by oral gavage. The animals were observed for clinical signs of toxicity and mortality immediately after dosing and daily thereafter for two days (until approximately 48 hours post-dosing).

3. Definitive micronucleus assay

Each group of male and female mice (5/sex/dose/sacrifice time for low- and intermediate-doses; 7/sex/sacrifice time for the high-dose) was administered a single dose of 500, 1000, or 2000 mg indoxacarb/kg bw, or vehicle or positive control substances by oral gavage at a dose volume of 10 mL/kg bw. Approximately 24 and 48 hours after dosing, treated animals were sacrificed, and the bone marrow removed. Additional male and female animals were treated with the highest concentration to allow for unexpected mortality. The vehicle and positive control groups were administered a single dose by oral gavage the same day as groups receiving test-substance treatment. CP was administered at a concentration of 30 mg/kg body weight at a dose volume of 10 mL/kg. The positive control group was sampled approximately 24 hours after dosing.

Mice were weighed immediately before dose administration. Mice were observed after dose administration and throughout the course of the study for clinical signs of toxicity.

4. Preparation of bone marrow smears

Immediately after sacrifice at 24 or 48 hours after dose administration, femoral bone marrow was collected and smears were prepared. Bone marrow smears prepared from animals treated with the test substance at 500, 1000, and 2000 mg/kg at 24 hours post-dose and smears prepared from animals treated with the vehicle control and 2000 mg/kg at 48 hours post-dose were stained with acridine orange stain and microscopically evaluated for the presence of micronucleated polychromatic erythrocytes (MPCEs), biomarkers of test substance genotoxic potential.

5. Slide evaluation

Coded slides were scored for the presence of micronuclei in 2000 polychromatic erythrocytes (PCEs) per animal using fluorescent microscopy. Cells containing more than one micronucleus were scored as a single MPCE. The number of PCEs per 1000 erythrocytes, and ratio of PCEs to normochromatic erythrocyte (NCEs) were also recorded to assess bone marrow toxicity.

6. Statistical methods

Data for the proportion of PCEs among 2000 polychromatic erythrocytes and the proportion of PCEs among 1000 erythrocytes (MNPCE and PCE frequency, respectively) were transformed prior to analysis using an arcsine square root or Freeman-Tukey function. This transformation was appropriate for proportions since the distribution of the transformed data more closely approximates a normal distribution than does the nontransformed proportion. Transformed data were analysed separately for normality of distribution and equal variance using the Shapiro-Wilk and Levene's tests, respectively.

For those data that were normally distributed and had equal variance, parametric statistics (*e.g.*, analysis of variance (ANOVA) and Dunnett's test) were performed using the transformed data. For those data that were normally distributed but had unequal variance, a robust ANOVA and unequal variance Dunnett's test was done. For those data that were not normally distributed, nonparametric statistics (*e.g.*, Kruskal-Wallis and Dunn's test) utilizing non-transformed data were performed. The individual animal was considered the experimental unit. All data analyses were one-tailed and conducted at a significance level of 5%.

No statistics were conducted on body weights or clinical signs.

7. Evaluation criteria

The test substance would have been considered to induce a positive response if the incidence of MPCEs at one or more doses was statistically elevated relative to the vehicle control ($p \leq 0.05$, binomial distribution, Kastenbaum-Bowman Tables). The test substance was judged negative if no statistically significant increase in the incidence of MPCEs in the test substance groups relative to the concurrent negative (vehicle) groups was observed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The analytical method is validated (see Volume 3B5). Indoxacarb was present at acceptable concentrations in the dosing solutions (within 4% of nominal concentrations). Indoxacarb was shown to be stable in the dosing solutions under the conditions of the study. Indoxacarb was not found in the 0 mg/mL samples.

B. DOSE RANGE-FINDING STUDY

The test substance vehicle (negative) control was polyethylene glycol. Three male mice were administered 2000 mg indoxacarb/kg bw by oral gavage and observed for approximately 48 hours. No mortality was observed. Clinical signs of toxicity were observed in all three animals. Approximately 1 hour post dosing, lethargy (1/3), tremors (1/3), and hyperreactivity (1/3) were observed. The clinical signs observed at approximately 3-5 hours post dose were lethargy (2/3) and spasms (1/3). Approximately 24 hours after the initial dose, lethargy (1/3) and spasms (1/3) persisted. By 48 hours, no adverse signs of clinical toxicity could be observed. An average weight loss of approximately 15% was recorded. On the basis of these tests, the doses for the definitive micronucleus assay were 500, 1000, and 2000 mg/kg.

C. DEFINITIVE MICRONUCLEUS ASSAY

1. Toxicity

No mortality was observed for any of the treatment groups. Clinical signs of toxicity were observed in both male and female mice. At the 2000 mg/kg dose group, the observations included prostration (1/14 male and 4/14 females), head tilt (7/14 males and 4/14 females), tremors (5/14 males and 1/14 female), spasm (1/14 male and 2/14 females), and lethargy (6/14 males and 4/14 females). In addition, male animals expressed signs of toxicity as ruffled fur (2/14), abnormal gait (1/14), convulsions (1/14), and stained fur/skin (1/14) and females exhibited splayed limb (1/14), abnormal posture (1/14), and hyperreactivity (2/14). For the 1000 mg/kg dose group, lethargy was observed (4/10 males and 4/10 females). In addition, male animals exhibited tremors (1/10), and female animals exhibited head tilt (1/10) and abnormal gait (1/10). At 500 mg/kg, male animals exhibited tremors (1/10), spasm (2/10), hyperreactivity (1/10), and hyperactivity (1/10). Females exhibited head tilt (1/10), abnormal posture (1/10), spasm (2/10), lethargy (4/10), and hyperreactivity (2/10).

Body weight losses were observed in both male and female mice. The largest reductions in weight, approximately 15%, were observed at 2000 mg/kg in both male and female mice. Smaller reductions in body weight were observed in males and females at the other dose levels, with the exception of male mice at 1000 mg/kg (no weight loss was noted for this group). The weight reductions were considered test substance-related and represent biologically significant signs of systemic toxicity.

2. PCE ratio

Small, but statistically non-significant depressions in the frequency of PCEs and the PCE/NCE ratio were detected at the 24-hour time point in male mice at the 2000 mg indoxacarb/kg and in female mice at all three test substance dose levels. A statistically significant depression in the PCE and PCE/NCE ratio was observed in both male and female mice administered 2000 mg indoxacarb/kg and sacrificed at the 48-hour time point. Although within the historical laboratory negative control range, the observed decreases may be indicative of bone marrow toxicity.

3. Micronucleated polychromatic erythrocytes

There were no statistically significant increases in MPCE frequency in male or female mice administered indoxacarb. The positive and vehicle controls fulfilled the requirements for a valid test.

Table B.6.4.2-1
Summary of micronucleus results, PCE frequency

Time (h)	Sex	Number of animals	Mean PCE/total erythrocyte ratio \pm standard deviation				
			Vehicle control 0 mg/kg	Low dose 500 mg/kg	Mid dose 1000 mg/kg	High dose ^a 2000 mg/kg	Positive control 30 mg/kg CP
24	Male	5	530 \pm 137	518 \pm 133	573 \pm 102	483 \pm 148	571 \pm 102
24	Female	5	677 \pm 51	588 \pm 162	611 \pm 81	611 \pm 133	631 \pm 73
48	Male	5	622 \pm 62	^b	^b	429 \pm 94 ^d	^c
48	Female	5	610 \pm 16	^b	^b	518 \pm 70 ^d	^c

^a Group contained seven animals per sex per time point.

^b Group not evaluated at this time point.

^c Group not included at this time point.

^d Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test

CP = Cyclophosphamide

Vehicle control = polyethylene glycol

Table B.6.4.2-2
Summary of micronucleus results, MPCE frequency

Time (h)	Sex	Number of animals	Mean MPCE/2000 PCE \pm standard deviation				
			Vehicle control 0 mg/kg	Low dose 500 mg/kg	Mid dose 1000 mg/kg	High dose ^a 2000 mg/kg	Positive control 30 mg/kg CP
24	Male	5	1.6 \pm 1.5	1.6 \pm 1.5	1.0 \pm 1.4	2.2 \pm 0.8	35.2 \pm 14.5 ^d
24	Female	5	2.2 \pm 1.5	2.6 \pm 0.9	1.4 \pm 1.5	1.0 \pm 0.7	29.2 \pm 6.1 ^d
48	Male	5	3.2 \pm 1.3	^b	^b	1.4 \pm 0.5	^c
48	Female	5	2.0 \pm 1.9	^b	^b	1.6 \pm 0.9	^c

^a Group contained seven animals per sex at this time point.

^b Group not evaluated at this time point.

^c Group not included at this time point.

^d Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test

CP = Cyclophosphamide

Vehicle control = polyethylene glycol

III. CONCLUSION

All criteria for a valid study were met. Under the conditions of the study conducted as described in this report, a single oral administration of indoxacarb at doses up to and including 2000 mg/kg did not induce a significant increase in the incidence of MPCE in bone marrow. Therefore, indoxacarb was concluded to be negative in the micronucleus test using male and female Crl:CD-1[®] mice.

Previous evaluation:	In DAR (2000)
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Report: [REDACTED] (1997): DPX-MP062 Technical (Approximately 75% DPX-KN128, 25% DPX-KN127): Mouse Bone Marrow Micronucleus Assay. DuPont Report No. HLR 1046-96. Published: No.

Guidelines: EEC B.12; OECD 474; US EPA 84-2; MAFF Japan 1985. Deviations: Treatment solutions/suspensions were not analysed for composition, concentration, uniformity, or stability of the test and control articles. The procedures used by trained personnel to prepare the treatment solutions/suspensions were intended to ensure that this did not affect the validity of the study.

GLP: Yes. Certified Laboratory: No (laboratories in the USA are not certified by any governmental agency but are subject to regular GLP inspections from the US EPA).

Materials and methods:

DPX-MP062 technical (purity 94.5%), was evaluated for its ability to induce micronucleated polychromatic erythrocytes (MNPCEs) in the bone marrow of Crl:CD[®]-1(ICR)BR mice (5-6 mice/sex/group at each of 3 time points). Male mice received single doses of 0, 3000, or 4000 mg/kg by oral intubation. Doses in females were 0, 1000, or 2000 mg/kg. The test substance was suspended in corn oil at a concentration of 200 mg/ml. Cyclophosphamide (40 mg/kg) was used as the positive control. In-life observations included clinical signs and body weight determinations. Bone marrow smears were prepared approximately 24, 48, and 72 hours after dosing. Two thousand PCEs per animal were scored for micronuclei. The number of PCEs per 1000 erythrocytes was also recorded to assess toxic effects on the bone marrow. Mice evaluated for micronuclei included those administered 4000 mg/kg (males) and 2000 mg/kg (females), sacrificed 24, 48, or 72 hr after dosing, and those administered 3000 mg/kg (males) and 1000 mg/kg (females), sacrificed 24 hr after dosing.

Findings:

The frequencies of MNPCEs in the treated, control, and positive control groups are summarised in the following table.

Table B.6.4.2-3
Summary of micronucleus findings with DPX-MP062

DPX-MP062 Dose (mg/kg)	Sample Time (hr)	Sex	N	%MNPCE Mean (SEM)
0	24	M	5	0.25 (0.02)
0	24	F	5	0.17 (0.05)
4000	24	M	5	0.38 (0.05)
2000	24	F	5	0.11 (0.04)
3000	24	M	5	0.02 (0.01)
1000	24	F	5	0.05 (0.03)
0	48	M	5	0.29 (0.05)
0	48	F	5	0.08 (0.01)
4000	48	M	5	0.51 (0.14)
2000	48	F	5	0.13 (0.05)
0	72	M	5	0.16 (0.05)
0	72	F	4	0.05 (0.03)
4000	72	M	5	0.20 (0.06)
2000	72	F	5	0.11 (0.05)
CP, 40	24	M	5	2.00 (0.85) ^{a*}
CP, 40	24	F	5	1.99 (0.14)*

^a Median value and interquartile range reported

* Significant difference from control, alpha = 0.05

MNPCE = micronucleated polychromatic erythrocyte; SEM = standard error of mean;

CP, 40 = 40 mg/kg cyclophosphamide; N = number of animals

Clinical signs in all dosed groups of male and female mice included convulsions, ataxia, tremors, vocalisation, lethargy, ruffled fur, salivation, and abnormal gait. Signs were noted 2 hours post-dosing and most were absent by the 72-hour observation period. Approximately 24 hr post-dosing, 1 male from the 3000 mg/kg was found dead. Statistically significant mean body weight losses were observed at the 24, 48, and 72 hr time points in

male mice administered 3000 and 4000 mg/kg of DPX-MP062. There were no mean weight changes in female mice treated with the test substance.

There were no statistically significant increases in MNPCE frequency in male or female mice administered DPX-MP062. In addition, no statistically significant depressions in the proportion of PCEs among 1000 erythrocytes were observed.

No direct proof of exposure of the bone marrow was observed in this study. Nevertheless, taking into account the ADME data on DPX-MP062 and its distribution in plasma, red blood cells, whole blood and bone marrow, it can be expected that the bone marrow was exposed during this *in vivo* micronucleus study.

Conclusion: The test substance, DPX-MP062, did not induce an increase in micronuclei in bone marrow cells of mice; the material is considered negative in this *in vivo* assay.

B.6.4.3. In vivo studies in germ cells

Since indoxacarb (DPX-KN128) and DPX-MP062 were negative in all *in vitro* and *in vivo* studies, the test in germ cells is not needed.

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

The chronic toxicity and/or carcinogenicity of DPX-JW062 (50:50) were evaluated in rats and mice. These studies were already available in the first DAR (2000). According to the applicant, since DPX-JW062 (50:50) was not carcinogenic in rats or mice, and DPX-MP062 (75:25) and DPX-KN128 (99:1) technical actives were not genotoxic, and the primary metabolites IN-JT333 and IN-KG433 were also not genotoxic, chronic studies were not repeated with DPX-MP062 (75:25) or DPX-KN128 (99:1) technical actives.

Table B.6.5-1
Summary of long-term toxicity studies for indoxacarb (DPX-JW062)

Type of study	Dose range tested	NOAEL		LOAEL		Target organ(s) and effects	Reference
		ppm	mg/kg/d	ppm	mg/kg/d		
Oral (Feeding), 2 - year Rat DPX-JW062 (50:50)	M: 0, 20, 40, 60, 125, 250 ppm – 0, 0.798, 1.59, 2.40, 5.03, 10.0 mg/kg bw/d F: 0, 10, 20, 40, 60, 125 ppm – 0, 0.554, 1.04, 2.13, 3.60, 7.83 mg/kg bw/d	M: 40 F: 20	M: 1.59 F: 1.04	M: 60 F: 40	M: 2.40 F: 2.13	At LOAELs: haemolytic effects on circulating RBC and histopathological effects in the liver and spleen At higher doses: decreased body weights, body weight gains, histopathological findings in the kidney and bone marrow, increased spleen weights No carcinogenic potential.	1997a HLR 1174-96, Revision No. 1

Oral (Feeding), 18-month Mouse DPX-JW062 (50:50)	M: 0, 20, 100, 200/150/125 – 0, 2.63, 13.8, 32.2/22.7/17.0 mg/kg bw/d F: 0, 20, 100, 200/150/125 ppm – 0, 3.99, 20.3, 44.1/31.4/23.7 mg/kg bw/d	M: 20 F: 20	M: 2.63 F: 3.99	M: 100 F: 100	M: 13.8 F: 20.3	At LOAELs: decreased body weights, body weight gains and food efficiency, clinical signs indicative of neurotoxicity At higher doses: mortality, histopathological findings in heart (myocardial necrosis and haemorrhage in males) and brain (neuronal degeneration/necrosis in males and females) No carcinogenic potential.	1997 HLR 799- 96
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DPX-JW062 (50:50) did not elicit an oncogenic response in rats or mice at any dose level tested.

According to the co-RMS (ES), “*The toxicokinetics and overall metabolism of DPX MP062 and DPX JW062 showed quantitative differences; moreover, according to the available data DPX-MP062 (75:25) seems to be more absorbed in female rats than DPX-JW062 (50:50). From a theoretical point of view, the greater amount of the S-isomer in DPX-KN128 would lead to an increase of metabolite IN-JT333 and consequently prolonged and more markedly effects in female rats could be expected. In view of the available data for long-term and carcinogenesis (there are only studies with the 50:50 proportion of the enantiomers) the carcinogenic potential of indoxacarb can't totally excluded given the higher proportion of S-enantiomer in DPX-KN128.*”

The RMS acknowledged that carcinogenicity studies were only performed with the racemic mixture DPX-JW062 (50:50). Nevertheless, given that:

- no neoplastic lesions were observed in the rat and mouse long-term studies with DPX-JW062 (50:50);
- DPX-KN128 (99:1) and DPX-MP062 (75:25) did not show genotoxic potential;
- the metabolite IN-JT333 did not show genotoxic potential;
- DPX-JW062 (50:50), DPX-MP062 (75:25) and DPX-KN128 (99:1) showed similar adverse effects at similar range of dose levels. No additional target organ was identified with the pure enantiomer DPX-KN128;

it is considered unlikely that DPX-KN128 would be a carcinogenic substance.

In rats, test substance-related effects comprised haematological effects indicative of haemolytic anaemia, i.e., decreases in RBC counts, Hb and Ht, accompanied with indications of increased hematopoiesis (spleen), increased haemosiderin accumulation (liver, spleen, kidneys), splenic congestion, increased spleen weights and bone marrow hyperplasia. The decreases in RBC, Hb, and Ht were generally greater than 10% of the control values in the 40, 60 and 125 ppm female groups during the first 18 months of the study. In males, decreases of red blood cell mass parameters were more pronounced at 125 and 250 ppm the first 6 months of treatment. After 24-months mean RBC, Hb and Ht were comparable with or higher than control values.

Increased mortality was seen in females of the top dose group (125 ppm equivalent to 7.83 mg/kg bw/d) prior to interim sacrifice. Decedent females showed bone marrow atrophy, thymic necrosis and splenic lymphoid depletion. Decreased body weights and body weight gains were observed from 60 ppm in females and 125 ppm in males.

The NOAEL of this study is set at 20 ppm in females (1.04 mg/kg bw/d) and 40 ppm in males (1.59 mg/kg bw/d).

In mice, effects at 100 ppm (13.8 mg/kg bw/d in males and 20.3 mg/kg bw/d in females) and above comprised decreased body weight, body weight gain, and food efficiency, and clinical signs suggestive of neurotoxicity (abnormal gait and/or mobility, and tilt head) in male and female mice. Effects at the top dose level included increased mortality and histopathological findings in heart (myocardial necrosis and haemorrhage in males) and brain (neuronal degeneration/necrosis in males and females). No substance-related effects were seen at 20 ppm (equal to 2.63 mg/kg bw/day in males and 3.99 mg/kg bw/day in females).

Based on the finding of myocardial necrosis observed in this long-term mouse study from ≥ 17 mg/kg bw/d, a classification as STOT-RE was adopted by the ECHA Committee for Risk Assessment (2011).

Previous evaluation:	HLR 1174-96 : In DAR (2000) HLR 1174-96 Revision No.1 : Submitted for the purpose of renewal (see reasons for revisions below)
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CA 5.5/01

Report: [REDACTED] (1997a); Combined chronic toxicity/oncogenicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) two-year feeding study in rats

DuPont Report No.: HLR 1174-96, Revision No. 1

Guidelines: OECD 453, USEPA 83-5, 59 Nohsan No. 4200, Directive 87/302/EEC

Deviations: Absence of weekly assessment of detailed clinical observations in an open field arena. However, reconducting the study is unlikely to produce a different result since the careful observations conducted at the cage-site would have detected any gait abnormalities.

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 1174-96, Revision No. 1

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

Reason for Revision No. 1:

1. Clarification of nomenclature regarding the distinction between the insecticidally active enantiomer DPX-KN128, the insecticidally inactive enantiomer, and the racemic mixture of enantiomers.
2. Discussions of hematology and microscopic findings were updated following re-evaluation.
3. Statements regarding results from 90-day studies that were revised due to re-assessment of the hematology and microscopic findings in these studies.
4. Addition of literature citations regarding the re-assessment of hematology and microscopic findings.
5. Addition of historical control data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062 technical
 Lot/Batch #: JW062-106
 Purity: 95.03%
 Description: Off-white solid
 CAS #: 144171-61-9
 Stability of test compound: Analyses confirmed that test material was stable in feed for at least 14 days at room temperature or refrigerated, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.
2. Vehicle and/or positive control: Test item was mixed with acetone and then added to feed. Untreated diet was mixed with acetone.
3. Test animals
 Species: Rat
 Strain: CrI:CD[®](SD)BR
 Age at dosing: Approximately 49 days old
 Weight at dosing: 262.4–264.2 g for males; 168.0–170.3 g for females
 Source: XX
 Acclimation period: 20 days
 Diet: Purina[®] Certified Rodent Chow[®] (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
 Water: Tap water, *ad libitum*
 Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
 Temperature: 23 ± 2°C
 Humidity: 50 ± 10%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 17-October-1994 to 22-October-1996
2. Methods
 In a 2-year chronic toxicity and oncogenicity feeding study, DPX-JW062 (purity 95.03%) was administered to male and female CrI:CD[®](SD)BR rats (approximately 72 rats/sex/concentration). Dietary concentrations in male rats were 0, 20, 40, 60, 125, or 250 ppm; female rats were fed dietary concentrations of 0, 10, 20, 40, 60, or 125 ppm. Ten rats per group were sacrificed after approximately 1 year of study and all surviving rats were sacrificed after approximately 2 years of study. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, clinical pathology, ophthalmology, organ weights, and gross and microscopic pathology.

II. RESULTS AND DISCUSSION

A. FINDINGS

Findings from the 2-year feeding study in rats with DPX-JW062 are summarised in the following table.

Table B.6.5-1
Summary of results of the 2-year feeding study in rats with DPX-JW062

Dosage	<u>Male</u>						
	Dietary conc.:	0	20	40	60	125	250 ppm
	Mean daily intake:	0	0.798	1.59	2.40	5.03	10.0 mg/kg/day
	<u>Female</u>						
	Dietary conc.:	0	10	20	40	60	125 ppm
	Mean daily intake:	0	0.554	1.04	2.13	3.60	7.83 mg/kg/day
Analytical	The test substance was distributed homogeneously and was stable in the diet. Measured concentrations of DPX-JW062 were from 89.3 to 106.4% of nominal for all concentrations throughout the study.						
	Dose Group (ppm)	Results					
General observations	nad ^a						
Mortality	125 f ^b :	deaths of undetermined cause increased during first year					
Body weight	≥125 m:	decreased ^c (11–14%)					
	≥60 f:	decreased (16–18%)					
Body weight gain	≥125 m:	decreased (17–22%)					
	≥60 f:	decreased (23–28%)					
Food consumption	≥125 m:	decreased (6–10%)					
	≥60 f:	transient decrease (especially Weeks 0–2 of the study)					
Food efficiency	250 m:	transient decrease (especially Week 1 of the study)					
	≥60 f:	transient decrease (11–38% over first year)					
Ophthalmology	nad						
Haematology	≥40 f:	Mild haemolytic anaemia (through 18 months, not at 24 months) Evidence of regeneration: MCV transiently increased, reticulocytes transiently increased					
Clinical chemistry	nad						
Urinalysis	nad						
Gross pathology	nad						
Organ weights	125 m, 60 f:	spleen weights increased					
Histopathology		Changes secondary to haemolytic effects:					
	≥60 m, ≥60 f:	haemosiderin in spleen increased					
	≥250 m, ≥40 f:	haemosiderin in liver increased					
	125 f:	haemosiderin in kidney (not present at 1 year)					
	≥125 m:	haemopoiesis in spleen increased (at 1 year only)					
	250 m, 125 f:	haemopoiesis in bone marrow increased (at 1 year only)					
	≥60 m, f	splenic congestion (at 1 year only except 250 ppm males)					
		Changes limited to early death animals:					
	125 f:	bone marrow atrophy, thymic necrosis and splenic lymphoid depletion					

^a nad = no abnormalities detected that were considered test substance-related and/or biologically adverse

^b f = female; m = male

^c All increases or decreases are relative to controls unless otherwise noted.

B. OBSERVATIONS

1. Clinical signs of toxicity

In female rats, a statistically significant increase in alopecia was present in the 125 ppm group (35/74) compared to controls (16/72). In the absence of other clinical or histopathological evidence of dermatotoxicity, this finding is not considered to be biologically adverse.

2. Mortality

No statistically significant differences in survival between treated groups relative to controls were present for either sex. Overall survival in males was 37%, 44%, 32%, 28%, 37% and 48% in the 0, 20, 40, 60, 125 and 250 ppm groups respectively. In females, survival was 36%, 33%, 35%, 46%, 39% and 43% in the 0, 10, 20, 40, 60 and 125 ppm groups respectively.

However, although there were no effects on overall survival rates, a small but statistically significant increase in deaths of undetermined cause was present in 125 ppm females during the first year of the study (incidences of 0/72, 1/72, 0/71, 0/72, 1/72 and 7/73 in the 0, 10, 20, 40, 60 and 125 ppm groups respectively). These undetermined causes of death were likely test substance related based on their increased incidence relative to controls. These deaths were associated with the microscopic findings of bone marrow atrophy, splenic lymphoid depletion and thymic necrosis.

C. BODY WEIGHT AND BODY WEIGHT GAIN

Statistically and/or biologically significant test substance-related decreases in body weight and body weight gain relative to controls were present in male rats at 125 and 250 ppm, and in female rats at 60 and 125 ppm. These test substance-related effects on body weight and nutritional parameters were generally limited to, or were most severe when evaluated over the first year of the study. The decreases in body weight and body weight gain were correlative with decreases in food consumption and, except in 125 ppm males, with decreases in food efficiency.

Transient statistically significant changes in mean body weight were observed in female rats at 40 ppm during the first 6 months of the study. Nevertheless, the changes were small (around 5%) and were not associated with statistically significant changes over the entire study period. Therefore, they were not considered to be test substance related.

D. HAEMATOLOGY

In females, at each sampling time during the first 18 months of the study the group means for the indicators of circulating red blood cell mass (red blood cell count, haemoglobin concentration, haematocrit) were decreased. The decreases were generally greater than 10% of the control values in the 40, 60 and 125 ppm groups. In males, decreases of red blood cell mass parameters were more pronounced at 125 and 250 ppm the first 6 months of treatment.

There was some haematologic evidence that the anaemia had elicited a bone marrow regenerative response based on increased reticulocyte counts and erythrocyte macrocytosis. The presence of a regenerative anaemia (along with secondary histologic changes, such as increased iron storage and haemopoiesis in the spleen) indicates that the anaemia was due to haemolysis.

By the 24-month sampling time, changes in group means for all indicators of red cell mass were similar to controls and not statistically significant despite continued exposure to the test compound. The absence of red cell effects at 24 months, as well as the presence of a regenerative response, confirm the reversible nature of the test-substance-induced haematologic changes.

Table B.6.5-2
2-Year feeding study in rats with DPX-JW062: Summary of changes in the indicators of circulating erythrocyte mass in female rats

Concentration (ppm)	RBC		HB		HT	
	(RBC x 10 ⁶ /μL)	% Change	(g/dL)	% Change	(%)	% Change
3 months						
0	7.62		15.4		45	
10	7.16	-6%	14.7 ^a	-5%	43	-4%
20	7.21	-5%	14.6 ^a	-5%	43	-4%
40	7.16	-6%	14.6 ^a	-5%	44	-2%
60	6.82 ^a	-10%	14.3 ^a	-7%	43 ^a	-4%
125	6.52 ^a	-14%	13.8 ^a	-10%	41 ^a	-9%
6 months						
0	8.21		16.3		50	
10	7.55 ^a	-8%	15.2 ^a	-7%	46 ^a	-8%
20	7.67	-7%	15.0 ^a	-8%	47	-6%
40	7.03 ^a	-14%	14.1 ^a	-13%	43 ^a	-14%
60	6.66 ^a	-19%	13.6 ^a	-17%	42 ^a	-16%
125	6.50 ^a	-21%	13.5 ^a	-17%	42 ^a	-16%
12 months						
0	7.59		16.0		47	
10	7.25	-4%	15.2 ^a	-5%	46	-2%
20	7.23	-5%	15.0 ^a	-6%	45	-4%
40	6.83 ^a	-10%	14.8 ^a	-8%	45	-4%
60	6.71 ^a	-12%	14.4 ^a	-10%	43 ^a	-9%
125	6.40 ^a	-16%	13.8 ^a	-14%	42 ^a	-11%
18 months						
0	7.27		15.3		47	
10	6.65	-8%	14.0 ^a	-8%	43	-8%
20	7.01	-4%	14.6	-5%	45	-4%
40	6.86	-6%	14.1 ^a	-8%	43	-8%
60	6.68	-8%	13.9 ^a	-9%	43	-8%
125	6.34 ^a	-13%	13.4 ^a	-12%	42	-11%
24 months						
0	6.42		13.6		40	
10	5.97	-7%	12.9	-5%	37	-8%
20	6.86	7%	14.7	8%	43	8%
40	6.46	1%	13.7	1%	41	3%
60	6.30	-2%	13.7	1%	41	3%
125	6.63	3%	14.0	3%	44	10%

^a Statistically significant difference from control at p < 0.05.

Table B.6.5-3
2-Year feeding study in rats with DPX-JW062: Summary of changes in the indicators of
circulating erythrocyte mass in male rats

Concentration (ppm)	RBC		HB		HT	
	(RBC x 10 ⁶ /μL)	% Change	(g/dL)	% Change	(%)	% Change
3 months						
0	8.37		16.1		48	
20	8.11	-3%	15.0 ^a	-7%	45 ^a	-6%
40	8.34	0%	15.3 ^a	-5%	46	-4%
60	7.85 ^a	-6%	14.8 ^a	-8%	45 ^a	-6%
125	7.69 ^a	-8%	14.4 ^a	-11%	44 ^a	-8%
250	7.45 ^a	-11%	14.7 ^a	-9%	44 ^a	-8%
6 months						
0	8.38		15.4		46	
20	8.38	0%	15.1	-2%	45	-2%
40	8.45	1%	15.3	-1%	47	2%
60	7.96	-5%	14.9	-3%	45	-2%
125	7.72 ^a	-8%	14.4 ^a	-6%	44	-4%
250	7.33 ^a	-13%	13.9 ^a	-10%	42 ^a	-9%
12 months						
0	8.04		15.4		46	
20	8.32	3%	15.5	1%	47	2%
40	7.98	-1%	15.4	0%	46	0%
60	7.93	-1%	15.9	3%	48	4%
125	7.60	-5%	14.7	-5%	45	-2%
250	7.34	-9%	14.5	-6%	45	-2%
18 months						
0	6.33		12.5		38	
20	6.82	8%	13.0	4%	40	5%
40	7.60 ^a	20%	13.9	11%	44 ^a	16%
60	7.08	12%	13.8	10%	43	13%
125	7.12	12%	14.1	13%	44 ^a	16%
250	7.11	12%	13.6	9%	43	13%
24 months						
0	6.43		13.0		38	
20	6.28	-2%	12.6	-3%	37	-3%
40	6.63	3%	13.3	2%	38	0%
60	7.05	10%	13.9	7%	41	8%
125	6.49	1%	12.8	-1%	38	0%
250	6.04	-6%	12.2	-6%	36	-5%

^a Statistically significant difference from control at p <0.05.

E. ORGAN WEIGHTS

1. One-year interim sacrifice

Statistically significant increases in spleen weight parameters occurred in males and females at the high doses (250 and 125 ppm, respectively) and not statistically significant increase in spleen weight of about 15-20% was also observed in the 125 ppm male group. Decreased body weights accentuated the increases in relative spleen weight (organ/body weight). The splenic weight changes correlated with secondary test substance-related microscopic changes observed in the spleen at those dietary concentrations.

Increased relative brain weight occurred in 250 ppm males, which was considered secondary to the decreased final body weights. Similarly, statistically significant differences in absolute and relative organ weight parameters were observed in 125 ppm females, secondary to the decreased body weight which included: changes in liver, kidney, heart, ovaries, and brain weight parameters. None of these body-weight associated organ weight changes were associated with test substance-related pathological findings, and were not considered to be indicative of primary target organ toxicity.

2. Two-year sacrifice

Consistent with findings at the 1-year interim sacrifice, statistically significant increases in spleen weight parameters occurred in males and females at the high doses (250 and 125 ppm, respectively). In addition, although not statistically significant, spleen weight relative to body weight was increased in 60 ppm females. The splenic weight changes correlated with secondary test substance-related microscopic changes observed in the spleen.

All other organ weight changes in the high-intermediate and high-concentration groups were secondary to the body weight effects that occurred in these groups, and were not associated with test substance-related microscopic findings. A statistically significant decrease in absolute and relative – to-brain heart weight in 20 ppm males was not associated with primary dose-related changes and was considered to be spurious.

Increases of 350%, 444% and 340% of absolute adrenal glands weight, adrenal glands to body and brain weight ratios respectively were noted in the high dose female group at terminal sacrifice. Nevertheless, these apparent increases are due to 1 female having a considerably higher adrenal weight than the other animals of the group (10.753g compared to 0.069-0.256g), certainly due to a mass of 3 cm diameter confirmed to be a carcinoma at histopathological examination.

Table B.6.5-4
2-Year dietary study in rats: Organ weights

Parameter	0 ppm	10 ppm	20 ppm	40 ppm	60 ppm	125 ppm	250 ppm
Males:							
12-Month							
Absolute Liver weight (% control)	23.054 (control)	na ^d	20.913 (90.7)	20.577 (89.3)	19.700 (85.5)	20.219 (87.7)	19.529 (84.7)
Relative ^a Liver weight (% control)	3.061 (control)	na	2.917 (95.3)	2.711 (88.6)	2.723 (89.0)	2.827 (92.4)	2.905 (94.9)
Liver to brain weight (% control)	10.219 (control)	na	9.246 (90.5)	9.107 (89.1)	8.570 (83.9)	8.967 (87.7)	8.788 (86.0)
Absolute Kidneys weight (% control)	4.502 (control)	na	4.946 (110)	4.696 (104)	4.395 (97.6)	4.644 (103)	4.602 (102)
Relative ^a Kidneys weight (% control)	0.601 (control)	na	0.701 (117)	0.627 (104)	0.612 (102)	0.654 (109)	0.688 (115)
Kidneys to brain weight (% control)	1.995 (control)	na	2.190 (110)	2.078 (104)	1.916 (96.0)	2.056 (103)	2.070 (104)
Absolute Heart weight (% control)	1.884 (control)	na	1.870 (99.3)	1.879 (99.7)	1.863 (98.9)	1.934 (103)	1.803 (95.7)
Relative ^a Heart weight (% control)	0.251 (control)	na	0.264 (105)	0.251 (100)	0.259 (103)	0.272 (108)	0.269 (107)
Heart to brain weight (% control)	0.835 (control)	na	0.829 (99.3)	0.831 (99.5)	0.813 (97.4)	0.856 (103)	0.811 (97.1)
Absolute Spleen weight (% control)	1.034 (control)	na	1.157 (112)	1.121 (108)	1.020 (98.6)	1.192 (115)	1.321 ^b (128)
Relative ^a Spleen weight (% control)	0.137 (control)	na	0.162 (118)	0.150 (110)	0.142 (104)	0.167 (122)	0.195 ^b (142)
Spleen to brain weight (% control)	0.460 (control)	na	0.511 (111)	0.496 (108)	0.445 (96.7)	0.530 (115)	0.594 ^b (129)
Absolute Adrenal Glands weight (% control)	0.065 (control)	na	0.069 (106)	0.066 (102)	0.062 (95.4)	0.069 (106)	0.063 (96.9)
Relative ^a Adrenal Glands weight (% control)	0.009 (control)	na	0.010 (111)	0.009 (100)	0.009 (100)	0.010 (111)	0.009 (100)
Adrenal Glands to brain weight (% control)	0.029 (control)	na	0.030 (103)	0.029 (100)	0.027 (93.1)	0.031 (107)	0.028 (96.6)
Absolute Testes weight (% control)	3.530 (control)	na	3.542 (100)	3.616 (102)	3.603 (102)	3.744 (106)	3.609 (102)
Relative ^a Testes weight (% control)	0.472 (control)	na	0.505 (107)	0.486 (103)	0.501 (106)	0.528 (112)	0.539 (114)
Testes to brain weight (% control)	1.564 (control)	na	1.572 (101)	1.600 (102)	1.573 (101)	1.656 (106)	1.621 (104)
Absolute Brain weight (% control)	2.257 (control)	na	2.256 (100)	2.262 (100)	2.293 (102)	2.261 (100)	2.226 (98.6)
Relative ^a Brain weight (% control)	0.302 (control)	na	0.321 (106)	0.302 (100)	0.318 (105)	0.319 (106)	0.333 (110)

Table B.6.5-4
2-Year dietary study in rats: Organ weights (continued)

Parameter	0 ppm	10 ppm	20 ppm	40 ppm	60 ppm	125 ppm	250 ppm
24-Month							
Absolute Liver weight (% control)	20.181 (control)	na	20.481 (102)	20.746 (103)	18.202 (90.2)	18.232 (90.3)	17.026 ^b (84.4)
Relative ^a Liver weight (% control)	2.952 (control)	na	2.971 (101)	2.859 (96.8)	2.758 (93.4)	2.870 (97.2)	2.841 (96.2)
Liver to brain weight (% control)	8.770 (control)	na	8.787 (100)	9.074 (104)	7.827 (89.2)	7.799 (88.9)	7.378 ^b (84.1)
Absolute Kidneys weight (% control)	5.959 (control)	na	5.687 (95.4)	5.720 (96.0)	5.563 (93.4)	5.317 (89.2)	5.129 (86.1)
Relative ^a Kidneys weight (% control)	0.875 (control)	na	0.826 (94.4)	0.795 (90.9)	0.850 (97.1)	0.847 (96.8)	0.860 (98.3)
Kidneys to brain weight (% control)	2.595 (control)	na	2.438 (93.9)	2.497 (96.2)	2.398 (92.4)	2.270 (87.5)	2.222 ^c (85.6)
Absolute Heart weight (% control)	2.452 (control)	na	2.232 ^b (91.0)	2.272 (92.7)	2.281 (93.0)	2.171 ^b (88.5)	2.158 ^b (88.0)
Relative ^a Heart weight (% control)	0.358 (control)	na	0.321 (89.7)	0.315 (88.0)	0.348 (97.2)	0.347 (96.9)	0.362 (101)
Heart to brain weight (% control)	1.066 (control)	na	0.956 ^b (89.7)	0.989 (92.8)	0.981 (92.0)	0.928 ^b (87.1)	0.935 ^b (87.7)
Absolute Spleen weight (% control)	1.465 (control)	na	1.281 (87.4)	1.659 (113)	1.423 (97.1)	1.395 (95.2)	1.516 (104)
Relative ^a Spleen weight (% control)	0.211 (control)	na	0.185 (87.7)	0.225 (107)	0.215 (102)	0.217 (103)	0.253 ^c (120)
Spleen to brain weight (% control)	0.637 (control)	na	0.548 (86.0)	0.722 (113)	0.611 (95.9)	0.600 (94.2)	0.658 (103)
Absolute Adrenal Glands weight (% control)	0.109 (control)	na	0.197 (181)	0.126 (116)	0.103 (94.5)	0.108 (99.1)	0.139 (128)
Relative ^a Adrenal Glands weight (% control)	0.016 (control)	na	0.027 (169)	0.018 (113)	0.016 (100)	0.018 (113)	0.022 (138)
Adrenal Glands to brain weight (% control)	0.047 (control)	na	0.085 (181)	0.055 (117)	0.044 (93.6)	0.046 (97.9)	0.061 (130)
Absolute Testes weight (% control)	3.488 (control)	na	3.405 (97.6)	3.603 (103)	3.344 (95.9)	3.511 (101)	3.719 (107)
Relative ^a Testes weight (% control)	0.501 (control)	na	0.492 (98.2)	0.503 (100)	0.502 (100)	0.559 (112)	0.626 ^c (125)
Testes to brain weight (% control)	1.512 (control)	na	1.459 (96.5)	1.574 (104)	1.429 (94.5)	1.501 (99.3)	1.608 (106)
Absolute Brain weight (% control)	2.308 (control)	na	2.335 (101)	2.296 (99.5)	2.333 (101)	2.346 (102)	2.311 (100)
Relative ^a Brain weight (% control)	0.344 (control)	na	0.338 (98.3)	0.319 (92.7)	0.357 (104)	0.381 (111)	0.390 ^c (113)

Table B.6.5-4
2-Year dietary study in rats: Organ weights (continued)

Parameter	0 ppm	10 ppm	20 ppm	40 ppm	60 ppm	125 ppm	250 ppm
Females:							
12-Month							
Absolute Liver weight (% control)	10.982 (control)	11.407 (104)	10.791 (98.3)	10.435 (95.0)	11.012 (100)	9.111 (89.0)	na
Relative ^a Liver weight (% control)	2.539 (control)	2.758 (109)	2.550 (100)	2.697 (106)	2.699 (106)	3.088 ^b (122)	na
Liver to brain weight (% control)	5.497 (control)	5.587 (102)	5.306 (96.5)	4.962 (90.3)	5.348 (97.3)	4.368 ^b (79.5)	na
Absolute Kidneys weight (% control)	2.795 (control)	2.814 (101)	2.839 (102)	2.622 (93.8)	2.829 (101)	2.352 (84.2)	na
Relative ^a Kidneys weight (% control)	0.648 (control)	0.684 (106)	0.675 (104)	0.693 (107)	0.699 (108)	0.798 ^b (123)	na
Kidneys to brain weight (% control)	1.401 (control)	1.378 (98.4)	1.395 (99.6)	1.246 (88.9)	1.372 (97.9)	1.128 ^b (80.5)	na
Absolute Heart weight (% control)	1.314 (control)	1.311 (99.8)	1.348 (103)	1.274 (97.0)	1.312 (99.8)	1.142 ^b (86.9)	na
Relative ^a Heart weight (% control)	0.306 (control)	0.319 (104)	0.321 (105)	0.334 (109)	0.324 (106)	0.387 ^b (127)	na
Heart to brain weight (% control)	0.658 (control)	0.642 (97.6)	0.662 (101)	0.606 (92.1)	0.637 (96.8)	0.548 ^b (83.3)	na
Absolute Spleen weight (% control)	0.652 (control)	0.651 (99.8)	0.644 (98.8)	0.662 (102)	0.701 (108)	0.751 (115)	na
Relative ^a Spleen weight (% control)	0.152 (control)	0.159 (105)	0.152 (100)	0.176 (116)	0.173 (114)	0.255 ^b (168)	na
Spleen to brain weight (% control)	0.326 (control)	0.319 (97.9)	0.316 (96.9)	0.315 (96.6)	0.339 (104)	0.359 (110)	na
Absolute Adrenal Glands weight (% control)	0.082 (control)	0.094 (115)	0.079 (96.3)	0.076 (92.7)	0.093 (113)	0.072 (87.8)	na
Relative ^a Adrenal Glands weight (% control)	0.019 (control)	0.023 (121)	0.019 (100)	0.020 (105)	0.023 (121)	0.024 (126)	na
Adrenal Glands to brain weight (% control)	0.041 (control)	0.046 (112)	0.039 (95.1)	0.036 (87.8)	0.045 (110)	0.034 (82.9)	na
Absolute Ovaries weight (% control)	0.106 (control)	0.094 (88.7)	0.108 (102)	0.101 (95.3)	0.109 (103)	0.096 (90.6)	na
Relative ^a Ovaries weight (% control)	0.025 (control)	0.023 (92.0)	0.025 (100)	0.027 (108)	0.027 (108)	0.032 ^b (128)	na
Ovaries to brain weight (% control)	0.053 (control)	0.046 (86.8)	0.053 (100)	0.048 (90.6)	0.053 (100)	0.046 (86.8)	na
Absolute Brain weight (% control)	2.004 (control)	2.044 (102)	2.038 (102)	2.106 (105)	2.066 (103)	2.088 (104)	na
Relative ^a Brain weight (% control)	0.469 (control)	0.501 (107)	0.487 (104)	0.561 ^b (120)	0.513 (109)	0.710 ^b (151)	na

Table B.6.5-4
2-Year dietary study in rats: Organ weights (continued)

Parameter	0 ppm	10 ppm	20 ppm	40 ppm	60 ppm	125 ppm	250 ppm
24-Month							
Absolute Liver weight (% control)	14.024 (control)	13.750 (98.0)	12.511 (89.2)	13.857 (98.8)	11.626 ^b (82.9)	12.031 (85.8)	na
Relative ^a Liver weight (% control)	3.048 (control)	3.237 (106)	2.799 (91.8)	2.931 (96.2)	2.990 (98.1)	3.222 (106)	na
Liver to brain weight (% control)	6.876 (control)	6.563 (95.4)	5.967 (86.8)	6.628 (96.4)	5.601 ^b (81.5)	5.707 ^b (83.0)	na
Absolute Kidneys weight (% control)	3.438 (control)	3.722 (108)	3.481 (101)	3.481 (101)	3.295 (95.8)	3.210 (93.4)	na
Relative ^a Kidneys weight (% control)	0.783 (control)	0.893 (114)	0.798 (102)	0.760 (97.1)	0.871 (111)	0.862 (110)	na
Kidneys to brain weight (% control)	1.686 (control)	1.783 (106)	1.658 (98.3)	1.663 (98.6)	1.587 (94.1)	1.521 (90.2)	na
Absolute Heart weight (% control)	1.684 (control)	1.659 (98.5)	1.675 (99.5)	1.602 (95.1)	1.559 (92.6)	1.522 (90.4)	na
Relative ^a Heart weight (% control)	0.377 (control)	0.393 (104)	0.379 (101)	0.347 (92.0)	0.407 (108)	0.409 (109)	na
Heart to brain weight (% control)	0.825 (control)	0.791 (95.9)	0.797 (96.6)	0.764 (92.6)	0.752 (91.2)	0.721 (87.4)	na
Absolute Spleen weight (% control)	0.837 (control)	0.805 (96.2)	0.829 (99.0)	0.882 (105)	0.872 (104)	1.385 ^c (166)	na
Relative ^a Spleen weight (% control)	0.184 (control)	0.189 (103)	0.181 (98.4)	0.190 (103)	0.225 (122)	0.366 ^c (199)	na
Spleen to brain weight (% control)	0.409 (control)	0.383 (93.6)	0.394 (96.3)	0.421 (103)	0.419 (102)	0.653 ^c (160)	na
Absolute Adrenal Glands weight (% control)	0.150 (control)	0.151 (101)	0.136 (90.7)	0.133 (88.7)	0.121 (80.7)	0.525 (350)	na
Relative ^a Adrenal Glands weight (% control)	0.034 (control)	0.037 (109)	0.032 (94.1)	0.030 (88.2)	0.033 (97.1)	0.151 (444)	na
Adrenal Glands to brain weight (% control)	0.073 (control)	0.073 (100)	0.065 (89.0)	0.063 (86.3)	0.059 (80.8)	0.248 (340)	na
Absolute Ovaries weight (% control)	0.150 (control)	0.152 (101)	0.159 (106)	0.153 (102)	0.147 (98.0)	0.141 (94.0)	na
Relative ^a Ovaries weight (% control)	0.033 (control)	0.037 (112)	0.037 (112)	0.032 (97.0)	0.038 (115)	0.038 (115)	na
Ovaries to brain weight (% control)	0.073 (control)	0.072 (98.6)	0.076 (104)	0.072 (98.6)	0.071 (97.3)	0.067 (91.8)	na
Absolute Brain weight (% control)	2.044 (control)	2.098 (103)	2.098 (103)	2.102 (103)	2.080 (102)	2.111 (103)	na
Relative ^a Brain weight (% control)	0.460 (control)	0.509 (111)	0.491 (107)	0.464 (101)	0.551 (120)	0.571 ^b (124)	na

^a Relative weight is defined as the organ to body weight ratio.

^b Significantly different from control by Dunnett's test, $p < 0.05$.

^c Significantly different from control by Dunn's test, $p < 0.05$.

^d Not applicable.

F. MICROSCOPIC FINDINGS

1. Neoplastic lesions:

Dietary exposure to DPX-JW062 did not produce neoplasms in either male or female rats at any of the concentrations tested.

2. Non-neoplastic lesions:

One-year interim sacrifice:

Increased incidences of pigment (hemosiderin) in the liver were observed in 125 ppm females. In the spleen, increased incidences of congestion, extramedullary hematopoiesis, and pigment were observed in males and females at 60 ppm and above. Increased incidences of bone marrow hematopoiesis (hyperplasia) were observed in 250 ppm males, and 125 ppm females. These changes noted in the liver, spleen, and bone marrow were secondary physiological responses to test substance-related hemolysis.

Two-year sacrifice:

Increased incidences of pigment in the hepatic Kupffer cells were observed in females at 40 ppm and above, and in males at 250 ppm. The increased incidence of pigment was a secondary physiological marker of increased hemolysis.

Increased incidences of splenic pigment were observed in males at 20 ppm and above (not dose-related), and in females at 60 ppm and above. The increased incidence of pigment (hemosiderin) was minimal to mild in severity, and was a secondary physiological marker of increased hemolysis. Increased incidence of splenic congestion (associated with hemolysis and extramedullary hematopoiesis) was slightly increased in 250 ppm males.

Increased incidences of renal tubular pigment were observed in 125 ppm females, which was secondary to hemolysis.

Increased incidences of bone marrow atrophy, splenic lymphoid depletion, and thymic necrosis were observed in 125 ppm females due to increased incidences of these lesions among the 7 females in which test substance-related mortality occurred.

In summary, secondary test substance-related microscopic observations were observed in the liver, spleen, bone marrow, kidneys, and thymus. Except for lesions seen in the seven high-concentration (125 ppm) female early deaths (bone marrow atrophy, splenic lymphoid depletion, and thymic necrosis), all of the test substance-related lesions were related to the haematological effects (hemolysis) of DPX-JW0621-106. The bone marrow atrophy, splenic lymphoid depletion, and thymic necrosis were considered to be nonspecific lesions secondary to the general toxicity of the test substance at the high concentration.

Table B.6.5-5
2-Year dietary study in rats: Microscopic findings

DPX-JW062 (ppm)	0	10	20	40	60	125	250
Males: 12-Month							
Number of animals/group:	10	na^f	10	10	10	10	10
Liver							
Pigment, increased Kupffer cell	0 ^a	na	0	0	0	0	1
Spleen							
Congestion	0	na	1	1	7 ^b	10 ^b	10 ^b
EMH, increased	1	na	1	2	3	8 ^b	5 ^b
Macrophages, pigment	0	na	2	2	5 ^b	5 ^b	5 ^b
Bone Marrow							
Hyperplasia, mixed	0	na	3	2	3	2	6 ^b
Males: 24-Month							
Number of animals/group:	62	na	62	62	62	62	62
Liver							
Pigment, increased Kupffer cell	4	na	4	2	3	3	11
Spleen							
Congestion	2	na	1	2	9	3	13 ^b
Macrophages, pigment	1	na	15 ^c	8	15 ^b	25 ^b	27 ^b
Depletion, lymphoid	2	na	1	0	0	4	3
Bone Marrow							
Atrophy	0	na	1	0	3	3	0
Kidneys							
Pigment, tubular	3	na	7	0	0	3	2
Thymus ^d							
Necrosis	0	na	ne ^g	ne	ne	ne	0
Females: 12-Month							
Number of animals/group:	10	10	10	10	10^e	10	na
Liver							
Pigment, increased Kupffer cell	0	0	0	0	0	7 ^b	na
Spleen							
Congestion	0	0	0	2	6 ^b	10 ^b	na
EMH, increased	4	2	3	3	4	3	na
Macrophages, pigment	2	4	3	6	6 ^b	8 ^b	na
Bone Marrow							
Hyperplasia, mixed	0	0	1	0	1	3 ^b	na

Table B.6.5-5
2-Year dietary study in rats: Microscopic findings (continued)

DPX-JW062 (ppm)	0	10	20	40	60	125	250
Females: 24-Month							
Number of animals/group:	62	62	61	62	62	63	na
Liver							
Pigment, increased Kupffer cell	2	4	2	11 ^b	12 ^b	23 ^b	na
Spleen							
Congestion	1	0	0	2	1	0	na
Macrophages, pigment	27	30	26	39	50 ^b	49 ^b	na
Depletion, lymphoid	0	0	1	2	0	7 ^b	na
Bone Marrow							
Atrophy	0	2	0	2	2	9 ^b	na
Kidneys							
Pigment, tubular	4	3	3	5	8	10 ^b	na
Thymus ^d							
Necrosis	0	ne	ne	ne	ne	5 ^{c, d}	na

^a Number of organs with microscopic change.

^b Statistically significant by the Cochran-Armitage criteria, $p < 0.05$.

^c Statistically significant by the Fisher's exact test, $p < 0.05$.

^d Thymic necrosis was considered to be a secondary test substance related microscopic finding only in high concentration females.

^e Only 9 spleens available for microscopic examination in this group.

^f Not applicable

^g Not evaluated

EMH : Extramedullary haemopoiesis

Conclusions from the original DAR (2000, RMS NL):

Test substance-related effects comprised changes in body weight, and haematological effects indicative of haemolytic anaemia, i.e., decreases in RBC counts, Hb and Ht, accompanied at higher dose levels with indications of increased hematopoiesis (spleen, bone marrow), increased hemosiderin accumulation (liver, spleen), splenic congestion, increased spleen weights, increased MCV (females), incidentally increased reticulocyte (only after 3-months, not dose-related) and decreased monocyte count (after 1 year). The decreases in RBC, Hb, and Ht were most pronounced in females after 6 months. After 24-months mean RBC, Hb and Ht were comparable with or higher than control values. Slightly increased mortality was seen in females of the top dose group prior to interim sacrifice, and organ weight changes considered to be secondary to body weight changes occurred in males and females of the two highest dose levels. Treatment-related effects were present from the lowest dose examined, i.e., in 20 mg/kg food males (0.80 mg/kg bw/day), and in 10 mg/kg food females (0.55 mg/kg bw/day).

The authors of the report established a NOAEL of 60 and 40 mg/kg food for male and female rats, respectively. In males, the NOAEL was based on body weight and nutritional effects at 125 mg/kg food and higher, and in females on body weight and nutritional effects, and haematological changes at 60 mg/kg food and higher. The authors considered the significant decreases noted in some indicators of circulating erythrocyte mass (i.e., RBC count, Hb, Ht) in all male treatment groups and in 40 mg females to be biologically irrelevant, because the magnitude of the changes was $\leq 10\%$ as compared to controls and not accompanied with changes indicative of a regenerative bone marrow response (increase in reticulocyte count, erythrocyte macrocytosis).

The reviewer does not agree with the NOAELs established by the authors of the study. The reviewer considers a statistically significant and prolonged decrease in one or more indicators of circulating erythrocyte mass as an adverse effect of the test substance, even though this decrease is $\leq 10\%$. Therefore, based on haematological changes in low dose males and females, the NOAEL is set at < 20 mg/kg food for males (equal to < 0.80 mg/kg bw/day), and < 10 mg/kg food for females (equal to < 0.55 mg/kg bw/day), resulting in an overall NOAEL < 0.55 mg/kg bw/day. Because the effects noted at the lowest dose level were considered to be only marginal, this dose level was considered to be a marginal-observed-adverse-effect-level (MOAEL).

The test substance did not induce tumours in rats.

Conclusions from the Post-Annex I Addendum (2007, RMS NL):

Test substance-related effects comprised changes in body weight, and haematological effects indicative of haemolytic anaemia, i.e., decreases in RBC counts, Hb and Ht, accompanied at higher dose levels with indications of increased hematopoiesis (spleen, bone marrow), increased haemosiderin accumulation (liver, spleen), splenic congestion, increased spleen weights, increased MCV (females), incidentally increased reticulocyte (only after 3-months, not dose-related) and decreased monocyte count (after 1 year). The decreases in RBC, Hb, and Ht were most pronounced in females after 6 months. After 24-months mean RBC, Hb and Ht were comparable with or higher than control values. Slightly increased mortality was seen in females of the top dose group prior to interim sacrifice, and organ weight changes considered to be secondary to body weight changes occurred in males and females of the two highest dose levels. Treatment-related effects were present from the lowest dose examined, i.e., in 20 mg/kg food males (0.80 mg/kg bw/day), and in 10 mg/kg food females (0.55 mg/kg bw/day).

The authors of the report established a NOAEL of 60 and 40 mg/kg food for male and female rats, respectively. In males, the NOAEL was based on body weight and nutritional effects at 125 mg/kg food and higher, and in females on body weight and nutritional effects, and haematological changes at 60 mg/kg food and higher. The authors considered the significant decreases noted in some indicators of circulating erythrocyte mass (i.e., RBC count, Hb, Ht) in all male treatment groups and in 40 mg females to be biologically irrelevant, because the magnitude of the changes was $\leq 10\%$ as compared to controls and not accompanied with changes indicative of a regenerative bone marrow response (increase in reticulocyte count, erythrocyte macrocytosis).

The reviewer does not agree with the NOAELs established by the authors of the study. The reviewer considers a statistically significant and prolonged decrease of more than 10% in one or more indicators of circulating erythrocyte mass as an adverse effect of the test substance. Therefore, based on haematological changes in low dose females, the NOAEL is set at 20 mg/kg food for females (equal to 1.04 mg/kg bw/day).

The test substance did not induce tumours in rats.

The JMPR concluded in their 2005 evaluation: The NOAEL was 60 ppm (2.4 mg/kg bw per day) for males and 40 ppm (2.1 mg/kg bw per day) for females, on the basis of decreased body-weight gain and food consumption and haemolysis at the higher doses.

Conclusions proposed by the applicant (2015):

The no-observed-adverse-effect level (NOAEL) was 60 ppm for males and 40 ppm for females, equivalent to 2.4 and 2.13 mg/kg/day, respectively. In male rats, the NOAEL was based on body weight and nutritional effects at 125 ppm and above. In females, the NOAEL was based on body weight and nutritional effects, and mild but reversible, changes in haematologic parameters at 60 ppm and above. Under the conditions of this study, DPX-JW062 was not an oncogen.

RMS FR assessment (2016):

The NOAEL of this study is set at 20 ppm in females (1.04 mg/kg bw/d) and 40 ppm in males (1.59 mg/kg bw/d). At the LOAEL of 40 ppm in females (2.13 mg/kg bw/d), decreases in circulating erythrocyte mass parameters by more than 10% were observed during the first 18-month of the study and haemosiderin was noted in the liver. At highest dose levels, other histopathological findings indicative of haemolytic anemia were noted in males and females in the spleen, the liver, the kidneys and the bone marrow. Increased spleen weights were also observed. Mortality was evident in females of the highest treated group (125 ppm equivalent to 7.83 mg/kg bw/d) and decedent females showed bone marrow atrophy, thymic necrosis and splenic lymphoid depletion. Decreased body weights and body weight gains were observed from 60 ppm in females and 125 ppm in males.

Previous evaluation:	In DAR (2000)
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CA 5.5

Report: [REDACTED] (1997); Oncogenicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) eighteen-month feeding study in mice

DuPont Report No.: HLR 799-96

Guidelines: USEPA 83-2, 59 Nohsan No. 4200, OECD 451, Directive 87/302/EEC **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 799-96

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:
Lot/Batch #: DPX-JW062 technical
Purity: JW062-106
Description: 95.03%
CAS #: Off-white solid
Stability of test compound: 144171-61-9
Analyses confirmed that test material was stable in feed for 7 or 14 days at room temperature or 14 days refrigerated, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.
2. Vehicle and/or negative control: Untreated diet mixed with acetone under the same conditions as test diets
3. Test animals
Species: Mouse
Strain: Crl:CD[®]-1(ICR)BR
Age at dosing: Approximately 51 days old
Weight at dosing: 29.4–29.7 g for males; 22.3–22.6 kg for females
Source: XX
Acclimation period: 22 days
Diet: Purina[®] Certified Rodent Chow[®] (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water: Tap water, *ad libitum*
Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
Temperature: 23 ± 2°C
Humidity: 40–60%
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed
01-February-1995 to 14-August-1996
2. Methods
In an 18-month oncogenicity feeding study, DPX-JW062 (purity 95.03%) was administered to male and female Crl:CD[®]-1(ICR)BR mice (approximately 70 mice/sex/concentration) at dietary concentrations of 20, 100, or 200 ppm. The concentration of DPX-JW062 in diets fed to high-level mice was reduced twice, from 150 ppm on Day 126 to 125 ppm on Day 287, because of excessive mortality. Parameters evaluated included body weight/body weight gain, food consumption, food efficiency, clinical signs, haematology, ophthalmology, organ weights, and gross and microscopic pathology.

II. RESULTS AND DISCUSSION

A. FINDINGS

Findings from the 18-month feeding study in mice with DPX-JW062 are summarised in the following table.

Table B.6.5-6
Summary of results of the 18-month feeding study in mice with DPX-JW062

Dosage	Male			
	Dietary conc.:	0	20	100
	Mean daily intake:	0	2.63	13.8
	200/150/125 ^a ppm			
	Female			
	Dietary conc.:	0	20	100
	Mean daily intake:	0	3.99	20.3
	44.1/31.4/23.7 mg/kg/day			
Analytical	The test substance was distributed homogeneously and was stable in the diet under the study conditions. Measured concentrations of DPX-JW062 were from 89.5 to 102.7% of nominal for all concentrations throughout the study.			
	Dose Group (ppm)	Results		
General observations	≥125 m ^b :	abnormal gait, head tilt, pallor		
	≥100 m:	weakness, stained fur		
	≥100 f:	abnormal gait, hunched over, hyper-reactive, and/or head tilt		
Mortality	≥150 m, f: 125 f:	decreased ^c overall survival test substance-related mortality		
Body weight	≥100 m:	decreased (5–10%)		
	≥100 f:	decreased (10–12%)		
Body weight gain	≥100 m:	decreased (21–29%)		
	≥100 f:	decreased (28–32%)		
Food consumption	200 m:	decreased (11%)		
	200 f:	decreased (20%)		
Food efficiency	100 and 200 m, f:	decreased (25%)		
Ophthalmology	nad ^d			
Haematology	nad			
Gross pathology	≥125 m:	fluid in pleural cavity (correlative to microscopic heart lesions)		
Organ weights	nad except for changes attributable to body weight effects			
Histopathology	≥125 m, ≥100 f:	Low incidences of neuronal necrosis:		
	m:	2/70 at ≥125 ppm		
	f:	2/70 at ≥125 ppm and 1/70 at 100 ppm		
	125 f:	Residual vacuolation of brain in 2 mice		
	>125 m:	Myocardial necrosis and haemorrhage in 12/70 male mice		

^a The 200 ppm group was reduced to 150 ppm on Day 126 and to 125 ppm on Day 287.

^b m = male; f = female

^c All increases or decreases are relative to controls unless otherwise noted.

^d nad = no abnormalities detected that were considered test substance-related and/or biologically adverse

B. OBSERVATIONS

1. Clinical signs of toxicity

Test substance-related clinical observations suggestive of neurotoxicity, including abnormal gait/mobility and head tilt, were significantly increased in male mice in the high-concentration group and in female mice in the 100 ppm and high-concentration groups.

2. Mortality

Survival was 70%, 80%, 68% and 31% in males and 72%, 73%, 79% and 59% in females in the control, low, mid and high-dose groups respectively. When evaluated over the entire course of the

study, test substance-related decreases in survival were present in high-concentration male and female mice, but were statistically significant only in males. In addition, although overall survival was not decreased, deaths likely attributable to ingestion of the test substance occurred at 100 ppm in female mice. Test substance-related causes of death included central nervous system disorders in males and females, and heart inflammation/necrosis in males only.

C. BODY WEIGHT AND BODY WEIGHT GAIN

Test substance-related decreases, relative to controls, in body weight and body weight gain were present in male and female mice at 100 ppm and at the high-concentration (200/150/125 ppm). These body weight effects in the high-concentration groups were most severe during the 200 ppm exposure period.

D. FOOD CONSUMPTION AND FOOD EFFICIENCY

Decreases in food consumption occurred in high-concentration males and females while receiving the 200 ppm diet. Reductions in mean food efficiency were present in both male and female mice in the 100 ppm and high-concentration groups when evaluated over the entire study period.

E. GROSS PATHOLOGY AND HISTOPATHOLOGY

The only test-substance related gross necropsy findings were an increase in the incidence of fluid in the pleural cavity of male mice at the high dose levels (1/70, 0/69, 0/69 and 6/70 for the 0, 20, 100 and 200/150/125 ppm, respectively) and the presence of haemorrhage in the pleural cavity of 1 male mouse at the high dose level. In all these mice, the fluid was red and occurred in mice with heart lesions characterized by necrosis, haemorrhage and inflammation (as did the single incidence of pleural cavity haemorrhage in the top dose male), whereas in the control male the fluid was clear and associated with chronic progressive nephropathy.

There were no test substance-related neoplastic changes at any of the dietary concentrations evaluated. Low incidences of neuronal degeneration/necrosis occurred in the brain of male and female mice at the highest dietary level, and in a female mouse at 100 ppm. The primary sites affected were the piriform cortex and the hippocampus. A more chronic brain lesion, diagnosed as residual vacuolation, was present in the piriform cortex of two high-concentration females sacrificed at the termination of the study.

In male mice, minimal to severe myocardial necrosis of the heart, with associated haemorrhage and inflammation, occurred in 12/70 animals at the highest dietary level.

Lymphoid depletion was present in the spleen of both male and female mice at the highest dietary level. This finding is commonly seen as a non-specific reaction to stress in mice that are found dead or sacrificed *in extremis*.

According to the study author, although the pathogenesis of the test substance-related brain and heart lesions is not known, the distribution of microscopic brain lesions (in the hippocampus and piriform cortex) is suggestive of effects secondary to seizures. Furthermore, heart lesions in high-concentration males may represent neurogenic, rather than primary, myocardial necrosis. Neurogenic myocardial necrosis is known to occur in some central nervous system disorders and all high-concentration males with heart lesions also exhibited one or more neurological signs.

Conclusions from the original DAR (2000, RMS NL):

The test substance did not elicit an oncogenic response in male or female mice. Effects at 100 mg/kg food and above comprised decreased body weight, body weight gain, and food efficiency, and clinical signs suggestive of neurotoxicity (abnormal gait and/or mobility, and tilt head) in male and female mice. Effects at the top dose level included increased mortality and neuronal degeneration/necrosis in the brain of males and females, and myocardial necrosis and haemorrhage in male mice. No substance-related effects were seen at 20 mg/kg food equal to 2.63 and 3.99 mg/kg bw/day for males and females, respectively.

Conclusions proposed by the applicant (2015):

The no-observed-adverse-effect level (NOAEL) was 20 ppm for males and females, equivalent to 2.63 and 3.99 mg/kg/day, respectively. In male mice, the NOAEL was based on decreased body weight, body weight gain, and food efficiency at 100 ppm and above. Mortality, myocardial necrosis, and evidence of neurotoxicity were present in males in the high-concentration group. In females, the NOAEL was based on the following changes at 100 ppm and above: decreased body weight and body weight gain, decreased food efficiency, neurotoxicity, and mortality. Under the conditions of this study, DPX-JW062 was not an oncogen.

RMS FR assessment (2016):

The NOAEL is set at 20 ppm, equivalent to 2.63 mg/kg bw/d in males and 3.99 mg/kg bw/d in females. At 100 ppm (13.8 mg/kg bw/d in males and 20.3 mg/kg bw/d in females) and above, decreased body weights, body weight gains and food efficiency were observed, as well as clinical signs indicative of neurotoxicity. At the top dose level, mortality and histopathological findings in heart (myocardial necrosis and haemorrhage in males) and brain (neuronal degeneration/necrosis in males and females) were reported.

B.6.6. REPRODUCTIVE TOXICITY

A two-generation reproduction study was conducted in rats with DPX-JW062 (50:50). Developmental studies in the rat and rabbit were conducted with DPX-JW062 (50:50). Developmental studies in rats were also conducted with DPX-MP062 (75:25) and DPX-KN128 (99:1).

Table B.6.6-1
Summary of reproductive toxicity data for indoxacarb (DPX-KN128, DPX-MP062 and DPX-JW062)

Type of study and test substance	Doses/concentrations tested	NOAEL	LOAEL	Target organ(s) and effects	Reference
Multigeneration reproduction Rat DPX-JW062 (50:50)	0, 20, 60, 100 ppm in the diet Eq. to 1.2, 3.7 and 6.1 mg/kg bw/d	Parental: 20 ppm = 1.2 mg/kg bw/d Repro/Fertility: 100 ppm 6.1 mg/kg bw/d Offspring: 20 ppm = 1.2 mg/kg bw/d	Parental: 60 ppm = 3.7 mg/kg bw/d Repro/Fertility: >100 ppm > 6.1 mg/kg bw/d Offspring: 60 ppm = 3.7 mg/kg bw/d	Parental: decreased body weight gains and food consumption in F0 females, increased spleen weight in F0 and F1 females Offspring: decreased F1 pup body weights during lactation	1997 HLO 115-96, Revision No. 2
Developmental Rat DPX-KN128 (99:1)	0, 0.5, 1, 2, 3.5 mg/kg/day In PEG by gavage GD 6-20	Maternal: 0.5 mg/kg bw/d Developmental: 2 mg/kg bw/d	Maternal: 1 mg/kg bw/d Developmental: 3.5 mg/kg bw/d	Maternal: decreased body weight gains Developmental: decreased fetal weights	2004 DuPont-12748*
Developmental Rat DPX-MP062 (75:25)	0, 0.5, 1, 2, 4 mg/kg/day In PEG by gavage GD 7-21	Maternal: 2 mg/kg bw/d Developmental: 2 mg/kg bw/d	Maternal: 4 mg/kg bw/d Developmental: 4 mg/kg bw/d	Maternal: decreased body weights, body weight gains and food consumption Developmental: decreased fetal weights	2005 HL-1997-00202, Revision No. 2
Developmental Rat DPX-JW062 (50:50)	0, 10, 100, 500, 1000 mg/kg/day In methylcellulose by gavage GD 7-21	Maternal: 10 mg/kg bw/d Developmental: 10 mg/kg bw/d	Maternal: 100 mg/kg bw/d Developmental: 100 mg/kg bw/d	Maternal: decreased body weight, body weight gains and food consumption, clinical signs, mortality and GI tract macroscopic findings Developmental: decreased mean number of live foetuses per litter. At higher doses: decreased mean fetal weights	1997 HL-1997-00049

Developmental Rabbit DPX-JW062 (50:50)	0, 250, 500, 1000 mg/kg/day In methylcellulose by gavage GD 7-28	Maternal: 500 mg/kg bw/d Developmental: 500 mg/kg bw/d	Maternal: 1000 mg/kg bw/d Developmental: 1000 mg/kg bw/d	Maternal: decreased body weights, body weight gains and food consumption Developmental: decreased mean fetal weights and retarded sternebral ossification	1995 HLR 587- 95
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* Studies newly submitted

In an oral 2-generation reproduction toxicity study with DPX-JW062 (50:50) in rats, the NOAEL for parental toxicity was established at 1.2 mg/kg bw/day, based on effects on body weight, food consumption, and organ weights (especially the spleen) observed at higher dose levels. Because of decreased pup weights during lactation, the NOAEL for offspring toxicity was set at 1.2 mg/kg bw/day as well. No reproduction toxicity was observed up to 100 ppm (6.1 mg/kg bw/day).

Prenatal developmental toxicity studies were performed in rats with DPX-KN128 (99:1), DPX-MP062 (75:25) and DPX-JW062 (50:50) and in rabbits with DPX-JW062 (50:50). No indications for a teratogenic potential of the test compounds were observed in these studies.

In the rat study conducted with DPX-KN128 (99:1), the maternal NOAEL is set at 0.5 mg/kg bw/d, based on the decreased body weight gains observed from the dose level of 1 mg/kg bw/d (-62% during GD6-8 and -11% during GD6-21). The developmental NOAEL is 2 mg/kg bw/d, based on decreased fetal weight observed at the dose level of 3.5 mg/kg bw/d.

Maternal and developmental NOAELs were determined to be 2 mg/kg bw/d for DPX-MP062 (75:25), based on decreased body weights, body weight gains and food consumption in the dams and decreased weights of the fetuses.

With DPX-JW062 (50:50), maternal and developmental NOAELs were set at 10 mg/kg bw/d. From the dose level of 100 mg/kg bw/d, decreased body weight, body weight gains and food consumption, as well as clinical signs, mortality and GI tract macroscopic findings were observed in dams and decreased number of live fetuses per litter was also noted. Decreased mean fetal weights occurred at 500 mg/kg bw/d and above.

In an oral developmental toxicity study in rabbits with DPX-JW062 (50:50), the NOAEL for maternal and developmental toxicity were both established at 500 mg/kg bw/day, based on decreased body weight, body weight gains and food consumption in dams and decreased fetal weights and retarded sternebral ossification in fetuses.

It is important to note that the differences in NOAELs observed between DPX-MP062 (75:25) and DPX-JW062 (50:50) in the teratogenicity studies in rats may be due to differences in bioavailability influenced by the dosing vehicle. Indeed, in two pilot teratogenicity studies in rats in which both compounds were dissolved in polyethylene glycol (PEG 400) comparable (provisional) NOAELs were established for both maternal and developmental toxicity (DPX-MP062 (75:25) respective NOAELs 1 and 4 mg/kg bw/d; DPX-JW062 (50:50) respective NOAELs 1.5 and 6 mg/kg bw/d), which are in the same order of magnitude than NOAELs obtained with DPX-KN128 (99:1) and DPX-MP062 (75:25) dissolved in PEG. On the contrary, NOAELs determined for DPX-JW062 (50:50) dissolved in methylcellulose amount to 10 mg/kg bw/d.

Developmental studies with DPX-JW062 (50:50) administered in methylcellulose demonstrated that the rat was more susceptible than the rabbit to the systemic toxicity of the compound. The NOAELs for both maternal and foetal toxicity in the rat was 10 mg/kg/day as compared to 500 mg/kg/day in the rabbit.

It is noted that haematological effects, identified as the critical endpoint in oral short-term and long-term toxicity studies, were not addressed in the reproduction and teratogenicity studies.

B.6.6.1. Generational studies

Previous evaluation:	HLO 115-96: In DAR (2000) HLR 115-96 Revision No.2 : Submitted for the purpose of renewal (see reasons for revisions below)
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CA 5.6.1/01

Report: [REDACTED] (1997); Two generation reproduction/fertility study with DPX-JW062-106 in rats

DuPont Report No.: HLO 115-96, Revision No. 2

Guidelines: USEPA 83-4, OPPTS 870.3800

Deviations: Estrus cyclicity was not measured; offspring sexual maturation and functional observation parameters were not evaluated, and primordial follicle counts were not evaluated. This study was conducted before several changes (e.g., estrus cyclicity, sperm parameters, and sexual maturation) were made to EEC and international reproduction toxicity guidelines. However, reconducting this study is unlikely to yield a significantly different result because the gonads have not been identified as target organs in other repeated-dose studies. In addition, a developmental neurotoxicity study is available in which offspring development was evaluated.

Testing Facility: [REDACTED]

Testing Facility Report No.: 125-041

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

Reason for Revision Nos. 1 and 2:

1. Method section was revised to provide additional details on methods of semen analyses and statistical analyses.
2. Changes in format and organization of results, discussion, summary, tables, appendices
3. Compound consumption was re-calculated, and tables and text were revised to reflect the recalculated values.
4. Appendix L was updated with gestational food consumption data instead of lactational food consumption data for the 20 ppm group

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062 technical
 Lot/Batch #: JW062-106
 Purity: 95.3%
 Description: Off-white solid
 CAS #: 144171-61-9
 Stability of test compound: Analyses confirmed that test material was stable in feed for at least ten days at room temperature, was distributed uniformly in the feed, and was present in the feed at targeted concentrations. Batches were prepared at weekly intervals.
2. Vehicle and/or negative control: Diet mixed with acetone
3. Test animals
 Species: Rat
 Strain: CrI:CD[®] VAF/Plus[®]
 Age at initial dosing: Approximately 63 days old
 Weight at initial dosing (week 0): 275–322 g for males; 182–224 g for females
 Source: [REDACTED]
 Acclimation period: 14 days
 Diet: PMI[®] Feeds, Inc., Certified Rodent Chow[®] (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
 Water: Tap water, *ad libitum*
 Housing: Animals were housed singly during non-mating periods in stainless steel, wire-mesh cages suspended above cage boards. During cohabitation periods, rats were housed as breeding pairs in wire mesh. After cohabitation periods, females without evidence of copulation were housed singly in plastic cages with wood-chip bedding. Females with evidence of copulation were housed singly in stainless steel, wire-mesh cages suspended above cage boards for Days 0 to 20 of gestation and in plastic cages with wood-chip bedding for Days 20 to delivery. After delivery and through lactation, females were housed with their litters in plastic cages with the same bedding.
4. Environmental conditions
 Temperature: 19–22°C
 Humidity: 40 to 70%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiation/completion
 05-December-1994 to 18-August-1995
2. Animal assignment and treatment
 Animals were assigned to dose groups by computerised, stratified randomisation. The experimental design is described in the following tables. For treatment groups and dietary concentrations, see Table B.6.6.1-1. Dose levels were based on results from a 90-day, one generation pilot reproduction study and a 28-day feeding study of DPX-JW062 and preliminary results from the early weeks of a 2-year chronic study of DPX-JW062, all in rats. Effects from the dose levels of 0, 20, 60, and 100 ppm DPX-JW062 were expected to range from no-observed-adverse-effect to induction of toxicity without excessive mortality. For treatment schedule and sacrifice schedule, see the following tables.

Table B.6.6.1-1
Two-generation reproductive study in the rat: Treatment groups and dietary concentrations

Dosage group	P ₁ Generation number of rats per sex	F ₁ Generation number of rats per sex	Dietary concentration (ppm) ^a	
I	26	26	0	(Control)
II	26	26	20	(Low-intermediate)
III	26	26	60	(High-intermediate)
IV	26	26	100	(High)

Note: The test substance was considered 95.3% pure for the purpose of dosage calculations.

^a Weight/weight concentration of DPX-JW062

Table B.6.6.1-2
Two-generation reproductive study in the rat: Treatment schedule

Generation	Approximate age at start of feeding (days)	Approximate number of study days before mating	Duration of feeding
P ₁	63	70	Until sacrifice
F ₁	21	77 ^a	Until sacrifice

^a Premating period was at least 77 days.

Table B.6.6.1-3
Two-generation reproductive study in the rat: Sacrifice schedule

Animals	Generation	Schedule
Adult males	P ₁ , F ₁	P ₁ : After siring litters and completion of parturition—test Days 107–115 F ₁ : After siring litters and completion of parturition—test Days 240–248
Pregnant females	P ₁ , F ₁	On day of weaning litters—postpartum Day 22
Nonpregnant females	P ₁ , F ₁	Approximately Day 25 after the end of cohabitation
Day 4 culled pups	F ₁ , F ₂	Day 4 of lactation
Weanlings	F ₁ , F ₂	On day of weaning—postnatal Day 22 (except F ₁ rats selected for parental rats)

3. Diet preparation and analysis

The test substance, dissolved in acetone, was added to 500 grams of the rodent diet and thoroughly mixed for 10 minutes. The resulting premix was then added to additional diet and mixed for 10 more minutes. Control diets were mixed for the same period of time. All diets were prepared weekly and stored at room temperature until used. The stability, homogeneity, and concentration of DPX-JW062 in the dietary mixtures were checked by analysis using HPLC study weeks 1 through 4, then every four weeks thereafter. The test substance was at target concentrations (97 to 98%) homogeneous (1.9–3.1 relative standard deviation) throughout the feed, and was stable (97 to 99%) for up to 10 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. Statistics

Table B.6.6.1-4
Statistics: Two-generation reproductive study in the rat

Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Food consumption Parental body weights and body weight changes Sperm analysis Organ weights Copulatory interval Implantation sites Total born per litter Stillborns per litter Live pups per litter	One-way analysis of variance (ANOVA)	None	Step-down, ^a orthogonal, linear-trend tests
Mean pup weights	Analysis of covariance (ANCOVA)	None	Step-down, orthogonal, linear-trend test
Pup sex ratio Copulatory indices Fecundity indices Fertility indices Gestational indices	None	Step-down, Cochran-Armitage trend test	
Pup survival	None	Transform the percent values for each dam to ranks then analyse the rank using the step-down process	

^a Used to establish statistically significant differences between the control group and each treatment group. If a significant dose response was detected, data from the top-dose group was excluded and a subsequent trend test was conducted. This process was repeated until a non-significant trend test was observed or only the control group remained. Significance was judged at $p < 0.05$.

All statistical analyses compared the treatment groups with the control group. All means were accompanied by standard deviations. SAS statistical software, as well as in-house software, were utilised for analyses. In all cases, the litter was used as the unit of analysis.

C. METHOD

A two-generation reproduction study, involving the production of one set of litters in each generation, was conducted with DPX-JW062. CrI:CD[®] VAF/Plus[®] rats (26/sex/concentration) were fed diets containing 0, 20, 60, and 100 ppm DPX-JW062. Following 70 days (P₁) or minimum 77 days (F₁) of diet administration (premating), the P₁ and F₁ generation males and females were cohoused within their respective treatment groups, to produce F₁ and F₂ litters, respectively. Vaginal smears were collected daily from all females during mating until evidence of copulation was observed or until the mating period ended. Dams were allowed to deliver and rear their offspring until weaning (postpartum Day 22). F₁ and F₂ litters were culled to 4 pups/sex/litter (litter size permitting) on postnatal Day 4. At weaning, 26 male and 26 female F₁ rats/group were randomly selected to comprise the F₁ generation and were given the same concentration level as their respective P₁ generation sires and dams.

Animals were observed for mortality and overt toxicity twice per day. Clinical observations, body weight, food consumption and compound consumption were determined weekly throughout the study. Litter examinations (live, dead or missing pups, individual pup weights, clinical observations) were determined at birth (Day 0), Day 4 and weekly during the lactation period. Oestrous cycle parameters (percent days in diestrus, proestrus, and estrus) and oestrous cycle length were evaluated for 3 weeks prior to cohabitation in P₁ and F₁ female rats. The age at either vaginal opening or preputial separation was recorded for the F₁

generation. For P₁ and F₁ male parental rats, sperm motility was determined from a sample from the vas deferens; sperm morphology and concentration were determined from a sample from the cauda epididymis.

Pups that died or were sacrificed *in extremis* before scheduled termination were examined for cause of death or moribund condition, given a gross post-mortem examination, and were retained in 10% neutral buffered formalin. All pups culled on Day 4 were sacrificed, given a gross post-mortem examination, and gross lesions were retained. On Day 22 postpartum, F₁ weanlings not selected to comprise the F₁ generation and all F₂ weanlings were given a gross post-mortem examination and discarded. The brain, spleen, adrenals, kidneys, liver, and thymus were weighed then discarded from one weanling/sex/litter from F₁ and F₂ litters. The testes and ovaries were weighed and retained from one weanling/sex/litter from F₁ and F₂ litters; the femur and sternum were retained but not weighed. After litter production, all P₁ and F₁ parental rats were sacrificed and given a gross pathological examination. Testes, epididymides, prostate, seminal vesicles (with coagulating glands), ovaries, uterus (with oviducts and cervix), vagina, bone (femur; P₁ female and F₁ male and F₁ female only), bone (sternum; P₁ female and F₁ male and F₁ female only) and gross lesions were collected. The testes, epididymides, seminal vesicles (with coagulating glands and their fluids), prostate, ovaries, uterus (with cervix), brain, spleen, adrenal glands, kidneys, liver and thymus were weighed.

Tissues and gross lesions from all P₁ and F₁ rats and target organs from F₁ weanlings in the control and 100 ppm groups were examined microscopically. All gross lesions in F₁ and F₂ weanlings were examined microscopically.

II. RESULTS AND DISCUSSION

A. P₁ AND F₁ PARENTAL RATS

1. General observations

Clinical signs/mortality:

There were no apparent test substance-related clinical signs observed during the course of this study.

During the P₁ lactation period, 2 dams from the 100 ppm group were euthanized *in extremis* (Days 4 and 5 of lactation respectively) along with the remaining live pups from their respective litters. They experienced one or more of the following clinical signs: dehydration, cold to touch, increased salivation, mydriasis, ataxia, impaired righting reflex, convulsions, and leaning to the right. A whole litter loss was noted for another dam from this group. This dam did not experience the clinical observations noted for the 2 other dams. A dam from the 60 ppm group also lost her entire litter which contained only 2 pups (1 pup died on lactation day 15 and the other was missing and presumed cannibalized on lactation day 17). These observations were not repeated in the F₁ generation animals at 100 ppm and could not be definitively attributed to treatment. One F₁ female in the 20 ppm group was found dead at 4 weeks old. No remarkable findings were observed at necropsy.

Body weights – P₁ generation:

No statistically significant, treatment-related effects on body weights were observed in P₁ males at 20 or 60 ppm. Mean body weights for the 100 ppm group males were decreased throughout most of the treatment period with statistically significant decreases noted at Week 1 through Week 6 and Weeks 8 and 11. Towards the end of the exposure period, the body weights of the 100 ppm males recovered and were slightly greater than those of the control group on Weeks 15 and 16.

No treatment-related effects on body weight or body weight gain were noted in the 20 ppm group P₁ females at any time during the study. During the pre-mating period, the mean body weights of the 60 and 100 ppm P₁ females were decreased compared to the control group, with statistically significant decreases noted on Weeks 2, 4, and 6 through 10 at 60 ppm and on Weeks 1 through 10 at 100 ppm.

The P₁ female gestational mean weights for the 60 ppm (Days 0, 7, 14) and 100 ppm (Days 0, 7, 14, 21) groups were statistically significantly lower than the control group. Gestation body weight change was also statistically significantly decreased during the Day 0 to 7 interval for the 100 ppm

group. However, the body weight change at 100 ppm was recovered and was slightly increased relative to the control group at later gestational intervals and during the entire gestation period (Days 0 to 21). No effects on maternal body weight change during gestation were observed at 20 or 60 ppm.

P₁ mean body weights during lactation showed a similar trend, with lower mean body weights for the 60 ppm group and statistically significant reductions in mean body weights for the 100 ppm group through Day 14 of lactation, as compared to the control group. Examination of the mean maternal P₁ lactation body weight changes revealed no statistically significant effects for the 20 and 60 ppm groups, whose body weight changes were similar to the control group from Day 0 to Day 21 of lactation. The 100 ppm group gained approximately twice as much weight as the control group during the lactation period and gained a statistically significant amount of weight from Days 14 to 21 and 0 to 21 of lactation.

Overall, mean body weights and body weight changes were significant at 100 ppm in P₁ males and at 60 and 100 ppm in P₁ females and were considered to be related to treatment with the test article.

Body weights – F₁ generation:

The mean body weights for the F₁ male and female treated groups were either comparable to or greater than those of the control group during each measurement interval from Week 4 to 21 of the F₁ study period. Mean body weights were statistically significantly greater than the control group for F₁ males from the 60 and 100 ppm groups from Week 10 to Week 21 and for F₁ males from the 20 ppm group from Weeks 16 and 17.

Mean body weights for the F₁ females from the treated groups were comparable with the control group from Week 4 to Week 15, and there were no statistically significant differences noted for the treated groups when compared with the control group. Mean body weights and mean body weight changes for the F₁ females from the treated groups were comparable with the weights for the control group during the gestation and lactation periods.

Food consumption – P₁ generation:

The mean P₁ male food consumption for the 100 ppm group was statistically significantly ($p < 0.05$ or $p < 0.01$) lower than the control group when analysed by the linear-trend test for the intervals of Weeks 0 to 6 and for the interval of Week 7 to 8. Food consumption for the P₁ males for the 60 ppm group was significantly lower at one time point, Week 0 to 1, compared with the control group. The food consumption for the remaining weeks for the 60 and 100 ppm P₁ males was comparable with that of the control group.

There were statistically significant decreases in P₁ female mean food consumption for the 60 ppm group from Weeks 0 to 1, 2 to 4, and 7 to 8 and for the 100 ppm group from Week 0 to Week 10, as compared with the control group.

Mean food consumption for P₁ females during gestation and lactation was statistically significantly ($p \leq 0.01$ or $p \leq 0.05$) lower for the 60 and 100 ppm groups compared with the control group from Days 0 to 7 and 7 to 14 of gestation and for the 100 ppm group during Days 0 to 7 of lactation. Food consumption for the 60 and 100 ppm P₁ females during the remaining weeks of gestation and lactation was not statistically different from the control group.

Food consumption – F₁ generation:

Food consumption for the F₁ treated group males averaged above the food consumption of the control group and was statistically significantly ($p < 0.01$ or $p < 0.05$) higher for Weeks 6 to 9 and the interval of Week 12 to 13 for the 100 ppm group, Weeks 6 to 8 for the 60 ppm group, and Week 6 to 7 for the 20 ppm group.

Mean food consumption for the F₁ females in the treated groups was comparable with the control group during the premating, gestation, and lactation periods.

Table B.6.6.1-5
Two-generation reproduction study: Body weights, P₁ parental rats

Parameter ^a	0 ppm	20 ppm	60 ppm	100 ppm
Males:				
Week 0 body weight (g)	298.5 (control)	296.3 (99.3%) ^b	292.2 (97.9%)	295.2 (98.9%)
Week 1 body weight (g)	343.2 (control)	345.7 (101%)	335.7 (97.8%)	333.3 ^c (97.1%)
Week 2 body weight (g)	371.7 (control)	380.2 (102%)	365.6 (98.4%)	361.0 ^c (97.1%)
Week 3 body weight (g)	398.7 (control)	413.1 (104%)	395.5 (99.2%)	385.2 ^c (96.6%)
Week 4 body weight (g)	421.6 (control)	439.8 (104%)	418.6 (99.3%)	410.5 ^d (97.4%)
Week 5 body weight (g)	447.5 (control)	464.7 (104%)	439.0 (98.1%)	433.0 ^d (96.8%)
Week 6 body weight (g)	467.5 (control)	485.2 (104%)	460.0 (98.4%)	451.8 ^d (96.6%)
Week 8 body weight (g)	498.7 (control)	523.8 (105%)	494.6 (99.2%)	486.5 ^d (97.6%)
Week 11 body weight (g)	520.7 (control)	549.3 (105%)	514.9 (98.9%)	509.4 ^d (97.8%)
Week 16 body weight (g)	567.4 (control)	619.2 (109%)	570.5 (101%)	578.3 (102%)
Females:				
Week 0 body weight (g)	203.7 (control)	206.7 (102%)	202.2 (99.3%)	201.7 (99.0%)
Week 1 body weight (g)	219.8 (control)	222.8 (101%)	215.9 (98.2%)	209.8 ^d (95.5%)
Week 2 body weight (g)	232.3 (control)	234.4 (101%)	224.6 ^c (96.7%)	216.6 ^d (93.2%)
Week 3 body weight (g)	241.1 (control)	242.2 (101%)	233.7 (96.9%)	223.8 ^d (92.8%)
Week 4 body weight (g)	249.1 (control)	252.1 (101%)	239.2 ^c (96.0%)	228.7 ^d (91.8%)
Week 5 body weight (g)	259.3 (control)	262.0 (101%)	251.0 (96.8%)	237.8 ^d (91.7%)
Week 6 body weight (g)	263.7 (control)	268.0 (102%)	253.0 ^c (95.9%)	241.0 ^d (91.4%)
Week 7 body weight (g)	272.0 (control)	275.8 (101%)	261.9 ^c (96.3%)	249.2 ^d (91.6%)
Week 8 body weight (g)	276.6 (control)	279.8 (101%)	263.8 ^c (95.4%)	250.2 ^d (90.5%)
Week 9 body weight (g)	279.2 (control)	285.5 (102%)	267.6 ^c (95.8%)	252.0 ^d (90.3%)
Week 10 body weight (g)	287.8 (control)	291.6 (101%)	275.0 ^c (95.6%)	258.5 ^d (89.8%)
Body weight at start of gestation (g)	287.4 (control)	291.7 (101%)	273.4 ^c (95.1%)	253.6 ^d (88.2%)

Table B.6.6.1-5
Two-generation reproduction study: Body weights, P₁ parental rats (continued)

Parameter ^a	0 ppm	20 ppm	60 ppm	100 ppm
Females:				
Body weight on Day 7 of gestation (g)	317.2 (control)	325.7 (103%)	301.3 ^c (95.0%)	278.6 ^d (87.8%)
Body weight on Day 14 of gestation (g)	344.0 (control)	351.0 (102%)	327.4 ^c (95.2%)	306.9 ^d (89.2%)
Body weight at end of gestation (g)	415.0 (control)	429.4 (103%)	399.2 (96.2%)	386.4 ^d (93.1%)
Body weight at start of lactation (g)	313.5 (control)	326.3 (104%)	310.3 (99.0%)	287.2 ^d (91.6%)
Body weight on Day 7 of lactation (g)	331.5 (control)	337.1 (102%)	320.0 (96.5%)	303.0 ^d (91.4%)
Body weight on Day 14 of lactation (g)	348.5 (control)	359.2 (103%)	338.6 (97.2%)	329.0 ^d (94.4%)
Body weight at end of lactation (g)	337.2 (control)	343.0 (102%)	328.8 (97.5%)	331.0 (98.2%)

^a The first and last week's data are listed here, along with any week with any significance. Any week with no significant difference was omitted.

^b Values in parentheses are percent of control.

^c Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.05$.

^d Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.01$.

Table B.6.6.1-6
Two-generation reproduction study: Body weights, F₁ parental rats

Parameter ^a	0 ppm	20 ppm	60 ppm	100 ppm
Males:				
Week 4 body weight (g)	99.4 (control)	100.6 (101%) ^b	100.7 (101%)	96.7 (97.3%)
Week 10 body weight (g)	407.0 (control)	422.2 (104%)	429.8 ^c (106%)	432.1 ^d (106%)
Week 11 body weight (g)	435.3 (control)	452.0 (104%)	461.7 ^c (106%)	465.7 ^d (107%)
Week 12 body weight (g)	461.1 (control)	480.3 (104%)	491.0 ^d (107%)	493.0 ^d (107%)
Week 13 body weight (g)	478.5 (control)	499.1 (104%)	514.8 ^d (108%)	513.2 ^d (107%)
Week 14 body weight (g)	494.7 (control)	515.9 (104%)	530.4 ^d (107%)	524.0 ^d (106%)
Week 15 body weight (g)	505.7 (control)	531.3 (105%)	546.8 ^d (108%)	540.1 ^d (107%)
Week 16 body weight (g)	504.8 (control)	535.8 ^c (106%)	552.2 ^d (109%)	550.3 ^d (109%)
Week 17 body weight (g)	519 (control)	546.2 ^c (105%)	566.9 ^{cd} (109%)	561.2 ^d (108%)
Week 18 body weight (g)	540.0 (control)	561.3 (104%)	581.5 ^d (108%)	575.9 ^d (107%)
Week 19 body weight (g)	551.4 (control)	571.4 (104%)	596.5 ^d (108%)	588.9 ^d (107%)
Week 20 body weight (g)	560.4 (control)	577.6 (103%)	597.5 ^d (107%)	598.0 ^d (107%)
Week 21 body weight (g)	569.7 (control)	593.2 (104%)	619.0 ^d (109%)	615.0 ^d (108%)

^a The first and last week's data are listed here, along with any week with any significance; i.e., any week with no significant difference was omitted.

^b Values in parentheses are percent change from control.

^c Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.05$.

^d Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.01$.

Table B.6.6.1-7
Two-generation reproduction study: Food consumption P₁ male parental rats

Parameter ^a	0 ppm	20 ppm	60 ppm	100 ppm
Food consumption, FC (g/day)				
Week 0 to 1	26.4 (control)	26.5 (100%) ^b	25.2 ^c (95.5%)	24.0 ^d (90.9%)
Week 1 to 2	25.8 (control)	26.7 (103%)	25.3 (98.1%)	24.3 ^d (94.2%)
Week 2 to 3	26.5 (control)	27.6 (104%)	26.1 (98.5%)	25.0 ^d (94.3%)
Week 3 to 4	27.0 (control)	28.0 (104%)	26.5 (98.1%)	25.8 ^c (95.6%)
Week 4 to 5	27.2 (control)	28.9 (106%)	26.5 (97.4%)	26.3 ^c (96.7%)
Week 5 to 6	28.2 (control)	28.6 (101%)	27.1 (96.1%)	26.5 ^d (94.0%)
Week 7 to 8	28.2 (control)	28.7 (102%)	27.2 (96.5%)	26.9 ^c (95.4%)
Week 15 to 16	28.6 (control)	30.7 (107%)	27.6 (96.5%)	28.6 (100%)

^a The first and last week's data are listed here, along with any week with any significance; i.e., any week with no significant difference was omitted.

^b Values in parentheses are percent of control.

^c Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.05$.

^d Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.01$.

Table B.6.6.1-8
Two-generation reproduction study: Food consumption F1 male parental rats

Parameter ^a	0 ppm	20 ppm	60 ppm	100 ppm
Food consumption, FC (g/day)				
Week 3 to 4	13.3 (control)	13.4 (101%) ^b	13.5 (102%)	13.7 (103%)
Week 6 to 7	26.8 (control)	27.9 ^c (104%)	28.4 ^d (106%)	28.5 ^d (106%)
Week 7 to 8	28.6 (control)	29.6 (104%)	30.1 ^c (105%)	31.3 ^d (109%)
Week 8 to 9	29.4 (control)	29.8 (101%)	30.1 (102%)	31.1 ^d (106%)
Week 12 to 13	28.8 (control)	30.0 (104%)	30.6 (105%)	30.0 ^d (104%)
Week 20 to 21	28.2 (control)	28.8 (102%)	29.8 (106%)	29.2 (104%)

^a The first and last week's data are listed here, along with any week with any significance; i.e., any week with no significant difference was omitted.

^b Values in parentheses are percent of control.

^c Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.05$.

^d Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.01$.

Table B.6.6.1-9
Two-generation reproduction study: Body weight gain/food consumption P₁ female parental rats

Parameter	0 ppm	20 ppm	60 ppm	100 ppm
Body weight gain, WG (g)				
Gestation Day 0–21	127.7 (control)	137.7 (108%) ^a	125.7 (98.4%)	132.7 (104%)
Lactation Day 0–21	23.8 (control)	16.8 (70.6%)	18.5 (77.7%)	40.7 ^b (171%)
Food consumption, FC (g/day)				
Premating Week 0–1	17.8 (control)	18.0 (101%)	16.5 ^b (92.7%)	14.2 ^c (79.8%)
Premating Week 1–2	18.4 (control)	18.4 (100%)	17.0 (92.4%)	14.1 ^c (76.6%)
Premating Week 2–3	18.8 (control)	19.0 (101%)	17.0 ^c (90.4%)	15.8 ^c (84.0%)
Premating Week 3–4	19.0 (control)	19.6 (103%)	17.6 ^b (92.6%)	16.1 ^c (84.7%)
Premating Week 4–5	18.8 (control)	19.5 (104%)	18.3 (97.3%)	16.7 ^c (88.8%)
Premating Week 5–6	19.0 (control)	19.6 (103%)	17.9 (94.2%)	16.6 ^c (87.4%)
Premating Week 6–7	19.1 (control)	19.4 (102%)	18.2 (95.3%)	16.4 ^c (85.9%)
Premating Week 7–8	18.9 (control)	19.1 (101%)	17.5 ^b (92.6%)	16.5 ^c (87.3%)
Premating Week 8–9	19.4 (control)	20.3 (105%)	18.7 (96.4%)	16.6 ^c (85.6%)
Premating Week 9–10	18.9 (control)	19.9 (105%)	18.9 (100%)	16.7 ^c (88.4%)
Gestation Day 0–21	24.5 (control)	24.9 (102%)	23.0 (93.9%)	21.8 (89.0%)
Lactation Day 0–21	50.2 (control)	52.0 (104%)	48.1 (95.8%)	48.4 (96.3%)

^a Values in parentheses are percent of control.

^b Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.05$.

^c Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.01$

Note: Food consumption was not recorded during mating periods.

Table B.6.6.1-10
Two-generation reproduction study: Body weight gain F₁ female parental rats

Parameter	0 ppm	20 ppm	60 ppm	100 ppm
Body weight gain, WG (g)				
Gestation Day 0–21	131.9 (control)	137.1 (104%)	136.1 (103%)	140.5 (107%)
Lactation Day 0–21	18.5 (control)	8.4 (45.4%)	6.0 (32.4%)	17.2 (93.0%)

^a Values in parentheses are percent change from control.

^b Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.05$.

^c Significantly different from control by the one-way analysis of variance (ANOVA) criteria, $p < 0.01$

The achieved daily intake (ranges and mean) were reported in the following tables.

Table B.6.6.1-11
DPX-JW062 rat reproduction study consumption ranges (mg/kg/day) from DuPont Report Number
HLO 115-96, Revision No. 2

	20 ppm		60 ppm		100 ppm	
Interval	Low	High	Low	High	Low	High
F0 Males – Premating	1.040	1.536	3.142	4.504	5.317	7.211
F0 Females - Premating	1.365	1.612	4.015	4.552	6.387	7.067
F0 Females – Gestation	1.180	1.466	3.823	4.249	6.464	7.162
F0 Females – Lactation	1.896	4.137	5.424	11.606	8.919	19.601
F1 Males – Premating	0.936	2.671	2.872	8.098	4.763	14.145
F1 Females – Premating	1.496	2.775	4.284	8.433	7.381	14.585
F1 Females – Gestation	1.204	1.658	3.607	4.791	6.272	7.562
F1 Females – Lactation	1.695	3.535	5.478	10.333	9.379	18.319

Table B.6.6.1-12
Summary of mean consumption values (mg/kg/day) for DPX-JW062 rat reproduction study

	20 ppm	60 ppm	100 ppm
F0 males, premating (test weeks 1-10)	1.234	3.678	6.079
F0 females, premating (test days 1-71)	1.482	4.302	6.743
F0 females, gestation (GD 7-21)	1.361	4.090	6.769
F0 females, lactation (LD 7-14)	3.041	8.741	14.815
F1 males, premating (test Days 29-148)	1.521	4.584	7.817
F1 females, premating (test Days 29-141)	1.797	5.440	9.337
F1 females, gestation (GD 7-21)	1.434	4.324	6.961
F1 females, lactation (LD 7-14)	2.727	7.920	14.266

2. Reproductive parameters

F0 and F1 Estrous Cycle: No treatment-related or statistically significant effects on estrus, diestrus, or proestrus were observed in F0 or F1 females administered any dietary concentration of the test substance.

F0 and F1 Semen Analyses: No treatment-related or statistically significant effects on sperm motility, epididymal sperm concentration, or sperm morphology were observed in F0 or F1 males administered any dietary concentration of the test substance. Mean epididymal sperm concentration was slightly decreased : 964.7, 844.8, and 852.4×10^6 (per gram caudal tissue) for the 20, 60, and 100 ppm groups as compared to the control value of 1003.1×10^6 . However, these values were not statistically significant, did not exhibit a dose response relationship and were within the laboratory historical

control range (803.3 to 1621.7×10^6 per gram caudal tissue), and was not considered to be test substance-related.

F0 and F1 Reproductive Performance: No test substance-related statistically significant effects were observed on male or female mating, fertility, or fecundity indices, copulatory interval, duration of gestation, or gestation index in F0 or F1 males and females at any dietary concentration. The lower fertility and fecundity indices observed in F1 animals in the 100 ppm group were within the historical control range of the test facility (50-100% and 52-100%, respectively). In addition, since there were no effects on sperm or estrus cyclicity parameters or tissue morphology, the lower fertility and fecundity indices in the 100 ppm F1 animals was considered to be within the normal biological variation of this strain of rats.

F0 and F1 Litter Parameters: No test substance-related effects were observed on the mean number of pups delivered per litter, liveborn per litter, stillborn per litter, number of live pups per litter on lactation days 4, 7, 14, or 21, or survival indices were observed in F0 or F1 animals administered any dietary concentration of the test substance. The lower postculling survival index for the 100 ppm group in the F1 litters was due to a single whole-litter loss and was not observed in the F2 generation. For the F2 litters, the Day 4 viability index for 20, 60, and 100 ppm groups (90.77, 88.81, and 92.29%, respectively) was lower compared to the control value (98.39%) however, a dose-response relationship was not evident and the values were within the historical control range (83.5-100%) for the testing facility.

Table B.6.6.1-13
Summary of F₀ oestrous cycle abnormalities

	0 ppm (Untreated control)		20 ppm		60 ppm		100 ppm	
	No.	(%) ^a	No.	(%) ^a	No.	(%) ^a	No.	(%) ^a
Treated pre-mating								
Long dioestrus ^b	5	(19)	3	(12)	2	(8)	3	(12)
Short dioestrus ^c	4	(15)	4	(15)	7	(27)	12	(46)
Long oestrous ^d	24	(92)	25	(96)	25	(96)	26	(100)
Long pro-oestrus ^e	2	(8)	0	(0)	0	(0)	0	(0)
Treated mating								
Long dioestrus	4	(15)	1	(4)	2	(8)	0	(0)
Short dioestrus	2	(8)	2	(8)	0	(0)	1	(4)
Long oestrous	1	(4)	1	(4)	1	(4)	1	(4)
Long pro-oestrus	1	(4)	1	(4)	0	(0)	0	(0)

^a % Incidence per 26 females per group

^b Long dioestrus = Dioestrus occurring for more than 3 consecutive days

^c Short dioestrus = Dioestrus occurring for less than 2 consecutive days

^d Long oestrous = Oestrous occurring for 2 or more consecutive days

^e Long pro-oestrus = Pro-oestrus occurring for more than one day

Table B.6.6.1-14
Summary of F₁ oestrous cycle abnormalities

	0 ppm (Untreated control)		20 ppm		60 ppm		100 ppm	
	No.	(%) ^a	No.	(%) ^b	No.	(%) ^a	No.	(%) ^a
Treated pre-mating								
Long dioestrus ^c	5	(19)	4	(16)	2	(8)	3	(12)
Short dioestrus ^d	18	(69)	19	(76)	21	(81)	15	(58)
Long oestrus ^e	26	(100)	25	(100)	26	(100)	26	(100)
Treated mating								
Long dioestrus	2	(8)	3	(12)	2	(8)	1	(4)
Short dioestrus	5	(19)	5	(20)	3	(12)	3	(12)
Long oestrus	2	(8)	6	(24)	3	(12)	3	(12)

^a % Incidence per 26 females per group

^b % Incidence per 25 females for this group

^c Long dioestrus = Dioestrus occurring for more than 3 consecutive days

^d Short dioestrus = Dioestrus occurring for less than 2 consecutive days

^e Long oestrous = Oestrous occurring for 2 or more consecutive days

Table B.6.6.1-15
Summary of F₀ male sperm parameter data

	0 ppm (Untreated control)			20 ppm			60 ppm			100 ppm		
	Mean ^a	S.D. ^b	N ^c	Mean ^a	S.D.	N	Mean ^a	S.D.	N	Mean ^a	S.D.	N
Sperm motility %	66.42	10.13	26	65.19	10.94	26	63.88	14.89	26	63.08	11.87	26
Sperm concentration × 10 ⁶ per g caudal tissue	1003.12	376.27	26	964.70	438.54	26	844.75	344.44	26	852.37	314.78	26
Percent abnormal	0.81	1.13	26	1.23	2.90	26	0.77	1.34	26	0.85	2.39	26

^a No statistical significance observed.

^b S.D. = Standard deviation

^c N = Number of animals

Table B.6.6.1-16
Summary of F₁ male sperm parameter data

	0 ppm (Untreated Control)			20 ppm			60 ppm			100 ppm		
	Mean ^a	S.D. ^b	N ^c	Mean ^a	S.D.	N	Mean ^a	S.D.	N	Mean ^a	S.D.	N
Sperm motility %	69.81	8.60	26	68.48	10.40	25	71.80	10.57	25	72.20	9.01	25
Sperm concentration × 10 ⁶ per g caudal tissue	1273.80	271.67	26	1284.61	441.33	26	1296.61	361.64	25	1214.86	294.15	25
Percent abnormal	2.15	2.38	26	2.04	3.55	26	1.60	1.89	25	1.56	2.16	25

^a No statistical significance observed

^b S.D. = Standard deviation

^c N = Number of animals

Table B.6.6.1-17
Summary of F₀ cohabitation data

		0 ppm (Untreated control) ^a	20 ppm ^a	60 ppm ^a	100 ppm ^a
Females placed with males	N	26	26	26	26
Total number inseminated	N	25	26	26	26
Female mating index	%	96.2	100.0	100.0	100.0
Pregnant	N	22	24	23	22
Female fecundity index	%	88.0	92.3	88.5	84.6
Female fertility index	%	84.6	92.3	88.5	84.6
Males placed with females	N	26	26	26	26
Mated	N	25	26	26	26
Male mating index	%	96.2	100.0	100.0	100.0
Females pregnant	N	22	24	23	22
Male fecundity index	%	88.0	92.3	88.5	84.6
Male fertility index	%	84.6	92.3	88.5	84.6
Mating days until Day 0 pc ^b	Mean	3.22	3.92	2.88	3.00
(copulatory interval)	S.D.	2.49	3.39	2.60	1.81

^a No statistical significance observed

^b pc = post-coitus

Table B.6.6.1-18
Natural F₀ adult delivery data and F₁ litter data summary

		0 ppm (Untreated control)	20 ppm	60 ppm	100 ppm
Females on study	N	26	26	26	26
Females with live born	N	22	24	23	22
Gestation index	%	100.0	100.0	100.0	100.0
Duration of gestation (days)	Mean	22.30	21.57	22.13	22.09
	S.D.	0.47	2.00	0.46	0.29
Females with entire live born litter dying and/or missing, cannibalized, culled					
Days 0–21	N	0	0	1	3
	%	0.0	0.0	4.3	13.6
Pups delivered (total)	N	279	314	251	279
Total pups born/litter	Mean	12.68	13.08	10.91	12.68
	S.D.	3.50	2.10	3.90	1.64
	N	22	24	23	22
Liveborn Live birth index	Mean %	98.10	97.89	95.58	97.77
	S.D.	3.89	5.75	6.68	6.41
	N	22	24	23	22
Stillborn	Mean	0.27	0.29	0.39	0.32
	S.D.	0.55	0.75	0.58	0.95
	N	22	24	23	22
Pups surviving 4 days ^b	Mean %	95.81	97.51	97.11	93.36
Viability index	S.D.	6.26	4.19	6.70	9.87
Pups surviving 7 days	Mean %	100.00	100.00	98.91	93.75
	S.D.	0.00	0.00	3.60	22.76
	N	22	24	23	20
Pups surviving 14 days	Mean %	100.00	100.00	98.91	93.75
	S.D.	0.00	0.00	3.60	22.76
	N	22	24	23	20
Pups surviving 21 days Lactation index	Mean %	100.00	100.00	93.94	93.75
	S.D.	0.00	0.00	20.97	22.76
	N	22	24	23	20
Implantation sites per litter	Mean	13.59	14.63	13.04	13.86
	S.D.	3.55	1.53	3.81	1.46
	N	22	24	23	22
Live pups/litter					
Day 0	Mean	12.41	12.79	10.52	12.36
	S.D.	3.38	2.21	4.05	1.56
	N	22	24	23	22
Day 4 Preculling	Mean	11.86	12.46	10.13	11.67
	S.D.	3.33	2.13	3.83	1.98
	N	22	24	23	21
Day 4 Postculling	Mean	7.59	7.96	7.13	7.86
	S.D.	1.30	0.20	1.63	0.36
	N	22	24	23	21
Day 7	Mean	7.59	7.96	7.04	7.40
	S.D.	1.30	0.20	1.61	1.82
	N	22	24	23	20
Pup weight/litter (grams) Day 0	Mean	6.48	6.40	6.51	6.14
	S.D.	0.71	0.54	0.64	0.63
	N	22	24	23	22
Day 4 Preculling	Mean	10.21	9.95	9.70 ^a	8.62 ^b

	S.D.	1.27	1.26	1.39	1.08
	N	22	24	23	21
Day 4 Postculling	Mean	10.24	9.92	9.83	8.68 ^b
	S.D.	1.24	1.35	1.51	1.12
	N	22	24	23	20
Day 7	Mean	16.79	16.31	15.21 ^a	13.72 ^b
	S.D.	1.48	1.98	2.32	1.59
	N	22	24	23	19
Day 14	Mean	33.98	33.62	31.92	30.25 ^b
	S.D.	2.82	3.3	5.55	2.08
	N	22	24	23	19
Day 21	Mean	55.05	54.85	53.71	50.70 ^b
	S.D.	3.93	5.38	5.03	3.76
	N	22	24	22	19
Sex ratio (male pups/total pups)					
Day 0	N	142	171	128	130
	%	52.0	55.7	52.9	47.8
Day 21	N	83	104	81	68
	%	49.7	54.5	50.9	45.9

^a Significantly different from the control group; $p \leq 0.05$

^b Significantly different from the control group; $p \leq 0.01$

Table B.6.6.1-19
Summary of F₁ cohabitation data

		0 ppm (Untreated control) ^a	20 ppm ^a	60 ppm ^a	100 ppm ^a
Females placed with males	N	26	25	26	26
Total number inseminated	N	26	23	25	23
Female mating index	%	100.0	92.0	96.2	88.5
Pregnant	N	22	18	21	15
Female fecundity index	%	84.6	78.3	84.0	65.2
Female fertility index	%	84.6	72.0	80.8	57.7
Males placed with females	N	26	25	25	25
Mated	N	26	23	24	23
Male mating index	%	100.0	92.0	96.0	92.0
Females pregnant	N	22	18	21	15
Male fecundity index	%	84.6	78.3	87.5	65.2
Male fertility index	%	84.6	72.0	84.0	57.7
Mating days until Day 0 pc ^b	Mean	2.96	2.38	2.67	2.65
(copulatory interval)	S.D.	1.76	1.24	1.66	1.75

^a No statistical significance observed

^b pc = post-coitus

Table B.6.6.1-20
Natural F₁ adult delivery data and F₂ litter data summary

		0 ppm (Untreated control)	20 ppm	60 ppm	100 ppm
Females on study	N	26	25	26	26
Females with liveborn	N	22	18	21	15
Gestation index	%	100.0	100.0	100.0	100.0
Duration of gestation (days)	Mean	22.40	22.19	22.20	22.07
	S.D.	0.50	0.54	0.41	0.46
Females with entire liveborn litter dying and/or missing, cannibalized, culled					
Days 0–21	N	0	1	1	0
	%	0.0	5.6	4.8	0.0
Pups delivered (total)	N	274	229	235	209
Total pups born/litter	Mean	12.45	12.72	11.19	13.93
	S.D.	3.61	3.74	4.12	4.03
Liveborn Live birth index	Mean %	97.37	97.79	97.14	95.27
	S.D.	6.59	7.44	4.34	12.87
	N	22	18	21	15
Stillborn	Mean %	0.32	0.28	0.38	0.33
	S.D.	0.78	0.96	0.59	0.62
	N	22	18	21	15
Pup surviving 4 days ^a Viability index	Mean %	98.39	90.77	88.81	92.29 ^b
	S.D.	4.70	23.34	29.92	7.76
	N	22	18	21	15
Pups surviving 7 days	Mean %	98.86	98.53	98.13	98.33
	S.D.	3.68	4.15	6.12	4.40
	N	22	17	20	15
Pups surviving 14 days	Mean %	98.86	98.53	98.13	98.33
	S.D.	3.68	4.15	6.12	4.40
	N	22	17	20	15
Pups surviving 21 days Lactation index	Mean %	98.86	98.53	98.13	97.50
	S.D.	3.68	4.15	6.12	5.18
	N	22	17	20	15
Implantation sites per litter	Mean	13.82	14.06	13.00	14.53
	S.D.	4.11	4.30	3.77	3.76
	N	22	18	21	15

Table B.6.6.1-20
Natural F₁ adult delivery data and F₂ litter data summary (continued)

		0 ppm (Untreated control)	20 ppm	60 ppm	100 ppm
Live pups/litter					
Day 0	Mean	12.14	12.44	10.81	13.60
	S.D.	3.67	3.87	3.93	4.34
	N	22	18	21	15
Day 4 Preculling	Mean	11.91	11.78	10.33	12.47
	S.D.	3.60	3.87	4.44	4.00
	N	22	18	21	15
Day 4 Postculling	Mean	7.68	7.50	6.95	7.60
	S.D.	1.29	1.89	2.36	1.55
	N	22	18	21	15
Day 7	Mean	7.59	7.39	6.81	7.47
	S.D.	1.30	1.88	2.34	1.55
	N	22	18	21	15
Day 14	Mean	7.59	7.39	6.81	7.47
	S.D.	1.30	1.88	2.34	1.55
	N	22	18	21	15
Day 21	Mean	7.59	7.39	6.81	7.40
	S.D.	1.30	1.88	2.34	1.55
	N	22	18	21	15
Pup weight/litter (grams) Day 0	Mean	6.48 (6.42) ^d	6.26 (6.29)	6.47 (6.42)	5.89 (6.04)
	S.D.	0.77	0.64	0.61	0.56
	N	22	18	21	14
Day 4 Preculling	Mean	10.13 (10.04)	9.71 (9.85)	9.95 (9.93)	9.02 (9.02)
	S.D.	1.30	1.38	1.83	1.22
	N	22	17	20	15
Day 4 Postculling	Mean	10.13 (10.06)	9.76 (9.70)	10.00 (10.19)	9.09 (9.03)
	S.D.	1.31	1.37	1.83	1.18
	N	22	17	20	15
Day 7	Mean	16.22 (16.08)	16.01 (15.77)	16.31 (16.72)	15.20 (15.19)
	S.D.	1.40	1.78	3.23	1.61
	N	22	17	20	15
Day 14	Mean	32.74 (32.46)	33.88 (33.22)	33.19 (34.08)	31.74 (31.73)
	S.D.	2.81	3.17	6.10	3.27
	N	22	17	20	15
Day 21	Mean	54.62 (54.16)	55.13 (54.18)	55.19 (56.41)	53.56 (53.68)
	S.D.	4.22	5.30	9.18	5.41
	N	22	17	20	15
Sex ratio (male pups/total pups)					
Day 0	N	137	103	104	102
	%	51.3	46.0	45.8	50.0
Day 21	N	82	66	69	55
	%	49.1	49.6	48.3	49.5

^a Before culling

^b Significantly different from the control group; $p \leq 0.01$

^c No statistical significance observed

^d Values in parentheses are least square means for analysis of covariance

3. Gross pathology, organ weights, and histopathology

An increased incidence of enlarged spleen was observed in the 100 ppm F₁ females only. The mean absolute spleen weights were significantly increased in P₁ 100 ppm males and in P₁ 20, 60 and 100

ppm females. The mean relative spleen weights were also increased for these groups and reached statistical significance in all but the 20 ppm group females. Mean absolute and/or relative spleen weights were also significantly increased in F₁ 100 ppm males and in F₁ 60 and 100 ppm females.

These weight changes were considered to be a possible test substance-related effect in view of similar splenic weight increases in both generation animals. The repeatability of these weight changes between generations and the correlation of macroscopic findings (in 3/26 100 ppm F₁ females only) suggest a possible test substance-related effect, but the potential for marked variations in the weight of the spleen due to variations in the completeness of exsanguination at necropsy and the lack of microscopic correlates (however, it is noted that the spleen was not examined for all animals) make the changes difficult to interpret.

Microscopic examination of the spleen was only carried out for 1 F₀ male, 1 F₀ female, 1 F₁ male and 1 F₁ female of the 60 ppm group, as well as for 1 F₀ male and 3 F₁ females of the 100 ppm group. In these animals, mild to moderate extramedullary hematopoiesis and mild to moderate brown pigmentations were observed. Congestion was also noted in the 3 F₁ females of the 100 ppm group. These microscopic findings are consistent with results of the short- and long-term toxicity studies and are likely to be related to haemolytic anemia (hematology parameters not investigated in this 2-generation study).

The increase in absolute spleen weight at 20 ppm in F₀ females was not considered toxicologically significant in view of the lack of an effect on the relative weight of this organ, the relatively slight magnitude of the effect and the lack of an effect at this dose level in the F₁ animals.

There were no test substance-related microscopic observations in the P₁, F₁ or F₂ animals.

Table B.6.6.1-21
Two-generation reproduction study: Spleen weights, P₁ and F₁ parental rats

Parameter	0 ppm	20 ppm	60 ppm	100 ppm
P₁ Males:				
Absolute spleen weight (% control)	0.79 (control)	0.84 (106)	0.82 (104)	0.89 ^b (113)
Relative ^a spleen weight (% control)	0.141 (control)	0.142 (125)	0.146 (128)	0.160 ^b (140)
Spleen to brain weight (% control)	0.378 (control)	0.410 (108)	0.394 (104)	0.432 (114)
P₁ Females:				
Absolute spleen weight (% control)	0.57 (control)	0.65 ^c (114)	0.76 ^b (133)	0.90 ^b (158)
Relative spleen weight (% control)	0.180 (control)	0.199 (111)	0.247 ^b (137)	0.294 ^b (163)
Spleen to brain weight (% control)	0.297 (control)	0.328 (111)	0.388 (131)	0.469 (158)
F₁ Males:				
Absolute spleen weight (% control)	0.88 (control)	0.85 (96.6)	0.97 (110)	1.00 ^b (114)
Relative spleen weight (% control)	0.153 (control)	0.144 (94.1)	0.158 (103)	0.165 (108)
Spleen to brain weight (% control)	0.438 (control)	0.421 (96.1)	0.471 (108)	0.518 (118)
F₁ Females:				
Absolute spleen weight (% control)	0.58 (control)	0.61 (105)	0.80 ^b (138)	0.92 ^b (159)
Relative spleen weight (% control)	0.187 (control)	0.195 (104)	0.253 ^b (135)	0.291 ^b (156)
Spleen to brain weight (% control)	0.287 (control)	0.307 (107)	0.394 (137)	0.477 (166)

^a Relative weight is defined as the organ to body weight ratio.

^b Significantly different from control by the one-way analysis of variance (ANOVA) criteria, p <0.01.

^c Significantly different from control by the one-way analysis of variance (ANOVA) criteria, p <0.05.

B. F₁ AND F₂ LITTER AND PUP DATA

1. General observations

The mean body weights of F₁ pups in the 60 and 100 ppm groups were decreased in a dose-related manner throughout lactation. These decreases were consistent with the effects on P₁ maternal body weight during the premating and gestation periods and were dose-related, i.e. increasing severity and frequency of weight depression with increasing dose, and therefore were attributed to treatment.

Mean body weights of F₂ pups in the 100 ppm group were slightly reduced at birth and remained reduced throughout the lactation period. However, these differences were not statistically significant. No effects were noted at 20 or 60 ppm. There were no effects on incidences of clinical observations in either F₁ or F₂ pups.

There were no test substance-related or statistically differences noted in the time required to reach of either of the two developmental indices, balanopreputial cleavage or vaginal opening (indices only measured in F₁ control and 100 ppm groups). All animals evaluated for balanopreputial separation met the criteria by day 45 of lactation, the first day of evaluation. Vaginal opening was reached in a mean of 31.7 days for the 100 ppm group, compared with a mean of 31.9 days for the control group.

Table B.6.6.1-22
Summary of F₁ pup developmental indices

			0 ppm (Untreated control)				100 ppm							
Index			Mean day ^{a,b}		S.D. ^c		N ^d		Mean day ^{a,b}		S.D. ^c		N ^d	
Vaginal opening			31.91		1.97		22		31.74		1.82		19	
Cumulative number of offspring positive														
Day of Age														
	21		24		27		30		33		36			
	No. of off-spring	%	No. of off-spring	%	No. of off-spring	%	No. of off-spring	%	No. of off-spring	%	No. of off-spring	%		
Dosage Level														
0 ppm	0	0.0	0	0.0	1	3.8	9	34.6	25	96.2	26	100		
100 ppm	0	0.0	0	0.0	2	7.7	9	34.6	26	100	—	—		
			0 ppm (Untreated Control)					100 ppm						
Balanopreputial cleavage														
Number of pups			25					26						
Pups reaching criteria on Day 45 (%)			100					100						

^a Mean day calculation based on the day all pups in a litter passed a test or exhibited a developmental index

^b No statistical significance observed

^c S.D. = Standard deviation

^d N = Number of litters

3. Gross pathology, organ weights, histopathology

There were no treatment related effects noted for gross pathology, organ weight or histopathology parameters for any F₁ or F₂ litters at any treatment level.

Conclusions from the original DAR (2000, RMS NL):

In F0 and F1 parental animals, significant effects on body weight gain, food consumption and organ weights were observed at ≥60 mg/kg food. The increase in absolute spleen weights observed in 20 mg/kg food F0 female parental animals was not considered toxicologically relevant, since at this dose level the effect was not accompanied by a significant change in relative spleen weights, and was absent in F1 parental animals. Based on these considerations, the NOAEL for parental toxicity was set at 20 mg/kg food, equal to 0.7 mg/kg bw/day. The NOAEL for developmental toxicity was set at 20 mg/kg food, equal to 0.7 mg/kg bw/day, based on significantly decreased body weights and survival index in higher dose groups observed in F1 pups. The test substance did not induce reproduction toxicity.

Conclusions proposed by the applicant (2015):

The NOAELs in the two-generation reproduction study in rats were as follows:

- Parental toxicity: 20 ppm (1.140 mg/kg bw/day for males; 1.361 mg/kg bw/day for females), based on reduced weight gain and food consumption in P₁ males and females at 60 ppm and above and on potential treatment-related changes in spleen weights in F₁ males and females at 60 ppm and above.
- Reproduction and fertility: 100 ppm (5.785 mg/kg bw/day for males; 6.743 mg/kg bw/day for females), as there were no treatment-related observable effects on reproduction or fertility.
- Pup growth and development: 20 ppm, based on the reduced mean pup weights in F₁ pups during lactation at 60 ppm and above. Effects were likely secondary to altered growth and nutrition in the dams.

RMS FR assessment (2016):

The parental NOAEL is 20 ppm equivalent to 1.2 mg/kg bw/d, based on decreased body weight gains and food consumption in F0 females and increased spleen weight in F0 and F1 females at the dose level of 60 ppm equivalent to 3.7 mg/kg bw/d.

The offspring NOAEL is 20 ppm equivalent to 1.2 mg/kg bw/d, based on decreased F1 pup body weights during lactation at the dose level of 60 ppm equivalent to 3.7 mg/kg bw/d.

In the absence of reproductive toxicity in this multigeneration study, the reproductive NOAEL is 100 ppm equivalent to 6.1 mg/kg bw/d.

It is to be noted that the above mentioned mean daily intakes of indoxacarb correspond to mean daily intake during pre-mating period for F0 males as a worst case assumption.

B.6.6.2. Developmental toxicity studies

Pilot developmental toxicity studies in rats

Previous evaluation:	In DAR (2000)
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CA 5.6.2/03

Report: [REDACTED] (1997a); DPX-JW062-112: Pilot developmental toxicity study (no. 2) in rats

DuPont Report No.: HL-1997-01050

Guidelines: Not reported **Deviations:** Not applicable

Testing Facility: [REDACTED]

Testing Facility Report No.: HL-1997-01050

GLP: No

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062 technical
 Lot/Batch #: JW062-112
 Purity: Not stated in the report
 Description: Not stated in the report
 CAS #: Not stated in the report
 Stability of test compound: Not evaluated in this study
2. Vehicle and/or positive control: Undiluted polyethylene glycol
3. Test animals
 Species: Rat
 Strain: CrI:CD (SD)IGS BR female rats (not stated in the report, however, this is the standard strain used at the laboratory)
 Age at initial dosing: Not stated in the report
 Weight at initial dosing: 252.0-310.5 g for females
 Source: Not stated in the report; however, [REDACTED] has historically been the supplier for these studies>
 Acclimation period: Not stated in the report
 Diet: Not stated in the report; however, PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum* has historically been used
 Water: Not stated in the report; however, Tap water, *ad libitum* has historically been used.
 Housing: Not stated in the report; however, historically animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards
4. Environmental conditions
 Temperature: Not reported
 Humidity: Not reported
 Air changes: Not reported
 Photoperiod: Not reported; however, historically alternating 12-hour light and dark cycles are used at the laboratory

B. STUDY DESIGN

1. In-life initiated/completed
 The in-life initiation date was not stated in the report. The completed date is 04-November-1997.
2. Animal assignment and treatment
 In a developmental toxicity study, DPX-JW062 was administered by oral gavage to mated female rats (eight/dose group) on gestation Days 7-21. Doses of 0, 1.5, 3, 6, and 12 mg/kg bw/day were selected for the main study. Animals were assigned to control and experimental groups, using a computerised randomisation procedure designed to produce a homogeneous distribution of body weights across groups within each breeding lot according to standard operating procedures used for these type studies. The test substance was administered in polyethylene glycol (molecular weight 400). The dose volume was not stated in the report; however, a volume of 2 mL/kg bw is typically used for this vehicle, based on the most recent body weight. A negative control group received PEG400 alone.

Group no.	Exposure (mg/kg bw/day)	Number of time-mated females
I	0 ^b	8
II	1.5	8
III	3	8
IV	6	8
V	12	8

3. Dosing suspensions, preparation, and analysis

Although not stated in the report, suspensions of test substance in polyethylene glycol (molecular weight 400) were prepared once and refrigerated until used, based on similarly conducted studies.

4. Statistics

The pregnancy rate data, adult mortality data, and the total resorption data were statistically analysed using Cochran-Armitage test. All litter mean data (except foetal weight and sex ratio) were analysed using Jonckheere's test. Foetal weight and sex ratio data were analysed using linear contrast of least squares from ANCOVA.

C. METHOD

1. Observations

Clinical signs were recorded at least once daily on gestation Days 1 through 22.

2. Body weights

All dams were weighed on gestation Days 1, 7, 9, 11, 13, 15, 17, 19, 21, and 22.

3. Food consumption

Food consumption was measured on gestation Days 1, 7, 9, 11, 13, 15, 17, 19, 21, and 22.

4. Sacrifice and pathology

At termination (gestation Day 22), animals were sacrificed, and gross examinations were performed on all main study animals. The uterus of each pregnant rat was removed and dissected to permit examination of the uterine contents.

5. Reproductive outcomes

The following reproductive parameters were evaluated: pregnancy rate, total resorption rate, mean number of implantations, live foetuses, dead foetuses, corpora lutea counts, and pup sex ratio.

6. Evaluation of foetuses

Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external alterations.

II. RESULTS AND DISCUSSION

A. MATERNAL EFFECTS

1. Clinical signs of toxicity

Clinical observations of salivation (8/8 animals), stained fur, and alopecia (6/8) were significantly increased in the 12 mg/kg bw/d dose level. Salivation was significantly increased in the 6 mg/kg group (6/8), and was seen in 2/8 animals in the 3 mg/kg bw/d dose group. The 1.5 mg/kg bw/d group did not show any dose related clinical signs.

2. Mortality

There was no test substance-related effect on survival. One female rat in the 6 mg/kg bw/d dose group was accidentally killed by gavage trauma.

3. Body weight and body weight gain

The 12 mg/kg and 6 mg/kg bw/d dose groups had significant reductions in maternal body weight and maternal body weight gain. Body weight losses were observed at the beginning of treatment (GD7-9). There were significant test substance-related reductions on body weight gain at the 3 mg/kg bw/d dose group.

4. Food consumption

There were test substance-related reductions on food consumption for animals in the 12 mg/kg bw/d, 6 mg/kg bw/d, and 3 mg/kg bw/d groups.

Table B.6.6.2-1
Prenatal developmental toxicity study in rats: Maternal body weights (g)

Day	0 mg/kg bw/d	1.5 mg/kg bw/d	3 mg/kg bw/d	6 mg/kg bw/d	12 mg/kg bw/d
Day 1	248.4	247.5	249.5	250.4	247.9
Day 7	281.3	281.4	287.6	284.2	284.7
Day 9	288.3	285.7	292.4	284.0	280.8
Day 11	297.9	292.6	297.3	288.8	282.0 ^a
Day 13	305.7	297.6	303.9	288.0 ^a	285.0 ^a
Day 15	311.9	308.3	308.2	297.3	288.5 ^a
Day 17	329.3	328.6	327.7	316.9	304.5 ^a
Day 19	355.4	351.8	354.9	344.0	330.7 ^a
Day 21	390.8	387.2	383.8	372.6	358.9 ^a
Day 22	414.9	403.1	403.4	393.2	372.2 ^a

^a Significant trend (linear contrast of means).

Table B.6.6.2-2
Prenatal developmental toxicity study in rats: Maternal body weight gain/adjusted body weight gain/food consumption

Parameter	0 mg/kg bw/d	1.5 mg/kg bw/d	3 mg/kg bw/d	6 mg/kg bw/d	12 mg/kg bw/d
Body weight gain, gestation Days 7-22(g)	133.6	121.7	115.9	109.0 ^b	87.5 ^b
Adjusted ^a body weight gain, gestation Days 7-22 (g)	33.5	23.5	19.8 ^b	15.2 ^b	-2.5 ^b
Food consumption, gestation Days 7-22 (g/day)	24.3	23.9	23.7	21.9	19.6 ^b

^a Weight change using final body weight minus products of conception

^b Significant trend (linear contrast of means).

5. Reproductive outcomes

There were no adverse, test substance-related effects on reproductive parameters during the course of this study. One 3 mg/kg bw/d dose group female rat was found not pregnant.

Table B.6.6.2-3
Prenatal developmental toxicity study in rats: Maternal reproductive effects

Parameters observed	0 mg/kg bw/d	1.5 mg/kg bw/d	3 mg/kg bw/d	6 mg/kg bw/d	12 mg/kg bw/d
Number pregnant	8	8	7	7	8
Number delivered early	0	0	0	0	0
Number with total resorptions	1	2	1	3	3
Mean corpora lutea	15.9	16.1	15.7	16.0	16.0
Mean implants/Litter	15.3	15.6	15.1	15.1	15.9
Mean resorptions/Litter	0.1	0.5	0.1	0.4	0.9
Mean live foetuses/Litter	15.1	15.1	15.0	14.7	15.0
Mean dead foetuses/Litter	0	0	0	0	0
Foetal sex ratio ^a	8.3/15.1	7.3/15.1	5.6/15.0	6.7/14.7	8.4/15.0

^a Number of male foetuses/total foetuses per litter.

6. Gross pathology

No test substance-related gross lesions were observed at necropsy.

B. FOETAL EFFECTS

The mean foetal weight was significantly reduced in the 12 mg/kg bw/d dose group.

Table B.6.6.2-4
Prenatal developmental toxicity study in rats: Foetal effects

Parameters observed	0 mg/kg bw/d	1.5 mg/kg bw/d	3 mg/kg bw/d	6 mg/kg bw/d	12 mg/kg bw/d
Mean foetal weight (g)	5.23	5.09	5.09	5.07	4.79 ^a

^a Significant trend (linear contrast of least square means from ANCOVA), $p \leq 0.05$

Examinations for external alterations did not reveal any findings.

Conclusions from the original DAR (2000, RMS NL):

The study was not performed in accordance with OECD guideline 414, because e.g. only 8 pregnant females/dose were studied and skeletal and visceral examination of the fetuses was not performed. The study was designed as a pilot study, and is acceptable as such. The study should be considered as supplementary.

The test substance induced maternal toxicity (decreased body weight, body weight gain and food consumption, and clinical signs) at ≥ 3 mg/kg bw/d. Developmental toxicity (decreased fetal weights) was observed in the highest dose group (12 mg/kg bw/d). Therefore, a provisional NOAEL for DPX-JW062 for maternal and developmental toxicity in rats was set at 1.5 and 6 mg/kg bw/day, respectively.

Conclusions proposed by the applicant (2015):

The NOAEL was 1.5 mg/kg bw/day for maternal effects, and 6 mg/kg bw/day for foetal effects. The maternal NOAEL is based on reductions in body weight gain and food consumption at the LOAEL of 3 mg/kg bw/day. The foetal NOAEL is based on reductions in mean foetal weight at the LOAEL of 12 mg/kg bw/day.

RMS FR assessment (2016):

The RMS agrees with the previous conclusions.

Previous evaluation:	In DAR (2000)
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CA 5.6.2/04

Report: [REDACTED] (1997b); DPX-MP062 technical: Pilot developmental toxicity study (no. 2) in rats

DuPont Report No.: HL-1997-01051

Guidelines: Not reported **Deviations:** Not applicable

Testing Facility: [REDACTED]

Testing Facility Report No.: HL-1997-01051

GLP: No

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-------------------------------------|--|
| 1. Test material: | DPX-MP062 technical |
| Lot/Batch #: | 21793-02 |
| Purity: | 94.5% |
| Description: | Not stated in the report |
| CAS #: | Not stated in the report |
| Stability of test compound: | Not evaluated in this study |
| 2. Vehicle and/or positive control: | Polyethylene glycol (molecular weight 400): PEG400 |
| 3. Test animals | |
| Species: | Rat |
| Strain: | CrI:CD BR female rats |
| Age at initial dosing: | Approximately 63 days old upon receipt |
| Weight at initial dosing: | 242.4–309.5 g for females |
| Source: | [REDACTED] |
| Acclimation period: | At least 6 days |
| Diet: | Purina® Certified Rodent Chow®, <i>ad libitum</i> . |
| Water: | Water from United Water Delaware, <i>ad libitum</i> |
| Housing: | Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards |
| 4. Environmental conditions | |
| Temperature: | 23°C ± 1°C |
| Humidity: | 50% ± 10% |
| Air changes: | Not reported |
| Photoperiod: | Alternating 12-hour light and dark cycles |

B. STUDY DESIGN

- In-life initiated/completed
02-October-1996 to 18-October-1996
- Animal assignment and treatment
In a developmental toxicity study, DPX-MP062 Technical was administered by oral gavage to mated CrI:CD BR female rats (eight/dose group) on gestation Days 7–21. Doses of 0, 1.0, 2.0, 4.0, and

8.0 mg/kg bw/day were selected for this pilot study. Animals were assigned to control and experimental groups, using a randomisation procedure designed to produce a homogeneous distribution of body weights across groups within each breeding lot. The test substance was administered in PEG400 at a volume of 2 mL/kg bw based on the most recent body weight. A negative control group received PEG400 alone.

Table B.6.6.2-5
Study design: Prenatal developmental toxicity test by the oral route in the rat

Group no.	Exposure (mg/kg bw/day) ^a	Test formulation concentration (mg/mL)	Number of time-mated females
I	0 ^b	0 (control)	8
II	1.0	0.5	8
III	2.0	1.0	8
IV	4.0	2.0	8
V	8.0	4.0	8

^a Formulations of test substance in PEG400 were administered once daily by oral gavage on Days 7-21G at a dosing volume of 2 mL/kg bw.

^b The control group received PEG400 only at 2 mL/kg.

3. Dosing preparation, and analysis

Suspensions of test substance in PEG400 were prepared once and stored refrigerated until used. The stability and concentration of DPX-MP062 in the dosing solutions were checked by analyses using at the beginning and end of the study. Three sets of samples were taken. The first was from the initial large formulated batch, the second was taken from one of the aliquots taken from the initial large batch, and the third was taken from an aliquot at the end of the study.

4. Statistics

Table B.6.6.2-6
Statistics: Prenatal developmental toxicity test by the oral route in the rat

Parameter	Trend test
Maternal body weight, maternal body weight gain, maternal food consumption	Linear contrast of means
Live foetuses, dead foetuses, resorptions, nidations, <i>corpora lutea</i> , incidence of foetal alterations	Jonckheere's test
Incidence of pregnancy, clinical observations, maternal mortality, females with total resorptions, early deliveries	Cochran-Armitage test
Foetal weight (covariates: litter size, sex ratio), sex ratio (covariate: litter size)	Linear contrast of least square means from ANCOVA

C. METHOD

1. Observations

Clinical signs were recorded with body weights before the study began, each morning on Days 1-22 of gestation and each afternoon on Days 7-21. Clinical signs observed at other times were recorded by exception.

2. Body weights

All dams were weighed on Days 1 and 1, 7, 9, 11, 13, 15, 17, 19, 21 and 22 of gestation.

3. Food consumption

Food consumption was measured on gestation Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 22.

4. Sacrifice and pathology

At termination (gestation Day 22), animals were sacrificed by carbon dioxide inhalation and exsanguinated. Gross examinations were performed on all main study animals. Abdominal and thoracic viscera were examined. The uterus of each pregnant rat was removed and dissected to permit examination of the uterine contents. The intact and the empty uterus of each dam having at least one viable foetus was weighed to permit calculation of maternal body weight adjusted to exclude the products of conception.

5. Reproductive outcomes

The following reproductive parameters were evaluated: pregnancy rate, early delivery rate, total resorption rate, mean number of implantations, live foetuses, dead foetuses, *corpora lutea* counts, and pup sex ratio.

6. Evaluation of foetuses

Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, head, and skeletal malformations and variations.

II. RESULTS AND DISCUSSION

A. MATERNAL EFFECTS

1. Clinical signs of toxicity

Salivation was seen in 2/8 animals at the 2 mg/kg dose level. In the 4 mg/kg bw/d and 8 mg/kg bw/d dose groups, there was a significant increase in the incidence of salivation (7/8 and 8/8 animals respectively), as well as wet (8 mg/kg bw/d group only) and stained fur (both 4 mg/kg bw/d and 8 mg/kg bw/d dose groups).

2. Mortality

There was no effect on survival.

3. Body weight and body weight gain

There were significant test substance-related reductions in body weight gains in the 2, 4, and 8 mg/kg bw/d dose groups. In both the 4 mg/kg bw/d and 8 mg/kg bw/d dose groups, there were also significant reductions in body weights.

4. Food consumption

In the 4 mg/kg bw/d and 8 mg/kg bw/d dose groups, there were significant test substance-related reductions in food consumption.

Table B.6.6.2-7
Prenatal developmental toxicity study in rats: Maternal body weights (g)

Day	0 mg/kg bw/d	1.0 mg/kg bw/d	2.0 mg/kg bw/d	4.0 mg/kg bw/d	8.0 mg/kg bw/d
Day1	242.4	245.1	244.5	247.5	244.3
Day 7	278.3	277.3	279.1	277.4	276.8
Day 9	283.9	280.0	281.8	276.2	274.3
Day 11	289.0	288.0	289.4	282.9	278.2
Day 13	299.0	294.7	297.7	287.6	280.1 ^a
Day 15	308.2	305.5	305.1	291.8 ^a	283.3 ^a
Day 17	324.2	319.4	317.9	304.6 ^a	294.1 ^a
Day 19	356.1	352.3	347.7	328.9 ^a	320.1 ^a
Day 21	386.4	384.5	383.6	355.0 ^a	335.8 ^a
Day 22	408.3	407.0	395.9	371.6 ^a	337.0 ^a

^a Significantly different from control by the linear contrast of means criteria, $p \leq 0.05$.

Table B.6.6.2-8
Prenatal developmental toxicity study in rats: Maternal body weight gain/adjusted body weight gain/food consumption

Parameter	0 mg/kg bw/d	1.0 mg/kg bw/d	2.0 mg/kg bw/d	4.0 mg/kg bw/d	8.0 mg/kg bw/d
Body weight gain, gestation Days 7–22 (g)	129.9	129.8	116.9	94.2 ^b	60.2 ^b
Adjusted ^a body weight gain, gestation Days 7–22 (g)	35.5	34.1	24.2 ^b	6.0 ^b	-22.6 ^b
Food consumption, gestation Days 7–22 (g/day)	24.6	23.6	23.5	20.7 ^b	17.4 ^b

^a Weight change using final body weight minus products of conception

^b Significantly different from control by the linear contrast of means criteria, $p \leq 0.05$.

5. Reproductive outcomes

There were no adverse, test substance-related effects on reproductive parameters during the course of this study.

Table B.6.6.2-9
Prenatal developmental toxicity study in rats: Maternal reproductive effects

Parameters observed	0 mg/kg bw/d	1.0 mg/kg bw/d	2.0 mg/kg bw/d	4.0 mg/kg bw/d	8.0 mg/kg bw/d
Number pregnant	7	8	8	8	7
Number delivered early	0	0	0	0	0
Number with total resorptions	0	0	0	0	0
Mean <i>corpora lutea</i>	15.4	15.4	15.4	15.0	16.1
Mean implants/Litter	15.4	15.0	15.0	14.3	15.9
Mean resorptions/Litter	0.7	0	0.3	0.3	0
Mean live foetuses/Litter	14.7	15.0	14.8	14.0	15.9
Mean dead foetuses/Litter	0	0	0	0	0
Foetal sex ratio ^a	7.9/14.7	8.3/15.0	5.6 ^b /14.8	6.5 ^b /14.0	6.7 ^b /15.9

^a Number of male foetuses/total foetuses per litter.

^b Significant trend (linear contrast of least square means from ANCOVA), $p \leq 0.05$.

6. Gross pathology

No gross lesions were observed at necropsy.

B. FOETAL EFFECTS

Mean foetal weights were significantly reduced in the 8 mg/kg bw/d dose group. No effects on the incidence of foetal malformations or variations were detected.

Table B.6.6.2-10
Prenatal developmental toxicity study in rats: Foetal effects

Parameters observed	0 mg/kg bw/d	1.0 mg/kg bw/d	2.0 mg/kg bw/d	4.0 mg/kg bw/d	8.0 mg/kg bw/d
Mean foetal weight (g)	5.05	5.06	4.91	5.01	4.21 ^a
Developmental variations - Mean % affected	0	0	0	0	0
Variations due to retarded development – Mean % affected	0	0	0	0	0
Malformations - Mean % affected	0	0	0	0.01 [#]	0

^a Significant trend (linear contrast of least square means from ANCOVA), $p \leq 0.05$

[#] one foetus with imperforate anus and absent tail (unable to sex)

Conclusions from the original DAR (2000, RMS NL):

The study was not performed in accordance with OECD guideline 414, because e.g. only 8 pregnant females/dose were studied and skeletal and visceral examination of the fetuses was not performed. The study was designed as a pilot study, and is acceptable as such. The study should be considered as supplementary.

The test substance induced maternal toxicity (decreased body weight, body weight gain and food consumption, and clinical signs) at ≥ 2 mg/kg bw/d. Developmental toxicity (decreased fetal weights) was observed in the highest dose group (8 mg/kg bw/d). Therefore, a provisional NOAEL for maternal and developmental toxicity in rats was set at 1 and 4 mg/kg bw/d, respectively. Based on these findings the dose levels applied in the main study were 0, 0.5, 1, 2, and 4 mg/kg bw/d.

Conclusions proposed by the applicant (2015):

The NOAEL was 1 mg/kg bw/d for maternal effects, and 4 mg/kg bw/d for foetal effects. The maternal NOAEL is based on a decrease in maternal body weight gain and increased incidence of salivation at the LOAEL of 2 mg/kg bw/day. The foetal NOAEL is based on a reduction in mean foetal weight at the LOAEL of 8 mg/kg bw/day.

RMS FR assessment (2016):

The RMS agrees with the previous conclusions.

Teratogenicity test by the oral route in the rat

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.6.2/02

Report: [REDACTED] (2004); Indoxacarb (DPX-KN128) technical: Developmental toxicity study in rats

DuPont Report No.: DuPont-12748

Guidelines: 59 NohSan No. 4200 (1985), Directive 87/302/EEC Part B (1987), U.S. EPA 870.3700 (1998), OECD 414 (1981) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: DuPont-12748

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

- | | |
|-------------------------------------|--|
| 1. Test material: | Indoxacarb technical |
| Lot/Batch #: | KN128-098 |
| Purity: | 95.47% |
| Description: | White solid |
| CAS #: | 173584-44-6 |
| Stability of test compound: | Stable over the duration of the study. |
| 2. Vehicle and/or positive control: | Polyethylene glycol (PEG) 400 |
| 3. Test animals | |
| Species: | Rat |
| Strain: | Crl:CD [®] (SD)IGS BR female rats |
| Age at dosing: | Approximately 63 days old |
| Weight at dosing: | 245.8-299.6 g on gestation day 6 for females |
| Source: | |
| Acclimation period: | Approximately 3 days |
| Diet: | PMI [®] Nutrition International, LLC Certified Rodent LabDiet [®] (#5002), <i>ad libitum</i> . |
| Water: | Tap water, <i>ad libitum</i> |
| Housing: | Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards. |
| 4. Environmental conditions | |
| Temperature: | 18-26°C |
| Humidity: | 30-70% |
| Air changes: | Not recorded |
| Photoperiod: | Alternating 12-hour light and dark cycles |

B. STUDY DESIGN

1. In-life initiated/completed
10-August-2003 to 28-August-2003
2. Animal assignment and treatment
In a developmental toxicity study, indoxacarb was administered by oral gavage to time-mated Crl:CD[®](SD)IGS BR female rats (22/dose group) on gestation Days 6–20. Based on a previously conducted pilot study, doses of 0, 0.5, 1.0, 2.0, and 3.5 mg/kg bw/day were selected. The test substance was administered in PEG at a volume of 2 mL/kg bw based on the most recent body weight. A negative control group received PEG alone.

Table B.6.6.2-11
Study design: Prenatal developmental toxicity test by the oral route in the rat

Group No.	Exposure (mg a.s./kg bw/day) ^a	Test formulation concentration (mg a.s./mL)	Number of time-mated females
I	0 ^b	0 (control)	22
II	0.5	0.25	22
III	1.0	0.5	22
IV	2.0	1.0	22
V	3.5	1.75	22

^a Formulations of test substance in PEG were administered once daily by oral gavage on Days 6–20G at a dosing volume of 2 mL/kg bw.

^b The control group received PEG only at 2 mL/kg.

3. Dosing formulations, preparation and analysis

Formulations of the test substance in PEG were prepared once and stored refrigerated until used. The stability, homogeneity, and concentration of indoxacarb in the dosing formulations were checked by analyses using HPLC. Homogeneity, concentration, and 5-hour stability at room temperature were verified in samples collected near the beginning of the study. Concentration was verified in additional samples collected at the middle, and near the end of study. Based on this information, it can be concluded that the animals received the targeted concentrations of test substance during the study. The analytical method is validated (see Volume 3B5).

4. Statistics

Table B.6.6.2-12
Statistics: Prenatal developmental toxicity test by the oral route in the rat

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Maternal body weight, maternal body weight gain, maternal food consumption, live fetuses, dead fetuses, resorptions, implantations	Levene's test and Shapiro-Wilk test ^a	One-way analysis of variance followed with Dunnett's test	Kruskall-Wallis test followed with Dunn's test
Incidence of pregnancy, clinical observations, maternal mortality, females with total resorptions, early deliveries	None	Cochran-Armitage test for trend	
Incidence of foetal alterations	None	Exact Mann-Whitney with a Bonferroni-Holm adjustment	
Foetal weight (covariates: litter size, sex ratio), Sex ratio (covariate: litter size)	None	Analysis of covariance	Dunnett-Hsu

^a If the Shapiro-Wilk test is not significant, but Levene's test is significant, a robust version of Dunnett's test will be used.

If the Shapiro-Wilk test is significant, Kruskal-Wallis test is followed with Dunn's test.

Significance was judged at $p < 0.05$.

C. METHOD

1. Observations

Clinical signs were recorded once daily and twice daily on Days 6-20G.

2. Body weights

All dams were weighed daily.

3. Food consumption

Food consumption was measured on Days 4, 6, 8, 10, 12, 14, 16, 18, 20, and 21G.

4. Sacrifice and pathology

At termination (Gestation Day 21), animals were sacrificed by carbon dioxide anaesthesia. Gross examinations were performed on all main study animals. Abdominal and thoracic viscera were examined. The uterus of each pregnant rat was removed and dissected to permit examination of the uterine contents.

5. Reproductive outcomes

The following reproductive parameters were evaluated: pregnancy rate, early delivery rate, total resorption rate, mean number of implantations live foetuses, dead foetuses, and corpora lutea counts, and pup sex ratio.

6. Evaluation of foetuses

Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, head, and skeletal malformations and variations.

II. RESULTS AND DISCUSSION

A. MATERNAL EFFECTS

1. Clinical signs of toxicity

No statistically significant or biologically significant changes in the incidence of clinical signs of toxicity were observed for any test concentration.

2. Mortality

There was no test substance-related effect on survival.

3. Body weight and body weight gain, food consumption

Test substance-related reductions in body weight and body weight gain were evident in the 1, 2, and 3.5 mg/kg bw/d groups.

At 1 and 2 mg/kg bw/d, a transient reduction in bodyweight gain occurred on GD6-8 (-62% and -67% compared to the control group respectively). This decrease did not affect mean final body weights but affected the mean adjusted weight gain over the course of the study, although not statistically significantly (-11% and -12% compared to the control group respectively). Considering also the dose-response relationship, it cannot be excluded that this effect is treatment-related and adverse.

At 3.5 mg/kg bw/d, the reduction in weight gain persisted beyond GD6-8 and resulted in mean weight gain over the course of the study that was 10% lower than the control group. When the final body weights were adjusted for the weight of the uterine contents, the mean weight gain for this group for GD6-21 was 28% lower than the control group. These persistent reductions in maternal body weight gain resulted in lower or significantly lower mean maternal body weights beginning on GD10 and persisting for the duration of the study; mean final body weights were 4% lower than the control group.

They were test substance-related reductions in maternal food consumption at 3.5 mg/kg bw/d. These reductions were more pronounced at the beginning of the study, reaching statistical significance on GD6-8, 8-10 and 10-12 (-11% to -13% compared to controls). The mean food consumption over the entire period of the study is 7% lower than the control group, without statistical significance.

Table B.6.6.2-13
Prenatal developmental toxicity study in rats: Mean maternal body weights (g)

Day	0 mg /kg bw/d	0.5 mg /kg bw/d	1 mg /kg bw/d	2 mg /kg bw/d	3.5 mg /kg bw/d
Day 0	241.8	240.4	240.2	241.0	241.5
Day 4	255.8	256.2	256.5	255.0	257.0
Day 6	270.3	269.6	269.8	271.2	269.2
Day 8	278.7	275.8	273.0	274.0	272.0
Day 10	288.4	285.2	283.7	283.7	278.1*
Day 12	300.1	297.7	295.3	296.8	288.5*
Day 14	309.3	306.5	303.3	305.2	298.5
Day 16	327.6	325.9	321.3	322.4	316.0
Day 18	355.4	354.6	349.5	353.8	345.7
Day 20	384.6	383.3	377.9	384.4	372.4
Day 21	408.1	407.3	400.2	407.0	393.8
Day 21C ^a	316.2	313.7	310.8	311.8	302.4*
% control		-0.8%	-1.7%	-1.4%	-4.4%

^a Corrected final maternal body weight = final maternal body weight – gravid uterine weight + empty uterine weight

* Significantly different from control by the Dunnett/Tamhane-Dunnett criteria, p <0.05.

Table B.6.6.2-14
Prenatal developmental toxicity study in rats: Mean maternal body weight gains (g)

Day	0 mg /kg bw/d	0.5 mg /kg bw/d	1 mg /kg bw/d	2 mg /kg bw/d	3.5 mg /kg bw/d
0-4	14.0	15.8	16.3	14.0	15.4
4-6	14.5	13.4	13.4	16.3	12.2
6-8	8.4	6.2	3.2* -62%	2.8* -67%	2.8* -67%
8-10	9.7	9.4	10.7	9.7	6.1
10-12	11.7	12.5	11.6	13.1	10.4
12-14	9.2	8.9	8.0	8.4	10.0
14-16	18.2	19.4	17.9	17.2	17.5
16-18	27.8	28.6	28.3	31.4	29.7
18-21	52.7	52.7	50.7	53.2	48.1
6-21	137.8	137.7	130.4	135.8	124.6
6-21C	45.9	44.1	41.0	40.6	33.2*
% control		-3.9%	-10.7%	-11.5%	-27.7%

^a Corrected final maternal body weight = final maternal body weight – gravid uterine weight + empty uterine weight

* Significantly different from control by the Dunnett/Tamhane-Dunnett criteria, p <0.05.

Table B.6.6.2-15
Prenatal developmental toxicity study in rats:
Maternal body weight gain/adjusted body weight gain/food consumption

Parameter	0 mg /kg bw/d	0.5 mg /kg bw/d	1 mg /kg bw/d	2 mg /kg bw/d	3.5 mg /kg bw/d
Body weights 6-21G (g)	408.1	407.3	400.2	407.0	393.8
% compared to control group		-0.2%	-2%	-0.3%	-4%
Adjusted ^a body weights 6-21G (g)	316.2	313.7	310.8	311.8	302.4*
% compared to control group		-0.8%	-2%	-1%	-4%
Body weight gain 6-21G (g)	137.8	137.7	130.4	135.8	124.6
% compared to control group		-	-6%	-2%	-10%
Adjusted ^a body weight gain 6-21G (g)	45.9	44.1	41.0	40.6	33.2*
% compared to control group		-4%	-11%	-12%	-28%
Food consumption 6-21G (g/day)	24.4	24.8	24.1	24.3	22.8
% compared to control group		+2%	-1%	-	-7%

^a Weight/weight change using final body weight minus products of conception

* Significantly different from control by the Dunnett/Tamhane-Dunnett criteria, $p < 0.05$.

4. Reproductive outcomes and litter data

There was a small, statistically significant ($p = 0.045$) reduction (3.5% lower than control group mean) in mean foetal weight at 3.5 mg/kg bw/day, which was considered possibly test substance-related and secondary to maternal toxicity. There were no other adverse test substance-related effects on reproductive parameters during the course of this study.

Table B.6.6.2-16
Prenatal developmental toxicity study in rats: Maternal reproductive effects

Parameters observed	0 mg /kg bw/d	0.5 mg /kg bw/d	1 mg /kg bw/d	2 mg /kg bw/d	3.5 mg /kg bw/d
Number pregnant	21	22	21	22	22
Number aborted	0	0	0	0	0
Number delivered early	0	0	0	0	0
Number deaths	0	0	0	0	0
Number with total resorptions	0	0	0	0	0
Number litters	21	22	21	22	22
Number live foetuses	268	288	265	293	291
Number dead foetuses	0	0	0	0	0
Number foetal resorptions	9	13	8	5	1
Mean corpora lutea	14.8	15.2	15.0	15.3	15.2
Mean implants/Litter	13.2	13.7	13.0	13.5	13.3
Mean resorptions/Litter	0.4	0.6	0.4	0.2	0.0
Mean live foetuses/Litter	12.8	13.1	12.6	13.3	13.2
Mean dead foetuses/Litter	0	0	0	0	0
Foetal sex ratio	0.52	0.52	0.50	0.56	0.56
Mean foetal weight ^a (g)					
Total	5.74	5.65	5.59	5.70	5.54*
Males	5.89	5.78	5.73	5.85	5.66
Females	5.57	5.50	5.44	5.51	5.39

^a Statistical analyses are only conducted on the total mean foetal weight. The means for males and females are presented for information only. * Statistically significant at $p < 0.05$.

5. Gross pathology

There were no test substance-related gross lesions were observed at necropsy.

B. FOETAL EFFECTS

No test substance-related effect on the incidence of foetal malformations or foetal variations was detected at any dosage.

The incidence of the variation, “supernumerary ribs” was increased in the 3.5 mg/kg bw/day group, as compared to the control incidence. The incidence was not statistically significant, and was within the laboratory historical control range. Furthermore, according to the study author, the supernumerary ribs observation in this group consisted of extra ossification site(s) of pinpoint size, which are not permanent and are generally not considered indicative of developmental toxicity. In addition, the low incidences of rudimentary cervical rib, and retarded ossification of sternebra, were also within the historical control ranges.

Table B.6.6.2-17
Prenatal developmental toxicity study in rats: Foetal effects

Parameters observed	0 mg /kg bw/d	0.5 mg /kg bw/d	1 mg /kg bw/d	2 mg /kg bw/d	3.5 mg /kg bw/d
Mean foetal weight (g)	5.74	5.65	5.59	5.70	5.54*
Developmental variations - Number affected foetuses (litters)	10 (7)	11 (9)	7 (5)	14 (10)	24 (13)
Variations due to retarded development – Number affected foetuses (litters)	55 (14)	98 (20)	49 (17)	63 (18)	59 (16)
Malformations – Number affected foetuses (litters)	1 (1)	5 (3)	1 (1)	0	0

* Statistically significant trend by Cochran-Armitage test, $p < 0.05$

Table B.6.6.2-18
Incidence of foetal variations in the rat developmental study with DPX-KN128

Number of foetuses affected (number of litters affected)					
Observation	0 mg/kg bw/d	0.5 mg/kg bw/d	1.0 mg/kg bw/d	2.0 mg/kg bw/d	3.5 mg/kg bw/d
Rudimentary cervical rib	0	1(1)	1(1)	2(2)	1(1)
Supernumerary rib	10(7)	10(9)	6(4)	12(10)	23(13)
Retarded ossification of sternebra	0	2(2)	1(1)	0	2(1)
Historical control rudimentary cervical rib	1-17 (1-9) ^a	-	-	-	-
Historical control supernumerary rib	3-25 (3-12) ^b	-	-	-	-
Historical control retarded ossification of sternebra	1-50 (1-15)	-	-	-	-

^a Historical control data are presented as the range of minimum – maximum observed number of foetuses per litter (minimum-maximum number of litters) for 78 developmental studies conducted at [REDACTED] (no indication on date of studies and strains of rats)

^b Historical control data from 9 studies conducted at [REDACTED] between 2000 and 2002

Conclusions from the original DAR (2000, RMS NL):

New study in the context of this renewal.

Conclusions proposed by the applicant (2015):

The maternal LOAEL in the prenatal developmental toxicity study in rats was 3.5 mg/kg bw/day based on reduction in body weight and weight gain. The foetal LOAEL was 3.5 mg/kg bw/d based on slight reduction in foetal body weight. The NOAEL was 2 mg/kg bw/d for both maternal and foetal effects.

RMS FR assessment (2016):

The maternal NOAEL is set at 0.5 mg/kg bw/day based on the decreased in body weight gains observed from the dose level of 1 mg/kg bw/d (GD6-8 and overall mean). The developmental NOAEL is 2 mg/kg bw/d, based on decreased fetal weight observed at the dose level of 3.5 mg/kg bw/d.

Previous evaluation:	HL-1997-00202: In DAR (2000) HL-1997-00202 Revision No.2: Submitted for the purpose of renewal (see reasons for revisions below)
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CA 5.6.2/01

Report: [REDACTED] (2005); DPX-MP062 (approximately 75% DPX-KN128, 25% IN-KN127): Developmental toxicity study in rats

DuPont Report No.: HL-1997-00202, Revision No. 2

Guidelines: OECD 414 (1981), USEPA 83-3, 59 Nohsan No. 4200 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HL-1997-00202, Revision No. 2

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

Reasons for Revision Nos. 1 and 2:

1. Clarification of nomenclature regarding the insecticidally active and insecticidally in-active isomer.
2. Additional rationale for selection of dosing vehicle was added.
3. Clarification of interpretation regarding observations considered to be incidental findings.
4. Corrections to reference of the pilot study.
5. Correction of misspelled word.

A. MATERIALS

- | | | |
|----|----------------------------------|---|
| 1. | Test material: | DPX-MP062 technical |
| | Lot/Batch #: | MP062-51A |
| | Purity: | 94.5% |
| | Description: | Off-white solid |
| | CAS #: | None for DPX-MP062 |
| | Stability of test compound: | DPX-MP062-51A consists of approximately 75% DPX-KN128 and approximately 25% IN-KN127. The CAS number for DPX-KN128, the insecticidally active isomer, is 173584-44-6. The test substance was present at target concentrations and was stable for up to 5 hours at room temperature. |
| 2. | Vehicle and/or negative control: | Undiluted polyethylene glycol (PEG400) |
| 3. | Test animals | |
| | Species: | Rat |
| | Strain: | CrI:CD [®] (SD) BR female rats |
| | Age: | 63 days old at arrival, (approximately 76 days old at dosing) |
| | Weight at initial dosing: | 230.9–338.7 g |
| | Source: | [REDACTED] |
| | Acclimation period: | 6 days |
| | Diet: | Purina [®] Certified Rodent Chow [®] (#5002), <i>ad libitum</i> . |
| | Water: | Tap water, <i>ad libitum</i> |
| | Housing: | Animals were housed singly in, stainless steel, wire-mesh cages suspended above cage boards. |
| 4. | Environmental conditions | |
| | Temperature: | 22–24°C |
| | Humidity: | 40–60% |
| | Air changes: | Not recorded |
| | Photoperiod: | Alternating 12-hour light and dark cycles |

B. STUDY DESIGN

1. In-life initiated/completed
09-February-1997 to 09-April-1997

- ## 2. Animal assignment and treatment

In a developmental toxicity study, DPX-MP062 was administered by oral gavage to mated Crl:CD®(SD)BR female rats (25/dose group) on gestation Days 7–21. Based on a range-finding preliminary test, doses of 0.0, 0.5, 1.0, 2.0, and 4.0 mg/kg bw/day were selected for the main study. Animals were assigned to control and experimental groups, using a computerised randomisation procedure designed to produce a homogeneous distribution of body weights across groups within each breeding lot. The test substance was administered in undiluted polyethylene glycol (PEG400) at a volume of 2 mL/kg bw based on the most recent body weight. A negative control group received PEG400 alone.

Table B.6.6.2-19
Study design: Prenatal developmental toxicity test by the oral route in the rat

Group No.	Exposure (mg/kg bw/day) ^a	Test formulation concentration (mg/mL) ^c	Number of time-mated females
I	0 ^b	0 (control)	25
II	0.5	0.25	25
III	1.0	0.5	25
IV	2.0	1.0	25
V	4.0	2.0	25

^a Formulations of test substance in PEG400 were administered once daily by oral gavage on Days 7–21G at a dosing volume of 2 mL/kg bw.

^b The control group received PEG400 only at 2 mL/kg.

^c Adjusted for purity of 94.5%.

3. Dosing solutions, preparation, and analysis

Solutions of test substance in PEG400 were prepared once and stored refrigerated until used. The stability, homogeneity, and concentration of DPX-MP062 in the dosing solutions were checked by analyses using HPLC. The test substance was at target concentrations $\pm 10\%$, homogeneous (92.8% to 103.0%), and stable (88.0% to 100.0% of nominal) for up to 5 hours at room temperature. Based on this information, it can be concluded that the animals received the targeted concentrations of test substance during the study.

4. Statistics

Table B.6.6.2-20
Statistics: Prenatal developmental toxicity test by the oral route in the rat

Parameter	Trend Test
Maternal body weight Maternal body weight gain Maternal food consumption	Linear contrast of means
Live foetuses Dead foetuses Resorptions Implantations Corpora lutea Incidence of foetal alterations	Jonckheere's test
Incidence of pregnancy Clinical observations Maternal mortality Females with total resorptions Abortions/early deliveries	Cochran-Armitage test
Foetal weight (covariates: litter size, sex ratio) Sex ratio (covariate: litter size)	Linear contrast of least square means

Significance was judged at $p \leq 0.05$.

If a significant dose-response was detected, data from the top dose group were excluded and the test repeated until no significant trend was detected.

For litter parameters, the proportion of affected foetuses per litter or the litter mean was the experimental unit for statistical evaluation. Where the data were tied and the standard large sample version of Jonckheere's test was not applicable, exact p values were calculated using permutation methodology.

C. METHODS

1. Observations

Clinical signs were recorded once daily on Days 1-6G and Day 22 and twice daily on Days 7-21G.

2. Body weights

Prior to the start of dosing, females were weighed at least twice. During the dosing period (Gestation day 7-22), all dams were weighed daily.

3. Food consumption

Food consumption was measured on Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 22G.

4. Sacrifice and pathology

At termination (gestation Day 22), animals were sacrificed by carbon dioxide anaesthesia. Gross examinations were performed on all main study animals. Abdominal and thoracic viscera were examined. The uterus of each pregnant rat was removed and dissected to permit examination of the uterine contents, and weighed.

5. Reproductive outcomes

The following reproductive parameters were evaluated: pregnancy rate, early delivery rate, total resorption rate, mean number of implantations, live foetuses, dead foetuses, corpora lutea counts, and pup sex ratio.

6. Evaluation of foetuses

Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, head, and skeletal malformations and variations.

II. RESULTS AND DISCUSSION

A. FINDINGS

Findings from the developmental toxicity study with DPX-MP062 in rats are summarised in the table below.

No dose-related mortality occurred during the study although 2 rats from the 4 mg/kg bw/d group died prior to scheduled sacrifice. One rat was found dead on GD18 and was observed to have sustained injuries consistent with gavage trauma at the post-mortem evaluation. One rat was sacrificed on GD16 and was observed to have bilateral hydronephrosis at the gross post-mortem examination. This condition is not believed to be treatment-related. This is supported by the fact that there was no mortality seen in the pilot study in animals dosed at levels up to 8 mg/kg bw/d.

Reduced maternal body weight gains were observed at the beginning of the treatment period in all tested groups but the statistical significance is reached at the highest tested dose only (maternal body weight changes during GD7-9 are 6.9, 4.0, 4.1, 3.5, -5.2 grams in the 0, 0.5, 1, 2 and 4 mg/kg bw/day groups respectively). Furthermore, this had no impact on the overall (adjusted or not) maternal body weight gains (GD7-22) in the 0.5, 1 and 2 mg/kg bw/d groups (+3%, +8%, -9% compared to controls respectively). Dams in the 4 mg/kg group had adjusted body weight loss over the course of the dosing period (adjusted body weight changes are 32.4, 33.4, 35.0, 29.3 and -0.2 grams in the 0, 0.5, 1, 2 and 4 mg/kg bw/day groups respectively). Similarly, foetal body weights were slightly statistically significantly decreased in the 4 mg/kg/day dose group (mean fetal weights of 5.12, 5.10, 5.20, 5.01 and 4.79 grams in the 0, 0.5, 1, 2 and 4 mg/kg bw/day groups respectively).

Alopecia was present at 1 mg/kg/day and above (incidences of 4, 6, 9, 10, 10 out of 25 females in the 0, 0.5, 1, 2 and 4 mg/kg bw/day groups respectively). However, although clearly dose-related (the incidence and the overall extent of alopecia increased with increasing dose), in the absence of other evidence of primary dermatotoxicity, this finding was not considered biologically adverse. According to the study author, close

examination of the individual animal data suggests that this alopecia may actually be the result of barbering and grooming.

Table B.6.6.2-21
Summary of the results of the developmental toxicity study in rats with DPX-MP062

Dosage	Mean daily intake: 0	0.5	1.0	2.0	4.0 mg/kg bw/day
Analytical	Measured concentrations of DPX-MP062 in dosing solutions indicated that the solutions were at the expected levels, ranging from 90 to 103% of nominal. The dosing solutions were stable as concentrations were 88 to 100% of nominal after holding the samples at room temperature for 5 hours. Analysis indicated that the test substance was distributed homogeneously in the dosing solutions.				
Dose Group (mg/kg/day)		Results			
Maternal effects					
General observations	>1.0:	alopecia			
Mortality	nad ^a				
Body weight change	4.0:	decreased ^b over Days 7-22 (34%)			
Adjusted ^c body weight change	4.0:	adjusted body weight loss over Days 7-22			
Food consumption	4.0:	decreased over Days 7-22 (18%)			
Reproductive outcomes ^d	nad				
Gross pathology	nad				
Foetal effects					
Mortality	nad				
Body weights	4.0:	slight but statistically significant decrease (6%)			
Malformations	nad	See below			
Variations	nad	See below			

^a nad = no abnormalities detected that were considered test substance-related and/or biologically adverse

^b All increases or decreases are relative to controls unless otherwise noted.

^c Calculated using final body weights minus the weight of the products of conception

^d Pregnancy rate, abortion rate, early delivery rate, total resorption rate, mean corpora lutea, mean number of implantations and live foetuses, and sex ratio

There was a statistically significant increase in the incidence of wavy ribs at 4 mg/kg bw/day. The reported incidences in foetuses/litters were 0/0, 0/0, 0/0, 1/1, 2/2 in the 0, 0.5, 1, 2 and 4 mg/kg bw/day groups respectively. According to the study author, the biological significance of this finding is questionable given the low incidence relative to historical control values for this finding. Indeed, the study author reported incidences for 15 studies conducted from 1991-1996: “this finding occurred in anywhere from 0 to 8 control group foetuses from 0 to 2 litters”. Therefore this finding is not considered to be treatment related.

Anophthalmia was reported in one foetus from one litter at 4 mg/kg/day and microphthalmia was reported in a separate foetus from a different litter for a total of 2 foetuses from 2 litters with these eye malformations in the 4.0 mg/kg bw/day group. The incidence for either of these findings in control groups was 2 foetuses from 2 litters. Therefore, the study incidence is within the range of test facility historical control data.

Table B.6.6.2-22
Incidence of foetal malformations in the rat developmental study with DPX-MP062

Number of foetuses affected (number of litters affected)					
Observation	0 mg/kg bw/d	0.5 mg/kg bw/d	1.0 mg/kg bw/d	2.0 mg/kg bw/d	4.0 mg/kg bw/d
Anophthalmia	0	0	0	0	1(1)
Microphthalmia	0	0	1(1)	0	1(1)
Historical control anophthalmia	1-2 (1-2) ^a	-	-	-	-
Historical control microphthalmia	1-2 (1-2)	-	-	-	-

^a Historical control data are presented as the range of minimum – maximum observed number of foetuses per litter (minimum-maximum number of litters) for 78 developmental studies conducted at [REDACTED] (no indication on date of studies and strains of rats)

Conclusions from the original DAR (2000, RMS NL):

Although the incidence and severity of hair loss displayed a clear dose-response relationship, it was not considered toxicologically relevant. There was no clear pattern in affected body locations. Therefore, it was concluded that the test substance induced maternal (decreased body weights and food consumption) and developmental toxicity (decreased fetal weights) in the highest dose group. Therefore, the NOAEL for maternal as well as developmental toxicity in rats was set at 2 mg/kg bw/day. The test substance was not teratogenic.

Conclusions proposed by the applicant (2015):

The no-observed-adverse-effect level (NOAEL) was 2 mg/kg bw/d for both maternal and foetal effects. The maternal NOAEL was based on decreased maternal weight changes and food consumption relative to controls at 4 mg/kg. The foetal NOAEL was based on decreased foetal weight at 4 mg/kg bw/d. DPX-MP062 was not teratogenic and was not uniquely toxic to the rat conceptus.

RMS FR assessment (2016):

The RMS agrees with the previous conclusions. The maternal NOAEL is set at 2 mg/kg bw/d based on decreased body weights, body weight gains and food consumption at 4 mg/kg bw/d. The developmental NOAEL is 2 mg/kg bw/d based on decreased fetal weights at the LOAEL of 4 mg/kg bw/d.

Previous evaluation:	In DAR (2000)
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CA 5.6.2

Report: [REDACTED] (1997); DPX-JW062-112 (50% DPX-KN128, 50% DPX-KN127): Developmental toxicity study in rats

DuPont Report No.: HL-1997-00049

Guidelines: OECD 414, USEPA 83-3, 59 Nohsan No. 4200 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HL-1997-00049

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|----------------------------------|--|
| 1. | Test material: | DPX-JW062 technical |
| | Lot/Batch #: | JW062-112 |
| | Purity: | 94.76% |
| | Description: | Not provided in the report |
| | CAS #: | 144171-61-9 |
| | Stability of test compound: | The test substance was present at target concentrations and was stable for up to 5 hours at room temperature. Dosing solutions/suspensions were prepared daily |
| 2. | Vehicle and/or positive control: | 0.5% methylcellulose |
| 3. | Test animals | |
| | Species: | Rat |
| | Strain: | CrI:CD [®] (SD)BR female rats |
| | Age:: | 63 days on the day after arrival |
| | Weight at initial dosing: | 236.6–317.5 g |
| | Source: | |
| | Acclimation period: | 6 days |
| | Diet: | Purina [®] Certified Rodent Chow [®] (#5002), <i>ad libitum</i> |
| | Water: | Tap water, <i>ad libitum</i> |
| | Housing: | Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards. |
| 4. | Environmental conditions | |
| | Temperature: | 23 ± 1°C |
| | Humidity: | 50 ± 10% |
| | Air changes: | Not recorded |
| | Photoperiod: | Alternating 12-hour light and dark cycles |

B. STUDY DESIGN

1. In-life initiated/completed
17-November-1996 to 14-March-1997

- ## 2. Animal assignment and treatment

In a developmental toxicity study, DPX-JW062 was administered by oral gavage to mated Crl:CD®(SD)BR female rats (25/dose group) on gestation Days 7–21. Doses of 0, 10, 100, 500, and 1000 mg/kg bw/day were selected for the main study. Animals were assigned to control and experimental groups, using a computerised randomisation procedure designed to produce a homogeneous distribution of body weights across groups within each breeding lot. The test substance was administered in 0.5% methylcellulose at a volume of 10 mL/kg bw based on the most recent body weight. A negative control group received 0.5% methylcellulose alone.

Table B.6.6.2-23
Study design: Prenatal developmental toxicity test by the oral route in the rat

Group no.	Exposure (mg/kg bw/day) ^a	Test formulation concentration (mg/mL) ^b	Number of time-mated females ^c
I	0 ^d	0.0 (control)	25
II	10	1.0	25
III	100	10.0	25
IV	500	50.0	25
V	1000	100.0	25

^a Formulations of test substance in 0.5% methylcellulose were administered once daily by oral gavage on Days 7–21G at a dosing volume of 10 mL/kg bw.

^b Adjusted purity for 94.76%.

^c Copulation confirmed.

^d Vehicle only (0.5% methylcellulose).

3. Dosing suspensions, preparation, and analysis

Suspensions of test substance in 0.5% methylcellulose were prepared daily. The stability, homogeneity, and concentration of DPX-JW062 in the dosing suspensions were checked by analyses using HPLC at the beginning of the study. The concentration of DPX-JW062 in the dosing suspensions was also checked at the middle and end of the study. The test substance was at target concentrations (77.1–107.6%), homogeneous (CV = 1.1–3.2%), and stable (84.9–96.7% of nominal) for up to 5 hours at room temperature. Based on this information, it can be concluded that the animals received the targeted concentrations of test substance during the study.

4. Statistics

Sequential trend testing was applied to the developmental toxicity data for each parameter as tabulated below. If a significant dose-response was detected, data from the top dose group was excluded and the test repeated until no significant trend was detected. For litter parameters, the proportion of affected fetuses per litter or the litter mean was the experimental unit for statistical evaluation. The level of significance selected was $p < 0.05$.

Table B.6.6.2-24
Statistics: Prenatal developmental toxicity test by the oral route in the rat

Parameter	Trend test
Maternal body weight, maternal body weight gain, maternal food consumption	Linear contrast of means
Live fetuses, dead fetuses, resorptions, implantations, corpora lutea, incidence of foetal alterations	Jonckheere's test
Incidence of pregnancy, clinical observations, maternal mortality, females with total resorptions, abortions/early deliveries	Cochran-Armitage test
Foetal weight (covariates: litter size, sex ratio), sex ratio (covariate: litter size)	Linear contrast of least square means

Where the data were tied and the standard large sample version of Jonckheere's test was not applicable, exact p values were calculated using permutation methodology.

C. METHOD

1. Observations

Clinical signs were recorded once daily on Days 1–6G and twice daily on Days 7–21G.

2. Body weights

All dams were weighed on Days 1 and 7–22G.

3. Food consumption

Food consumption was measured on Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 22G.

4. Sacrifice and pathology

At termination (gestation Day 22), animals were sacrificed by carbon dioxide asphyxiation. Gross examinations were performed on all main study animals. Abdominal and thoracic viscera were examined. The uterus of each pregnant rat was removed and dissected to permit examination of the uterine contents.

5. Reproductive outcomes

The following reproductive parameters were evaluated: pregnancy rate, early delivery rate, total resorption rate, mean number of implantations, live foetuses, dead foetuses, corpora lutea counts, and pup sex ratio.

6. Evaluation of foetuses

Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, head, and skeletal malformations and variations.

II. RESULTS AND DISCUSSION

A. MATERNAL FINDINGS

1. Mortality

Significant mortality was produced by dose levels of 100 mg/kg bw/d and higher. Of the 25 animals assigned to each group: 4, 17, and 15 rats died prior to scheduled sacrifice on GD 15 to 21 from the 100, 500, and 1000 mg/kg groups, respectively.

2. Body weights and weight changes

Among the rats which survived until scheduled sacrifice, there were significant reductions in mean maternal body weights, weight changes, adjusted final body weights, and weight changes calculated using the adjusted final weight at 100 mg/kg/day and above. At 10 mg/kg bw/d, there were no effects on any maternal weight parameter; all values were comparable to the control group values.

At 100 mg/kg bw/d, weight changes were reduced over Days 7–9, 13–15, 17–19, 19–21, 21–22, and 7–22G. In addition to those listed for the 100 mg/kg bw/d group, there was a significant reduction over Days 9–11G at 500 mg/kg bw/d. At 1000 mg/kg bw/d, weight changes were significantly reduced at every measured interval over the entire dosing period and when averaged over the entire dosing period (Days 7–22G).

At 100 mg/kg bw/d and above, weight changes calculated using the final body weight adjusted for the products of conception were significantly reduced (Days 1–22 and 7–22G).

At 100 mg/kg bw/d, maternal body weights including the adjusted final weights were significantly reduced starting on Day 17G and persisting for the remainder of the study. At 500 mg/kg bw/d, body weights were significantly reduced for the dosing period starting on Day 13G. At 1000 mg/kg bw/d, this reduction was seen on Day 11G and again, persisted for the duration of the study.

3. Food consumption

Significant, dose-related reductions in mean maternal food consumption were observed at 100 mg/kg bw/d and above. At 500 and 1000 mg/kg, all measured intervals during the dosing period were affected (Days 7–9, 9–11, 11–13, 13–15, 15–17, 17–19, 19–21, 21–22, and 7–22G). At 100 mg/kg bw/d, all intervals except for Days 9–11G were affected. At 10 mg/kg, there was one interval during which food consumption was statistically significantly reduced (Days 11–13G). This reduction was

not considered adverse; nor was it considered toxicologically relevant. The magnitude of change relative to the control group was very small (21.1 g for the 10 mg/kg group versus 22.7 g for the control group or 93% of the control group value). In addition, there was no other corroborative evidence of maternal toxicity at 10 mg/kg bw/d.

4. Clinical observations

Clinical signs indicative of toxicity were significantly increased at 100 mg/kg bw/d and above. At 1000 mg/kg bw/d, the following observations were significantly increased: abnormal gait/mobility, alopecia, hunched over posture, stained fur (perinasal, periocular, perioral, and perineal areas), general weakness, and inability to stand. At 500 mg/kg bw/d, all of these observations were significantly increased except for stained fur (periocular area), and inability to stand. Despite the lack of statistical significance, there were occurrences of these findings at 500 mg/kg bw/d which were most likely dose-related. At 100 mg/kg bw/d, the incidence of animals with abnormal gait/mobility was significantly increased. In addition, there were non-statistically significantly increased occurrences of alopecia, hunched over posture, general weakness; these findings are likely to be toxicologically relevant as well. There were no dose-related adverse clinical observations seen at 10 mg/kg bw/d.

5. Postmortem findings

The gross postmortem examinations revealed signs of toxicity at 100 mg/kg bw/d and above, especially among those animals which died prior to scheduled sacrifice. For affected animals, the entire digestive tract or portions of the digestive tract (including the stomach, small and/or large intestine) were the most severely affected. Typical observations that were recorded included: distended condition, unusual contents (white, yellow, or orange-coloured pasty contents), haemorrhage or ulcerated areas, and lack of formed faeces. There were no dose-related, adverse postmortem findings seen at 10 mg/kg bw/d.

B. REPRODUCTIVE EFFECTS

There were no compound-related effects at any dose level on the following reproductive outcome parameters: dams with either total resorptions or that delivered early, or the mean number of implantations.

The mean number of live foetuses per litter was significantly reduced at 100 mg/kg bw/d and above. This observation is likely to be indicative of decreased foetal viability.

C. FOETAL FINDINGS

1. Mortality

There were no statistically significant effects indicative of embryonic or foetal mortality (resorptions, or dead foetuses). However, at 1000 mg/kg bw/d, the incidence of resorptions per litter, especially late resorptions, was slightly increased.

2. Body weight

Mean foetal weight was markedly and significantly reduced at 500 and 1000 mg/kg bw/d and unaffected at 100 and 10 mg/kg bw/d.

3. Malformations

There were no compound-related effects on the incidence of foetal malformations.

4. Variations

There were no compound-related effects on the incidence of foetal variations.

Conclusions from the original DAR (2000, RMS NL):

The NOAEL for maternal toxicity was set at 10 mg/kg bw/day, based on decreased body weights, food consumption, clinical signs and macroscopic abnormalities occurring at ≥ 100 mg/kg bw/day. Based on decreased

numbers of live fetuses in the ≥ 100 mg/kg bw/day groups, the NOAEL for developmental toxicity was also set at 10 mg/kg bw/day. The test substance did not induce teratogenic effects.

Conclusions proposed by the applicant (2015):

Under the conditions of this study, maternal and developmental toxicity were observed at 100 mg/kg bw/d and above. The maternal and developmental no-observed-effect level (NOEL) was 10 mg/kg bw/d. Thus, the results of this study indicate that DPX-JW062 is not uniquely toxic to the rat conceptus.

RMS FR assessment (2016):

The NOAEL for maternal and developmental toxicity was set at 10 mg/kg bw/d based on findings noted at 100 mg/kg bw/d and above: decreased body weight, body weight gains and food consumption, clinical signs, mortality and GI tract macroscopic findings in females and decreased mean number of live foetuses per litter. At higher dose levels, mean foetal weight was decreased. The test substance is not teratogenic in this study.

Teratogenicity test by the oral route in the rabbit

Previous evaluation:	In DAR (2000)
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CA 5.6.2

Report: [REDACTED] (1995); Developmental toxicity study of DPX-JW062-112 in rabbits

DuPont Report No.: HLR 587-95

Guidelines: OECD 414, USEPA 83-3, 59 Nohsan No. 4200, Directive 87/302/EEC **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 587-95

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-JW062 technical
 Lot/Batch #: JW062-112
 Purity: 94.76%
 Description: Off-white solid milled into a fine powder
 CAS #: 144171-61-9
 Stability of test compound: The test substance was present at target concentrations and was stable for up to 5 hours at room temperature. Dosing solutions/suspensions were prepared daily
2. Vehicle and/or positive control: 0.5% Aqueous methylcellulose, 1% Tween[®] 80 (except for dosing days 1 and 2, which did not contain the 1% Tween[®] 80)
3. Test animals
 Species: Rabbit
 Strain: Hra:(NZW)SPF female rabbits
 Age at initial dosing: Young adult, approximately 184-193 days old based on age at arrival of 180 or 187 days old.
 Weight at Gestation day 0: 2913–4128 g
 Source: XXXXXXXXXX
 Acclimation period: 4 days
 Diet: Purina[®] Certified Rabbit Chow[®] (#5325), approximately 150 g/day
 Water: Tap water, *ad libitum*
 Housing: Animals were housed singly in wire-mesh, stainless steel cages suspended above cage boards.
4. Environmental conditions
 Temperature: 20 ± 1°C
 Humidity: 50 ± 10%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed
 15-May-1995 to 13-September-1995

2. Animal assignment and treatment

In a developmental toxicity study, DPX-JW062 was administered by oral gavage to mated Hra:(NZW)SPF female rabbits (23/dose group) on gestation Days 7–28. Based on three range-finding studies, doses of 0, 250, 500, or 1000 mg/kg bw/day were selected for the main study. Animals were assigned to control and experimental groups, using a computerised randomisation procedure designed to produce a homogeneous distribution of body weights across groups within each breeding lot. The test substance was administered in 0.5% methylcellulose, 1% Tween[®] 80 at a volume of 10 mL/kg bw based on the most recent body weight. A negative control group received 0.5% methylcellulose, 1% Tween[®] 80 alone. Tween[®] 80 was added to the dosing solution beginning dosing day 3.

Table B.6.6.2-25
Study design: Prenatal developmental toxicity test by the oral route in the rabbit

Group no.	Exposure (mg/kg bw/day) ^a	Test formulation concentration (mg/mL) ^b	Number of time-mated females
I	0 ^c	0.0 (control)	23
II	250	25.0	23
III	500	50.0	23
IV	1000	100.0	23

^a Formulations of test substance in 0.5% methylcellulose, 1% Tween[®] 80 were administered once daily by oral gavage on Days 7–28G at a dosing volume of 10 mL/kg.

^b Adjusted for purity.

^c Vehicle only (0.5% methylcellulose, 1% Tween[®] 80).

3. Dosing suspensions, preparation, and analysis

Suspensions of test substance in 0.5% methylcellulose, 1% Tween[®] 80 were prepared daily. Tween[®] 80 was added to the dosing solution beginning dosing Day 3. The stability, homogeneity, and concentration of DPX-JW062 in the dosing suspensions were checked by analyses using HPLC at beginning of study. The results of these analyses indicated that the test material was not homogeneously distributed throughout the vehicle. Therefore, on the third and all subsequent dosing days, 1% Tween[®] 80 was added to the vehicle to aid in dispersing the test material. To ensure that this change in vehicle addressed the homogeneity issue, samples were taken from the fourth day's batch of dosing suspensions. Additional analyses for concentration, homogeneity, and stability confirmed that the homogeneity problem had been resolved. For the third and fourth samplings, analyses addressed concentration. The test substance was at target concentrations (90.6–115%), homogeneous (CV = 1.4–3.5%), and stable (67.6–124% of nominal) for up to 5 hours at room temperature. Based on this information, it can be concluded that the animals received the targeted concentrations of test substance during the study.

4. Statistics

Sequential trend testing was applied to the data for each parameter as tabulated below. If a significant dose-response was detected, data from the top dose group was excluded and the test repeated until no significant trend was detected. For litter parameters, the proportion of affected fetuses per litter or the litter mean was the experimental unit for statistical evaluation. The level of significance selected was $p \leq 0.05$.

Table B.6.6.2-26
Statistics: Prenatal developmental toxicity test by the oral route in the rabbit

Parameter	Trend test
Maternal body weight, maternal body weight gain, maternal food consumption	Linear contrast of means from ANOVA
Live fetuses, dead fetuses, resorptions, nidations, corpora lutea, incidence of foetal alterations	Jonckheere's test
Incidence of pregnancy, clinical observations, maternal mortality, incidence of abortion, females with total resorptions, early deliveries	Cochran-Armitage test
Foetal weight (covariates: litter size, sex ratio), sex ratio (covariate: litter size)	Linear contrast of least square means from ANOVA

Where the data were tied and the standard large sample version of Jonckheere's test was not applicable, exact p values were calculated using permutation methodology.

C. METHODS

1. Observations

Clinical signs were recorded once daily on Days 4–6 and twice daily on Days 7–29G.

2. Body weights

All dams were weighed on Days 4 and 7–29G.

3. Food consumption

Food consumption was measured on Days 4–29G.

4. Sacrifice and pathology

At termination, animals were sacrificed by intravenous injection of an approved commercial euthanasia solution and exsanguination. Gross examinations were performed on all main study animals. Abdominal and thoracic viscera were examined. The uterus of each pregnant rabbit was removed and dissected to permit examination of the uterine contents.

5. Reproductive outcomes

The following reproductive parameters were evaluated: pregnancy rate, early delivery rate, total resorption rate, live foetuses, dead foetuses, corpora lutea counts, and pup sex ratio.

6. Evaluation of foetuses

Parameters evaluated in foetuses were body weight, incidences of dead foetuses and/or foetal resorptions, and incidences of external, visceral, head, and skeletal malformations and variations.

II. RESULTS AND DISCUSSION

A. MATERNAL EFFECTS

1. Mortality

There was no compound-related mortality in this study.

However, a total of five animals died prior to scheduled sacrifice from dosing-related injuries (gavage trauma). The distribution across dose groups was 0, 1, 2, and 2 from the 0, 250, 500, and 1000 mg/kg bw/day groups, respectively.

2. Body weight and weight changes

Mean maternal body weight changes were significantly reduced at 1000 mg/kg/day over Days 21–23G and when averaged over the entire dosing period (Days 7–29G, -27% compared to controls). Weight changes were unaffected at 500 mg/kg bw/day and lower.

No dose-related effects were detected at any dose level on maternal body weights, maternal adjusted body weights, or on weight changes calculated using this adjusted weight.

3. Food consumption

Mean maternal food consumption was significantly reduced at 1000 mg/kg bw/day over Days 13–15, 15–17, 21–23, 23–25G, and when averaged over the entire dosing period (Days 7–29G, -6.3% compared to controls).

No dose-related effects on maternal food consumption were observed at 500 mg/kg bw/day or lower.

4. Clinical observations

The incidence of green-coloured stools was significantly increased for the 1000 mg/kg bw/day group over Days 7–29G. Otherwise, there were no dose-related increases in the incidence of any clinical observation.

5. Postmortem findings

There were no compound-related increases in the incidence of any gross postmortem finding.

B. REPRODUCTIVE EFFECTS

There were no compound-related effects on reproductive parameters (pregnancy rate, abortion rate, early delivery rate, total resorption rate, mean corpora lutea, mean number of implantations and live fetuses, and sex ratio) at any level.

C. FOETAL FINDINGS

1. Mortality

No dose-related increases in foetal mortality were observed. The incidence of early, late, or total resorptions was comparable across dose groups. There were no dead fetuses.

2. Body weight

Mean foetal weight was significantly reduced at 1000 mg/kg bw/day (-9.6% compared to control group). No effect on foetal weight was observed at 500 mg/kg bw/day or lower.

3. Malformations

No compound-related effect on the incidence of any foetal malformation was detected.

4. Variations

There was a significant dose-related increase in the incidence of retarded sternebral ossification at 1000 mg/kg bw/day (number of fetuses (litters) affected: 35(10), 32(11), 20(9) and 44(16)* in the control, 250, 500 and 1000 mg/kg bw/day groups respectively). No compound-related effect on the incidence of any foetal variation was detected at 500 mg/kg bw/day or lower.

Conclusions from the original DAR (2000, RMS NL):

The test substance induced maternal (decreased body weights and food consumption) and developmental toxicity (decreased fetal weights and retarded sternebral ossification) in the highest dose group. Therefore, the NOAEL for maternal as well as developmental toxicity in rabbits was set at 500 mg/kg bw/day. The test substance was not teratogenic.

Conclusions proposed by the applicant (2015):

In the present study, significant maternal and developmental toxicity was demonstrated at a daily dose level of 1000 mg/kg bw/day. No evidence of either maternal or developmental toxicity was detected at 500 mg/kg bw/day. Thus, the maternal and developmental no-observed-effect level (NOEL) was 500 mg/kg bw/day. Therefore, under these experimental conditions, DPX-JW062 was not uniquely toxic to the rabbit conceptus.

RMS FR assessment (2016):

Maternal and developmental NOAELs were set at 500 mg/kg bw/d. At 1000 mg/kg bw/d, decreased body weights, body weight gains and food consumption were observed in dams. At this dose level, developmental toxicity was evidenced by decreased mean fetal weights and retarded sternebral ossification.

B.6.7. NEUROTOXICITY

An acute and a 90-day rat neurotoxicity studies were performed on DPX-MP062 (75:25). A newly submitted rat developmental neurotoxicity study was carried out on DPX-KN128 (99:1).

Table B.6.7-1
Summary of neurotoxicity studies for indoxacarb (DPX-KN128 and DPX-MP062)

Type of study and test substance	Doses/concentrations tested	NOAEL	LOAEL	Target organ(s) and effects	Reference
Acute neurotoxicity (Gavage), Rat DPX-MP062 (75:25)	M: 0, 25, 100, 200 mg/kg F: 0, 12.5, 50, 100 mg/kg	Neurotoxicity: M: 100 mg/kg bw F: 50 mg/kg bw Systemic: M: 100 mg/kg bw F: 12.5 mg/kg bw	Neurotoxicity: M: 200 mg/kg bw F: 100 mg/kg bw Systemic: M: 200 mg/kg bw F: 50 mg/kg bw	Neurotoxicity: decreased forelimb grip strength and decreased foot splay in males; decreased motor activity in females Systemic: decreased body weight gains, body weights and food consumption	2001 HLR 1117-96, Revision No. 2
Subchronic neurotoxicity (Feeding), 90-d Rat DPX-MP062 (75:25)	M: 0, 10, 100, 200 ppm – 0, 0.569, 5.62, 11.9 mg/kg bw/d F: 0, 10, 50, 100 ppm – 0, 0.685, 3.30, 6.09 mg/kg bw/d	Neurotoxicity: M: 200 ppm 11.9 mg/kg bw/d F: 100 ppm 6.09 mg/kg bw/d Systemic: M: 10 ppm 0.57 mg/kg bw/d F: 10 ppm 0.685 mg/kg bw/d	Neurotoxicity: M: >200 ppm >11.9 mg/kg bw/d F: >100 ppm >6.09 mg/kg bw/d Systemic: M: 100 ppm 5.62 mg/kg bw/d F: 50 ppm 3.30 mg/kg bw/d	Neurotoxicity : no effect Systemic: decreased body weights, body weight gains and food consumption. Higher dose: mortality in F	1997 HLR 1116-96, Revision No. 1
Developmental Neurotoxicity Rat DPX-KN128 (99:1)	0, 0.5, 1, 1.5, 3.0 mg/kg/day In PEG by gavage GD 6 – LD10 for dams PND 11-20 for F1 pups	Maternal: 1 mg/kg bw/d Developmental: 1.5 mg/kg bw/d	Maternal: 1.5 mg/kg bw/d Developmental: 3.0 mg/kg bw/d	Maternal: decreased body weight gains. At 3 mg/kg bw/d: mortality, clinical signs of neurotoxicity Developmental: increased number of stillborn pups, increased pup mortality on PND1 to 4 and decreased pup weight per litter on PND0	2006a and 2006b DuPont 15150 and DuPont 15150 Supplement No. 1*

* Studies newly submitted

In an acute study and in a 90-day feeding study, neurotoxicity of DPX-MP062 (75:25) was assessed in rats using a functional observational battery, motor activity measurements, and neuropathological examinations of nerve tissues and muscle.

No evidence of neurotoxicity was observed in the 90-day study whereas clinical signs indicating neurodysfunction were reported in the acute study and consisted of decreased forelimb grip strength and decreased foot splay in males at 200 mg/kg bw and decreased motor activity in females at 100 mg/kg bw. According to RAC opinion, although these effects were observed at doses below guidance value for STOT SE 1 (<300 mg/kg bw), they occurred at doses that are also relevant to induce lethality. Indeed, in the acute oral toxicity study with DPX-KN128 (99:1), the LD50 is 179 mg/kg bw in females and clinical signs included hypoactivity, ataxia or impaired righting reflex. It can be expected that these effects indicate severe moribundity and are mortality-related. As indoxacarb is already classified for acute oral toxicity, data were not considered to justify a classification for STOT-SE.

It should be noted that neurotoxicity findings were observed in the repeated-dose toxicity studies performed in mice with the racemic mixture DPX-JW062 (50:50). Indeed, clinical signs indicative of neurotoxicity were reported in the 18-month study at 100 ppm and above in males and females (13.8 mg/kg bw/d), in the 90-d study at 150 ppm and above in females (30 mg/kg bw/d) and at 300 ppm in males (44 mg/kg bw/d) and in the 28-day study at 118 ppm and above in males and females (17.9 mg/kg bw/d). In the long-term mouse study, low incidences of neuronal degeneration/necrosis occurred in the brain of male and female mice at the highest dietary level, and in a female mouse at 100 ppm (20.3 mg/kg bw/d). The primary sites affected were the piriform cortex and the hippocampus. A more chronic brain lesion, diagnosed as residual vacuolation, was present in the piriform cortex of two high-concentration (23.7 to 44 mg/kg bw/d) females sacrificed at the termination of the study.

As a consequence, indoxacarb was classified as STOT RE for effects on the nervous system by the ECHA Committee for Risk Assessment (2011).

Clinical signs of neurotoxicity were also observed in rats in the newly submitted developmental neurotoxicity study conducted with DPX-KN128 (99:1). Indeed, dams administered the dose level of 3 mg/kg bw/d from GD6 to PND10 showed decreased motor activity, hunched posture, head tilt or lost righting reflex during gestation and ataxia or abnormal autonomic function during the lactation period. Mortality was also reported in three of the dams showing clinical signs of neurotoxicity on gestation days 19-20 and lactation day 3. As no neurotoxic effects were observed in rats during other repeated-dose studies, it could be argued that the dams seem to be more susceptible to neurotoxic effects induced by DPX-KN128 (99:1) than non-pregnant females. Nevertheless, such clinical signs were not reproduced in the prenatal developmental rat toxicity study with DPX-KN128 (99:1) at the same range of dose levels. It is to note that the highest tested dose levels in rats in other repeated-dose studies (up to approx. 15 mg/kg bw/d) were below the dose levels leading to neurotoxic effects in the mouse studies and in the acute rat neurotoxicity study. Moreover, specific neurofunctional testing was not performed in all available rat studies.

The systemic NOAELs of the acute and 90-d neurotoxicity studies were based on decreased body weights, body weight gains and food consumption observed at the LOAEL. It is noted that no haematology measurement was performed in the 90-day study, whereas other studies have indicated the occurrence of effects on red blood cell parameters to be critical. Mortality was reported in 3 out of 12 female rats in the subchronic neurotoxicity study on test days 9 to 12.

In the newly submitted developmental neurotoxicity study, the offspring NOAEL is 1.5 mg/kg bw/day based on increased number of stillborn pups, increased pup mortality on PND1 to 4 and decreased pup weight per litter on PND0 at the dose level of 3 mg/kg bw/day. Rat offsprings were not more susceptible to potential neurotoxicity effects of indoxacarb based on the absence of neurobehavioral effects at the highest dosage tested of 3 mg/kg/day. The maternal NOAEL is set at 1 mg/kg bw/d based on decreased body weight gains observed during gestation at the dose level of 1.5 mg/kg bw/d.

B.6.7.1. Neurotoxicity studies in rodents

Previous evaluation:	HLR 1117-96 : In DAR (2000) HLR 1117-96 Revision No.2 : Submitted for the purpose of renewal (see reasons for revisions below)
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CA 5.7.1/01

Report: [REDACTED] (2001); Acute oral neurotoxicity study of DPX-MP062 (approximately 75% DPX-KN128; 25% DPX-KN127) in rats

DuPont Report No.: HLR 1117-96, Revision No. 2 (original report and revision No. 1: Christoph; G.R. (1997))

Guidelines: OPPTS 870.6300 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 1117-96, Revision No. 2

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

Reason for Revision Nos. 1 and 2:

1. Clarification of the nomenclature regarding the distinction between the insecticidally active enantiomer, and the insecticidally inactive enantiomer.

2. Summary, text, and conclusions were revised to present separate NOAELs for both systemic toxicity and neurotoxicity, and clarify that the endpoints were determined for the racemic mixture, instead of the insecticidally active enantiomer.
3. Rationale for selection of the dosing vehicle was added
4. Discussion section was revised regarding the comparison of results to a study in which corn oil was used as a vehicle, and clarification of the duration of effects.
5. Individual footsplay data in the appendix for control females were corrected due to an error. This did not affect the summary table or text.
6. Protocol amendment 3 was added to change the study director for the purpose of revising the report.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MP062 technical
 Lot/Batch #: MP062-51A
 Purity: 94.5%
 Description: Off-white solid
 CAS #: None for DPX-MP062
 Stability of test compound: The test material was stable for at least five hours at room temperature.
2. Vehicle and/or negative control: 99.575% polyethylene glycol (0.050% 3-t-butyl-4-hydroxyanisole and 0.375% L-ascorbic acid 6-palmitate were added as preservatives since this vehicle was shared with the rat developmental study, and prepared once to supply both studies)
3. Test animals
 Species: Rat
 Strain: CrI:CD®BR
 Age at dosing: 56–57 days old
 Weight at dosing: 230.1–305.5 g for males; 163.6–224.1 g for females
 Source: [REDACTED]
 Acclimation period: 6 days
 Diet: Purina® Certified Rodent Chow® (#5002), *ad libitum*
 Water: Tap water, *ad libitum*
 Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
 Temperature: 23 ± 1°C
 Humidity: 50 ± 10%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 24-October-1996 to 29-April-1997
2. Brief description of dose prep method, and analysis
 Briefly, the method used for preparation involved adding acetonitrile to weighed quantities of DPX-MP062 technical with sonication until the DPX-MP062 technical was dissolved. The

acetonitrile was evaporated under nitrogen and then the components of the vehicle were added with subsequent sonication. The formulations were frozen after preparation and thawed prior to use in this study.

3. Animal assignment and treatment

Following a range-finding preliminary test in which groups of female rats received 0, 100, 250, 500, or 1000 mg/kg bw of the test substance, doses of 0, 25, 100, and 200 mg/kg bw were selected for the main study with males and 0, 12.5, 50, and 100 mg/kg bw were selected for the main study with females. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex.

Rats were given a single dose of DPX-MP062 by gavage. The test substance was administered in 99.575% polyethylene glycol, 0.050% 3-*t*-butyl-4-hydroxyanisole, and 0.375% L-ascorbic acid 6-palmitate at a volume of 6.25 mL/kg bw for males and 3.13 mL/kg bw for females. The animals were weighed on test Days 1 (prior to dosing), 2, 8, and 15. A neurobehavioral test battery, consisting of motor activity and functional observational battery assessments, was conducted on all study rats prior to dosing, on test Day 1, 2- 4 hours after dosing and on Days 8 and 15. Other parameters evaluated included body weight, body weight gain, food consumption, clinical signs, and gross pathology. On test Day 16, surviving animals were euthanized. Six rats per sex per group were necropsied to detect grossly observable evidence of organ or tissue damage, and perfused *in situ* with fixative. The remaining six rats per sex per group were discarded without further evaluation. A microscopic neuropathological evaluation of the central and peripheral nervous system and selected muscle tissues from the control and high dose group was conducted. Tissue included brain (forebrain, cerebrum, midbrain, cerebellum, pons, and medulla), spinal cord (cervical and lumbar regions), gasserian ganglia, cervical and lumbar dorsal root ganglia, dorsal and ventral root fibers, sciatic nerve, tibial nerve, and gastrocnemius muscle.

4. Functional observational battery and motor activity

FOB and MA assessments were conducted on all rats prior to exposure (baseline), on test Day 1 approximately 2–4 hours post-dosing, and on test Days 8 and 15. The experimenter conducting the FOB was unaware of the treatment group of each animal. The following parameters were evaluated in the FOB: home cage (posture, palpebral closure, writhing, circling and biting), removal from cage (ease of removal, vocalisations, ease of handling, piloerection, muscle tone, fur/skin appearance, bite marks, palpebral closure, lacrimation, exophthalmus, salivation), open field evaluation (arousal, grooming, convulsions, gait/coordination, locomotion, tremors, laboured breathing, defecation, diarrhoea, urination righting reflex, palpebral closure, and vocalisations), sensory function (approach/touch, auditory stimulus, tail pinch), grip strength, and foot splay. Duration of movement and number of movements were evaluated in six consecutive blocks of 10 minutes each, as well as for the total 60-minute session. Upon removal from the MA chambers, defecation, urination and pupillary response were assessed.

5. Sacrifice and neuropathology

At termination, six animals/sex/group were sacrificed by pentobarbital anaesthesia, exsanguination, given a gross examination, and subjected to whole body *in situ* perfusion. Tissue samples from the nervous system and skeletal muscle were saved from all groups: brain (forebrain, cerebrum, midbrain, pons, medulla, and cerebellum), spinal cord (cervical and lumbar), sciatic nerve, tibial nerve, gasserian ganglia, cervical and lumbar dorsal root fibres and ganglia (DRF&G), cervical and lumbar ventral root fibres (VRF), and gastrocnemius muscle. Tissues collected from animals receiving the highest dose (100 or 200 ppm) and control dose (0 ppm) were processed to slides and evaluated microscopically.

6. Statistics

Incidences of gross observations and incidences and severity of microscopic observations are reported for the neuropathology data. No statistical tests were performed on these data. Clinical observation incidence data, as well as descriptive FOB parameters, were evaluated by the Cochran Armitage test for Trend. When significant, this test was repeated sequentially dropping off the highest dosage level until no further significance was found. When the trend test was not significant but a significant lack

of fit was evident, Fisher's Exact test was used to determine statistically significant differences from the control group.

Body weights, body weight gains, food consumption, grip strength, and foot splay data were analysed as parametric data. For the grip strength and foot splay, Bartlett's test for homogeneity of variances was used to estimate the probability that the groups had different variances. When Bartlett's test was not significant, these data as well as the body weight and food consumption data were then analysed via univariate analysis of variance, with Dunnett's test used to identify which dosage groups, if any, were significantly different from the control group. When Bartlett's test was significant, non-parametric tests (Kruskal-Wallis followed by Dunn's Test) were used. Separate analyses were performed on the data collected on each test day and for each gender. Motor activity data was examined with the Shapiro Wilk's Test and Levene's Test to detect deviations from a normal distribution and equality of variance, respectively. Non parametric tests (Kruskal-Wallis followed by Dunn's Test) were used because of deviation from the assumptions of homogeneity of variance or normal distribution. Significance levels were judged at $\alpha < 0.05$.

II. RESULTS AND DISCUSSION

A. MORTALITY

One female rat in the 100 mg/kg group was found dead on test Day 12. The interval between death and compound administration is similar to that in pilot studies, and the death is regarded as a toxicological effect of the test substance. Clinical observations of the rat on the day prior to its death included abnormal gait or mobility and hunched posture. Except for a notation of alopecia, gross necropsy revealed no remarkable findings. The rat was discarded after necropsy.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity most often observed in female rats included pallor and alopecia, with significantly greater incidence in the 100 mg/kg group than the control group. Other clinical signs of toxicity observed in female rats of this group included abnormal gait or mobility, hunched posture, hyperactivity, and cage licking. They developed on test day 3 or later. A significantly greater incidence of alopecia was also identified in the 50 mg/kg bw group. None of the clinical observations of male rats indicated statistically significant, test substance-related effects.

C. BODY WEIGHT

In male rats there were no statistically significant effects of the test substance on mean body weight during the study, but there was a significant reduction (36%) in mean body weight gain of male rats in the 200 mg/kg group during the interval between test Days 2 and 8 relative to control. Weight gains for the 25 mg/kg and 100 mg/kg groups were unaffected.

Mean female body weights were significantly reduced on test day 8 for the 50 mg/kg bw group (7%) and the 100 mg/kg bw group (15%). Mean body weight gains were significantly reduced for the interval between test days 2 and 8 for the 50 mg/kg bw group. In the 100 mg/kg bw group, body loss was observed for the interval days 2-8. There appeared to be a compensatory rebound in body weight gain for the interval days 8-15 such that these females gained significantly more weight than controls. During the entire study (test day interval 1-15) females in the 100 mg/kg group had significantly lower mean weight gain than control females (45%). There were no effects on body weight parameters for the 12.5 mg/kg group.

Table B.6.7.1-1
Acute neurotoxicity study in rats: Body weights (g)

Males:	0 mg/kg		25 mg/kg		100 mg/kg		200 mg/kg	
Day 1	267.3	(18.2) ^a	267.4	(25.9)	264.8	(13.4)	265.4	(17.6)
Day 2	267.1	(17.1)	265.6	(25.1)	264.2	(14.5)	263.2	(16.8)
Day 8	312.7	(28.0)	308.6	(39.3)	301.5	(22.1)	292.7	(20.6)
Day 15	352.2	(37.0)	347.5	(53.3)	343.6	(25.7)	340.6	(30.1)
Females:	0 mg/kg		12.5 mg/kg		50 mg/kg		100 mg/kg	
Day 1	195.8	(9.3)	197.8	(12.2)	192.9	(14.3)	195.1	(10.9)
Day 2	197.4	(10.2)	197.4	(14.3)	190.9	(14.7)	196.1	(10.4)
Day 8	216.5	(12.1)	217.9	(13.6)	200.3 ^b	(20.7)	185.1 ^b	(12.0)
Day 15 ^c	231.2	(15.0)	235.9	(19.1)	221.3	(21.1)	213.9	(14.0)

^a Standard deviation is reported in parentheses

^b Statistically significant differences relative to control by Dunnett's Test at p <0.05.

^c N = 11 for the 100 mg/kg group for this test day because one rat was found dead on Test Day 12.

Table B.6.7.1-2
Acute neurotoxicity study in rats: Body weight gain (g)

Males:	0 mg/kg		25 mg/kg		100 mg/kg		200 mg/kg	
Days 1–2	-0.2	(4.2) ^a	-1.8	(3.5)	-0.6	(3.3)	-2.1	(4.6)
Days 2–8	45.6	(11.9)	42.9	(15.6)	37.3	(11.0)	29.4 ^b	(10.6)
Days 8–15	39.4	(10.1)	38.9	(15.0)	42.1	(7.4)	48.0	(13.5)
Days 1–15	84.8	(19.8)	80.1	(29.2)	78.8	(15.1)	75.3	(19.1)
Females:	0 mg/kg		12.5 mg/kg		50 mg/kg		100 mg/kg	
Days 1–2	1.6	(5.3)	-0.3	(5.4)	-2.1	(5.7)	1.0	(5.5)
Days 2–8	19.1	(5.1)	20.4	(7.4)	9.4 ^b	(10.2)	-11.0 ^b	(9.3)
Days 8–15 ^c	14.7	(5.6)	18.1	(7.7)	20.9	(6.3)	28.7 ^b	(11.5)
Days 1–15 ^c	35.4	(8.8)	38.2	(10.1)	28.3	(9.6)	19.5 ^b	(10.8)

^a Standard deviation is reported in parenthesis

^b Statistically significant differences relative to control by Dunnett's Test at p <0.05.

^c N = 11 for the 100 mg/kg group for this interval because one rat was found dead on Test Day 12.

D FOOD CONSUMPTION AND FOOD EFFICIENCY

Males administered 100 and 200 mg/kg bw had lower food consumption compared to controls during the interval between test Days 1 and 2 (22% and 26% lower, respectively). Females administered 50 mg/kg bw had lower food consumption compared to controls (22% lower) during the interval between test Days 1 and 2. For the intervals between test Days 8 and 15, mean food consumption was significantly reduced (17%) for only the 100 mg/kg female rats and not for the other dosage groups.

Table B.6.7.1-3
Acute neurotoxicity study in rats: Food consumption

Males	0 mg/kg	25 mg/kg	100 mg/kg	200 mg/kg
Males:				
Food consumption, Day 1–2 (g/day)	19.2	16.2	14.9 ^a	14.2 ^a
Food consumption, Day 2–8 ^b (g/day)	-	-	-	-
Food consumption, Day 8–15 (g/day)	26.3	26.2	25.8	27.7
Females:				
	0 mg/kg	12.5 mg/kg	50 mg/kg	100 mg/kg
Food consumption, Day 1–2 (g/day)	16.3	14.7	12.7 ^a	13.6
Food consumption, Day 2–8 ^b (g/day)	-	-	-	-
Food consumption, Day 8–15 ^c (g/day)	19.6	20.1	19.9	16.2 ^a

^a Significantly different from control by the Dunnett's Test criteria, $p < 0.05$.

^b An unknown amount of food was added to the rats' cages during this interval; therefore, an accurate determination of food consumption could not be determined for this interval.

^c N = 11 for the 100 mg/kg group for this interval because one rat was found dead on Test Day 12.

E FUNCTIONAL OBSERVATIONAL BATTERY AND MOTOR ACTIVITY

1. Functional observation battery

Male rats that received 200 mg/kg of the test substance had significantly lower forelimb grip strength during test Day 15, but not during other time points. Forelimb grip strength in female rats was not significantly affected by administration of test substance.

Hindlimb grip strength in male rats of the intermediate dose group was significantly greater than control during test day 8. In the absence of a dose-dependency, this change was not considered as adverse. Hindlimb grip strength in female rats was not significantly affected by administration of test substance.

In male rats that received 200 mg/kg, mean hindlimb foot splay was significantly less than control during test Day 15. All other foot splay values for all treatment groups were not significantly different than control. Mean foot splay values in female rats were not significantly affected by administration of test substance.

The remainder of the FOB parameters for males and females were toxicologically unimportant. Only one type of assessment revealed significant differences: male rats had a significantly reduced incidence of defecation in the open field on test day 1 relative to control in all tested groups (incidences of defecation of 8/12, 3/12, 3/12 and 3/12 in the 0, 25, 100 and 200 mg/kg bw groups respectively). Nevertheless, as the defecation parameter for the same rats on the same day in the motor activity monitor was not affected, this finding was not considered as toxicologically important.

2. Motor activity

For male rats neither the mean duration of movement nor the mean number of movements was affected by administration of the test substance during the study. During test Days 1 and 8, however, females that received 100 mg/kg had significantly reduced mean duration of activity during the first 10-minute block of the sessions, and these differences are regarded as treatment-related effects of the test substance because they occurred in the high dose group. No significant effects on motor activity were identified during test Day 15 in female rats.

Several 10-minute blocks within some baseline sessions revealed significant differences between groups of female rats, but these differences were not related to treatment since the test compound had not been yet administered.

Table B.6.7.1-4
Acute neurotoxicity study in rats: Functional observational battery

Males	0 mg/kg N = 12		25 mg/kg N = 12		100 mg/kg N = 12		200 mg/kg N = 12	
Forelimb grip strength (kg)								
Baseline	0.53	(0.09) ^a	0.52	(0.12)	0.53	(0.10)	0.49	(0.10)
Day 1	0.51	(0.12)	0.62	(0.17)	0.59	(0.11)	0.55	(0.13)
Day 8	0.58	(0.16)	0.57	(0.18)	0.63	(0.18)	0.66	(0.17)
Day 15	0.72	(0.11)	0.63	(0.14)	0.64	(0.09)	0.57 ^b	(0.11)
Hindlimb grip strength (kg)								
Baseline	0.44	(0.07)	0.44	(0.06)	0.46	(0.06)	0.43	(0.07)
Day 1	0.53	(0.11)	0.50	(0.13)	0.58	(0.12)	0.50	(0.07)
Day 8	0.59	(0.12)	0.64	(0.08)	0.73 ^b	(0.15)	0.69	(0.13)
Day 15	0.76	(0.15)	0.76	(0.09)	0.77	(0.10)	0.76	(0.18)
Hindlimb foot splay (cm)								
Baseline	6.8	(1.1)	6.4	(1.5)	6.6	(1.5)	6.6	(1.6)
Day 1	7.1	(1.2)	6.9	(0.9)	6.7	(1.6)	6.6	(1.3)
Day 8	6.9	(1.3)	5.9	(1.4)	5.6	(1.8)	6.1	(1.4)
Day 15	7.3	(1.1)	6.7	(1.5)	6.9	(1.6)	5.8 ^b	(1.1)
Females	0 mg/kg N = 12		12.5 mg/kg N = 12		50 mg/kg N = 12		100 mg/kg N = 12; N = 11 ^c	
Forelimb grip strength (kg)								
Baseline	0.46	(0.08)	0.47	(0.06)	0.47	(0.12)	0.47	(0.03)
Day 1	0.49	(0.09)	0.54	(0.12)	0.48	(0.12)	0.50	(0.14)
Day 8	0.56	(0.14)	0.52	(0.18)	0.48	(0.15)	0.52	(0.14)
Day 15	0.55	(0.10)	0.47	(0.07)	0.44	(0.14)	0.47	(0.12)
Hindlimb grip strength (kg)								
Baseline	0.45	(0.08)	0.44	(0.06)	0.46	(0.07)	0.47	(0.05)
Day 1	0.52	(0.07)	0.52	(0.10)	0.53	(0.12)	0.50	(0.08)
Day 8	0.65	(0.18)	0.61	(0.11)	0.65	(0.12)	0.64	(0.11)
Day 15	0.70	(0.13)	0.59	(0.08)	0.7	(0.21)	0.68	(0.14)
Hindlimb foot splay (cm)								
Baseline	5.4	(1.3)	5.9	(1.4)	5.9	(1.3)	6.8 ^b	(1.4)
Day 1	6.7	(1.6)	6.9	(1.1)	6.6	(2.1)	6.1	(1.5)
Day 8	6.1	(1.4)	5.9	(1.3)	6.3	(1.8)	5.8	(1.9)
Day 15	6.1	(1.9)	6.0	(1.4)	5.3	(1.5)	5.0	(1.5)

^a Standard deviation is presented in parentheses.

^b Statistically significant differences relative to control by Barlett's test, One-Way Analysis of variance and Dunnet's Test at p < 0.05.

^c N = 11 on Day 15 because one rat was found dead on Test Day 12.

Table B.6.7.1-5
Acute oral neurotoxicity study in rats: Summary of motor activity, mean duration of movement in females (seconds)

Baseline								
Successive 10-minute interval	0 mg/kg N = 12		12.5 mg/kg N = 12		50 mg/kg N = 12		100 mg/kg N = 12	
1	372	(39) ^a	397	(49)	381	(48)	384	(30)
2	293	(91)	312	(67)	293	(92)	271	(72)
3	145	(75)	218	(105)	246 ^b	(77)	199	(106)
4	102	(101)	231 ^b	(62)	177	(132)	117	(82)
5	152	(114)	152	(80)	71	(87)	85	(95)
6	115	(83)	30 ^b	(38)	40 ^b	(66)	69	(85)
TOTAL	1178	(342)	1341	(311)	1208	(357)	1125	(277)
Day 1								
Successive 10-minute interval	0 mg/kg N = 12		12.5 mg/kg N = 12		50 mg/kg N = 12		100 mg/kg N = 12	
1	347	(79)	340	(74)	323	(66)	218 ^b	(98)
2	186	(85)	195	(91)	179	(106)	120	(88)
3	105	(87)	134	(76)	78	(79)	79	(76)
4	42	(55)	81	(84)	47	(80)	75	(71)
5	11	(16)	43	(67)	10	(23)	29	(23)
6	18	(30)	25	(38)	13	(23)	37	(66)
TOTAL	710	(213)	817	(207)	649	(287)	560	(222)
Day 8								
Successive 10-minute interval	0 mg/kg N = 12		12.5 mg/kg N = 12		50 mg/kg N = 12		100 mg/kg N = 12	
1	403	(62)	417	(29)	381	(79)	319 ^b	(45)
2	244	(122)	318	(78)	254	(102)	241	(74)
3	135	(109)	199	(120)	157	(111)	145	(81)
4	103	(89)	114	(131)	90	(126)	113	(73)
5	72	(69)	78	(94)	71	(93)	74	(66)
6	124	(132)	154	(151)	64	(67)	65	(90)
TOTAL	1082	(396)	1279	(384)	1017	(436)	957	(193)
Day 15								
Successive 10-minute interval	0 mg/kg N = 12		12.5 mg/kg N = 12		50 mg/kg N = 12		100 mg/kg N = 11	
1	386	(67)	401	(54)	401	(55)	335	(41)
2	224	(88)	236	(84)	276	(92)	197	(84)
3	182	(146)	194	(121)	185	(96)	162	(82)
4	146	(134)	199	(101)	108	(112)	125	(94)
5	100	(98)	152	(102)	41	(61)	69	(91)
6	101	(95)	114	(110)	97	(113)	58	(66)
TOTAL	1138	(428)	1295	(486)	1107	(336)	946	(270)

^a Standard deviation is presented in parentheses.

^b Statistically significant differences relative to control by Kruskal-Wallis and Dunn's Test at p <0.05.

F. GROSS PATHOLOGY AND NEUROPATHOLOGY

Extensive neuropathological evaluation of brain, spinal cord, and peripheral nerves did not reveal any test substance-related structural damage.

Conclusions from the original DAR (2000, RMS NL):

Neurotoxic effects were observed in the high dose males (decreased forelimb grip strength and foot splay) and females (decreased motor activity). In the mid-dose groups, food consumption was significantly decreased in males, whereas in females significantly decreased body weight and alopecia was observed. Based on these observations, the NOAEL was set at 50 mg/kg bw for acute neurotoxicity and at 12.5 mg/kg bw for acute systemic toxicity.

Conclusions proposed by the applicant (2015):

The NOAEL for neurotoxicity was 100 mg/kg bw for males based on reduced forelimb grip strength and decreased foot splay in males at 200 mg/kg bw (LOAEL). The NOAEL for females was 50 mg/kg bw based on transient decreased in motor activity in females at the LOAEL, 100 mg/kg bw. The NOAEL for systemic toxicity for males was 100 mg/kg bw based on reduced food consumption in males at the LOAEL, 200 mg/kg bw. The NOAEL for females was 12.5 mg/kg bw based on reduced body weight and reduced body weight gain in females at the LOAEL, 50 mg/kg bw.

RMS FR assessment (2016):

The NOAEL for neurotoxicity is set at 100 mg/kg bw for males, based on decreased forelimb grip strength and decreased foot splay observed at the dose level of 200 mg/kg bw. In females, the NOAEL for neurotoxicity is set at 50 mg/kg bw based on decreased motor activity observed at 100 mg/kg bw.

Systemic toxicity was reported in males at 200 mg/kg bw (decreased body weight gains and food consumption) and in females at 50 mg/kg bw (decreased body weights and body weight gains). Therefore, systemic NOAEL is set at 100 mg/kg bw in males and 12.5 mg/kg bw in females.

Previous evaluation:	In DAR (2000)
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CA 5.7.1

Report: [REDACTED] (1997); Subchronic oral neurotoxicity study of DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127) in rats

DuPont Report No.: HLR 1116-96, Revision No.1

Guidelines: USEPA 82-7 **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 1116-96, Revision No. 1

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|----------------------------------|--|
| 1. | Test material: | DPX-MP062 technical |
| | Lot/Batch #: | MP062-51A |
| | Purity: | 94.5% |
| | Description: | Off-white solid |
| | CAS #: | None for DPX-MP062 technical |
| | Stability of test compound: | The test substance was homogeneously distributed and was stable in the diet. |
| 2. | Vehicle and/or negative control: | The test substance was dissolved in acetone and mixed with untreated diet. The same amount of acetone was added to control diet. |
| 3. | Test animals | |
| | Species: | Rat |
| | Strain: | CrI:CD [®] BR |
| | Age at dosing: | Approximately 34 days of age |
| | Weight at dosing: | 194.6–255.4 g for males; 145.4–192.9 g for females |
| | Source: | |
| | Acclimation period: | 6 days |
| | Diet: | Purina [®] Certified Rodent Chow [®] (#5002), <i>ad libitum</i> (except during neurobehavioral evaluations) |
| | Water: | Tap water, <i>ad libitum</i> (except during neurobehavioral evaluations) |
| | Housing: | Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards. |
| 4. | Environmental conditions | |
| | Temperature: | 23 ± 1°C |
| | Humidity: | 50 ± 10% |
| | Air changes: | Not recorded |
| | Photoperiod: | Alternating 12-hour light and dark cycles |

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
24-June-1996 to 24-September-1996
2. Animal assignment and treatment
In a 90-day neurotoxicity feeding study, DPX-MP062 (purity 94.5%) was administered to male and female CrI:CD[®]BR rats (12 rats/sex/concentration). Dietary concentrations in male rats were 0, 10, 100, or 200 ppm. Female rats were fed dietary concentrations of 0, 10, 50, or 100 ppm. Parameters evaluated included body weight/body weight gain, food consumption/efficiency, clinical signs, a neurobehavioral test battery consisting of motor activity and functional observational battery assessments, and microscopic pathology of the nervous system and muscle. Tissues for light microscopy were fixed by whole-body in situ perfusion.

II. RESULTS AND DISCUSSION

Findings from the 90-day neurotoxicity study in rats with DPX-MP062 in rats are summarised in the following table.

Test substance-related mortality occurred in three of 12 females in the 100 ppm group. Test substance-related mortality did not occur in male rats at any dietary concentration. Male rats in the 100 and 200 ppm groups and females in the 50 and 100 ppm groups had test substance-related decrements in body weight and body weight gain relative to controls which were correlative to decreases in food consumption and food efficiency. There were no test substance-related effects in either males or females on forelimb or hindlimb grip strength, foot

splay, or any of the 34 other parameters evaluated by the functional observational battery. In addition, there was no evidence of neurotoxicity during evaluation of motor activity, clinical signs, or morphologic neuropathology.

Table B.6.7.1-6
Summary of results of the 90-day neurotoxicity study in rats with DPX-MP062

Dosage	Male	Dietary conc.:	0	10	100	200 ppm
		Mean daily intake:	0	0.569	5.62	11.9 mg/kg/day
	Female	Dietary conc.:	0	10	50	100 ppm
		Mean daily intake:	0	0.685	3.30	6.09 mg/kg/day
Analytical	The test substance was homogeneously distributed and was stable in the diet. The measured concentrations of DPX-MP062 technical from analysis of samples taken on Test Days 39 and 80 were 92.0 to 95.6% and 93.6 to 96.1% of nominal, respectively.					
		Dose Group (ppm)	Results			
General observations		nad				
Mortality		100 f:	3/12 on Test Days 9-12			
Body weight		≥100 m:	Decreased ^b (12-16%)			
		≥50 f:	Decreased (10-14%)			
Body weight gain		≥100 m:	Decreased (18-28%)			
		≥50 f:	Decreased (23-35%)			
Food consumption		≥100 m:	Decreased (12-16%)			
		≥50 f:	Decreased (16-26%)			
Food efficiency		≥100 m:	Decreased (8-13%)			
		≥50 f:	Decreased (9-12%)			
Functional observation battery						
Forelimb grip strength		nad ^c				
Hindlimb grip strength		nad				
Hindlimb foot splay		nad				
Other endpoints ^a		nad				
Motor activity		nad				
Gross pathology		nad				
Neuropathology		nad				

^a series of 34 other qualified behavioural evaluations

^b all increases or decreases are relative to controls unless otherwise noted

^c nad = no abnormalities detected that were considered test substance-related and/or biologically adverse; m = male; f = female

Conclusions from the original DAR (2000, RMS NL):

No neurotoxic effects were observed. Significantly decreased body weights and food consumption, and alopecia were observed in the middle and high dose animals. Based on these effects, the NOAEL for systemic toxicity was set at 10 mg/kg food (equal to 0.57 mg/kg bw/day). The NOAEL for semichronic neurotoxicity was established at ≥100 mg/kg food (equal to ≥6.09 mg/kg bw/day).

Conclusions proposed by the applicant (2015):

The no-observed-adverse-effect level for neurotoxicity (NOAEL) was 200 ppm for males and 100 ppm for females, (the highest dietary concentrations tested). These dietary concentrations were equivalent to 11.9 and 6.09 mg/kg/day, respectively. The NOAEL for systemic toxicity was 10 ppm for male and female rats, equivalent to 0.569 and 0.685 mg/kg/day. This NOAEL was based on decrements in body weight, weight gain, food consumption, and food efficiency relative to controls. The relatively low NOAELs for systemic toxicity in this study are a reflection of dose selection, specifically the spacing between the intermediate- and low-dose levels. The actual NOAEL following 90-day dietary exposure to DPX-MP062 was better demonstrated in the 90-day feeding study conducted at approximately the same time as the 90-day neurotoxicity study. In the subchronic feeding study with DPX-MP062, the NOAEL in female rats (the more sensitive gender) was determined to be 2.13 mg/kg/day.

RMS FR assessment (2016):

In the absence of neurotoxicity effects, the neurotoxicity NOAEL is greater than 100 ppm in females (equivalent to 6.09 mg/kg bw/d) and 200 ppm in males (equivalent to 11.9 mg/kg bw/d)

The systemic NOAEL is set at 10 ppm (0.57 mg/kg bw/d in males, 0.685 mg/kg bw/d in females) based on decreased body weights, body weight gains and food consumption observed at 50 ppm (3.30 mg/kg bw/d) and above in females and at 100 ppm (5.62 mg/kg bw/d) and above in males. At 100 ppm (6.09 mg/kg bw/d), mortality occurred in females.

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.7.2/01 and CA 5.7.2/02

Report: [REDACTED] (2006a and 2006b); Oral (gavage) developmental neurotoxicity study of DPX-KN128 (Indoxacarb) technical in Crl:CD (SD)IGS BR VAF/Plus rats

DuPont Report No.: DuPont-15150 and DuPont-15150, Supplement No. 1

Guidelines: OPPTS 870.6300 **Deviations:** None

RMS comment: It is to be noted that OECD Guideline 426 was adopted in 2007 i.e. after the study. It is noted that administration of the test substance in dams was stopped on PND10 although the OECD guideline recommends extending the administration until PND21. Nevertheless, as pups were also treated from PND11 to PND20, this is not considered to impact the results of the study.

Some other parameters are different between the 2 guidelines, nevertheless, they are not judged to impact the results of this study which is considered acceptable.

Testing Facility: [REDACTED]

Testing Facility Report No.: 104-026

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

A. MATERIALS

- ## B. STUDY DESIGN

- Prepared formulations were administered once daily beginning on Day 6 of presumed gestation (DG 6) and continuing until either scheduled sacrifice on DG 25 (dams that did not deliver a litter) or Day 10 of the lactation/postpartum period (DL 10), inclusive. The F₁ generation pups were similarly

administered the formulations via oral gavage between postnatal Days 11 through 20 (PNDs 11 through 20).

For sacrifice schedule, see Table B.6.7.1-10. Animal housing and husbandry were in accordance with the provisions of the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources [1996]).

Table B.6.7.1-7
Developmental neurotoxicity study in the rat: Treatment groups and dosages

Dosage group	F ₀ Generation number of female rats	F ₁ Generation number of rats per sex	Dietary concentration			
			Dosage ^{a,b} (mg/kg/day)	Concentration (mg/mL)	Dosage volume (mL/kg)	
I	25	100	0 (Vehicle)	0	2	(Control)
II	25	100	0.5	0.25	2	(Low-intermediate)
III	25	100	1	0.50	2	(Intermediate)
IV	25	100	1.5	0.75	2	(High-intermediate)
V	25	100	3.0	1.5	2	(High)

^a The test substance was considered 95.47% pure for the purpose of dosage calculations.

^b The dosages were adjusted daily on the basis of individual body weights recorded before intubation.

Table B.6.7.1-8
Developmental neurotoxicity study in the rat: Initiation of dosing

Generation	Initiation of dosing	Last day of dosing
F ₀	Gestation Day 6	Lactation Day 10
F ₁	Lactation Day 11	Lactation Day 21

Table B.6.7.1-9
Developmental neurotoxicity test by the oral route in the rat: Offspring subset designation

Subset	Evaluation type	Postnatal Day	Number of pups per sex per litter	Number of pups per group
1	Brain weight and neurohistology	21	1	10 per sex
2	Watermaze	58-62 and 65-69	1	20
2	Passive avoidance	22-24 and 29-31	1	20
2, 3, 4	Sexual maturation	Beginning 27 for females and 38 for males	all	all
3	Motor activity	13, 17, 21, and 58-62	1	20
3	Acoustic startle habituation	22 and 61-65	1	20
4	Behaviour, Brain weight and neurohistology	70	1	10 per sex
5	hematology	21	1	20

Table B.6.7.1-10
Developmental neurotoxicity study in the rat: Sacrifice schedule

Animals	Generation	Schedule
Pregnant/Lactating females	F ₀	On day of weaning litters—postpartum Day 22
Nonpregnant females	F ₀	Approximately Day 25 after the end of cohabitation
Day 4 culled pups	F ₁	Day 4 of lactation
Weanlings – Subset 1 Neuropathology	F ₁	On postnatal day 21
Weanlings – Subset 2 Passive avoidance, water maze, sexual maturation	F ₁	After postpartum Day 69 evaluation of passive avoidance and water maze performance
Weanlings – Subset 3 Motor activity, sexual maturation	F ₁	After postpartum Day 60-63 evaluation of motor activity and acoustic startle performance
Weanlings – subset 4 Evaluation of autonomic dysfunction and behaviour, sexual maturation, neuropathology	F ₁	After postpartum Day 60 evaluation of autonomic dysfunction, posture, behaviour, appearance
Weanlings – subset 5 Hematology	F ₁	Approximately postpartum Day 21

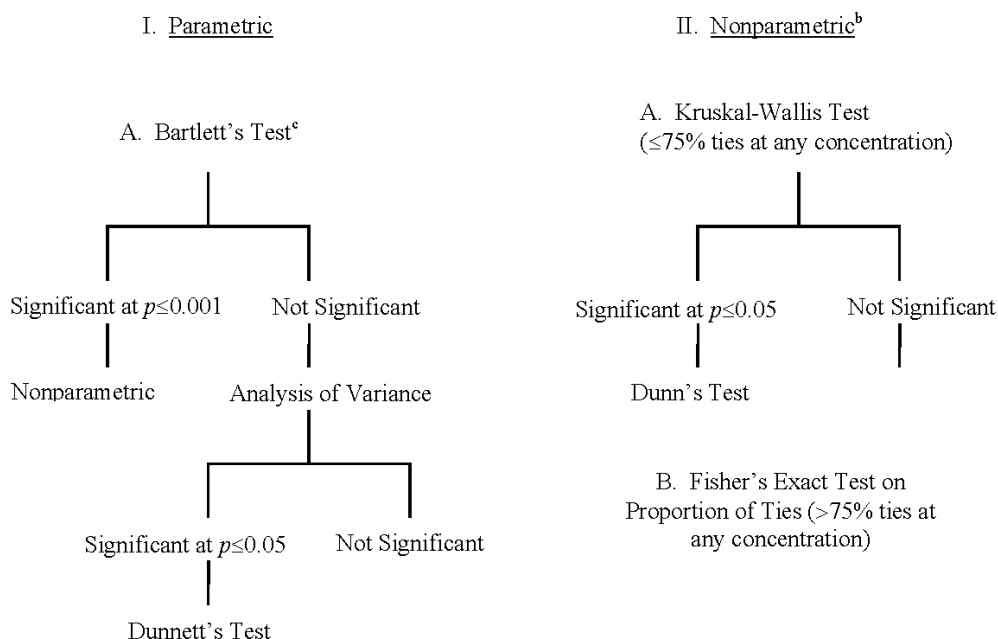
3. Formulation preparation and analysis

Formulations were prepared once by the [REDACTED] and were supplied to the In-Life Testing Facility. The vehicle was polyethylene glycol (molecular weight 400). The formulations were aliquoted into daily portions prior to shipping. Prior to each daily dosage administration, one container of formulation for each dosage level was removed from the refrigerator and placed in a sonicator until all solid material was re-solubilized. The re-solubilized formulations were then stirred continuously during dosage. Prepared formulations were stored refrigerated (1 to 8°C). The stability, homogeneity, and concentration of indoxacarb in the formulations were checked by analysis using HPLC at the beginning and end of the study. The test substance was at target concentrations (± 4.3 – 4.8% of nominal), homogeneous (CVs = 0–4), and was stable for up to 5 hours at room temperature. Based on this information, it can be concluded that the animals received the targeted concentrations of test substance during the study. The analytical method is validated (see Volume 3B5).

4. Statistics

Data generated during the course of this study were recorded either by hand or using the Argus Automated Data Collection and Management System, the Vivarium Temperature and Relative Humidity Monitoring System, the Coulbourn Instruments Passive Infrared Motor Activity System, the Coulbourn Instruments Acoustic Startle System, and/or the Coulbourn Instruments Spatial Delayed Alternation System. All data were tabulated, summarised, and/or statistically analysed using the Argus Automated Data Collection and Management System, the Vivarium Temperature and Relative Humidity Monitoring System, Microsoft® Excel (part of Microsoft® Office 97/2000/XP), Quattro Pro 8, and/or The SAS System (version 6.12).

Averages and percentages were calculated. Litter values were used where appropriate. The following schematic represents the statistical analyses of the data:

Type of Test^aIII. Test for Proportion Data

Variance Test for Homogeneity of the Binomial Distribution

- a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
 b. Proportion data are not included in this category.
 c. Test for homogeneity of variance.

Clinical observations and other proportional data were analysed, using the Variance Test for Homogeneity of the Binomial Distribution.

Clinical pathology data were analysed as described in Table . Significance was judged at $p < 0.05$. Separate analyses were performed on the data collected for each sex.

Table B.6.7.1-11
Statistics: Developmental neurotoxicity study in the rat

Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Clinical pathology ^a	Levene's test for homogeneity and Shapiro-Wilk test for normality ^b	One-way analysis of variance followed with Dunnett's test	Kruskall-Wallis test followed with Dunn's test

^a When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.1 , 0.05 was used for any calculations performed with that data. When an individual observation was recorded as being greater than a certain value, calculations were performed on the recorded value. For example, if specific gravity was reported as >1.083 , 1.083 was used for any calculations performed with that data.

^b If the Shapiro-Wilk test was not significant, but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed with Dunn's test.

C. METHOD

A developmental neurotoxicity study, involving the production of one set of litters, was conducted with indoxacarb. CrI:CD[®](SD)IGS BR VAF/Plus[®] rats (25 female rats/concentration for the F₀ generation) were dosed at 0, 0.5, 1, 1.5, and 3.0 mg/kg/day from GD6 to PND10. The dosages were adjusted daily on the basis of individual body weights recorded before intubation. The F₀ rats were presumed pregnant following a cohabitation period with untreated breeder males. The F₁ generation pups may have been exposed *in utero* during gestation or via maternal milk during the maternal postpartum dosage period. In addition, the F₁ generation pups were similarly administered the formulations via oral gavage between PNDs 11 through 20. Dams were allowed to deliver and rear their offspring until weaning (postpartum Day 21). F₁ litters were culled to five pups/sex/litter (litter size permitting) on PND 4. After standardisation, five male and five female pups (when possible) from each litter were assigned to one of five subsets. One male and one female pup were assigned to each evaluation as follows: PND 21 brain weights and neurohistological evaluations (Subset 1); watermaze and passive avoidance (Subset 2); motor activity and acoustic startle habituation (Subset 3); behaviour, brain weights, and neurohistological evaluations at sacrifice (Subset 4), and to standardise litter size to ten pups (five male and five female pups per litter) from PNDs 4 to 21 and for haematology evaluations (Subset 5). Sexual maturation was also evaluated on all offspring in subsets 2-4.

For the F₀ maternal generation, clinical observations and general appearance were observed weekly and on DG 0 during the predosage period and prior to and approximately 60 ± 10 minutes after dosage administration during the dosage period. F₀ generation body weights were determined weekly during the predosage period, on DG 0, daily during the dosage period, on PNDs 11, 14, 17, and 21, and at sacrifice. Food consumption for the F₀ generation was determined on DG 0 and daily during the dosage period. Because pups began to consume maternal feed at approximately PND 13, food consumption values were not recorded after PND 13. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter sizes (all pups delivered), live litter size (live born pups only) and pup viability at birth. Maternal behaviour was evaluated on PNDs 0, 4, 7, 13, and 21. Variations from expected maternal behaviour were recorded, if and when present, on all other days of the postpartum period. Each litter was evaluated for viability at least twice daily. The pups in each litter were counted daily. Rats were observed for viability at least twice daily during the postweaning period. Clinical observations were recorded once daily during the predosage period, daily before administration during the dosage period, and weekly during the postdosage period. Pup body weights were recorded on PND 0 (birth), PND 4 (both pre- and post-standardisation), PND 7, and daily during the dosage period. Body weights were recorded weekly during the postdosage period. Food consumption values were recorded weekly throughout the postweaning period. The age at either vaginal opening or preputial separation was recorded for the female rats in Subsets 2 through 4 and male rats in Subsets 2 through 4, respectively.

Beginning at PNDs 22 to 24 and retested on Days 30 to 31 postpartum, one male rat and one female rat from each litter, where possible, were evaluated in a passive avoidance test for learning, short-term retention, long-term retention, and hyperactivity. Each rat was tested twice. The test sessions were separated by a 1-week interval, and the criterion was the same for both days of testing. Dosage groups were compared for the following dependent measures: the number of trials to the criterion in the first session (this measure was used to compare groups for overall learning performance), the latency (in seconds) to enter the “dark” compartment from the “bright” compartment on trial 1 in the first session (this measure was used to compare groups for activity levels and exploratory tendencies in a novel environment), the latency (in seconds) to enter the “dark” compartment from the “bright” compartment on trial 2 in the first test session (this measure was used to compare groups for short-term retention), the number of trials to the criterion in the second test session (this measure was used to compare groups for long-term retention), and the latency (in seconds) to enter the “dark” compartment from the “bright” compartment on trial 1 in the second session (this value was another indication of long-term retention).

Beginning at PNDs 58 to 62 and retested on Days 65 to 69 postpartum, one male rat and one female rat from each litter, where possible, were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning, and memory. Each rat was tested twice. The test sessions were separated by a 1-week interval. The correct goal and the criterion were the same for both test sessions. Dosage groups were

compared for the following dependent measures: the number of trials to criterion on the first day of testing (this measure was used to compare groups for overall learning performance), the average number of errors (incorrect turns in the maze) for each trial on the first day of testing (this measure was also used to compare groups for overall learning performance), the latency (in seconds) to reach the correct goal on trial 2 of the first day of testing (this measure was used to compare groups for short-term retention), the number of trials to criterion on the second day of testing (this measure was used to compare groups for long-term retention), the average number of errors on each trial on the second day of testing (this measure was also used to compare groups for long-term retention), and the latency (in seconds) to reach the correct goal on trial 1 of Day 2 of testing (this was another indicator of long-term retention).

Motor activity (MA) assessments were conducted on one rat/sex/litter (when possible) from Subset 3 on PNDs 13, 17, and 21 before dosage administration and on PND 60 ± 2 days. MA (duration of movement and number of movements) was evaluated in six consecutive blocks of 10 minutes each, as well as for the total 60-minute session. Acoustic startle habituation was evaluated on PND 22 and again on PND 61 to 63. One male and one female rat (when possible) from each litter were tested on both days for reactivity to auditory stimuli and habituation of responses with repeated presentation of stimuli.

Acoustic startle habituation was evaluated on PNDs 22 and again on PND 61 to 63. One male and one female rat (when possible) from each litter were tested on both days for reactivity to auditory stimuli and habituation of responses with repeated presentation of stimuli. The rats were tested in sets of four within a sound attenuated chamber. Each rat was placed inside a small cage situated above a platform containing a force transducer in its base. A microcomputer sampled the output of the force transducer and controlled the test session. The rats initially underwent an adaptation period of 5 minutes. During the last minute of this period, ten “blank” trials were conducted to sample the baseline force in the absence of a stimulus. The rats were then presented with 30 millisecond 120dB bursts of noise at ten-second intervals for 50 trials. An additional ten “blank” trials followed. The peak amplitude and latency to peak of each response was recorded, and the average response on the baseline trials subtracted to calculate the response magnitude. The average response magnitude and the pattern of responses over ten trial blocks were compared among the dosage group.

On PND 4 (post-standardisation), 11 (before dosage administration), 21, 35, 45, and 60, all rats assigned to Subset 4 were examined outside of the home cage. Each rat was examined for signs of autonomic dysfunction, abnormal postures, abnormal movements or abnormal behaviour patterns, and unusual appearance by an individual unaware of the dosage group for each rat.

On the day of scheduled sacrifice (PND 21), 40 non-fasted pups (20 male and 20 female pups) in each dosage group (designated Subset 5) were selected for blood collection for haematological evaluations. Whole blood samples were collected after sacrifice via the *vena cava* for haematological evaluations. Approximately 1 mL of blood was collected into EDTA-coated (lavender top) tubes. Within 1 hour of collection, samples were analysed for methemoglobin (MetHb). After analyses of MetHB were completed, the remaining whole blood was transported on wet ice or refrigerated. The samples were analysed for erythrocyte count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) on the day of collection.

After completion of the 22-day postpartum period, F₀ female rats were sacrificed by carbon dioxide asphyxiation, and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The number and distribution of implantation sites was recorded. Rats that did not deliver a litter were sacrificed on DG 25 and examined for gross lesions. Uteri were examined while being pressed between glass plates to confirm the absence of implantation sites. Dams with no surviving pups were sacrificed by carbon dioxide asphyxiation after the last pup was found dead or missing, presumed cannibalised. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The number and distribution of implantation sites was recorded. Dams that delivered a litter but were not selected for continued observation on PND 4 were sacrificed by carbon dioxide asphyxiation subsequent to this selection process. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The number and distribution of implantation sites were recorded. Rats that were found dead or were sacrificed because of moribund condition were examined for the cause of death or moribund condition on the day the observation was made. These rats were examined for gross lesions. The pregnancy status and uterine contents of F₀ generation female rats were

recorded. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites.

Pups dead before initial examination were evaluated to determine if they were stillborn pups or liveborn pups that died shortly after birth. All pups culled on Day 4 were sacrificed, given a gross post-mortem examination, and gross lesions were retained. On PND 21, F₁ pups selected to comprise the Subset 1 were perfused in situ with neutral buffered 10% formalin, given a gross post-mortem examination. The brain was weighed, and brain, head, spinal column, and nerve tissue from hindlimbs were evaluated for morphological measurements and neurohistology. Rats assigned to Subset 1 that were not selected for these procedures and all other surviving rats were sacrificed by carbon dioxide asphyxiation and given a gross pathological examination of thoracic, abdominal, and pelvic viscera.

The remaining F₁ generation rats were sacrificed after completion of all the postweaning behavioural evaluations. Selected rats assigned to Subset 4 were perfused in situ with neutral buffered 10% formalin, given a gross post-mortem examination. The brain was weighed, and brain, head, spinal column, and nerve tissue from hindlimbs were evaluated for morphological measurements and neurohistology. Rats assigned to Subset 4 that were not selected for these procedures and all other surviving rats were sacrificed by carbon dioxide asphyxiation, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed.

Rats that died were examined for the cause of death on the day the observation was made. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed.

II. RESULTS AND DISCUSSION

A. F₀ PARENTAL RATS

1. General observations

Mortality

A total of one, one, and four rats were found dead (or sacrificed in moribund condition) in the 0.5, 1, and 3.0 mg/kg/day dosage groups, respectively. One rat in each of the 0.5 and 1 mg/kg/day dosage groups were found dead during the gestation period (on GD20 and GD22 respectively). At 3.0 mg/kg/day, two rats were found dead during the gestation period (GD19 and 20), one rat was moribund sacrificed during the lactation period (LD3), and one rat was found dead during the lactation period (LD10). The mortality of three of the four rats in the 3.0 mg/kg/day dosage group was considered test substance-related because it was accompanied by clinical signs of neurotoxicity. The other death in the 3.0 mg/kg/day dosage group (on LD10) and the death of the rat in the 0.5 mg/kg/day dosage group were the result of intubation errors. The death in the 1 mg/kg/day dosage group was considered to be an incidental event and unrelated to the test substance because it was a single, non dosage-dependent occurrence. All other rats survived until scheduled sacrifice.

Clinical observations

Adverse clinical signs considered to be test substance-related were primarily observed in the rats in the 3.0 mg/kg/day dosage group that were subsequently either found dead or were moribund sacrificed during the gestation or lactation periods. These observations included chromorhinorrhea, dehydration, soft or liquid faeces, decreased motor activity, coldness to the touch, hunched posture, lost righting reflex, rales, and lacrimation. Head tilt, piloerection, splayed forelimbs, and low carriage were also observed during the gestation period and ptosis during the lactation period. There was a significant increase ($p \leq 0.01$) in the number of rats in the 3.0 mg/kg/day dosage group observed with ataxia during the lactation period (3/22 animals). There was also a significant increase ($p \leq 0.01$) in the number of rats in the 3.0 mg/kg/day dosage group observed with abnormal autonomic functions during the lactation period (11/22 animals).

All other clinical observations were considered unrelated to the test substance because: the observations were not dosage-dependent; the observation occurred in only one or two rats; and/or the observations are common in this strain of rat. These observations included red or pink perioral substance, sparse hair coat, pale extremities, chromodacryorrhea, impaired righting reflex, swollen snout, red perivaginal substance, excess salivation, localised alopecia (limbs, neck and underside), missing/broken and/or misaligned incisors, mass (right side of the back), urine-stained abdominal fur,

tip-toe walk, paleness, gasping, and emaciation. There was a significant increase ($p \leq 0.01$) in the number of rats in the 0.5 mg/kg/day dosage group observed with abnormal autonomic functions during the lactation period. This increased incidence was not considered to be test substance-related because it was not dosage-dependent.

Body weight, body weight gain, and food consumption

Gestation period:

No test substance-related effects on body weight or weight gain and food consumption were observed at maternal dosages of 0.5 or 1.0 mg/kg/day.

Test substance-related decreases in maternal body weight gain occurred in the 3.0 mg/kg/day dosage group. Maternal body weight gains were reduced or significantly reduced in the 3.0 mg/kg/day dosage group at all intervals following the initiation of dosage administration, including the gestation dosage period (DGs 6 through 20, -23% compared to the control group) and the entire gestation period (DGs 0 through 20, -16%). The average maternal body weights were also significantly reduced in the 3.0 mg/kg/day dosage group on DGs 10 through 20 (-5 to -9% compared to the control group).

Test substance-related decreases in maternal body weight gain were also observed in the 1.5 mg/kg/day dosage group during the gestation period. Body weight gains were reduced or significantly reduced on DGs 6 to 9 (-28% compared to the control group), 9 to 12 (-23%) and 12 to 15 (-20%). Although the statistical significance was not reached, maternal body weight gains were reduced on DGs 6 through 20 (-8%) and DGs 0 through 20 (-10%). The average maternal body weights were statistically significantly reduced on DGs 10 through 20 (-4 to -6% compared to the control group). Considering the statistical significances and the magnitude of body weight declines, the decreases in body weight and body weight gain were considered to be adverse.

At 3.0 mg/kg/day, the absolute and relative feed consumption values were significantly reduced at all intervals following the initiation of dosage administration, including the gestation dosage period and the entire gestation period. At 1.5 mg/kg/day, absolute feed consumption values were significantly decreased on DGs 9 to 12 and 12 to 15 as well as during the gestation exposure period (DGs 6 through 20) and the entire gestation period (DGs 0 through 20). Relative feed consumption values were significantly reduced on DGs 9 to 12.

Lactation period:

The average maternal body weights during lactation were reduced or significantly reduced in the 1.5 and 3.0 mg/kg/day dosage groups on DLs 0 through 4. However, maternal body weight gains were increased in the 1.5 and 3.0 mg/kg/day dosage groups during the lactation dosage period (DLs 0 to 10) and postdosage period (DLs 11 to 21). At 3.0 mg/kg/day, there was a significant increase in maternal body weight gain during the entire lactation period (DLs 0 to 21). Due to the variability of the body weight changes during the lactation period, they were not considered to be adverse. Test substance-related decreases in absolute and relative feed consumption values occurred in the 3.0 mg/kg/day dosage group throughout the gestation period.

Table B.6.7.1-12
Developmental neurotoxicity study: Mean body weight/ body weight gain/food consumption
P₀ maternal rats

Dosage (mg/kg bw/day)	0	0.5	1	1.5	3.0
Gestational Parameters					
Body weight (g) GD 20	388.2	379.4	374.3	371.1 (-4.4%)	355.4** (-8%)
Body weight gain (g) GD 6-9	11.7	10.6 (-9.4%)	11.9 (+1.7%)	8.4 (-28%)	5.4** (-54%)
Body weight gain (g) GD 9-12	19.7	18.7 (-5.1%)	16.9 (-14%)	15.1* (-23%)	11.1** (-44%)
Body weight gain (g) GD 12-15	20.6	21.0 (+1.9%)	16.0 (-22%)	16.4 (-20%)	13.2 (-20%)
Body weight gain (g) GD 6-20	115.8	111.3	106.5	106.1 (-8.4%)	89.0** (-23%)
Food consumption (g) GD 6-20	24.0	23.9	22.9	22.2* (-7.5%)	19.8** (-18%)
Lactational Parameters					
Body weight (g) LD 0	298.7	291.0	289.7	282.9* (-5.3%)	275.2** (-8%)
Body weight (g) LD 21	341.8	338.1	338.4	332.0	337.9
Body weight gain (g) LD 0-21	41.8	47.4	46.8	50.4	58.4** (+40%)
Food consumption (g) LD 0-13 ^b	47.1	46.8	47.7	47.2	47.6

* Significantly different from control by Dunnett's criteria, $p < 0.05$

** Significantly different from control by Dunnett's criteria, $p < 0.01$.

^a Calculated through LD 14 because it was presumed that pups would start consuming diet after LD 14.

Nb in brackets: % compared to control group

2. Reproductive parameters and litter observations (pups PND 0 to 10)

Natural delivery observations were based on 21 to 25 pregnant rats in the five dosage groups. All pregnant rats that survived the gestation period delivered litters.

Values for the numbers of dams delivering litters, the duration of gestation, averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant), and the numbers of dams with stillborn pups were comparable among the five groups. The values for surviving pups/litter and mean live litter size at weighing were also comparable among the five dosage groups.

There was a significant increase ($p \leq 0.01$) in the number of stillborn pups in the 3.0 mg/kg/day dosage group. However, the stillborn pups were represented in two litters (13 of the 15 pups born in one litter and one pup from the second litter). Maternal toxicity was evident in the litter in which there were 13 stillborn pups, and the remaining two pups from this litter were found dead prior to weighing on the day of birth. Therefore, the increase in the number of stillborn pups in the 3.0 mg/kg/day dosage group can be attributed to the large number of stillborn pups observed in one litter and the subsequent significant decrease ($p \leq 0.01$) in the percent of liveborn pups in this dosage group.

There was also a significant increase ($p \leq 0.01$) in the number of pups found dead, presumed cannibalised, or moribund sacrificed on PNDs 1 to 4 in the 3.0 mg/kg/day dosage group. This increase was based primarily on two litters in which all of the pups died or were sacrificed *in extremis*. These pups were the offspring from dams that also exhibited clinical signs of toxicity and mortality. The death of these two litters resulted in a significant decrease ($p \leq 0.01$) in the viability index (number of live pups on Day 4 [preculling] postpartum/number of liveborn pups on Day 0 postpartum). The mortality and reduced survival observed in the 3.0 mg/kg/day dosage group is secondary to maternal toxicity since they were associated with significant maternal toxicity and mortality.

There was a significant decrease ($p \leq 0.05$) in the average pup weight per litter in the 3.0 mg/kg/day dosage group at PND 0. There was also a slight increase in the number of litters in the 3.0 mg/kg/day dosage group observed with coldness to the touch and dehydration from birth to day 10 postpartum.

Nevertheless, these findings are not considered to be treatment-related. Indeed, there is no dose-related trend. Furthermore, control rats are similarly affected by dehydration than the highest dose group.

No adverse natural delivery or litter observations in the F₁ generation pups were attributable to exposure to the test substance at dosages as high as 1.5 mg/kg/day. There was a significant increase ($p \leq 0.01$) in the lactation index (number of live pups on Day 10 postpartum/ number of live pups on Day 4 [postculling] postpartum) observed in all dosage groups administered the test substance. These increases were the result of the increased mortality observed in the pups in the 0 mg/kg/day dosage group from PNDs 4 to 10 and were not considered to be the result of the test substance. There was also a significant increase ($p \leq 0.05$ or $p \leq 0.01$) in the percent male pups per number of pups sexed in the 0.5 mg/kg/day dosage group on PNDs 0 and 4 (preculling) and in the 1.5 mg/kg/day dosage group on PNDs 0, 4 (pre- and postculling), and 7. This was not considered to be test substance-related because the incidences were not dosage-dependent.

Table B.6.7.1-13
Developmental neurotoxicity study: Litter observations F₁ rats

Indoxacarb (mg/kg bw/day)	0	0.5	1	1.5	3.0
Litter parameters					
Live born(%)	99.7	99.1	99.7	100.0	95.8**
Live born (n)	298/299	339/342	325/326	349/349	321/335
Still born (%)	0.3	0.9	0.3	0.0	4.2**
Still born (n)	1/299	3/342	1/326	0/349	14/335**
FD, cannibalized, sacrificed in extremis day 0	1/298	1/339	3/325	0/349	3/321
FD, cannibalized, sacrificed in extremis days 1-4	3/297	5/338	5/322	7/349	34/318**
FD, cannibalized, sacrificed in extremis days 5-7	3/204	0/221	0/226	0/232	1/194
FD, cannibalized, sacrificed in extremis days 8-10	1/201	0/221	1/226	0/232	0/193
Viability index (%) ^a	98.6	98.2	97.5	98.0	88.5**
Lactation index (%) ^b	98.0	100.0**	99.6**	100.0**	99.5**
% male pups per number of pups sexed Day 0	46.3	55.7*	54.9	59.6**	52.4
pup weight/litter day 0 (g)	6.4	6.4	6.5	6.4	5.9*
pup weight/litter day 4 (post culling) (g)	9.2	9.4	9.7	9.3	8.8
pup weight/litter day 7 (g)	14.2	14.4	14.7	14.6	13.8
weight gain days 11-21 (g) males	23.8	25.0	24.9	25.2	24.6
weight gain days 11-21 (g) females	23.4	24.0	23.9	24.1	23.8
Weight gain days 21-76 (g) males	396.7	385.2	401.2	394.0	408.0
Weight gain days 21-76 (g) females	198.4	206.9	212.7	204.4	216.1
Food consumption days 21-76 (g) males	23.2	23.7	24.4	23.5	25.1**
Food consumption days 21-76 (g) females	17.0	18.2*	18.3*	17.7	19.0**
Clinical observations (total frequency (days x pups)/litters with observation):					
Cold to touch	0/0	3/2	11/3	0/0	15/4
Dehydrated	15/1	6/2	2/2	3/1	16/3

* Significantly different from control by Dunnett's criteria, $p < 0.05$.

** Significantly different from control by Dunnett's criteria, $p < 0.01$.

^a # of live pups on Day 4 (preculling) postpartum/# liveborn pups on Day 0 postpartum

^b # of live pups on Day 10 postpartum/# of live pups on Day 4 (postculling) postpartum

FD = found dead

3. Gross pathology, organ weights, and histopathology

No test substance-related necropsy observations occurred.

With the exception of those observations that occurred in the rats found dead, gross lesions occurred only in one rat in the 0 mg/kg/day dosage group (red and thick walls of the urinary bladder and containing two calculi), one 1.5 mg/kg/day dosage group rat (clear fluid filled cyst in the left horn of

the uterus), and three 3.0 mg/kg/day dosage group rats. In the 3.0 mg/kg/day dosage group, one female had two masses in the right atrium of the heart; one female had a tan area in the left kidney extending into the parenchyma; and one female was observed with numerous firm, green masses in the spleen.

B. F₁ LITTER AND PUP DATA

1. General observations

There were no test substance-related effects on mortality among the F₁ male and female pups during the lactation period. There was a significant increase in mortality among the 0.5 and 1.5 mg/kg/day dosage group male rats compared to the vehicle control group. However, these deaths were not considered to be test substance-related because the majority of the deaths were the result of intubation errors, and the frequency was not dosage dependent.

There were no test substance-related adverse clinical signs or abnormal autonomic functions. All clinical signs were considered unrelated to the test substance since the frequency was not dosage dependent, the observations were associated with accidental deaths due to intubation errors, the observation occurred in only 1 or 2 rats and/or were commonly observed for this strain of rat.

There were no test substance related effects on body weights or body weight gains during the dosage (PND 11-21) or postdosage period (PND 22-76). Significant increases in body weight gains and absolute and relative feed consumption values in the 3.0 mg/kg/day F₁ generation rats was observed throughout the postdosage period, mainly in males. As these changes were sporadic and represented less than 10% variation compared to the control group, they are not considered to be adverse.

2. Sexual maturation

There were no test substance-related effects on vaginal patency or preputial separation. The average age of sexual maturation was 46.2, 46.0, 45.1, 44.9 and 45.2 in the 0, 0.5, 1.0, 1.5 and 3.0 mg/kg bw/d groups respectively as determined by preputial separation in males and 32.1, 32.2, 32.2, 32.6 and 32.3 in the 0, 0.5, 1.0, 1.5 and 3.0 mg/kg bw/d groups respectively as determined by vaginal patency in females. The average day of preputial separation was significantly reduced in the 1 mg/kg/day group, compared to the control value. However, this observation was not dosage dependant, and was not considered related to the test substance.

3. Motor activity

There were no test substance-related effects in either males or females on motor activity evaluated on PNDs 13, 17, 21, and again between 58 and 62 for either number of movements or time spent moving.

4. Acoustic startle

There were no test substance-related effects in either males or females on for either response magnitude or average response magnitude when evaluated on PNDs 23 and again between 61 and 65.

5. Passive avoidance

There were no test substance-related effects in either males or females on learning and memory as evaluated in a passive avoidance paradigm at approximately 22 days of age, and again one week later. There was a significant increase in the latency to trial 1 in session 2 for the 1 mg/kg/day females which was not considered to be test substance related since it was not dosage dependant.

6. Watermaze

There were no test substance-related effects on learning or memory as evaluated in the watermaze paradigm. In addition, there were no effects on the errors per trial, latency, failure to learn, or any endpoint during the retention phase of the paradigm.

A significant increase in the number of Trials to Criterion for 3.0 mg/kg/day males occurred during the learning phase (session 1), which was within the control range for the concurrent study, and within the testing facility historical control range of 7.3-10.7 trials. In addition, there were no effects on

errors per trial, latency, failure to learn, or any endpoint during the retention phase of the paradigm. Therefore, this statistical difference was not considered to be dosage related.

There was a significant increase in the Trials to Criterion for Session 1 for 1.0 and 1.5 mg/kg/day females. However, these increases were not considered to be test substance-related since they were not dosage dependant, the control value was below the testing facility historical control value for Trials to Criterion for Session 1, and the values for the 1.0 and 1.5 mg/kg/day females were within the testing facility historical control range.

Table B.6.7.1-14
Summary of watermaze performance in F₁ male and female rats in the developmental neurotoxicity study

Endpoints	0 mg/kg/d	0.5 mg/kg/d	1.0 mg/kg/d	1.5 mg/kg/d	3.0 mg/kg/d
Male: Session 1 (Learning Phase)					
Trials to Criterion	8.2 (2.4) ^a	9.0 (3.1)	8.3 (2.6)	7.8 (2.1)	10.4 (2.7)*
Errors per Trial	0.36 (0.22)	0.36 (0.24)	0.37 (0.17)	0.33 (0.17)	0.45 (0.23)
Latency Trial (sec)	12.4 (6.4)	10.8 (5.6)	12.3 (8.0)	11.6 (6.3)	19.4 (17.0)
Failed to Learn	1 (5.9)	1 (7.1)	1 (5.3)	0 (0)	1 (5.6)
Male: Session 2 (Learning Phase)					
Trials to Criterion	6.1 (1.7)	6.8 (2.0)	6.7 (2.5)	6.3 (2.0)	7.4 (3.4)
Errors per Trial	0.09 (0.11)	0.13 (0.16)	0.14 (0.14)	0.11(0.14)	0.14(0.16)
Latency Trial (sec)	11.0 (4.6)	9.5 (7.8)	9.8 (6.6)	11.3 (12.4)	10.0 (8.4)
Female: Session 1 (Learning Phase)					
Trials to Criterion	7.0 (1.8)	8.7 (2.8)	8.9 (2.2)*	8.9 (2.0)*	8.9 (2.8)
Errors per Trial	0.32(0.19)	0.42 (0.31)	0.36 (0.17)	0.35 (0.12)	0.37 (0.16)
Latency Trial (sec)	10.8 (4.4)	14.6 (8.8)	13.5 (7.2)	12.9 (5.2)	13.2 (9.1)
Failed to Learn	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.6)
Female: Session 2 (Learning Phase)					
Trials to Criterion	7.8 (3.3)	6.7 (1.9)	7.9 (3.2)	7.9 (3.2)	6.9 (2.8)
Errors per Trial	0.16 (0.14)	0.20 (0.17)	0.21 (0.21)	0.19 (0.12)	0.10 (0.12)
Latency Trial (sec)	11.2 (7.2)	13.8 (9.0)	13.8 (9.4)	12.3 (7.7)	9.8 (4.9)

^a Standard deviation

7. Clinical pathology

There were no test substance-related effects in either males or females on percent methemoglobin or selected red cell parameters.

Table B.6.7.1-15
Developmental neurotoxicity study: Summary of haematology values for F₁ offspring

	Group I^a 0 mg/kg/day	Group II^a 0.5 mg/kg/day	Group III^a 1 mg/kg/day	Group IV^a 1.5 mg/kg/day	Group V^a 3 mg/kg/day
Males					
RBC (×10 ⁶ /μL) DAY 22	4.23 ^a 0.27(14)	4.22 0.39(15)	4.40 0.29(17)	4.29 0.41(19)	4.48 0.31(15)
HGB (g/dL) DAY 22	7.9 0.9(14)	8.2 1.2(15)	8.2 0.9(17)	8.4 1.1(19)	8.5 0.8(15)
HCT (%) DAY 22	28.2 2.6(14)	29.2 4.2(15)	29.7 3.0(17)	29.9 4.0(19)	30.2 2.8(15)
MCV (fL) DAY 22	66.6 4.1(14)	68.9 5.8(15)	67.5 5.3(17)	69.5 4.3(19)	67.6 3.7(15)
MCH (pg) DAY 22	18.6 1.5(14)	19.4 2.0(15)	18.7 1.7(17)	19.5 1.3(19)	19.0 1.3(15)
MCHC (g/dL) DAY 22	27.9 0.7(14)	28.1 1.1(15)	27.6 0.7(17)	28.0 0.6(19)	28.0 0.9(15)
METH (%) DAY 22	0.5 0.2(14)	0.6 0.2(15)	0.6 0.2(17)	0.5 0.2(19)	0.6 0.2(15)
Females					
RBC (×10 ⁶ /μL) DAY 22	4.19 0.37(18)	4.46 0.47(16)	4.43 0.32(14)	4.42 0.37(14)	4.51 0.38(16)
HGB (g/dL) DAY 22	8.0 0.9(18)	8.6 1.2(16)	8.3 1.0(14)	8.6 0.9(14)	8.7 1.0(16)
HCT (%) DAY 22	28.9 3.0(18)	30.8 4.5(16)	30.4 3.4(14)	30.7 3.4(14)	31.4 3.6(16)
MCV (fL) DAY 22	68.9 3.6(18)	68.9 5.4(16)	68.4 4.8(14)	69.3 5.3(14)	69.4 2.9(16)
MCH (pg) DAY 22	19.1 1.4(18)	19.2 1.7(16)	18.7 1.7(14)	19.4 1.7(14)	19.3 0.9(16)
MCHC (g/dL) DAY 22	27.7 0.9(18)	27.8 0.7(16)	27.3 0.7(14)	28.0 0.9(14)	27.8 0.8(16)
METH (%) DAY 22	0.6 0.1(18)	0.6 0.1(16)	0.7 0.1(15)	0.6 0.1(14)	0.7 0.2(16)

^a Data arranged as: Mean Standard deviation (Number of values included in calculation)

Note: There were no statistically significant differences from control at p <0.05.

8. Brain weight parameters

Terminal body weights, brain weights, and ratio of brain weight to terminal body weight on PND 21 and 69 were similar to control values for all dosages in both males and females.

9. Brain morphometry and neuropathology

Microscopic examinations of brains from juvenile and adult rats from the control and high dose groups were performed, as well as examination of sections of spinal cord and peripheral nervous system (including eyes and skeletal muscle) from the adult rats. There were no test substance related effects on gross linear brain measurements, or neuropathological lesions in F₁ males and females on either PND 21 or 69.

Conclusions from the original DAR (2000, RMS NL):

Newly submitted study in the context of the renewal.

Conclusions proposed by the applicant (2015):

The NOAELs in the developmental neurotoxicity study in rats were as follows:

Parental toxicity: 1.5 mg/kg/day, based on transient and minimal maternal effects observed in the 1.5 mg/kg/day dosage group, and on mortality, decreases in body weight gain, and reductions in feed consumption values observed in the 3.0 mg/kg/day dosage group.

Pup growth and development: 1.5 mg/kg/day for the F₁ generation male rats and 3.0 mg/kg/day for the F₁ generation female rats. There was an increase in body weight gains and feed consumption values during the postdosage period in the 3.0 mg/kg/day dosage group male rats. There were no test substance-related effects on motor activity, learning and memory, neuromorphometry, or neurohistopathology. Therefore, indoxacarb is not a developmental neurotoxicant.

RMS FR assessment (2016):

The NOAEL for maternal toxicity is 1 mg/kg bw/d based on effects in body weight gains in females from the dose level of 1.5 mg/kg bw/d. Mortality and clinical signs of neurotoxicity were observed at the dose level of 3 mg/kg bw/day.

The offspring NOAEL is 1.5 mg/kg bw/day based on increased number of stillborn pups, increased pup mortality on PND1 to 4 and decreased pup weight per litter on PND0 at the dose level of 3 mg/kg bw/day.

B.6.7.2. Delayed polyneuropathy studies

A delayed neurotoxicity study was not required for indoxacarb (DPX-KN128). None of the subchronic and chronic studies conducted with DPX-JW062, DPX-MP062, or indoxacarb, in rats, mice, or dogs, demonstrated any effects suggestive of delayed neurotoxicity. The acute and subchronic (90-day) neurotoxicity studies in rats also did not show any evidence of delayed neurotoxicity.

B.6.8. OTHER TOXICOLOGICAL STUDIES

B.6.8.1. Toxicity studies on metabolites and relevant impurities

Summary data for IN-KG433:

The oral LD₅₀ of IN-KG433 was estimated to be 174 mg/kg bw in female rats.

IN-KG433 is negative in an Ames test and in an *in vitro* gene mutation assay in mammalian cells. An *in vitro* UDS assay was performed in mammalian cells, nevertheless, this test is not considered sensitive enough and the OECD test guideline 482 was deleted in April 2014.

An *in vitro* chromosomal aberration assay is lacking for this metabolite to assess its genotoxic potential. Nevertheless, IN-KG433 is not a metabolite found in groundwater or in residues at significant level. Therefore, this test is not considered needed in the context of the renewal of approval of the active substance.

Table B.6.8.1-1
Summary of toxicity data for IN-KG433

Type of study	Test conditions	Results	Reference
IN-KG433			

Oral LD50	Rat Males: 5000 mg/kg bw Females: 250, 500, 2000 mg/kg bw	M: LD50 > 5000 mg/kg bw F: LD50 = 174 mg/kg bw	1997 HLO-1997-00469
Ames test	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, and TA97a <i>Escherichia coli</i> strain WP2 uvrA (pKM101) Up to 5000 µg/plate in the presence and absence of S9 activation	Negative	Wagner and Reece 1997 HLO-1997-00254
<i>In vitro</i> gene mutation assay in mammalian cells	Chinese Hamster Ovary cells (CHO K1 BH4) Locus HGPRT Up to 5000 µg/mL in the presence and absence of S9 activation	Negative	San and Clarke 1997 HLO-1997-00405
<i>In vitro</i> UDS assay in mammalian cells	Primary rat hepatocytes Up to 1000 µg/mL	Negative	San and Sly 1997 HLO-1997-00406

Summary data for IN-JT333:

The oral LD₅₀ of IN-JT333 was estimated to be 39 mg/kg bw in female rats.

IN-JT333 is negative in an Ames test, in an *in vitro* gene mutation assay in mammalian cells and in an *in vitro* chromosomal aberration assay. It can thus be concluded that IN-JT333 is not an *in vitro* genotoxicant.

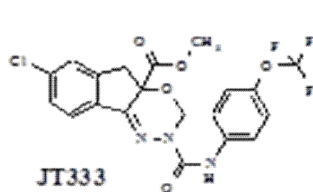
A 14-day oral toxicity study with IN-JT333 is available in the rats. Nevertheless, this test being not GLP and not OECD guideline compliant and showing some deficiencies in its protocol (e.g. haematological parameters, clinical chemistry, and histopathology were not performed), comparison of the toxicity profile to that of the active substance indoxacarb is not possible.

Table B.6.8.1-2
Summary of toxicity data for IN-JT333

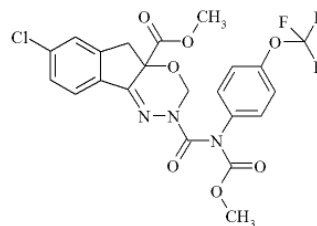
Type of study	Test conditions	Results	Reference
IN-JT333			
Oral LD50	Rat Males and females 10, 30, 50, 100 and 200 mg/kg bw	M: LD50 = 52 mg/kg bw F: LD50 = 39 mg/kg bw	1996 HLR 927-96
Ames test	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, and TA97a <i>Escherichia coli</i> strain WP2 uvrA (pKM101) Up to 5000 µg/plate in the presence and absence of S9 activation	Negative	Mathison 1996 HLR 830-96
<i>In vitro</i> gene mutation assay in mammalian cells	Chinese Hamster Ovary cells (CHO K1 BH4) Locus HGPRT Up to 125 µg/mL in the presence and absence of S9 activation	Negative	San and Clarke 1997 HLO 925-96
<i>In vitro</i> chromosome aberrations assay	Human peripheral blood lymphocytes Up to 2000 µg/mL	Negative	Gudi and Schadley 1996 HLO 951-96
14-day oral study	Rat Feeding 0, 2, 10, 40, 100 ppm Males: 0.19, 0.88, 3.0, 5.6 mg/kg bw/d Females: 0.18, 0.87, 2.9, 4.5 mg/kg bw/d	Study not considered acceptable	1992 HLR 475-91

IN-JT333 is a residue metabolite. Therefore, this metabolite should be assessed in order to compare its toxicity to the toxicity of the parent compound.

The chemical structures of each compound are shown below.



IN-JT333



Indoxacarb

IN-JT333 is not genotoxic *in vitro*. IN-JT333 is more acutely toxic than indoxacarb with a LD₅₀ of 39 mg/kg bw in females (LD₅₀ are equal to 179 mg/kg bw and 268 mg/kg bw for DPX-KN128 (99:1) and DPX-MP062 (75:25) respectively). No acceptable repeated-dose toxicity study is available with IN-JT333 to compare the respective toxicities of IN-JT333 and indoxacarb.

As described in B.6.1, DPX-MP062 (75:25) and DPX-JW062 (50:50) are extensively metabolized in male and female rats, with the predominant distribution of total radioactivity to the fat for both DPX-MP062 (75:25) and DPX-JW062 (50:50). IN-JT333 was the most important metabolite found in fat, representing approximately >95% of the total radioactive residue in that compartment in females following a single oral dose of 5 mg/kg DPX-JW062 (50:50) or DPX-MP062 (75:25).

Table B.6.8.1-3
Percentage (%) of total administered dose in male and female rats administered a dosage of 5 mg/kg/bw/day of indoxacarb

	Males DPX-JW062 (50:50)	Female DPX-JW062 (50:50)	Male DPX-MP062 (75:25)	Female DPX-MP062 (75:25)
Total tissue residue	3.4	7.8	4.4	12.9
Total Fat residue	1.76	4.7	2.6	8.76
Total IN-JT333 residue in fat (% total radioactive residue in fat)	92 <i>n=1</i>	96-99.5 mean = 98.2 <i>n=5</i>	93 <i>n=1</i>	91.5-98.4 mean = 96.4 <i>n=5</i>

In females, which is the most sensitive sex following administration of indoxacarb or IN-JT333 (as determined in the acute oral study), IN-JT333 is present at approx. 4.6% in the fat following administration of DPX-JW062 (50:50) ((4.7*98.2)/100) and 8.5% in the fat following administration of DPX-MP062 (75:25) ((8.8*96.4)/100). As described in B.6.1, DPX-KN128 (99:1) was shown to be the preferred substrate for the enzymatic removal of the N-carboxymethyl group in the parent compound, leading to the formation of the metabolite IN-JT333. Therefore, it can be expected that a greater amount of IN-JT333 would be present in the fat of rats administered the pure S-enantiomer DPX-KN128.

Nevertheless, IN-JT333 was not found in the urine of rats and the quantity of this metabolite sequestered in the fat would not be bioavailable.

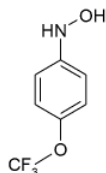
Overall, a short-term repeated dose toxicity study is considered useful to compare the toxicity of the active substance and this residue metabolite and to set reference values for this compound.

At a late stage during the assessment of the active substance, the applicant agreed with the RMS to conduct a guideline repeated-dose toxicity study with IN-JT333 and proposed to perform a 28-day study (OECD 407).

Meanwhile, as a worst-case approach, it is proposed to use the TTC approach. IN-JT333 is not genotoxic in *in vitro* genotoxicity studies. Therefore, the TTC value for non-genotoxic Cramer class III substances can be used: 0.0015 mg/kg bw/d.

Summary data for IN-MT713:

The chemical structure of IN-MT713 (N-hydroxy-4-trifluoromethoxyaniline) is as follows:



IN-MT713

IN-MT713 (N-hydroxy-4-trifluoromethoxyaniline) was not found in the rat after administration of indoxacarb. On the contrary, 4-trifluoromethoxyaniline (IN-P0036) was found to be associated with erythrocytes of rats following administration of indoxacarb. According to the study authors, by analogy to other haemolytic arylamines, which need to be converted to their N-hydroxylated metabolites to exert their haemolytic activity, IN-MT713 is thought to be the metabolite ultimately responsible for indoxacarb-induced haemolytic effects.

IN-MT713 had a dose-dependent haemolytic potential *in vitro* in erythrocytes of rats, dogs and humans, demonstrated by the oxidative effect on glutathione in erythrocytes. Based on the *in vitro* results, humans are likely to be less sensitive to the haemolytic effects of the metabolite than rats or dogs. Furthermore, the *in vitro* haemolytic potential of IN-MT713 was investigated in erythrocytes of glucose-6-phosphate dehydrogenase (G6PDH) normal and deficient humans. G6PDH-deficient individuals may be slightly more sensitive to the oxidative effects of the metabolite than G6PDH-normal individuals.

Nevertheless, based on these results, it cannot be stated with certainty that humans would be less sensitive to haemolysis induced *in vivo* after administration of indoxacarb. Indeed, the mode of action of indoxacarb is not clearly established (see B.6.8.2). Furthermore, in contrast, published data showed that dogs and humans are more sensitive than rats to e.g. the formation of MetHb², which was demonstrated to occur in the only 90-d rat study where this parameter was measured.

IN-KG433

Previous evaluation:	In DAR (2000)
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CA 5.8.1

Report: [REDACTED] (1997); Acute oral toxicity study with IN-KG433 technical in male and female rats

DuPont Report No.: HLO-1997-00469

Guidelines: USEPA 81-1 (1984), 59 NohSan No. 4200 (1985), EEC Method B.1. (1992), OECD 401 (1987)

Deviations: None

Testing Facility: [REDACTED]

Testing Facility Report No.: WIL-189109

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

² Muller et al. 2006 – Hazard classification of chemicals inducing haemolytic anemia: an EU regulatory perspective. Regulatory Toxicology and Pharmacology 45 (2006) 229-241

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KG433 technical
 Lot/Batch #: KG433-3
 Purity: 98.0%
 Description: Off-white solid
 CAS #: None available
 Stability of test compound: The test substance was assumed to be stable under the conditions of administration.
2. Vehicle: Corn oil
3. Test animals
 Species: Rat
 Strain: Crl:CD[®](SD)BR
 Age at dosing: 52 days (males); 73–92 days (females)
 Weight at dosing: 215–226 g for males; 205–220 g for females
 Source: XX
 Acclimation period: At least 7 days
 Diet: PMI[®] Feeds Inc. Certified Rodent LabDiet[®] (#5002), *ad libitum* except when fasted
 Water: Tap water, *ad libitum*
 Housing: Animals were housed singly in stainless steel, wire-mesh cages.
4. Environmental conditions
 Temperature: 22.2–23.2°C
 Humidity: 34.9–48.6%
 Air changes: Not recorded
 Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 13-March-1997 to 22-April-1997
2. Animal assignment and treatment
 IN-KG433 (purity 98.0%) in corn oil was administered by gastric intubation to fasted male Crl:CD[®](SD)BR rats at a concentration of 5000 mg/kg and female rats were dosed at concentrations of 250, 500, or 2000 mg/kg. Surviving animals were observed for clinical signs of toxicity and mortality over a 14-day observation period.

II. RESULTS AND DISCUSSION

A. FINDINGS

The results of the oral LD₅₀ study in rats with IN-KG433 are summarised in the following table.

Table B.6.8.1-2
Acute oral toxicity in rats with IN-KG433: Clinical signs and mortality

Dose (mg/kg bw)	Toxicological results ^a	Duration of signs	Time of death	LD ₅₀ (14 days)
Male rats				
5000	0/5/5	0 – 13 d	--	>5000 mg/kg bw
Female rats				
250	4/5/5	0 – 8 d	1 – 7 d	174 mg/kg bw
500	2/5/5	0 – 14 d	1 – 7 d	
2000	5/5/5	0 – 6 d	1 – 6 d	

^a number of animals which died/number of animals with clinical signs/number of animals in dose group

B. CLINICAL OBSERVATIONS

Clinical findings were noted in all dose groups. Clinical findings most often observed included ataxia, hypoactivity, and various external matting/material/staining. Additional findings included abnormal excretion, impaired righting reflex, hair loss on the urogenital area, emaciation, convulsions, clear ocular discharge, prostration, tremors, opacity in the eyes, missing upper incisor(s), and a missing digit on the left forepaw. With the exception of three 500 mg/kg females, all surviving animals appeared normal by Day 14 or earlier.

C. NECROPSY AND GROSS PATHOLOGY

Gastrointestinal abnormalities, including distended stomachs and dark red or clear gastrointestinal content, were noted for seven rats that died during the study. A single animal had dark red lungs. Various external matting was observed for all 11 rats. There were no other gross necropsy findings for animals that were found dead during the study.

III. CONCLUSION

The oral LD₅₀ for IN-KG433 in rats was greater than 5000 mg/kg body weight in males and the LD₅₀ in females was 174 mg/kg.

Previous evaluation:	In DAR (2000)
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CA 5.8.1

Report: Wagner, V.O., Reece, J.D. (1997); IN-KG433 technical: Mutagenicity testing in the *Salmonella typhimurium* and *Escherichia coli* plate incorporation assay

DuPont Report No.: HLO-1997-00254

Guidelines: none stated, but the protocol followed the OECD guidances 471 and 472 in place at that time
Deviations: None

Testing Facility: Microbiological Associates, Inc., Rockville, Maryland, USA

Testing Facility Report No.: G97AG74.502011

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KG433 technical metabolite
 Lot/Batch #: KG433-3
 Purity: 98.0%
 Description: Off-white solid
 CAS #: None
 Stability of test compound: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
 Solvent: Dimethyl sulfoxide (DMSO)
2. Control materials
 Negative/Solvent control DMSO at 100 µL/plate

Positive control, non activation:

Positive control	Dose (µg/plate)	Strain(s)
2-Nitrofluorene	1.0	TA98
Sodium azide	1.0	TA100, TA1535
9-aminoacridine	75	TA97a
Methyl methanesulfonate	1000	WP2 <i>uvrA</i>

Positive control, activation:

Positive control	Dose (µg/plate)	Strain
2-Aminoanthracene	1.0	TA98, TA100, TA1535
	2.0	TA97a
	10	WP2 <i>uvrA</i>

3. Activation: Rat liver S9 from male Sprague-Dawley rats induced with Aroclor 1254.
 Lot number: Not available
 Source: Male Sprague-Dawley rats induced with Aroclor 1254.
 Protein content:
 Characterisation: Each bulk preparation of S9 was assayed for its ability to metabolise 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to metabolites mutagenic to *Salmonella typhimurium* TA100.
 S9 mix composition
 Sodium phosphate buffer (pH 7.4): 100 mM
 Glucose-6-phosphate: 5 mM
 NADP: 4 mM
 KCl: 33 mM
 MgCl₂: 8 mM
 S9 homogenate: 10% (v/v)
4. Test organisms
Salmonella typhimurium strains TA98, TA100, TA1535, and TA97a and *Escherichia coli* strain WP2 *uvrA* (pKM101) were properly maintained.

5. Test concentrations for plate incorporation assay

Trial 1: 33, 100, 333, 560, 1667, and 5000 µg IN-KG433/plate in triplicate in the presence and absence of S9 activation.

Trial 2: 100, 333, 561, 1667, and 5000 µg IN-KG433/plate in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

13-February-1997 to 11-March-1997

2. Methods

IN-KG433 (purity 98.0%) was evaluated for mutagenicity in *Salmonella typhimurium* strains TA100, TA1535, TA97a, and TA98, and in *Escherichia coli* strain WP2 *uvrA* (pKM101) with and without an exogenous metabolic activation system (S9). Nominal concentrations of 33, 100, 333, 560 or 561, 1667, and 5000 µg/plate were evaluated in two trials (the 33 µg/plate concentration was not used in the second trial). The test substance was dissolved in DMSO. Positive indicators were 2-aminoanthracene (2AA), 2-nitrofluorene (2NF), sodium azide (NAAZ), 9-aminoacridine (9AAc), and methyl methanesulfonate (MMS). A test substance was classified as positive when the average number of revertants in any strain at any test substance concentration was at least two times greater than the negative control and occurred in a dose response relationship.

II. RESULTS AND DISCUSSION

A. FINDINGS

Concentrations of 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 µg IN-KG433/plate in single cultures in the presence and absence of S9 activation were tested in a preliminary cytotoxicity assay. Precipitate was observed at concentrations >667 µg IN-KG433/plate, however, there was no indication of toxicity.

Findings from the mutagenicity test with IN-KG433 in the *Salmonella typhimurium* and *Escherichia coli* plate incorporation assay are summarised in the following table.

In the mutagenicity assay, no positive response was observed. Precipitate was generally observed at ≥1667 µg per plate but no appreciable toxicity was observed.

Table B.6.8.1-3
Summary of average revertants/plate for IN-KG433

Compound	Conc. µg/plate	TA100		TA1535		TA97a		TA98		WP2 <i>uvrA</i>	
		Trial I ^a	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
Average revertants/plate without activation											
IN-KG433	0	106	115	13	12	117	120	14	11	221	242
	33	106	ne ^b	11	ne	110	ne	10	ne	242	ne
	100	119	108	12	10	113	146	8	13	224	265
	333	118	113	12	13	115	152	10	19	214	315
	560	106	ne	14	ne	118	ne	4	ne	223	ne
	561	ne	115	ne	11	ne	140	ne	15	ne	293
	1667	111	128	10	14	109	133	5	14	187	254
	5000	110	109	13	7	119	136	5	13	170	217
NAAZ ^c	1	536	687	413	523	ne	ne	ne	ne	ne	ne
2AA ^d	1	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
9AAc ^e	100	ne	ne	ne	ne	2354	1682	ne	ne	ne	ne
2NF ^f	1	ne	ne	ne	ne	ne	ne	178	152	ne	ne
MMS ^g	1000	ne	ne	ne	ne	ne	ne	ne	ne	1972	1848
Average revertants/plate with activation											
IN-KG433	0	118	130	13	18	134	139	19	17	211	299
	33	121	ne	18	ne	140	ne	16	ne	212	ne
	100	124	139	16	16	137	144	19	19	190	311
	333	124	126	21	21	144	137	20	22	209	319
	560	144	ne	11	ne	124	ne	15	ne	195	ne
	561	ne	126	ne	23	ne	155	ne	24	ne	332
	1667	148	131	15	16	146	141	22	17	222	351
	5000	126	118	18	12	153	136	15	13	237	279
2AA	1	887	899	79	77	ne	ne	582	711	ne	ne
	2	ne	ne	ne	ne	1249	1273	ne	ne	ne	ne
	10	ne	ne	ne	ne	ne	ne	ne	ne	1666	1655

^a Average of three replicates per trial

^b ne = not evaluated

^c NAAZ = sodium azide

^d 2AA = 2-aminoanthracene

^e 9 AAc = 9-aminoacridine

^f 2NF = 2-nitrofluorene

^g MMS = methyl methanesulfonate

III. CONCLUSION

IN-KG433 was negative for mutagenic activity in the *in vitro* bacterial gene mutation assay.

Previous evaluation:	In DAR (2000)
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CA 5.8.1

Report: San, R.H.C., Clarke, J.J. (1997); IN-KG433 technical: *In vitro* mammalian cell gene mutation test (CHO/HGPRT) with an independent repeat assay

DuPont Report No.: HLO-1997-00405

Guidelines: 87/302/EEC Part B, USEPA 84-2, OECD 476 **Deviations:** None

Testing Facility: Microbiological Associates, Inc., Rockville, Maryland, USA

Testing Facility Report No.: G97AG74.782001

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KG433 technical
Lot/Batch #: KG433-3
Purity: 98.0%
Description: Off-white solid
CAS #: None available
Stability of test compound: Test substance appeared to be stable under the conditions of the study; no evidence of instability was observed
Solvent: Dimethyl sulfoxide (DMSO)
2. Control materials
Negative (solvent) control: DMSO
Positive, non-activation: Ethyl methanesulfonate (EMS) in DMSO at a final concentration of 0.2 µL/mL
Positive, activation: Benzo(a)pyrene (BaP) in DMSO at a final concentration of 4.0 µg/mL
3. Activation: Rat liver S9 from male Sprague-Dawley rats induced with Aroclor 1254
Lot number: Not available
Source: Sprague-Dawley rats induced with Aroclor 1254
Protein content: Not provided in the report
Characterisation: The metabolic activation ability of the S9 was characterised using varying S9 and positive control concentrations.
S9 mix composition
Sodium phosphate buffer (pH 8.0): 50 mM
Glucose-6-phosphate: 5 mM
NADP: 4 mM
KCl: 30 mM
MgCl₂: 10 mM
Calcium chloride: 10 mM
S9 homogenate: 100 µL/mL cofactor pool)
4. Test cells
Chinese Hamster Ovary cells (CHO-K₁-BH₄) were cleansed, frozen, and checked for mycoplasma contamination. Cells used in the mutation assay were within four subpassages from frozen stock in order to assure karyotypic stability.
5. Culture medium
F12FBS5-Hx (Ham's F12 medium without hypoxanthine supplemented with 5% dialyzed foetal bovine serum (FBS), 100 units penicillin/mL, 100 µg streptomycin/mL, and 2 mM L-glutamine/mL.

6. Locus examined

Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) – the selection agent used was 10 μ M 6-thioguanine (TG, 2-amino-6-mercaptopurine).

7. Test compound concentrations used

Preliminary cytotoxicity

Trial 1: 0.5, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μ g IN-KG433/mL in the presence and absence of S9 activation.

Mutagenesis assay

Trial 2: 25, 50, 75, 150, 250, and 500 μ g IN-KG433/mL in duplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion:

11-February-1997 to 20-April-1997

2. Preliminary cytotoxicity assay

CHO cells were exposed for 5 hours to vehicle alone and nine concentrations of test substance ranging from 0.5 to 5000 μ g/mL in both the absence and presence of S9-activation for evaluation of test substance effect on colony-forming efficiency (CE).

3. Mutagenesis Assay

CHO cells were exposed for 5 hours to the vehicle alone, appropriate positive controls, and six concentrations of test substance in duplicate in both the absence and presence of S9-activation. After 5 hours, the cells were washed with Ca^{++} and Mg^{++} – free Hanks' balanced salt solution (CMF-HBSS) and cultured for an additional 18–24 hours. At this time, the cells were subcultured to assess cytotoxicity and to initiate the phenotypic expression period.

Cytotoxicity: The replicates from each treatment condition were detached using trypsin and subcultured in triplicate at a density of 100 cells/60 mm dish. After 7–10 days incubation, the colonies were rinsed with HBSS, fixed with methanol, stained with 10% aqueous Giemsa, counted, and cloning efficiency determined.

Phenotypic expression/selection: The replicates from each treatment condition were detached using trypsin and subcultured in duplicate at a density no greater than 10^6 cells/100 mm dish. Subculturing at 2- to 3-day intervals was employed for the 7- to 9-day expression period. For selection of the TG-resistant phenotype, the replicates from each treatment condition were trypsinised and replated, in quintuplicate, at a density of 2×10^5 cells/100 mm dish in medium containing 10 μ M TG. For cloning efficiency determination at the time of selection, 100 cells/60 mm dish were plated in triplicate. After 7–10 days of incubation, the colonies were fixed, stained, and counted for both cloning efficiency and mutant selection.

4. Statistics

The data did not warrant statistical analysis.

5. Evaluation criteria

The test substance was considered to induce a positive response if there was a concentration-related increase in mutant frequencies with at least two consecutive doses showing mutant frequencies of >40 mutants per 10^6 clonable cells. If no culture exhibited a mutant frequency of >40 mutants per 10^6 clonable cells, the test substance was considered negative.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Dimethyl sulfoxide (DMSO) was determined to be the solvent of choice based on solubility of the test article and compatibility with the target cells. The test article was soluble in dimethyl sulfoxide at a concentration of 500 mg/mL, the maximum concentration tested.

B. PRELIMINARY CYTOTOXICITY ASSAY

CHO cells were exposed to solvent alone and nine concentrations of test article ranging from 0.5 to 5000 µg/mL in the absence and presence of S9 reaction mixture. Visible precipitate was observed in treatment medium at concentrations ≥500 µg/mL. Treatment medium was cloudy with no visible precipitate at a concentration of 150 µg/mL. Concentrations ≤50 µg/mL were not cloudy in treatment medium. The osmolality of the solvent control was 407 mmol/kg, and the osmolality of the highest soluble dose, 50 µg/mL, was 398 mmol/kg. Cloning efficiency relative to the solvent controls (RCE) was 105% at 5000 µg/mL without activation and 92% at 5000 µg/mL with S9 activation. Based on the results of the toxicity test, the doses chosen for the initial mutagenesis assay ranged from 25 to 2000 µg/mL for both the non-activated and S9-activated cultures.

C. MUTAGENESIS ASSAY

Due to excessive contamination, the results of the first trial of the initial mutagenesis assay were recorded but not reported. In both the non-activated and S9-activated systems in the initial mutagenesis assay, cultures treated with concentrations of 25, 50, 75, 150, 250, and 500 µg/mL were cloned. Visible precipitate was observed in treatment medium at concentrations ≥500 µg/mL. Treatment medium was cloudy with no visible precipitate at concentrations of 150 and 250 µg/mL in the second trial without activation and at 250 µg/mL in the second trial with S9 activation. Concentrations ≤75 µg/mL in the second trial without activation and ≤150 µg/mL in the second trial with S9 activation were not cloudy in treatment medium. Cultures treated with 1000 and 2000 µg/mL without S9 activation, and with 1000 µg/mL with S9 activation, were discontinued prior to cloning due to insolubility. Relative cloning efficiency was 91% and 98% at the highest dose tested in the non-activated and S9-activated systems, respectively. None of the treated cultures exhibited mutant frequencies of greater than 40 mutants per 10⁶ clonable cells.

In both the non-activated and S9-activated systems in the independent repeat assay, cultures treated with concentrations of 25, 50, 75, 150, 250, and 500 µg/mL were cloned. Visible precipitate was observed in treatment medium at concentrations ≥500 µg/mL. Treatment medium was cloudy with no visible precipitate at a concentration of 250 µg/mL. Concentrations ≤150 µg/mL were not cloudy in treatment medium. Cultures treated with 1000 µg/mL were discontinued prior to cloning due to insolubility. Relative cloning efficiency was 69% and 100% at the highest dose tested in the non-activated and S9-activated systems, respectively. None of the treated cultures exhibited mutant frequencies of greater than 40 mutants per 10⁶ clonable cells.

III. CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the CHO/HGPRT Mutation Assay indicate that, under the conditions of this study, IN-KG433 technical did not cause a positive response in the non-activated and S9-activated systems and was concluded to be negative.

Previous evaluation:	In DAR (2000)
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CA 5.8.1

Report: San, R.H.C., Sly, J.E. (1997); IN-KG433 technical: Unscheduled DNA synthesis in mammalian cells *in vitro* with an independent repeat assay

DuPont Report No.: HLO-1997-00406

Guidelines: 87/302/EEC Part B, USEPA 84-4, OECD 482 (as reported in the study protocol, wrongly mentioned OECD 476 in the study report), 59 Nohsan No. 4200 **Deviations:** None

Testing Facility: Microbiological Associates, Inc., Rockville, Maryland, USA

Testing Facility Report No.: G97AG74.380001

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:
Lot/Batch #: IN-KG433 technical metabolite
Purity: KG433-3
Description: 98.0%
CAS #: Off-white solid
Stability of test compound: None
The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Solvent used: Dimethyl sulfoxide (DMSO)
2. Control materials
Negative (solvent) control/final concentration: DMSO, 1%
Positive control: Dimethylbenz(a)anthracene (DMBA), diluted in DMSO to 300 and 1000 µg/mL

3. Primary culture preparation

For each trial, primary rat hepatocytes were derived from the liver of a normal adult male Sprague-Dawley rat obtained from Harlan Sprague-Dawley, Inc. (Frederick, MD). The rat was anaesthetised and a midventral incision made to expose the liver. The liver was perfused with 0.5 mM ethylene glycol-bis (β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) solution followed by collagenase solution (80–100 units Type I collagenase/mL culture medium). The liver was removed, transected, and shaken in a dilute collagenase solution to release the hepatocytes. The cells were pelleted by centrifugation, resuspended in complete WME (Williams' Media E buffered with 0.01 M HEPES, supplemented with 2 mM L-glutamine, 50 µg/mL gentamicin, and 10% foetal bovine serum). Approximately 5×10^5 cells were seeded into 35 mm tissue culture dishes containing complete WME. The cultures were incubated for 90 to 180 minutes, washed with complete medium, refed with serum-free medium, and used in the test.

4. Test compound concentrations used

Preliminary toxicity test

Concentrations of 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, and 5000 µg IN-KG433/mL were evaluated in duplicate.

Unscheduled DNA synthesis test

Trial 1: 7.8, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 µg IN-KG433/mL in triplicate.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

12-February-1997 to 24-April-1997

2. Methods

IN-KG433 (purity 98.0%) was tested in the unscheduled DNA synthesis (UDS) assay using primary cultures of rat hepatocytes. Based on the results of a preliminary toxicity assay, concentrations tested were 7.8, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 µg/mL. Evaluations for UDS were conducted at dose levels of 125 µg/mL and below. For both the initial and independent repeat assays, the test substance was dissolved in DMSO. DMBA and DMSO served as positive and solvent controls, respectively. Hepatocyte cultures were exposed to their respective concentrations of the test substance (or positive or solvent control), along with 10 µCi ³H-thymidine/mL, for approximately 18 to 21 hours. Mean net nuclear grain counts and percent of cells in repair (cells with ≥5 net nuclear grains) were obtained for each group from autoradiography slides. Cytotoxicity was assessed based on lactate dehydrogenase (LDH) determinations.

II. RESULTS AND DISCUSSION

A. FINDINGS

Findings from the UDS assay with IN-KG433 are summarised in the following table.

Cytotoxicity was observed in cells treated with 250, 500, and 1000 µg/mL. This toxicity precluded evaluation for UDS at these concentrations. UDS was not observed in either trial as a result of treatment of primary rat hepatocytes with IN-KG433 at any concentration evaluated. As expected, the positive indicator, DMBA, did induce UDS.

Table B.6.8.1-4
Summary of UDS assay with IN-KG433

Treatment	Concentration	Mean net grains/nucleus ^a		% of cells in repair ^b	
		Initial assay	Repeat assay	Initial assay	Repeat assay
DMSO ^c	10 µL/mL	-2.4 (2.2) ^d	-1.9 (2.2)	0	0
IN-KG433	7.8 µg/mL	-3.4 (2.9)	-2.5 (1.9)	0	0
	15.6 µg/mL	-5.1 (3.4)	-2.3 (2.5)	0	1
	31.3 µg/mL	-2.1 (2.6)	-2.8 (2.5)	0	0
	62.5 µg/mL	-2.1 (2.5)	-3.0 (2.7)	0	0
	125 µg/mL	-0.8 (2.5)	-20 (2.3)	3	0
DMBA ^e	10 µg/mL	13.8 (7.6) ^f	16.1 (6.5) ^f	93	91

^a Represents the grand mean of three slides per treatment; 50 nuclei per slide were counted

^b Cells in repair = those with five or more net nuclear grains

^c DMSO = dimethyl sulfoxide

^d Standard deviation in parenthesis

^e DMBA = dimethylbenz(a)anthracene

^f Significant based on increase of ≥5 net nuclear grain counts relative to control

III. CONCLUSION

IN-KG433 did not cause a significant increase in unscheduled DNA synthesis and was concluded to be negative.

IN-JT333

Previous evaluation:	In DAR (2000)
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CA 5.8.1

Report: [REDACTED] (1996); Acute oral toxicity study with IN-JT333-20 in male and female rats

DuPont Report No.: HLR 927-96

Guidelines: USEPA 81-1 (1984), 59 NohSan No. 4200 (1985), EEC Method B.1. (1992), OECD 401 (1987)
Deviations: None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 927-96

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:
Lot/Batch #: IN-JT333 technical metabolite
Purity: JT333-20
Description: 98.7%
CAS #: Beige solid
Stability of test compound: 144171-39-1
The test substance was assumed to be stable under the conditions of the study.
2. Vehicle: Mazola[®] corn oil
3. Test animals
Species: Rat
Strain: Crl:CD[®]BR
Age at dosing: 53–57 days (males); 60–71 days (females)
Weight at dosing: 204.1–237.9 g for males; 184.1–224.6 g for females
Source: [REDACTED]
Acclimation period: Approximately 1 week
Diet: Purina[®] Certified Rodent Chow[®] (#5002), *ad libitum*, except when fasted
Water: Tap water, *ad libitum*
Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
Temperature: 22–24°C
Humidity: 40–60%
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
03-July-1996 to 15-August-1996
2. Animal assignment and treatment
Doses of 10, 30, 50, 100, and 200 mg/kg body weight were selected for this study. Following an overnight fast (approximately 18 hours), five male and five female rats per dose group were given a single dose of IN-JT333-20 by gavage. The test substance was administered in Mazola[®] corn oil at a volume of 10 mL/kg bw. Animals were weighed and observed for mortality and signs of illness, injury, or abnormal behaviour daily, except for animals in the 30 mg/kg dosage group, which were not weighed or observed for clinical signs of toxicity on test Days 10 or 11. On test Day 14, surviving

animals were euthanised, and all animals were necropsied to detect grossly observable evidence of organ or tissue damage.

3. Statistics

The LD₅₀ values were calculated from the mortality data using the probit analysis method of Finney (Finney, 1971).

II. RESULTS AND DISCUSSION

The results of the oral LD₅₀ study in rats with IN-JT333 are summarised in the following table.

Table B.6.8.1-5
Acute oral toxicity in rats with IN-JT333: Clinical signs and mortality

Dose (mg/kg bw)	Toxicological results ^a	Duration of signs	Time of death	LD ₅₀ (14 days)
Male rats				
10	0/4/5	2–4 d	—	52 mg/kg bw
30	0/2/5	2–15 d	—	
50	2/5/5	2–15 d	7–11 d	
100	5/5/5	1–6 d	2–6 d	
200	5/5/5	1–4 d	3–5 d	
Female rats				
10	0/2/5	2–4 d	—	39 mg/kg bw
30	0/2/5	2–9 d	—	
50	5/5/5	2–10 d	6–11 d	
100	5/5/5	1–11 d	4–12 d	
200	5/5/5	1–4 d	2–5 d	
Oral LD ₅₀ for males and females combined:				47 mg/kg bw

^a Number of animals which died/number of animals with clinical signs/number of animals in dose group

Clinical signs: Clinical signs of toxicity most commonly observed in fatally exposed rats from the 50, 100, and 200 mg/kg dose groups included ataxia, piloerection, hunched posture, splayed rear legs, general spasms, tremors, and ruffled fur. The surviving male rats from the 50 mg/kg dose group exhibited hunched posture and piloerection. Ruffled fur, diarrhoea, and high carriage were also observed in some of these rats. Male and female rats from the 10 and 30 mg/kg dose groups exhibited very few clinical signs of toxicity; these included piloerection, hunched posture, ruffled fur, and diarrhoea. Other clinical signs observed sporadically or infrequently during the study included oral and ocular discharges, stained nose or face, exophthalmus, wet perineum, alopecia, salivation, animal leaning to left, aggressive behaviour, not eating, weakness, low carriage, stained mouth, abnormal gait or mobility, missing toe, sore tail, and sore on left leg.

Gross necropsy: No test substance-related gross lesions were observed at necropsy.

III. CONCLUSION

The oral LD₅₀ for IN-JT333 in rats was 52 and 39 mg/kg body weight for males and females, respectively.

Previous evaluation:	In DAR (2000)
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CA 5.8.1

Report: Mathison, B.H. (1996); IN-JT333-20: Mutagenicity testing in the Salmonella typhimurium and Escherichia coli plate incorporation assay

DuPont Report No.: HLR 830-96

Guidelines: 92/69/EEC Methods B.13 and B.14, OECD 471 and 472, 59 Nohsan No. 4200, USEPA 84-2
Deviations: None

Testing Facility: DuPont Haskell Laboratory, Newark, Delaware, USA

Testing Facility Report No.: HLR 830-96

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JT333 technical metabolite
Lot/Batch #: JT333-20
Purity: 98.7%
Description: Beige solid
CAS #: 144171-39-1
Stability of test compound: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Solvent: Dimethyl sulfoxide (DMSO)
2. Control materials
Negative/Solvent control/final concentration: DMSO at 0.1 mL/plate

Positive control, non activation:

Positive control	Dose (µg/plate)	Strain(s)
Sodium azide (NAAZ)	2	TA100, TA1535
ICR 191 Acridine	2	TA97a
2-nitrofluorene (2NF)	25	TA98
Methyl methanesulfonate (MMS)	1000	WP2 <i>uvrA</i>

Positive control, activation:

Positive control	Dose (µg/plate)	Strain
2-aminoanthracene (2AA)	1	TA100, TA97a
	2	TA1535, TA98
	25	WP2 <i>uvrA</i>

3. Activation: Rat liver S9 from male Sprague-Dawley rats induced with Aroclor 1254.
 Lot number: -
 Source: Molecular Toxicology, Annapolis, Maryland
 Protein content: 1.6 mg S9 protein/ 1.0 mL S9 Mix
 Each lot of manufactured S9 is characterized by performed Ames assays using TA98 and TA100 against both 2-aminoanthracene (1 µg/plate) and benzo(a)pyrene (5 µg/plate).
- Characterisation: Not provided in the report.
 S9 mix composition
 Sodium phosphate buffer (pH 7.4): 100 mM
 Glucose-6-phosphate: 5 mM
 NADP: 4 mM
 KCl: 33 mM
 MgCl₂: 8 mM
 S9 homogenate: 1.6 mg S9 protein/ 1.0 mL S9 Mix)
4. Test organisms
Salmonella typhimurium strains TA98, TA100, TA1535, and TA97a and *Escherichia coli* strain WP2 *uvrA* (pKM101) were properly maintained.
5. Test concentrations for plate incorporation assay
Trial I: 0.0, 10, 50, 100, 500, 1000, 2500, and 5000 µg IN-JT333/plate in triplicate in the presence and absence of S9 activation.
Trial II: 0.0, 10, 50, 100, 500, 1000, 2500, and 5000 µg IN-JT333/plate in triplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
 18-June-1996 to 23-September-1996

2. Methods

IN-JT333 (purity 98.7%) was evaluated for mutagenicity in *Salmonella typhimurium* strains TA100, TA1535, TA97a, and TA98 and in *Escherichia coli* strain WP2*uvrA* (pKM101) with and without an exogenous metabolic activation system (S9). Nominal concentrations of 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate were evaluated in two trials. The test substance was dissolved in DMSO. Positive indicators were 2-aminoanthracene (2AA), 2-nitrofluorene (2NF), sodium azide (NAAZ), ICR-191 Acridine (ICR-191), and methyl methanesulfonate (MMS). A test substance was classified as positive when the average number of revertants in any strain at any test substance concentration was at least two times greater than the negative control and occurred in a dose-response relationship.

II. RESULTS AND DISCUSSION

A. FINDINGS

Findings from the mutagenicity test with IN-JT333 in the *Salmonella typhimurium* and *Escherichia coli* plate incorporation assay are summarised in the following tableTable .

Toxicity was observed at 1000 µg/plate and higher in Trial I with activation in strain TA97a. However, no toxicity was observed in this strain in Trial II. The number of revertants at all concentrations of the test substance, both with and without activation, was not two times increased compared to controls.

Historical control data, not reported in the study report (it was not a test guideline requirement at the time the study was performed), were provided by the applicant. They demonstrated the validity of controls when tested both in the presence and in the absence of S9 metabolic activation.

Table B.6.8.1-6
Summary of average revertants/plate with IN-JT333

Compound	Conc µg/plate	TA100		TA1535		TA97a		TA98		WP2 <i>uvrA</i>	
		Trial I ^a	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
Average revertants/plate without activation											
IN-JT333	0	194	103	21	32	82	131	19	33	145	191
	10	184	91	19	32	95	140	19	29	174	191
	50	196	97	24	25	97	144	18	32	168	209
	100	215	98	17	28	100	131	19	35	165	205
	500	210	86	18	23	96	162	19	32	147	201
	1000	208	104	16	24	77	169	23	32	173	199
	2500	206	92	21	30	112	150	16	34	176	230
	5000	208	162	26	24	111	220	26	30	199	304
NAAZ ^b	2	703	922	601	936	ne ^c	ne	ne	ne	ne	ne
2AA ^d	1	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ICR-191 ^e	2	ne	ne	ne	ne	2450	1102	ne	ne	ne	ne
2NF ^f	25	ne	ne	ne	ne	ne	ne	1528	968	ne	ne
MMS ^g	1000	ne	ne	ne	ne	ne	ne	ne	ne	1728	1252
Average revertants/plate with activation											
IN-JT333	0	181	137	15	24	106	169	24	28	183	190
	10	193	133	15	24	113	180	26	29	179	192
	50	190	149	10	28	109	197	25	30	184	197
	100	193	151	15	28	105	221	21	33	144	192
	500	191	136	15	29	107	226	18	32	174	191
	1000	207	138	10	21	23	211	21	33	169	182
	2500	255	148	11	22	0	202	20	35	169	177
	5000	235	161	13	27	0	230	7	39	238	188
2AA	1	1120	1480	ne	ne	684	1306	ne	ne	ne	ne
	2	ne	ne	600	480	ne	ne	2102	2207	ne	ne
	250	ne	ne	ne	ne	ne	ne	ne	ne	1340	1989

^a Average of three replicates per trial

^b NAAZ = sodium azide

^c ne = not evaluated

^d 2AA = 2 aminoanthracene

^e ICR-191 = ICR-191 Acridine

^f 2NF = 2-nitrofluorene

^g MMS = Methyl methanesulfonate

Table B.6.8.1-7
Historical control data based on 16 Ames tests at DuPont Haskell Laboratory (1988-1997)

Tester	Control	Exogenous metabolic	Mean	SD	Range	
Strain	(Positive control)	Activation system			Minimum	Maximum
TA97	Negative	Absent	113	15	86	154
	Negative	Present	139	20	100	196
	Positive	Absent	1918	390	1120	2888
	Positive	Present	1511	324	501	2249
TA98	Negative	Absent	19	6	9	40
	Negative	Present	31	6	18	47
	Positive	Absent	1564	221	1073	2109
	Positive	Present	1972	373	1109	2662
TA100	Negative	Absent	116	19	83	166
	Negative	Present	122	16	95	164
	Positive	Absent	809	177	397	1136
	Positive	Present	1833	434	764	2632
TA1535	Negative	Absent	17	5	8	34
	Negative	Present	15	4	7	23
	Positive	Absent	584	155	230	873
	Positive	Present	296	66	151	468
WP2 <i>uvrA</i>	Negative	Absent	151	11	140	167
	Negative	Present	193	11	176	205
	Positive	Absent	1774	221	1506	2051
	Positive	Present	2039	113	1890	2146

III. CONCLUSION

IN-JT333 was negative for mutagenic activity in the *in vitro* bacterial gene mutation assay.

Previous evaluation:	In DAR (2000)
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CA 5.8.1

Report: San, R., Clarke, J.J (1997); IN-JT333-20: *In vitro* mammalian cell gene mutation test (CHO/HGPRT) with an independent repeat assay

DuPont Report No.: HLO 925-96

Guidelines: OECD 476, 87/302/EEC Part B, USEPA 84-2 **Deviations:** None

Testing Facility: Microbiological Associates, Inc., Rockville, Maryland, USA

Testing Facility Report No.: G96AW62.782001

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JT333 technical
 Lot/Batch #: JT333-20
 Purity: 98.7%
 Description: Beige solid
 CAS #: 144171-39-1
 Stability of test compound: Test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
 Solvent: Dimethylsulfoxide
2. Control materials
 Negative (solvent) control: DMSO
 Positive, non-activation: Ethyl methanesulfonate (EMS) in DMSO at a final concentration of 0.2 µL/mL
 Positive, activation: Benzo(a)pyrene (BaP) in DMSO at a final concentration of 4 µg/mL
3. Activation: Rat liver S9 from male Sprague-Dawley rats induced with Aroclor 1254
 Lot number: No lot number assigned
 Source: Sprague-Dawley rats induced with Aroclor 1254
 Protein content: Not provided
 Characterisation: The metabolic activation ability of the S9 was characterised using varying S9 and positive control concentrations.
 S9 mix composition
 Sodium phosphate buffer (pH 8.0): 50 mM
 Glucose-6-phosphate: 5 mM
 NADP: 4 mM
 KCl: 30 mM
 MgCl₂: 10 mM
 Calcium chloride: 10 mM
 S9 homogenate: 1 mL S9 per 25 cm² flask
4. Test cells
 Chinese Hamster Ovary cells (CHO-K₁-BH₄) were cleansed, frozen, and checked for mycoplasma contamination. Cells used in the mutation assay were within four subpassages from frozen stock in order to assure karyotypic stability.
5. Culture medium
 F12FBS5-Hx (Ham's F12 medium without hypoxanthine supplemented with 5% foetal bovine serum (FBS), 100 units penicillin/mL, 100 µg streptomycin/mL, and 2 mM L-glutamine/mL).
6. Locus examined
 Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) – the selection agent used was 10 µM 6-thioguanine (TG, 2-amino-6-mercaptopurine).
7. Test compound concentrations used
Preliminary cytotoxicity
 Trial 1: 0.5, 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg IN-JT333-20/mL in the presence and absence of S9 activation.
Mutagenesis assay
 Trial 2: 7.8, 16, 31, 63, and 125 µg IN-JT333-20/mL in duplicate in the presence and absence of S9 activation.

Independent repeat assay

Trial 2: 3.9, 7.8, 16, 31, 63, and 125 µg IN-JT333-20/mL in duplicate in the presence and absence of S9 activation.

B. STUDY DESIGN AND METHODS

1. Experimental start/completion:
06-June-1996 to 19-August-1996

2. Preliminary cytotoxicity assay

CHO cells were exposed for 5 hours to vehicle alone and nine concentrations of test substance ranging from 0.5 to 5000 µg/mL in both the absence and presence of S9-activation for evaluation of test substance effect on colony-forming efficiency (CE).

3. Mutagenesis Assay

CHO cells were exposed for 5 hours to the vehicle alone, appropriate positive controls, and five concentrations of test substance in duplicate in both the absence and presence of S9-activation. After 5 hours, the cells were washed with Ca^{++} and Mg^{++} – free Hanks' balanced salt solution (CMF-HBSS) and cultured for an additional 18–24 hours. At this time, the cells were subcultured to assess cytotoxicity and to initiate the phenotypic expression period.

Cytotoxicity: The replicates from each treatment condition were detached using trypsin and subcultured in triplicate at a density of 100 cells/60 mm dish. After 7–10 days incubation, the colonies were rinsed with HBSS, fixed with methanol, stained with 10% aqueous Giemsa, counted, and cloning efficiency determined.

Phenotypic expression/selection: The replicates from each treatment condition were detached using trypsin and subcultured in duplicate at a density no greater than 10^6 cells/100 mm dish. Subculturing at 2- to 3-day intervals was employed for the 7- to 9-day expression period. For selection of the TG-resistant phenotype, the replicates from each treatment condition were trypsinised and replated, in quintuplicate, at a density of 2×10^5 cells/100 mm dish in medium containing 10 µM TG. For cloning efficiency determination at the time of selection, 100 cells/60 mm dish were plated in triplicate. After 7–10 days of incubation, the colonies were fixed, stained, and counted for both cloning efficiency and mutant selection.

4. Statistics

The data did not warrant statistical analysis.

5. Evaluation criteria

The test substance was considered to induce a positive response if there was a concentration-related increase in mutant frequencies with at least two consecutive doses showing mutant frequencies of >40 mutants per 10^6 clonable cells. If no culture exhibited a mutant frequency of >40 mutants per 10^6 clonable cells, the test substance was considered negative.

II. RESULTS AND DISCUSSION**A. ANALYTICAL DETERMINATIONS**

Dimethylsulfoxide was determined to be the solvent of choice based on solubility of the test article and compatibility with the target cells. The test article was soluble in dimethylsulfoxide at a concentration of 500 mg/mL, the maximum concentration tested.

B. PRELIMINARY CYTOTOXICITY ASSAY

CHO cells were exposed to solvent alone and nine concentrations of test article ranging from 0.5 to 5000 µg/mL in the absence and presence of S9 reaction mixture. Visible precipitate was observed in treatment medium at concentrations of 1500 and 5000 µg/mL. The test article was soluble but cloudy in treatment medium with no visible precipitate at a concentration of 500 µg/mL. Concentrations of ≤150 µg/mL were soluble in treatment medium. The osmolality of the solvent control was 409 mmol/kg, and the osmolality of the highest soluble dose, 150 µg/mL, was 420 mmol/kg. Cloning efficiency relative to the solvent controls (RCE) was 87% at 5000 µg/mL without activation and 74% at 5000 µg/mL with S9 activation. Based on the results of the toxicity test, the doses chosen for the initial mutagenesis assay ranged from 12.5 to 500 µg/mL for both the non-activated and S9-activated cultures. The first trial of the initial mutagenesis assay was discontinued immediately after treatment because there were too many soluble but cloudy doses in treatment medium. The doses chosen for the second trial of the initial mutagenesis assay ranged from 0.98 to 500 µg/mL.

C. MUTAGENESIS ASSAY

Visible precipitate was observed in treatment medium at a concentration 500 µg/mL in the initial mutagenesis assay. The test article was soluble but cloudy in treatment medium with no visible precipitate at concentrations of 125 and 250 µg/mL. Concentrations of ≤63 µg/mL were soluble in treatment medium. Based on these observations, only cultures treated with concentrations of 7.8, 16, 31, 63, and 125 µg/mL were cloned in both the non-activated and S9-activated systems. Relative cloning efficiency was 100% and 90% at the highest dose cloned in the non-activated and S9-activated systems, respectively. One of the cloned cultures, 16 µg/mL without S9 activation, exhibited a mutant frequency of greater than 40 mutants per 10⁶ clonable cells.

Visible precipitate was observed in treatment medium at a concentration 500 µg/mL in the independent repeat assay. The test article was soluble but cloudy in treatment medium with no visible precipitate at concentrations of 31, 63, 125, and 250 µg/mL. Concentrations of ≤16 µg/mL were soluble in treatment medium. Based on these observations, only cultures treated with concentrations of 3.9, 7.8, 16, 31, 63, and 125 µg/mL were cloned in both the non-activated and S9-activated systems. Relative cloning efficiency was 84% and 121 % at the highest dose cloned in the non-activated and S9-activated systems, respectively. None of the cloned cultures exhibited mutant frequencies of greater than 40 mutants per 10⁶ clonable cells.

III. CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the CHO/HGPRT Mutation Assay indicate that, under the conditions of this study, IN-JT333-20 did not cause a positive response in the non-activated and S9-activated systems and was concluded to be negative.

Previous evaluation:	In DAR (2000)
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CA 5.8.1

Report: Gudi, R., Schadley, E. (1996); IN-JT333-20: *In vitro* evaluation for chromosome aberrations in human peripheral blood lymphocytes

DuPont Report No.: HLO 951-96

Guidelines: USEPA 84-2, EEC 92/69 Method B.10, OECD 473 **Deviations:** None

Testing Facility: Microbiological Associates, Inc., Rockville, Maryland, USA

Testing Facility Report No.: G96AW62.342003

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:
Lot/Batch #: IN-JT333
Purity: JT333-20
Description: 98.7%
CAS #: Beige powder
Stability of test compound: 144171-39-1
Test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Solvent: Dimethylsulfoxide (DMSO)
2. Control materials
Negative (Solvent) control/final concentration: DMSO
Positive, non-activation: Mitomycin C (MMC) in water at 0.25 µg/mL
Positive, activation: Cyclophosphamide (CP) in water at 25 mg/mL
3. Activation: Rat liver S9 from male Sprague-Dawley rats induced with Aroclor 1254
Lot number: Not provided in the report
Source: Microbiological Associates, Rockville, Maryland
Protein content: Not provided in the report
Characterisation: Not provided in the report.
S9 mix composition
Sodium phosphate buffer (pH 7.4): Not provided in the report
Glucose-6-phosphate: 1 mM
NADP: 1 mM
KCl: 6 mM
MgCl₂: 2 mM
S9 homogenate: 20 µL/mL medium
4. Test cells
Human lymphocytes obtained from human blood from normal, healthy donors.
5. Culture medium
RPMI 1640 serum-free, supplemented with 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine.

6. Test compound concentrations used in the chromosome aberration assay
- | | |
|---|--|
| Non-activated 4-hour exposure group, initial assay, 20-hour harvest | 0.5, 1.5, 5, 15, 50, 150, 500, and 1500, and 5000 µg IN-JT333-20/mL in duplicate in the absence of S9 activation. Data from this assay were not used, and the assay was repeated due to poor slide quality. |
| S9-activated 4-hour exposure group, initial assay, 20-hour harvest | 0.5, 1.5, 5, 15, 50, 150, and 500, 1500, and 5000 µg IN-JT333-20/mL in duplicate in the presence of S9 activation. Data from 0.5, 1.5, 5, and 15 µg assays was not needed for analysis. 5000µg was insoluble |
| Non-activated 4-hour exposure group, repeat assay, 20-hour harvest | 62.5, 125, 250, 500, 1000, 1500, and 2000 µg IN-JT333-20/mL in duplicate in the absence of S9 activation. |
| S9-activated 4-hour exposure group, repeat assay, 20-hour harvest | 15.7, 31.3, 62.5, 125, 250, 500, and 1000 µg IN-JT333-20/mL in duplicate in the presence of S9 activation. |
| Non-activated 4-hour exposure group, repeat assay, 44-hour harvest | 500, 1000, 1500, and 2000 µg IN-JT333-20/mL in duplicate in the absence of S9 activation. |
| S9-activated 4-hour exposure group, repeat assay, 44-hour harvest | 125, 250, 500, and 1000 µg IN-JT333-20/mL in duplicate in the presence of S9 activation. |

B. STUDY DESIGN AND METHODS

1. Experimental start/completion
20-June-1996 to 30-August-1996

2. Initial chromosome aberration assay

The initial chromosome aberration test was performed for the purpose of establishing the dose range for testing and evaluating the clastogenic potential of the test substance. The cells were exposed to solvent alone and to concentrations of the test substance ranging from 15 to 500 µg/mL for 4 hours in the presence of S9 activation and from 50 to 1500 µg/mL for 4 hours in the absence of S9 activation.

3. Repeat chromosome aberration assay

Cell treatment: Cells were exposed to test compound, solvent, or positive control for 4 h (non-activated) or 4 h (activated).

Spindle inhibition: Two hours prior to the scheduled cell harvest at approximately 20 h or at 44 h after treatment initiation, Colcemid® was added to the cell cultures at a final concentration of 0.1 µg/mL.

Cell harvest: Two hours after the addition of Colcemid®, metaphase cells were harvested for both the activated and non-activated studies by centrifugation at 1200 rpm for about 5 minutes. The cell pellet was resuspended in 5 mL of 0.075 M KCl and incubated at 37 ± 1°C for 20 minutes. At the end of the KCl treatment and immediately prior to centrifuging, the cells were gently mixed and approximately 0.5 mL of fixative (methanol:glacial acetic acid, 3:1 v/v) was added to each tube. The cells were collected by centrifugation, the supernatant aspirated, and the cells were fixed with two washes with approximately 3–5 mL of fixative and stored in fixative overnight or longer at approximately 2–6°C.

Slide preparation: Fixed cells were centrifuged at approximately 1200 rpm for 5 minutes. The supernatant was aspirated, and the cells were suspended in 1 mL cold fresh fixative. The cells were

collected by centrifugation and the supernatant aspirated, leaving 0.1 to 0.3 mL fixative above the cell pellet. An aliquot of the cell suspension was dropped onto a glass slide and allowed to air dry overnight. The dried slides were stained with 5% Giemsa, air dried and permanently mounted.

Evaluation of metaphase cells: Metaphase cells with 46 centromeres were examined under oil immersion without prior knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase-spreads (100 per duplicate [replicate] treatment condition) were examined for chromatid-type and chromosome-type aberrations. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverised chromosome(s), pulverised cells, and severely damaged cells (>10 aberrations) also were recorded. Chromatid gaps and isochromatid gaps were recorded but not included in the analysis. The mitotic index was recorded as the percentage of cells in mitosis per 500 cells counted. The percent polyploid cells were recorded per 100 metaphase cells.

4. Statistics

Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test ($p \leq 0.05$). In the event of a positive Fisher's exact test at any test substance concentration, the Cochran-Armitage test was used to measure dose-responsiveness.

5. Evaluation criteria

The test substance was considered to induce a positive response if the percentage of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the concurrent negative control group ($p \leq 0.05$) or if a reproducible and significant increase in the percentage of cells with aberrations occurred at a single dose level relative to concurrent negative controls. Test substances not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

II. RESULTS AND DISCUSSION

Findings from the *in vitro* mammalian cytogenetic test for chromosome aberrations with IN-JT333 are summarised in the following table.

Table B.6.8.1-8
Summary of chromosome aberration data with IN-JT333

Compound	Conc µg/mL	Initial assay		Repeat assay					
		% of cells with structural aberrations		% of cells with structural aberrations				% of cells with polyploidy	
		S9-	S9+	S9-		S9+		S9-	S9+
		20 hr	20 hr	20 hr	44 hr	20 hr	44 hr	44 hr	44hr
Untreated	—	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0
DMSO	—	0.5	0.0	0.5	0.5	2.0	0.0	0.0	0.0
IN-JT333-20	15	ne ^a	1.0	ne	ne	ne	ne	ne	ne
	50	0.0	1.0	ne	ne	ne	ne	ne	ne
	125	ne	ne	ne	ne	0.0	0.5	ne	0.0
	150	0.0	1.0	ne	ne	ne	ne	ne	ne
	250	ne	ne	ne	ne	2.0	0.0	ne	0.0
	500	0.0	0.0	1.0	2.0	1.0	0.0	0.5	1.0
	1000	ne	ne	2.0	0.0	1.5	0.5	0.0	0.0
	1500	0.5	ne	0.5	1.0	ne	ne	0.5	ne
	2000	ne	ne	2.0	0.5	ne	ne	0.0	ne
MMC ^b	0.25	7.5 ^c	ne	6.5 ^c	7.0 ^c	ne	ne	0.0	ne
CP ^d	25	ne	11.0 ^c	ne	ne	12.8 ^c	10.0 ^c	ne	0.0

^a Not evaluated or data not collected

^b Mitomycin C

^c Statistically significant ($p \leq 0.01$; Fisher's exact test)

^d Cyclophosphamide

In the initial chromosome aberration assay, dose level 5000 µg/mL in the non-activated study and dose levels 1500 and 5000 µg/mL in the S9-activated study were not analysed due to insufficient number of scorable metaphase cells. No statistically significant increases in structural or numerical chromosome aberrations were observed in either trail, with or without activation.

III. CONCLUSION

IN-JT333 was negative for structural and numerical chromosome aberrations in the non-activated and S9-activated test systems in the *in vitro* mammalian cytogenetics test using human peripheral blood lymphocytes.

Previous evaluation:	Submitted for the first inclusion of indoxacarb but not included in the DAR (2000).
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CA 5.8.1

Report: [REDACTED] (1992); Repeated dose oral toxicity: 14-Day feeding study with in JT333-1 in male and female rats

DuPont Report No.: HLR 475-91

Guidelines: none stated **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: HLR 475-91

GLP: No

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:
Lot/Batch #: JT333-1
Purity: >95%
Description: White solid
CAS #: Not supplied in the report
Stability of test compound: Not determined
2. Vehicle and/or negative control: Untreated diet with acetone
3. Test animals
Species: Rat
Strain: CrI:CD[®]BR
Age at initial dosing: Approximately 6 weeks old
Weight at initial dosing: 135.8–211.2 g for males; 142.3–172.4 g for females
Source: [REDACTED]
Acclimation period: Approximately one week
Diet: Ground Purina[®] Certified Rodent Chow[®] (#5002), *ad libitum*.
During the test period, test substance was incorporated into the feed of all animals except negative controls.

Water: Tap water, *ad libitum*
Housing: Animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4. Environmental conditions
Temperature: 21–25°C
Humidity: 40–60%
Air changes: Not recorded
Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed
23-January-1991 to 06-February-1991
2. Animal assignment and treatment
Five groups of 5 or 9 animals/sex/concentration were administered concentrations of IN-JT333-1 in feed daily for 14 days. Males received 0, 2, 10, 40, and 100 ppm and females received 0, 2, 10, 40, and 100 ppm. Dose levels were selected to duplicate those used to evaluate other insecticidal compounds such that relative toxicity associated with similar pre-developmental candidates could be determined. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet.

Table B.6.8.1-9
Study design: 14-day feeding study in rats

Males				Females			
Group no.	No./group ^b	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw	Group no.	No./group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw
I	9	0 (control)	0 (control)	II	9	0 (control)	0 (control)
III	5	2	0.19	IV	5	2	0.18
V	9	10	0.88	VI	9	10	0.87
VII	9	40	3.0	VIII	9	40	2.9
IX	9	100	5.6	X	9	100	4.5

^a Weight/weight concentration of test substance

^b 4 animals per group were sacrificed on test day 8 for potential genotoxicity testing.

3. Diet preparation and analysis

The test substance (dissolved in acetone) was added to the rodent diet and thoroughly mixed for 3 minutes. Control diets were mixed for the same period of time. All diets were prepared once and stored refrigerated until used. Samples of the diet were taken on the day they were prepared but were not analysed. In the absence of visible evidence to the contrary, the test substance was assumed to be stable under the conditions of administration.

4. Statistics

Body weights and body weight gains were analysed by a one-way analysis of variance. When the test for differences among test group means (F test) was significant, pairwise comparisons between test and control groups were made with the Dunnett's test. Significance was judged at $p = 0.05$.

C. METHODS

1. Observations

Animals were observed at least once daily for mortality and morbidity and for signs of abnormal behaviour and appearance. On days when they were weighed, each animal was individually handled, examined for abnormal behaviour and appearance.

2. Body weights

All animals were weighed at least twice per week.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded weekly for each animal. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Genotoxicology assessment

Four rats/sex from the control and the 10, 40, and 100 ppm IN JT333-1 treated groups were sacrificed on Day 8. Bone marrow samples were prepared for the potential assessments of micronucleus induction in polychromatic erythrocytes (PCEs), as well as the relative proportion of normochromic erythrocytes (NCEs) to PCEs, however, analyses was not conducted.

5. Sacrifice and pathology

At termination, animals were sacrificed by chloroform anaesthesia and exsanguination. Gross examinations were performed on all main study animals (except animals sacrificed on test Day 8 for potential genotoxicity evaluation). The heart, liver, kidneys, testes (male), ovaries (female), and brain were weighed in those animals that were sacrificed by design at the end of the feeding period.

II. RESULTS AND DISCUSSION

A. FINDINGS

Findings from the 14-day feeding study with IN-JT333 in rats are summarised in the following table.

All 100 ppm female rats were either found dead or sacrificed in extremis on Test Days 7 or 8. Clinical signs including lethargy, hunched posture, and hindlimb extension, the latter suggestive of central nervous system (CNS) effects, were present in male and female rats in the 100 ppm groups. Hunched posture was also observed in females of the 40 ppm dose group. Piloerection was noted in both male and female rats treated at 10 ppm and above. The biological significance of this finding is not known. The mean body weights, body weight gains, food consumption, and/or food efficiency were decreased in males and females at 40 ppm and above.

Table B.6.8.1-10
Summary of results of the 14-day feeding study in rats with IN-JT333

Dosage	Male					
	Dietary conc.:	0	2	10	40	100 ppm
	Mean daily intake:	0	0.19	0.88	3.0	5.60 mg/kg/day
	Female					
	Dietary conc.:	0	2	10	40	100 ppm
	Mean daily intake:	0	0.18	0.87	2.9	4.5 mg/kg/day
Dose Group (ppm)		Results				
General observations	≥10 m, f ^a	exhibited piloerection after first week of the study				
	≥40 f	hunched posture				
	100 m, f	lethargy, hunched posture, and hindlimb extension				
Mortality	100 f	All found dead or sacrificed in extremis on Test Days 7 or 8				
Body weight	≥40 m	decreased ^b (9-38%) not statistically significant at 40 ppm				
	≥40 f	decreased (11-38%) not statistically significant at 40 ppm				
Body weight gain	≥40 m	decreased (21-93%) not statistically significant at 40 ppm				
	≥40 f	decreased (38% at 40 ppm; weight loss at 100 ppm)				
Food consumption	≥40 m	decreased (22-58%)				
	≥40 f	decreased (25-70%)				
Food efficiency	>100 m, f	negative food efficiency over first week of study				
Gross pathology	100 f	small liver and spleen in 1 rat				

^a m = male; f = female

^b All increases or decreases are relative to controls unless otherwise noted.

Conclusions from the original DAR (2000, RMS NL):

This study was submitted for the first inclusion of indoxacarb but its summary was not included in the original DAR.

Conclusions proposed by the applicant (2015):

The no-observed-adverse-effect level (NOAEL) was 10 ppm for males and females (0.88 and 0.87 mg/kg/day, respectively). This NOAEL was based on body weight and nutritional effects at 40 ppm and above. Mortality occurred in female rats at the high dose (100 ppm).

RMS FR assessment (2016):

This study was not performed according to GLP and did not follow any OECD guidance. Several parameters were not investigated, e.g. haematological parameters, clinical chemistry, and histopathology. Therefore, based on this study, comparison of the toxicity profile to that of the active substance indoxacarb is not possible.

IN-MT713

Previous evaluation:	In Addendum to DAR (2005)
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CA 5.8.1

Report: Kemper, R.A. (2002); *In vitro* hemolytic potential of IN-MT713 in erythrocytes from normal and glucose-6-phosphate dehydrogenase-deficient humans

DuPont Report No.: DuPont-11842 FR

Guidelines: Not applicable **Deviations:** None

Testing Facility: DuPont Haskell Laboratory, Newark, Delaware, USA

Testing Facility Report No.: DuPont-11842 FR

GLP: No

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

I. MATERIALS AND METHODS

A. MATERIALS

- | | | |
|----|-----------------------------|-----------------------------------|
| 1. | Test material: | IN-MT713 technical metabolite |
| | Lot/Batch #: | Not reported |
| | Purity: | Not reported |
| | Description: | Not reported |
| | CAS #: | Not reported |
| | Stability of test compound: | Not reported |
| | Solvent used: | Acetonitrile |
| 2. | Control materials | |
| | Negative (solvent) control: | Acetonitrile |
| | Positive control: | Dapsone-N-hydroxylamine (DDS-NOH) |

B. STUDY DESIGN

1. Experimental start/completion

The experimental start date was not included in the report. The report completion date is 08 December 2002

2. Methods

The haemolytic potential of IN-MT713, the ultimate haemolysin of DPX-MP062, was evaluated in G6PDH-normal and G6PDH-deficient humans using an *in vitro* glutathione (GSH) oxidation assay. DDS-NOH was used as a positive control, since its haemolytic potential and *in vitro* oxidative potential are well characterised. Blood samples were obtained from a total of 15 volunteers. Genetic analysis of the donor pool indicated that the experimental group was composed of five G6PDH-normal individuals, seven Med⁻ G6PDH-deficient (Mediterranean variant) individuals, and three A⁻ G6PDH-deficient (African variant) individuals. The investigators conducting the GSH oxidation and *in situ* haemolysis experiments were blind with respect to G6PDH status of the samples. Erythrocyte suspensions from each individual were incubated with IN-MT713 (5-250 µM), DDS-NOH (150 µM), or vehicle (acetonitrile) with orbital shaking. At selected time points (0-120 min), three aliquots were removed from each well for analysis of GSH, total oxidised glutathione (GSSX), and total haemolysis. Areas under the time vs. concentration curves (AUCs) for GSSX were calculated and used as an index of oxidative stress. Plots of AUC vs. IN-MT713 concentration were constructed and the data were fit using a sigmoidal dose-response model. EC₅₀ values, defined as the

concentration of IN-MT713 producing a 50% maximal response, were determined from the dose-response curves and compared to estimate relative sensitivity of the test populations to haemolytic effects of IN-MT713.

II. RESULTS AND DISCUSSION

A. FINDINGS

IN-MT713 produced a dose-dependent decrease in the concentration of GSH in G6PDH-normal individuals and in both G6PDH-deficient phenotypes. The positive control DDS-NOH produced a precipitous drop in GSH in all sample groups, indicating that the assay system was performing as expected. The decrease in GSH concentration following exposure to IN-MT713 was accompanied by a dose-dependent increase in total oxidised glutathione (GSSX). EC_{50} values were determined for each individual dose-response relationship. The EC_{50} values determined for Med⁻ and A⁻ phenotypes were quite similar (55.5 ± 21.1 and 57.7 ± 7.9 , respectively). These values, although not statistically significant, were approximately 33% lower than the EC_{50} for G6PDH-normal samples (75.5 ± 25.5). No significant changes in supernatant haemoglobin concentration were observed following exposure to IN-MT713 or DDS-NOH.

Table B.6.8.1-11
Estimated EC_{50} values for individual dose-response relationships for oxidation of GSH by IN-MT713

Red Blood Cell Phenotype	Number Evaluated	Mean EC_{50}^a (μM)	Standard Deviation
Normal	5	75.5	25.5
Med ⁻ G6PDH deficiency	7	55.5	21.1
A ⁻ G6PDH deficiency	3	57.7	7.9

^a For each individual sample, the maximum IN-MT713-induced *in vitro* GSH oxidation was determined. The EC_{50} value represents the *in vitro* concentration that produces 50% of this maximum oxidation.

III. CONCLUSION

IN-MT713 produced dose- and time-dependent increases in oxidation of intracellular GSH in G6PDH-normal subjects and both phenotypes of G6PDH-deficient subjects *in vitro*. As expected, increased GSH oxidation occurred at a lower concentration of IN-MT713 in G6PDH-deficient erythrocytes compared to normal erythrocytes. The mean EC_{50} values for oxidation of intracellular GSH were approximately 33% lower in G6PDH-deficient erythrocytes compared to erythrocytes from normal individuals. Overall, the results of the study suggest that G6PDH-deficient individuals may be slightly more sensitive to the oxidative effects of IN-MT713 than G6PDH-normal individuals. IN-MT713 did not produce *in situ* haemolysis at any dose level in any G6PDH phenotype. Therefore, IN-MT713 would not be expected to cause intravascular haemolysis in humans.

Previous evaluation:	In Addendum to DAR (2005)
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CA 5.8.1

Report: Kemper, R.A. (2004); *In vitro* hemolytic potential of N-hydroxy-4-(trifluoromethoxy)aniline (IN-MT713) in rats, dogs, and humans

DuPont Report No.: DuPont-12062, Revision No. 1

Guidelines: Not applicable

Testing Facility: DuPont Haskell Laboratory, Newark, Delaware, USA

Testing Facility Report No.: DuPont-12062

GLP: No

Study design

The *in vitro* haemolytic potential of N-hydroxy-4-trifluoromethoxyaniline (IN-MT713), a metabolite of indoxacarb with putative haemolytic properties, was investigated in a comparative study with erythrocytes of rats, dogs and humans, using the oxidative effect on glutathione in erythrocytes as parameter. The experimental set up is as follows:

Exposure	: 0 – 120 min incubation at 37°C of erythrocytes, suspended at a concentration of 25% (w/v) in Dulbecco's phosphate buffered saline (1% saline)
Doses	: 5 – 250 µM IN-MT713; 150 µM DDS-NOH (positive control); acetonitrile (negative control)
Vehicle	: acetonitrile

The glutathione status was analysed at different time points by HPLC following derivatisation with the fluorophore OPA (*o*-phthalaldehyde). Reduced glutathione (GSH) was determined directly in the deproteinated erythrocyte lysate, total oxidised glutathione (GSSX) was analysed following reduction with dithiothreitol. The area under the curve (AUC) for GSSX was determined as an index of oxidative stress and dose-response curves were used to determine species differences.

Results

In all three species a dose-dependent depletion of GSH by IN-MT713 was demonstrated. The decrease of GSH concentration was generally accompanied with an increase in GSSX. In rats the GSSX increased rapidly, reaching a plateau level after approximately 15 min. In dog erythrocytes the plateau levels were not reached at the end of the experiment (120 min). In human samples only the highest concentrations (125 and 250 µM) had a significant effect. The rate of GSH oxidation was much slower than in the rat (\pm 60min).

The sensitivity of erythrocytes GSH oxidation by *in vitro* incubation with IN-MT713 was rat > dog > humans, whereas the magnitude of species differences increased with increasing concentrations of the test compound. Human red blood cells were the least sensitive to GSH oxidation. They were up to 2-3 times less sensitive than dog RBCs and 4-5 times less sensitive than rat RBCs.

Conclusions

In all three species investigated the indoxacarb metabolite IN-MT713 had a dose-dependent *in vitro* haemolytic potential (determined as glutathione oxidation) in erythrocytes. Species differences were demonstrated, indicating that humans are likely to be less sensitive than either rats or dogs to the *in vitro* haemolytic action of the compound.

B.6.8.2. Supplementary studies on the active substance

Distribution of the radioactivity in erythrocytes of rats administered a single oral dose of 111-113 mg/kg bw DPX-JW062 (50:50) by gavage showed that the major identified compound was IN-P0036.

In a 28-day immunotoxicity study in mice, DPX-KN128 (99:1) was not shown to induce adverse effect on the humoral immune response.

Table B.6.8.2-1
Summary of supplementary studies on indoxacarb (DPX-KN128 and DPX-JW062)

Type of study and test substance	Doses/concentrations tested	NOAEL	LOAEL	Target organ(s) and/or effects	Reference
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Distribution of erythrocytes (Gavage) Rat DPX-JW062 (50:50)	M: 111-113 mg/kg bw Single dose	Not applicable	Not applicable	IN-P0036 was the single radioactive species associated with erythrocytes 72-h after administration	1999 DuPont-1952
28-day immunotoxicity (Feeding), Mouse DPX-KN128 (99:1)	F: 0, 10, 25, 100 ppm – 0, 2, 5, 11, 23 mg/kg bw/d	Immunotoxicity: 100 ppm 23 mg/kg bw/d Systemic: 100 ppm 23 mg/kg bw/d	Immunotoxicity: >100 ppm >23 mg/kg bw/d Systemic: >100 ppm >23 mg/kg bw/d	Immunotoxicity: no effect on the humoral immune response Systemic: no effect	2011 DuPont-29280*

* Studies newly submitted

Postulated mode of action of indoxacarb:

The racemic mixture DPX-JW062, the 75:25 S:R enantiomeric blend DPX-MP062 and the pure S-enantiomer DPX-KN128 induced effects on red blood cell parameters and histopathological findings in the spleen, the bone marrow, the liver and/or the kidney in short-term and long-term toxicity studies in all tested species.

In rats, as demonstrated in ADME studies with DPX-MP062 (75:25) and DPX-JW062 (50:50), one of the metabolic pathways involved the opening of the oxadiazine ring and subsequent cleavages, resulted in the formation of aniline analogs metabolites. Aniline and related compounds are well-known to be responsible of haemolytic anemia with associated changes in spleen, bone marrow and liver of rats.

In particular, indoxacarb is metabolised to an arylamine metabolite IN-P0036 (4-trifluoromethoxyaniline). This metabolite is found at low level in the rat urine but is an intermediate in the metabolic pathway leading to the formation of 2 major urine metabolites (MC218 representing 21-24% of the administered dose and MG195 representing 14-17% of the administered dose after DPX-JW062 (50:50) administration). IN-P0036 was also shown to be associated with erythrocytes following administration of DPX-JW062 (50:50) in rats. The haemolytic potential of this metabolite was not investigated in further studies.

Nevertheless, according to the applicant, the mechanism of arylamine-induced oxidant effects on red cells has been determined for a number of compounds in this class and is dependent upon biotransformation of the arylamine to its N-hydroxylamine. Therefore, the haemolytic potential of IN-MT713, the N-hydroxy derivative of 4-trifluoromethoxyaniline, although not detected in the rat metabolism, has been investigated. IN-MT713 had a dose-dependent *in vitro* haemolytic potential, determined as glutathione oxidation, in erythrocytes of rats, dogs and humans.

Therefore, according to this postulated mode of action, indoxacarb-induced haematologic effects are indirect, requiring conversion of the parent molecule to IN-P0036 and subsequent conversion of that metabolite to its hydroxylamine IN-MT713.

According to the applicant, it is interesting to note that the arylamine metabolite, produced from the trifluoromethoxyphenyl portion of the parent compound, does not contain the chiral center of the molecule. Therefore, if haematological effects observed after administration of indoxacarb are related to the formation of this metabolite, it could be expected that haematological effects produced following exposure to either the racemic, the 75:25 enantiomer blend or the pure S-enantiomer would be similar irrespective of the isomeric blend. This assumption is also supported by the results of a regression analysis of changes in red cell mass parameters following subchronic dietary exposure to the 3 compounds.

The RMS FR considered that indoxacarb could produce haematological effects *via* this postulated mode of action although some uncertainties remained (e. g. IN-MT713 was not detected in the rat metabolism, conversion of IN-P0036 to IN-MT713 was not demonstrated). Moreover, it cannot be excluded that the formation of this aniline metabolite could be influenced by the ratio of isomers. As an example, as shown in the ADME studies, the S-isomer DPX-KN128 was found to be the preferred substrate for the enzymatic reaction leading to the formation of the metabolite IN-JT333. Therefore, given the uncertainties in the enzymatic reaction, the speculated absence of difference of toxicity due to the formation of a non-chiral metabolite is questionable. However, according to the results of the three available 90-day toxicity studies in rats, it was shown that the NOAELs/LOAELs of the three enantiomeric blends (ratios of approx. 99:1, 75:25 and 50:50 of isomers S and R respectively) were similar *in vivo*.

Previous evaluation:	In Addendum to DAR (2001)
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CA 5.8.2

Report: [REDACTED] (1999); ¹⁴C-DPX-JW062 (a racemic mixture of DPX-KN128 and IN-KN127); Distribution of erythrocytes of rats

DuPont Report No.: DuPont-1952

Guidelines: 59 NohSan No. 4200 (1985) **Deviations:** None

Testing Facility: [REDACTED]

Testing Facility Report No.: DuPont-1952

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the US EPA.

A. MATERIALS

- ## B. STUDY DESIGN AND METHODS

1. In-life initiated/completed
29-December-1998 to 15-February-1999

3. Main study

Six male Crl:CD[®](SD)IGS BR rats were used for this study. Five rats were administered a single oral dose of 130 mg/kg of [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062, and one rat was administered a dose of 111 mg/kg due to a limitation in the amount of dosing solution available. The animals were sacrificed at approximately 72 hours after dosing, and whole blood was collected into heparinised vials. Erythrocytes were obtained by centrifugation of whole blood and analysed using a liquid scintillation analyser (LSA). Erythrocytes were further analysed for subcellular distribution by fractionation. Metabolic profiling was performed using solvent extraction and pepsin digestion and analysed by LSA and HPLC.

II. RESULTS AND DISCUSSION

Following oral (*via* gavage) dosing of rats with [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062, a significant portion of the dosed radioactivity was associated with the erythrocytes. Washed erythrocytes from the blood obtained from rats 72 hours following dosing with 130 mg/kg [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 contained on average 29.6 µg/g [trifluoromethoxyphenyl(U)-¹⁴C]DPX-JW062 equivalents. Most of the radioactivity was distributed approximately evenly between the intracellular fluid and haemoglobin fractions; only traces of radioactivity were associated with the toluene and stroma fractions.

Table B.6.8.2-1
Distribution of radioactivity in erythrocyte fractions

Fraction	% Total radioactive residue
Toluene	0.2
Stroma	2.9
Intracellular Fluid	44.1
Haemoglobin	52.8
Total	100

The major identified radioactive species in erythrocytes was the trifluoromethoxyaniline, [¹⁴C]IN-P0036. During characterisation of erythrocyte-associated radioactivity, recovery of total radioactivity was 53.3%. Lost radioactivity was believed due to loss of a volatile component, possibly [¹⁴C]IN-P0036 itself.

III. CONCLUSION

IN-P0036 was the single radioactive species associated with erythrocytes of rats 72 hours after gavage dosing with radiolabeled DPX-JW062.

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.8.2/01

Report: Green, J.W. (1999); DPX-MP062, DPX-JW062, and DPX-KN128: Regression analysis of changes in red cell mass parameters following subchronic dietary exposure

DuPont Report No.: DuPont-2780

Guidelines: Not applicable **Deviations:** Not applicable

Testing Facility: DuPont Haskell Laboratory, Newark, Delaware, USA

Testing Facility Report No.: DuPont-2780

GLP: No

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

The comparative hematologic effects produced following subchronic dietary exposure to DPX-MP062 (90-d study HL-1997-00056), DPX-JW062 (90-d studies HLR 751-93 and HL-1998-01200 and 90-d results of the 2-y study HLR 1174-96), and indoxacarb (DPX-KN128) (90-d study HLR 301-94) were assessed in female rats. The subchronic studies with these compounds (total of 5 studies) were examined with regard to changes in RBC, Hb, and Ht following 90 days of exposure to the respective test materials. In order to compare the relative toxicity of these three compounds in the five studies, mathematical models were fit to each response using all data from the five studies. The absolute measures of each of these three endpoints was modelled, using a third-degree polynomial with separate intercepts, separate slopes, separate curvatures (*i.e.* quadratic terms), and separate inflections (*i.e.* cubic terms) with respect to intake. By a backward regression procedure, non-significant terms were eliminated to produce the best fit consistent with parsimony. The absolute change from control was estimated from the model for each study. Tests of between-study differences in change from control at a given dose level were conducted at doses ranging from 0.2 to 10.0 mg/kg/day.

There were no statistically significant differences in the absolute change from control for any red cell mass parameter in DPX-MP062 study compared to the studies with DPX-JW062 or DPX-KN128. Any differences along the modeled dose-response curves for red cell mass parameters among the 5 studies were the result of between-study differences in control values.

The dose responses for changes in red cell mass parameters in subchronic feeding studies with DPX-MP062, DPX-JW062, and DPX-KN128 were modelled using a third degree polynomial model. There were no statistically significant differences in the absolute change from control for any red cell mass parameter in DPX-MP062 study compared to the studies with DPX-JW062 or DPX-KN128. These results suggest that the hematologic effects observed following 90-days exposure to the three isomer blends are equivalent.

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.8.2/02

Study submitted to the EU for the first time in this submission and listed under “Documents Submitted”.

Report: Green, J.W. (2002); A statistical analysis of indoxacarb-induced hematologic changes in rats and dogs using historical control data

DuPont Report No.: DuPont-6122, Revision No. 1

Guidelines: Not applicable **Deviations:** None

Testing Facility: DuPont Haskell Laboratory, Newark, Delaware, USA

Testing Facility Report No.: DuPont-6122, Revision No. 1

GLP: No

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

For the 90-day and 1-year studies in dogs (HLO 494-95, Revision No. 3 and HLO 885-96 Revision No. 1) with DPX-JW062, and the 90-day study in rats with DPX-MP062 (HL-1997-00056, Revision No. 1), effects on red cell mass parameters (RBC, HB, HT) relative to study controls were statistically analysed in the context of historical variances for these parameters.

Specifically, the historical variance for each red cell mass parameter was estimated using data derived from the relevant historical control population. The historical variances were then used in a statistical analyses of treated and control groups from the 90-day study with DPX-MP062 and the dog studies with DPX-JW062.

There were no statistically significant changes in red cell mass parameters in male or female rats fed dietary concentrations of 10 ppm. With the exception of decreased hemoglobin at one time point, there were no statistically significant decreases in these parameters in males and females fed 50 and 25 ppm, respectively. At higher dietary concentrations, statistically significant decreases in red cell mass parameters were generally present in one or more parameters at either or both the 45 and 90-day time points. Since more detailed historical information was available, the pooled or ANOVA within-group variance from the DPX-MP062 study was compared to the within-study variance from the historical control data. One noteworthy observation was that for all three endpoints, RBC, HB and HT, the DPX-MP062 within study variance was less than the historical within-study variance. The implications of this are two-fold. First, the DPX-MP062 data analysis was more sensitive than would be expected from the historical data, meaning that there was an inflated chance of a treatment effect being declared statistically significant than would appear to be justified historically. Second, the between-study variance, as a percent of the within-study variance, varies from 7% to 50% for RBC and HB and from 78% to 151% for HT. This suggests, especially for HT, that some between group differences in the DPX-MP062 study that appear statistically significant, may in reality be manifestations of meaningless background variation.

There were no statistically significant changes in any red cell mass parameter, relative to study controls, in male or female dogs in either the 90-day or 1-year study at 40 ppm. At 80 ppm, statistically significant changes in red cell mass parameters were present at some, but not all, time points in the 1-year study. At 640 and 1280 ppm statistically significant decreases in red cell mass parameters were present across most time points.

Previous evaluation:	Submitted for the purpose of renewal
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CA 5.8.2/03

Report: [REDACTED] (2011); Indoxacarb (DPX-KN128) technical: 28-Day immunotoxicity feeding study in mice

DuPont Report No.: DuPont-29280

Guidelines: OPPTS 870.7800 (1998)

Deviations: According to guideline OPPTS 870.7800, “*The test substance, vehicle, or positive control substance shall be administered for at least twenty eight days for the anti-SRBC assay.*” In this study, the positive control cyclophosphamide is administered for 5 consecutive days only following injection of sRBC. The applicant provided the following statement which is considered acceptable by the RMS.

“The purpose of using a positive control is to provide evidence that the test system is capable of detecting the endpoint examined, which, in the case of OPTTS 870.7800, is immunosuppression. Please note that of the 11 references listed in this guideline, 3 of them were from our laboratory (Ladics and Loveless, 1994; Ladics et al, 1994; Ladics et al, 1996 and can be provided upon request). Following the 1998 publication of the guideline, there was debate amongst immunotoxicologists on a number of issues, including the need to dose positive control animals for 28 days vs 5 days. As you will see in the table below (i.e. Table B.6.8.2-7), dosing animals with 25 mg/kg/day cyclophosphamide (CP) for 5 days results in a suppression of the SRBC antibody response by 35-40%. Dosing animals with 2 mg/kg/day of CP for 30 days resulted in a 28% suppression in a study DuPont published in 1995 (Ref #7 in OPTTS 870.7800 Guideline). In the early 2000s, we shared our data comparing suppression by CP after 5 or 28 days of dosing and asked Dr. Yung G. Yang (Toxicology and Epidemiology Branch, Health Effects Division, US-EPA) if the 5-day dosing protocol would meet the intent of the OPTTS guidelines with respect to a positive control. He agreed and we have employed this dosing regimen ever since. The table also compares the positive control data from the Indoxacarb Technical immunotox study with the positive control data generated in 4 subsequent immunotox studies in 2011. One can see that the Log2 values for the vehicle control were quite similar (11.45 vs 11.27), as were those for the CP positive control (7.18 vs 7.01), resulting in similar % suppressions (37 vs 38). We feel these data provide confidence that the testing system was capable of detecting immunosuppression and none was observed following 28 days of Indoxacarb Technical exposure.”

Testing Facility: [REDACTED]

Testing Facility Report No.: DuPont-29280

GLP: Yes

Certifying Authority: Laboratories in the USA are not certified by any governmental agency, but are subject to regular inspections by the U.S. EPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Indoxacarb technical
 Lot/Batch #: KN128-215
 Purity: 98.4%
 Description: Solid
 CAS #: 173584-44-6
 Stability of test compound: The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed. The stability of the test substance was confirmed by analysis near the beginning and end of the study.
2. Vehicle and/or positive control: Vehicle: Diet treated with acetone
 Positive control: Cyclophosphamide Monohydrate
3. Test animals
 Species: Mouse
 Strain: CrI:CD[®]1(ICR)
 Age at dosing: 64 days
 Weight at dosing: 21.2–27.8 g
 Source: XX
 Acclimation period: 7 days
 Diet: PMI[®] Nutrition International, LLC Certified Rodent LabDiet[®] (#5002), *ad libitum*. During the test (exposure) period, test substance was incorporated into the feed at concentrations specified by study design.
 Water: Tap water, *ad libitum*
 Housing: Animals were group housed in solid bottom caging with bed-o-cobs[®] bedding and nestlets as enrichment.
4. Environmental conditions
 Temperature: 18–26°C
 Humidity: 30–70%
 Air changes: Not recorded in report
 Photoperiod: Approximate 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed
 25 May 2010 to 22 June 2010
2. Animal assignment and treatment
 Five groups of 10 female mice/concentration were administered concentrations of 0, 10, 25, 50, and 100 ppm indoxacarb in feed daily for 28 days. An additional group of 10 female mice were assigned to the cyclophosphamide positive control group. The dietary concentrations for this study were selected based on the results of a 90-day study in mice with DPX-JW062 and an 18-month study in mice with DPX-JW062. Animals were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means. A negative control group received untreated diet. Animal housing and husbandry were in accordance with the provisions of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Table B.6.8.2-2
Study design: 28-Day feeding study in female mice

Group no.	No./ group	Conc. in diet (ppm) ^a	Mean daily intakes mg/kg bw
1	10	0 (control)	0
2	10	10	2
3	10	25	5
4	10	50	11
5	10	100	23

^a Weight/weight concentration of test substance (adjusted for sponsor-supplied purity of active ingredient).

3. Diet preparation and analysis

Indoxacarb, dissolved in acetone, was added to the diet and thoroughly mixed for a period of time that, by experience or pretest determination, was adequate to ensure homogeneous distribution in the diet. The same amount of acetone was added to the control diets and similarly mixed for the same period of time. Diets were prepared once and refrigerated until used. A minimum of 2 samples of all dietary concentrations were collected at the initial diet preparation and analysed to verify the concentration and homogeneity of indoxacarb in the diets. A minimum of 2 samples were taken from the lowest and highest dietary concentration of the initial diet preparation and analysed to verify the stability of indoxacarb in the diets. The stability, homogeneity, and concentration of indoxacarb in the dietary mixtures were checked by analysis using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The test substance was at target concentrations $\pm 10.0\%$ of nominal, homogeneous (RSD $\leq 6\%$) throughout the feed and was stable when stored at room temperature for the whole study period. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study. The analytical method is validated (see Volume 3B5).

4. Statistics

Table B.6.8.2-3
Statistics: 28-Day feeding study in female mice

Parameter	Preliminary test	Method of Statistical Analysis	
		If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Food consumption Food efficiency Humoral immune function data ^a Organ weights	Levene's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance followed with Dunnett's test	Transforms of the data to achieve normality and variance homogeneity were used. The order of transforms attempted was Log, square-root, and rank-order. If the log and square-root transforms failed, the rank-order was used.

^a sRBC-specific serum IgM antibody titer data were transformed to Log2 to obtain normality or homogenous variances.

Significance was judged at $p < 0.05$.

C. METHODS

1. Observations

Animals were observed at least once daily for mortality and morbidity and examined weekly for clinical signs of toxicity.

2. Body weights

All animals were weighed once per week.

3. Food consumption, food efficiency, and daily intake

Food consumption was recorded for each animal over the weighing interval. Food efficiency and daily intake were calculated from food consumption and body weight data.

4. Humoral immune function

On test Day 23, animals were injected intravenously in the lateral tail vein with 0.2 mL of 5×10^8 sRBC/mL. Following the IV injection of sRBC, mice in the positive control group were injected intraperitoneally for 5 consecutive days with 25 mg/kg/day of the known immunosuppressive agent, cyclophosphamide monohydrate in deionized water, at a dose volume of 10 mL/kg body weight. Five days after injection (test Day 28), the animals were euthanized by isoflurane anesthesia and exsanguination. The maximum amount of blood was collected from the abdominal vena cava and processed to serum. The serum was frozen at $\leq 60^\circ\text{C}$ until analyzed. Humoral immune function was evaluated by examining serum from individual control and test substance treated animals by quantitative measurement of mouse anti-sRBC IgM levels with an enzyme-linked immunosorbent assay (ELISA). The serum from each animal was assayed as 3 serial, 2-fold dilutions, with 1 replicate per dilution. The optical density (OD) of the serum samples was measured at 450 nm and the \log^2 of the mean result of the serial diluted serum sample was reported. Serum samples collected from mice injected with cyclophosphamide monohydrate were run concurrently with the study samples as a positive control.

6. Anatomic pathology evaluation

After approximately 28 days of the study, the surviving female mice from each exposure level were sacrificed and necropsied. The order of sacrifice for scheduled deaths was stratified between treatment groups. Mice were euthanized by isoflurane anesthesia and exsanguination. The maximum volume of blood was collected at sacrifice from the abdominal vena cava, while the animal was under isoflurane anesthesia. Approximately 100 μL of blood were placed in a blood collection tube containing EDTA and the remaining blood was placed in a tube with no anticoagulant, processed to serum for humoral immune function. Blood smears stained with new methylene blue were prepared from the EDTA anticoagulated blood, but were not required to support experimental findings.

Gross examinations were performed on all mice, and final body weights were recorded. Following gross examination, the spleen, thymus, and brain from all animals in Groups 1-5 were weighed. Relative organ weights (relative to final body weight; relative to brain weight) for weighed organs were calculated. Final body weights determined just prior to necropsy were used in the assessment of organ weight changes. The spleen, thymus, bone marrow (collected along with femur and sternum), and any gross lesions were placed in 10% buffered formalin, routinely trimmed and processed to slides. Spleen, thymus and bone marrow from mice in the control and high-dose group (Group 5) and gross lesions were evaluated microscopically by a veterinary pathologist.

Table B.6.8.2-4
28-Day immunotoxicity feeding study in female mice: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic findings with lesion grades
Brain	X	
Bone marrow		X
Spleen	X	X
Thymus	X	X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No statistically significant or biologically significant changes in the incidence of clinical signs of toxicity were observed for any dietary concentration in the mice.

2. Mortality

Test substance-related mortality did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no test substance-related changes in mean body weight and body weight gains in female mice fed 0, 10, 25, 50 or 100 ppm. Mean body weight gain in female mice fed 10 ppm was decreased (80% lower than control) during Days 7-14, however, subsequent weighings were similar to the control and not statistically significant.

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND DAILY INTAKE

There were no test substance-related effects in food consumption, food efficiency or intake in female mice fed 10, 25, 50 or 100 ppm. A statistically significant increase in food consumption was observed at test days 21-28 in mice fed 100 ppm (33% above the control) and an overall increase in food consumption in female mice fed 50 or 100 ppm (14.9% and 16.6% above control, respectively). This increase was considered to be unrelated to the test-substance and non-adverse since increases in mean daily food consumption are within the expected biological variation of the test system.

The overall mean daily intake of the test substance for female mice in the 0, 10, 25, 50, or 100 ppm groups was calculated to be 0, 2.12, 4.53, 11.12, and 22.95 mg/kg/day, respectively.

Table B.6.8.2-5

28-Day immunotoxicity feeding study in female mice: Body weight gain/food consumption/food efficiency

Parameter	0 ppm	10 ppm	25 ppm	50 ppm	100 ppm	CP 25 mg/kg
Females:						
Body weight gain, Day 0-28 (% control)	2.8	3.3 (19.6)	4.2 (50.7)	3.8 (37)	3.3 (18.1)	4.3 (55.1)
Food consumption, Day 0-28 (% control)	5.2	5.7 (9.2)	4.9 (-5.6)	6.0* (14.9)	6.1* (16.6)	5.0 (-4.3)
Food efficiency, Day 0-28 (% control)	0.020	0.025 (27.5)	0.031 (58.2)	0.023 (18.6)	0.019 (-1.3)	0.031 (60.9)

Units for table values are grams.

Note: Mean food efficiency = body weight gain (g)/ food consumed (g)

* Statistical Test: Dunnett Non-Parametric 2 Sided p <0.05

D. IMMUNOTOXICITY EVALUATION

No adverse or statistically significant effects were observed on the humoral immune response for female mice at any concentration tested.

The humoral immune response from mice dosed with 25 mg/kg/day cyclophosphamide monohydrate (positive control) for 5 days was lower than the study control group. Therefore, the sRBC-specific ELISA test system was valid for this feeding study with indoxacarb technical.

It is to be noted that a statistical comparison between vehicle and positive controls was not performed at the time of the report submission. According to the applicant, when the vehicle and positive controls are compared in a t-test, a significant difference is detected ($p < 0.0001$).

Table B.6.8.2-6
28-Day immunotoxicity feeding study in female mice: Primary humoral immune response

Parameter	0 ppm	10 ppm	25 ppm	50 ppm	100 ppm	Positive control
IgM (Log ₂)	11.45	11.48	11.29	10.98	10.73	6.75 ^a 8.25 6.55

^a Mean values from 3 different 96-well plates

Table B.6.8.2-7
Historical control data for [REDACTED] compared to indoxacarb

IgM Response- Historical Controls 2011		Indoxacarb technical		4 Subsequent Studies 2011	
Control Group		IgM Log ²		IgM Log ²	
Anti-SRBC IgM - ELISA		Mean: 11.45		Mean: 11.27	
Instrument: Grifols Triturus		SD: 0.83		SD: 1.21	
Kit: Life Diagnostics Inc. Cat 4200-1 (mouse)					
Age: ~28 days on study		Min: 9.83		Min: 7.94	
		Max: 12.38		Max: 13.45	
		N: 7		N: 39	
25 mg/kg/day Cyclophosphamide Monohydrate	Indoxacarb technical		4 Subsequent Studies 2011		
	Log ²	% Inhibition	Log ²	% Inhibition	
Anti-SRBC IgM - ELISA	Mean: 7.18	Mean: 37	Mean: 7.01	Mean: 38	
Instrument: Grifols Triturus	SD:0.93		SD:1.12		
Kit: Life Diagnostics Inc. Cat 4200-1 (mouse)					
Age: ~28 days on study	Min: 6.55	-	Min: 5.55	-	
	Max: 8.25	--	Max: 9.68	-	
	N: 3 ^a	-	N: 40 ^b	-	

^a Each experimental microtiter plate contained serum from one randomly selected positive control mouse

^b Serum titers were calculated for all positive control mice

E. ANATOMIC PATHOLOGY EVALUATION

1. Organ weight data

There were no test substance-related effects on absolute and relative (% body weight and % brain weight) spleen and thymus weight.

2. Gross observations

There were no test substance-related gross observations.

3. Microscopic findings

There were no test substance-related microscopic observations in the spleen, thymus, or bone marrow.

Table B.6.8.2-8
28-Day Immunotoxicity feeding study in female mice: Organ weights

Parameter	0 ppm	10 ppm	25 ppm	50 ppm	100 ppm
Terminal Body weight (g)	28.2	28.5 (1.2) ^b	29.3 (4)	29.2 (3.4)	28.0 (-0.5)
Brain weight (g) (% control)	0.475	0.498 (4.7)	0.478 (0.5)	0.496 (4.2)	0.500 (5.3)
Relative ^a brain weight (% control)	1.691	1.750 (3.5)	1.636 (-3.2)	1.710 (1.1)	1.790 (5.8)
Spleen weight (g) (% control)	0.126	0.151 (20.3)	0.150 (19.1)	0.133 (6.3)	0.131 (4)
Spleen to brain weight (% control)	26.355	30.478 (15.6)	31.521 (19.6)	26.895 (2)	26.137 (-0.8)
Relative ^a spleen weight (% control)	0.446	0.531 (19)	0.510 (14.2)	0.458 (2.7)	0.465 (4.2)
Thymus weight (g) (% control)	0.043	0.049 (15.8)	0.051 (19.3)	0.048 (13.6)	0.054 (27.1)
Thymus to brain weight (% control)	8.953	9.861 (10.1)	10.700 (19.5)	9.836 (9.9)	10.774 (20.3)
Relative ^a thymus weight (% control)	0.151	0.172 (13.8)	0.172 (13.8)	0.168 (10.7)	0.193 (27.2)

^a Relative weight is defined as the organ to body weight ratio.

^b percent difference compared to control

Table B.6.8.2-9
28-Day immunotoxicity feeding study in female mice:
Incidences of microscopic findings with lesion grades in mice

Indoxacarb (ppm):	0	10	25	50	100
Number of female mice/group:	10	10	10	10	10
Bone Marrow					
Examined	10	0	0	0	10
No visible Lesions	10	-	-	-	10
Spleen					
Examined	10	1	0	0	10
No Visible Lesions	6	0	-	-	5
Hematopoiesis, extramedullary, increased	4	1	-	-	5
....minimal	2	0	-	-	4
....mild	2	1	-	-	1
Thymus					
Examined	10	0	0	0	10
No Visible Lesions	10	-	-	-	10

III. CONCLUSION

The no-observed-adverse-effect level (NOAEL) in the 28-day feeding study in female mice was 100 ppm, the highest concentration tested. This concentration is equivalent to 23 mg/kg/day in female mice. The NOAEL is based on the lack of adverse test substance-related effects on any in-life or anatomic pathology parameter or on the humoral immune response in female mice fed up to 100 ppm indoxacarb. The humoral immune response NOAEL of indoxacarb was 100 ppm. Indoxacarb technical is not considered to be an immunotoxicant.

B.6.8.3. Studies on endocrine disruption

No histopathological effects were observed on endocrine-related tissues in the repeated-dose toxicity studies with DPX-JW062 (50:50), DPX-MP062 (75:25), or DPX-KN128 (99:1). In addition, no effects on reproduction were observed in the multigeneration reproduction study conducted with DPX-JW062 (50:50), in the developmental studies in rats with DPX-JW062 (50:50), DPX-MP062 (75:25), and DPX-KN128 (99:1), or the developmental neurotoxicity study with DPX-KN128 (99:1).

Two publications dealing with potential ED effects of several substances, including indoxacarb, were retrieved after the literature review. These publications were provided and considered relevant and reliable by the applicant (but no summary was submitted).

- In a publication by Orton *et al.* (2011), indoxacarb (racemic form was not specified in the publication) was tested in the MDA-kb2 assay for potential androgen activity. There were no effects at a cytotoxic concentration of 11.3 μ M.

- In a publication by Sipes *et al.* (2013), indoxacarb was tested in a battery of assays used for the U.S. Environmental Protection Agency ToxCast Program. This publication only presented the test results for compounds which were positive in the suite of assays. The results for indoxacarb were not discussed in the manuscript, indicating that positive responses were not obtained for indoxacarb.

Therefore, in the absence of effects which could be mediated by an ED mode of action in apical studies and in the absence of relevant effects in literature review, additional studies for potential endocrine effects are not warranted.

B.6.9. MEDICAL DATA AND INFORMATION

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

Indoxacarb (DPX-KN128) technical and its end use products are produced on a commercial scale. No illnesses have been attributed to exposure associated with the handling, testing, or manufacturing of indoxacarb (DPX-KN128) technical or its end use products at the facilities where the technical material is manufactured or formulated into the end use products.

B.6.9.2. Data collected on humans

The following databases were searched for published reports of clinical cases and poisoning incidents linked to indoxacarb use:

AGRICOLA:	A bibliographic database containing selective worldwide coverage of agriculture and related fields.
CABA:	Covers worldwide literature from all areas of agriculture and related applied and life sciences.
CAPLUS:	Covers worldwide literature from all areas of chemistry, biochemistry, chemical engineering, and related sciences.
FROSTI:	A bibliographic database containing 1972-present coverage of the Foodline® Science database.
TOXCENTER:	A bibliographic, abstract, CAS RN database covering toxicological, food, environmental and waste topics from 1907.

PubMed: Data base contains the most recent published journals that have not yet been entered in STN's catalog. Therefore, it is searched to verify that the most recent publications are reviewed for toxicology.

The results of the search contained 5 possible incidents from intentional ingestion and the references are considered to be relevant, and reliable since they were published in respected medical and technical journals. Brief summaries of the incidents are provided below.

	Reference	Effects Observed	Treatment and Outcome
CA 5.9.2/03	Prasanda L., Rao SM, Singh V, Kujur R, and Gowrishankar. Indoxacarb Poisoning: An Unusual Presentation as Methemoglobinemia. Indian J. Critical Care Med. 12(4): 198-200, 2008	Patient had increased methemoglobin, vomiting, drowsiness, hypoventilation following a suicide attempt from ingestion of Indoxacarb (product name not specified).	Methylene blue, vitamin C, ventilation support, iv fluid administration. Patient fully recovered.
CA 5.9.2/02	Park JS, Hoon K, Lee SW, and Min JH. Successful treatment of methemoglobinemia and acute renal failure after indoxacarb poisoning. Clinical Toxicology 49: 744-746, 2011	Patient had labored breathing, altered mental status, low blood pressure, low heart rate, metabolic acidosis, methemoglobinemia with high anion gap metabolic acidosis following intentional ingestion of approximately 100 mL of Steward® Gold containing 5 g of Indoxacarb in 85% propylene glycol. After initial treatment with methylene blue, the methemoglobinemia improved, however, she developed oliguria followed by anuria.	Gastric lavage, activated charcoal, iv fluid, mechanical ventilation, Methylene blue (1 mg/kg i.v). followed by sodium bicarbonate. Due to deterioration of kidney function, continuous veno-venous hemofiltration, iv fluid, sodium bicarbonate, norepinephrine and dopamine were administered and continued for 6 days. Patient fully recovered.
CA 5.9.2/04	Wu, Y.-J., Lin, Y-Li, Huang, H-Y, and Hsu, B-G. Methemoglobinemia induced by indoxacarb intoxication. Clinical Toxicology 48: 766-767, 2010	Patient had increased methemoglobin, dizziness, headache, fatigue, cyanotic lips and nails following a suicide attempt from ingestion of 50 mL Avatar® (14.5% indoxacarb, 79.5% inert ingredients).	Methylene blue 2 mg/kg by iv administration, followed by a second dose 4 hours later. Patient fully recovered.
CA 5.9.2/05	Shih, P-C and Tsai, T-H. Methemoglobinemia following ingestion of Indoxacarb: a case report. J. Acute Medicine 1(2): 55-57, 2011	Patient had vomiting, diarrhea, shortness of breath, lip cyanosis, wheezing, discoloured blood (muddy brown) following a suicide attempt from ingestion of approximately 100 mL of a formulation containing 14.5% Indoxacarb, 6% related isomers, and 79.5% inert ingredients.	Gastric lavage, activated charcoal, oxygen, methylene blue (1 mg/kg by i.v. administration, followed by a second dose. Patient fully recovered.
CA 5.9.2/01	Jin, K. Rhabdomyolysis, methemoglobinemia and acute kidney injury after indoxacarb poisoning Clinical Toxicology 50: 227, 2012	Patient had cyanosis of lips and nails, breathing difficulty, increased methemoglobin, oliguria, increased CPK and LDH following ingestion of approximately 250 mL of Steward® Gold (5 g Indoxacarb in 85% propylene glycol)	Gastric lavage, charcoal, isotonic saline, oxygen, methylene blue (1 mg/kg iv at intervals of 8 hours, with 1 g Vitamin C. Patient fully recovered.

B.6.9.3. Direct observation

As described above, five incidents of over-exposure to indoxacarb have been reported in humans. In all 5 cases, increased methemoglobin and secondary clinical signs improved following gastric lavage, activated charcoal, intravenous fluid, oxygen/mechanical ventilation, intravenous treatment with methylene blue and, in some cases, Vitamin C. In addition, symptoms of kidney effects were treated with continued fluids, sodium bicarbonate, and/or continuous ventilation, and other supportive measures. In all 5 cases, the patients recovered.

B.6.9.4. Epidemiological studies

There have been no epidemiological studies conducted with indoxacarb (DPX-KN128). Although there have been 5 incidences of over-exposure to indoxacarb since the beginning of commercial use, there are no reports of adverse effects on human health following standard use as described on the product label or from handling the technical material or formulated product during manufacturing.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

Based on the clinical reports from intentional over-exposure in humans (suicide) and on acute toxicity studies in animals, an oral overdose may cause clinical signs of neurotoxicity, altered breathing rate, vomiting, methemoglobinemia, altered mental status, cyanosis of the lips and/or nails, and renal injury. Repeated oral exposure to doses in excess of 100 fold above the ADI may result in decreased body weight, and reversible altered hematology/clinical chemistry parameters. Clinical tests for diagnosis include blood oxygen saturation, methemoglobin, hematology indices, creatine, and LDH.

Given the low dermal and inhalation toxicity, it is unlikely for accidental or intentional overexposure to occur by these routes.

Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion

Based on 5 incidents of over-exposure in humans, and animal testing results, serious acute oral over-exposure might lead to symptoms of neurotoxicity, methemoglobinemia, and/or renal failure. Repeated over-exposure greater than 100 fold above the ADI by the oral route would be expected to result in decreased body weight, and reversible anaemia.

Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment

Effects of human exposure to indoxacarb should be transitory and resolved by approximately 1 week after exposure, following prompt treatment.

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

There is no specific antidote. For clinical evidence methemoglobinemia, treat with methylene blue, and supportive therapy (vitamin C, IV fluids, ventilation support, oxygen). For other clinical manifestations, treat symptomatically. The following recommendations are standard protocol procedures for over exposure to chemicals:

- a. Inhalation: Move to fresh air. Provide oxygen or artificial respiration if needed. Consult a physician after significant exposure.
- b. Skin contact: Wash off immediately with soap and plenty of water.

- c. Eye contact: Rinse eye immediately with plenty of water. Also rinse under the eyelids. Keep eye wide open while rinsing. If eye irritation persists, consult a specialist.
- d. Ingestion: Call a physician immediately. Drink 1 or 2 glasses of water. Do not induce vomiting without medical advice. Never give anything by mouth to an unconscious person.

Note to physician: Efforts should be directed toward alleviation of any symptoms of illness and to prevent further absorption of indoxacarb.

B.6.10. REFERENCES RELIED ON

REVIEW OF SCIENTIFIC OPEN LITERATURE

The general methodology used for literature search is presented in Volume 1, Level 2.

The searches were performed with a date range of March 2005 to March 2013 and March 2013 to November 2014. The following databases were used for mammalian toxicology: AGRICOLA, CABA, CPlus, FROSTI, TOXCENTER and PubMed Online.

For mammalian toxicology endpoint, the review of the published literature identified 7 relevant studies and studies of unclear relevance: 5 of them were discussed in B.6.9.2 (Data collected on humans) and the 2 others were included in B.6.8.3 (Studies on Endocrine Disruption). All these relevant publications were considered as reliable by the applicant.

It is noted that the relevance criteria used by the applicant seem to be too restrictive. Nonetheless, based on the titles and/or abstracts of excluded published papers, the RMS considers that these documents would not give further relevant information on the toxicological profile of indoxacarb.

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation

CA, 5.1.1		1997a	¹⁴ C-DPX-MP062 (a 3:1 mixture of DPX-KN128 and IN-KN127): Metabolism in the rat HL-1997-00439 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.1.1		1997b	¹⁴ C-DPX-JW062 (a racemic mixture of DPX-KN128 and IN-KN127): Metabolism in the rat HLR 283-96 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.1.1/01		2000	¹⁴ C-DPX-JW062 (a racemix mixture of DPX-KN128 and IN-KN127): Metabolism in the rat HLR 283-96, Supplement No. 1 GLP: Yes -No Published: No	Y	Y <u>Comment from RMS:</u> this study is a supplemental of the previous one 1997b. Moreover, this is not a GLP study. So, it cannot grant any data protection.	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. ^a
CA, 5.2.1		2005	Oral approximate lethal dose (ALD) in mice HL-1997-00432,	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

			Revision No. 1 Published: No					
CA, 5.2.1/01		1997	Acute oral toxicity study with DPX- KN128 technical in male and female rats [REDACTED] HLO-1997- 00055 GLP: Yes Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. <u>Comment from RMS:</u> Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.2.1		1996a	Acute oral toxicity study with DPX-MP062 technical (approximately 75% DPX- KN128, 25% DPX-KN127) in male and female rats [REDACTED] HLR 910-96 Published: No	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.2.2/01		2003	Indoxacarb (DPX-KN128) technical: Acute dermal toxicity study in rats [REDACTED] DuPont-13019 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.2.2		1996b	Acute dermal toxicity study with DPX-MP062 technical	Y	N		DuPont	Study previously reviewed for EU approval in the 2000

			(approximately 75% DPX-KN128, 25% DPX-KN127) in rats [REDACTED] HLR 798-96 Published: No					DAR.
CA, 5.2.3	[REDACTED]	1995	Inhalation median lethal concentration (LC ₅₀) study with DPX-JW062-112 in rats [REDACTED] HLR 70-95. Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR
CA, 5.2.4/01	[REDACTED]	2003	Indoxacarb (DPX-KN128) technical: Acute dermal irritation study in rabbits [REDACTED] DuPont-13164 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.2.4	[REDACTED]	1997a	Primary dermal irritation study with DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127) in rabbits [REDACTED] HLR 589-96 Published: No	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

CA, 5.2.5/01		2003	Indoxacarb (DPX-KN128) technical: Acute eye irritation study in rabbits DuPont-13020 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.2.5		1997b	Primary eye irritation study with DPX-MP062 technical (approximately 75% DPX- KN128, 25% DPX-KN127) in rabbits HLR 588-96 Published: No	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

CA, 5.2.6/01		2003	Indoxacarb (DPX-KN128) technical: Dermal sensitization - Magnusson- Kligman maximization method DuPont-13018 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.2.6/02		2006	Indoxacarb (DPX-KN128) technical: Dermal sensitization test - Buehler method DuPont-18915 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.2.6		1996	Guinea pig dermal sensitization - Magnusson- Kligman maximization test with DPX- MP062 technical (approximately 75% DPX- KN128, 25% DPX-KN127). HLO 388-96 Published: No	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

CA, 5.2.7/01	Markell, L.K.	2015	Indoxacarb (DPX-KN128) technical: <i>In vitro</i> 3T3 NRU phototoxicity test DuPont Haskell Laboratory DuPont-43522 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.3.1	[REDACTED]	1993a	Repeated dose oral toxicity: 28-day feeding study with DPX-JW062-34 in male and female rats [REDACTED] HLR 403-93 Published: No	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.3.1	[REDACTED]	1993b	Repeated dose oral toxicity: 28-Day feeding study with DPX-JW062-34 in male and female mice [REDACTED] HLR 406-93, Revision No. 1 Published: No	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.

CA, 5.3.2		1997	Subchronic oral toxicity: 90-Day study with DPX-JW062-34 (50% DPX-KN128, 50% DPX-KN127) feeding study in mice HLR 750-93, Revision No. 1 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.3.2		1998	DPX-JW062 technical: Subchronic toxicity; 90-day feeding study in rats HL-1998-01200 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.3.2/01		1997	Subchronic oral toxicity: 90-Day study with DPX-MP062 (approximately 75% DPX-KN128, 25% DPX-KN127) feeding study in rats HL-1997-00056, Revision No. 1 GLP: Yes Published: No	Y	Y <u>Comment from RMS:</u> No new experimental results are available in the Revision No 1 of this previously submitted study. So it cannot grant any new data protection.	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. <u>Comment from RMS:</u> Study HL-1997-00056 previously reviewed for EU approval in the 2000 DAR.
CA, 5.3.2/02		1997a	Subchronic oral toxicity: 90-Day study with DPX-JW062-69 (99.7% DPX-KN128) feeding study in rats	Y	Y <u>Comment from RMS:</u> No new experimental results are available in the Revision No 2 of this	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously	DuPont	N.A. <u>Comment from RMS:</u> Study HLR 301-94 Revision No. 1 previously reviewed for EU approval in the 2000

			HLR 301-94, Revision No. 2 GLP: Yes Published: No		previously submitted study. So it cannot grant any new data protection.	protected the period of data protection has not expired at the time of submission of this dossier.		DAR.
CA, 5.3.2/03		1997b	Subchronic oral toxicity: 90-Day study with DPX-JW062-34 (50% DPX-KN128, 50% DPX-KN127) feeding study in rats HLR 751-93, Revision No. 2 GLP: Yes Published: No	Y	Y <u>Comment from RMS:</u> No new experimental results are available in the Revision No 2 of this previously submitted study. So it cannot grant any new data protection.	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. <u>Comment from RMS:</u> Study HLR 751-93 Revision No. 1 previously reviewed for EU approval in the 2000 DAR.
CA, 5.3.2/04		1997a	Subchronic oral toxicity: 90-Day study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) feeding study in dogs HLO 494-95, Revision No. 3 GLP: Yes Published: No	Y	Y <u>Comment from RMS:</u> No new experimental results are available in the Revision No 3 of this previously submitted study. So it cannot grant any new data protection.	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. <u>Comment from RMS:</u> Study HLO 494-95 previously reviewed for EU approval in the 2000 DAR.
CA, 5.3.2/05		1997b	Chronic toxicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) one year feeding study in dogs HLO 885-96, Revision No. 1 GLP: Yes	Y	Y <u>Comment from RMS:</u> No new experimental results are available in the Revision No 1 of this previously submitted study. So it cannot grant any	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of	DuPont	N.A. <u>Comment from RMS:</u> Study HLR 885-96 previously reviewed for EU approval in the 2000 DAR.









			Published: No		new data protection.	submission of this dossier.		
CA, 5.3.2/06		1995	Six-week palatability and toxicity study with DPX-JW062-106 feeding study in dogs HLO 162-95 GLP: Yes Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. <u>Comment from RMS:</u> Study previously reviewed for EU approval in the 2001 Addendum to DAR.
CA, 5.3.3		1999	DPX-MP062 technical: Repeated -dose dermal toxicity 28-day study in rats DuPont-2813 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (Addendum to the DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study submitted in the EU Dossier in 2001 and included in the first EU approval review (2001 Addendum to DAR).
CA, 5.4.1	Donner E.M.	2005	mutagenicity testing in the <i>Salmonella typhimurium</i> plate incorporation assay DuPont Haskell Laboratory HL-1997-00253, Revision No. 1 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.4.1	San, R.H.C., Sly, J.E.	1997b	DPX-MP062 (approximately 75% DPX-KN128, 25% DPX-KN127): Unscheduled DNA synthesis in mammalian cells <i>in vitro</i>	N	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.

			with an independent repeat assay Microbiological Associates, Inc. HLO-1997-00033 Published: No		protected anymore			
CA, 5.4.1/01	Gudi, R., Rao, M.	2004	Indoxacarb (DPX-KN128) technical: <i>In vitro</i> mammalian chromosome aberration study in human peripheral blood lymphocytes BioReliance DuPont-13022, Revision No. 1 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.4.1/02	San, R.H.C., Clarke, J.	2003	Indoxacarb (DPX-KN128) technical: <i>In vitro</i> mammalian cell gene mutation test (CHO/HGPRT test) BioReliance DuPont-13023 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.4.1/03	Wagner, V.O., Klug, M.L.	2004	Indoxacarb (DPX-KN128) technical: bacterial reverse mutation test BioReliance DuPont-14332 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has	DuPont	N.A.

						not expired at the time of submission of this dossier.		
CA, 5.4.1	Mathison, B.H.	1997	DPX-MP062 (approximately 75% DPX-KN128, 25% DPX-KN127): Mutagenicity testing in the <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> plate incorporation assay DuPont Haskell Laboratory HLR 831-96 Superseded by DuPont-14332 Published: No	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.4.1	Gudi, R., Schadley, E.	1996	DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127): <i>In vitro</i> mammalian cytogenetic test using human peripheral lymphocytes Microbiological Associates, Inc. HLO 979-96 Superseded by DuPont-13022, Revision No. 1 Published: No	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.4.1	San, R.H.C., Clarke, J.J.	1997	DPX-MP062 (approximately 75% DPX-KN128, 25% DPX-KN127): <i>In vitro</i> mammalian cell gene mutation test with an independent	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.


			repeat assay Microbiologica l Associates, Inc. HLO-1997- 00030 Superseded by DuPont-13022, Revision No. 1 Published: No					
CA, 5.4.2/01		2003	Indoxacarb (DPX-KN128) technical: Mouse bone marrow micronucleus test DuPont-13021 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.4.2		1997	DPX-MP062 (approximately 75% DPX-KN128, 25% DPX- KN127): Mouse bone marrow micronucleus assay HLR 1046-96, Revision No. 1 Superseded by DuPont-13021 Published: No	Y	N		DuPont	Study previously reviewed for EU approval in the 2000 DAR.



CA, 5.5		1997	Oncogenicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) eighteen-month feeding study in mice HLR 799-96 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.5/01		1997	Chronic toxicity study with DPX-JW062-106 (50% DPX-KN128, 50% DPX-KN127) one year feeding study in dogs HLR 1174-96, Revision No. 1 GLP: Yes Published: No	Y	Y <u>Comment from RMS:</u> No new experimental results are available in the Revision No 1 of this previously submitted study. So it cannot grant any new data protection.	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. <u>Comment from RMS:</u> Study HLR 1174-96 previously reviewed for EU approval in the 2000 DAR.
CA, 5.6.1/01		2005	Two generation reproduction/fertility study with DPX-JW062-106 in rats HLO 115-96, Revision No. 2 GLP: Yes Published: No	Y	Y <u>Comment from RMS:</u> No new experimental results are available in the Revision No 2 of this previously submitted study. So it cannot grant any new data protection.	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. <u>Comment from RMS:</u> Study HLR HLO 115-96 previously reviewed for EU approval in the 2000 DAR.
CA, 5.6.2		1997	DPX-JW062-112 (50% DPX-KN128, 50% DPX-KN127): Developmental toxicity study in rats	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so	Data protection is on a country by country basis as the status of data protection may vary	DuPont	Study previously reviewed for EU approval in the 2000 DAR.

			 HL-1997-00049 Published: No		this study is not protected anymore	depending on the MS		
CA, 5.6.2		1995	Developmental toxicity study of DPX-JW062-112 in rabbits  HLR 587-95 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.6.2/01		2005	DPX-MP062 (approximately 75% DPX-KN128, 25% IN-KN127): Developmental toxicity study in rats  HL-1997-00202, Revision No. 2 GLP: Yes Published: No	Y	Y <u>Comment from RMS:</u> No new experimental results are available in the Revision No 2 of this previously submitted study. So it cannot grant any new data protection.	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A. <u>Comment from RMS:</u> Study HLR HL-1997-00202 previously reviewed for EU approval in the 2000 DAR.
CA, 5.6.2/02		2004	Indoxacarb (DPX-KN128) technical: Developmental toxicity study in rats  DuPont-12748 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.6.2/03		1997a	DPX-JW062-112: Pilot developmental toxicity study (no. 2) in rats	Y	N		DuPont	N.A. <u>Comment from RMS:</u> Study


			<div></div> <p>HL-1997-01050 GLP: No Published: No</p>					previously reviewed for EU approval in the 2000 DAR.
CA, 5.6.2/04	<div></div>	1997b	<p>DPX-MP062 technical: Pilot developmental toxicity study (no. 2) in rats</p> <div></div> <p>HL-1997-01051 GLP: No Published: No</p>	Y	N		DuPont	<p>N.A.</p> <p><u>Comment from RMS:</u> Study previously reviewed for EU approval in the 2000 DAR.</p>
CA, 5.7.1	<div></div>	1997	<p>Subchronic oral neurotoxicity study of DPX-MP062 technical (approximately 75% DPX-KN128, 25% DPX-KN127) in rats</p> <div></div> <p>HLR 1116-96, Revision No. 1 Published: No</p>	Y	<p>Y</p> <p><u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore</p>	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.7.1/01	<div></div>	2001	<p>Acute oral neurotoxicity study of DPX-MP062 (approximately 75% DPX-KN128; 25% DPX-KN127) in rats</p> <div></div> <p>HLR 1117-96, Revision No. 2 GLP: Yes Published: No</p>	Y	<p>Y</p> <p><u>Comment from RMS:</u> No new experimental results are available in the Revision No 2 of this previously submitted study. So it cannot grant any new data protection.</p>	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	<p>N.A.</p> <p><u>Comment from RMS:</u> Study HLR 1117-96 previously reviewed for EU approval in the 2000 DAR.</p>
CA, 5.7.2/01	<div></div>	2006a	<p>Oral (gavage) developmental neurotoxicity study of DPX-KN128</p>	Y	Y	The study is necessary for the regulatory decision, conducted	DuPont	N.A.

			(Indoxacarb) technical in CrI:CD (SD)IGS BR VAF/Plus rats [REDACTED] DuPont-15150 GLP: Yes Published: No			according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		
CA, 5.7.2/02	[REDACTED]	2006b	Oral (gavage) developmental neurotoxicity study of DPX- KN128 (Indoxacarb) technical in CrI:CD (SD)IGS BR VAF/Plus rats [REDACTED] DuPont-15150, Supplement No. 1 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.	DuPont	N.A.
CA, 5.8.1	Gudi, R., Schadley, E.	1996	IN-JT333-20: <i>In vitro</i> evaluation for chromosome aberrations in human peripheral blood lymphocytes Microbiologica l Associates, Inc. HLO 951-96 Published: No	N	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.8.1	Kemper, R.A.	2002	In vitro hemolytic potential of IN- MT713 in erythrocytes from normal and glucose-6-phos phate dehydrogenase -deficient humans DuPont	N	N		DuPont	Study previously reviewed for EU approval in the 2005 Addendum to DAR.

			Haskell Laboratory DuPont-11842 FR Published: No					
CA, 5.8.1	Kemper, R.A.	2004	In vitro hemolytic potential of N-hydroxy-4-(trifluoromethoxy)aniline (IN-MT713) in rats, dogs, and humans DuPont Haskell Laboratory DuPont-12062, Revision No. 1 Published: No	N	N		DuPont	Study previously reviewed for EU approval in the 2005 Addendum to DAR.
CA, 5.8.1		1997	Acute oral toxicity study with IN-KG433 technical in male and female rats  HLO-1997-00469 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.8.1	Mathison, B.H.	1996	IN-JT333-20: Mutagenicity testing in the <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> plate incorporation assay DuPont Haskell Laboratory HLR 830-96 Published: No	N	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.8.1	San, R., Clarke, J.J	1997	IN-JT333-20: <i>In vitro</i> mammalian cell gene mutation test (CHO/HGPRT) with an independent repeat assay Microbiologica 1 Associates,	N	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2000 DAR.

			Inc. HLO 925-96 Published: No		anymore			
CA, 5.8.1	San, R.H.C., Clarke, J.J.	1997	IN-KG433 technical: <i>In vitro</i> mammalian cell gene mutation test (CHO/HGPRT)) with an independent repeat assay Microbiologica l Associates, Inc. HLO-1997- 00405 Published: No	N	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPon t	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.8.1	San, R.H.C., Sly, J.E.	1997	IN-KG433 technical: Unscheduled DNA synthesis in mammalian cells <i>in vitro</i> with an independent repeat assay Microbiologica l Associates, Inc. HLO-1997- 00406 Published: No	N	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPon t	Study previously reviewed for EU approval in the 2000 DAR.
CA, 5.8.1		1996	Acute oral toxicity study with IN-JT333- 20 in male and female rats  HLR 927-96 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPon t	Study previously reviewed for EU approval in the 2000 DAR
CA, 5.8.1	Wagner, V.O., Reece, J.D.	1997	IN-KG433 technical: Mutagenicity testing in the <i>Salmonella</i> <i>typhimurium</i> and <i>Escherichia</i> <i>coli</i> plate incorporation assay Microbiologica l Associates,	N	Y <u>Comment from RMS:</u> Study from the original submission (DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPon t	Study previously reviewed for EU approval in the 2000 DAR.

			Inc. HLO-1997- 00254 Published: No					
CA, 5.8.2		1999	¹⁴ C-DPX- JW062 (a racemic mixture of DPX-KN128 and IN- KN127): Distribution of erythrocytes of rats DuPont-1952 Published: No	Y	Y <u>Comment from RMS:</u> Study from the original submission (Addendum to the DAR), so this study is not protected anymore	Data protection is on a country by country basis as the status of data protection may vary depending on the MS	DuPont	Study previously reviewed for EU approval in the 2001 Addendum to DAR.
CA, 5.8.2/01	Green, J.W.	1999	DPX-MP062, DPX-JW062, and DPX-KN128: Regression analysis of changes in red cell mass parameters following subchronic dietary exposure DuPont Haskell Laboratory DuPont-2780 GLP: No Published: No	N	N		DuPont	N.A.
CA, 5.8.2/02	Green, J.W.	2002	A statistical analysis of indoxacarb- induced hematologic changes in rats and dogs using historical control data DuPont Haskell Laboratory DuPont-6122, Revision No. 1 GLP: No Published: No	N	N		DuPont	N.A.
CA, 5.8.2/03		2011	Indoxacarb (DPX-KN128) technical: 28-Day immunotoxicit	Y	Y	The study is necessary for the regulatory decision, conducted	DuPont	N.A.

			y feeding study in mice  DuPont-29280 GLP: Yes Published: No			according to GLP and has not previously been protected or if previously protected the period of data protection has not expired at the time of submission of this dossier.		
CA, 5.8.3/01	Orton, F., Rosivatz, E., Scholze, M., Kortenkam p, A.	2011	Widely used pesticides with previously unknown endocrine activity revealed as <i>in vitro</i> antiandrogens Envir Health Persp Vol 119(6), 794- 600 (2011) GLP: No Published: Yes	N	N		Author s	N.A.
CA, 5.8.3/02	Sipes, N.S., Martin, M.T., Kothiya, P., Reif, D.M., Judson, R.S., Richard, A.M., Houck, K. A., Dix, D.J., Kavlock, R.J., Knudsen, T.B.	2013	Profiling 976 toxcast chemicals across 331 enzymatic and receptor signaling assays 'Chem Res Tox, Vol 26, 878-895 (2013) GLP: No Published: Yes	N	N		Author s	N.A.
CA, 5.9.2/01	Jin, K.	2012	Rhabdomyolys is, methemoglobin emia and acute kidney injury after indoxacarb poisoning Clin Toxicol, Vol 50, 227 (2012) GLP: No Published: Yes	N	N		Author s	N.A.

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^a N.A. = not applicable, as this is a new study submitted for the first time at EU level for the purpose of renewal