INDOXACARB

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Explanation

Indoxacarb is the International Organization for Standardization (ISO) approved name for a new oxadiazine insecticide, methyl (*S*)-*N*-[7-chloro-2,3,4a,5-tetrahydro-4a-(methoxycarbonyl)-indeno[1,2-*e*][1,3,4]oxadiazin-2-ylcarbonyl]-4′-(trifluoromethoxy)carbanilate (the International Union of Pure and Applied Chemistry, IUPAC), also known as methyl (4a*S*)-7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy)-phenyl]amino]carbonyl]indeno[1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylate (Chemical Abstracts Service, CAS).

The indoxacarb racemate contains two enantiomers (*S* : *R*), designated DPX-KN128 and DPX-KN127, but only the *S* enantiomer has insecticidal activity. The ISO approved common name applies only to the insecticidally active *S* enantiomer. The indoxacarb racemate DPX-JW062 has been used in several toxicological studies. Subsequent refinements in the chemical synthesis process have enabled commercial production of a mixture enriched approximately 3 : 1 with the insecticidally active enantiomer. This enriched mixture has the code DPX-MP062 and is the active ingredient in all currently formulated products. The database contains a series of studies with DPX-MP062 to demonstrate its toxicological equivalence with DPX-JW062.

Indoxacarb has not previously been considered by the Meeting.

All pivotal studies were performed by good laboratory practice (GLP)-certified laboratories and complied with the relevant OECD test guidelines.

Evaluation for acceptable daily intake

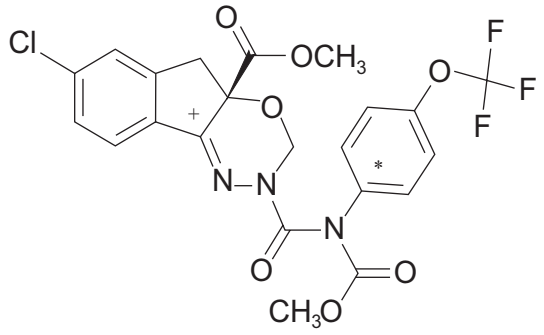
Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant OECD test guideline(s) or similar guidelines of the European Union or United States Environmental Protection Agency (EPA). As these guidelines specify the tissues normally examined and the clinical pathology tests normally performed, only significant exceptions to these guidelines are reported here, to save repetitive listing of study parameters.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution, metabolism and excretion of DPX-JW062 (Himmelstein, 1997a) and DPX-MP062 (Himmelstein, 1997b) were evaluated in male and female Crl:CD(SD)BR (Sprague-Dawley) rats. The structure of DPX-JW062/DPX-MP062 and the location of the ^{14}C radiolabels used in the studies of metabolism are shown in Figure 1.

Figure 1. Structure of DPX-MP062/DPX-JW062 and position of radiolabels



\+ Indanone label

* Trifluoromethoxyphenyl label

Although only sparingly soluble in aqueous media (0.2 μg/ml), racemic indoxacarb (DPX-JW062 or DPX-MP062) was suspended in polyethylene glycol (PEG-400) to give a fixed dosage volume of 10 ml/kg bw. The rats received indoxacarb suspensions containing radiolabelled indoxacarb at a dose of 5 or 150 mg/kg bw (15–40 μCi, 555–1480 MBq, per rat) by gavage. An additional group of female rats received 5 mg/kg bw per day for 14 days, to determine the potential for bioaccumulation.

Indoxacarb was slowly absorbed from the gastrointestinal tract after administration of 5 mg/kg bw, but at 150 mg/kg bw it appeared that absorption was saturable despite the presence of an absorption enhancer (PEG-400). Absorption in both sexes at 5 mg/kg bw was similar and estimated to be approximately 69–81% based on the radioactivity excreted in urine, bile and that found in tissues. At 150 mg/kg bw, the absorption was reduced to about 8–13.5%. Biliary excretion was slightly greater in males (23%) than in females (17%) for both labels at 5 mg/kg bw, but was more pronounced at 150 mg/kg bw, i.e. 6.4% and 1.8% respectively. At 5 mg/kg bw, the T_{max} in plasma was similar in males and females (males, 5 h; females, 8 h), while at 150 mg/kg bw the difference was greater (males, 3 h; females, 27 h). The elimination half-lives at 5 mg/kg bw were 35 h and 52 h for the indanone label and 92 h to 114 h for the trifluoromethoxyphenyl label in males and females respectively.

At 150 mg/kg bw, the half-lives for the indanone label in both sexes were similar (45–59 h) while they were much longer for the trifluoromethoxyphenyl label (i.e. 92 h and 114 h for males and females respectively), suggesting that some biotransformation of indoxacarb had occurred and that the elimination of the resulting radiolabelled metabolite was slow. An inspection of the distribution and retention profile of radioactivity in tissues 168 h after oral dosing suggested that most of the retained metabolites were lipophilic (see Table 1). The similarity of the tissue to plasma ratios for the two labels in fat, adrenals and ovaries suggests that the metabolites present in these organs are likely to be similar. In contrast, the tissue to plasma ratio was markedly different for the two labels in erythrocytes and therefore it seems likely that the metabolites would be different from those observed in fat, adrenals and ovaries.

In both sexes, most of the radiolabel (85–100%) in fat was associated with the biologically more active metabolite (methyl 7-chloro-2,5-dihydro-2-[[[4-(trifluoromethoxy)phenyl]amino]-carbonyl] (IN-JT333), which is formed via hydrolysis of the carboxymethyl group from the amino

Table 1. Tissue to plasma ratio of indoxacarb in selected tissues of rats 168 h after administration of radiolabelled indoxacarb as a single oral dose

Tissue	Indanone label			Trifluoromethoxyphenyl label		
	Male		Female	Male		Female
	Enantiomer ratio *(S : R)*					
	1:1	3:1	1:1	3:1	1:1	1:1
5 mg/kg bw						
Fat	11	31	29	63	16	32
Adrenals	3	7	5	16	9	5
Ovaries	—	—	3	10	—	3
Erythrocytes	2	4	2	2	123	61
150 mg/kg bw						
Fat	6	—	22	—	5	18
Adrenals	2	—	5	—	4	3
Ovaries	—	—	8	—	—	2
Erythrocytes	1.5	—	1.3	—	88	52

From Himmelstein (1997a)

nitrogen of the trifluoromethoxyphenyl moeity in the parent compound (see proposed metabolic pathway, Figure 2). Data from rat hepatic microsome preparations in vitro showed that while females metabolized indoxacarb (5 µmol/l) more slowly than did males (i.e. $t_{1/2}$ = 25.5 and 67.6 min respectively), almost tenfold more IN-JT333 was formed. These in vitro data support the observation made in vivo that females had a twofold greater body burden of IN-JT333 in fat than males (Himmelstein, 1997a). It was speculated that the metabolite that was selectively retained in erythrocytes and contributed to the long half-life of the trifluoromethoxyphenyl radiolabel involved only the trifluoromethoxyphenyl portion of the indoxacarb molecule (i.e. metabolite KB-687; see proposed metabolic pathway, Figure 2). In a follow-up study, the metabolite was characterized as being a related metabolite, arylamine 4-trifluoromethoxyaniline (IN-P0036) (see section 2.6; Anderson, 1999).

The distribution of radioactivity for rats given indoxacarb with an indanone label as a single oral dose at 5 mg/kg bw was: urine, 37–41%; faeces, 44%; and tissues, 3.4–7.8%. For rats given indoxacarb with a trifluoromethoxyphenyl label as a single oral dose at 5 mg/kg bw, the distribution of radioactivity was: urine, 47–55%; faeces, 27–30%; and tissues, 10–17%. Females retained more tissue residues than did males (Table 1).

Chiral high-performance liquid chromatography (HPLC) analysis indicated that the active enantiomer of IN-JT333, namely IN-KN125, was preferentially retained in fat with the approximate ratio of 2 : 1. Additional support for stereospecific kinetics was from the observation that the ratio of active (IN-KN125) to inactive enantiomer (IN-KN124) was approximately 6 : 1 7 days after dosing with indoxacarb (DPX-MP062) with an enantiomer ratio of 3:1 (i.e. DPX-KN125 : DPX-KN124) at 5 mg/kg bw. The absorption, distribution and elimination kinetics of the active enantiomer-enriched indoxacarb (DPX-MP062) was similar to that for racemic indoxacarb.

As anticipated from the long elimination half-life in plasma, there was evidence of bioaccumulation in body fat after repeated daily administration at 5 mg/kg bw per day for 14 days. In female rats dosed with trifluoromethoxyphenyl label at 5 mg/kg bw per day for 14 days, the total radioactive residues in fat, liver and brain increased and evidence for a steady state during days 8–14 was equivocal. The elimination half-lives for total radioactive residues in tissues were between 7.8 days for plasma and 18 days for fat. (Himmelstein, 1997a; Anderson, 1999; Himmelstein, 2000).

In studies of metabolism with DPX-MP062, no significant differences in the mean pharmacokinetic parameters were observed relative to those for DPX-JW062. The elimination half-lives at 5 mg/kg bw were 39 h and 49 h for the indanone label in males and females respectively. Although the oral absorption of DPX-JW062 was calculated from residues in urine, tissues and bile, this was not possible for DPX-MP062 because elimination in bile was not measured. However, data from the study of metabolism with DPX-JW062 in rats indicated that biliary elimination of DPX-MP062 could be estimated from total residues of faecal metabolites. Therefore, absorption values for DPX-MP062 were determined from residues in urine and tissue, as well as that portion of faecal residues comprised of metabolites. On the basis of these comparisons, the oral absorption of DPX-MP062 and DPX-JW062 were similar and were approximately 70–80% (Frame, 2002).

After dosing with indanone-labelled DPX-MP062 (5 mg/kg bw), male rats excreted slightly less label in the urine (35%) and more in the faeces (47%), while in females more was present in urine (45%) and less in faeces (33%). In both sexes, the excretion was greatest within 72 to 96 h after dosing. As with DPX-JW062, the retention of residues in tissues was greater in females (12.9%) than in males (4.4%) 168 h after dosing. The increased retention was attributable to increased residues (IN-JT333) in fat. For rats dosed with DPX-MP062, the percentage of the administered dose retained in the fat was 2.6% and 8.8% in males and females, respectively. For rats dosed with DPX-JW062, male rats retained 1.8% of the administered dose in fat compared with 4.7% in female rats.

1.2 Biotransformation

The metabolic profiles for DPX-MP062 and DPX-JW062 were similar. The proposed metabolic pathway of indoxacarb in the rat is shown in Figure 2. The major metabolites in faeces were IN-JT333 (0.4–3.3%), formed by hydrolysis of the carboxymethyl group from the amino nitrogen of the trifluoromethoxyphenyl portion of the parent compound, and 5-HO-JW062 in two stereoisomeric forms (3.5–12.7%), formed by hydroxylation of the indanone ring. An oxadiazine ring-opened metabolite (IN-KG433) formed by hepatic microscomal enzymes is likely to be a precursor for several metabolites found in urine. The major urinary metabolite from rats given indoxacarb labelled with indanone was IN-MU716, and from rats given indoxacarb labelled with trifluoromethoxyphenyl was IN-MG195 and IN-MC218. Eight minor urinary metabolites were identified, all present at < 5% of the administered dose. No parent compound was detected in bile, and no single metabolite accounted for more than 4% of the administered dose.

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with indoxacarb are summarized in Table 2.

(a) Oral administration

Groups of five male and five female fasted Crl:CD BR rats were given indoxacarb (DPX-MP062-51; purity, 94.5%) at a dose of 1000, 3000 or 5000 mg/kg bw by gavage in corn oil (500 mg/ml). Two additional groups of 10 females were dosed at 100 or 250 mg/kg bw. The animals were observed daily for mortality and clinical signs for up to 24 days after dosing. Body weight was recorded daily. All rats found dead and those that survived the observation period underwent gross pathological examination.

All rats at 3000 and 5000 mg/kg bw died. Deaths occurred up to 20 days after dosing. Body weights fluctuated throughout the recovery period; individual weight losses were generally less than 7% of the previous day's weight. By the end of the recovery period, two out of five surviving males and the surviving female at 1000 mg/kg bw weighed less than their starting body weights. The remaining surviving animals had gained weight by the end of the recovery period. Clinical signs of toxicity included ataxia, ruffled fur, hunched posture and stained perineum. Signs generally occurring after day 5 included general spasms, pallor, ocular discharge, immobility, lethargy, piloerection, wet perineum, tremors, salivation and head or facial stains. The gross pathological examinations revealed no specific organ toxicity (Sarver, 1996a).

(b) Dermal administration

A single dose of indoxacarb (DPX-MP062-51; purity, 94.5%) at 5000 mg/kg bw, moistened to a paste with water, was applied to the shaved intact skin of five male and five female Crl:CD BR rats. After semi-occlusion of the application site for 24 h, the test substance was removed and the skin was washed with warm water and dried. The animals were observed for 2.5 h after dosing and then daily for mortality and clinical signs. Body weights were recorded on study days 0, 7 and 14. At the end of the 14-day recovery period, all rats underwent gross pathological examination. No rats died and no clinical signs of toxicity were observed during the study. Weight losses of up to about 9% of initial body weight were observed in all rats 1 day after application of the test substance. The weight losses were attributed to collars and wrappings that restricted the animals' access to food during the first 24 h (although food intake during this periodwas not recorded). No dermal irritation was observed and no gross lesions were found at necropsy (Sarver, 1997a).

(c) Exposure by inhalation

Groups of five male and five female Crl:CD BR rats received a single nose-only exposure to indoxacarb (DPX-JW062-112; purity, 94.8%) at a chamber concentration of 3300 or 5500 mg/m^3, and additional groups of 10 females were exposed to indoxacarb at 450, 2300 or 4000 mg/m^3 for 4 h. The mass median aerodynamic diameter of the dust in the chamber ranged from 1.7 to 3.2 μm, with 8–22% of the particles being < 1 μm, 47–77% being < 3 μm, and 91–99% being < 10 μm. The animals were observed daily for mortality and clinical signs for 14 days after dosing, and were weighed daily. All rats found dead and those that survived the observation period underwent gross pathological examination. Exposure to indoxacarb at 5400 or 3300 mg/m^3 caused three out of five and two out of five deaths in females, respectively, and no deaths in male rats. Subsequent exposure of females to indoxacarb at 450, 2300 and 4000 mg/m^3 caused zero out of ten, four out of ten, and four out of ten deaths, respectively. Surviving males and females exhibited slight to severe weight loss up to 2 days after exposure to indoxacarb; although some rats showed transient weight loss during the remainder of the recovery period, all surviving rats showed an overall weight gain by the end of the 14-day recovery period. Animals of both sexes showed clinical signs of nasal/ocular discharge and stained/wet perineum during the 14-day observation period, and females showed alopecia, hunched posture, abnormal gait and lethargy. Gross pathological examination revealed no specific organ toxicity in any rats. The LC_{50} was > 5400 mg/m^3 in males and 4200 mg/m^3 in females (> 5.4 and 4.2 mg/l air, respectively) (O'Neill, 1995).

(d) Dermal and ocular irritation

A single application of 500 mg of indoxacarb (DPX-MP062-51, purity, 94.5%), moistened to a paste with water, was applied to the shaved intact skin of six male New Zealand White rabbits. After occlusion of the application site for 4 h, the test substance was removed and the skin was washed with warm water and dried. The test sites were examined at 1, 24, 48 and 72 h after removal of the patches, and animals were observed for clinical signs of toxicity at the same times. There was no evidence of erythema or oedema at any time-point in any animal. Indoxacarb is not a skin irritant in rabbits (Sarver, 1997b).

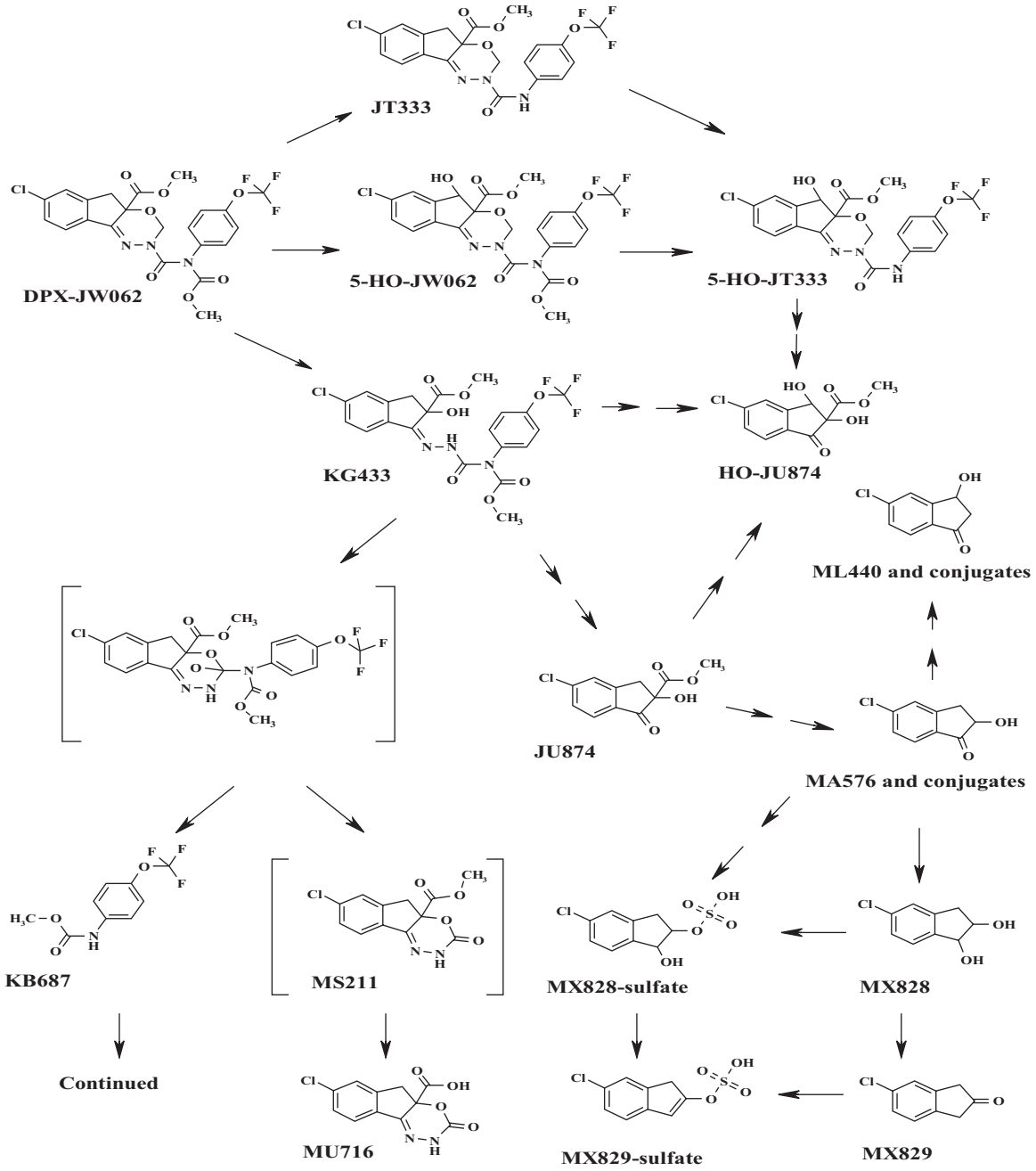
Approximately 48 mg of indoxacarb (DPX-MP062; purity, 94.5%; volume, approximately 0.1 ml in water), was applied to the lower conjunctival sac of the right eyes of six female New Zealand White rabbits. The untreated left eye served as a control. The eyes were not washed after treatment. The rabbits were observed for signs of irritation and clinical signs at 1, 24, 48 and 72 h after application of the test substance.

Indoxacarb produced blistering of the conjunctiva in all rabbits within 1 h. Corneal opacity, iritis, and conjunctival redness, chemosis and discharge were observed during the study, but ocular irritation had cleared by 72 h. No adverse clinical signs were noted. Indoxacarb is therefore considered to be a moderate eye irritant in rabbits (Sarver, 1997c)

(e) Dermal sensitization

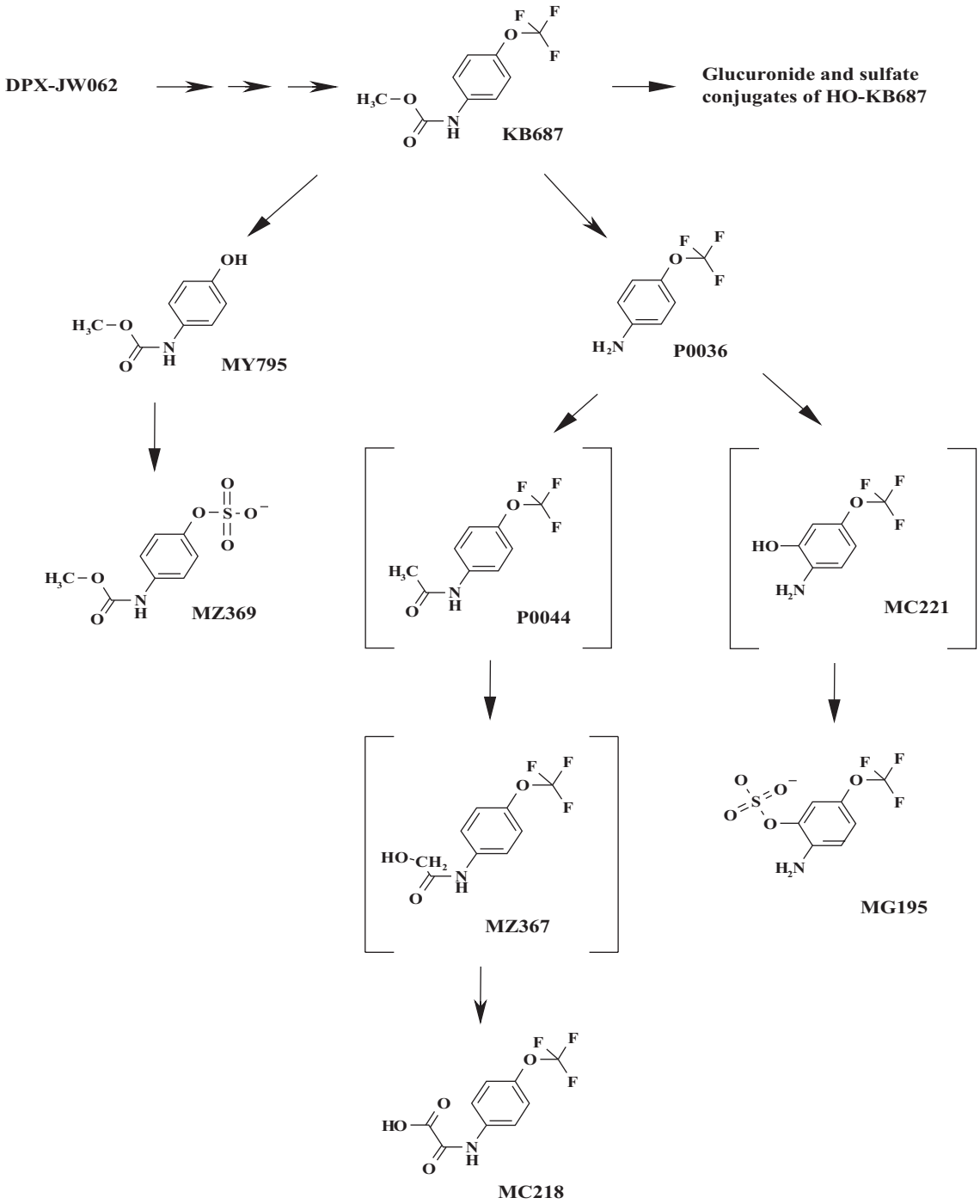
Using the Magnusson & Kligman test protocol, groups of 20 male Hartley guinea-pigs received appropriate induction and challenge doses (0.1 ml in water) of indoxacarb. On day 8, 500 mg of indoxacarb (DPX-MP062; purity, 94.5%), moistened with propylene glycol, was applied to the intradermal test area. The sites were occluded with plastic wrap and tape for 48 h. After the tape was removed, the site was cleansed with water and propylene glycol. The sites were examined at 24 h and 48 h after challenge. Appropriate positive (dinitrochlorobenzene) and vehicle control groups were included, and gave satisfactory responses. After challenge with indoxacarb, 14 out of 20 animals that had been sensitized with indoxacarb showed mild to moderate redness at 28 h or 48 h, and reaching intense redness in one animal at 48 h. Therefore indoxacarb is considered to be a skin sensitizing agent (Moore, 1997).

Figure 2. Proposed metabolic pathway of DPX-MP062/DPX-JW062 in rats



[ ] Proposed intermediates

Figure 2. Proposed metabolic pathway of DPX-MP062/DPX-JW062 in rats (continued)



[ ] Proposed intermediates

Table 2. Acute toxicity of DPX-MP062

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Reference
Rat	Crl:CD (SD)BR	Male	Oral	1730	—	Sarver (1996a)
		Female		268	—	
		Male	Dermal	> 5000	—	Sarver (1997a)
		Female		> 5000	—	
		Male	Inhalation[a]	—	> 5.4	O'Neill (1995)
		Female			4.2	

[a] Indoxacarb racemate (DPX-JW062) dust was used owing to the inability to generate respirable particles of the mix that is enriched in the insecticidally active enantiomer (DPX-MP062).

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female Cr1:CD(ICR)BR mice received diets containing racemic indoxacarb (DPX-JW062; purity, 94.7%) at a concentration of 0, 12, 29/400, 59, 118, 235, 1225 or 2450 ppm for 28 days. One group was started on diet containing indoxacarb at 29 ppm, which was then increased to 400 ppm on day 8. The animals were observed twice weekly for clinical signs, body weight and food consumption. On day 29, the surviving animals were killed and examined for gross pathology. Organ weights were recorded for liver, kidney, brain and testes. No microscopic examinations were conducted. The mean daily intake of indoxacarb in the groups at 12, 59, 118 and 235 ppm was 2.06, 10.8, 17.9 and 34.0 mg/kg bw per day for males, and 2.52, 11.8, 21.5 and 35.3 mg/kg bw per day for females.

Owing to excessive toxicity, mice at 1225 ppm and 2450 ppm were killed after 7 days. In the group at 235 ppm, one male and one female died at 8 and 27 days, respectively. At 235 and 400 ppm, there were clinical signs suggestive of neurotoxicity (abnormal gait, head tilt and tremors). Mice at 1225 ppm and 2450 ppm lost 21–24% of their initial body weight before early sacrifice. Significant decreases in body-weight gain occurred in males at 118, 235 and 400 ppm (12.2%, 15.2% and 25%, respectively) and in females at 235 and 400 ppm (14.2% and 21.9%, respectively), with a concomitant decrease in food consumption and food efficiency. There were no compound-related gross pathological lesions present in mice examined at necropsy. Significant changes in relative organ weights were attributable to decreased final mean body weights.

The no-observed-adverse-effect level (NOAEL) was 59 ppm for males and 118 ppm for females, equal to 10.8 and 21.5 mg/kg bw per day, respectively. In male mice, the NOAEL was identified on the basis of reduced body-weight gain and food consumption at 118 ppm (17.9 mg/kg bw per day) and above. In female mice, the NOAEL was identified on the basis of reduced body-weight gain at 235 ppm (35.3 mg/kg bw per day) and above (Reynolds, 1993a).

Groups of 10 male and 10 female Cr1:CD-1(ICR)BR mice were fed diets containing racemic indoxcarb (DPX-JW062; purity, 94.7%) at a concentration of 0, 10, 35, 75 or 150 ppm for 90 days. From day 42, mice at 10 ppm were changed to diets containing indoxacarb at 300 ppm. The animals were observed daily for clinical signs, and food consumption and body weights were measured weekly. All mice received an ophthalmoscopic examination before and at the end of the study. Haematology evaluations were performed on days 45 and 90. The mean daily intakes of indoxacarb by male mice in the groups at 10/300, 35, 75 and 150 ppm were 1.7/44, 5.5, 12 and 23 mg/kg bw per day, respectively, and by female mice were 2.1/51, 7.0, 16 and 30 mg/kg bw per day, respectively. One possible compound-related death occurred in a male

mouse at 300 ppm, and six other accidental or sporadic deaths unrelated to treatment occurred in the remaining groups. There were no ophthalmological findings related to treatment. Clinical signs suggestive of neurotoxicity (abnormal posture/gait/ mobility) occurred in five out of ten male mice at 300 ppm and four out of ten, and ten out of ten females at 150 and 300 ppm, respectively. Mice in these groups showed lower body-weight gain, food consumption and food efficiency than the control groups; at the end of the study, animals at 300 ppm showed a mean overall body-weight loss of 8% (males) and 3% (females). At the 90-day sampling time, eight out of eight, and nine out of nine male mice at 150 and 300 ppm, respectively, and seven out of nine female mice at 300 ppm had an increased incidence of Heinz bodies (oxidized/denatured haemoglobin precipitation within erythrocytes). Males at 75 ppm and above and females at 300 ppm had increased reticulocyte counts, although the increases were only statistically significant in males at 75 and 300 ppm (but not at 150 ppm). Other erythrocyte parameters (erythrocyte count, haemoglobin, erythrocyte volume fraction (EVF)) in the treated groups were not significantly different from those of controls. Male and female mice at 300 ppm had leukocytosis, characterized by an approximate doubling of neutrophil (males only) and lymphocyte counts. The haematological pattern of leukocytosis with minimal effects on erythrocyte parameters is typical of mild haemolysis in mice. Females at 300 ppm had small spleens with microscopic evidence of lymphoid depletion. There were no other significant organ-weight changes. A minimal to mild increased incidence of splenic pigment (haemosiderin) was noted in males and females at 75 ppm and above, with increased splenic erythropoiesis at 150 and 300 ppm. Minimal to mild increased hepatic haemosiderin was observed at 300 ppm.

The NOAEL in mice was 35 ppm, equal to 5.5 mg/kg bw per day for males and 7 mg/kg bw per day for females, on the basis of increased reticulocyte counts in males and microscopic evidence of mild haemolysis in both sexes at the higher doses (≥ 12 mg/kg bw per day) (Malek, 1997a).

Rats

Groups of five male and five female Cr1:CD BR rats received diets containing racemic indoxacarb (DPX-JW062; purity, 94.7%) at a concentration of 0, 8/400, 12, 29, 59, 118 or 235 ppm for 28 days. One group started receiving a diet containing indoxacarb at 8 ppm and this was increased to 400 ppm on day 17. The animals were observed twice weekly for clinical signs, body weight and food consumption. On day 29, surviving rats were killed and examined for gross pathology. Organ weights were recorded for liver, kidney, brain and testes. No microscopic examinations were conducted. The mean daily intake of indoxacarb at 12, 29, 59, 118 and 235 ppm was 1.02, 2.47, 5.89, 8.85 and 20.6 mg/kg bw per day for males and 1.08, 2.61, 4.72, 9.29 and 23.5 mg/kg bw per day for females. Three out of five females at 400 ppm and two out of five at 235 ppm died during days 8–28. Females at 400 ppm showed abnormal gait and dehydration and, at 235 ppm had pallor and ruffled fur. No males died and there were no clinical signs of toxicity in the males. Male rats at 8/400 and 235 ppm showed a significant decrease in mean body-weight gain; at the end of the study, body weights were 4.4% and 10.7%, respectively, lower than those of the controls. Female body weights at 59, 118, 235 and 8/400 ppm were significantly lower than those of the controls at the end of the study (6.5%, 15.0%, 23.6% and 35.5%, respectively). Food consumption and food efficiency were not significantly affected in males, but in females food consumption and food efficiency were reduced at 118 and 235 ppm, respectively. The only compound-related gross pathological change was a thin body for a female at 235 ppm. Changes in absolute and/or relative organ weights were attributable to decreased final mean body weights.

The NOAEL in rats was 118 ppm for males and 29 ppm for females, equal to 8.85 and 2.61 mg/kg bw per day, respectively, on the basis of body-weight effects in males at 235 ppm (20.6 mg/kg bw per day) and in females at 59 ppm (4.72 mg/kg bw per day) and above (Reynolds, 1993b).

Groups of 10 male and 10 female Crl:CD BR rats were fed diets containing racemic indoxacarb (DPX-JW062; purity, 94.7%) at a concentration of 0, 15 (females only), 30, 60, 125 or 250 (males only) ppm for 90 days. The animals were observed daily for clinical signs, and food consumption and body weight were recorded weekly. All rats received an ophthalmoscopic examination at the start and end of the study. Haematology, clinical chemistry and urine analysis evaluations were performed on days 48 and 90. All animals found dead, or killed at the end of the study, were examined for gross pathological effects and selected tissues were examined microscopically. The mean daily intake of indoxacarb by male rats in the groups at 30, 60, 125 and 250 ppm was 1.9, 3.9, 8.0 and 16 mg/kg bw, respectively, and by female rats in the groups at 15, 30, 60 and 125 ppm was 0.99, 2.3, 4.6 and 9.5 mg/kg bw, respectively.

There were no treatment-related deaths, clinical signs or ophthalmological abnormalities in any group. Males at 250 ppm and females at 125 ppm showed reduced body-weight gain with decreased food consumption and/or food efficiency throughout the study; mean overall body-weight gains were 16% (males at 250 ppm) and 34% (females at 125 ppm) lower than controls. At both the 45-day and 90-day sampling times, animals at 30 ppm and above showed a reduction in erythrocyte count, haemoglobin and/or EVF, with increases in mean corpuscular volume (MCV) and reticulocyte count, indicative of mild haemolysis (Table 3). These effects were statistically significant in males at 30 ppm at 45 days and at 60 ppm at 90 days; in females, the effects were statistically significant at 60 ppm at the 45-day time-point and at 30 ppm at 90 days. At dietary concentrations below 250 ppm (males) and 125 ppm (females), the parameters were not decreased more than 10% from the control group and reticulocyte counts were not consistently increased in these groups. Female rats at 125 ppm had slightly decreased total protein and globulin concentrations at both sampling times. There were no significant urine analysis or organ weight findings. Histopathology revealed increases in haemosiderin in macrophages of the liver at 250 ppm (males) and 125 ppm (females), and in haemosiderin in the spleen of male and female rats at 30 ppm and above, with increased erythrocytic hyperplasia in the spleen of males and females at 60 ppm and above, and in the bone marrow in males at 250 ppm and females at 125 ppm (Table 4).

Female rats at 125 ppm had slightly decreased total protein and globulin concentrations at both sampling times. There were no significant urine analysis or organ weight findings. Histopathology revealed increased haemosiderin in macrophages of the liver at 250 ppm (males) and 125 ppm (females), and increased haemosiderin in the spleen of male and female rats at 30 ppm and above, with increased erythrocytic hyperplasia in the spleen of males and females at 60 ppm and above, and in the bone marrow in males at 250 ppm and females at 125 ppm.

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) (Malek, 1997b).

Groups of 10 male and 10 female Cr:CD(SD) BR rats were fed diets containing indoxacarb (DPX-MP062-51; purity, 94.5%) at a concentration of 0, 10, 25 (females only), 50, 100, or 200 (males only) ppm for 90 days. The animals were observed daily for clinical signs, and food consumption and body weight were measured weekly. All rats received an ophthalmoscopic examination at the start and end of the study. Haematology, clinical chemistry and urine analysis evaluations were performed on days 45 and 90. All animals found dead, or killed at the end of the study, were examined for gross pathological effects and selected tissues were examined microscopically. Mean daily intake of indoxacarb by male rats in the groups at 10, 50, 100 ppm and 200 ppm was 0.62, 3.09, 6.01 and 15.0 mg/kg bw per day, respectively, and by female rats in the groups at 10, 25, 50 and 100 ppm was 0.76, 2.13, 3.78 and 8.94 mg/kg bw per day, respectively.

Table 3. Summary of significant findings at 90 days in rats fed diets containing indoxacarb

Parameter	Sex	Dietary concentration (ppm)					
		0	15	30	60	125	250
Erythrocytes (10^6/ml)	M	8.49	—	8.36	8.10	7.47*	7.10*
	F	8.12	7.83	7.53*	7.27*	6.89*	—
Hb	M	16.3	—	15.6	15.0*	14.8*	14.5*
(g/dl)	F	16.3	15.6	14.9*	15.1*	14.5*	—
EVF	M	0.44	—	0.43	0.41*	0.41*	0.40*
	F	0.44	0.44	0.42	0.43	0.41*	—
Reticulocytes	M	100	—	128	121	136	224*
(10^6/µl)	F	54	66	106	165*	147*	—
MCV	M	52	—	51	51	55	56*
(µm^3)	F	55	56	56	59*	60*	—
MCHC	M	37	—	37	36	37	36
(g/dl)	F	37	36	35*	36*	35*	—

From Malek (1997b)

*Significantly different from control ($p < 0.05$)

EVF, erythrocyte volume fraction; F, female; Hb, haemoglobin; M, males; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration.

Five female rats dosed at 100 ppm were either found dead or were killed in extremis during the second or third week of dietary exposure. The cause of death was not determined, but the rats showed body-weight loss and weakness before death. Surviving females at 100 ppm (and, to a lesser extent, at 50 ppm) also demonstrated decreased food consumption, food efficiency and body-weight loss during the first 2 weeks; females at 100 ppm also had weakness and ataxia during weeks 1 to 3. All females subsequently gained weight and demonstrated partial recovery from early toxic effects, but body weights at 100 ppm at the end of the study were 20% lower than those of the controls. Body-weight gain at 100 and 50 ppm were 58% and 62% of concurrent controls respectively. In male rats, exposure to 200 ppm produced significant body-weight loss during the first week and a decrease in body-weight gain during subsequent weeks, with decreased food consumption and food efficiency, especially during the first week. By the end of the study, body weights in this group were 30% lower than controls. No compound-related effects on body weight were seen in males at ≤ 50 ppm. No ocular lesions were detected in any group.

At the 45-day and 90-day sampling times, male rats at 100 ppm and female rats at 50 ppm showed a statistically significant reduction in erythrocyte count, haemoglobin and/or EVF, indicative of mild haemolysis (Table 5). Increased mean cell volume, suggestive of a bone marrow regenerative response, was also observed in these groups; however, reticulocyte counts were not increased. Male rats at 200 ppm had slightly decreased total protein and globulin concentrations at both sampling times. There were no significant findings related to urine analysis or organ weight.

Histopathology revealed minimal to mild increased haematopoiesis and increased haemosiderin in the spleen of male rats at 50 ppm and above and females at all doses, with increased haemosiderin in macrophages of the liver at 200 ppm (males) and 50 ppm (females) (Table 6). These effects were not dose-related and were likely to be secondary to mild haemolysis.

Table 4. Incidences of microscopic changes at 90-day sampling time in rats fed diets containing indoxacarb

Parameter	Dietary concentration (ppm)					
	0	15	30	60	125	250
No. of rats per group	10	10	10	10	10	10
Males						
Liver: pigment increased	0	—	0	0	0	4
Spleen: pigment increased	2	—	7	10	10	10
Haematopoiesis increased	0	—	0	4	10	10
Bone marrow:						
Hyperplasia, mixed	0	—	1	2	3	4
Females						
Liver: pigment increased	0	0	1	2	7	—
Spleen: pigment increased	1	1	10	10	10	—
Haematopoiesis increased	0	0	1	3	4	—
Bone marrow:						
Hyperplasia, mixed	1	1	1	1	5	—

From Malek (1997b)

Table 5. Summary of significant haematological findings at 90 days in rats fed diets containing indoxacarb

Parameter	Sex	Dietary concentration (ppm)					
		0	10	25	50	100	200
Erythrocytes (10^6/ml)	M	8.54	8.78	—	8.29	7.78*	7.19*
	F	8.19	7.64	7.71	7.36*	7.00*	—
Hb	M	16.1	16.2	—	15.1*	14.8*	14.4*
(g/dl)	F	16.0	15.4	15.1*	14.9*	14.7*	—
EVF	M	0.47	0.48	—	0.45	0.44*	0.43*
	F	0.49	0.46*	0.46*	0.45*	0.45*	—
MCV	M	55	55	—	55	57	60*
(μm^3)	F	60	60	60	61	65*	—

From MacKenzie (1997)

*Significantly different from control ($p < 0.01$)

EVF, erythrocyte volume fraction; Hb, haemoglobin; MCV, mean corpuscular volume.

Female rats found dead or killed in extremis (100 ppm) showed atrophy of the spleen, thymus and/or bone marrow, due to loss of lymphoid and haematopoietic cells. Haemoglobin pigment was also observed in renal tubule cells and/or lumens of early-death rats, but was not observed in rats killed at the end of the study. This suggests that rats dying early may have had lysis of erythrocytes in the blood stream, while surviving rats were experiencing phagocytosis of erythrocytes by hepatic and splenic macrophages.

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).

Table 6. Incidences of microscopic changes at 90 days in rats fed diets containing indoxacarb

Parameter	Dietary concentration (ppm)					
	0	10	25	50	100	200
No. of rats per group	10	10	10	10	10[a]	10
Males						
Liver: pigment increased	0	0	—	0	0	1
Spleen: pigment increased	0	0	—	2	6	10
haematopoiesis increased	0	0	—	7	6	9
Females						
Liver: pigment increased	0	0	0	3	10	—
Kidney: haemoglobin pigment	0	0	0	0	5	—
Spleen: pigment increased	0	6	10	9	5	—
haematopoiesis increased	1	4	4	6	2	—

From MacKenzie (1997)

[a]Includes autopsy of five females found dead or killed in extremis between days 7 and 20; these rats had atrophy of the spleen, thymus and bone marrow, and increased pigment in the liver.

Groups of five male and five female Cr1:CD(SD) BR rats received indoxacarb technical (DPX-MP062-51; purity, 94.5%) moistened with water and applied at a dose of 0, 50, 500, 1000 or 2000 mg/kg bw per day to the shaved intact dorsal skin for 28 days. The skin sites were occluded for 6 h after each application, then washed with soap and water. Control animals received 0.5 ml of deionized water in a comparable regimen. The animals were observed daily for clinical signs and dermal irritation, twice weekly for body weight, weekly for food consumption, and haematology and clinical chemistry parameters were measured at necropsy. On day 29, all animals were killed, examined macroscopically, and selected organs were weighed and tissues taken for histopathology. Clinical findings included yellow matting in the urogenital region in males at 1000 and 2000 mg/kg bw per day and in females at 500, 1000 and 2000 mg/kg bw per day. Dermal irritation was noted in all treatment groups, although the incidences were sporadic, generally consisting of mild erythema without oedema. The severity was greater at the higher doses, reaching erythema Draize score 4 (severe) in one male and two females at 1000 mg/kg bw per day on days 6–7, 7–11 and 5–11, respectively, and in one male and two females at 2000 mg/kg bw per day on days 25, 5–18, and 19–21, respectively.

A dose-related reduction in body-weight gain occurred during the first 2 weeks of dosing; thereafter, body-weight gain was similar to or above control group values. Mean body-weight gain in males at 2000 mg/kg bw per day was lower than that of controls (76% of control; $p < 0.05$). The body-weight gain reductions observed among females at 2000, 1000, and 500 mg/kg bw per day were 53, 53, and 55% of the control value, respectively. Mean body weights of female rats in these groups were also lower than controls. Males treated at 2000 mg/kg bw per day had lower food consumption than controls (91% of control). Females treated with 500 mg/kg bw per day 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 females (64–65% of controls). All animals at 1000 and 2000 mg/kg bw per day had evidence of mild haemolysis, but statistically significant decreases (about 10%) in erythrocyte parameters (erythrocyte count , haemoglobin and EVF) were only found in females at 2000 mg/kg bw per day. There was some haematological evidence that the haemolysis had elicited a bone marrow regenerative response (increased MCV and polychromatophil numbers). There was histological evidence of non-suppurative inflammation in two males at 2000 mg/kg bw per day.

The NOAEL was 1000 mg/kg bw per day for males and 50 mg/kg bw per day for females. The NOAEL in males was based on reduced body-weight gain and food consumption in rats exposed to 2000 mg/kg bw per day. The NOAEL in female rats was 50 mg/kg bw per day based

on reduced body-weight gain, food consumption, and food efficiency in females exposed to 500 mg/kg bw per day and above (MacKenzie, 1999).

Dogs

Groups of four male and four female outbred beagle dogs were fed diets containing indoxacarb (DPX-JW062-106, purity, 95.03%) at a concentration of 0, 40, 80, 160 or 640 ppm for 90 days. The animals were observed twice daily for clinical signs, food consumption was recorded daily, and body weights were measured weekly. All dogs received an ophthalmoscopic examination at the start and end of the study. In addition to haematology, serum chemistry and urine analysis parameters, γ-glutamyl transferase, clotting parameters (PT, APTT) and erythrocyte morphology were evaluated and nitrite and leukocyte measurements were included in the urine analysis. Evaluations were performed before the study and during weeks 4, 9 and 13. Ocular examinations were conducted before the study and during week 13. Any animals found dead during the study and those killed at scheduled necropsy were examined for gross pathological effects, selected organs were weighed and tissues were examined microscopically. The average test substance consumption in dogs fed 40, 80, 160 or 640 ppm in the diet was 1, 2, 5 and 18 mg/kg bw per day for males and 1, 3, 5 and 17 mg/kg bw per day for females.

No animals died during the study and no treatment related clinical signs were noted in any group. In the females at 640 ppm, reduced food consumption was noted during the first 8 weeks and body-weight loss and/or reduced body-weight gain occurred sporadically throughout the study. Overall weight gain at the end of the study was 3.8% in this group, compared with 10.3% in the control group. No treatment-related ocular lesions were found. Mild anaemia (decreased erythrocytes, haemoglobin and EVF) was seen in the 640 ppm group and 160 ppm males at all evaluation time-points (Table 7). The pattern of changes in the mean values of erythrocyte indices 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 in these groups, indicating that a bone-marrow regenerative response had occurred. An increased number of Heinz bodies at 160 (females only) and 640 ppm indicated that oxidative denaturation of haemoglobin may have been the cause of haemolysis. Serum bilirubin concentrations in the 160 and 640 ppm groups were increased at all evaluation time-points. These increases were most likely secondary to haemolysis. Serum alkaline phosphatase activities were increased in females at 160 and 640 ppm at all time-points. Urine analysis parameters were unaffected by treatment. At necropsy, no macroscopic lesions were detected and there was no treatment-related effect on organ weights. Microscopically, haemosiderin was present in the spleen and liver of male and female dogs at 40 ppm and above (Table 8). 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 (Mertens, 1997a).

Groups of five male and five female beagle dogs were fed diets containing indoxacarb (DPX-JW062; purity, 95.03%) at a concentration of 0, 40, 80, 640 or 1280 ppm for 52 weeks. Animals were observed twice daily for mortality and clinical signs. Food consumption was recorded daily, and body weights were measured weekly. Haematology (including clotting parameters, erythrocyte morphology and Heinz bodies), serum chemistry (including γ-glutamyl transferase) and urine analysis were conducted before study start and during weeks 12, 25 and 51.

Table 7. Summary of significant findings at 90 days in dogs fed diets containing indoxacarb

Parameter	Sex	Dietary concentration (ppm)				
		0	40	80	160	640
Haematology						
Erythrocytes (10^6/ml)	M	6.17	5.76	5.82	5.01*	5.02*
	F	6.71	6.11	5.93	6.13	5.33*
Hb (g/dl)	M	14.1	13.1	13.4	11.8*	11.8*
	F	15.0	14.0	13.7	14.5	11.7*
Platelets (10^3/µl)	M	337	508*	554*	618*	550*
	F	427	490	538	600	702*
Reticulocytes (10^6/µl)	M	0.2	0.8	0.3	1.1	2.4*
	F	0.1	0.4	1.1	1.7	2.3*
MCV (µm^3)	M	69.4	73.6	73.0	74.3*	76.6*
	F	69.0	71.4	72.7	75.0*	78.0*
MCHC (g/dl)	M	32.9	31.0	31.5	31.8	30.7
	F	32.4	32.2	31.7	31.5	28.2*
Serum chemistry						
Bilirubin	M	0.2	0.3	0.3	0.3	0.4*
	F	0.2	0.2	0.2	0.3	0.4*
Alkaline phosphatase	M	98	87	84	94	81
	F	69	85	80	134	130

From Mertens (1997a)

*Significantly different from control ($p < 0.01$)

Hb, haemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration.

Table 8. Incidences of microscopic changes at 90 days in dogs fed diets containing indoxacarb

Observation	Dietary concentration (ppm)				
	0	40	80	160	640
No. of dogs per group	4	4	4	4	4
Males					
Liver: pigment increased	0	1	4	4	4
Kidneys: pigment increased	0	1	2	3	4
Spleen: pigment increased	0	4	4	4	4
Haematopoiesis increased	0	0	2	4	4
Bone marrow: pigment increased	0	0	2	4	4
Hyperplasia, erythrocytic [a]	0	0	2/1	0/4	0/4
Females					
Liver: pigment increased	0	1	4	4	4
Kidneys: pigment increased	0	0	1	3	3
Spleen: pigment increased	0	2	4	3	4
Haematopoiesis increased	0	3	1	3	3
Bone marrow: pigment increased	0	3	4	4	4
Hyperplasia, erythrocytic [a]	0	2/2	1/3	0/4	0/4

From Mertens (1997a)

[a] Minimal/mild

Ocular examinations were conducted before the study and during study week 51. At the end of the study, all animals were killed, examined macroscopically, and selected organs were weighed (excluding heart and spleen, including thyroid/parathyroid and epididymes) and tissues taken (except lachrymal gland and sternum) for histopathology. The mean daily intake of indoxacarb in the 40, 80, 640 and 1280 ppm groups was 1.1, 2.3, 17.5 and 33.6 mg/kg bw per day for males and 1.3, 2.4, 18.9 and 36.1 mg/kg bw per day for females, respectively. There were no deaths during the study, and no treatment-related clinical signs. Food consumption and mean body-weight gain were decreased in the 1280 ppm group, especially during the first 3 months of test article administration. Body-weight gain appeared to fluctuate throughout the study in all groups (including controls); by the end of the study, female weights were comparable to controls, but body weights of males at 1280 ppm were approximately 1 kg less than controls, representing a decrease in body-weight gain of about 30%. There were no effects in urine analysis and no ophthalmic or gross lesions indicative of indoxacarb toxicity.

There was a dose-related decrease in indicators of circulating erythrocytic mass (erythrocytes, haemoglobin and EVF) at all time-points in the groups at 640 and 1280 ppm, with increased numbers of Heinz bodies and increased mean reticulocyte counts (Table 9). Other haematological changes secondary to haemolysis in these groups included increased MCV, decreased MCHC, erythrocyte morphologic changes (Howell-Jolly bodies, polychromasia and hypochromasia) and/or increased mean platelet counts, and increased serum bilirubin. 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 chemistry findings.

Table 9. Summary of significant haematological findings at 51 weeks in dogs given diets containing indoxacarb

Parameter	Sex	Historical control range	Dietary concentration (ppm)				
			0	40	80	640	1280
Erythrocytes (10^6/ml)	M	5.41–7.73	7.18	6.21**	6.07**	5.34**	5.29**
	F	5.63–7.89	6.7	6.47	5.66*	5.6*	5.01**
Hb	M	12.6–17.2	16.3	14.6**	14.0**	12.8**	12.4**
(g/dl)	F	13.4–17.4	14.7	14.8	13.2**	13.3**	12.1**
EVF	M	0.365–0.511	0.475	0.430*	0.421**	0.382**	0.377**
	F	0.386–0.518	0.454	0.453	0.392*	0.398*	0.360**
MCV	M	63.0–70.8	66.3	69.3	69.4	71.7**	71.4**
(μm^3)	F	63.1–71.5	67.8	70.1	69.4	71.1	72.1*
MCHC	M	32.6–35.6	34.3	34.0	33.3*	33.5	32.9**
(g/dl)	F	32.1–36.1	34.3	33.8	33.7	33.6	33.5*
Reticulocytes (%)	M	—	0.4	0.3	0.9	0.9	1.5**
	F		0.1	0.6	0.6	1.6**	0.9
Heinz bodies (%)	M	—	0	0	4.4	11.0**	12.4**
	F		0	0.1	7.2	11.9**	14.8**

From Mertens (1997b)

*Significantly different from control, $p < 0.05$, Dunnett test; ** Significantly different from control, $p < 0.01$, Dunnett test

EVF, erythrocyte volume fraction; Hb, haemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin.

Mean absolute and relative liver weights in males at 640 ppm and in males and females at 1280 ppm were increased by up to 52% at scheduled necropsy. Microscopic changes were observed in the liver, spleen, kidneys and/or bone marrow of all treated groups, and consisted of minimal to mild increased haemosiderin in Kupffer cells, kidney tubule epithelium, spleen and bone marrow (Table 10). There was evidence of minimal to mild extramedullary haematopoiesis in the spleen and bone marrow hyperplasia, consistent with a secondary physiological response to indoxacarb-induced haemolysis. Both the haematological and microscopic changes, although mild, showed a clear dose-related trend.

On the basis of the haematological changes indicative of mild haemolysis together with Heinz body formation, the NOAEL was 40 ppm, equal to 1.1 mg/kg bw per day for males and 1.3 mg/kg bw per day for females) (Mertens, 1997b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 70 male and 70 female Cr1:CD(ICR)BR mice were fed diets containing indoxacarb (DPX-JW062; purity, 95.03%) at a concentration of 0, 20, 100 or 200 ppm for 18 months.

The concentration of indoxacarb in the diet at the highest dose was reduced twice, to 150 ppm on day 126 and to 125 ppm on day 287, because of excessive mortality. Animals were observed daily for mortality, and clinical signs, food consumption and body weights were recorded weekly. Haematology (except reticulocyte counts) and total plasma protein were evaluated in 10 mice per group at 3, 6, 12 and 18 months. Urine analysis was not performed.

Table 10. Incidences of microscopic changes at 51 weeks in dogs fed diets containing indoxacarb

Microscopic change	Dietary concentration (ppm)				
	0	40	80	640	1280
No. of dogs per group	5	5	5	5	5
Males					
Liver: pigment increased	0	2	4	5	5
Spleen:					
pigment increased	0	0	0	3	3
haematopoiesis increased	0	0	3	5	5
Kidney: haemoglobin pigment	0	3	5	4	5
Bone marrow:					
pigment increased	0	2	1	5	5
hyperplasia, mixed [a]	0	2/0	3/0	3/2	1/4
Females					
Liver: pigment increased	0	1	5	5	5
Kidney: haemoglobin pigment	0	0	3	5	4
Spleen:					
pigment increased	0	0	1	3	5
haematopoiesis increased	0	1	5	5	5
Bone marrow:					
pigment increased	0	0	4	5	5
hyperplasia, mixed[a]	0	4/0	1/4	2/3	1/4

From Mertens (1997b)

[a] Minimal/mild

Ocular examinations were conducted before the study and before scheduled sacrifice. All mice found dead during the study and those killed at the end of the study were necropsied. Selected organs were weighed and tissues taken for histopathology. The mean daily intake of indoxacarb in the 20, 100 and 200/150/125 ppm groups was 2.63, 13.8 and 22.1 mg/kg bw for males and 3.99, 20.3 and 30.8 mg/kg bw for females, respectively.

Clinical signs suggestive of neurotoxicity, including abnormal gait/mobility and head tilt, were observed in males at 200/150/125 ppm and females at 100 ppm and above. Food consumption was decreased in males and females (by 11% and 20%, respectively) while receiving indoxacarb at 200 ppm, and food efficiency was decreased by 25% in the groups at 100 and 200/150/125 ppm. Mean absolute body weights and weight gain were significantly lower at the two higher doses. In the group at 200/150/125 ppm, males and females showed mean body weight that were decreased by about 18%, 11% and 10% at the end of the periods of treatment at 200, 150 and 125 ppm, respectively. At 100 ppm, mean body weights at the end of the study were 5% and 10% less than controls for males and females, respectively. No ophthalmological abnormalities were found before necropsy. In both males and females, survival was significantly decreased at the end of the exposure regime at 200 and 150, but not 125 ppm. For the groups at 0, 20, 100 and 200/150/125 ppm, overall survival in males was 70%, 80%, 68% and 31%, respectively; overall survival in females was 72%, 73%, 79% and 59%, respectively. In mice dying during the study, the cause of death was attributed to central nervous system disorder or heart inflammation/necrosis (males only), which was of sufficient severity to cause death.

There were no significant treatment-related findings related to haematology or total protein parameters at any dose. At necropsy, no treatment-related changes in organ weights were found, but in 12 out of 70 male mice treated with indoxacarb at 200/150/125 ppm, red fluid was found in the pleural cavity; this finding was associated, in all instances, with necrotic, haemorrhagic, inflammatory heart lesions. In male and female mice (200/150/125 ppm), lymphoid depletion in the spleen was noted. Mild to moderate neuronal degeneration occurred in 2 out of 70 male and 2 out of 70 female mice at 200/150/125 ppm; minimal neuronal degeneration was seen in one female at 100 ppm. The primary sites affected were the piriform cortex and the hippocampus. In two female mice (200/150/125 ppm), chronic brain lesions in the piriform cortex consisted of empty, vacuolated spaces indicating previous necrosis and subsequent phagocytosis/clearing of necrotic material. There were no treatment-related neoplastic changes in any group.

The NOAEL was 20 ppm for males and females, equal to 2.63 mg/kg bw per day for males and 3.99 mg/kg bw per day for females, on the basis of decreased body-weight gain and food efficiency at the higher doses (Frame, 1997a).

Rats

Groups of 72 male and 72 female Cr1:CD(SD)BR rats were fed diets containing indoxacarb (DPX-JW062; purity, 95.03%) at a concentration of 0, 10 (females only) 20, 40, 60, 125 or 250 (males only) ppm for 24 months. The rats were observed daily for mortality and clinical signs. Food consumption and body weights were recorded weekly. Ten rats from each group were selected for haematology and clinical chemistry evaluations (including triglycerides and sorbitol dehydrogenase) at 3, 6 and 12 months, and a further remaining 10 rats per group were evaluated at 18 and 24 months. After the 12-month evaluation, the group of 10 rats was sacrificed and necropsied. Urine analysis (except for appearance and leukocytes) was performed before each blood sampling time. Ophthalmological examinations were conducted before the study and at 12 and 24 months. At 24 months, all surviving rats were necropsied, selected organs were weighed and tissues taken for histopathology. The mean daily intake of indoxacarb in males was 0.798, 1.59, 2.4, 5.03 and 10 mg/kg bw per day in the 20, 40, 60, 125 and 250 ppm groups, respectively. In female rats, the mean daily intake of indoxacarb was 0.554, 1.04, 2.13, 3.6 and 7.83 mg/kg bw per day in the 10, 20, 40, 60 and 125 ppm groups, respectively.

There were no significant differences in survival among treated groups. At 125 ppm a small (seven relative to one in controls) but statistically significant increase in deaths of undetermined cause was observed in females during the first year of the study. In females, a statistically

significant increase in alopecia was present at 125 ppm (35 out of 74) relative to controls (16 out of 72). Significant reductions in 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. The decreases in body-weight gain correlated with reductions in food consumption and, except in males at 125 ppm, with decreases in food efficiency.

At each sampling time, male and female rats had mild haemolysis, consisting of decreased erythrocytes, haemoglobin and EVF (Table 11).

The effect was greatest at the 6-month sampling time, and at the end of the study there was no difference in haematological parameters between treated groups and controls. In males, haemoglobin and erythrocyte values differed from controls by more than 10% only at 3 months and 6 months (at 125 ppm and 250 ppm, respectively). In females, the erythrocyte count, haemoglobin concentration and EVF differed by more than 10% from controls at doses ≥ 60 ppm after 6 and 12 months, and after 125 ppm at 18 months. There was some evidence of a bone marrow regenerative response as indicated by minimal increases in reticulocyte counts and erythrocyte macrocytosis in these groups. In males and females dosed at 125 ppm and above, increased spleen weights, splenic congestion and increased haemosiderin in macrophages of the spleen and liver were noted at the 1-year necropsy (Table 12).

Seven female (125 ppm) rats that died during the first year of the study had bone marrow atrophy, splenic lymphoid depletion and/or thymic necrosis. There were no treatment-related neoplastic changes in any group. At the 2-year necropsy, males had increased pigment in splenic macrophages (not dose-related) and females had increased pigment in liver Kupffer cells (40 ppm). Females at 125 ppm showed significant splenic lymphoid depletion, bone marrow atrophy and thymic necrosis.

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 (Frame, 1997b).

2.4 Genotoxicity

A battery of tests was conducted to determine the genotoxic potential of DPX-MP062 in vitro and in vivo. The results of these studies indicated that indoxacarb (DPX-MP062; 3 : 1 enantiomer ratio) is not genotoxic (Table 13).

Table 11. Summary of significant haematological findings at 12 months (n = 10 per group) in rats fed diets containing indoxacarb

Parameter	Sex	Normal range[a]	Dietary concentration (ppm)						
			0	10	20	40	60	125	250
Erythrocytes (10^6/ml)	M	6.8–8.4	8.04	—	8.32	7.98	7.93	7.60	7.34
	F	6.7–8.2	7.59	7.25	7.23	6.83*	6.71*	6.40*[b]	—
Hb (g/dl)	M	13.6–16.3	15.4	—	15.5	15.4	15.9	14.7	14.5*
	F	13.2–15.9	16.0	15.2*	15.0*	14.8*	14.4*	13.8*	—
EVF	M	0.395–0.470	0.460	—	0.470	0.460	0.480	0.450	0.450
	F	0.373–0.456	0.470	0.460	0.450	0.450	0.430*	0.420*	—

From Frame (1997b)

EVF, erythrocyte volume fraction; F, female; Hb, haemoglobin; M, male.

* Significantly different from control, $p < 0.05$, Dunnett test.

[a] Data from Charles River Breeding Laboratories (1989) for Sprague-Dawley rats aged 12 months

[b] Outside normal range.

Table 12. Incidences of microscopic changes in rats fed diets containing indoxacarb for 1 or 2 years

Microscopic change	Dietary concentration (ppm)					
	0	20	40	60	125	250
Males at 1-year sacrifice (*n* = 10)						
Liver: pigment increased	0	0	0	0	0	1
Spleen:						
congestion	0	1	1	7*	10*	10*
pigment increased	0	2	2	5	5*	5*
haematopoiesis increased	1	1	2	3	8*	5*
Bone marrow: hyperplasia	0	3	2	3	2	6*
Males at 2-year sacrifice (*n* = 62)						
Liver: pigment increased	4	4	2	3	3	11
Spleen:						
congestion	2	1	2	9	3	13*
macrophages—pigment	1	15**	8	15*	25*	27*
lymphoid depletion	2	1	0	0	4	3
Bone marrow: atrophy	0	1	0	3	3	0
Kidney: pigment, tubular	3	7	0	0	3	2
Thymus: necrosis	0	—	—	—	—	0
	Dietary concentration (ppm)					
	0	10	20	40	60	125
Females at 1-year sacrifice (*n* = 10)						
Liver: pigment increased	0	0	0	0	0	7*
Spleen:						
congestion	0	0	0	2	6*	10*
pigment increased	2	4	3	6	6*	8*
haematopoiesis increased	4	2	3	3	4	3
Bone marrow: hyperplasia	0	0	1	0	1	3*
Females at 2-year sacrifice (*n* = 62)						
Liver: pigment increased	2	4	2	11*	12*	23*
Spleen:						
congestion	1	0	0	2	1	0
macrophages—pigment	27	30	26	39	50*	49*
lymphoid depletion	0	0	1	2	0	7*
Bone marrow: atrophy	0	2	0	2	2	9*
Kidney: pigment, tubular	4	3	3	5	8	10*
Thymus: necrosis	0	—	—	—	—	5**

From Frame (1997b)

*Statistically significant (Cochran-Armitage); ** Statistically significant (Fisher's exact test)

Table 13. Results of studies of genotoxicity with indoxacarb

End-point	Test system	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation (Ames)	*S. typhimurium*, TA100, TA1535, TA97a and TA98 and *E. coli* strain WP2 *uvr*A (pKM101), plate incorporation; ± S9.	10–5000 µg/plate in DMSO	94.5	Negative	Mathison (1997)
Chromosomal aberration (clastogenicity)	Human lymphocytes; ± S9	15.7–1000 µg/ml in DMSO (125–1000 µg/ml in the repeat assay)	94.5	Negative	Gudi & Schadley (1996a)
Mammalian cell mutagenicity (HGPRT locus)	CHO cells; ± S9	3.1–250 µg/ml in DMSO	94.5	Negative	San & Clark (1997a)
Unscheduled DNA synthesis	Rat primary hepatocytes	1.56–200 µg/ml in DMSO	94.5	Negative	San & Sly (1997a)
In vivo					
Micronucleus formation	Mouse bone marrow	Males: 0, 3000, 4000 mg/kg bw per day in corn oil Females: 0, 1000, 2000 mg/kg bw per day in corn oil	94.5	Negative	Cox (1997

DMSO, dimethylsulfoxide; S9, 9000 × *g* supernatant of rodent liver

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 26 male and 26 female Cr1:CD VAF/Plus rats were fed diets containing racemic indoxacarb (DPX-JW062; purity, 95.3%) at a concentration of 0, 20, 60 or 100 ppm (0, 1.3, 4.0, or 6.7 mg/kg bw per day), from 70 days before mating until euthanasia (males after mating and females at weaning). The F_1 offspring selected for mating received the test article in the diet beginning at weaning (day 21 of lactation) for a minimum of 77 days before mating and until euthanasia. All animals were observed for mortality and clinical signs twice daily. Food consumption and body weights were recorded at least once weekly. The F_0 and F_1 parental reproductive performance was assessed, and the F_1 and F_2 litters were evaluated for viability and growth. All parental animals and offspring were subjected to gross necropsy at scheduled euthanasia or time of death. Selected organs were weighed and tissues taken for histopathology. Sperm analysis was conducted for all adult males.

There were no definitive, treatment-related clinical signs in either generation. Parental toxicity in the F_0 generation was indicated at dosage levels of 60 ppm (females) and 100 ppm (males and females) by significant reductions in body-weight gain and food consumption of 10% (males) and 30% (females). During gestation, body-weight gain in treated females was similar to controls, although absolute body weights were about 12% lower. There were no observable effects on gonad function, estrous cycling, or mating behaviour in either the F_0 or F_1 animals. The mating and fertility indices for the F_0 animals were not affected by treatment. In the F_1 adults, the fertility and fecundity indices for the group at 100 ppm were slightly reduced compared with

controls, but the result was not statistically significant. There were no compound-related effects on pup survival. The weights of F_1 pups in the groups at 60 and 100 ppm were reduced by about 15% during lactation. Mean pup weights in the F_2 litters were not affected at any dose. An increase in absolute and relative spleen weights of 56%, 13%, 37% and 63% was noted in F_1 females at 100 ppm, F_0 males at 100 ppm, and F_0 females at 60 and 100 ppm, respectively. Microscopic examination was only carried out in one male and three females from the group at 100 ppm; in these animals, mild to moderate splenic extramedullary haematopoiesis and haemosiderin pigmentation was found. There were no other significant macroscopic or microscopic findings.

The NOAEL for parental toxicity was 20 ppm (1.3 mg/kg bw per day) on the basis of lower body weights and food intake at higher doses. The NOAEL for pup development was 20 ppm (1.3 mg/kg bw per day) on the basis of lower body weights of F_1 pups during lactation at the next higher dose of 60 ppm. There were no effects on the reproductive success of parental animals at the highest dose of 100 ppm (Breslin, 1997).

(b) Developmental toxicity

Rats

In a pilot study of developmental toxicity, groups of 8 mated Cr1:CD(SD)BR female rats received indoxacarb (DPX-JW062; purity, 94.76%) at a dose of 0, 1.5, 3, 6, or 12 mg/kg bw per day by gavage in polyethylene glycol (PEG 400) on days 7 to 21 of gestation. Parameters evaluated in dams were body weight, body-weight gain and adjusted body-weight gain, food consumption, survival, clinical signs, reproductive outcomes, and gross pathology. Parameters evaluated in fetuses were body weights, mortality, incidences of resorptions, and incidences of external alterations.

Adjusted maternal body weights were decreased at 3 and 6 mg/kg bw per day, and adjusted body-weight loss occurred at 12 mg/kg bw per day over the course of the dosing period. Fetal body weights were slightly decreased in the group at 12 mg/kg bw per day.

The NOAEL was 1.5 mg/kg bw per day and 6 mg/kg bw per day for maternal and fetal effects, respectively. The maternal NOAEL was based on decreased maternal weight at 3 mg/kg bw per day. The fetal NOAEL was based on decreased fetal weight at 12 mg/kg bw per day (Munley, 1997a).

In a pilot study of developmental toxicity, groups of 8 mated Cr1:CD(SD)BR female rats received indoxacarb (DPX-MP062; purity 94.5%) at a dose of 0, 1, 2, 4, or 8 mg/kg bw per day by gavage in polyethylene glycol (PEG 400) on days 7 to 21 of gestation. Parameters evaluated in dams were body-weight gain and adjusted body-weight gain, food consumption, survival, clinical signs, reproductive outcomes, and gross pathology. Parameters evaluated in fetuses were body weights, mortality, incidences of resorptions, and incidences of external alterations.

Adjusted maternal body weights were decreased at 2 and 4 mg/kg bw per day and adjusted body-weight loss occurred at 8 mg/kg bw per day over the course of the dosing period. Fetal body weights were decreased at 8 mg/kg bw per day.

The NOAEL was 1 mg/kg bw per day and 4 mg/kg bw per day for maternal and fetal effects, respectively. The NOAEL for maternal toxicity was based on decreased adjusted maternal weight changes relative to controls at 2 mg/kg bw per day. The NOAEL for fetal toxicity was based on decreased fetal weight at 8 mg/kg bw per day (Munley, 1997b).

Groups of 25 mated Cr1:CD(SD)BR rats received indoxacarb (DPX-MP062-51; purity, 94.5%) at a daily dose of 0, 0.5, 1, 2 or 4 mg/kg bw by gavage in polyethylene glycol (PEG 400) from days 7 to 21 of gestation. Animals were observed one to two times daily for mortality and clinical signs. Body weight was recorded weekly except for days 7 to 22 of gestation, when it was

recorded daily. Food consumption was recorded every second day. On day 22 of gestation, the rats were killed and necropsied. The uteri were examined, the fetuses removed and the weight, sex, external, visceral, head and skeletal alterations were recorded.

There were no compound-related maternal deaths, and no effect of treatment on the number of resorptions, fetal viability or sex ratio. Maternal body-weight gain and food consumption was reduced by about 50% at 4 mg/kg bw per day. Alopecia was noted in 10/25 rats from each of the groups at 2 and 4 mg/kg bw per day. Fetal mortality was unaffected by treatment; however, at 4 mg/kg bw per day there was a significant decrease in mean fetal weight (4.79 g) compared with controls (5.12 g). There was no treatment-related increase in the incidence of fetal malformations.

The NOAEL for maternal and developmental toxicity was 2 mg/kg bw per day on the basis of lower maternal and fetal body weight at 4 mg/kg bw per day (Munley, 1997c).

Rabbits

Groups of 25 mated New Zealand White rabbits were given indoxacarb technical (DPX-JW062-112, purity, 94.8%) at a daily dose of 0, 250, 500 or 1000 mg/kg bw by gavage in 0.5% methyl cellulose from days 7 to 28 of gestation. Animals were observed one to two times daily for mortality and clinical signs. Body weight was recorded weekly except for days 7 to 22 of gestation, when it was recorded daily. Food consumption was recorded every second day. On day 22 of gestation, the rabbits were killed and necropsied. The uteri were examined, the fetuses removed and the weight, sex, external, visceral, head and skeletal alterations were recorded.

There were no compound-related maternal deaths, and no effect of treatment on the fetal viability or sex ratio. At 1000 mg/kg bw per day, maternal body-weight gain was reduced by about 30%, food consumption was also reduced, and green-coloured stools were noted throughout the dosing period. Mean fetal weight was significantly reduced at 1000 mg/kg bw (36.9 g) compared with controls (40.8 g) and there was a significant increase in retarded sternebral ossification (44/186; 23.7%) compared with controls (35/201; 17.4%). No evidence of maternal or developmental toxicity was observed at 250 or 500 mg/kg bw per day.

The NOAEL for maternal and developmental toxicity in rabbits was 500 mg/kg bw per day on the basis of lower maternal and fetal body weight and retarded fetal ossification at the higher dose (Munley, 1995).

2.6 Special studies

(a) Reversibility of haemolytic and body-weight changes

Groups of 10 male and 10 female Cr:CD BR rats were fed diets containing racemic indoxacarb (DPX-JW062; purity, 95.03%) at a concentration of 0, 15 (females only) 30, 60, 125, or 250 (males only) ppm for 98 days. This was followed by a 28-day recovery period to monitor the reversibility of the haematological effects. The animals were observed daily for clinical signs, and food consumption and body weight were recorded weekly. Ophthalmoscopic examinations were not undertaken because no treatment-related effects were observed in other 90-day studies in rats. Haematology evaluations were performed on days 45 and 90 and on days 21 and 22 (males and females, respectively) of the recovery period. No clinical chemistry or organ weight data were collected as these were considered in other short-term studies. Similarly, no histopathology analysis was performed. The mean daily intake of indoxacarb by male rats in the groups at 30, 60, 125 and 250 ppm was 1.8, 3.7, 7.5, and 15 mg/kg bw, respectively, and by female rats at 15, 30, 60, and 125 ppm was 1.2, 2.5, 4.9, and 12 mg/kg bw, respectively

There were no treatment-related deaths or clinical signs in any group. Reductions in body-weight gain appeared to be dose-related, but only achieved statistical significance in males at 125 and 250 ppm and in females at 60 and 125 ppm during days 0–98. The mean body-weight gain in males at 125 and 250 ppm was 13% and 18% lower than controls, respectively. In females at 60 and 125 ppm, the body-weight gain was 21% and 32% lower than controls, respectively. During

the recovery period (days 98–126), there were dose-related increases in body-weight gain, with males showing a 18%, 18%, 33%, and 46% increase at 30, 60, 125, and 250 ppm respectively relative to controls, but only the increases at 125 and 250 ppm achieved statistical significance. In females the effect was more pronounced with the body-weight gain increasing by 27%, 49%, 56%, and 64% at 15, 30, 60 and 125 ppm respectively. However, similar to males, statistical significance was only achieved at the two higher doses, namely 60 and 125 ppm. Paradoxically the weekly food consumption for males was significantly lower on several occasions during treatment and achieved significance at 125 and 250 ppm over the test period. In contrast, females showed sporadic weekly reductions in food intake so that after 98 days of treatment the overall reduction was not statistically significant at any dose. Food efficiency in males was not significantly different during treatment, but it did increase sufficiently to achieve significance at 125 and 250 ppm during recovery. In females the food efficiency at 125 ppm was significantly lower than concurrent controls during days 0–98, but it was significantly higher at 30, 60 and 125 ppm during days 98–126.

Males and females at the 45-day and 90-day sampling times showed a reduction in erythrocyte count, haemoglobin and/or EVF, with increases in MCV and MCH, indicative of mild haemolysis (Table 14). The severity of the haemolysis appeared to increase with duration of exposure as the values on day 45 were less than on day 90. Although the effects were mild, statistically significant erythrocytic changes occurred at doses ≥ 60 ppm in males and ≥ 30 ppm in females.

However, by day 21/22 of recovery, all haematological values were similar to those in the concurrent controls. The NOAEL was 60 ppm for males and 30 ppm in females (equal to 3.7 mg/kg bw per day for males and 2.5 mg/kg bw per day for females) on the basis of reductions in body-weight gain and evidence of mild haemolysis at the higher doses (Sarver, 1998).

(b) Distribution of ^{14}C-DPX-JW062 in erythrocytes of rats

In a study of the distribution of DPX-JW962 in erythrocytes, five fasted male Crl:CD (SD)IGS BR rats received [trifluoromethoxyphenyl(U)-^{14}C]DPX-JW062 as a single oral gavage dose at 130 mg/kg bw. Owing to a limited supply of the radiolabel, a sixth rat received a dose of only 111 mg/kg bw. The rats were sacrificed at approximately 72 h after dosing and whole blood was collected. Radioactivity associated with the erythrocytes was measured by liquid scintillation counting (LSC). Metabolic profiling was performed using solvent extraction and pepsin digestion and analysed by LSC and HPLC.

After dosing, a significant portion of the radioactivity was associated with erythrocytes with an average of 29.6 pg/g [trifluoromethoxy-phenyl(U)-^{14}C]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 stromal fractions. The major identified radioactive species in erythrocytes was the trifluoromethoxyaniline, [^{14}C]IN-P0036. During characterization of erythrocyte-associated radioactivity, recovery of total radioactivity was 53.3%. The loss of radioactivity was believed to be attributable to the presence of a volatile component, possibly some [^{14}C]IN-P0036. Based on the results of this study, IN-P0036 was the single radioactive species associated with erythrocytes of rats 72 h after gavage dosing with radiolabeled DPX-JW062 (Anderson, 1999).

(c) Haemolytic potential of N*-hydroxy-4-(trifluoromethoxy)aniline (INMT713) in rats, dogs, and humans in vitro*

The comparative haemolytic potential of *N*-hydroxyarylamine (IN-MT713), the putative ultimate haemolytic metabolite of indoxacarb, was assessed in vitro in erythrocytes derived from rats, dogs and humans. The haemolytic potential was assessed by measuring the oxidation of glutathione in erythrocytes by HPLC after derivatization with the fluorophore *o*-phthalaldehyde. Dapsone-*N*-hydroxylamine (DDS-NOH) served as a positive control. The samples of erythrocytes

Table 14. Summary of significant findings at 90 days in rats fed diets containing indoxacarb

Parameter	Sex	Dietary concentration (ppm)					
		0	15	30	60	125	250
Erythrocytes (10^6/ml)	M	8.56	—	8.27	8.09	7.89*	7.09*
	F	8.13	7.90	7.49*	7.29*	6.95*	—
Hb (g/dl)	M	16.8	—	15.8*	15.6*	15.7*	14.7*
	F	16.5	15.9	15.3*	14.9*	15.2*	—
EVF	M	0.50	—	0.48	0.47*	0.47*	0.44*
	F	0.51	0.49	0.47*	0.47*	0.46*	—
MCV	M	59	—	58	59	59	62*
(μm^3)	F	62	62	63	64	66*	—
MCH	M	20	—	19	20	20	21*
(pg)	F	20	20	21	21	22*	—

From Sarver (1998)

*Significantly different from control ($p < 0.05$)

EVF, erythrocyte volume fraction; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

were obtained from four adult male Sprague-Dawley rats, beagle dogs and human volunteers respectively. The erythrocyte suspensions from each individual were incubated with IN-MT713 (5–250 µmol/l), DDS-NOH (150 µmol/l) or vehicle (acetonitrile) at 37 °C for 2 h. Total oxidized glutathione (GSSX = GSSG + mixed disulfides) was analysed as glutathione (GSH) equivalentsafter reduction with dithiothreitol. Concentration-versus-time curves for GSSX were normalized to T_0 values, and areas under the curve (AUC) were calculated as an index of oxidative stress. Incubation of erythrocytes with IN-MT713 resulted in dose-dependent oxidation of GSH in all three species, and decreases in GSH generally mirrored increases in GSSX. The rank order of sensitivity of erythrocytes to GSH oxidation after in vitro exposure to IN-MT713 was rat >> dog > human. The magnitude of the differences in sensitivity between species increased with increasing concentration of IN-MT713 in vitro, with human erythrocytes being up to two- to threefold less sensitive than dog erythrocytes and four- to fivefold less sensitive than rat erythrocytes. The kinetics of the erythrocyte response to the positive control, DDS-OH, was similar in all species; however, the oxidative effect on human erythrocytes, as measured by AUC for GSSX, was approximately 3.5-fold less than that seen in the rat and dog. These results agree with previous studies that showed human erythrocytes to be two- to threefold less sensitive to the oxidant effects of DDS-NOH than were rat erythrocytes at comparable concentrations. In rats, the oxidative responses to IN-MT713 and DDS-NOH were quite similar; however, in dogs and humans, the oxidative response to IN-MT713 at a concentration of 125 mmol/l was about threefold and twofold less, respectively, relative to DDS-NOH at 150 mmol/l. Difference between species in the oxidative response to IN-MT713 exposure in vitro indicate that humans are likely to be less sensitive than either rats or dogs to the haemolytic action of this compound in vivo (Kemper, 2004).

(d) Haemolytic potential of IN-MT713 in vitro in erythrocytes from normal and glucose-6-phosphate dehydrogenase-deficient humans

The haemolytic potential of IN-MT713 (*N*-hydroxy-4-trifluoromethoxyaniline), the putative haemolysin of indoxacarb, was evaluated in glucose-6-phosphate dehydrogenase (G6PDH)-normal and G6PDH-deficient humans using an assay for glutathione (GSH) oxidation in vitro. DDS-NOH was used as a positive control. Blood samples were obtained from 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. Erythrocyte suspensions from each individual were incubated with IN-MT713 (5–250 μmol/l), DDS-NOH (150 μmol/l) or vehicle (acetonitrile). Areas under the time-versus-concentration curves (AUCs) for GSSX were calculated and used as an index of oxidative stress. EC_{50} values, defined as the concentration of IN-MT713 producing a 50% maximal response, were determined from the dose–response curves and compared with the relative sensitivity of the test populations to haemolytic effects of IN-MT713.

IN-MT713 produced a dose-dependent decrease in the concentration of GSH in G6PDH-normal individuals and in both G6PDH-deficient phenotypes. The decrease in GSH concentration after exposure to IN-MT713 was accompanied by a dose-dependent increase in total oxidized glutathione (GSSX). The EC_{50} values determined for Med- and A-phenotypes were quite similar (55.5 ± 21.1 μmol/l and 57.7 ± 7.9 μmol/l, respectively). These values, although not statistically significant, were approximately 33% lower than the EC_{50} for G6PDH-normal samples (75.5 ± 25.5 μmol/l). No significant changes in supernatant haemoglobin concentration were observed after exposure to IN-MT713 or DDS-NOH, indicating that no haemolysis in situ occurred at any dose in all G6PDH phenotypes. Overall, the results of the study suggest that G6PDH-deficient individuals are more sensitive to the oxidative effects of IN-MT713 than are G6PDH-normal individuals (Kemper, 2002).

(e) Neurotoxicity

Rats

In a study of acute neurotoxicity, groups of 12 male and 12 female Cr1:CD(SD)BR rats were given indoxacarb (DPX-MP062; purity, 94.5%) as a single dose by gavage in polyethylene glycol. The doses administered were 0, 12.5, 50 or 100 mg/kg bw in females and 0, 25, 100 or 200 mg/kg bw in males. The animals were observed daily for clinical signs, body weights were recorded on days 2, 8 and 15, and food consumption was measured during days 1–2 and 8–15. A battery of neurobehavioural tests was conducted on all rats before administration and 2–4 h after compound administration on days 1, 8 and 15. On day 16, six rats of each sex per group were anaesthetized and perfused in situ with fixative. Tissue samples from the nervous system (brain, spinal cord, sciatic and tibial nerves, gasserian ganglia, cervical and lumbar dorsal root fibres and ganglia, cervical and lumbar ventral root fibres), and gastrocnemius muscle were taken for histopathological examination.

Compound-related effects in female rats included alopecia, reduced body-weight gain, reduced food consumption (≥ 50 mg/kg bw) pallor and slightly reduced motor activity (100 mg/kg bw). Toxicity in male rats included reduced body-weight gain and food consumption, reduced forelimb grip strength and decreased foot splay (200 mg/kg bw). There was no histopathological evidence of neurotoxicity in males at 200 mg/kg bw or in females at 100 mg/kg bw.

The NOAEL in males was 100 mg/kg bw for both systemic and neurotoxicity. In females, the NOAELs were 12.5 mg/kg bw for systemic toxicity and 50 mg/kg bw for neurotoxicity (Christoph, 2001).

In a 90-day study of neurotoxicity, groups of 12 male and 12 female Cr1:CD(SD)BR rats were given diets containing DPX-MP062 (purity, 94.5%) at a concentration of 0, 10, 100 or 200 ppm for males and 0, 10, 50 or 100 ppm for females, for 90 days. The animals were observed daily for clinical signs, and body weights and food consumption were measured weekly. A neurobehavioural test battery, consisting of motor activity and functional observational battery (FOB) assessments, were conducted on all rats before the start of the study and at weeks 4, 8 and 13. At completion of the study, six rats of each sex per group were anaesthetized and perfused in situ with fixative. Tissue samples from the nervous system (brain, spinal cord, sciatic and tibial nerves, gasserian ganglia, cervical and lumbar dorsal root fibres and ganglia, cervical and lumbar ventral root fibres), and gastrocnemius muscle were taken for histopathological examination; only

tissues from the control group and the groups receiving the highest dose (200 and 100 ppm for male and female rats, respectively) were evaluated. The mean daily intakes of indoxacarb for 10, 100 and 200 ppm males were 0.57, 5.6 and 11.9 mg/kg bw per day, respectively, and for 10, 50 and 100 ppm females were 0.68, 3.3 and 6.1 mg/kg bw per day, respectively.

Three of 12 females at 100 ppm died between days 9 and 11; the cause of death was not established but was assumed to be substance-related. There were no dose-related clinical signs in surviving rats from any group, although alopecia was noted in some females at 50 and 100 ppm. Males at 100 and 200 ppm and females at 50 and 100 ppm showed reduced body-weight gain (periodically showing as body-weight loss); the overall weight gain over the study period was lower than controls by 18%, 28%, 23% and 35%, respectively, and was associated with reduced food consumption and food efficiency. There were no treatment-related effects on forelimb or hindlimb grip strength, foot splay or on any of 34 other parameters evaluated by the FOB. There was no evidence of neurotoxicity during evaluation of motor activity or morphologic neuropathology. There was no histopathological evidence of neurotoxicity in males at 200 ppm or in females at 100 ppm.

The NOAELs for neurotoxicity were 200 ppm (11.9 mg/kg bw per day) for males and 100 ppm for females, (6.1 mg/kg bw per day; the highest dietary concentrations tested). The NOAELs for systemic toxicity were 10 ppm for male and female rats, equal to 0.57 and 0.68 mg/kg bw per day, respectively. These NOAELs were based on a reduction in body-weight gain, food consumption, and food efficiency relative to controls (Malley, 1997).

(f) Studies on the insecticidally-inactive enantiomer

DPX-KN127 is the insecticidally-inactive enantiomer of indoxacarb (DPX-JW062 and DPX-MP062). Two studies of toxicity have been performed with DPX-KN127. These include a reverse mutation (Ames) assay (study abstract only provided) and a study of acute oral toxicity.

(i) Acute toxicity

Groups of five male and five female fasted Crl:CD BR rats were given DPX-KN127 (purity, 99.8%) at a dose of 255, 500, 1820, 3077, or 4000 mg/kg bw by gavage in corn oil. Owing to the viscosity of the resulting corn oil suspension, each dose was administered twice as a half dose (10 ml/kg each) 4 h apart. The animals were observed daily for mortality and clinical signs for up to 14 days after dosing.

All rats in the groups at 1820, 3077 and 4000 mg/kg bw died; deaths occurred from 2 to 11 days after dosing. Dose-related clinical signs of toxicity occurred in all groups and included hypoactivity, ataxia, and impaired righting reflex. Although food consumption was not measured there were reductions in body weight (0.3–12%) among survivors during the first week. Gross pathological examinations on those that died revealed dark red contents inside distended stomach and intestines. The oral LD_{50} of DPX-KN127 was 444 mg/kg bw and 480 mg/kg bw for males and females respectively (Kern, 1997a).

(ii) Genotoxicity

An abstract report of an Ames test indicated that at doses of up to 5000 µg per plate DPX-KN127 is not genotoxic (Anon., date not given).

(g) Studies on insecticidally-active enantiomer

DPX-KN128 (indoxacarb) is the insecticidally-active enantiomer of indoxacarb (DPX-JW062 and DPX-MP062). A number of studies of toxicity have been performed with DPX-KN128. These include testing for genotoxicity, studies of acute oral and dermal toxicity, a 90-day feeding study and a study of developmental toxicity.

(i) Acute toxicity

The acute LD_{50} values for DPX-KN128 are given in Table 15.

In the study of acute oral toxicity, clinical signs of toxicity most often observed in male and female rats included discharge, staining and matting of fur, and/or hair loss in the urogenital area. Other clinical signs of toxicity were abnormal excretion, hypoactivity and signs suggestive of neurotoxicity, including ataxia, impaired righting reflex and tremors. Studies of acute toxicity after inhalation were not performed because the physical properties of DPX-KN128 prevented the generation of a respirable dust.

(ii) Ocular and dermal irritation and dermal sensitization

DPX-KN128 produced no evidence of skin irritation in rabbits (Finlay, 2003b) and was not irritating to the eyes of rabbits (Finlay, 2003c). DPX-KN128 is a skin sensitizer based on the results of the Magnusson-Kligman maximization test in guinea-pigs (Moore, 2003).

(iii) Short-term studies of toxicity

Groups of 10 male and 10 female Cr:CD BR rats were fed diets containing the indoxacarb *S*-enantiomer (DPX-KN128; purity, 99.7%) at a concentration of 0, 3 (females only), 8, 20, 50, 100 or 200 (males only) ppm for 90 days. The animals were observed daily for clinical signs, and food consumption and body weight were recorded weekly. All rats received an ophthalmoscopic examination at the start and end of the study. Haematology, clinical chemistry and urine analysis evaluations were performed on days 45 and 92. All animals found dead, or killed at the end of the study, were examined for gross pathological effects and selected tissues were examined microscopically. The mean daily intake of indoxacarb by male rats in the 8, 20, 50, 100 and 200 ppm groups was 0.56, 1.4, 3.2, 6.6 and 14 mg/kg bw, respectively, and by female rats in the 3, 8, 20, 50 and 100 ppm groups was 0.25, 0.68, 1.7, 4.1 and 8.5 mg/kg bw, respectively.

One male rat at 200 ppm was sacrificed in extremis and necropsied. There were no other treatment-related deaths, clinical signs or ophthalmological abnormalities in any group. Male rats at 200 ppm and females at 50 and 100 ppm showed significantly reduced body-weight gain with decreased food consumption and/or food efficiency throughout the study. Mean overall body-weight gain in males at 200 ppm was 45% lower than controls, and in females at 50 and 100 ppm were 32% and 41% lower than controls, respectively. Males at 90 days and females at both the 45-day and 90-day sampling times, showed a reduction in erythrocyte count, haemoglobin and/or EVF, with increases in MCV and reticulocyte count, indicative of mild haemolysis (Table 16). Although the effects were mild, statistically significant erythrocytic changes occurred across all doses tested. There were no significant clinical chemistry, urine analysis or organ weight findings.

Histopathology revealed increased haemosiderin in macrophages of the liver at 200 ppm (males) and 100 ppm (females), and increased haemosiderin in the spleen of male rats at 50 ppm and above and female rats at 20 ppm and above, with increased erythrocytic hyperplasia in the spleen of males and females at 50 ppm and above (Table 17). An increased incidence of bone-marrow hyperplasia was observed in females at 50 ppm. Haematological effects observed at 50 ppm and above were considered to be adverse.

Table 15. Acute toxicity of DPX-KN128

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Reference
Rat	Crl:CD (SD)BR	Male	Oral	843	Kern (1997b)
		Female		179	
		Male	Dermal	> 5000	Finlay (2003a)
		Female		> 5000	

Table 16. Summary of significant findings at 90 days in rats given diets containing indoxacarb S-enantiomer (DPX-KN128)

Parameter	Sex	Dietary concentration (ppm)						
		0	3	8	20	50	100	200
Erythrocytes (10^6/ml)	M	8.13	—	8.22	7.80	7.93	7.49*	7.33*
	F	8.24	7.74*	7.75*	7.57*	7.09*	6.84*	—
Hb (g/dl)	M	16.3	—	15.9*	15.5	15.4*	15.2*	14.8*
	F	16.8	16.2	16.0*	15.6*	15.0*	15.1*	—
EVF	M	0.49	—	0.48	0.47	0.46	0.45	0.46
	F	0.52	0.49*	0.49*	0.48*	0.47*	0.46*	—
Reticulocytes (10^6/µl)	M	106	—	91	104	155	168	188*
	F	96	66	62	83	131	96	—
MCV (µm^3)	M	60	—	58	61	58	60	62*
	F	63	63	63	63	66*	67*	—
MCH (pg)	M	20	—	19	20	19	20	20
	F	20	21	21	21	21	22*	—

From Malek (1997c)

*Significantly different from control ($p < 0.05$)

EVF, erythrocyte volume fraction; F, female; Hb, haemoglobin; M, male; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

The NOAEL was 50 ppm for males and 20 ppm for females (equal to 3.2 mg/kg bw per day for males and 1.7 mg/kg bw per day for females) on the basis of reduced body-weight gain in females and haematological and microscopic evidence of mild haemolysis at the higher doses in both sexes (Malek, 1997c).

(iv) Genotoxicity

A battery of tests was conducted in vitro and in vivo to determine the genotoxic potential of DPX-KN128 (Table 18). The results of these studies indicate that DPX-KN128 is not genotoxic.

(v) Developmental toxicity

Groups of 22 pregnant Crl:CD(SD)IGS BR rats were given indoxacarb (DPX-KN128; purity, 95.5%) by gavage in polyethylene glycol 400 on days 6 to 20 of gestation. The doses administered to each group were 0, 0.5, 1, 2, or 3.5 mg/kg bw per day respectively. Animals were observed one to two times daily for mortality and clinical signs. Body weight was recorded weekly except for days 7 to 21 of gestation, when it was recorded daily. Food consumption was recorded every second day. On day 21 of gestation, the rats were killed and necropsied. The uteri were examined, the fetuses removed and the weight, sex, external, visceral, head and skeletal alterations were recorded.

Maternal toxicity was evident as 10% and 28% reductions in overall weight gain and corrected weight gain (excluding uterine contents), respectively, at 3.5 mg/kg bw per day. Developmental toxicity was limited to a statistically significant reduction (3.5% less than control) in mean fetal weight at 3.5 mg/kg bw per day, a finding that was considered to be test substance-related and secondary to maternal toxicity.

The NOAEL for both maternal and developmental toxicity on the basis of a reduced body weight in pups and dams was 2 mg/kg bw per day. DPX-KN128 was not teratogenic to the developing fetus (Mylchreest, 2004).

Table 17. Incidences of microscopic changes at 90 days in rats given diets containing indoxacarb* S-*enantiomer (DPX-KN128)

Microscopic change	Dietary concentration (ppm)						
	0	3	8	20	50	100	200
No. of rats per group	0	10	10	10	10	10	10
Males							
Liver: pigment increased	0	—	0	0	0	1	8
Spleen:							
pigment increased	0	—	1	2	6	10	10
haematopoiesis increased	0	—	0	1	8	9	8
Bone marrow: hyperplasia, mixed	3	—	5	3	4	6	5
Females							
Liver: pigment increased	0	0	0	0	1	6	—
Spleen:							
pigment increased	0	1	2	10	10	10	—
haematopoiesis increased	0	0	2	2	5	8	—
Bone marrow: hyperplasia, mixed	0	1	1	1	5	7	—

From Malek (1997c)

Table 18. Results of studies of genotoxicity with DPX-KN128

End-point	Test system	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation (Ames)	*S. typhimurium*, TA100, TA1535, TA97a & TA98 and *E. coli* strain WP2 *uvr*A (pKM101), plate incorporation; ± S9	2.5–5000 µg/plate in DMSO	95.5	Negative	Wagner & Klug (2004)
Chromosomal aberration (clastogenicity)	Human lymphocytes; ± S9	13.3–100 µg/ml in DMSO	95.5	Negative	Gudi & Rao (2004)
Mammalian cell mutagenicity (HGPRT locus)	CHO cells; ± S9	15–200 µg/ml in DMSO	95.5	Negative	San & Clark (2003)
In vivo					
Micronucleus formation	Mouse bone marrow	0, 500, 1000, and 2000 mg/kg bw per day in corn oil	95.5	Negative	Donner (2003)

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant of rodent liver

(h) Studies with metabolites

(i) IN-JT333

IN-JT333 (methyl 7-chloro-2,5-dihydro-2-[[[4-(trifluoromethoxy)phenyl]amino] carbonyl] = indeno[1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylate) is a metabolite formed in rats dosed with indoxacarb and is also present as an impurity in technical indoxacarb at concentrations of up to 1000 ppm (0.1%).

Acute toxicity

Groups of five male and five female fasted Crl:CD BR rats were given IN-JT333 (purity, 98.7%) at a dose of 10, 30, 50, 100, or 200 mg/kg bw by gavage in corn oil. The animals were observed daily for mortality and clinical signs for up to 14 days after dosing.

All rats in the groups at 100 and 200 mg/kg bw, and all females from the group at 50 mg/kg bw died; deaths occurred up to 12 days after dosing. Dose-related clinical signs of toxicity occurred in all groups and included ataxia, piloerection, hunched posture, splayed rear legs, spasms, tremors and ruffled fur. The gross pathological examinations revealed no specific organ toxicity. The oral LD_{50} of IN-JT333 was 52 mg/kg bw and 39 mg/kg bw for males and females respectively (Sarver, 1996b).

Short-term studies of toxicity

Groups of five male and five female Cr1:CD(SD)BR rats were fed diets containing IN-JT333 (purity, > 95%) at a concentration of 0, 2, 10, 40 or 100 ppm for 14 days. Groups of four rats of each sex were added to each level for potential genotoxicity (bone-marrow micronucleus formation) assessments; these rats were killed on day 8. The animals were observed daily for clinical signs, twice weekly for body weight, weekly for food consumption, and haematology and clinical chemistry parameters were measured at necropsy. All animals found dead, or killed at the end of the study, were examined for gross pathological effects. The mean daily intakes of IN-JT333 for the 2, 10, 40 and 100 ppm dose groups were approximately 0.19, 0.88, 3.0 and 5.6 mg/kg bw per day, and 0.18, 0.87, 2.9 and 4.5 mg/kg bw per day for male and female rats, respectively.

All females treated with IN-JT333 at 100 ppm died or were killed in extremis on days 7–8. Rats from all treatment groups showed reduced body-weight gain and food consumption, although values were statistically significant only at 40 and 100 ppm, in the first and/or second week of the study. Most animals treated above 2 ppm showed piloerection, generally after the first week of the study. Both male and female rats dosed at 100 ppm exhibited lethargy, hunched posture and hindlimb extension. No compound-related effects were observed in any animal submitted for gross pathological evaluation. Samples taken for histopathology or evidence of genotoxicity were not evaluated.

The NOAEL was 10 ppm for males and females (0.88 and 0.87 mg/kg bw per day, respectively) on the basis of body weight and nutritional effects at 40 ppm (approximately 3 mg/kg bw per day) and above (Malek, 1992).

Genotoxicity

A battery of tests was conducted in vitro to determine the genotoxic potential of IN-JT333 (Table 19). The results of these studies indicate that IN-JT333 is not genotoxic.

(ii) IN-KG433

IN-KG433 (methyl 5-chloro-2,3-dihydro-2-hydroxy-1-[[[(methoxycarbonyl) [4-(trifluoro-methoxy) phenyl]amino]carbonyl]hydrazono]-1*H*-indene-2-carboxylate) is a metabolite formed in rats after dosing with indoxacarb and is also present as an impurity in technical indoxacarb at concentrations of up to 10 000 ppm (1%).

Acute toxicity

Groups of five male and five female fasted Crl:CD BR rats were given IN-KG433 (purity, 98.0%) at a dose of 250, 500 or 2000 mg/kg bw in females and 5000 mg/kg bw in males by gavage in corn oil . The animals were observed daily for mortality and clinical signs for up to 14 days after dosing.

None of the males at 5000 mg/kg bw died; however, the incidence of deaths among females at 250, 500 and 2000 mg/kg bw was four out of five, two out of five and five out of five,

respectively. All deaths occurred during the first week after dosing. Clinical signs of toxicity occurred in all groups (including males) and included ataxia, hypoactivity, fur staining/matting, and abnormal excretion (decreased defaecation and urination, soft stool). Clinical signs in males

Table 19. Results of studies of genotoxicity with IN-JT333 in vitro

End-point	Test system	Concentration	Purity (%)	Result	Reference
Reverse mutation (Ames)	*S. typhimurium*, TA100, TA1535, TA97a & TA98 and *E. coli* strain WP2 *uvr*A (pKM101), plate incorporation; ± S9.	10–5000 µg/plate in DMSO	98.7	Negative	Mathison (1996)
Chromosomal aberration (clastogenicity)	Human lymphocytes; ± S9	62.5–2000 µg/ml –S9 in DMSO 15.7–1000 µg/ml in DMSO +S9	98.7	Negative	Gudi & Schadley (1996b)
Mammalian cell mutagenicity (CHO/HGPRT)	CHO cells; ± S9	3.9–125 µg/ml in DMSO	98.7	Negative	San & Clark (1997b)

DMSO, dimethylsulfoxide; S9, 9000 × g supernatant from rodent liver.

Table 20. Results of studies of genotoxicity with IN-KG433 in vitro

End-point	Test system	Concentration	Purity (%)	Result	Reference
Reverse mutation (Ames)	*S. typhimurium*, TA100, TA1535, TA97a and TA98 and *E. coli* strain WP2 *uvr*A (pKM101), plate incorporation; ±S9.	33–5000 µg/plate in DMSO	98	Negative	Wagner & Reese (1997)
Unscheduled DNA synthesis	Rat hepatocytes; ±S9	7.8–1000 µg/ml in DMSO	98	Negative	San & Sly (1997b)
Mammalian cell mutagenicity (HGPRT locus)	CHO cells; ± S9	25–2000 µg/ml in DMSO	98	Negative	San & Clark (1997c)

DMSO, dimethylsulfoxide ; S9, 9000 × g supernatant from rodent liver

were limited to the first week, but in females they persisted throughout the second week. The most common necropsy findings were distended stomachs and dark red or clear gastrointestinal contents. The oral LD_{50} of IN-KG433 was > 5000 mg/kg bw and 174 mg/kg bw for males and females respectively (Kern, 1997c).

Genotoxicity

A battery of tests was conducted in vitro to determine the genotoxic potential of IN-KG433 (Table 20). The results of these studies indicate that IN-KG433 is not genotoxic.

Comments

Biochemical aspects

The kinetics and metabolism of racemic or enantiomer-enriched indoxacarb appeared to be very similar in rats. Indoxacarb administered by gavage at low doses (5 mg/kg bw) is extensively,

albeit slowly, absorbed (69–81%), but at higher doses (150 mg/kg bw) saturation kinetics become evident (8–14% absorption). There was a considerable difference in the time required to achieve the maximal concentration in blood between the sexes. In males it was 5 h at a low dose and 3 h at a high dose, while in females it was 8 h and 27 h respectively. In-vitro evidence from rat hepatic microsome preparations showed that while females metabolized indoxacarb more slowly than males, they produced almost tenfold more of the toxic metabolite IN-JT333. This metabolite, which contains the chiral centre, showed evidence of stereospecific uptake into fat. Elimination (probably caused by the preferential accumulation of metabolites in fat and erythrocytes) was slow, with the half-life in plasma ranging between 92 h and 114 h in males and females respectively.

In rats, indoxacarb is biotransformed to yield the arylamine metabolite 4-trifluoromethoxyaniline. This metabolite, which does not contain a chiral centre, was present in the urine and erythrocytes. The *N*-hydroxy derivative of 4-trifluoromethoxyaniline, while not being detected in excreta or erythrocytes, has been implicated as the causative agent responsible for haemolytic effects observed in all repeat-dose studies because of its ability to effectively oxidize glutathione in erythrocytes in vitro. The haemolytic potential of the arylamine metabolite (4-trifluoromethoxyaniline) observed in the erythrocytes of treated rats was not tested.

The major metabolites in the faeces were formed by hydrolysis of the carboxymethyl group from the amino nitrogen of the trifluoromethoxyphenyl portion of the parent compound, and hydroxylation of the indanone ring. No parent compound was detected in bile and no single metabolite accounted for more than 4% of an administered dose. An oxadiazine ring-opened metabolite formed by hepatic microsomal enzymes is likely to be a precursor for several metabolites found in urine. The eight minor urinary metabolites in rats accounted in total for less than 5% of the administered dose.

Toxicological data

Indoxacarb (DPX-MP062) has low acute oral toxicity (LD_{50} = 1730 mg/kg bw) in male rats and moderate oral toxicity (LD_{50} = 268 mg/kg bw) in female rats, and low dermal (LD_{50} > 5000 mg/kg bw) and inhalation toxicity (DPX-JW062; LC_{50} = 4200 mg/m^3 (4.2 mg/l) in rats. The difference in oral toxicity between the sexes is thought to arise from the more efficient biotransformation of indoxacarb to an acutely toxic metabolite IN-JT333 in females (LD_{50} = 52 mg/kg bw and 39 mg/kg bw in males and females respectively). Purified indoxacarb (DPX-KN128) and its insecticidally-inactive enantiomer (DPX-KN127) are almost equally toxic by the oral route. Although DPX-KN128, like DPX-MP062, showed a difference in oral toxicity between the sexes (i.e. LD_{50} = 843 mg/kg bw and 179 mg/kg bw in males and females respectively), the absence of a sex difference for DPX-KN127 (LD_{50} = 444 mg/kg bw and 480 mg/kg bw in males and females respectively) may be attributable to the dose selection.

Indoxacarb (DPX-MP062) was a moderate eye irritant in rabbits, was not a skin irritant in rabbits, but was a skin sensitizing agent in the maximization test in guinea-pigs.

Although indoxacarb has been shown to block neuronal sodium channels in insects, clear evidence of neurotoxicity in mammals occurred only at high acute doses (200 mg/kg bw) at which ataxia, reduced motor activity, forelimb grip strength and decreased foot splay were observed in male rats. Clinical signs suggestive of neurotoxicity were noted in short-term repeat-dose dietary studies in mice and included abnormal gait/mobility and head tilt at high doses (30 mg/kg bw per day and greater). Long-term exposure to indoxacarb at doses of 22 mg/kg bw or greater in mice caused neuronal degeneration in the piriform cortex and hippocampus. Higher doses resulted in death. In contrast, a repeat-dose study of neurotoxicity in rats showed no effects on motor activity or functional observational battery assessments, and no histological evidence of neurotoxicity at doses of up to 12 mg/kg bw per day in males and 6 mg/kg bw per day in females.

In studies in mice, rats and dogs, the two main toxicological findings after repeated dosing with indoxacarb were mild haemolysis and reduced body-weight gain. Both effects occurred at similar doses in short-term repeat-dose studies, irrespective of the ratio of enantiomers. The reduction in body-weight gain was usually associated with a concomitant decrease in food

consumption and food efficiency. In long-term studies in dogs and rats, the effect levels were similar (NOAELs were approximately 1–2 mg/kg bw per day respectively, and LOAELs were approximately 3–4 mg/kg bw per day). In a long-term study, mice were found to be insensitive to haematological effects and slightly less sensitive to reductions in body-weight gain (the NOAEL was 2.6 mg/kg bw per day, and the LOAEL was 13.8 mg/kg bw per day). The mild haemolysis observed in rats and dogs was characterized by reduced erythrocyte count, EVF, haemoglobin concentration, and a secondary physiological response involving increased haematopoiesis 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. In rats, early mortalities in groups receiving the highest dose and necropsy at 2 years revealed haemosiderin pigment in renal tubule cells and/or lumens, suggesting that haemolysis may have been a factor; these animals showed atrophy of the spleen, thymus and/or bone marrow, which was attributable to loss of lymphoid and haematopoietic cells. In mice (short-term exposure only) and dogs, haemoglobin within erythrocytes was oxidized/denatured (Heinz bodies). At high doses (> 17 mg/kg bw per day), morphological changes (Howell-Jolly bodies, polychromasia and hypochromasia) of the erythrocytes were observed in dogs.

There was no evidence of carcinogenicity at dietary concentrations of up to 125 ppm (22–30 mg/kg bw per day) in mice and up to 125 ppm (females only) and 250 ppm (8 mg/kg bw per day) in rats.

Indoxacarb (DPX-MP062) and two of its major metabolites, IN-JT333 and IN-KG433, gave negative results in an adequate battery of studies of genotoxicity in vitro and in vivo.

In view of the absence of any carcinogenic potential in rodents and the lack of genotoxic potential in vitro and in vivo, the Meeting concluded that indoxacarb is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, adults given indoxacarb at a dose of 3.8 mg/kg bw per day had reduced body-weight gain and food consumption while the pups had lower body-weight gain during lactation. The NOAEL for effects in the parents and pups was 1.3 mg/kg bw per day. There were no effects on reproductive performance.

In studies of developmental toxicity in rats and rabbits, indoxacarb was not teratogenic but caused reduced fetal body weight when dams also showed reduced body weight and food consumption. The NOAEL for these effects was 2 mg/kg bw per day in rats and 1 mg/kg bw per day in rabbits.

The Meeting concluded that the existing database on indoxacarb was adequate to characterize the potential hazards to fetuses, infants and children.

In a study of acute neurotoxicity in rats, reduced body-weight gain and food consumption occurred at doses of 50 mg/kg bw and above in females and 200 mg/kg bw in males. The NOAEL was 12.5 mg/kg bw. In females, evidence of neurotoxicity, such as slightly reduced motor activity, was observed at 100 mg/kg bw. In males, a reduced forelimb grip strength and decreased foot splay was observed at 200 mg/kg bw.

In-vitro data indicated that glucose-6-phosphate dehydrogenase-deficient individuals were slightly more sensitive (the concentration of agonist that elicits a response that is 50% of the possible maximum, EC_{50} = 55.5 µmol/l relative to 75.5 µmol/l for controls) to the oxidative effects of *N*-hydroxy-4-trifluoromethoxyaniline. The Meeting considered that the application of the normal tenfold safety factor for intraspecies variability would also be protective for glucose-6-phosphate dehydrogenase-deficient individuals.

Toxicological evaluation

It should be recognized that the ADI and ARfD applies to indoxacarb (*S* enantiomer) and its *R* enantiomer. The Meeting established an ADI of 0–0.01 mg/kg bw per day based on a NOAEL of 1.1 mg/kg bw per day for erythrocyte damage 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 is 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 and food consumption in dams was observed. The pups lost body weight during lactation at this dose.

The Meeting established an ARfD of 0.1 mg/kg bw based on the NOAEL of 12.5 mg/kg bw for reduction in body-weight gain and food intake after a single administration of indoxacarb in a study of neurotoxicity in rats, and using 100-fold safety factor.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	3-month study of toxicity [a] (Indoxacarb 1:1 DPX-JW062)	Reduced body-weight gain; haemolysis	30 ppm, equal to 2.3 mg/kg bw per day	60 ppm, equal to 4.6 mg/kg bw per day
	3-month study of toxicity[a] (Indoxacarb 3:1 DPX-MP062)	Reduced body-weight gain; haemolysis	2 ppm, equal to 2.1 mg/kg bw per day	50 ppm, equal to 3.8 mg/kg bw per day
	3-month study of toxicity [a] (Indoxacarb 1:0 DPX-KN128)	Reduced body-weight gain; haemolysis	20 ppm, equal to 1.7 mg/kg bw per day	50 ppm, equal to 4.1 mg/kg bw per day
	2-year study of toxicity and carcinogenicity[a] (Indoxacarb 1:1 DPX-JW062)	Reduced body-weight gain; haemolysis	40 ppm, equal to 2.1 mg/kg bw per day	60 ppm, equal to 3.6 mg/kg bw per day
	Acute neurotoxicity[b] (DPX-MP062)	Reduced body-weight gain and food consumption	12.5 mg/kg bw	50 mg/kg bw
	Two-generation study of reproductive toxicity[a]	Maternal toxicity: reduced maternal body weight and food consumption Fetal toxicity: reduced maternal body weight during lactation	20 ppm, equal to 1.3 mg/kg bw per day	60 ppm, equal to 4 mg/kg bw per day
	Developmental toxicity[b]	Reduced maternal body-weight gain, food consumption and reduced fetal body weight	2 mg/kg bw per day	4 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Reduced maternal body-weight gain, food consumption, clinical signs, decreased weight and number of live fetuses	10 mg/kg bw per day	100 mg/kg bw per day
Dog	12-month study of toxicity[a] (Indoxacarb 1:1 DPX-JW062)	Haemolysis	40 ppm, equal to 1.1 mg/kg bw per day	80 ppm, equal to 2.3 mg/kg bw per day

[a] Dietary administration

[b] Gavage administration

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

0.1 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to indoxacarb

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, approximately 70–80% at 5 mg/kg bw. Absorption rate and extent declines with dose, i.e. saturation kinetics evident.
Distribution	Distributed throughout the body with the highest levels in fat and erythrocytes.
Rate and extent of excretion	In both sexes, most of the administered dose was excreted within 72 to 96 h after single oral doses. The elimination half-life in plasma after a single dose ranged between 92 h and 114 h.
Potential for accumulation	Up to 9% of the administered dose retained in fat 7 days after a single dose. The elimination half-life in fat after dosing for 14 days was 18 days.
Metabolism in mammals	Extensive, no unchanged indoxacarb excreted in bile or urine
Toxicologically significant compounds (animals, plants and the environment)	Parent compound (*S*, *R* enantiomers), racemic metabolites IN-JT333 and IN-KG433
Acute toxicity (DPX-MP062 tested except for inhalation toxicity, DPX-JW062)	
Rat LD_{50} oral	1730 mg/kg bw (males); 268 mg/kg bw (females)
Rat LD_{50} dermal	> 5000 mg/kg bw (no deaths)
Rat LC_{50} inhalation (dust)	4.2 mg/l (4200 mg/m^3)
Rabbit, skin irritation	Non-irritant
Rabbit, eye irritation	Moderate irritant
Skin sensitization (test method)	Sensitizer in guinea-pigs (Magnussen & Kligman)
Acute toxicity (enantiomers)	
DPX-KN128: rat LD_{50} oral	843 mg/kg bw (males); 179 mg/kg bw (females)
DPX-KN127: rat LD_{50} oral	444 mg/kg bw (males); 480 mg/kg bw (females)
DPX-KN128: rat LD_{50} dermal	> 5000 mg/kg bw
Short-term studies of toxicity	
Target/critical effect	Reduced body-weight gain, haemolysis in rats and dogs
Lowest relevant oral NOAEL	1.1 mg/kg bw per day (12-month study in dogs; DPX-JW062)
Lowest relevant dermal NOAEL	50 mg/kg bw per day in rats (DPX-MP062)
Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	Unlikely to pose a genotoxic risk in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body-weight gain and haemolysis

Lowest relevant NOAEL	2.1 mg/kg bw per day in a 2-year dietary study in rats (DPX-JW062)
Carcinogenicity	Not carcinogenic in rats or mice; unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproduction target/critical effect	Reduced pup weight gain at parentally toxic doses
Lowest relevant reproductive NOAEL	20 ppm, equal to 1.3 mg/kg bw per day
Developmental target/critical effect	Reduced fetal body weight at parentally toxic doses
Lowest relevant developmental NOAEL	2 mg/kg bw per day (rats)
Neurotoxicity/delayed neurotoxicity	
	Evidence of neurotoxicity at high doses (100 mg/kg bw in females and 200 mg/kg bw in males)
Lowest relevant NOAEL	12.5 mg/kg bw (for reduced body-weight gain and food consumption)
Other toxicological studies	
	Studies on a plant metabolite of indoxacarb indicated that it was no more toxic than the parent compound.
Medical data	
	No data

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Dog, 1-year study	100
ARfD	0.1 mg/kg bw	Rat, acute neurotoxicity	100

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