

MANDIPROPAMID

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Explanation

Mandipropamid is the ISO approved name for 4-chloro-*N*-[2-[3-methoxy-4-(2-propynyloxy)phenyl]ethyl]- α -(2-propynyloxy)-benzeneacetamide (CAS No. 374726-62-2). It is a new fungicide that belongs to the subset mandelamides in the class carboxylic acid amides. The proposed fungicidal

mode of action is by inhibition of phospholipid biosynthesis. Mandipropamid has not been evaluated previously by the JMPR and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR). All the pivotal studies contained certificates of compliance with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

The toxicokinetics of technical-grade mandipropamid have been studied in rats given oral doses of radiolabelled test material. Summaries of the relevant data are presented below.

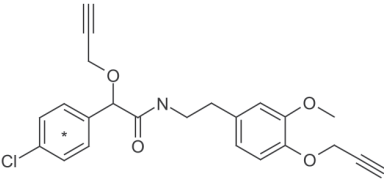
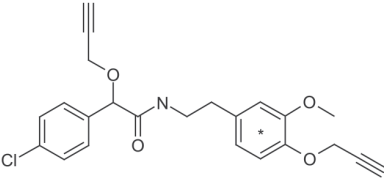
1.1 Absorption, distribution and excretion

Rats

In studies of metabolism, groups of three or four male and three to four female Alpk:APfSD (Wistar-derived) rats were given [^{14}C]mandipropamid (radiochemical purity, 95.2–96.8%) as a single oral dose at 3 or 300 mg/kg bw or as multiple doses of 3 mg/kg bw per day by gavage in 1% w/v aqueous carboxymethylcellulose for up to 14 days. Results were evaluated after treatment with [methoxyphenyl- ^{14}C]mandipropamid or [chlorophenyl- ^{14}C]mandipropamid (see Table 1). Bile-duct cannulation and tissue time-course studies were also performed.

Of the administered dose, 67–74% was absorbed by the rats at 3 mg/kg bw and 30–45% in the rats at 300 mg/kg bw, based on the sum of the radioactive residues found in the urine, bile, carcass and cage-wash 48 h after dosing. There was no clear difference in compound absorption between males and females. Absorption was decreased at the higher dose, suggesting saturation of the absorption kinetics. The T_{max} in blood at the lower dose was 8.5 h in males and 4.5 h in females, and at the higher dose was 24 h in males and 10 h in females. These data suggested that the rate of absorption is greater in females than in males, while the extent of the absorption is similar in males and females, and that the extent and rate of absorption was greater at the lower dose than at the higher dose.

Table 1. Radiolabelled forms of mandipropamid used in studies of absorption, distribution, metabolism and excretion: chemical structure and position of the radiolabel

Radiolabelled form	Structure and position of label
[Chlorophenyl- ^{14}C]mandipropamid	
[Methoxyphenyl- ^{14}C]mandipropamid	

* Position of radiolabel

Total recoveries at 168 h after dosing were 88–99% of the administered dose. The administered dose was mostly eliminated within 48 h. Apart from females at 3 mg/kg bw, most of the administered dose was excreted in the faeces regardless of the position of the radiolabel. In the females at 3 mg/kg bw, the amount excreted in the faeces was similar to that excreted in the urine. Regardless of sex, radiolabel position or dose, the amount of radiolabel isolated from the exhaled air was 0.16% or less of the administered dose, and the amount of radiolabel remaining in the body after 168 h was < 1.1% of the administered dose. In the multiple-dose study (methoxyphenyl label only), the excretion profile on day 2 after a single dose was similar to that on day 15 after 14 doses, indicating that pre-treatment did not influence the excretion profile. Elimination after 48 h in the bile was high (55–73% of the administered dose) at 3 mg/kg bw, but was only 22–28% of the administered dose at 300 mg/kg bw (at this dose, there were corresponding increases in the residues found in the faeces of males and urine of females).

The highest concentration of radiolabel was found in the liver at the initial measurement and all following measurements. At 8 h after dosing, concentrations were 0.64–1.25 µg equivalents/g (0.89 µg equivalents/g on day 4 in the multiple-dose study) at 3 mg/kg bw and 27–46 µg equivalents/g at 300 mg/kg bw. Concentrations of radiolabel in the liver of males were approximately double those in females. The second highest concentrations were usually found in the kidneys. More radiolabel was isolated in the plasma than in whole blood. The profile of tissue distribution was thus generally similar regardless of dose, radiolabel position, or sex; however, there were quantitative differences. Tissue concentrations did not increase proportionally with dose. An increase in dose of 100-fold resulted in a increase in concentrations in the liver of 17–56-fold. Results from the multiple-dose study indicated that bioaccumulation did not occur in any sampled tissue. Half-life values for the elimination of radiolabel from the blood were 18.4–20.2 h at 3 mg/kg bw and 24.8–32.7 h at 300 mg/kg bw (Roberts, 2005a, 2005b; Silcock & Duerden, 2005; Wake, 2005).

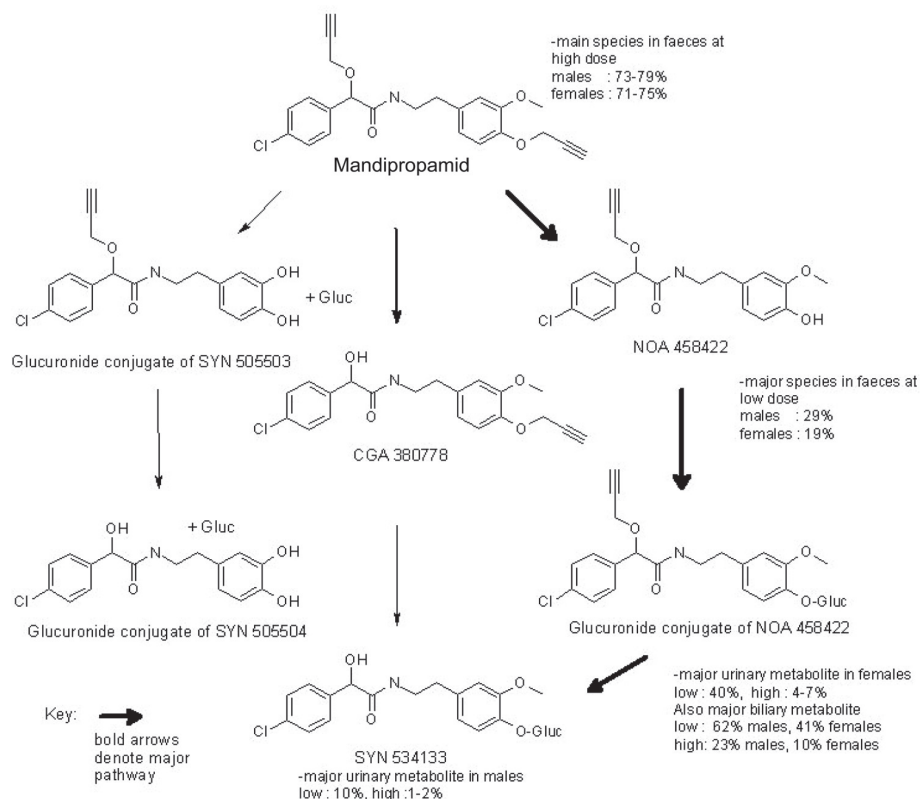
1.2 Biotransformation

Rats

In the main study of biotransformation in rats, identified compounds accounted for 65.7–93.5% of the administered dose in each group; however, 6–14% of the administered dose was unaccounted for after 168 h. Differences in the metabolite profile were found on the basis of sex, dose, and radiolabel position as described below and shown in [Figure 1](#).

Parent compound and the following metabolites were present in the urine, faeces or bile at concentrations of 5% or greater of the administered dose in rats at 3 or 300 mg/kg bw: NOA 458422, NOA 458422 glucuronide, SYN 534133, and CGA 380778. The quantity of each metabolite isolated in males and females at 3 mg/kg bw after sampling for 168 h differed. The amount of NOA 458422 glucuronide was almost three times greater in females than in males, while lesser amounts of parent and NOA 458422 were isolated in females than in males. Increasing the dose resulted in increasing amounts of radiolabel isolated as parent. In the rats treated with [chlorophenyl- ^{14}C] mandipropamid, only the parent was isolated in the faeces at > 5% of the administered dose, but other compounds were also isolated in rats treated with [methoxyphenyl- ^{14}C]mandipropamid. Thus, there were differences in the metabolite profile attributable to sex and dose. Each isolated unidentified compound accounted for < 5% of the administered dose. The major metabolic transformations involved loss of one or both of the propargyl groups followed by glucuronidation and *O*-demethylation (Wake, 2005).

Figure 1. Proposed metabolic pathway of mandipropamid in rats



Dermal absorption

In a study of dermal penetration, [Ethyl-1-¹⁴C]mandipropamid (radiochemical purity, > 97.9%) was applied to the skin (10 cm²) of groups of four male Alpk:AP₁SD rats. Nominal doses were 0.00152, 0.0076, or 2.54 mg/cm² skin, with water and/or a commercial formulation containing 23.7% (w/w) mandipropamid as a 250 g/l suspension concentrate (A12946B). The highest dose mimicked a formulation concentrate, and was included to assess exposure to mixer/loaders. The lower doses were aqueous dilutions representing typical in-use spray strength dilutions of 1/333 and 1/1667 v/v. The exposure duration was 6 h, after which four males per dose were sacrificed. The remaining groups of four males were sacrificed at 24, 72, or 120 h after dose application.

Recovery of the applied dose (mass balance) was 96–112%. Minimal absorption, based on the sum of residues in urine, faeces, cage-wash, gastrointestinal tract with contents, residual carcass, and blood, was observed (< 0.17 to 3.44% of the applied dose). Most of the administered dose (91–105%) was recovered from the skin wash at 6 h; 0.03–0.47% of the applied dose was retained at the application site, and 0.02–0.77% was found in the *stratum corneum*.

The greatest amount of absorption was noted in the 1/1667 aqueous dilution 114 h after the 6 h exposure interval. At this time 3.44% of the applied dose was absorbed. The amount available for absorption in the skin at the application site was less than the limit of detection (LOD) in all four rats (< 0.11%) with an additional 0.53% isolated in the *stratum corneum* (Silcock, 2005).

In a non-guideline study of dermal penetration in vitro, [ethyl-1-¹⁴C]mandipropamid (radiochemical purity, > 95%) was applied to excised rat skin in vitro. The formulation suspension

concentrate was included to assess exposure to mixer/loaders. The aqueous dilutions represented a typical in-use spray strength dilution of 1/333 and 1/1667 v/v. The applied nominal doses were 1.35, 6.69, or 2538 µg/cm² skin. Skin was used from freshly killed male Wistar rats. Skin membrane discs of approximately 3.3 cm in diameter were prepared from at least two rats, and were mounted in diffusion cells with an exposed membrane area of 2.54 cm². Each of the three doses were applied to the skin in a dose volume of 10 µl/cm² (n = 4–6) for a duration of 6 or 24 h. The cells were not occluded for the duration of exposure. Samples of receptor fluid were taken before treatment and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 h after dose application for those cells assigned an exposure of 24 h, or at 6 h for those cells assigned an exposure of 6 h. The original volume of the receptor fluid was restored by adding fresh receptor fluid after sampling. The test substance absorbed was considered to be the mandipropamid detected in the receptor fluid.

Recovery of the applied radioactivity was 99–105%. Absorption was poor. In the 1/1667 v/v spray dilution after 24 h of exposure (recovery, 104%), 13.1% of the radioactive compound was absorbed and 89.1% was found in the skin wash. At the other exposure conditions, < 0.03% to 6.00% of the radioactive compound was absorbed and 93.5–100% was isolated in the skin wash. The absorption rate was greatest during the first hour of exposure (0.05–0.06 µg/cm² per h for the aqueous spray dilutions). Absorption rates over the 24 h of exposure were < 0.04 µg/cm² per h in the concentrate formulation (concentrations measured below the quantifiable limit) and only 0.01 µg/cm² per h in the aqueous spray dilutions. The amount absorbed as a percentage of applied dose decreased with increasing dose (Davies, 2005a).

In a non-guideline study of dermal penetration in vitro, [ethyl-1-14C]mandipropamid (radiochemical purity, > 95%) was applied to excised human skin in vitro. The formulation concentrate was included to assess exposure to mixer/loaders. The aqueous dilutions represented a typical in-use spray strength dilution of 1/333 and 1/1667 v/v. The applied nominal doses were 1.35, 6.69, or 2538 µg/cm² skin. Skin membrane discs of approximately 3.3 cm in diameter were prepared from at least two humans, and were mounted in diffusion cells with an exposed membrane area of 2.54 cm². Each of the three doses were applied to the skin in a dose volume of 10 µl/cm² (n = 5–6) for a duration of 6 or 24 h. Samples of receptor fluid were taken before treatment and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 h after dose application for those cells assigned an exposure of 24 h or at 6 h for those cells assigned an exposure of 6 h. The test substance absorbed was considered to be the mandipropamid detected in the receptor fluid.

Recovery of the applied radioactivity was 95–102%. Absorption was minimal. Absorption was < 0.04% to 0.77%, and 93.9–102% of the applied radiolabel was isolated in the skin wash. The absorption rate was greatest during the first 1–2 h of exposure (0.003–0.004 µg/cm² per h in the aqueous spray dilutions). Absorption rates over the 24 h of exposure were < 0.04 µg/cm² per h in the concentrate formulation (concentrations measured below the quantifiable limit) and ≤ 0.001 µg/cm² per h in the aqueous spray dilutions. The amount absorbed as a percentage of applied dose decreased with increasing dose (Davies, 2005b).

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with mandipropamid technical are summarized in [Table 2](#). All studies complied with GLP.

(a) Lethal doses

In a study of acute oral toxicity, three female Sprague-Dawley rats were given a single oral dose of mandipropamid (purity, 96.5%) at 5000 mg/kg bw using the up-and-down procedure. The rats were inspected for mortality and clinical abnormalities during the first hours after dosing and at least once per day for up to 14 days thereafter. Body weights were obtained before dosing and again on days 7 and 14. A necropsy examination was performed on all rats.

All rats survived and gained weight throughout the study. One rat exhibited anogenital staining at 5 h after dosing. No other clinical abnormalities were observed. No gross abnormalities were observed at necropsy. The oral median lethal dose (LD_{50}) for females was estimated to be > 5000 mg/kg bw (Moore, 2004).

In a study of acute dermal toxicity, five male and five female Sprague-Dawley rats were exposed dermally to mandipropamid (purity, 96.5%) at a dose of 5050 mg/kg bw. On the day before study initiation, the dorsal trunk area was clipped free of fur. On the day of study initiation, each individual dose of the test substance was moistened with “a sufficient amount of corn oil (0.75 ml/g test substance)” and applied to the test site in a thin, uniform layer, and covered with a 2×4 inch gauze patch. The patch was secured with non-irritating adhesive tape. The trunk of each rat was wrapped with vet wrap and secured with non-irritating adhesive tape to prevent ingestion of the test material. After 24 h, all binding materials were removed. The test sites were washed with tap water at room temperature and a clean cloth was used to remove any residual test substance. The rats were observed for signs of toxicity or clinical abnormalities at least three times on the day of dosing (day 0) and at least once per day thereafter for 14 days. Body weights were measured just before dosing and again on days 7 and 14. Observations for dermal irritation at the test site were taken on days 1, 4, 7, 11 and 14. A necropsy examination was performed on all rats tested.

All rats survived to study termination. No clinical abnormalities or skin irritation were observed. Body weight was unaffected by mandipropamid. No gross abnormalities were observed at necropsy. There were no deaths at 5050 mg/kg bw (Kuhn, 2005).

In a study of acute toxicity on inhalation, five male and five female Alpk:AP_rSD rats were exposed “nose-only” via inhalation to mandipropamid (purity, 96.5%) at a gravimetric concentration of 5.19 ± 0.55 mg/l air. The rats were observed for clinical signs frequently during and at the end of exposure and daily until study termination. Body weights were recorded on study days -1, 1, 8 and 15. A necropsy examination was performed on all rats tested.

All rats (five out of five males and five out of five females) survived to study termination. Clinical abnormalities observed during exposure included wet fur, stains around the nose, slight salivation, and respiratory-tract irritation (increased breathing depth). Immediately after exposure, the rats continued to exhibit wet fur and stains around the nose. Some rats also exhibited chromodacryorrhoea, slight salivation, and irritation of the upper respiratory tract (abnormal respiratory noise). All females recovered by study day 2 and males by study day 4. All rats gained normal body weight by

Table 2. Acute toxicity with mandipropamid^a

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Reference
Rat	Sprague-Dawley	Females	Oral	> 5000	—	Moore (2004)
	Sprague-Dawley	Males & females	Dermal	> 5050	—	Kuhn (2005)
	Alpk:AP _r SD	Males & females	Inhalation	—	> 5.19	Kilgour (2003)

^a Technical mandipropamid of purity 96.5% was used in all the studies of acute toxicity.

study termination. At necropsy, one male exhibited a pelvic dilation of the kidney. The study author stated that this is common for the age and strain of the rat used and did not attribute this observation to administration of the test substance. No other abnormalities were observed at necropsy. The median lethal concentration (LC_{50}) was $> 5.19 \pm 0.55$ mg/l air (Kilgour, 2003).

(b) *Ocular irritation*

Rabbits

In a study of primary eye irritation in New Zealand White rabbits, two males and one female received approximately 100 mg of undiluted mandipropamid (purity, 98.5%) into the conjunctival sac of the left eye. The right eyes were left untreated to serve as negative controls. Ocular irritation was graded according to the Draize scale at 1 h and additionally at 1, 2, 3, 4 and 7 days after instillation.

No corneal opacity was observed during the study. Iritis was observed in two out of three rabbits at 1 h only. Positive signs of conjunctivitis were observed in three out of three rabbits at 1 h, resolving within 4 days. Mandipropamid was minimally irritating to the eyes of New Zealand White rabbits (Johnson, 2004a).

(c) *Dermal irritation*

Rabbits

In a study of primary skin irritation in New Zealand White rabbits, two males and one female received approximately 500 mg of mandipropamid (purity, 98.5%) applied topically to shaved skin sites on the back. The test site was covered with gauze and a sheet of rubber for 4 h after which all coverings were removed and the site was washed with water and wiped dry with clean tissue paper. Each test site was evaluated for irritation potential according to the Draize scale at 1 h and additionally at 1, 2, 3, 4 and 7 days after application. Very slight (grade 1) erythema was observed in one out of three rabbits at 1 h, resolving within 7 days. This rabbit also exhibited slight desquamation. No oedema was observed during the study. Mandipropamid was minimally irritating to the skin of New Zealand White rabbits (Johnson, 2004b).

(d) *Dermal sensitization*

In a study of dermal sensitization with mandipropamid (purity, 96.5%), groups of four male CBA/Ca/Ola/Hsd mice were tested for a dermal sensitization response using the local lymph node assay. The mice received mandipropamid as 10%, 25%, or 50% w/v preparations in dimethylformamide (DMF). A study using the positive control hexylcinnamaldehyde was conducted within 6 months of the main study. Approximately 25 μ l of a 1%, 3% or 10% w/v preparation of hexylcinnamaldehyde in acetone was applied, and a vehicle-control group was similarly treated using acetone. The test substance produced a stimulation index of < 3 in all groups of mice. Therefore, mandipropamid was not considered to be a sensitizer (defined as producing a positive response) (Johnston, 2004c).

Table 3. Irritation and skin sensitization potential of mandipropamid

Species	Strain	Sex	End-point	Purity (%)	Result	Reference
Rabbit	NZW	M/F	Eye irritation	98.5	Minimally irritating	Johnson (2004a)
Rabbit	NZW	M/F	Skin irritation	98.5	Minimally irritating	Johnson (2004b)
Mouse	CBA/CA/Ola/Hsd	M	Skin sensitization (local lymph node assay)	96.5	Not sensitizing	Johnston (2004c)

F, females; M, males; NZW, New Zealand White.

2.2 Short-term studies of toxicity

(a) Oral administration

Rats

Groups of 10 male and 10 female Alpk:APfSD rats were given diets containing mandipropamid (purity, 96.5%) at a concentration of 0, 100, 500, 3000 or 5000 ppm (equal to 0, 8, 41, 260 or 435 mg/kg bw per day in males and 0, 9, 45, 260 or 444 mg/kg bw per day in females) for 90 days.

No adverse treatment-related effects were observed on mortality, clinical signs, neurological evaluation, food consumption, ophthalmoscopic examinations, haematology, urine analysis, or gross pathology. Decreased body weights were observed in the males at 3000 ppm during weeks 2–14 (decrease, 1–10%) and at 5000 ppm during weeks 2–5, 11, and 13–14 (decrease, 2–7%). Differences in cumulative body-weight gains generally paralleled the differences in body weights. Decreases of 11–28% in body-weight gain were noted in the males at dietary concentrations of 3000 ppm or greater during weeks 1–7, 7–14, and overall (weeks 1–14). Decreased food utilization was noted in males at 3000 ppm or greater during weeks 1–4, 5–8, 9–13, and 1–13 (decrease, 9–29%), except in males 5000 ppm males during weeks 5–8. Indications of slight hepatotoxicity were observed.

Plasma gamma-glutamyl transferase was increased in females at dietary concentrations of 3000 ppm or greater (increase, 58–105%). Absolute and relative liver weights were increased by 14–36% in males and females at 3000 ppm or greater. Minimal to slight periportal hypertrophy/eosinophilia in the liver was noted in males at 5000 ppm (8 out of 10) and the females at 3000 ppm or greater (10 out of 10 treated) compared with 0 out of 10 in the controls and other dose groups. In the erythropoietic system, toxicity was demonstrated as decreases in a number of erythrocyte parameters (see Table 4), and increased kidney weight (males) and an increased incidence of tubular basophilia at the highest dose only were indicative of renal effects.

The no-observed-adverse-effect level (NOAEL) was 500 ppm, equal to 41 mg/kg bw per day in males and 45 mg/kg per day in females, on the basis of decreased body weight, body-weight gain and food utilization at 3000 ppm (Pinto, 2005a).

Table 4. Selected haematology findings in rats given diets containing mandipropamid for 90 days (means)

Parameter (mean)	Dietary concentration (ppm)									
	Males					Females				
	0	100	500	3000	5000	0	100	500	3000	5000
Haemoglobin (g/dl)	14.8	15.1	14.7	14.7	14.2*	15.3	15.1	15.0	14.5**	14.2**
Haematocrit	0.465	0.474	0.464	0.469	0.456	0.473	0.468	0.463	0.454*	0.445**
Mean cell volume (fl)	51.4	51.4	51.0	49.9**	48.9**	53.9	54.0	52.9*	52.0**	51.7**
Mean cell haemoglobin (pg)	16.3	16.4	16.2	15.6**	15.3**	17.4	17.4	17.1	16.6**	16.5**
Mean cell haemoglobin concentration (g/dl)	31.7	31.8	31.7	31.3*	31.2*	32.3	32.3	32.4	31.8*	31.9*

From Pinto (2005a)

* $p < 0.05$ (Student t-test, two-sided)

** $p < 0.01$ (Student t-test, two-sided)

Dogs

In a 90-day study of oral toxicity, groups of four male and four female beagle dogs were given capsules containing mandipropamid (purity, 96.5%) at a concentration of 0, 5, 25, 100, or 400 mg/kg bw per day.

There were no mortalities and no adverse effects of treatment on clinical observations, food consumption, ophthalmoscopy, haematology, urine analysis, or gross pathology. There were no treatment-related effects on any parameter at 5 or 25 mg/kg per day. Body-weight gains and body weights were decreased in females at 100 mg/kg per day beginning at week 8 and in dogs at 400 mg/kg per day beginning at week 3; however, these decreases were not considered to be adverse because they were minimal (body weights were only decreased by 2–5% and body-weight gains by 9–15%). Note that similar decreases ($\leq 12\%$) in body weights were observed throughout the long-term study of toxicity (Brammer, 2005b) at 400 mg/kg per day, but were associated with more substantial decreases of 23–56% in body-weight gains for weeks 1–13 than in this short-term study.

The liver was a target organ for toxicity. At 400 mg/kg per day, increases were reported in cholesterol concentration (increase, 27–49%), alkaline phosphatase activity (increase, 37–113%), and alanine aminotransferase (ALT) activity (increase, 43–238%, not significant at week 4) were observed in males and females throughout the study, compared with controls. At 100 mg/kg per day, cholesterol concentration and alkaline phosphatase activity were only increased by 22–53% and were not consistently observed throughout the study. ALT, the most definitive indicator of liver toxicity, was only increased by 35–37% at week 13.

Increases (10–19%) in absolute, and relative (to body weight) liver weights were observed in males and females at doses of 100 mg/kg bw per day or greater. At 100 mg/kg bw per day, minimal pigmentation of the hepatocytes and of the Kupffer cells were observed in one out of four males and two out of four females, compared with zero controls. At 400 mg/kg bw per day, the incidence of this finding increased in all dogs (four out of four males and four out of four females), and the severity increased from minimal at 100 mg/kg bw per day to minimal to slight at 400 mg/kg bw per day. It was stated that this brown pigment had a predominantly centrilobular distribution and was negative for haemosiderin and lipofuscin. It was bi-refrangent when viewed under polarized light and appeared to be consistent with porphyrin. Minimal centrilobular hepatocyte vacuolation was noted in females at 100 mg/kg bw per day (one out of four) and females at 400 mg/kg bw per day (three out of four) compared with 0 out of 4 controls. The microscopic findings at this dose were minimally increased in severity and/or incidence over controls.

The NOAEL was 25 mg/kg bw per day on the basis of evidence of liver toxicity (increased cholesterol, alkaline phosphatase activity, ALT activity, liver weights and microscopic pigment in the hepatocytes and Kupffer cells in males and females and centrilobular hepatocyte vacuolation in females) (Brammer, 2005a).

In a 1-year study, groups of four male and four female beagle dogs were given capsules containing mandipropamid (purity, 96.54%) at a dose of 0, 5, 40 or 400 mg/kg bw per day.

There were no mortalities and no effects of treatment on ophthalmoscopy, haematology, urine analysis, or gross pathology. There were no treatment-related effects on any parameter in dogs at 5 mg/kg per day. One female at 40 mg/kg bw per day was slightly thin beginning at week 6, and one female at 400 mg/kg per day was slightly to moderately thin beginning at week –2. Additionally at 400 mg/kg bw per day, two females exhibited slight to moderate salivation during dosing with the capsule (83 incidences beginning at week 3) and at non-dosing observation intervals (96 occasions beginning at week 1). These clinical findings continued until study termination. At 400 mg/kg bw per day, decreases of 1–12% in body weights were observed in males and females throughout the study. Body-weight gains for weeks 1–13 were decreased by 23% compared with controls in the males and by 30% in the females. Body-weight gains for the study overall (weeks 1–53) remained decreased at

this dose in males (decrease, 15%) and females (decrease, 45%) (Table 5). There were no effects on body weight at 5 or 40 mg/kg bw per day. Additionally, in females at 400 mg/kg bw per day, minor ($\leq 7\%$ compared with controls) but consistent (43 out of 52 weeks) decreases in food consumption were observed.

The liver was the target organ for toxicity. At doses of 40 mg/kg bw per day or greater, alkaline phosphatase activity was increased throughout the study in males (57–174%) and females (27–273%). These increases attained significance, except in females at 40 mg/kg bw per day at week 13. In the males, ALT activity was increased throughout the study at 40 mg/kg bw per day (89–150%) and 400 mg/kg bw per day (315–445%). ALT activity was also increased (182–352%) in females at 400 mg/kg per day throughout the study, attaining significance at week 52. Increases in absolute liver weight (7–15%), relative (to body weight) liver weight (20–27%), and liver weight adjusted for terminal body weight¹ (16–19%) were observed in males and females at 400 mg/kg bw per day, with adjusted liver weight in the males attaining significance. At 40 mg/kg bw per day, minimal increased pigment in the liver was observed in two out of four males and one out of four females, compared with zero out of four controls. At 400 mg/kg bw per day, the incidence of this finding increased to three out of four males and three out of four females, and the severity was minimal to moderate in the males and minimal to slight in the females. It was stated that this pigment appeared to be consistent with porphyrin.

The NOAEL was 5 mg/kg per day on the basis of liver toxicity (increased incidence and severity of pigment, increased activities of alkaline phosphatase and ALT) at 40 mg/kg bw per day (Brammer, 2005b).

Table 5. Comparison of body weights of dogs given capsules containing mandipropamid for up to 1 year

Week ^a	Body weight (kg)							
	Dose (mg/kg bw per day)							
	Males				Females			
	0	5	40	400	0	5	40	400
1	9.20	9.30	8.88	9.18	7.95	8.03	7.80	7.73
4	9.94	9.85	9.71	9.57*	8.34	8.30	8.24	8.23
8	10.65	10.64	10.35	10.09*	8.79	8.67	8.64	8.43
12	11.15	11.23	10.77	10.60*	9.06	9.12	8.99	8.58
18	11.76	11.76	11.26	11.17	9.61	9.58	9.46	8.87*
24	12.06	12.13	11.31	11.47	9.82	9.91	9.66	9.02
48	12.38	12.85	11.51	11.91	10.38	10.54	10.17	9.28
53	12.60	13.02	11.68	12.06	10.51	10.78	10.39	9.22

From Brammer (2005b)

^a Adjusted mean values shown for week 4 onwards.

* $p < 0.05$ (Student t-test, two-sided)

** $p < 0.01$ (Student t-test, two-sided)

- 1 Group mean organ weight corrected for intergroup differences in the group mean terminal body weight. The adjusted means are calculated statistically using analysis of covariance. The size of the adjustment is determined by the size of the intergroup differences in the group mean terminal body weight and the strength of the relationship between organ weight and terminal body weight.

(b) *Dermal administration*

Rats

In a study of dermal toxicity, groups of 10 male and 10 female Wistar rats received mandipropamid (purity, 96.5%) at a dose of 0, 250, 500, or 1000 mg/kg bw per day (limit dose) applied to the shaved skin for 6 h per day for 5–6 days per week (a total of 21 total doses) during a 28-day period.

No compound-related effects were observed on mortality, clinical signs of toxicity, body weight, body-weight gain, food consumption, a functional observational battery (FOB), motor activity, ophthalmoscopic examinations, haematology, clinical chemistry, urine analysis, absolute or relative organ weights, or gross or microscopic pathology in males or females. There was an increased incidence of slight dermal irritation (erythema, oedema, and desquamation of the application site) at doses of 250 mg/kg bw per day and above. The erythema and oedema were generally observed throughout the study and the desquamation was observed between days 3–15 in the treated rats.

The NOAEL was 1000 mg/kg per day, the highest dose tested (Lees, 2005).

2.3 *Long-term studies of toxicity and carcinogenicity*

Mice

Groups of 50 male and 50 female C57BL/10J_{CD-1} mice were given diets containing mandipropamid (purity, 96.5%) at a concentration of 0, 100, 500, or 2000 ppm (equal to 0, 10.6, 55.2 or 222.7 mg/kg bw per day in males and 0, 13.2, 67.8 or 284.6 mg/kg bw per day in females) for up to 80 weeks.

No adverse treatment-related effects were observed on mortality, food consumption, haematology (i.e. leukocyte differential), gross pathology, or histopathology. An increased frequency of circling behaviour was observed in females at 500 and 2000 ppm. However, this behaviour was only slightly increased in incidence at 2000 ppm compared with controls (two, one, two, and four mice at 0, 100, 500 and 2000 ppm, respectively).

Dose-related increases in the number of mice with a torn left ear were observed in males and females at 500 and 2000 ppm. The number of mice with a torn right ear was minimal in incidence (≤ 1 mouse per group) and did not show a pattern with dose. It was stated that the mice were identified by ear tags, and it is likely that these identification tags were placed in the left ear. The mice were housed together in groups and could have removed the tags (and selectively torn the left ear) of their cage-mates via fighting or other interactions. However, this would not explain why the incidence of this finding was dose-related. The possibility that the test substance increased fighting or some other behaviour that led to the left ear being torn cannot be ruled out. However, because there were no dose-related incidences in scabs or other signs of fighting and because there were no clinical signs of neurotoxicity in this study or in other studies of acute neurotoxicity or short-term studies of neurotoxicity in rats, the Meeting considered that the toxicological importance of this finding was equivocal.

At 2000 ppm, body weights were slightly decreased in males generally from weeks 19–35 and in the females during weeks 4, 5, and 13–81. Cumulative weekly body-weight gains were decreased at this dose in males during weeks 19–81 and in females during weeks 4–6 and 11–81. The decreased body-weight gains attained statistical significance throughout the study, except at week 81 in males and at week 79 in females. Food utilization was decreased in males during weeks 9–13 (decrease, 20%) and weeks 1–13 (decrease, 5%) and in the females for weeks 1–4 (decrease, 12%) and reflected the decrease in body weights at this dose. Absolute, relative-to-body-weight, and adjusted-for-initial-body-weight liver weights were increased in males at 500 ppm and in males and females at 2000 ppm. These increases were statistically significant, except for absolute weights in males at 2000 ppm (relative liver weights were not examined statistically). In the absence of any microscopic findings in the liver, the relatively small increases in liver weights were not considered to be adverse,

but were likely to be an adaptive response to the presence of the test substance. At the doses tested, there were no effects of treatment on the incidence or time to onset of any tumour types. Therefore, there was no evidence of a carcinogenic effect. Dosing was considered to be adequate on the basis of decreased body weight and body-weight gain.

The NOAEL was 500 ppm, equivalent to 55 mg/kg bw per day in males and 68 mg/kg bw per day in females, on the basis of decreased body-weight gain in males and females and food utilization in males at 2000 ppm (Milburn, 2005a).

Rats

In a long-term study of combined toxicity and carcinogenicity, groups of 52 male and 52 female Alpk:AP_fSD rats were given diets containing mandipropamid (purity, 96.5%) at a concentration of 0, 50, 250, or 1000 ppm (equal to 0, 3.0, 15.2 or 61.3 mg/kg bw per day in males and 0, 3.5, 17.6 or 69.7 mg/kg bw per day in females) for up to 2 years. Additional groups of 12 males and 12 females were treated similarly and terminated after 1 year.

No treatment-related effects were observed on mortality, clinical signs, neurological evaluation, food consumption, ophthalmoscopic examination, haematology, clinical chemistry, urine analysis, or organ weights. No treatment-related pathological findings were noted at 12 months. In males at 1000 ppm, decreased body weights were generally observed during weeks 2–15 and 67–103 (decrease, 1–6%). A similar effect was observed on cumulative body-weight gain (decrease, 3–7%). Overall (weeks 1–105) body-weight gain was decreased by 6% (not statistically significant). Food utilization was decreased during weeks 1–4, 5–8, 9–13, and 1–13 by 5–7% ($p \leq 0.01$; except not statistically significant at weeks 9–13). An increased incidence of a roughened surface was observed (13 out of 64 treated vs 5 out of 64 controls) in the kidney of males at 1000 ppm. The severity of chronic progressive nephropathy was increased in males receiving mandipropamid, with an increased incidence of moderate to marked severity (38, 41, 47 and 53% in the control group and at 50, 250 and 1000 ppm, respectively). Associated increases in the incidences of minimal to marked renal osteodystrophia fibrosa (19% treated vs 8% controls) and minimal to marked parathyroid hyperplasia (28% treated vs 17% controls) were also noted. At the doses tested, there was no treatment-related increase in tumour incidence when compared with controls. Dosing was considered to be adequate on the basis of decreased body-weight gain and food utilization and increased nephrotoxicity in the males.

The NOAEL was 250 ppm, equivalent to 15.2 mg/kg bw per day in males and 17.6 mg/kg bw per day in females, on the basis of decreased body-weight gain and food utilization and increased nephrotoxicity in males (Pinto, 2005b).

2.4 Genotoxicity

(a) In vitro

In three independent trials of an assay for reverse gene mutation, *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strains WP2P and WP2PuvrA were exposed to mandipropamid (purity, 96.5%) at concentrations of 0, 100, 200, 500, 1000, 2500, or 5000 µg/plate in dimethyl sulfoxide (DMSO) with or without metabolic activation from the S9 fraction derived from livers of male Sprague-Dawley rats induced with phenobarbital/β-naphthoflavone. The study was complied with OECD guideline 471. The standard plate incorporation method was used in all except one trial (in the presence of metabolic activation) in which a pre-incubation step was added. Standard strain-specific mutagens served as positive controls. Precipitation of the test material was observed at concentrations of 2500 µg/plate and above in trial 1 (with or without metabolic activation) and at 1000 µg/plate and above in trials 2 (without metabolic activation) and 3 (with metabolic activation). No precipitation was noted at any concentration in the pre-incubation assay. No evidence of cytotoxicity was observed in any strain in the presence or absence of metabolic

activation. There were no treatment-related increases in the mean number of revertants per plate in any strain. The positive controls induced marked increases in the number of revertant colonies compared with controls in all strains in the presence and absence of metabolic activation. There was no evidence for an increased induction of mutant colonies over background (Callander, 2005).

In three independent trials of an assay for mammalian cell gene mutation at the *Tk*^{+/−} locus, L5178Y mouse lymphoma cells cultured in vitro were exposed to mandipropamid (purity, 96.5 at concentrations of 0, 257, 515, 1030, 2060, or 4119 µg/ml (trials 1 and 2, with or without metabolic activation) or 0, 1, 10, 50, 100, 250, or 500 µg/ml (trial 3, with or without metabolic activation) in DMSO for 4 h. The study complied with OECD guideline 476. The S9 fraction used for metabolic activation was derived from the livers of male Sprague-Dawley rats induced with phenobarbital and β-naphthoflavone. Ethylmethane-sulfonate (EMS) and Benzo(α)pyrene served as positive controls in the absence and presence of metabolic activation, respectively.

Cytotoxicity (decreased mean relative survival) was observed at all doses in the presence and absence of metabolic activation in trials 1 and 2. No statistically significant or biologically relevant increases in the frequency of mutants were observed in the absence of metabolic activation. However, in the presence of metabolic activation, statistically significant increases ($p < 0.05$) in mutant frequency were noted at 2060 g/ml in trial 1 and 1030 g/ml in trial 2. These values were less than twice the values for the negative controls and were not dose-dependent, and were thus not considered to be biologically significant or treatment-related. The positive controls induced the appropriate response both in the presence and absence of metabolic activation. There was no evidence for increased induction of mutant colonies over background in the presence or absence of metabolic activation (Clay, 2002).

In two independent assays for chromosomal aberration in mammalian cells, lymphocyte cultures were prepared from human peripheral blood and exposed to mandipropamid (purity, 96.5%) at a concentration of 0, 10, 50, 100, 250, 500, 1000, 2000, 3000 or 4119 µg/ml (in the presence or absence of metabolic activation) for 3 h followed by a 17 h recovery period (trial 1); 0, 1, 2.5, 5, 10, 25, 50, 100, or 250 µg/ml for 3 h with a 17 h recovery period (in the presence of metabolic activation, trial 2); or 0, 1, 2.5, 5, 10, 25, 50, 100, or 250 µg/ml for 20 h with no recovery period (in the absence of metabolic activation, trial 2). The study complied with OECD guideline 473.

Excessive cytotoxicity was observed at concentrations of 250 µg/ml and above (in the presence or absence of metabolic activation, trial 1), 100 µg/ml and above (in the presence of metabolic activation, trial 2), and 50 µg/ml and above (in the absence of metabolic activation, trial 2). Therefore, the following concentrations were selected for evaluation of chromosomal aberrations: trial 1: 10, 50, and 100 µg/ml (in the presence or absence of metabolic activation); trial 2: 2.5, 10, and 25 µg/ml (in the absence of metabolic activation); and trial 2: 5, 25, and 50 µg/ml (in the presence of metabolic activation).

No significant increases in the mean percentage of aberrant cells were observed in either trial in the presence or absence of metabolic activation. The positive controls induced increases ($p < 0.001$) in the number of aberrant cells in the presence and absence of metabolic activation in both trials. There was no evidence of increased frequency of chromosome aberrations over background in the presence or absence of metabolic activation (Fox, 2002).

(b) *In vivo*

In an assay for micronucleus formation in bone marrow, groups of five male Wistar rats (age 6–7 weeks) were given a single dose of mandipropamid (purity, 96.5%) at 0 or 2000 mg/kg bw by gavage (dose volume, 10 ml/kg bw) in aqueous 0.5% methylcellulose. The study complied with OECD guideline 474. Bone-marrow cells were harvested at 24 h and 48 h after dosing.

No compound-related mortalities or clinical signs of toxicity were observed. A small decrease ($p < 0.05$) in the percentage of polychromatic erythrocytes (PCE) was observed at 48 h after dosing,

indicating that mandipropamid was slightly toxic to the bone marrow. No treatment-related increases in the frequency of micronucleated polychromatic erythrocyte (MPCE) were observed in the treated rats at either sacrifice time compared with controls. The positive control induced the appropriate response (Fox, 2005).

In an assay for unscheduled DNA synthesis assay *in vivo/in vitro*, rat hepatocyte cultures were prepared from groups of two to three male Wistar rats given a single oral dose of mandipropamid (purity, 96.5%) at 0 or 2000 mg/kg bw by gavage (dose volume, 10 ml/kg bw) in 0.5% carboxymethylcellulose. The study was conducted in compliance with OECD guideline 486. Hepatocytes were harvested at 2 h or 16 h after dosing.

The net nuclear grain (NNG) counts in the treated rats (−4.8 to −4.7) were well below the threshold of 0 or more NNG required for a positive response, and the mean percentage of cells in repair (> 5 NNG/cell) in the treated rats was only 1% in both trials. The positive controls induced the appropriate response in both trials. There was no evidence that unscheduled DNA synthesis, as determined by radioactive-tracer procedures (nuclear silver grain counts) was induced (Clay, 2005).

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 26 male and 26 female Alpk:APfSD (Wistar-derived) rats were given diets containing mandipropamid (purity, 96.5%) at a concentration of

Table 6. Results of studies of genotoxicity with mandipropamid

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
<i>In vitro</i>					
Reverse mutation ^{a,b}	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 <i>E. coli</i> WP2P and WP2P <i>uvrA</i>	100–5000 µg/plate ± S9, in DMSO	96.5	Negative ±S9	Callander (2005)
Forward mutation ^{b,c}	Mouse lymphoma cells (<i>Tk</i> ^{+/−} locus)	1–4119 µg/ml ±S9, in DMSO	96.5	Negative ±S9	Clay (2002)
Chromosomal aberration ^{d,e}	Human lymphocytes	Trial 1: 10–4119 µg/ml +S9 Trial 2: 1–250 µg/ml ±S9	96.5	Negative ±S9	Fox (2002)
<i>In vivo</i>					
Micronucleus formation ^{f,g}	Wistar rats (five males and five females per dose)	0 or 2000 mg/kg bw (single oral dose by gavage in methylcellulose)	96.5	Negative	Fox (2005)
Unscheduled DNA synthesis ^f	Rat primary hepatocytes	0 or 2000 mg/kg bw (single oral gavage dose)	96.5	Negative	Clay (2005)

DMSO, dimethyl sulfoxide; S9, 9000 × g supernatant from livers of male rats induced with phenobarbital and β-naphthoflavone.

^a: Positive and negative controls included.

^b: Tested in triplicate; positive and negative controls included.

^c Cytotoxicity observed at all doses ± S9.

^d Tested in duplicate; positive and negative controls included.

^e Cytotoxicity observed at concentrations of ≥ 250 µg/ml ± S9 in trial 1 and at ≥ 100 µg/ml ± S9 in trial 2.

^f Positive and negative controls included.

^g Aberrations analysed in five males and five females; no clinical signs of toxicity were observed.

0, 50, 250, or 1500 ppm (equal to 0, 4.7, 22.9 or 146.3 mg/kg bw per day in males and 0, 5.0, 24.5 or 148.2 mg/kg bw per day in females, on the basis of mean pre-mating food intake of F_0 and F_1 rats). The parental (F_0) generation rats were fed the test diets for 10 weeks before mating to produce the F_1 litters. Upon weaning, F_1 parents were fed the test diets for 10 weeks before mating to produce the F_2 a litters. Because of equivocal differences in live birth index, whole litter losses, and live litter size between the control and 1500 ppm groups, the F_1 parents were mated a second time to produce the F_2 b litters.

There were no effects of treatment on parental mortality, clinical signs, estrus cycle duration or periodicity; sperm parameters, or gross pathology. Additionally, there were no treatment-related effects on body weights, body-weight gains, food consumption, or food utilization during gestation or lactation.

During pre-mating in the F_0 generation, food consumption was increased by 5–10% in males at 1500 ppm during weeks 1–4, while body weights were similar to those of the controls. Food utilization of these rats was decreased by 8% during weeks 1–4, resulting in a decrease (4%) in food utilization overall for the pre-mating period (weeks 1–10). At 1500 ppm in the F_1 generation, body weights were decreased by 2–8% in males throughout pre-mating, attaining significance in 7 out of 11 weeks. Weekly cumulative body-weight gains in these rats were decreased by 6–9% throughout pre-mating, resulting in a decrease of 6% in body-weight gain for the overall (weeks 1–11) pre-mating period. Food consumption in these males was decreased by 5% during weeks 9–10, and food utilization was decreased by 8% during weeks 1–4 and by 6% during weeks 5–8, resulting in a decrease of 4% for overall food utilization (weeks 1–10). At 1500 ppm, absolute and adjusted liver weights were increased in the F_0 males (17–19%) and females (7–8%) and in the F_1 males and females (11–17%). These increases attained significance ($p \leq 0.05$) except for the absolute liver weight in the F_0 females. Because there were no microscopic findings in the liver and clinical-chemistry analyses were not performed, the increased liver weights were considered to be equivocal. Similar findings in the liver were noted in the short-term (Pinto, 2005a) and combined long-term/oncogenicity (Pinto, 2005b) studies in rats.

Absolute and adjusted adrenal weights were increased by 13–23% at 1500 ppm compared with those of controls in the F_0 males and F_1 males and females. Absolute adrenal weights were also increased (by 11%) in F_1 females at 250 ppm. An increase in the severity of vascular ectasia was observed in females at 1500 ppm (eight dams with minimal to moderate severity) compared with controls (seven dams with minimal severity). However, vascular ectasia was only observed in zero to four rats per group at 1500 ppm in P-generation male and females and in F_1 -generation males. The sponsor stated that vascular ectasia in the adrenal is a common spontaneous age-related lesion that is seen predominantly in female rats. It was also stated that the severity and incidence observed in the F_1 dams at 1500 ppm was less than the maximum for historical controls at 1-year interim sacrifice. Although data were not available, it was stated that the incidence of this finding can be as high as 90% at 1 year and varies widely, as does severity. Therefore, the findings in the adrenal gland were considered to be equivocal.

There were no treatment-related effects on viability, clinical signs, or anogenital distance. In the F_2 a litter, the live birth index was decreased at 1500 ppm (86.8%) compared with values for controls (97.7%), and the litter size at postnatal day 1 was decreased by 22% at 1500 ppm compared with values for controls. However, when the numbers of whole litter losses were excluded, these effects were not evident. At 1500 ppm, adjusted pup weights were decreased by 7–14% in male and female F_1 and F_2 b pups. In the F_2 a litter, pup weights of the treated males and females were comparable to those of the controls. There were no effects of treatment on total litter weight. In the F_1 parental males, the time until preputial separation was longer at 1500 ppm (44.8 days) than in the controls (43.7 days), indicating a slight delay in sexual maturation that was likely to be related to the decreased pup body weights beginning on postnatal day 15. However, the time to vaginal opening was unaffected by treatment. At 1500 ppm, relative liver weights (adjusted for body weight) were increased by 9–17%

in the F₁, F_{2a}, and F_{2b} pups. Also at this dose, absolute liver weights were increased by 14% in the F_{2a} females. However, because no clinical chemistry or histopathology analyses were performed, the increased liver weights were considered to be equivocal.

There were no effects of treatment on the pre-coital interval, number of females pregnant, number of complete litter resorptions, mating success, post-implantation loss, or duration of gestation in either generation. There were no effects of treatment on whole litter losses in the F₀ generation. However, in the F₁ generation, the number of whole litter losses was increased in the F_{2a} litter at 1500 ppm compared with controls. Therefore, the F₁ dams were mated a second time to produce the F_{2b} litters, and there was no effect on the number of whole litter losses, indicating that the finding in the F_{2a} litter was likely to be incidental.

The NOAEL for parental toxicity was 250 ppm (equivalent to 22.9 mg/kg bw per day in males and 24.5 mg/kg per day in females) on the basis of decreased body weights, body weight gains, food consumption, and food efficiency in males. The NOAEL for reproductive toxicity was 1500 ppm (equivalent to 146.3 mg/kg bw per day in males and 148.2 mg/kg bw per day in females) as no LOAEL was identified. The NOAEL for offspring toxicity was 250 ppm (equivalent to 22.9 mg/kg bw per day in males and 24.5 mg/kg per day in females), on the basis of decreased pup weights at 1500 ppm (Milburn, 2005b).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 24 time-mated female Alpk:AP₁SD (Wistar-derived) rats were given mandipropamid, (purity, 96.5%) at a dose of 0, 50, 200, or 1000 mg/kg bw per day by oral gavage in 0.5% aqueous carboxymethylcellulose in a treatment volume of 1 ml/100 g bw from day 5 until day 21 of gestation. All dams were killed on day 22 of gestation; fetuses were removed by cesarean section and examined.

All females survived to scheduled sacrifice, and there were no clinical signs of toxicity. There were no treatment-related, adverse effects on body weight, body-weight gains, food consumption, clinical chemistry, liver weights, or gross pathology at any dose.

There were no abortions, premature deliveries, or complete litter resorptions and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, resorptions (early or late) or on fetal sex ratio, or post-implantation loss, fetal body weights, gravid uterine weights, or skeletal ossification in the fetuses. There was no treatment-related effect on the ossification scores of the paws. There were no treatment-related external, visceral, or skeletal variations or malformations.

The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Moxon, 2005a).

Rabbits

In a study of developmental toxicity, 24 time-mated female New Zealand White rabbits were given mandipropamid (purity, 96.5%) at a dose of 0, 50, 250, or 1000 mg/kg bw per day by oral gavage in 0.5% aqueous carboxymethylcellulose in a treatment volume of 4 ml/kg bw from day 5 until day 29 of gestation. All surviving rabbits were killed on day 30 of gestation; fetuses were removed by caesarean section and examined.

There were no treatment-related mortalities and no adverse clinical signs of toxicity. There were no treatment-related, adverse effects on body weight, body-weight gains, food consumption, or gross pathology at any dose.

At 250 mg/kg bw per day, one female aborted; otherwise, there were no premature deliveries or complete litter resorptions and no effects of treatment on the numbers of litters, live fetuses, dead

fetuses, resorptions (early or late) or on fetal body weights, sex ratio, or post-implantation loss, fetal body weights, gravid uterine weights, or skeletal ossification in the fetuses. There were no treatment-related external, visceral, or skeletal variations or malformations. There was no treatment-related effect on the ossification scores of the paws.

The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Moxon, 2005b).

2.6 *Special studies*

(a) *Acute neurotoxicity*

In a study of acute neurotoxicity, groups of 10 male and 10 female fasted Alpk:AP_fSD (Wistar-derived) rats were given mandipropamid (purity, 96.5%) as a single dose at 0, 200, 600, or 2000 mg/kg bw by gavage in 0.5% carboxymethylcellulose (dose volume, 10 ml/kg bw) and were sacrificed on day 15 of the study. FOB and motor activity testing were performed on all rats before exposure, and on days 1 (at 2–4 h after dosing), 8, and 15. At study termination, five rabbits per group were anaesthetized and perfused in situ for neuropathological examination. The brain and peripheral nervous system tissues collected from the perfused animals in the control group and the group at 2000 mg/kg bw were subjected to histopathological evaluation.

No treatment-related effects were observed on mortality, clinical signs, body weight, body-weight gains, food consumption, FOB parameters, motor activity, or neuropathology.

The NOAEL was 2000 mg/kg bw, the highest dose tested (Milburn, 2005c).

(b) *Short-term study of neurotoxicity*

In a short-term study of neurotoxicity, groups of 12 male and 12 female Alpk:AP_fSD rats were given diets containing mandipropamid (purity, 96.5%) at a concentration of 0, 100, 500 or 2500 ppm (equal to 0, 7.4, 37.3 or 192.5 mg/kg bw per day in males and 0, 8.4, 41.0 or 206.7 mg/kg bw per day in females) for at least 90 consecutive days. Neurobehavioral assessment (FOB and motor activity testing) was performed in all rats at weeks –1, 2, 5, 9, and 14. At study termination, five males and five females per group were anaesthetized and perfused in situ for neuropathological examination. The tissues from the perfused animals in the control group and in the group at 2500 ppm were subjected to histopathological evaluation.

No adverse, treatment-related effects were observed on mortality, clinical signs, food consumption, functional observational battery parameters, motor activity, brain weights, or neuropathology. In males at 2500 ppm, body weights were decreased by 3% initially at week 1 and by 2–7% throughout the study, and decreased cumulative body weight (10–11%) was observed from week 10 until termination. Decreased food utilization (16%) was noted during weeks 5–8. Decreases were also observed during weeks 1–4 (6%) and weeks 9–13 (12%), leading to a decrease in overall food utilization in weeks 1–13 (9%). The effect on food utilization was of a similar magnitude to that observed on body weight and body-weight gain. There were no effects on body weight, body-weight gain, food consumption or food utilization in females. Slight increases of 13–26% were observed at 2500 ppm adjusted liver weight after perfusion in females, and in absolute and adjusted liver weights (without perfusion) in males and females. The effect on the liver was considered equivocal in the absence of any other evidence supporting hepatotoxicity. No neurological effects were observed at any dose in males or females.

The NOAEL was 500 ppm, equal to 37 mg/kg bw per day in males and 41 mg/kg bw per day in females, as no LOAEL was identified (Pinto, 2005c).

3. Observations in humans

No information was available.

Comments

Biochemical aspects

The extent of absorption of radiolabelled mandipropamid was similar in male and female rats dosed by gavage. Absorption was incomplete, with 67–74% of the administered dose being absorbed at the lower dose (3 mg/kg bw) and only 30–45% absorbed at the higher dose (300 mg/kg bw). Absorption was more rapid in females, with peak blood concentrations occurring at 4.5 h for the lower dose and 10 h at the higher dose, while for males the values were 8.5 h and 24 h, respectively. Little or no radioactivity was recovered in the expired air (less than 0.16% of the administered dose). Excretion was predominantly via the bile (lower dose) and faeces and > 90% of the administered dose was eliminated within 168 h. In males, a greater proportion of the administered dose was excreted in the faeces, while in females a significantly greater proportion was excreted via the urine. The greater extent of biliary elimination in males than in females was consistent with faecal excretion being the major route of elimination in males (73%) and of lesser importance in females (55%) at the lower dose. Some reabsorption of biliary metabolites was apparent at both doses. Tissue retention of the radiolabelled material was low, even after multiple doses. The total concentration of tissue residues including the carcass was < 0.3%, therefore demonstrating no evidence of bioaccumulation. The highest concentration of residues was found in the liver.

The biotransformation of mandipropamid was relatively simple since no cleavage of the molecule was observed. The major metabolic transformations involved loss of one or both of the propargyl groups of the molecule, followed by glucuronidation and *O*-demethylation, to produce six major metabolites. While the qualitative metabolite profile was largely independent of sex and dose, quantitative differences were found. Increasing the dose resulted in increasing amounts of radioactivity isolated as parent, indicating saturation of metabolic processes.

Toxicological data

Mandipropamid was of low acute toxicity in rats given a single dose orally ($LD_{50} > 5000$ mg/kg bw), dermally ($LD_{50} > 5000$ mg/kg bw) or by inhalation ($LC_{50} > 5.19$ mg/l). Mandipropamid was minimally irritating to the skin and eyes and was not found to be a dermal sensitizer (local lymph node assay in mice).

In short-term studies of toxicity with mandipropamid, the target organ was the liver, at doses that also resulted in decreased body weight and body-weight gain.

In a 90-day dietary study in rats, decreased body weight, body-weight gain and food consumption were observed at doses of 3000 ppm (260 mg/kg bw per day) and above. Various erythrocyte parameters (haemoglobin, erythrocyte volume fraction, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration) were decreased in males and females at doses of 3000 ppm (260 mg/kg bw per day) and above. Increases in liver weight (both sexes), plasma gamma-glutamyl transferase (58–105% in females only) at doses of 3000 ppm (260 mg/kg bw per day) and above were not considered to be adverse in the absence of findings of liver toxicity. The periportal hypertrophy/eosinophilia observed in the liver was considered to be treatment-related, but of questionable toxicological significance given the minimal to slight severity and the lack of any evidence of progression in the long-term study. The NOAEL for toxicity in rats was 500 ppm (41 mg/kg bw per day).

In dogs given capsules containing mandipropamid, increases in liver weights, cholesterol concentration, alkaline phosphatase and ALT activity were seen after 13 weeks at 100 mg/kg bw per day.

The NOAEL in the 90-day study in dogs was 25 mg/kg bw per day. In the 1-year study in dogs, body-weight gain and food consumption were decreased at 400 mg/kg bw per day, together with increased liver weight. At 40 mg/kg bw per day or above, there was also increased alkaline phosphatase and ALT activity and minimal to moderate pigmentation of the liver by porphyrin. The NOAEL in the 1-year study in dogs was 5 mg/kg bw per day. Considering the spacing of doses used and on the basis of the similarity of effects observed in the 90-day and 1-year studies in dogs, the overall NOAEL for dogs was 25 mg/kg bw per day.

In an 80-week study, mice were given diets containing mandipropamid at a concentration of 0, 100, 500 or 2000 ppm, equal to 0, 11, 55 or 223 mg/kg bw per day. There were no treatment-related changes in survival, or the incidence of tumours or non-neoplastic lesions. The only significant findings were observed at the highest dose: reductions in body weight and food conversion efficiency. There was no evidence of carcinogenicity with mandipropamid in this study. The NOAEL was 55 mg/kg bw per day.

In a 2-year (104-week) study, rats were given diets containing mandipropamid at a concentration of 0, 50, 250 or 1000 ppm, equal to 0, 3, 15 or 61 mg/kg bw per day. There were no treatment-related changes in survival or the incidence of tumours. The only significant findings were in males at the highest dose: reductions in body weight, body-weight gain and food conversion efficiency, gross and histopathological changes in the kidneys (roughened surface, and increased severity of chronic progressive nephropathy), and associated osteodystrophia fibrosa and histopathological changes in the parathyroid (increased severity of hyperplasia). Mandipropamid was not carcinogenic in this study. The NOAEL was 15 mg/kg bw per day.

Mandipropamid gave negative results in an adequate range of studies of genotoxicity in vitro and in vivo. The Meeting concluded that mandipropamid was unlikely to be genotoxic.

On the basis of the absence of carcinogenicity in rodents and the absence of genotoxicity, the Meeting concluded that mandipropamid is unlikely to pose a carcinogenic risk to humans.

In a multigeneration study of reproductive toxicity in rats, the target organ was also the liver. No reproductive effects were observed. The NOAEL for parental systemic toxicity was 250 ppm, equal to 22.9 mg/kg bw per day, on the basis of slightly lower body weight and body-weight gain in F₁ males during premating and increased absolute and relative liver weights in male and female parental animals sexes and in F₁ females. The NOAEL for reproductive toxicity was 1500 ppm, equal to 146.3 mg/kg bw per day, the highest dose tested. Toxicity observed in offspring at 1500 ppm included decreased pup weight from day 15 of lactation, increased liver weights in both generations and an increased time to preputial separation in male F₁ pups. The NOAEL for offspring toxicity was 250 ppm, equal to 22.9 mg/kg bw per day.

In a study of developmental toxicity in rats, the NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested. No developmental toxicity or teratogenicity was observed. In rabbits, no effects were observed in dams or fetuses at up to the limit dose of 1000 mg/kg bw per day.

In a study of acute neurotoxicity in rats, mandipropamid exhibited no systemic toxicity or evidence of neurotoxicity at 2000 mg/kg bw. In a 13-week study of neurotoxicity in rats, systemic toxicity was observed at 2500 ppm, equal to 192 mg/kg bw per day, as reductions in body weight, body-weight gain and food efficiency. No evidence of neurotoxicity was observed. The NOAEL was 37 mg/kg bw per day.

There were no reports of adverse health effects in manufacturing-plant personnel or in operators and workers exposed to mandipropamid formulations.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.2 mg/kg bw based on the NOAEL of 15.2 mg/kg bw per day, identified on the basis of decreased body weight and kidney effects (increased severity of chronic progressive nephropathy and associated osteodystrophia fibrosa) at 61.3 mg/kg bw per day in the long-term dietary study in rats and using a safety factor of 100.

The Meeting noted that mandipropamid was not acutely toxic after short-term dosing, that there were no adverse findings in a study of acute neurotoxicity and that mandipropamid did not exhibit developmental toxicity. The Meeting concluded that the establishment of an acute reference dose (ARfD) was unnecessary.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year studies of toxicity and carcinogenicity ^a	Toxicity	500 ppm, equal to 55 mg/kg bw per day	2000 ppm, equal to 223 mg/kg bw per day
		Carcinogenicity ^d	2000 ppm, equal to 223 mg/kg bw per day	—
Rat	Two-year studies of toxicity and carcinogenicity ^a	Toxicity	250 ppm, equal to 15 mg/kg bw per day	1000 ppm, equal to 61 mg/kg bw per day
		Carcinogenicity ^d	1000 ppm, equal to 61 mg/kg bw per day	—
	Multigeneration study of reproductive toxicity ^a	Parental toxicity	250 ppm, equal to 23 mg/kg bw per day	1500 ppm, equal to 146 mg/kg bw per day
		Offspring toxicity	250 ppm, equal to 23 mg/kg bw per day	1500 ppm, equal to 146 mg/kg bw per day
		Reproduction ^d	1500 ppm, equal to 146 mg/kg bw per day	—
	Developmental toxicity ^{a,b}	Maternal toxicity ^d	1000 mg/kg bw per day	—
		Embryo and fetal toxicity ^d	1000 mg/kg bw per day	—
Rabbit	Developmental toxicity ^b	Maternal toxicity ^d	1000 mg/kg bw per day	—
		Embryo and fetal toxicity ^d	1000 mg/kg bw per day	—
Dog	90-day and 1-year study of toxicity ^c	Toxicity	25 mg/kg bw per day ^e	40 mg/kg bw per day

^a Dietary administration.

^b Gavage administration.

^c Capsule administration.

^d Highest dose tested.

^e Based on an overall NOAEL from the two studies.

Estimate of acceptable daily intake for humans

0–0.2 mg/kg bw

Estimate of acute reference dose

Unnecessary

Information that would be useful for continued evaluation of the compound

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

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

<i>Absorption, distribution, excretion, and metabolism in mammals</i>	
Rate and extent of oral absorption	Rapid, extent dependent on dose, 67–74% at the lower dose, 30–45% at the higher dose
Distribution	Highest concentrations in the liver and kidney
Potential for accumulation	No evidence
Rate and extent of excretion	High, virtually complete by 168 h
Metabolism in animals	Mainly glucuronidation (> 50% of excreted dose mandipropamid glucuronide)
Toxicologically significant compounds (animals, plants and environment)	Parent
<i>Acute toxicity</i>	
Rat, LD ₅₀ , oral	> 5000 mg/kg bw
Rat, LD ₅₀ , dermal	> 5050 mg/kg bw
Rat, LC ₅₀ , inhalation	> 5.19 mg/l air
Rabbit, dermal irritation	Minimal irritation
Rabbit, ocular irritation	Minimal irritation
Mouse, dermal sensitization	Not sensitizing (local lymph node assay)
<i>Short-term studies of toxicity</i>	
Target/critical effect	Liver, body weight
Lowest relevant oral NOAEL	25 mg/kg bw per day (90-day and 1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day study in rats, highest dose tested)
Lowest relevant inhalation NOAEL	No data
<i>Genotoxicity</i>	
	Not genotoxic

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Body weight, kidney, parathyroid
Lowest relevant NOAEL	15 mg/kg bw per day (rats)
Carcinogenicity	Not carcinogenic in rats and mice

Reproductive toxicity

Reproduction target/critical effect	None
Lowest relevant reproductive NOAEL	146 mg/kg bw per day (rats, highest dose tested)
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	1000 mg/kg bw per day (rats, rabbits, highest dose tested)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity and studies of short-term neurotoxicity	No indications of neurotoxicity in studies of acute toxicity or repeat-dose studies
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Medical data

No occupational or accidental poisoning reported

Summary

	<i>Value</i>	<i>Study</i>	<i>Safety factor</i>
ADI	0–0.2	Rat, 2-year study	100
ARfD	Unnecessary	—	—

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