

MANDIPROPAMID (addendum)

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Explanation

Mandipropamid was reviewed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for the first time in 2008, when an acceptable daily intake (ADI) of 0–0.2 mg/kg body weight (bw) was established and it was concluded that an acute reference dose (ARfD) was not necessary.

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, mandipropamid was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review.

A 90-day feeding study in mice, an immunotoxicity study in mice and several mechanistic studies performed with the parent compound were submitted. In addition, an acute toxicity study and a number of genotoxicity studies with the metabolite (4-chlorophenyl)-prop-2-ynyloxy-acetic acid (SYN500003) were provided.

Evaluation for acceptable intake

1. Toxicological studies with mandipropamid

1.1 Short-term studies of toxicity

Groups of 10 male and 10 female C57BL/10JfCD-1 mice per dose were fed diets containing mandipropamid (batch no. SEZ2BP007; purity 96.5%) at a concentration of 0, 300, 800, 2000 or 5000 parts per million (ppm) (equal to 0, 37.2, 98.0, 247.6 and 624.3 mg/kg bw per day for males and 0, 47.3, 128.9, 315.8 and 800.5 mg/kg bw per day for females, respectively) for 90 days. The main purpose of the study was to identify appropriate doses for a subsequent carcinogenicity study, which was evaluated by JMPR in 2008. Over the course of the 90-day study, the animals were monitored for clinical signs and weighed regularly, and feed consumption was measured. At scheduled termination, cardiac blood samples were taken for haematology (clinical chemistry parameters were not measured). The mice were killed and grossly examined. Selected organs were weighed, and a

representative selection of organs and tissues was taken for histopathology. Microscopic examination of tissues and organs was confined to the control and high-dose groups, except for the livers, which were also examined in the other treated groups.

No premature mortality or clinical signs of toxicity were noted. The in-life findings attributed to treatment comprised slight, but dose related, reductions in body weight and body weight gain in male mice, which were statistically significant at the two highest doses. A statistically significantly lower mean terminal body weight in low-dose males was not part of a dose–response relationship and thus was most likely not related to treatment (see Table 2 below). In females, an initial (day 2) weight loss was seen in the two highest dose groups, whereas the only statistically significant effect at later stages of the study was a reduced body weight at the highest dose at termination (see Table 2 below). Lower feed consumption compared with the control groups was occasionally observed in both sexes at 5000 ppm and in males at 2000 ppm.

At termination, microcytic and hypochromic anaemia was apparent in both sexes at 5000 ppm and in females also at 2000 ppm, as haematocrit, mean cell volume and haemoglobin were statistically significantly reduced. Red blood cell count was not statistically significantly lower in males and not affected in females at all, suggesting a possible iron or cobalamin (B₁₂) deficiency behind the anaemia. In line with that, statistically significantly lower mean cell haemoglobin and lower erythrocyte size were noted in males of the 2000 and 5000 ppm groups and in females in all treatment groups (Table 1). However, the effects in females at 300 and 800 ppm, even though statistically significant, were minor. As the differences relative to the control values were less than 5%, these effects were considered to be of uncertain toxicological relevance. Furthermore, haematocrit and total haemoglobin were not affected in these female groups.

Table 1. Haematological parameters^a in the 90-day study with mandipropamid in mice

Parameter	0 ppm	300 ppm	800 ppm	2 000 ppm	5 000 ppm
Males					
Number examined	10	9	10	10	9
Red blood cell count ($\times 10^{12}/L$)	9.43 $\pm$ 0.20	9.57 $\pm$ 0.37	9.48 $\pm$ 0.25	9.45 $\pm$ 0.31	9.24 $\pm$ 0.30
Mean cell volume (fL)	50.1 $\pm$ 0.7	49.6 $\pm$ 0.6	49.6 $\pm$ 0.5	48.9** $\pm$ 1.0	49.0** $\pm$ 0.5
Haematocrit (%)	47.3 $\pm$ 1.3	47.4 $\pm$ 2.1	47.0 $\pm$ 1.4	46.2 $\pm$ 1.6	45.3** $\pm$ 1.3
Haemoglobin (g/dL)	14.4 $\pm$ 0.4	14.5 $\pm$ 0.6	14.3 $\pm$ 0.4	14.1 $\pm$ 0.5	13.7** $\pm$ 0.4
Mean cell haemoglobin (pg)	15.2 $\pm$ 0.1	15.1 $\pm$ 0.2	15.1 $\pm$ 0.2	14.9** $\pm$ 0.2	14.8** $\pm$ 0.3
Females					
Number examined	8	10	10	9	10
Red blood cell count ($\times 10^{12}/L$)	9.41 $\pm$ 0.29	9.67 $\pm$ 0.31	9.54 $\pm$ 0.32	9.29 $\pm$ 0.34	9.46 $\pm$ 0.23
Mean cell volume (fL)	50.2 $\pm$ 0.6	49.4* $\pm$ 0.5	48.4** $\pm$ 0.8	48.1** $\pm$ 0.8	47.8** $\pm$ 0.7
Haematocrit (%)	47.2 $\pm$ 1.5	47.7 $\pm$ 1.6	46.2 $\pm$ 1.9	44.7** $\pm$ 1.9	45.2** $\pm$ 1.1
Haemoglobin (g/dL)	14.5 $\pm$ 0.4	14.5 $\pm$ 0.5	14.1 $\pm$ 0.6	13.6** $\pm$ 0.6	13.8** $\pm$ 0.4
Mean cell haemoglobin (pg)	15.4 $\pm$ 0.2	15.0** $\pm$ 0.1	14.8** $\pm$ 0.2	14.6** $\pm$ 0.2	14.6** $\pm$ 0.2

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (analysis of variance)

^a Mean $\pm$ standard deviation.

Source: Milburn (2005)

There were no gross findings that could be attributed to treatment in any group. Dose-related increases in absolute liver weight were seen in both sexes at the two highest doses (Table 2). “Adjusted” organ weights, rather than relative organ weights, were the subject of statistical analysis

by the sponsor; this approach is based on a publication by Shirley (1977). As the increase in adjusted organ weights was statistically significant from 800 ppm onwards and, at 2000 and 5000 ppm, exceeded 10%, it is considered adverse. The liver weight increase was accompanied by cytoplasmic eosinophilia of periportal hepatocytes in most female mice in the 2000 and 5000 ppm groups and in half of the males at 5000 ppm. Surprisingly, no hypertrophy was reported.

Table 2. Organ weights^a in the 90-day study with mandipropamid in mice

Parameter	0 ppm	300 ppm	800 ppm	2 000 ppm	5 000 ppm
Males					
Number examined	10	10	10	10	10
Terminal body weight (g)	32.0 ± 2.8	29.9** ± 0.8	31.0 ± 1.4	30.5** ± 2.0	30.2** ± 1.3
Absolute liver weight (g)	1.35 ± 0.15	1.29 ± 0.08	1.40 ± 0.13	1.55** ± 0.17	1.71** ± 0.19
Relative liver weight (%)	4.22 ± 0.21	4.32 ± 0.23	4.51 ± 0.25	5.07 ± 0.35	5.66 ± 0.41
Liver weight adjusted for body weight (g)	1.27	1.34	1.38*	1.56**	1.75**
Absolute spleen weight (mg)	75 ± 6	69 ± 7	77 ± 11	73 ± 7	71 ± 9
Relative spleen weight (%)	0.235 ± 0.014	0.232 ± 0.021	0.248 ± 0.031	0.238 ± 0.020	0.235 ± 0.022
Spleen weight adjusted for body weight (mg)	72	72	76	73	73
Females					
Number examined	10	10	10	10	10
Terminal body weight (g)	24.4 ± 1.3	25.0 ± 1.0	24.5 ± 0.9	24.4 ± 1.3	23.4** ± 0.8
Absolute liver weight (g)	1.04 ± 0.10	1.09 ± 0.08	1.15 ± 0.15	1.21** ± 0.14	1.28** ± 0.10
Relative liver weight (%)	4.27 ± 0.26	4.36 ± 0.26	4.67 ± 0.48	4.95 ± 0.44	5.47 ± 0.31
Liver weight adjusted for body weight (g)	1.04	1.03	1.13*	1.20**	1.36**
Absolute spleen weight (mg)	93 ± 23	85 ± 7	82* ± 10	78** ± 7	74** ± 8
Relative spleen weight (%)	0.381 ± 0.091	0.338 ± 0.019	0.334 ± 0.037	0.319 ± 0.024	0.316 ± 0.034
Spleen weight adjusted for body weight (mg)	93	83	82*	78**	76**

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (analysis of variance and/or covariance, as proposed by the sponsor; based on Shirley, 1977); relative organ weights not statistically analysed

^a Mean ± standard deviation.

Source: Milburn (2005)

It is worth noting that a statistically significant, dose-related decrease in spleen weight was observed only in females from 800 ppm onwards (Table 2). The lower spleen weight was not in parallel to body weight, which was only marginally affected. However, a high variability in the control group was also noticed. No conclusion can be drawn if this organ weight change was

somehow related to the haematological findings. No evidence of extramedullary haematopoiesis in the liver or in the spleen was found. In one female at 5000 ppm, an increased amount of haemosiderin was detected in the spleen, whereas histopathological spleen findings were not reported for males. On balance, the toxicological relevance of the decrease in spleen weight is equivocal, and this finding is not considered a reliable basis for identifying the no-observed-adverse-effect level (NOAEL).

Based on haematological and liver weight effects at 2000 ppm (equal to 247.6 mg/kg bw per day), the NOAEL was 800 ppm (equal to 98.0 mg/kg bw per day). This NOAEL is higher than those identified in the short-term studies in dogs and rats that were previously reviewed by JMPR. Target organs in this study were the liver and the erythropoietic system, with possible involvement of the spleen. In addition, there were rather weak effects on body weight at higher doses. The liver findings were well in line with what was seen in rats and dogs. Effects on red blood cells resembled those noted in rats, also with regard to the doses at which they were observed (Milburn, 2005).

1.2 *Special studies*

(a) *Changes in the liver*

Biochemical and pathological changes in the liver subsequent to dietary administration of mandipropamid for up to 28 days were investigated in mice and rats.

In the first study, 20 male and 20 female C57BL/10Jf/Alpk mice (5 weeks old at the start of the study) received mandipropamid via their diet at a concentration of 0, 800, 2000 or 5000 ppm (equivalent to 0, 120, 300 and 750 mg/kg bw per day, respectively) for up to 28 days. Five males and five females per dose were killed following 7, 14 or 21 days of treatment or at study termination after 28 days. Three days before the respective scheduled terminations, the animals were surgically implanted with Alzet osmotic mini-pumps containing 5-bromo-2'-deoxyuridine (BrdU) at a concentration of 15 or 30 mg/mL (contradictory information given in the report) in 0.9% saline to facilitate assessment of possible hepatic proliferation.

Once a day, the animals were monitored for clinical signs. Body weights and feed consumption were recorded on a weekly basis. Urine was collected from the same five males and five females scheduled for termination on days 7, 14, 21 and 28. At interim or terminal kill, blood was taken by cardiac puncture, and a number of clinical chemistry parameters were determined. The liver was removed, weighed and subjected to histopathology. The number of apoptotic bodies per 1000 hepatocytes was measured by means of a histochemistry technique (terminal deoxynucleotidyl transferase deoxyuridine triphosphate [dUTP] nick end labelling [TUNEL]). Subcellular liver fractions were prepared, and total protein and cytochrome P450 (CYP) content, activities of cytochrome P450-mediated monooxygenases (ethoxyresorufin *O*-deethylase [EROD] and 7-pentoxeresorufin *O*-depentylase [PROD]) and glutathione *S*-transferases (GSTs), non-protein sulfhydryl levels and the extent of testosterone hydroxylation were determined in these samples. Apparently, most of these biochemical investigations (for total cytochrome P450 content, PROD, EROD and GST activities) were also performed on liver samples from another study. In this range-finding experiment, mandipropamid had been administered to mice (five of each sex per group) for 28 days at a dietary concentration of 700, 2100 or 7000 ppm.

There were no clinical signs of toxicity, but body weight tended to be lower at the highest dose from day 15 onwards. A liver weight increase was seen after 7 days of treatment in high-dose males and in females at the intermediate and high doses. In the animals killed at later time points, liver weight was consistently increased in the groups receiving 2000 or 5000 ppm. Clinical chemistry analysis in plasma revealed increases in cholesterol, gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST) at the high dose, but, surprisingly, decreases in alanine aminotransferase (ALT) and alkaline phosphatase (ALP). However, these findings were not consistent with regard to sex or duration of treatment. The most noteworthy histological finding in the liver was eosinophilia of the periportal region in both sexes at the intermediate and high doses, which was noted after 7 days of substance administration. After 28 days, but not before, apoptosis was reduced in females receiving 5000 ppm. Increases in EROD, PROD and GST activities in both sexes at 2000 and 5000 ppm

suggested an induction of xenobiotic metabolizing enzymes in the mouse liver. However, there was no evidence of increased liver cell proliferation or cytotoxicity. In the animals that had been treated for 28 days in the second study, increases in PROD and GST activities were observed in both sexes at 2100 and 7000 ppm, whereas EROD activity was lower in females at 700 and 2100 ppm but was not different from the control value at 7000 ppm (Dow, 2007a).

The same methods as in the study in mice (Dow, 2007a) were applied in two studies in Alpk:APfSD (Wistar-derived) rats, the results of which were reported together. In the first study, 20 male and 20 female rats were administered mandipropamid at a dietary concentration of 0, 100, 500 or 3000 ppm (equivalent to 0, 10, 50 and 300 mg/kg bw per day, respectively). Five males and five females from each dose group were killed after 7, 14, 21 or 28 days of treatment. In the second study, the only dietary concentration was 5000 ppm (equivalent to 500 mg/kg bw per day), and only male rats were employed. Ten animals from this treatment group and the control group were killed after 3, 7 or 14 days of treatment.

In addition, *in vitro* metabolism of mandipropamid was investigated in rat liver microsomes, partly following exposure to the cytochrome P450 inhibitors α -naphthaflavone, proadifen and furafylline.

There were no clinical signs of toxicity, and only a marginal decrease in body weight was noted in male rats receiving the test substance at 3000 ppm for 28 days. Liver weight was increased at 3000 and 5000 ppm, and this effect became more pronounced with duration of treatment. Liver cell hypertrophy was noted in the group receiving 3000 ppm after a minimum exposure of 21 days. This finding was more common and occurred earlier in females. No evidence of increased cell proliferation, cytotoxicity or an impact on apoptosis frequency was observed. Alterations in blood clinical chemistry parameters comprised increases in cholesterol, total protein and albumin in plasma at 3000 ppm, whereas triglycerides, AST and ALP were decreased. In the group receiving 5000 ppm, a small, transient increase in plasma glutamate dehydrogenase activity was observed after 3 and 7 days, whereas triglycerides were marginally decreased. Changes were generally small and not entirely consistent with regard to sex and time points of measurement.

PROD and GST activities in plasma were increased at 3000 and 5000 ppm. Higher GST activities were also noted in males at 500 ppm. In males, non-protein sulfhydryl levels were increased at 500 and 3000 ppm after 28 days of treatment. A rather strong increase in the activity of GGT in liver homogenates was observed in females at 3000 ppm. Thus, as in the mouse, there is good evidence for an induction of liver enzymes in the rat.

In vitro experiments revealed the abundance of at least four distinct metabolites, two of which could be identified and were the same as or similar to metabolites found *in vivo*. CYP2B and/or CYP1A2 were at least partially responsible for biotransformation in rat microsomes (Dow, 2007b).

In another study, liver cell proliferation was investigated in female Alpk:APfSD (Wistar-derived) rats. Groups of 30 animals either were fed a diet containing 5000 ppm mandipropamid (equal to 424 mg/kg bw per day) or were kept on basal diet for up to 15 days. Ten females per group were terminated on day 4, 8 or 15. Three days prior to first kill on day 4 or 7 days prior to termination on day 8 or 15, mini-pumps releasing BrdU at a rate of 10 μ L/hour were surgically implanted.

Throughout the study, the animals were monitored for clinical signs, body weight development and feed consumption. At termination, cardiac blood samples were taken for clinical chemistry measurements. Liver weight was determined, and liver samples were taken for histopathology, examination of BrdU incorporation or, by means of the TUNEL technique, determination of apoptosis frequency.

Apart from slight initial reductions in body weight and feed intake, treatment was well tolerated. The only alteration in clinical chemistry parameters that could be attributed to treatment was an increase in cholesterol levels in the mandipropamid-treated group from day 8 onwards, confirming

previous findings in male rats (Dow, 2007b) and in mice (Dow, 2007a). As was occasionally seen in these other studies, activities of ALT and AST were decreased after 2 weeks of feeding, but such an effect, even if treatment related, would not be considered adverse.

There were no gross lesions, apart from an enlarged liver in one animal in the 5000 ppm group. Mean liver weight (when adjusted for body weight) was 7%, 22% or 17% higher than in the control animals at termination on days 4, 8 and 15, respectively. However, histopathology of the liver did not reveal any findings that could be attributed to treatment. There was no impact on BrdU labelling index or on the number of apoptotic bodies. On balance, the results suggested that the test compound did not induce or stimulate liver cell proliferation, did not affect the cell cycle and had no effect on apoptosis (Lees, 2006).

(b) *Endocrine disrupting potential*

The endocrine disrupting potential of mandipropamid was examined in vitro and in vivo.

Estrogenic, anti-estrogenic, androgenic and anti-androgenic properties were investigated in recombinant *Saccharomyces cerevisiae* strains by testing the ability of mandipropamid to interact with the human estrogen receptor (ER) or androgen receptor (AR), which had been integrated in the yeast. 17 β -Estradiol (ER agonist), hydroxytamoxifen (ER antagonist), dihydrotestosterone (AR agonist) and flutamide (AR antagonist) were used as positive control substances; they all produced the expected effects, with which the activity of mandipropamid was compared. Activation of ER or AR would have become apparent by the stimulation of β -galactosidase owing to concomitant expression of the reporter gene *LacZ*, eventually resulting in a colour reaction that can be measured by absorbance. Antagonist activity was determined by the inhibition of agonistic effects of 17 β -estradiol and dihydrotestosterone.

Mandipropamid did not exhibit estrogenic, androgenic or anti-estrogenic activity at concentrations up to 1 mmol/L. It is not clear why testing was not performed at higher concentrations, as concentrations above 10 mmol/L were included for investigation of anti-androgenic properties, revealing equivocal responses. For clarification, an AR competitive binding assay was performed in which 17 α -methyltrienolone was used as the positive control substance. No evidence of AR binding was obtained, but the highest concentration was 0.5 mmol/L. These data are not considered suitable to confirm or dismiss the possible anti-androgenic effect at very high concentrations. On balance, this poorly reported study is considered unreliable and inconclusive (Moffat, 2001).

A uterotrophic assay was performed using six groups of 10 immature female Alpk:APfSD (Wistar-derived) rats. Two groups received mandipropamid by gavage at a dose of 500 or 1000 mg/kg bw per day for 3 days. In two additional groups, estradiol benzoate at a dose of 0.4 mg/kg bw per day was given together with the mandipropamid doses. The positive control group received only estradiol benzoate at 0.4 mg/kg bw per day for 3 days, and the negative control group was administered the vehicle (i.e. 1% carboxymethylcellulose in water) for 3 days.

The animals were observed for clinical signs, and body weight was recorded daily. Approximately 24 hours after the final dose, the rats were killed and grossly examined. The uteri were removed, and their wet weights were recorded.

No signs of toxicity were noted, and body weight was not affected. There were no gross findings at necropsy. Uterus weights were not increased over the vehicle control values when only mandipropamid had been given. Parallel administration of estradiol benzoate, in contrast, resulted in a marked increase in uterine weight that was similar to that seen in the positive control group.

To conclude, mandipropamid proved negative in this assay. The results suggest that the substance is neither estrogenic nor anti-estrogenic (Twomey, 2001).

(c) *Immunotoxicity*

An immunotoxicity study was conducted in which groups of 10 female CD-1 mice were fed mandipropamid for 28 days at a concentration of 0, 300, 1000 or 3000 ppm (equal to 0, 56, 187 and 649 mg/kg bw per day, respectively). A positive control group received cyclophosphamide by oral gavage at a dose of 10 mg/kg bw per day for 28 days. On day 25 of treatment, all animals received a single intravenous injection of 2×10^8 sheep red blood cells as the antigen. Blood samples were collected on day 29 for antibody detection. At termination on the same day, liver with gall bladder, spleen and thymus were taken and weighed.

Treatment with mandipropamid was well tolerated. There were no unscheduled deaths, clinical signs or effects on body weight, feed consumption or organ weights in any dose group. No remarkable findings were noted at necropsy. Specific immunoglobulin M (IgM) levels in serum were not different from those in the negative control group.

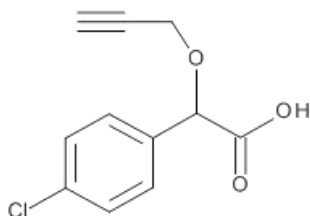
In contrast, an immunosuppressive effect of the positive control substance was indicated by a statistically significant decrease in serum antibodies (IgM) to the sheep red blood cells. In addition, mean absolute spleen weight was reduced, but this finding was most likely related to a somewhat lower body weight in this group, which might be treatment related. One animal from this group died on test.

On balance, there was no evidence that mandipropamid might affect the T cell-mediated immunological response in mice (Donald & Marr, 2011).

2. Toxicological studies with metabolite SYN500003

The chemical structure of metabolite SYN500003 is shown in Fig. 1.

Fig. 1. Chemical structure of SYN500003



2.1 Acute toxicity

An acute oral toxicity study using the “up-and-down procedure” was performed with female CD (CrL:CD (SD) IGS BR) rats. In total, 11 nulliparous animals 8–12 weeks of age at the start of the study were used. The test substance SYN500003 (batch no. KI-6311/5; purity not given) was suspended in arachis oil (as it did not dissolve in distilled water) and administered to fasted rats by oral gavage at a dosing volume of 10 mL/kg bw. Treatment was conducted in sequence, with at least a 48-hour interval between dosing of individual animals. Because no toxicity was expected, as suggested by available information, a limit test was first performed in five rats that received an oral dose of 2000 mg/kg bw. Based on unexpected deaths in this part of the study, three additional females were administered 2000 mg/kg bw, and three more received a dose of only 550 mg/kg bw. This latter dose was selected because it had been recommended by the statistical program used.

Rats were monitored for mortality and clinical signs, weighed and, after death on test or scheduled termination after the 2-week post-observation period, subjected to gross necropsy.

Three of eight rats receiving the high dose (2000 mg/kg bw) died within 1 day post-dosing, and three more females had to be killed for humane reasons on day 1 or 3. Signs of intoxication comprised hunched posture, lethargy, piloerection, diarrhoea, diuresis, dehydration, ataxia or tiptoe

gait, laboured respiration or a slower respiration rate, distended abdomen and pallor of the extremities. In the two surviving high-dose animals, signs had disappeared by day 4 or 5, with hunched posture being the most persistent. No deaths or clinical signs were noted in the animals receiving the low dose (550 mg/kg bw).

All animals that died on test or had to be killed prematurely lost weight, whereas all survivors gained weight over the post-observation period. In one of the two females surviving high-dose administration, body weight gain was observed only in the second week, but it was the highest among all animals.

In the premature decedents, common gross pathological findings comprised haemorrhagic or at least abnormally red lungs, dark liver and kidney. Gaseous distention of the stomach was also observed in one animal, whereas the non-glandular region of the stomach was haemorrhagic in another one. No abnormalities were seen in the survivors, irrespective of the dose administered.

A median lethal dose (LD₅₀) of 1049 mg/kg bw was calculated for female rats, with 95% confidence limits of 550 and 2000 mg/kg bw (Pooles, 2006). Based on this study, the metabolite is of moderate acute oral toxicity, but is more toxic than the parent mandipropamid, which has an LD₅₀ of greater than 5000 mg/kg bw (Moore, 2004).

2.2 Genotoxicity

SYN50003 was evaluated in three genotoxicity studies in vitro and one study in vivo. These studies are summarized in Table 3, and additional details are given below.

Table 3. Summary of genotoxicity studies with SYN500003

Type of study	Test system	Concentration/ dose range tested	Metabolic activation	Batch/ purity (%)	Result	Reference
In vitro						
Reverse gene mutation test in bacteria (Ames test)	<i>Salmonella typhimurium</i> TA98, TA100, TA1535 and TA1537 and <i>Escherichia coli</i> WP2 (pKM101) and WP2uvrA (pKM101)	100–5 000 µg/plate	±S9	KI-631/5, 99	Negative	Callander (2006)
Gene mutation in mammalian cells (mouse lymphoma assay)	Mouse lymphoma L5178Y cells (TK ^{+/−})	140.4–2 246.0 µg/mL	±S9	AMS 1201/2, 99.4	Negative, cytotoxic at maximum concentrations	Wollny (2013)
Chromosomal aberration	Human lymphocytes	14.6–2 250 µg/mL	±S9	AMS 1201/2, 99.4	Positive without activation, negative with activation	Bohnenberger (2013)
In vivo						
Micronucleus assay	Bone marrow cells of male NMRI mice	500–2 000 mg/kg bw (single dose)		AMS 1201/2, 99.4	Negative	Dony (2013)

bw: body weight; S9: 9000 × g supernatant fraction from rat liver homogenate; TK: thymidine kinase

No evidence of genotoxicity was obtained in the bacterial reverse gene mutation (Ames) assay in four *Salmonella typhimurium* and two *Escherichia coli* strains when tested according to the standard plate incorporation or the preincubation test protocols, with and without metabolic activation. The incubation period for all experiments was 3 days. All the experiments, with and without metabolic activation, were negative. In contrast, appropriate positive controls caused a distinct increase in mutation frequency (Callander, 2006).

Likewise, no increase in gene mutation frequency was observed in the in vitro thymidine kinase assay in L5178Y mouse lymphoma cells, either with or without metabolic activation. An exposure time of 4 hours was chosen for all experiments, followed by a 48-hour incubation of the cultures for expression and exponential growth. The positive control substances (methyl methane sulfonate in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) caused genotoxicity, proving the correct performance of the method and the test system. As no increase in small colonies of TK^{-/-} cells was noted with the test compound, no evidence of chromosomal alterations was found in this in vitro cell system. In contrast, both positive control substances produced an increase not only in general mutation frequency, but also in small colonies (Wollny, 2013).

In contrast, SYN500003 caused structural chromosomal aberrations in human lymphocytes in the absence of metabolic activation in one of two experiments. This finding achieved statistical significance from 734.2 µg/mL onwards and was dose related. The response was nearly equivalent to that of the positive control (Table 4). Substantial cytotoxicity, as evidenced by a more than 50% reduction in mitotic index, was confined to the highest concentration and thus cannot explain the positive response pattern. Moreover, there is no well-established link between mitotic index and frequency of aberrant cells. Some precipitation of the test item in the culture medium was observed only in the second experiment with activation at the maximum concentration. It should be noted that in the positive study, exposure time was longer (22 hours) and cells were immediately harvested, in contrast to the other experiments, with an exposure time of only 4 hours followed by an 18-hour recovery period before harvest and slide preparation. Another difference was that blood had been taken from a 29-year-old male donor for the first (negative) experiment, but from a 36-year-old female donor for the second (positive) one.

Correct performance of the test method and system was shown by clear increases produced by the positive control substances ethyl methane sulfonate and cyclophosphamide. No evidence of numerical aberrations (polyploidy) was observed in any experiment. The positive finding in vitro must be further investigated in vivo before a final conclusion on the clastogenic potential of this metabolite may be drawn (Bohnenberger, 2013).

Table 4. Structural chromosomal aberrations in the second experiment with SYN500003

Test item (concentration in µg/mL)	Without activation (22-hour exposure, immediate harvest)			With activation (4-hour exposure, 18-hour recovery)		
	Mitotic index (%)	Aberrant cells including gaps (%)	Aberrant cells excluding gaps (%)	Mitotic index (%)	Aberrant cells including gaps (%)	Aberrant cells excluding gaps (%)
Solvent control (DMSO)	100	0.5	0.5	100	1.0	1.0
EMS (770) ^a	29.4	53.0	51.0**	—	—	—
CPA (7.5)	—	—	—	52.9	13.0	12.5**
SYN500003 (419.8)	84.7	2.0	2.0	—	—	—
SYN500003	62.5	5.0	5.0*	88.6	1.5	1.5

Test item (concentration in µg/mL)	Without activation (22-hour exposure, immediate harvest)			With activation (4-hour exposure, 18-hour recovery)		
	Mitotic index (%)	Aberrant cells including gaps (%)	Aberrant cells excluding gaps (%)	Mitotic index (%)	Aberrant cells including gaps (%)	Aberrant cells excluding gaps (%)
(734.2)						
SYN500003 (1285.7)	53.3	8.0	7.5**	84.4	1.0	1.0
SYN500003 (2 250.0) ^a	33.1	38.0	38.0**	68.5	0.0	0.0

CPA: cyclophosphamide; DMSO: dimethyl sulfoxide; EMS: ethyl methane sulfonate; *: $P < 0.005$; **: $P < 0.001$ (Fisher's exact test)

^a In the absence of activation, only 50 instead of 100 metaphases per culture were evaluated, due to the strong clastogenic effect.

Source: Bohnenberger (2013)

In an in vivo micronucleus assay in mouse bone marrow, SYN500003 proved negative. The test item was suspended in 1% carboxymethylcellulose and, in the main study, administered once by oral gavage at a dosing volume of 10 mL/kg bw to seven male NMRI mice per dose and sampling time. Animals were killed, and bone marrow cells were collected at either 24 or 48 hours post-dosing. The doses were 500, 1000 and 2000 mg/kg bw for the 24-hour preparation interval, whereas the animals to be killed after 48 hours received only the limit dose of 2000 mg/kg bw. The negative (vehicle) and positive control groups consisted of five animals. The positive control substance was cyclophosphamide, applied at a dose of 40 mg/kg bw. Two negative control groups (killed 24 or 48 hours after treatment) but only one positive control group (killed at 24 hours post-dosing) were included.

Animals were monitored for mortality and the occurrence of clinical signs. Bone marrow was sampled, and slides were prepared for microscopic evaluation for signs of cytotoxicity and micronucleus formation. In 2000 erythrocytes per slide, the ratio between normochromatic erythrocytes (NCEs) and polychromatic erythrocytes (PCEs) was determined. Two thousand PCEs per animal were analysed for the presence of micronuclei, using the same slides.

In the high-dose group, one male mouse was found dead 24 hours after treatment with SYN500003. This single death may have been treatment related. The animal was discarded and not used for evaluation of clastogenicity. Signs of toxicity in the high-dose group comprised ruffled fur and a reduction in spontaneous activity. In a pretest, hunched back and eyelid closure had also been observed at the same dose. No evidence of systemic toxicity was obtained in the low- and mid-dose groups.

The micronucleus incidence in all the groups receiving SYN500003 was not different from the frequency noted in the negative control groups. In contrast, a clear increase in micronuclei was observed in the positive control group. The range of micronucleus occurrence in test group animals was 0–6 per 2000 PCEs, compared with 1–4 in the negative control animals but 26–53 in the positive control animals. Thus, the outcome of this in vivo assay was negative. However, the PCE:NCE ratio was not skewed in any group. Thus, there was no evidence of bone marrow toxicity, and, in the absence of absorption, distribution, metabolism and excretion data for this metabolite, it could not be proven that the bone marrow had, in fact, been exposed to the test item (Dony, 2013).

For clarification on bone marrow exposure, additional studies were performed to demonstrate the presence of SYN500003 in mouse plasma following oral administration (Britton, 2018; Kosar, 2018).

An analytical method for the determination of SYN500003 in mouse plasma was validated. The metabolite was recovered from plasma by protein precipitation, and the processed samples were analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) detection. Validation was performed for a wide range of concentrations (10–4000 ng/mL). Sufficient precision, accuracy and specificity could be shown. Stability of the metabolite in mouse plasma was also demonstrated (Kosar, 2018).

Three male Crl:CD-1 (ICR) mice were dosed with SYN500003 (batch no. AMS 1201/2; purity 99.4%) in 1% carboxymethylcellulose by gavage at a dose of 1000 mg/kg bw and a dosing volume of 10 mL/kg bw. All animals were observed for any visible signs of reaction to treatment, and body weights were recorded. Blood samples were taken at 0.5, 1, 4 and 24 hours after dosing, either from the tail vein or, at termination, from the orbital sinus. Animals were killed following their final blood sample and discarded without necropsy.

There were no deaths, and no clinical signs were observed during the study. Systemic exposure to SYN500003 was confirmed in all three animals by detectable circulating concentrations of SYN500003 in plasma at 0.5, 1, 4 and 24 hours after dosing (Table 5). As validation of the analytical method had been performed up to a maximum concentration of 4000 ng/mL, the samples collected at 0.5, 1 and 4 hours were diluted, as indicated in the table notes.

Table 5. Plasma concentrations of SYN500003 in male mice following oral dosing at 1000 mg/kg bw

Animal number	Hours after application	Plasma concentration (ng/mL)
1	0.5	386 000 ^a
	1	438 000 ^a
	4	51 800 ^b
	24	2 850
2	0.5	363 000 ^a
	1	380 000 ^a
	4	127 000 ^b
	24	442
3	0.5	415 000 ^a
	1	583 000 ^a
	4	299 000 ^b
	24	285

^a Diluted to below 4000 ng/mL: 1:200 dilution.

^b Diluted to below 4000 ng/mL: 1:100 dilution.

Source: Britton (2018)

As rather high concentrations of the metabolite were detected in plasma and as bone marrow is well perfused, it can be considered as proven that the bone marrow was adequately exposed in the micronucleus test (Dony, 2013), in which an even higher oral dose of 2000 mg/kg bw had been applied. It is not clear why a different mouse strain than the one used in the original micronucleus assay was used, but this difference is not considered to alter the conclusion (Britton, 2018).

Comments

Toxicological data

In a 90-day feeding study in mice, target organs were the liver and the erythropoietic system, with possible involvement of the spleen. The NOAEL was 800 ppm (equal to 98.0 mg/kg bw per day), based on evidence of slight anaemia, higher liver weight and cytoplasmic eosinophilia of hepatocytes at 2000 ppm (equal to 247.6 mg/kg bw per day). A lower spleen weight in females from 800 ppm onwards was of equivocal toxicological relevance (Milburn, 2005). The effects resembled those observed in other species, and the NOAEL was higher than those identified in short-term studies in rats and dogs (Annex 1, reference 115).

In a 28-day study in female mice, the T cell-mediated immunological response was not affected by administration of mandipropamid up to the highest dose of 3000 ppm (equal to 649 mg/kg bw per day) (Donald & Marr, 2011).

The Meeting considered that the mechanistic studies submitted did not provide any information relevant to the evaluation of mandipropamid.

Toxicological data on metabolites and/or degradates

The potato metabolite of mandipropamid, SYN500003, was more toxic than the parent compound in an acute oral toxicity study in rats, with an LD₅₀ of 1049 mg/kg bw (Pooles, 2006), compared with greater than 5000 mg/kg bw for the parent compound (Moore, 2004).

SYN500003 gave negative results in gene mutation tests in bacteria (Callander, 2006) as well as in mammalian cells (Wollny, 2013). However, it gave a positive response in a chromosomal aberration study in human lymphocytes (Bohnenberger, 2013). A negative result was seen in a micronucleus assay in mouse bone marrow in which 2000 mg/kg bw, the highest dose tested, produced clinical signs of toxicity; one possibly treatment-related death was also noted (Dony, 2013). In a “proof of exposure” study, high concentrations of the metabolite were detected in plasma following oral administration of 1000 mg/kg bw to rats (Britton, 2018). The abundance of SYN500003 in plasma can be considered as evidence that a sufficient amount had reached the bone marrow in the micronucleus test.

The Meeting concluded that SYN500003 is unlikely to be genotoxic in vivo.

Toxicological evaluation

The Meeting concluded that no revision of the ADI for mandipropamid was necessary.

For the metabolite SYN500003, the ADI for mandipropamid is not applicable, as the metabolite is structurally different from the parent compound and is more acutely toxic. No separate ADI or ARfD can be established for the metabolite, as the database is not sufficient for this purpose.

For chronic toxicity, the threshold of toxicological concern (TTC) concept for non-genotoxic substances (Cramer class III) is applicable to this metabolite.

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