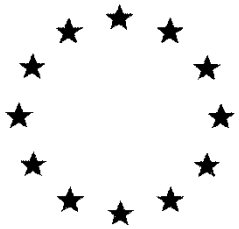
European Commission



Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

Napropamide-M

Volume 3 – B.6 (AS)

Rapporteur Member State: United Kingdom

Version History

When	What
June 2017	Initial DAR

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B.6. TOXICOLOGY AND METABOLISM DATA

Napropamide-M is a new active substance belonging to the chemical group of alkanamides. It is a selective systemic herbicide, absorbed by the roots and translocated acropetally. It inhibits root development and growth and is used for pre-emergence control of grasses and broad-leaved weeds in a wide range of crops.

Napropamide-M is the resolved isomer ((R)-(-)-N,N-diethyl-2-(1-naphthyloxy)propionamide) of the napropamide racemic mixture ((RS)-N,N-diethyl-2-(1-naphthyloxy)propionamide) containing the R and S isomers (also known as D and L forms, respectively) in a 1:1 ratio. The applicant believes that the R-isomer has 1.6 to 2 times the level of biological activity of the racemate.

A comprehensive database on napropamide racemate was evaluated for the approval of napropamide. The EFSA Conclusion (EFSA, 2010) was published on 29/4/2010. There were no data gaps, issues that could not be finalised or critical areas of concern relating to the toxicology assessment. The approval of the active entered into force on January 1st 2011 (Commission Directive 2010/83/EU of 30 November 2010).

The applicant for napropamide (racemate) was United Phosphorus. The applicant for napropamide-M is UPL Europe Limited, previously known as United Phosphorus. The RMS for napropamide was Denmark and wherever possible the text of the napropamide DAR has been used. In some cases no changes were necessary; however some changes were made to improve readability and to correct spelling mistakes and typographical or grammatical errors.

The following toxicology studies and additional information on napropamide-M were provided:

- ADME study in rats
- 28 day oral study in rats (dose range-finding study)
- 90 day oral study in rats
- Acute toxicity studies
- Genotoxicity studies
- Medical surveillance of manufacturing personnel.

These studies are new and have not previously been evaluated; they have been submitted by the applicant in support of the approval of napropamide-M. For all other end points no new data have been submitted. All study protocols for the new studies fully followed the respective OECD test guidelines, unless stated otherwise.

On the basis that the ADME and toxicity studies on napropamide racemate will have assessed the combination of both napropamide enantiomers, the applicant has developed a strategy to bridge to the existing ADME and toxicology studies on napropamide racemate for napropamide-M. The applicant's new active substance submission includes new studies conducted on napropamide-M to support bridging to the napropamide racemate database.

In summary, a metabolism study and a 90-day toxicity study in the rat on napropamide-M, with an equivalent high dose of napropamide racemate, demonstrated that both compounds exhibited comparable metabolism and toxicological properties. In addition the results of the acute toxicity studies for napropamide-M are consistent with those of napropamide racemate and they are therefore considered to have equivalent acute toxicity.

The opinion of the RMS is that napropamide-M (i.e. the R isomer) and the racemate have equivalent toxicity. Therefore, where appropriate, toxicity studies conducted on napropamide racemate have been used to satisfy a number of data points for napropamide-M.

Terminology

Note that napropamide racemate is also referred to as Devrinol® (technical) and R-7465 (technical) within this document.

Methods of analysis

Appropriate methods of analysis for all of the studies using napropamide-M have been provided (Report No. 228-2-13-6178, Raithatha, 2015; Report No. 228-2-13-7271, Raithatha, 2013; Report No. 228-2-14-7333, Sriram, 2014). These methods were fully validated in accordance with SANCO/3030/99 rev.4 (see Volume 3 CA

Section B.5). Procedural recoveries, where available, were checked and found to be acceptable. The methods of analysis using napropamide were considered valid for the approval of napropamide and have therefore been accepted.

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

The absorption, distribution, excretion and metabolism of napropamide racemate were investigated in rats by the oral route (single and repeat-dose). Four studies on napropamide racemate from the napropamide DAR are presented with some minor modifications to improve their readability.

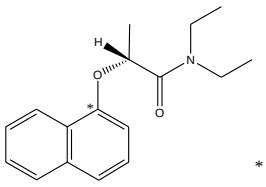
The absorption, distribution, excretion and metabolism of napropamide-M were investigated in rats by the oral route (single dose). This new study was submitted by the applicant in support of the approval of napropamide-M and has been evaluated for the first time.

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

B.6.1.1.1. Tissue distribution and elimination of napropamide-M in the rat – single oral study

Introduction

This is a new study; it was conducted to support the application for approval of napropamide-M and has been evaluated for the first time, here. This is the only available ADME study on napropamide-M.

Author(s):	[redacted] 2015
Study title:	Napropamide-M: the metabolism of [^{14}C] napropamide-M following oral administration in the rat. UPL Europe Ltd, Company Report No.: 34510 (unpublished) [redacted]
Test substance (non-radiolabelled):	Napropamide-M (CAS 41643-35-0)
Purity:	99.85 % (total D- + L-isomer)
Batch no.:	UPH-08/DNE-263/D-PURE/20121222
Test substance (radiolabelled):	Napropamide-M  * denotes position of ^{14}C
Purity:	Specific activity 56.10 mCi/mmol (7.60 MBq/mg), radiochemical purity 97.2 %
Batch no.:	7042CDB017-7
Test animals:	Sprague-Dawley rats ([redacted]) (weight: ♂: 238 g to 294 g, ♀:208 g to 267 g at dosing in main study, age: 7 – 9 weeks)
Groups:	4 rats/sex
Dose:	Phase 1 and 3: 30 mg/kg bw (range 29.2 - 33.3 mg/kg bw) Phase 2 and 4: 300 mg/kg bw (range 281 - 302 mg/kg bw)
Route:	Oral by gavage
Vehicle:	PEG 600
GLP:	Yes (certified laboratory)
Guideline:	OECD 417 (2010), EPA OPPTS 870.7485 (1998), JMAFF 12 Nohsan No 8147 (2000)
Deviation:	None
Acceptability:	Acceptable

Methods

The absorption, excretion and tissue distribution of radioactivity was investigated in rats administered a single oral gavage low or high dose of [^{14}C]-napropamide-M. The experimental groups were as follows:

Group	Number and Sex	Route and dose level of [^{14}C]-napropamide-M	
Phase 1	4 male, 4 female	Single oral dose (30 mg/kg)	Excreta, CO_2, collection for 4 days and selected tissues taken for analysis.
Phase 2	4 male, 4 female	Single oral dose (300 mg/kg)	
Phase 3	4 male, 4 female	Single oral dose (30 mg/kg)	Blood-sample collection for 4 days.
Phase 4	4 male, 4 female	Single oral dose (300 mg/kg)	

Rats used for the excretion phases were housed individually in metabolism cages. Rats used for all other phases were housed by sex in pairs in metabolism cages. In Phase 1 and 2, urine and faeces were collected at intervals over 4 days. Expired radioactivity (Phase 1 and 2 only) was also collected from 0 to 24 hours and 24 to 48 hours post dose. At termination of the study the following tissues were taken for radioactivity analysis: adrenals, muscle (leg), bone mineral, peri-renal fat, brain, plasma, gastrointestinal tract and contents, residual carcass, heart, spleen, kidneys, testes/ovaries, liver, thyroid, lung, uterus, whole blood.

In Phase 3 and 4, to investigate pharmacokinetics, serial blood samples were collected 5, 15 and 30 minutes, then 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours post dose.

All samples were counted for radioactivity by liquid scintillation counting either directly or following tissue digestion or sample oxidation. Combustion of standards showed that recovery efficiencies were in excess of 97 % throughout.

Findings

Absorption

In rats in the low-dose group, the apparent oral absorption, based on urinary excretion, the cage wash and the amount remaining in the tissues, amounted to 61-67 % of the administered dose.
In rats in the high-dose group, the apparent oral absorption, based on urinary excretion, the cage wash and the amount remaining in the tissues, amounted to 42-52 % of the administered dose.

Blood kinetics

Following a 30 mg/kg bw dose, radioactivity was detectable in plasma from the first sampling time of 5 minutes. Plasma concentrations continued to increase for several hours after dose administration, attaining a mean C_{max} of 4.12 µg equiv/mL at a median T_{max} of 6 hours. Thereafter, plasma concentrations gradually declined and by the last sampling time of 96 hours, mean plasma concentrations were below the limit of reliable measurement. The calculated mean half-life ($T_{1/2}$) was 14.8 hours. The calculated mean $AUC_{(0\ t)}$ and $AUC_{(0\text{-}inf)}$ values were 100 and 103 µg equiv.h/mL, respectively.

Following a 300 mg/kg bw dose, radioactivity was detectable in plasma at the first sampling time of 5 minutes. Plasma concentrations then increased for several hours after dose administration, attaining a mean C_{max} of 37.2 µg equiv/mL at a median T_{max} of 6 hours. Thereafter, mean plasma concentrations gradually declined and by the last sampling time of 96 hours, mean plasma concentrations were below the limit of reliable measurement. The calculated mean half-life ($T_{1/2}$) was 18.6 hours. The calculated mean $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}inf)}$ values were 773 and 803 µg equiv.h/mL, respectively (Table 6.1.1).

Table 6.1.1
Concentration of radioactivity in plasma and pharmacokinetic parameters in male rats administered a single oral dose of [^{14}C]-napropamide-M (µg equiv./g)

	30 mg/kg bw	**300 mg/kg bw**
5 m	0.70	1.5
15 m	1.62	4.5
30 m	2.07	9.3
1 h	2.08	16.0
2 h	2.32	22.5
4 h	3.65	30.1
6 h	4.06	35.4
8 h	3.17	32.0
12 h	2.91	19.6
24 h	1.69	13.1
48 h	0.50	3.0
72 h	0.15	1.3
96 h	0.07	0.9
C_{max} (µg.equiv./mL)	4.12	37.2
T_{max} (h)	6.0	6.0
$AUC_{(0-t)}$ (µg equiv.h/mL)	100	773
$AUC_{(0-inf)}$ (µg equiv.h/mL)	103	803
$T_{1/2}$ (h)	14.8	18.6

Excretion

At the low dose the main route of excretion was via urine (53 to 60 % of the administered dose) and 29 to 37 % in faeces. At the higher dose the main (marginally) route of excretion was via faeces (48 to 55 % of the administered dose) and 37 to 47 % in urine; there appears to be a difference in the pattern of excretion at the low and high dose.

Total recovery of radioactivity (low and high dose) was in the range 97 to 100 %. See Table 6.1.2, below.

Table 6.1.2
Excretion of radioactivity by male and female rats administered a single oral dose of [^{14}C]-napropamide-M (% administered dose)

	30 mg/kg bw				300 mg/kg bw			
	Male		Female		Male		Female	
Time (h)	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
0-6	10.6	9.3	16.3	6.9	4.2	30.8	3.9	28.2
6-24	25.8		31.4		21.4		30.4	
24-48	12.9	17.2	9.3	13.0	8.7	13.7	11.3	14.3
48-72	3.4	8.2	2.0	7.2	1.7	8.9	0.9	4.7
72-96	0.7	1.9	0.4	1.4	0.8	1.4	0.2	0.9
Total	53.3	36.6	59.5	28.5	36.9	54.8	46.7	48.1
Cage wash	6.8		6.8		4.4		4.7	
Expired air	0.0		0.0		0.0		0.0	
Total excreted	96.7		94.8		96.2		99.6	
Total recovery[a]	98.0		97.3		97.0		100.1	

[a] including dose present in tissues and residual carcass

At both dose levels and in both sexes, more than 90 % of the dose was excreted within 72 hours of dosing. Radioactivity measured in expired air was negligible.

Tissue Residues / Metabolism

For details of the codes used for metabolites (as well as their structures and synonyms) see Table 6.1.13**.**

30 mg/kg bw urine

At least 23 metabolites were detected in urine from male rats given the 30 mg/kg bw dose. Many of these were not fully resolved. The majority of the identified components were assigned as conjugates of hydroxy and dihydroxy DE-NPAM and glucuronide conjugates of hydroxylated napropamide-M. A sulphate conjugate of hydroxy-acid napropamide-M was only detected in urine from male rats given the 30 mg/kg bw dose. Two isomers of unconjugated hydroxy DE-NPAM and NOPAM were present. No other unconjugated metabolites were identified. The 11 metabolites that remained unassigned accounted for a total of 12.9 % of the administered dose. Individually, unassigned metabolites accounted for 0.6-1.9 % of the administered dose.

At least 15 metabolites were detected in urine from female rats given the 30 mg/kg bw dose. The most abundant metabolite was a glucuronide conjugate of a hydroxy napropamide-M isomer. Glucuronide conjugates of another hydroxy napropamide-M isomer and of dihydroxy napropamide-M were detected, as were unresolved hydroxy and dihydroxy DE-NPAM sulphate conjugates. A glucuronide conjugate of hydroxy DE-NPAM and two isomers of unconjugated hydroxy DE-NPAM were identified. Two isomers of unconjugated hydroxy napropamide-M and NOPAM were identified. The four metabolites that remained unassigned accounted for a total of 8.1 % of the administered dose with the individual unassigned metabolites ranging from 1.2 to 2.6 % of the administered dose.

30 mg/kg bw faeces
Napropamide-M was not detected in male rats given a 30 mg/kg bw dose and accounted for only 0.3 % of the applied dose in females at the same dose. The most abundant components were hydroxy napropamide-M. At least 11 components, which accounted for a total of 20.41 % of the administered dose, were detected in faeces from male rats given a 30 mg/kg bw dose but they were not identified, for analytical reasons. Individually, these unassigned metabolites ranged from 0.6 to 4.2 % of the administered dose. A broad region that eluted from *ca* 14-47 min was observed in faeces from female rats given 30 mg/kg bw and was deemed to comprise numerous

unresolved metabolites. These were not sufficiently resolved to allow quantification of individual components but the region was quantified as a whole and accounted in total for 12.1 % of the administered dose.

300 mg/kg bw urine

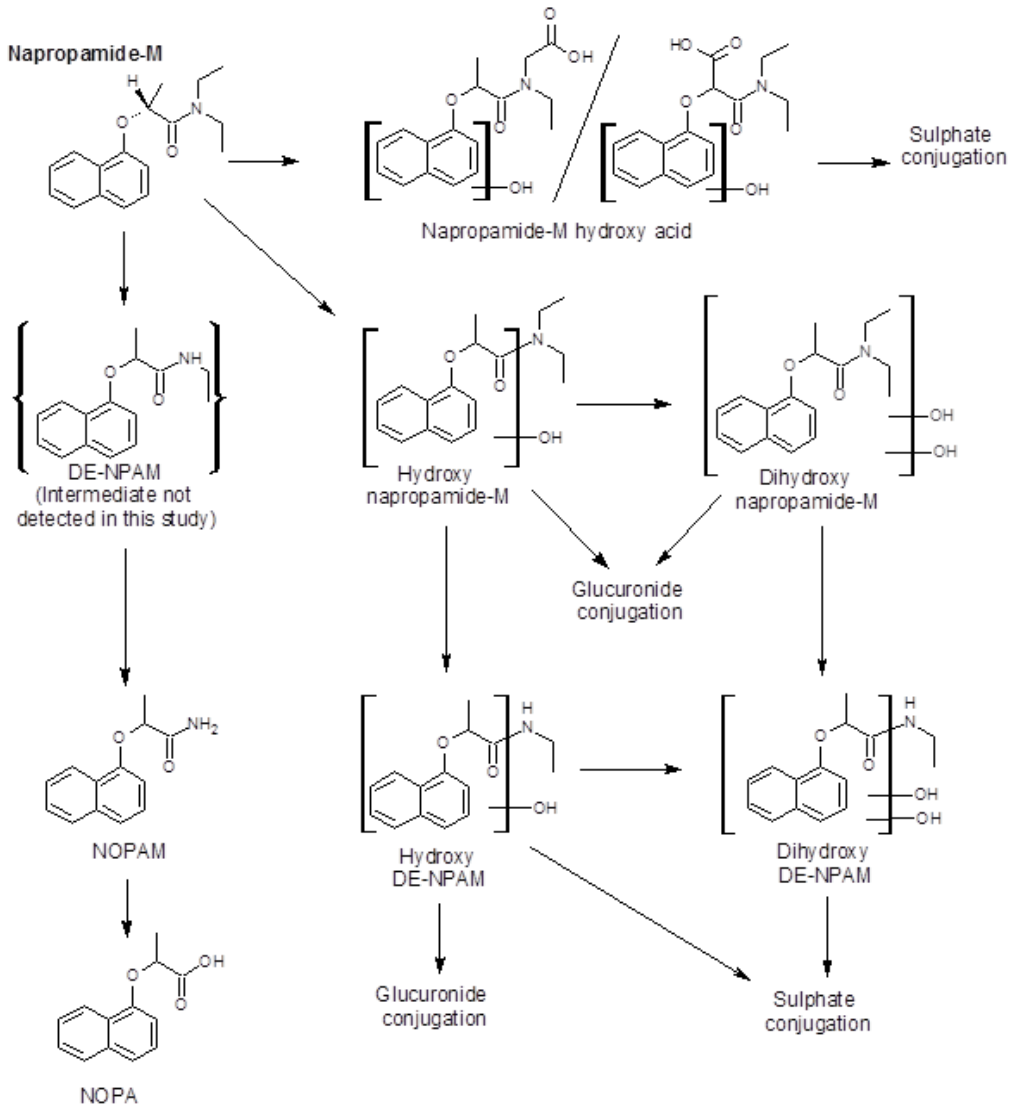
At least 13 metabolites were detected in urine from male rats. The majority of these were glucuronide and sulphate conjugates of hydroxy and dihydroxy DE-NPAM and glucuronide conjugates of hydroxy and dihydroxy napropamide-M. Two isomers of unconjugated hydroxy DE-NPAM were present. No other unconjugated metabolites were identified. The five metabolites that remained unassigned accounted for a total of 9.1 % of the administered dose. The majority accounted for 0.8 to 1.6 % of the dose while the most abundant which eluted in the wash-off phase accounted for 4.1 % of the dose.

At least 10 metabolites were detected in urine from female rats given the 300 mg/kg bw dose. The most abundant metabolite was a glucuronide conjugate of a hydroxy napropamide-M isomer. Glucuronide conjugates of another hydroxy napropamide-M isomer and of dihydroxy napropamide-M, unresolved hydroxy and dihydroxy DE-NPAM sulphate conjugates were present. A single isomer of unconjugated hydroxy DE-NPAM was identified. NOPAM was not detected. The four metabolites that remained unassigned accounted for a total of 10.9 % of the administered dose with the individual unassigned metabolites ranging from 1.4 to 4.2 % of the dose.

300 mg/kg bw faeces

Napropamide-M was present in faeces, accounting for a total of 54.8 and 48.1 % of the administered dose in males and females, respectively. The most abundant component in both sexes was a hydroxy napropamide-M isomer. A second hydroxy napropamide-M isomer was also present. Dihydroxy napropamide-M was detected in faeces from male rats but was not detected in faeces from female rats given the same dose. At least 16 components, which accounted for a total of 19.4 % of the applied dose, were detected in faeces from male rats but were not identified. Individually, unassigned metabolites ranged from 0.4 to 3.3 % of the dose. In faeces from female rats at least six metabolites were detected but not identified. In total these accounted for 10.3 % of the dose with individual unassigned metabolites in the range 1.25 to 2.74 % of the dose.

Figure 6.1-1
Napropamide-M: proposed metabolic pathway in rats



Conclusions

Napropamide-M was rapidly excreted and extensively metabolised at both dose levels and independent of sex. The excretion of radioactivity was approximately equally split between urine and faeces. The highest tissue concentrations of radioactivity, 96 hours after dosing, were present in the organs of metabolism and elimination: the liver and kidney. Peak plasma concentrations were achieved 6 hours after dosing. Generally, systemic exposure to total radioactivity increased approximately proportionally with dose.

The major metabolic routes were:

- Dealkylation to form NOPAM
- Oxidation of NOPAM to form NOPA
- Mono and di-hydroxylation of napropamide-M with subsequent dealkylation forming mono and di-hydroxy DE-NPAM
- Glucuronide conjugation of mono and di-hydroxylated napropamide-M
- Glucuronide and sulphate conjugation of hydroxylated DE-NPAM

With the exception of unassigned metabolites in faeces from female rats given a 30 mg/kg bw dose, unassigned metabolites were quantified and no individual metabolite accounted for >4.19 % of the administered dose.

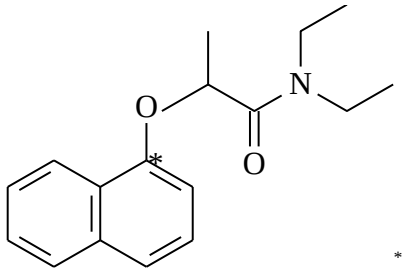
Chiral analysis of unchanged parent present in the excreta of rats administered napropamide-M shows that no systemic racemisation of the parent occurs.

B.6.1.1.2. Tissue distribution and elimination in the rat – single oral study

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed. The final conclusions drawn on the basis of all of the ADME studies on napropamide racemate have not changed and are as described in the EFSA Conclusion (EFSA, 2010).

Author(s): [redacted], 1970
Study title: Metabolism of R-7465-^{14}C[2-(µ-naphthoxy) N, N-diethylpropionamide]: balance and tissue residue elimination studies in the rat
Test substance: Napropamide, non-labelled and radio-labelled



* Position of ^{14}C

Purity: Specific activity 8.1 mCi/mmole, radiochemical purity ≥ 99 %
Purity of non-labelled napropamide not mentioned

Batch no.: Not mentioned
Test animals: Simonsen Albino rats ([redacted]) (weighing 139 – 181 g at dosing in main study)
Groups: 2 rats/sex in balance study; 6 rats/sex in tissue residue study
Dose: All rats were preconditioned with non-labelled napropamide administered in safflower oil by gavage once every day for 4 days prior to main study. The doses were 5.0 mg/kg bw/day or 25.0 mg/kg bw/day, but the report does not mention which rats and which dosage.
Balance study: Single dose of approx. 30 mg/kg bw (range 29.6 – 32.6)
Residue study: Single dose of approx. 195 mg/kg bw (range 158 – 214)
Route: Oral by gavage
Vehicle: Safflower oil
Statistics/ measurements: Statistics not performed
GLP: No (when this study was performed, GLP was not compulsory)
Guideline: None cited (this study was conducted before official guidelines were implemented. The study design, in part, resembles US EPA Guideline 85-1)
Deviation: Only material balance and tissue residue elimination phases were investigated. The use of 2 rats per sex in the balance study rather than 4 is considered scientifically acceptable when there is no sex difference. However, 2 rats/sex are generally not sufficient to demonstrate a sex difference.
Acceptability: Partially acceptable

Methods

Sixteen albino rats were preconditioned with non-radioactive napropamide in safflower oil by oral intubation at approximate dose levels of 25 mg/kg bw/day or 5.0 mg/kg bw/day for four days. A single oral dose of ^{14}C-napropamide was administered to two male and two female rats at the approximate dose level of 30 mg/kg bw. The animals were housed individually in metabolism cages to investigate elimination. The remaining 12 animals were administered a single oral dose of ^{14}C-napropamide at the approximate dose level of 195 mg/kg bw and housed in pairs (one male and one female). These animals were used to investigate tissue residue elimination.

Faeces, urine and NaOH from CO_2 traps were collected from rats from the balance study at intervals of 0-18, 18-28, 28-48, 48-72 and 72-96 hours. Urine and faeces samples from the tissue residue elimination study were kept frozen for future characterisation studies. Rats were sacrificed in groups of four on 1, 2, 4 and 8 days after dosing. The following tissues/organs were collected for analyses of radioactive residues: blood, brain, fat (visceral), gonads, hide (approx. 4 cm^2 from ventral side), kidneys, liver, hind leg muscle, spleen, intestine including stomach, and the remaining carcass. The radioactivity from urine, NaOH from CO_2 traps, faeces, organ/tissues and carcass was counted in a scintillation counter following appropriate homogenisation, solubilisation and/or dilution treatments.

Results

^{14}C-Napropamide was absorbed, metabolised and excreted rapidly. Primary routes of excretion were via urine and faeces. Approximately 46 % of the administered dose was excreted in the urine within 28 hours after dosing. Approximately 36.6 % of the administered dose was excreted in the faeces within 48 hours after dosing. No detectable radioactivity was found in the expired air. The excretion patterns for males and females were similar, and the average total recovery of administered radioactivity was 98.6 % (97.3 to 102.2 %). See Table 6.1.3.

The tissue residue elimination study showed no selective storage of ^{14}C-napropamide residues in any tissue/organ examined. The concentrations of radioactivity in tissues/organs at early intervals were not unusually high and could only be detected in blood, liver, kidney and carcass 8 days after dosing. The highest concentration 8 days after dosing was found in blood (2.6 µg equivalents/g in males), and the lowest detectable concentration was present in the residual carcass (0.1 µg equivalents/g in females) 8 days after dosing. According to the report, there was no difference in excretion pattern between rats receiving lower doses (30 mg/kg bw - excretion study) and those receiving higher doses (195 mg/kg bw - tissue residue elimination study) but excretion data for the high-dose rats were not reported. See Table 6.1.4.

Table 6.1.3: Recovery of radioactivity expressed in % of orally administered dose (30 mg/kg bw, 4 days preconditioning)

	Collection period					Min	Max
	0-18 h	0-28 h	0-48 h	0-72 h	0-96 h	0-96 h	0-96 h
Urine							
M	39.8	43.7	47.6	48.8	49.3	47.7	50.8
F	45.6	48.5	51.2	52.5	52.8	51.0	54.6
M+F	42.7	46.1	49.4	50.6	51.0	47.7	54.6
Cage wash							
M					5.6	1.6	9.5
F					10.1	6.5	13.6
M+F					6.5	1.4	13.6
Faeces							
M	35.0	36.9	38.8	41.3	42.7	37.4	48.0
F	30.8	31.8	34.4	37.8	38.7	30.6	46.7
M+F	32.9	34.3	36.6	39.6	40.7	30.6	48.0
Tissues							
M					0.37	0.27	0.47
F					0.46	0.37	0.54
M+F					0.41	0.27	0.54
Total							
M	74.8	80.6	86.4	90.1	99.8	97.4	102.3
F	76.3	80.2	85.6	90.3	97.5	95.1	99.8
M+F	75.6	80.4	86.0	90.2	98.6	95.1	102.3

Table 6.1.4: Summary of total ^{14}C residues in tissues and organs 1, 2, 4 and 8 days after single oral dose of 195 mg/kg bw (4 days preconditioning) (ppm, µg/g)

	Analysis intervals							
	24 h		48 h		96 h		8 days	
Sample	M	F	M	F	M	F	M	F
Blood	33.2	5.9	4.7	3.7	1.2	1.0	2.6	2.3
Brain	-	-	-	-	-	-	-	-
Fat	23.5	15.6	-	-	-	-	-	-
Gonads	8.4	16.7	-	-	-	-	-	-
Hide	9.3	9.5	1.8	1.3	0.2	1.3	-	-
Kidney	15.0	7.9	3.7	3.6	0.3	0.5	0.8	0.4
Liver	7.6	3.3	3.3	3.2	0.6	0.7	1.1	1.0
Muscle	1.1	0.7	-	-	-	-	-	-
Spleen	7.8	5.2	1.4	-	0.2	-	-	-
Intestine	80.0	145.5	45.0	23.6	0.1	0.4	-	-
Carcass	2.7	2.3	0.8	0.5	0.2	-	0.2	0.1
Total body	29.0	22.0	7.6	4.5	0.1	0.1	0.3	0.2

- Indicates radioactive residues below limit of detection for tissues

Conclusion

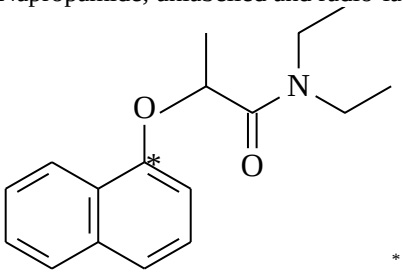
Radioactivity was rapidly absorbed and eliminated. Approximately 98.6 % of the administered radioactivity was eliminated in urine and faeces within 96 hours; 57.5 % in the urine and 40.7 % in the faeces. No detectable radioactivity was found in the expired air. Tissues and organs contained approximately 0.41 % of the administered radioactivity. The excretion patterns for males and females were similar, but 2 rats/sex in the balance study is too few animals for an adequate comparison between the sexes. Radioactivity associated with napropamide and its metabolites was eliminated rapidly and almost completely from faeces and urine of the rat. This indicates that no accumulation of the test compound occurs in animals.

B.6.1.1.3. Tissue distribution and elimination in the rat – single oral study

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed. The final conclusions drawn on the basis of all of the ADME studies on napropamide racemate have not changed and are as described in the EFSA Conclusion (EFSA, 2010).

Author(s): [redacted] (1988)
Study title: Napropamide: tissue distribution in animal
Test substance: Napropamide, unlabelled and radio-labelled



* Position of ^{14}C

Purity: Specific activity 23 mCi/mmole, radiochemical purity ≥ 99 %
Purity of unlabelled napropamide: 94.3 %

Batch no.: Not mentioned
0Test animals: Sprague-Dawley rats, 24/sex, 7 – 10 weeks of age and weighing 160 – 300 g (males 200-300 g, females 160-250 g) one week before the study

Groups:	12 rats/sex/dose group
Dose:	Single dose of 30 or 300 mg/kg bw. ^{14}C-napropamide was mixed with unlabelled napropamide
Route:	Oral by gavage
Vehicle:	5 % gum arabic solution
Statistics/ measurements:	Statistics not performed
GLP:	No (when this study was performed, GLP was not compulsory)
Guideline:	Japanese MAFF General Notice No. 59 NOHSAN 4200, 28 January 1985. US EPA Guideline 85-1
Deviation:	Only tissue distribution and excretion were investigated. Four groups of 3 male and 3 female rats were used at 2 dose levels. The use of 3 rats per sex rather than 4 is considered scientifically acceptable. Bone was not analysed, but since bone rarely contains radioactivity the omission of this tissue is not considered to be significant. The reason for not collecting 'expired air' is not indicated in the report but was analysed in a previous study and no radioactivity was found.
Acceptability:	Acceptable

Methods

Two groups each consisting of 12 male and 12 female Sprague Dawley rats were administered a single oral dose of either 30 or 300 mg/kg bw ^{14}C-napropamide. The dose was given in 5 % gum arabic (2 mL/kg bw) by gavage. Samples of urine and faeces were collected at 0-6, 6-24, 24-72 and 72-96 hours post-dosing. At 6, 24, 72 and 96 hours after dosing, 3 males and 3 females from each group were sacrificed and the following tissues/organs were collected: blood, plasma, brain, spinal cord, pituitary, eyeball, Harderian gland, submaxillary gland, thyroid gland, thymus, heart, lung, liver, spleen, pancreas, adrenal gland, kidney, skeletal muscle (hind leg), stomach, abdominal aorta, inferior vena cava, fat (peri-genital), skin, ovary, uterus, testes, carcass and bone marrow. The radioactivity of each collected sample was measured using a scintillation counter following the appropriate homogenisation, solubilisation and/or dilution treatments. In the main report, excretion data have been normalised to 100 % recovery. The actual excretion data have been included in a supplement to the report and have been used in this summary.

Results

The radioactivity was rapidly absorbed and excreted in urine and faeces. The excretion was almost complete within 72 hours after dosing with both low and high doses. The cumulative excretion was similar for male and female rats. The concentration of the administered radioactivity was highest in blood and tissues at 6 hours after dosing. The concentrations were dose dependent. The radioactivity from tissues declined gradually. The elimination of radioactivity from tissues/organs was almost complete 96 hours after dosing. No sex difference was evident in tissue concentrations. The highest concentrations were usually found in the intestines, especially within the first 72 hours. The results are summarised in Tables 6.1.5 to 6.1.7.

Table 6.1.5: Cumulative excretion of ^{14}C-napropamide in urine and faeces (%) after single oral administration (N=3)

Time (h)	30 mg/kg bw				300 mg/kg bw			
	Males		Females		Males		Females	
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
6	14.6±1.7	0.3±0.8	25.1±18.4	--	12.2±1.7	0.3±0.4	8.3±3.8	0.0±0.1
24	41.7±12.6	33.9±3.3	52.1±5.9	22.9±1.9	26.9±1.7	38.0±13.4	41.6±7.7	11.6±6.7
72	52.6±14.3	51.6±6.9	62.3±5.7	37.4±3.4	34.0±2.5	59.0±6.3	48.3±7.5	39.3±11.6
96	53.2±14.2	52.5±7.3	62.8±5.8	38.7±3.4	34.7±2.8	60.0±6.0	49.5±7.0	41.2±11.8
Total (96h)	105.6±7.9		101.5±8.0		94.7±3.8		90.7±9.8	

-- = No sample

Table 6.1.6: Mean tissue concentration of ^{14}C-napropamide (µg/g or ml) and recovery of radioactivity (%) in male rats after single oral administration (N=3)

TISSUE	30 mg/kg bw								300 mg/kg bw							
	6 h		24 h		72 h		96 h		6 h		24 h		72 h		96 h	
	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%
Plasma	4.0	0.5	1.1	0.1	0.1	0.0	0.1	0.0	35.3	0.4	5.4	0.06	0.9	0.01	0.5	0.01
Brain	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	1.9	0.0	0.5	0.0	0.3	0.0	0.2	0.0
Spinal cord	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	2.4	0.0	0.5	0.0	0.4	0.0	0.3	0.0
Hypophysis	1.1	0.0	0.3	0.0	0.2	0.0	0.5	0.0	9.2	0.0	2.7	0.0	1.6	0.0	3.0	0.0
Eyeballs	0.5	0.0	0.1	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.6	0.0	0.2	0.0	0.2	0.0
Harderian gland	1.1	0.0	0.2	0.0	0.1	0.0	0.1	0.0	7.7	0.0	1.9	0.0	0.6	0.0	0.6	0.0
Submaxillary gl.	1.1	0.01	0.2	0.0	0.1	0.0	0.1	0.0	8.1	0.0	1.7	0.0	0.7	0.0	0.6	0.0
Thyroid	1.8	0.0	0.8	0.0	0.4	0.0	0.4	0.0	21.2	0.0	6.5	0.0	6.4	0.0	4.3	0.0
Thymus	0.7	0.0	0.2	0.0	0.1	0.0	0.0	0.0	4.6	0.0	1.3	0.0	0.3	0.0	0.2	0.0
Heart	1.2	0.01	0.3	0.0	0.2	0.0	0.1	0.0	8.5	0.01	2.6	0.0	1.4	0.0	1.3	0.0
Lungs	2.2	0.03	0.8	0.01	0.4	0.01	0.2	0.0	20.1	0.03	6.3	0.01	2.0	0.0	1.9	0.0
Liver	30.0	3.9	3.5	0.6	0.5	0.1	0.9	0.1	132.2	1.7	36.4	0.6	7.1	0.2	9.3	0.1
Spleen	1.6	0.01	0.5	0.0	0.3	0.0	0.3	0.0	13.6	0.01	5.8	0.0	3.1	0.0	2.9	0.0
Pancreas	2.8	0.02	0.6	0.01	0.1	0.0	0.0	0.0	21.1	0.02	12.0	0.01	0.5	0.0	0.4	0.0
Adrenal	1.7	0.0	0.7	0.0	0.3	0.0	0.2	0.0	50.0	0.0	9.1	0.0	2.1	0.0	2.1	0.0
Kidneys	11.7	0.3	2.5	0.1	0.6	0.02	0.3	0.01	68.4	0.2	17.5	0.1	4.3	0.01	3.0	0.01
Testes	0.7	0.02	0.2	0.0	0.0	0.0	0.0	0.0	5.6	0.02	0.9	0.0	0.3	0.0	0.2	0.0
Abd. Aorta	3.0	0.0	4.1	0.0	0.4	0.0	0.5	0.0	199.1	0.0	39.2	0.0	7.3	0.0	2.6	0.0
Abd. Vein	3.8	0.0	2.2	0.0	0.4	0.0	0.6	0.0	118.5	0.0	58.5	0.0	7.6	0.0	6.3	0.0
Muscle	0.6	0.8	0.2	0.2	0.1	0.1	0.1	0.1	4.3	0.6	1.2	0.2	0.5	0.1	0.6	0.1
Fat	4.4	0.7	0.3	0.04	0.3	0.04	0.0	0.0	19.2	0.3	3.7	0.1	0.5	0.01	0.4	0.01
Stomach	14.9	0.2	2.4	0.03	0.2	0.0	0.1	0.0	736.7	0.9	9.8	0.01	2.6	0.0	0.8	0.0
Intestine	101.0	8.8	23.4	2.0	0.6	0.1	0.7	0.1	832.1	5.5	280.2	2.0	12.7	0.2	6.8	0.1
Blood	3.8	0.8	2.4	0.5	1.7	0.4	1.1	0.3	35.3	0.8	23.1	0.5	18.8	0.4	16.8	0.4
Marrow	1.1	0.0	0.4	0.0	0.2	0.0	0.3	0.0	16.5	0.0	3.3	0.0	2.4	0.0	2.4	0.0
Carcass	17.3	44.3	3.6	9.8	0.3	0.8	0.1	0.8	203.1	51.8	41.5	10.9	7.3	2.1	3.3	0.9
Skin	1.1	0.8	0.3	0.2	0.1	0.1	2.0	1.7	8.5	0.6	9.9	0.7	0.6	0.04	0.2	0.02
Total (%) [1)]	58.30±5.81		12.96±1.53		1.39±0.70		1.17±0.10		60.91±3.23		14.03±1.15		2.88±1.89		1.50±0.54	

[1)] Excluding plasma, muscle, fat and skin

Table 6.1.7: Mean tissue concentration of ^{14}C-napropamide (µg/g or ml) and recovery of radioactivity (%) in female rats after single oral administration (N=3)

TISSUE	30 mg/kg bw								300 mg/kg bw							
	6 h		24 h		72 h		96 h		6 h		24 h		72 h		96 h	
	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%	µg equ /g	%
Plasma	2.9	0.3	0.3	0.03	0.1	0.01	0.1	0.01	34.7	0.4	5.3	0.1	0.7	0.01	0.6	0.01
Brain	0.2	0.01	0.1	0.0	0.02	0.0	0.01	0.0	4.2	0.01	0.3	0.0	0.2	0.0	0.2	0.0
Spinal cord	0.3	0.0	0.03	0.0	0.01	0.0	0.03	0.0	4.9	0.0	0.5	0.0	0.1	0.0	0.3	0.0
Hypophysis	1.5	0.0	0.2	0.0	0.1	0.0	0.4	0.0	13.9	0.0	1.7	0.0	1.3	0.0	1.3	0.0
Eyeballs	0.5	0.0	0.04	0.0	0.01	0.0	0.02	0.0	6.3	0.0	0.7	0.0	0.2	0.0	0.2	0.0
Harderian gland	1.1	0.0	0.1	0.0	0.03	0.0	0.04	0.0	15.1	0.01	1.7	0.0	0.5	0.0	0.6	0.0
Submaxillary	1.0	0.01	0.1	0.0	0.02	0.0	0.04	0.0	11.0	0.01	1.2	0.0	0.5	0.0	0.4	0.0
Thyroid	2.4	0.0	0.4	0.0	0.1	0.0	0.1	0.0	28.2	0.0	5.8	0.0	3.5	0.0	2.6	0.0
Thymus	0.7	0.01	0.1	0.0	0.02	0.0	0.02	0.0	7.7	0.01	0.8	0.0	0.2	0.0	0.3	0.0
Heart	1.1	0.01	0.1	0.0	0.1	0.0	0.1	0.0	12.5	0.01	1.7	0.0	0.6	0.0	0.9	0.0
Lungs	2.0	0.03	0.2	0.0	0.2	0.0	0.2	0.0	21.2	0.04	4.4	0.01	1.6	0.0	2.5	0.0
Liver	16.0	2.4	2.9	0.4	25.5*	7.2*	0.8	0.1	110.0	1.8	49.8	0.9	12.9	0.2	9.5	0.1
Spleen	0.8	0.01	0.2	0.0	0.1	0.0	0.1	0.0	9.7	0.01	2.7	0.0	1.5	0.0	2.9	0.0
Pancreas	0.8	0.01	0.2	0.0	0.04	0.0	0.0	0.0	15.5	0.02	6.7	0.01	0.5	0.0	0.3	0.0
Adrenal	2.3	0.0	0.4	0.0	0.2	0.0	0.2	0.0	36.1	0.0	5.6	0.0	2.3	0.0	2.1	0.0
Kidneys	7.7	0.2	0.7	0.02	0.3	0.01	0.3	0.01	58.9	0.2	15.6	0.1	2.8	0.01	3.5	0.0
Ovaries	1.1	0.0	0.1	0.0	0.03	0.0	0.1	0.0	17.1	0.0	3.1	0.0	0.4	0.0	0.9	0.0
Uterus	1.6	0.01	0.2	0.0	0.1	0.0	0.03	0.0	43.2	0.03	7.4	0.01	0.6	0.0	0.5	0.0
Abd. Aorta	6.7	0.0	0.8	0.0	0.1	0.0	1.9	0.0	46.7	0.0	18.9	0.0	1.7	0.0	4.0	0.0
Abd. Vein	7.2	0.0	0.9	0.0	0.1	0.0	2.1	0.0	55.4	0.0	18.7	0.0	3.7	0.0	7.1	0.0
Muscle	0.7	0.9	0.1	0.1	0.02	0.03	0.02	0.03	7.5	1.0	0.8	0.1	0.5	0.1	1.5	0.2
Fat	2.6	0.4	0.2	0.03	0.1	0.01	0.03	0.01	47.1	0.8	6.7	0.1	0.5	0.01	0.2	0.0
Stomach	47.4	0.7	0.7	0.01	0.2	0.0	0.1	0.0	637.7	1.0	42.7	0.1	1.6	0.0	2.2	0.0
Intestine	59.6	7.2	15.6	1.7	1.0	0.2	0.8	0.1	1180.5	9.7	258.9	2.6	17.6	0.2	62.1	0.5
Blood	2.9	0.6	0.8	0.2	0.6	0.1	0.7	0.2	29.6	0.6	13.3	0.3	8.1	0.2	12.4	0.3
Marrow	2.2	0.0	0.2	0.0	0.04	0.0	0.5	0.0	21.8	0.0	1.5	0.0	2.4	0.0	3.0	0.0
Carcass	18.0	43.9	3.3	8.2	0.5	1.4	0.5	1.3	177.7	43.4	60.6	15.7	5.5	1.5	7.5	2.0
Skin	0.9	0.7	0.1	0.1	0.03	0.02	0.03	0.03	13.4	1.0	1.6	0.1	0.4	0.03	0.3	0.03
Total (%) [1)]	55.12±0.98		10.48±2.78		8.93±11.04*		1.67±0.30		56.82±5.75		19.54±4.95		2.13±0.92		2.98±0.63	

[1)] Excluding plasma, muscle, fat and skin

* The variation is mainly caused by a great variation in liver content (25.53±41.21 µg/g), which again is caused by a high concentration in one of the three rats (73.10 µg/g)

Conclusion

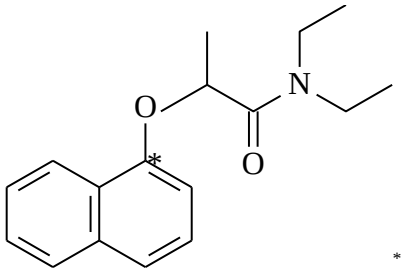
Most of the radioactivity was eliminated via urine and faeces within 72 hours after a single oral administration of 30 or 300 mg ^{14}C-napropamide/kg bw. The concentration of the administered radioactivity was highest in blood and tissues at 6 hours after dosing, and the concentrations were dose dependent. The radioactivity from tissues declined gradually, and the concentration of radioactive residues in tissues/organs were low 96 hours after dosing (about 1.5 % in males and 3.0 % in females). The highest concentrations were usually found in the intestines, especially within the first 72 hours. No sex difference was evident in excretion pattern or tissue concentrations.

B.6.1.1.4. Tissue distribution and elimination in the rat – repeated dose oral study

Introduction

This repeat-dose study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. This is the only repeat-dose ADME study. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed. The final conclusions drawn on the basis of all of the ADME studies on napropamide racemate have not changed and are as described in the EFSA Conclusion (EFSA, 2010).

Author(s): [redacted] (1991)
Study title: Napropamide: repeat dose study in the rat (30 mg/kg)
Test substance: Napropamide, unlabelled and radio-labelled



* Position of ^{14}C

Purity: Specific activity 58.1 mCi/mmole, radiochemical purity ≥ 99 %
Purity of unlabelled napropamide: 99.8 % according to an about 3.5 year old certificate of analysis at the time of the study
Batch no.: Not mentioned
Test animals: Sprague-Dawley rats, 8/sex, weighing 178 – 241 g at the start of the study
Groups: 8 rats/sex were dosed with unlabelled napropamide for 14 days. The rats were identified by a letter (A-H for males, I-P for females). Female I was mis-dosed on day 3 and excluded from study. The first 5 rats/sex, in alphabetically order received subsequently a single oral dose of ^{14}C-napropamide
Dose: 30 mg unlabelled napropamide/kg bw/day for 14 days, followed by a single oral dose of ^{14}C-napropamide at 30 mg/kg bw (0.054 mCi/kg) level in 5 % gum arabic suspension
Route: Oral by gavage
Vehicle: 5 % gum arabic solution
Statistics/ measurements: Statistics not performed
GLP: Yes
Guideline: None cited (the study complies in part with US EPA Guideline 85-1)
Deviation: Only repeat dosing (material balance and tissue distribution) phase was evaluated. It is uncertain whether the rats were randomized after a proper procedure.
Acceptability: Acceptable

Methods

A group of five male and five female Sprague Dawley rats were administered 14 daily single oral doses of unlabelled napropamide (purity 99.8 %) followed by a single oral dose of ^{14}C-napropamide (specific activity 58.1 mCi/mmol, radiochemical purity > 99 %) at 30 mg/kg bw level in 5 % gum arabic suspension. Urine was collected at 6 hours, and urine and faeces were collected at 12, 24, 36 and 48 hours and then at 24 hourly intervals until 7 days after dosing. Seven days after dosing, the rats were sacrificed, blood samples were

collected and the following tissues/organs were removed and retained: abdominal aorta, adrenals, brain, eyes, Harderian glands, heart, inferior vena cava, large and small intestines, kidneys, liver, lungs, ovaries, pancreas, pituitary gland, spinal cord, spleen, stomach, submaxillary salivary glands, testes, thymus, thyroid, uterus, bone marrow, muscle, fat, skin and residual carcass. All collected samples were counted for radioactivity in a scintillation counter following appropriate treatments (homogenisation, solubilisation and/or combustion).

Results

Most of the administered radioactivity was excreted during the first 72 hours after dosing (approximately 91 % for males and 90 % for females). Males excreted approximately 42 % of the administered dose in the urine and approximately 49 % of the dose in faeces within 72 hours after dosing. The corresponding values for females were 48 % (urine) and 42 % (faeces). Although overall recovery for males and females was similar at the end of the 7-day period, there was a slight difference in the amounts excreted in the urine and faeces. The amount of radioactivity excreted by males during the 7-day period was 42.9 % in urine and 49.9 % in faeces, while for females the corresponding values were 48.8 % and 43.0 %, respectively. The results are shown in Table 6.1.8.

Tissues contained less than 0.3 % of the administered dose seven days after dosing. In both sexes, the highest concentrations of radioactivity were present in the blood. Concentrations in plasma were lower indicating that the radioactivity in blood was associated with the cell fraction. Other tissues that contained comparatively high concentrations of radioactivity included blood rich organs like liver, spleen, thyroid (females only) and kidney. No significant differences in tissue distribution were seen between males and females. The results are shown in Table 6.1.9. The metabolite profiles from urine and faeces from this study were analysed during a later study (Macpherson and Jones, 1991) and were found to be similar to those for the single oral dosed rats at 30 mg/kg bw.

Table 6.1.8: Cumulative excretion of ^{14}C-napropamide in urine and faeces (%) after 14 days repeated oral administration of unlabelled napropamide followed by a single oral administration of radio-labelled napropamide (N=5)

Time (h)	30 mg/kg bw			
	Males		Females	
	Urine	Faeces	Urine	Faeces
6	10.0±3.9	--	15.0±6.2	--
12	23.0±6.9	0.7±1.0	28.5±5.0	5.9±5.7
24	35.4±6.1	30.8±6.0	41.5±5.6	30.1±3.9
72	42.3±7.2	48.6±7.7	48.0±6.2	42.3±7.4
168	42.9±7.3	49.9±7.6	48.8±6.3	43.0±7.4
Total, incl. Cage wash (168h)	92.9±2.1		92.1±1.6	

The cumulative excretion is calculated from individual figures in the report

-- = No sample

Table 6.1.9: Mean tissue concentration of radioactivity and % dose 7 days after giving a single oral dose of ^{14}C napropamide (30 mg/kg bw) to rats that had been given 14 daily oral doses of unlabelled napropamide

TISSUE	Male rats		Female rats	
	µg equiv/g	% Dose	Mg equiv/g	% Dose
Abdominal aorta	0.165	<0.001	0.139	<0.001
Adrenal glands	0.154	<0.001	0.144	<0.001
Brain	<0.050	<0.001	<0.038	<0.001
Eyes	0.026	<0.001	<LOD	<0.001
Harderian glands	0.035	<0.001	0.048	<0.001
Heart	0.143	0.002	0.156	0.002
Inferior vena cava	<0.317	<0.001	<0.244	<0.001
Intestines	0.049	0.006	0.047	0.007
Kidneys	0.195	0.005	0.190	0.005
Liver	0.354	0.053	0.356	0.053
Lungs	0.127	0.002	0.132	0.002

Ovaries	NA	NA	0.087	<0.001
Pancreas	0.075	<0.001	0.054	<0.001
Pituitary	<0.543	<0.001	<0.324	<0.001
Salivary gland	0.070	<0.001	0.056	<0.001
Spinal cord	0.124	0.001	0.121	0.001
Spleen	0.268	0.002	0.257	0.002
Stomach	<0.042	<0.001	<0.047	<0.001
Testes	0.057	0.002	NA	NA
Thymus	0.132	0.001	0.125	0.001
Thyroid	0.247	<0.001	<0.424	<0.001
Uterus	NA	NA	0.070	<0.001
Blood	0.798	-	0.655	-
Bone marrow	<0.493	-	<0.528	-
Muscle	0.073	-	0.048	-
Fat	<0.043	-	<0.039	-
Plasma	<LOD	-	<0.038	-
Skin	0.066	-	0.056	-
Residual carcass	0.075	0.200	0.078	0.202

Conclusions

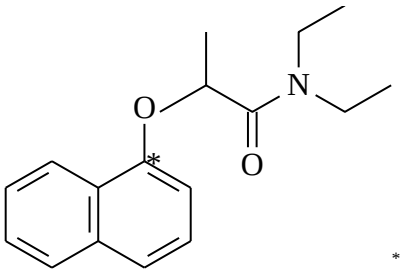
Fourteen days pre-treatment of male rats with napropamide had apparently no effect on the absorption, excretion and tissue retention of ^{14}C-napropamide (when compared with previous study, Y. Tanukura (1988)). The study director concluded that for female rats, pre-treatment with napropamide decreased urinary elimination and increased faecal excretion when compared with animals receiving the single dose. However, as this comparison is with animals in a study conducted at a different time in another laboratory, it is not strictly valid.

B.6.1.1.5. Biotransformation in the rat

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed. The final conclusions drawn on the basis of all of the ADME studies on napropamide racemate have not changed and are as described in the EFSA Conclusion (EFSA, 2010).

Author(s): [redacted] (1991)
Including first supplement to report: [redacted] (1993)
Study title: Napropamide: Biotransformation study in the rat.
Test substance: Napropamide, unlabelled and radio-labelled



* Position of ^{14}C

Purity: Specific activity 57.9 mCi/mmole, radiochemical purity ≥ 97 %
Purity of unlabelled napropamide: 99.8 % (total D- + L-isomer)

Batch no.: Not mentioned
Test animals: Rats, CD-1 strain, 11 males and 6 females, approx. 9 – 12 weeks old, weighing 198 – 375 g at the time of dosing

Groups & doses:		
	4 male rats (Nos. 1-4) &	A single oral dose of ^{14}C-napropamide at 300 mg/kg bw level in

	4 female rats (Nos. 5-8)	PEG 600
	2 bile duct cannulated male rats (Nos. 9-10)	A single oral dose of ^{14}C-napropamide at 300 mg/kg bw level in PEG 600
	3 male rats (Nos. 11-13)	A single oral dose of ^{14}C-napropamide at 300 mg/kg bw level in PEG 600
	2 male rats (Nos. 14-15) & 2 female rats (Nos. 16-18)	A single oral dose of ^{14}C-napropamide at 30 mg/kg bw level in 5 % gum arabic solution

Route: Oral by gavage
Vehicles: 5 % gum arabic solution and polyethylene glycol (PEG) 600
Statistics/ measurements: Statistics not performed
GLP: Yes, except that the stability of the test compound alone has not been reported
Guideline: None cited (the study complies, in part, with US EPA Guideline 85-1)
Deviation: Only biotransformation was investigated
Acceptability: Acceptable

Methods

A total of 11 male rats and 6 female rats were used in this study. Four male (Nos. 1-4) and four female (Nos. 5-8) rats were administered a single oral dose of ^{14}C-napropamide (specific activity 57.9 mCi/mmole, radiochemical purity of > 97 %) in PEG 600 at 300 mg/kg bw. Two bile duct-cannulated male rats (Nos. 9-10) and 3 male (Nos. 11-13) rats received similar single doses (300 mg/kg bw in PEG 600). In addition, two male (Nos. 14-15) and 2 female (Nos. 16-17) rats were administered single oral doses of ^{14}C-napropamide at 30 mg/kg bw in gum arabic suspension. Rat numbers 11-13 were housed together; all other animals were housed individually. Urine and faeces were collected from rat nos. 1-8 at 0-24, 24-48 and 48-72 hours after dosing. Urine and faeces samples, from the bile duct cannulated rats nos. 9-10, were collected at 0-24 and 24-48 hours after dosing. Bile was collected from these animals at hourly intervals of up to 7 hours and then at 7-12, 12-24, 24-30, 30-36 and 36-48 hours after dosing. For rat nos. 14-17, the urine and faeces samples were collected at 0-6 (urine only), 6-12 (urine only), 12-24, 24-36, 36-48 and 48-72 hours after dosing. Excreta were collected from group-housed rats (nos. 11-13) at daily intervals for 3 days after dosing. Samples of urine and faeces from a separately conducted repeat dose study (14 daily doses of 30 mg/kg bw of unlabelled napropamide followed by a single oral dose of ^{14}C-napropamide at 30 mg/kg bw – Hall and Howard, 1991) were also analysed for comparative profiling of metabolites. Radioactivity from urine, bile and faeces was measured using scintillation counting following appropriate homogenisation, dilution and/or combustion. Radioactivity from urine, bile and faeces was extracted in suitable solvents/partitioned/hydrolysed/derivatised and analysed for metabolites using TLC, Flash Column Chromatography and HPLC, GLC, MS, and NMR Spectroscopy. Metabolite identification was also made by comparing data with the available reference standards.

Results

Excretion data for male and female rats administered a single oral dose of 300 mg/kg bw show a slight sex difference in excretion profiles (Table 6.1.10). Males excreted 48 % of the dose in urine and 48 % via faeces over 72 hours. Similarly, females excreted approximately 57 % of the dose in the urine and 40 % in the faeces over the same period.

Bile-duct-cannulated rats eliminated approximately 78 % of the dose via bile over 48 hours. Almost the complete dose was eliminated within 24 hours after dosing. A mean of 15 % of the dose was excreted in the urine and less than 3 % in the faeces.

The excretion profile for group-housed rats (nos. 11-13, data not provided) given a 300 mg/kg bw dose was similar to single-housed rats (nos. 1-4).

Male rats given 30 mg/kg bw (nos. 14-17) excreted 45 % of the dose in urine and 37 % in the faeces while females from this group excreted 48 % in the urine and 36 % in the faeces in 72 hours (Table 6.1.11). These results were comparable to the rats from the repeated dose study (Hall and Howard, 1991). In the repeated dose study, males excreted 42 % in the urine and 49 % in the faeces and females excreted 48 % in the urine and 42 % in the faeces over 72 hours (Table 6.1.8).

A total of 15 metabolites were identified in urine and bile. Radioactivity in bile consisted mainly of conjugates. Approximately half of the biliary radioactivity was excreted via faeces and the remainder was reabsorbed for further metabolism and subsequent elimination in urine. The major metabolites were glucuronide conjugates, 4-

OGlu-NPAM, 4-OGlu-DE-NPAM, 4-OGlu-NOPAM and 4-OGlu-NOPA (Table 6.1.12). Eleven minor metabolites, each accounting for <3 % of the dose, were also present. The proportions of the metabolites identified in urine and faeces are shown in Table 6.1.12. Metabolite profiles in solvent extracts of faeces were qualitatively similar to urine profiles and were characterised only by co-chromatography since nearly all faecal metabolites were of biliary origin. No qualitative differences in metabolite profiles were apparent in the urine of rats given the high or low dose of napropamide and metabolism was unaffected by pre-treatment with napropamide. There were small sex differences in the proportion of metabolites excreted in the urine of rats given single or repeated low dose but this was not apparent at the higher dose level. The metabolites identified in this study include various permutations of mono- and di- de-ethylation of the alkyl side chain, hydrolysis of the propionamide to the carboxylic acid, hydroxylation of the naphthyl ring, primarily at the position 4, and subsequent glucuronidation. Because the major metabolite in bile is the glucuronide conjugate of 4-hydroxy napropamide, it is presumed in the biotransformation pathway that ring hydroxylation precedes N-de-ethylation. The proposed biotransformation pathway of napropamide is presented in Figure 6.1-2.

Table 6.1.10: Cumulative excretion of ^{14}C-napropamide in urine and faeces after a single oral administration (%)

Time (h)	300 mg/kg bw						
	Males [1)]		Females [1)]		Males [2)]		
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Bile
24	38.2±3.2	33.6±6.6	44.5±3.4	17.1±2.6	14.5	1.6	77.5
48	46.5±4.8	45.8±7.7	55.1±3.4	37.5±3.7	15.2	2.7	78.3
72	48.0±5.4	47.7±7.9	56.9±3.4	39.5±3.9			
Total (72h/48h)	95.7±2.7		96.4±1.3		96.3		

The cumulative excretion is calculated from individual figures in the report

[1)] Mean and standard deviation, N=4, [2)] Mean, N=2

Table 6.1.11: Cumulative excretion of ^{14}C-napropamide in urine and faeces after a single oral administration (%)

Time (h)	30 mg/kg bw			
	Males [1)]		Females [1)]	
	Urine	Faeces	Urine	Faeces
24	35.7	28.5	42.3	29.2
48	43.3	35.4	47.5	34.9
72	45.5	37.1	48.4	35.9
Total (72h)	82.5		84.2	

The cumulative excretion is calculated from individual figures in the report

[1)] Mean, N=2

Table 6.1.12: Amounts of metabolites (% dose) in bile and urine of rats given a single dose of 300 mg napropamide/kg bw

Metabolites			Male		Female
Abbreviation [1)]	Code[2)]	Chemical name	Bile	Urine	Urine
4-OGlu-NPAM	B1/U1	4-glucuronyl-(N,N-diethyl-2-(1-naphthoxy)) propionamide	29.5	7.7	18.4
4-OGlu-DE-NPAM	B2/U2	4-glucuronyl-(N-ethyl-2-(1-naphthoxy)) propionamide	8.4	5.1	5.9
4-OGlu-NOPAM + 4-OGlu-NOPA	B3 + B4	[*]glucuronyl-(1-naphthoxy) propionamide + 4-glucuronyl-(1-naphthoxy) propionic acid	14.5	12.3	9.8
Unknown	B16		Trace	-	-
NPAM	U5	Napropamide		<0.1	0.2
4-OH NPAM + 5-OH NPAM	U6 + U13	4-hydroxy (N,N-diethyl-2-(1-naphthoxy)) propionamide + 5-hydroxy (N,N-diethyl-2-(1-naphthoxy)) propionamide		0.2	0.2
4-OH DE-NPAM + 5-OH DE-NPAM	U7 + U14	4-hydroxy (N-ethyl-2-(1-naphthoxy)) propionamide + 5-hydroxy (N-ethyl-2-(1-naphthoxy)) propionamide		1.0	1.7
4-OH NOPAM	All codes U5 to U14 inclu-sive	[*]hydroxylated-(1-naphthoxy) propionamide	*ca* 8.0	0.3	-
4-OH NOPA	U9	4-hydroxy-(1-naphthoxy) propionic acid		0.6	-
DE-NPAM	U10	N-ethyl-2-(1-naphthoxy) propionamide (CAS No. 38641-90-6)		1.7	2.7
NOPAM	U11	(1-naphthoxy) propionamide		0.6	0.7
NOPA	U12	(1-naphthoxy) propionic acid (CAS No. 13949-67-2)		0.7	1.9
	U15	1,4-naphthoquinone	-	Minor	-
5-OH NOPA	B5	[**]5-hydroxy-(1-naphthoxy) propionic acid			

[1)] Abbreviation used in Figure 6.1-1

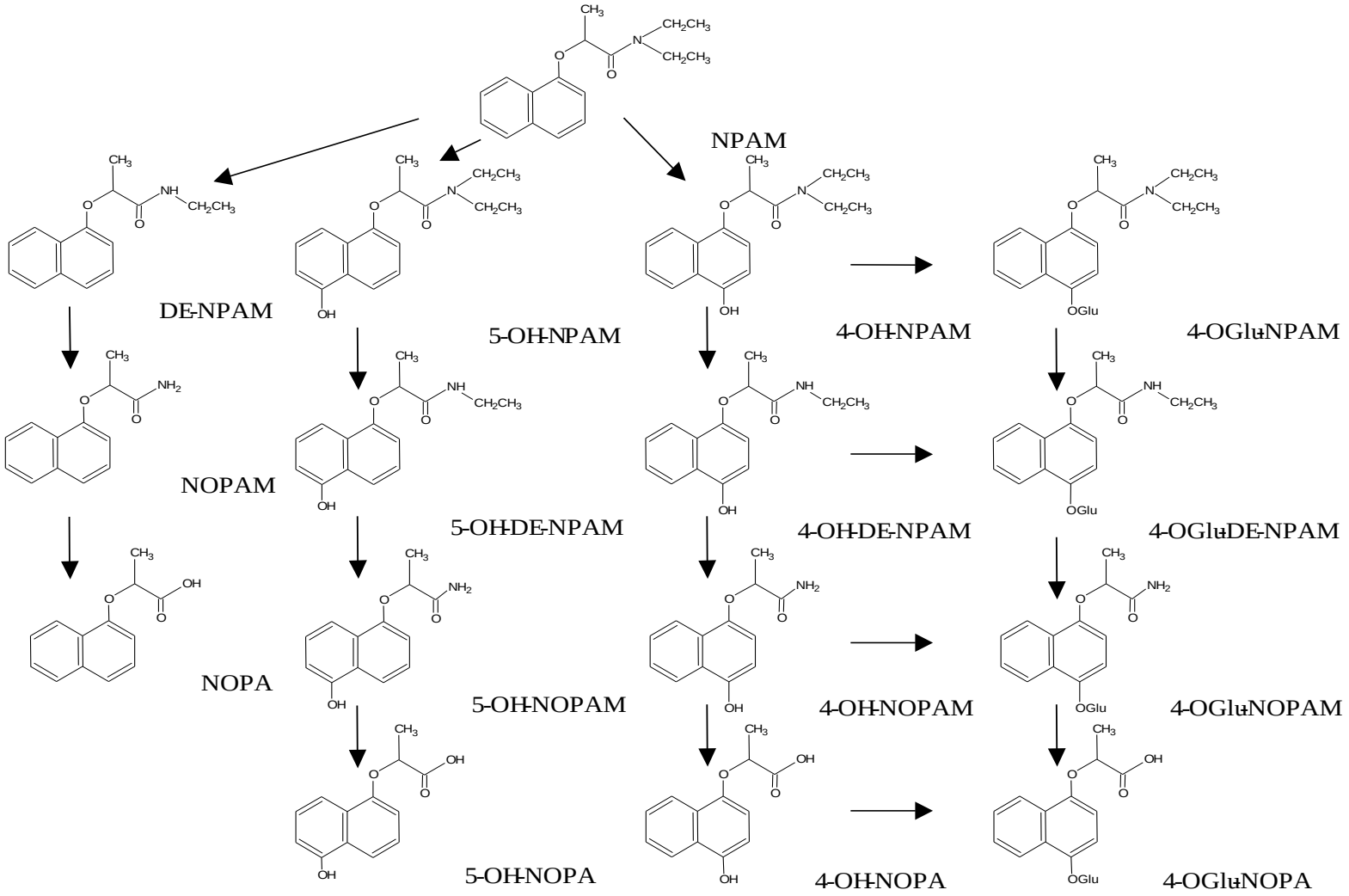
[2)] Code used in study report

- Not measurable or not characterised

[*] Position of hydroxylation unconfirmed

[**] Excreted as the glucuronic acid conjugate

Figure 6.1-2
Napropamide: proposed biotransformation pathway in rats



Conclusion

Napropamide was extensively metabolised and rapidly eliminated. No qualitative differences were apparent between the dose levels or between single and repeat-dose animals. There may be some small quantitative differences between sexes. Bile duct-cannulated rats appear to excrete a much lower proportion of the dose in urine. A mean of 15 % of the dose was excreted in the urine whereas in the other studies, all of which involved non-bile duct cannulated rats, the proportion of the dose excreted in urine ranged from approximately 35 % to 60 %. This suggests that enterohepatic circulation is an important process in the normal excretion of napropamide racemate. Biliary metabolites were mainly glucuronide conjugates of hydroxylated napropamide at position 4. Approximately 15 urinary metabolites were identified. Metabolite profile for faeces was similar to that of urine.

B.6.1.1.6. Overall summary on absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

Type of study	Dose levels (mg/kg b.w.)	Animal species, strain; sex	Substance	Findings	References
Metabolism of napropamide-M following oral administration in the rat	Phase 1 and 3: 30 mg/kg bw (range 29.2 - 33.3 mg/kg bw) Phase 2 and 4: 300 mg/kg bw (range 281 - 302 mg/kg bw)	Male and female Sprague-Dawley rats	^{14}C-napropamide-M and unlabelled napropamide-M	Napropamide-M was rapidly excreted and extensively metabolised at both dose levels. Excretion was approximately equally split between urine and faeces. Highest tissue concentrations, 96 hours after dosing, present in organs of metabolism and elimination: liver, kidney. Peak plasma concentration: 6 hours after dosing. Generally, systemic exposure to total radioactivity increased approximately proportionally with dose.	[redacted] 2015
Elimination (balance study) and tissue distribution (residue study) after oral administration (Single dose study)	Preconditioned with non-labelled test material 5 or 25 mg/kg bw/day for 4 days. Balance study: 30 mg/kg bw Residue study: 195 mg/kg bw Both with ^{14}C-napropamide	Male and female Simonsen Albino rats	^{14}C-napropamide and unlabelled napropamide	Approximately 98.6 % of the administered radioactivity was eliminated in urine and faeces within 96 hours; 57.5 % in the urine and 40.7 % in the faeces. No detectable radioactivity was found in the expired air. Tissues and organs contained approximately 0.41 % of the administered radioactivity 96 hours after administration. No sex difference was apparent for elimination and tissue distribution. Accumulation was not evident.	[redacted]. (1970)
Elimination and tissue distribution after oral administration (Single dose study)	Single dose of either 30 or 300 mg/kg bw	Male and female Sprague-Dawley rats	^{14}C-napropamide and unlabelled napropamide	Most of the radioactivity was eliminated via urine and faeces within 72 hours after a single oral administration of 30 (about 100 %) or 300 mg ^{14}C-napropamide/kg bw (about 91-95 %). The concentration of the administered radioactivity was highest in blood and tissues at 6 hours after dosing, and the concentrations were dose dependent. The radioactivity from tissues declined gradually, and the concentration of radioactive residues in tissues/organs were low 96 hours after dosing (about 1.5 % in males and 3.0 % in females). The highest concentrations were usually found in the intestines, especially within the first 72 hours. No sex difference was evident.	[redacted] (1988)
Elimination and tissue distribution after oral administration	Single dose of 30 mg/kg bw with radio-labelled napropamide following 14 days preconditioning	Male and female Sprague-Dawley rats	^{14}C-napropamide and unlabelled napropamide	Fourteen days of pre-treatment of male rats with napropamide had no effect on the absorption, excretion and tissue retention of ^{14}C-napropamide. Most of the radioactivity was eliminated via urine and faeces within 72 hours after dosing: males approx. 42 % (urine) and	[redacted] (1991)

Type of study	Dose levels (mg/kg b.w.)	Animal species, strain; sex	Substance	Findings	References
(Repeated dose study)	with 30 mg/kg bw/day of unlabelled test material			approx. 49 % (faeces), females 48 % (urine) and 42 % (faeces). Tissues contained less than 0.3 % of the administered dose seven days after dosing. The highest concentrations were found in blood, mainly associated with the blood cells. Comparatively high concentrations of radioactivity were found in liver, spleen, thyroid (females only) and kidney. No sex difference was evident in distribution.	
Biotransform-ation in the rat	Single dose of either 30 or 300 mg/kg bw	CD-1 rats	^{14}C-napropamide and unlabelled napropamide	Napropamide was extensively metabolised and rapidly eliminated. No qualitative differences were apparent between the sexes, the dose levels, or between single and repeat-dose animals. Biliary metabolites were mainly glucuronide conjugates of hydroxylated napropamide at position 4. Approximately 15 urinary metabolites were identified. Metabolite profile for faeces was similar to that of urine.	[redacted] 1991, 1993)

B.6.1.1.7. Overall conclusion

Five ADME studies are available to support napropamide-M. Four of the studies were done using napropamide racemate and were previously evaluated by Denmark for the approval of napropamide. Each of the studies involved investigation (single and repeat-dose) in the rat by the oral route. The summaries of these studies have not been changed (other than minor amendments to improve readability) and the conclusions have not changed. The final conclusions drawn on the basis of all of the ADME studies on napropamide racemate have not changed and are as described in the EFSA Conclusion (EFSA, 2010).

Only one of the studies was done using napropamide-M. This study was an investigation in the rat by the oral route (single dose) and was submitted by the applicant in support of the approval of napropamide-M; it has been evaluated for the first time, here.

The findings of the studies done using napropamide racemate and napropamide-M appear to be very similar. The ADME characteristics of napropamide and napropamide-M are considered to be sufficiently similar to extend the findings of the four napropamide studies to napropamide-M. The conclusions on the ADME of napropamide given in the EFSA Conclusion for napropamide (EFSA, 2010) are considered by the RMS to be equally applicable to napropamide-M.

A comparison of the studies on napropamide and napropamide-M (not involving bile-duct-cannulated rats) indicates that the percentage of napropamide (racemate) / napropamide-M excreted in urine is similar for both actives (approximately 50 – 60 %). This comparison is based on the single oral studies at a dose of 30 mg/kg bw:

- [redacted] 2015
- [redacted] 1970
- [redacted] (1991, 1993)
- [redacted] 1988

The comparison suggests that the results of the study with napropamide racemate can be applied to napropamide-M and in particular that the results of the study with bile-duct-cannulated male rats are applicable to napropamide-M.

Excretion via urine in the repeated-dose study ([redacted] 1991) was slightly lower (approximately 40-50 %) than the single-dose studies (which themselves all appear to be in agreement). In addition, bile duct-cannulated rats appear to excrete a much lower proportion of the dose in urine (mean of 15 % of the applied dose) than non-bile duct cannulated rats, in which the proportion of the dose excreted in urine ranged from approximately 35 % to 60 %. This suggests that enterohepatic circulation is an important process in the normal excretion of napropamide racemate.

Absorption
Napropamide (racemate) and napropamide-M are rapidly and extensively absorbed after oral administration. Based on a study (with napropamide racemate) with bile-duct-cannulated male rats, more than 90 % of the administered radioactivity was absorbed after oral administration.

Distribution
The concentrations of measured radioactivity in blood and tissue residues were dose dependent. The radioactivity from tissues was highest at the first time point of investigation (6 hours), after which it declined gradually. The elimination of radioactivity from tissues/organs was almost complete 4 days after dosing, and after 7 days tissues contained less than 0.3 % of the administered dose. No sex difference was evident in tissue concentrations, apart from early gastrointestinal distribution of radioactivity which is, however, difficult to interpret. The highest concentrations were usually found in the intestines, especially within the first 3 days. Other tissues that contained comparatively high concentrations of radioactivity included blood-rich organs, such as the liver, spleen and kidney. Seven days after oral administration, the highest concentrations of radioactivity were present in the blood of both sexes. At the 6-hour time point, the concentrations in plasma and blood were almost the same, but at all other time points the concentrations in plasma were lower than in blood. This indicates that the radioactivity in blood was associated with the cell fraction. No significant differences in tissue distribution were seen between males and females. Given the similarity of the findings of the study using napropamide-M with the studies on napropamide racemate, these conclusions are considered to be applicable to napropamide-M.

Metabolism Judged from the presence of very little (< 0.5 % of the administered dose) non-metabolised parent compound and the presence of approximately 15 metabolites identified in urine or faeces, napropamide was extensively metabolised. A study to investigate the metabolism of napropamide-M in rats confirmed that the isomer was extensively metabolised and rapidly excreted, with excretion being approximately equally split between urine and faeces. The major metabolic routes of both the isomer and the racemate were: dealkylation to form NOPAM; oxidation of NOPAM to form NOPA; mono and di-hydroxylation of napropamide-M with subsequent dealkylation forming mono and di-hydroxy DE-NPAM; glucuronide conjugation of mono and di-hydroxylated napropamide-M; glucuronide and sulphate conjugation of hydroxylated DE-NPAM. The metabolite profile for faeces was similar to that of urine. No qualitative differences were apparent between the sexes, the dose levels (30 mg/kg bw and 300 mg/kg bw), or between single and repeat-dose animals. In the study with napropamide, biliary metabolites were mainly glucuronide conjugates of hydroxylated napropamide at position 4 (not investigated in the napropamide-M study). A consideration of the findings of the study on napropamide-M with the studies on napropamide racemate indicates that their metabolism is comparable.

Elimination
The elimination of both napropamide M and napropamide racemate and their metabolites was fast with a cumulative excretion value of approximately 90 % after 3 days, but the majority of the excretion occurred within the first 24 hours. About 40-60 % was excreted in urine and about 29-55 % in faeces for both the isomer and the racemate. Considering the excretion of napropamide racemate in the bile (about 78 % of dose 48 hours after administration), an extensive enterohepatic circulation is presumed. No radioactivity was eliminated via expired air after administration of either the isomer or the racemate. The potential for bioaccumulation of both parents is considered to be low. The level of the parent compounds found in urine and faeces was very low (0.63 % / 5.6 % and ND / 7.1 % of the administered dose in the urine / faeces of males and females, respectively), and metabolites were mainly excreted as glucuronide conjugates.

The 14-day repeated dosing with napropamide racemate did not significantly influence absorption, elimination, distribution of the parent or metabolites in tissues.

In general, some minor variances in rate and extent of absorption, biotransformation and excretion may occur which could be attributed to sex and/or size of administered dose.

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

No studies on absorption, distribution, metabolism and excretion by other routes are available for napropamide racemate or for napropamide-M. There is no concern for toxicity following dermal exposure, compared with that following oral exposure. The vapour pressure of napropamide-M is $<10^{-2}$ Pascal and therefore studies on napropamide-M by the dermal or inhalation routes are not required.

The applicant has not conducted comparative *in vitro* metabolism studies because no agreed test methods have been published. In accordance with SANCO/10181/2013 rev 2.1 (13 May 2013) a waiver is considered acceptable until such methods are published in the form of an update of Commission Communications 2013/C 95/01 and 2013/C 95/02.

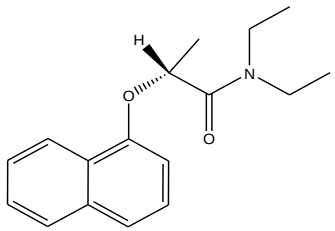
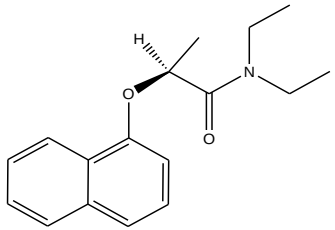
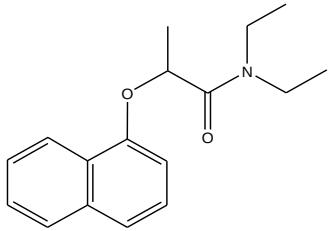
The metabolic pathways proposed for napropamide-M in the rat, N-dealkylation and aromatic ring hydroxylation with subsequent conjugation to form glucuronide and sulphate conjugates, are common metabolic steps and are likely to be conserved across species including humans. Furthermore, the extensive metabolism of napropamide-M in the rat indicates that it is a good substrate for the enzymes responsible for these metabolic pathways and it could be assumed that such rapid biotransformation and elimination via these routes reduces the likelihood of alternative pathways operating in humans.

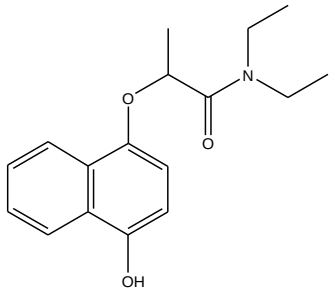
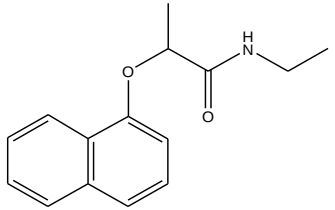
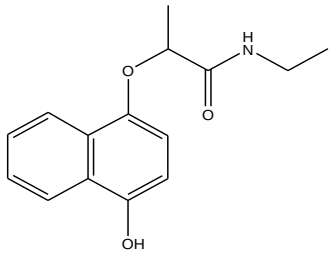
Table 6.1.13: Substances and metabolites; structures, codes, synonyms

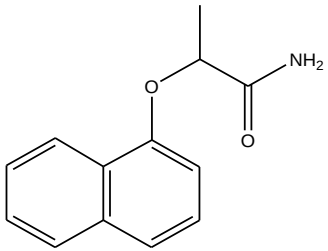
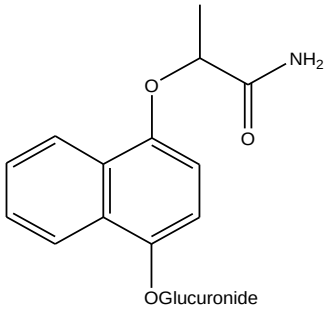
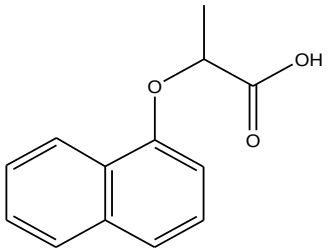
The main metabolites seen in the ADME study on napropamide-M are:

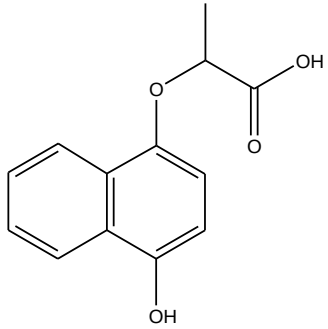
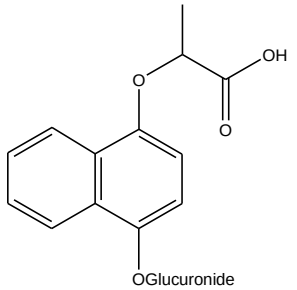
- DE-NPAM
- NOPAM
- NOPA

Other, minor, metabolites are also listed below.

Code Numbers and Trivial Names	Chemical Name	Synonyms	Compound found in:	Structure
Napropamide-M HBW07 Active substance (D-isomer)	(-)-*N*,*N*-diethyl-2-(1-naphthalenyloxy) propanamide	(R)-*N*,*N*-diethyl-2-(naphthalen-1-yloxy) propionamide	Crop (cabbage, tomato, potato) **Rat** Livestock (laying hen, lactating goat) Note: For parent, stereochemistry of applied active is considered to be conserved	
L-napropamide	(+)-*N*,*N*-diethyl-2-(1-naphthalenyloxy) propanamide	(S)-*N*,*N*-diethyl-2-(naphthalen-1-yloxy) propionamide		
Napropamide (racemate) Devrinol NPAM R7465/01 Compound 1 Compound II U5	*N*,*N*-diethyl-2-(1-naphthalenyloxy) propanamide	(2-(α-naphthoxy)-*N*,*N*-diethylpropamide) *N*,*N*-diethyl-2-(1-naphthoxy) propionamide (RS)-*N*,*N*-diethyl-2-(1-naphthyloxy) propionamide		

Code Numbers and Trivial Names	Chemical Name	Synonyms	Compound found in:	Structure
4-OH-NPAM R7465/05 4-Hydroxy-napropamide 4-Hydroxy devrinol Compound 5 Compound VI U6	*N*,*N*-diethyl-2-(4-hydroxy naphthalen-1-yloxy) propanamide	(2-(α-naphthoxy-4 hydroxy)-*N*,*N*-diethylpropamide) 4-hydroxy (*N*,*N*-diethyl-2-(1-naphthoxy)) propionamide	Crop (tomato, potato) Rat	
DE-NPAM R7465/07 Desethyl-napropamide DE napropamide Desethyl-devrinol Compound 7 Compound III U10	*N*-ethyl-2-(naphthalen-1-yloxy) propanamide	2-(α-naphthoxy)-*N*-ethyl propamide *N*-ethyl-2-(1-naphthoxy) propionamide	Crop (cabbage, tomato, potato) **Rat** Livestock (laying hen, lactating goat) Soil (aerobic, anaerobic and photolysis) Water/sediment	
4-OH-DE-NPAM R7465/08 4- Hydroxy-desethyl-napropamide Compound 8 U7	*N*-ethyl-2-(4-hydroxynaphthalen-1-yloxy) propanamide	(2-(α-naphthoxy-4-hydroxy)-*N*-ethylpropamide 4-hydroxy (N-ethyl-2-(1-naphthoxy)) propionamide	Crop (tomato) Rat Livestock (laying hen)	

Code Numbers and Trivial Names	Chemical Name	Synonyms	Compound found in:	Structure
NOPAM R7465/06 Naphthoxy-propionamide Compound 6 U11	2-(naphthalen-1-yloxy) propanamide	(2-(α-naphthoxy)-propionamide) naphthoxypropionamide 2-(1-naphthyloxy) propanamide (1-napthoxy) propionamide	Crop (cabbage) **<u>Rat</u>**	
4-OGlu-NOPAM U3, B3	O-glucuronide conjugate of: 2-(4-hydroxynaphthalen-1-yloxy)propanamide		Rat	
NOPA R7465/15 Compound 15 Compound VIII U12	2-(naphthalen-1-yloxy) propanoic acid	naphthoxypropionic acid 2-naphthoxypropionic acid 1-naphthoxypropionic acid 2-(α-naphthoxy)-propionic acid) 2-(1-napthyloxy) propionic acid	Crop (cabbage, tomato, potato) **<u>Rat</u>** Livestock (laying hen) Soil (photolysis) Water/sediment	

Code Numbers and Trivial Names	Chemical Name	Synonyms	Compound found in:	Structure
4-OH-NOPA R7465/22 Compound 22 U9	2-(4-hydroxynaphthalen-1-yloxy) propanoic acid	4-hydroxy-naphthoxypropionic acid 4-hydroxy-(1-naphthoxy) propionic acid	Crop (tomato) Rat	
4-OGlu-NOPA U4, B4	O-glucuronide of: 2-(4-hydroxynaphthalen-1-yloxy) propanoic acid	4-glucuronyl-(1-naphthoxy) propionic acid	Rat	

B.6.2. ACUTE TOXICITY

Introduction

The acute toxicity of napropamide-M has been investigated by the oral, dermal and inhalation routes. *In vivo* skin and eye irritation studies are available, whilst skin sensitisation has been investigated in a local lymph node assay. The table below gives an overview of the results of the studies on napropamide-M.

Type of study	Species	Result	Reference
Oral	Rat	LD_{50} >2000 mg/kg bw	CA 5.2.1/01 [redacted] (2010a)
Dermal	Rat	LD_{50} >2000 mg/kg bw	CA 5.2.2/01 [redacted] (2010b)
Inhalation	---[a]	---[a] >4.8 mg/l	CA 5.2.3/01 [redacted] (2011) CA 5.2.3/02 [redacted] (1989)
Skin irritation	Rabbit	non-irritant	CA 5.2.4/01 [redacted] (2010c)
Eye irritation	Rabbit	non-irritant	CA 5.2.5/01 [redacted] (2011)
Skin sensitisation (LLNA)	Mouse	non-sensitising	CA 5.2.6/01 [redacted] (2011)

a technically unfeasible

B.6.2.1. Oral

The acute-toxic-class method has been used to investigate the acute oral toxicity of napropamide-M.

Table 6.2.1. Summary of the acute oral toxicity of napropamide-M

Method Guideline, GLP status, reference	**Species, strain, sex, no./group**	**Test substance, dose levels, duration of exposure**	**LD50**	**Remarks**
OECD 423 (2001) (acute toxic class method) KCA 5.2.1/01 GLP [redacted], 2010a	Rats / Wistar / 3 females / group Observation period: 14 days	2000 mg/kg bw formulated in polyethylene glycol **Purity** Total D+L: 97.2 % D-isomer: 96.71 % L-isomer: 0.49 % Batch Number: UPV/714-181 /DEV/014	> 2000 mg/kg bw	No deaths. No clinical signs recorded during the entire observation period

In an acute oral toxicity study performed in accordance with the acute toxic class method, 2000 mg/kg bw napropamide-M was administered to three fasted female rats. As no deaths occurred in this group, the result was confirmed in three additional animals at the same dose level; none of these animals died.

No clinical signs were observed during the course of the study. The body weight of the animals was within the range commonly recorded for this strain and age. No macroscopic findings were recorded at necropsy. On the basis of this study, it is concluded that napropamide-M is not acutely toxic by the oral route (LD50 > 2000 mg/kg bw) and does not meet the criteria for classification.

B.6.2.2. Dermal

One acute dermal toxicity study is available, which was conducted in rats.

Table 6.2.2. Summary of the acute dermal toxicity of napropamide-M

Method Guideline, GLP status, reference	**Species, strain, sex, no./group**	**Test substance, dose levels, duration of exposure**	**LD50**	**Remarks**
OECD 402 (1987) GLP KCA 5.2.2/01 [redacted], 2010b GLP	Rat, Wistar, 5/sex Observation period: 14 days	2000 mg/kg bw formulated in polyethylene glycol, applied for 24 hours Batch Number: UPV/714-181 /DEV/014 **Purity** Total D+L: 97.2 % D-isomer: 96.71 % L-isomer: 0.49 %	> 2000 mg/kg bw	No deaths. No clinical signs recorded during the entire observation period.

In an acute dermal toxicity study, rats were exposed to a single limit dose of 2000 mg/kg bw for 24 hours under a semi-occlusive dressing. The application area was at least 10 % of the total body surface area. At the end of the 24-hour exposure period, the dressing was removed and the application site was rinsed with warm water. Local signs were recorded once daily during days 2 (following dressing removal) through to day 15. There were no deaths, signs of systemic toxicity, local skin effects or adverse macroscopic findings at necropsy with the exception of one female animal, which suffered an injury to the skin of the left flank caused by clipping. Two female animals lost body weight (-0.4 % to -0.7 %) during the first week after treatment. However, both animals then regained weight until the end of the observation period. Otherwise, the body weight of the animals was within the range commonly recorded for animals of this strain and age. No macroscopic findings were observed at necropsy.

On the basis of this study, it is concluded that napropamide-M is not acutely toxic by the dermal route (LD50 > 2000 mg/kg bw) and does not meet the criteria for classification.

B.6.2.3. Inhalation

Tests were conducted to investigate the acute toxicity of napropamide racemate and napropamide-M by the inhalation route in rats. A 4-hour inhalation exposure study was carried out for both test materials; however, in the most recent test an aerosol suitable for administration by inhalation at the requested aerosol concentration could not be generated because of the adhesive properties and the high particle size of the test item the test was therefore not conducted.

Table 6.2.3. Summary of studies to investigate the acute inhalation toxicity of napropamide-M

Method Guideline, GLP status, reference	**Species, strain, sex, no./group**	**Test substance, dose levels, duration of exposure**	**LC50**	**Remarks**
Acute inhalation toxicity OECD 403 (2009) GLP KCA 5.2.3/01 [redacted], 2009	N/A	napropamide-M Batch number: UPV/714-181/DEV/014 **Purity** Total D+L: 97.2 % D-isomer: 96.71 % L-isomer: 0.49 %	-	An aerosol suitable for administration by inhalation at the requested aerosol concentration could not be generated because of adhesive properties and the high particle size of the test item.
Acute inhalation toxicity Follows OECD 403 (1981) GLP KCA 5.2.3/02 [redacted], 1989	Rat, Wistar, 5/sex Nose only exposure 14 day observation period	napropamide racemate 0 and 5 mg/l air for 4 hours Batch: WRC 4921-27-27 Purity: 94.3 % (total D- + L-isomer)	>4.8 mg/l	No deaths. Clinical signs: salivation, tail erection, paw flicking, shaking, abnormal respiratory noise and mucoid nasal discharge. There was no significant effect on body weight or body weight gain.

Four technical trials were undertaken in an attempt to generate an aerosol suitable for administration by inhalation at the requested aerosol concentration. Such an aerosol could not be generated because of adhesive properties and the high particle size of the test item and therefore, since the objectives of this study could not be achieved, it was not conducted. Under normal production methods the physical form of the test item makes it unlikely to present an inhalation hazard. The smallest achievable MMAD in this study was 7.13µm.

In the second submitted study a group of 5 male and 5 female rats was exposed (nose-only) for a single four-hour period to napropamide racemate, at a target particulate concentration of 5 mg/l. A concurrent control group was similarly treated but was exposed only to air. The particulate concentration achieved was measured and found to be 5.1 mg/l (± 1.35). The atmospheric concentration of napropamide racemate, analysed using chromatography, was 4.8 g/l (± 1.30). The aerodynamic particle size distribution had a median size of 8.11 µm and a geometric standard deviation of 2.64.

There were no deaths. Observed clinical signs were not considered to be related to administration of the test substance and were likely to be due to the method of exposure. There were no effects on organ weights or organ to body weight ratios. There were no significant post mortem findings. The LC50 was > 4.8 mg/l. The MMAD of 8.11µm is above the range specified in the guideline (1-4 µm), but this was the lowest achievable MMAD for napropamide racemate.

It is noted that an acute inhalation toxicity study was conducted on the representative product (see Section B.6.1.3. of Volume 3 of B.6 (PPP)). On the basis of this study it was concluded that D-Devrinol 450 SC was not acutely toxic by the inhalation route (LC50 > 3.3 mg/l).

On the basis of the available information there are no concerns for inhalation toxicity.

B.6.2.4. Skin irritation

A primary skin irritation test in rabbits was performed to determine the irritancy potential of napropamide-M.

Table 6.2.4. Summary of the skin irritation studies with napropamide-M

Method Guideline, GLP status, reference	Test system	Test substance, dose levels, duration of exposure	Results
Primary Skin Irritation Study in Rabbits OECD 404 (2002) GLP Report KCA 5.2.4/01 [redacted], 2010c	Rabbit, New Zealand White, 3 males	0.5 g minimally moistened with water applied to intact skin for 4 hours under semi-occlusive dressing. Batch Number: UPV/714-181/DEV/014 **Purity** Total D+L: 97.2 % D-isomer: 96.71 % L-isomer: 0.49 %	Mean scores (averaged over 24, 48 & 72 hours) for each animal: 0, 0, 0 for erythema 0, 0, 0 for oedema Not a skin irritant.

The primary skin irritation potential of napropamide-M was investigated according to OECD test guideline 404. The test item was applied by topical semi-occlusive application of 0.5 g to the intact skin of the left flank of each of three young adult male New Zealand white rabbits. The duration of exposure was four hours.

The mean score was calculated separately for each animal across 3 scoring times (23, 48 and 72 hours after patch removal) for erythema/eschar grades and for oedema grades, respectively. The test item did not elicit any skin reaction at the application site of any animal at any of the observation times. The individual mean erythema, eschar and oedema score of the three animals was 0.

On the basis of this study, it is concluded that napropamide-M is not a skin irritant and thus does not meet the criteria for classification as an irritant.

B.6.2.5. Eye irritation

The eye irritation potential of napropamide-M has been investigated in one primary irritation study in rabbits.

Table 6.2.5. Summary of the eye irritation studies with napropamide

Method Guideline, GLP status, reference	Test system	Test substance, dose levels, duration of exposure	Results
Primary Eye Irritation in Rabbits OECD 405 (2002) GLP Report KCA 5.2.5/01 [redacted], 2011	Rabbit, New Zealand white, male, 3	0.1 g applied in one eye for 24 hours, followed by rinsing with tap water. Batch Number: UPV/714-181/DEV/014 **Purity** Total D+L: 97.2 % D-isomer: 96.71 % L-isomer: 0.49 %	Mean scores (averaged over 24, 48 & 72 hours) for each animal: 0, 0, 0 for corneal opacity 0, 0, 0 for iris light reflex 0.00, 0.00 and 0.33 for redness of the conjunctiva 0, 0, 0 for conjunctival chemosis All reactions reversible within 24 hours after application. No clinical signs. The test item did not induce significant or irreversible damage to the rabbit eye.

The primary eye irritation potential of napropamide-M was investigated according to OECD test guideline 405.

0.1 g of the test item was instilled into the left eye of each three young adult male New Zealand white rabbits.

The mean scores were calculated for each animal across three scoring times (24, 48 and 72 hours following test item instillation) for corneal opacity, iris light reflex, redness and chemosis of the conjunctivae, respectively. The individual mean scores for corneal opacity and iris light reflex were 0.00 for all three animals. The individual mean scores for the conjunctivae were 0.00, 0.00 and 0.33 for reddening and 0.00 for chemosis for all animals.

The instillation of napropamide-M into the eye resulted in mild, early onset and transient ocular changes, such as reddening of the conjunctivae and sclerae. These effects were reversible and were no longer evident 24 hours or 48 hours after treatment. There were no abnormal findings observed in the cornea or for the iris light reflex of any animals at any of the examinations. No corrosion was noted at any of the timing intervals. In addition no general clinical signs were observed.

Under the conditions of this study the test item did not lead to significant or irreversible damage to the rabbit eye. A classification for eye irritation is not required.

B.6.2.6. Skin sensitization

The skin sensitisation potential of napropamide M has been investigated in a local lymph node assay.

Table 6.2.6. Summary of the skin sensitisation studies with napropamide-M

Method, Guideline, GLP status, reference	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results
Local lymph node assay (LLNA) in mice OECD 429 (2010) GLP Report KCA 5.2.6/01 [redacted] 2011	Mice, CBA/CaOlaHsd, females, 5 in each group, 6 groups. 2 in pre-test. Total animals: 32	10, 25 and 50 % suspended in dimethylformamide Batch No.: UPV/714-181/DEV/014 **Purity** Total D+L: 97.2 % D-isomer: 96.71 % L-isomer: 0.49 %	Stimulation indices (S.I.) of 1.27, 1.00, and 1.41 were determined with the test item at concentrations of 10, 25, and 50 % in dimethylformamide. No symptoms of local toxicity and no systemic findings were observed during the study period.

A local lymph node assay was performed using napropamide-M formulated in dimethylformamide (DMF) at concentrations of 10, 25, and 50 % (w/v).

There were no animal deaths during the course of the study, nor did any animals show any clinical signs. Under the conditions of this study napropamide-M at concentrations of 10, 25 and 50 % in dimethylformamide elicited stimulation indices (S.I) of 1.27, 1.00 and 1.41, respectively. The S.I. determined with the positive control (concurrent, 25 % alpha-hexyl cinnamaldehyde in acetone:olive oil) was 10.03.

On the basis of this study, it is concluded that napropamide-M is not a skin sensitiser and thus does not meet the criteria for classification.

B.6.2.7. Phototoxicity

An *in vitro* 3T3 NRU Phototoxicity Test has not been conducted because napropamide-M does not meet the criteria in Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009. The active substance does not absorb electromagnetic radiation in the range 290-700 nm. The λ max (nm) values were ≤290 nm.

The levels of radioactivity in the eyes and skin (light exposed tissues) were negligible in terms of concentration and duration of exposure. Thus overall, the potential for any phototoxicity associated with napropamide-M is considered to be very low.

B.6.2.8. Conclusion

Napropamide-M shows very low acute oral and dermal toxicity, with LD_{50} values greater than the highest dose tested in each case.

For the inhalation endpoint, an aerosol with a respirable particle size could not be generated from napropamide-M, making the testing of this endpoint technically unfeasible ([redacted], 2011). It was therefore considered that under normal production methods the physical form of the test article makes it unlikely to present an inhalation hazard. The physical appearance of napropamide-M is very similar to that of napropamide racemate; they are described as a "beige crystalline solid" and a "light brown solid", respectively. It is considered appropriate to read across to the acute inhalation study on napropamide racemate (Hext, P.M. 1989) to satisfy this data point. This study gave an LC_{50} of >4.8 mg/l and therefore, it is concluded that napropamide-M does not require classification for acute inhalation toxicity.

The acute toxicity of napropamide racemate was investigated previously (EFSA, 2010). Other than the oral inhalation study (Hext, 1989), none of the acute toxicity studies on napropamide racemate have been presented here as they do not provide any additional information. The acute toxicity of napropamide-M is consistent with that of napropamide racemate and they are therefore considered to have equivalent acute toxicity.

Napropamide-M was neither an eye nor skin irritant nor sensitising in the LLNA skin sensitisation study. Therefore, napropamide-M does not meet the classification criteria for acute toxicity, irritation (skin or eye) or skin sensitisation endpoints according to Regulation 1272/2008.

B.6.3. SHORT-TERM TOXICITY

Introduction

The following short term studies are available on napropamide racemate and napropamide-M.

28-day studies
Rat
28-day dose-range-finding study on napropamide-M
28-day range finding study
Mouse
6-week dietary range-finding study
Dog
28 day oral dose range finding study

90-day
Rat
90-day dietary toxicity study on napropamide-M
Oral ninety days dietary toxicity study in rats: safety evaluation by dietary feeding to rats for 13 weeks

1 year
There are two 1-year studies in the dog.

There is also a 21-day dermal toxicity study in the rat.

All but two of the studies were done using napropamide racemate and were previously evaluated by Denmark for the approval of napropamide. The summaries of these studies presented below have not been changed (other than minor amendments to improve readability) and the conclusion has not changed. The final conclusions drawn on the basis of all of the short term studies on napropamide racemate have not changed and are as described in the EFSA Conclusion (EFSA, 2010).

The other two studies (28-day dose-range-finding study and a 90-day dietary toxicity study, both in the rat) are new, conducted using napropamide-M and have been evaluated for the first time, here.

The short term toxicity of napropamide-M was evaluated in a preliminary 28-day dose range finding study and a subsequent 90-day dietary toxicity study in the rat. Napropamide racemate was included at the same high dose level as napropamide-M in the 90-day study to facilitate a direct comparison of its toxic effects for the purposes of bridging to the napropamide racemate toxicology database.

B.6.3.1. Oral 28-day study

A 28-day dose-range finding study in rats has been conducted with napropamide M. The sub-acute toxicity of napropamide racemate has been investigated in 28-day studies in rats and dogs and a six-week study in mice.

B.6.3.1.1. Oral 28-day range finding study in rats

Introduction

This is a new study; it was conducted to support the application for approval of napropamide-M and has been evaluated for the first time, here.

Author(s):	[redacted] 2013
Study title:	28-day dose-range-finding dietary toxicity study of napropamide-M in Wistar rats Company Report No. 410-1-02-6144
Test substance:	napropamide-M
Purity:	Total D+L: 97.2 %, D-isomer: 96.71 %, L-isomer: 0.49 %
Batch no.:	UPH-08/DNE-263/Tech/20121226
Test animals:	Male and female RccHan: WIST strain rats
Groups:	5/sex/dose group.

Dose:	0, 1000, 5000, or 10 000 ppm
Vehicle/solvent:	None. Test article was admixed with diet
Route:	Oral via diet
Statistics/ measurements:	Yes. Statistical significance at 95 % confidence level All continuous data parameters were subject to Bartlett's test to check homogeneity of variance before conducting Analysis of Variance (ANOVA) and Dunnett's t-test. Where data did not meet homogeneity of variance, Student's t-test was performed to calculate significance
GLP:	Yes
Guideline:	Not applicable to a range finding study. Partially complied with OECD N° 407, "Repeated Dose 28-Day Oral Toxicity Study in Rodents" (October 2008). Partial compliance is acceptable for a range finding study.
Deviation:	Not applicable
Acceptability:	Acceptable

Method

Napropamide-M was administered on a continuous basis in the diet for 28 days to groups of 5 male and 5 female rats at dose levels of 0, 1000, 5000, or 10 000 ppm (equivalent to a maximum dose of 849 or 971 mg/kg bw/d for males and females respectively, see Table 6.3.1). At necropsy selected organs (adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, seminal vesicles, spleen, testes, thymus and uterus (horns and cervix) were weighed and selected tissues (brain, heart, kidneys, liver, lungs and spleen) were preserved for possible subsequent histological examination.

Table 6.3.1: Study design and dose received

Test group	Concentration in diet (ppm)	Mean dose (mg/kg bw/day)		Animals assigned	
		Male	Female	Male	Female
G1 (Control)	0	-	-	5	5
G2	1000	82.9	100	5	5
G3	5000	410	484	5	5
G4	10 000	849	971	5	5

Results

No clinical signs were observed throughout the study period. External and internal examination of terminally sacrificed animals did not reveal any abnormalities. Treatment-related reductions in mean terminal body weight were observed in mid dose (G3) and high dose (G4) treated animals when compared with the vehicle control animals (G1) (up to 12 %; Table 6.3.2). Mean food consumption of napropamide M - technical treated groups was comparable with the vehicle control group during the treatment period.

The absolute and relative weights of kidneys were higher in males at 5000 and 10 000 ppm and in females at 10 000 ppm compared with those of the control group, although statistical significance was only observed for relative weights. These findings were likely to be treatment related.

There were statistically significant increases in relative weights of the spleen (males 10 000 ppm; 30 %), liver (high-dose females: 17 %) and ovaries (females at 10 000 ppm: 28 %) compared with the vehicle control group, which, with the possible exception of the liver, were probably attributed to the decrease in terminal body weights, since the absolute weights were not affected. The slight increase in relative liver weight at the high dose is likely to indicate a treatment-related effect. In the absence of any difference from controls in relative weight, the lower absolute thymus weight observed in females at 10 000 ppm was probably related to the lower body weight.

As mentioned above, selected tissues were preserved for possible subsequent histological examination, however no examination, at the time of the report, had been conducted and therefore it is not possible to know if the effects on organs were associated with histopathological effects.

A summary of selected organ weights is given in Table 6.3.2.

Table 6.3.2: Absolute and relative weights of selected organs

Parameter	Male				Female			
Dose level (ppm)	0	1000	5000	10 000	0	1000	5000	10 000
Terminal b.w. g	360	349	341	319	221	213	208	197
Liver (abs) g	11.81	10.94	11.96	11.96	7.223	7.316	7.441	7.547
Liver (rel)	3.288	3.124	3.520	3.750	3.261	3.433	3.574	3.819*
Kidney (abs) g	2.301	2.306	2.498	2.557	1.524	1.566	1.557	1.554
Kidney (rel)	0.640	0.662	0.734**	0.801**	0.689	0.736	0.753	0.789*
Spleen (abs) g	0.748	0.733	0.892	0.863	0.596	0.513	0.540	0.523
Spleen (rel)	0.208	0.209	0.262	0.271*	0.270	0.242	0.258	0.265
Thymus (abs) g	0.481	0.469	0.545	0.444	0.479	0.408	0.451	0.388*
Thymus (rel)	0.134	0.134	0.159	0.139	0.218	0.194	0.217	0.197
Ovaries (abs) g	-	-	-	-	0.103	0.096	0.107	0.104
Ovaries (rel)	-	-	-	-	0.047	0.045	0.052	0.053*

abs: absolute weight
rel: organ weight relative to body weight (%)
* Significantly different from control; $p < 0.05$
** Significantly different from control; $p < 0.01$

Conclusion

A guideline-compliant 28-day dose range finding dietary toxicity study has been conducted in rats at doses of up to 10 000 ppm of napropamide-M, delivered in the diet for 28 consecutive days.

Based on the results obtained in this range-finding study, it is concluded that napropamide-M produced toxicity at the dose levels of 5000 ppm and 10 000 ppm. The degree of toxicity was dose dependent with slight toxicity observed at 5000 ppm. The observed toxic effects included reductions in mean body weight, increased relative kidney weights (males in the 5000 and 10 000 ppm dose group and females in the 10 000 ppm) and significant increases in relative weights of the spleen (only males in the 10 000 ppm dose group) and liver. Based on the limited information in the study (no clinical chemistry or histopathological examination) kidney, spleen and liver are potentially the target organs.

There was no evidence of toxicity at 1000 ppm.

On the basis of these results and in consideration of other information available on napropamide racemate, dose levels of 0, 600, 2500 and 10 000 ppm were selected for a subsequent 90-day dietary toxicity study of napropamide-M in Wistar rats.

Since this is a range-finding study, a NOAEL has not been set.

B.6.3.1.2. Oral 28-day range-finding study in rats

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] 1988a
Study title:	Memorandum Report for T-13275: 4-Week Dietary Range-Finding Study with R7465 in rats
Test substance:	Napropamide racemate
Purity:	Not specified in this report, but known from acute toxicity reports to be 94.3 % (total D- + L-isomer)
Batch no.:	EHC no 0952-17; WRC no 4921-27-27
Test animals:	Male and female [redacted]:CD® SD rats
Groups:	10/sex/dose group.
Dose:	0, 2500, 4200, 7100, 12000 and 20000 ppm
Vehicle/solvent:	None. Test article was admixed with diet
Route:	Oral via diet
Statistics/ measurements:	Yes. Statistically significance at 95 % or 99 % confidence level
GLP:	No.
Guideline:	None stated. No guideline applies to range-finding studies. The conduct of the study is largely in compliance with method B.7
Deviation:	Deviations: purity of substance not given. No histopathological examinations performed. Individual animal data not reported
Acceptability:	Acceptable

Method

Six groups of rats (10/sex/group) were administered napropamide (the batch was used in an acute toxicity test, where the purity was reported to be 94.3 %, total D- + L-isomer) in the diet for 4 weeks at constant concentrations of 0, 2500, 4200, 7100, 12000, or 20000 ppm.

Clinical signs were recorded daily, and body weights and food consumption were measured weekly. Haematology and blood chemistry tests were performed on samples taken at necropsy. Final body weight and organ weights (brain, liver, kidneys and gonads) were recorded for all animals at study termination.

Results

There were no mortalities throughout the study. The average dose levels based on nominal concentrations were 0, 181, 303, 502, 861, and 1577 mg/kg bw day for males and 0, 197, 320, 530, 873 and 1604 mg/kg bw day for females. Food consumption was significantly reduced in the first week of dosing in males of the two highest dose groups and in females of the high dose group. Body weight gain was significantly reduced in males and females administered 7100 ppm and above. At termination, body weights were significantly reduced by 12-15 % in males and 12-13 % in females in the three highest dose groups (7100 ppm and above, corresponding to 502 mg/kg bw/day and above in males and 530 mg/kg bw/day in females). Terminal body weights were also reduced at 4200 ppm (303 and 320 mg/kg bw/day in males and females, respectively), although these reductions were not statistically significant.

Clinical laboratory tests showed significant decreases in red blood cell, haemoglobin and haematocrit levels in rats administered 12 000 and 20 000 ppm (861 and 1577 mg/kg bw for males and 873 and 1604 mg/kg bw/day for females). Other statistically significant changes included a minor increase in platelet count at 4200 ppm and above (303 and 320 mg/kg bw/day and above in males and females, respectively). The effects on red blood cells, haemoglobin and haematocrit tended to be dose-related, whereas no dose-response was apparent for the effect on platelets.
Effects on clinical chemistry parameters included decreased glucose levels and increased cholesterol and gamma-glutamyl transferase in males administered 20 000 ppm (1577 mg/kg bw/day) and in females administered 12 000 and 20 000 ppm (873 and 1604 mg/kg bw/day). In addition, sorbitol dehydrogenase, alkaline phosphatase and globulin levels increased slightly in high-dose 20 000 ppm males (1577 mg/kg bw/day).

Napropamide induced an increase in absolute and/or relative liver weights at 4200 ppm and above in males (303 mg/kg bw/day) and in all dose groups in females. The magnitude of these findings was dose-related. In addition, kidney to body weight values were significantly increased in males at 4200 ppm (303 mg/kg bw/day) and above and in females at 20 000 ppm (1604 mg/kg bw/day). The increases in kidney weight were not dose-related in magnitude and were not accompanied by changes in absolute kidney weight.

Conclusion

This 28-day dietary study in rats with napropamide concentrations up to 20 000 ppm (1577/1604 mg/kg bw/day in males and females, respectively) was not conducted according to GLP. No OECD guideline applies to range-finding studies, but the study is largely in compliance with EU method B.7, although no histopathology was performed. The study is acceptable as supporting evidence for the evaluation of the short-term toxicity of napropamide. Findings from this range finding study suggest that dietary administration of napropamide to rats causes decreased body weight gain and haematological changes from dietary doses of around 300 mg/kg bw suggestive of mild anaemia, alterations of clinical chemistry parameters (increased serum cholesterol and gamma-glutamyl transferase activity) from around 860 mg/kg bw/day and increased liver weights from 303 mg/kg bw/day in males and from 197 mg/kg bw/day in females indicating the liver to be a target organ. Since this is a range-finding study, a NOAEL has not been set.

B.6.3.1.3. Oral 6-week range-finding study in mice

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] 1988b
Study title:	Memorandum report for T-13271: 6-Week dietary range-finding study with R-7465 in mice.
Test substance:	Napropamide racemate
Purity:	Not specified in this report, but the batch was indicated in acute toxicity study at a purity of 94.3 % (total D- + L-isomer)
Batch no.:	EHC no 0952-17; WRC no 4921-27-27
Test animals:	Male and female [redacted]:CD®-1 (ICR) BR mice
Groups:	10/sex/dose group
Dose:	0, 2500, 3750, 5000, 7000 and 14000 ppm
Vehicle/solvent:	None. Test article was admixed with basal diet
Route:	Oral via diet
Statistics/ measurements:	Yes. Significance at 95 and 99 % confidence limit reported for measured values. Method not given. No statistics on incidences
GLP:	No
Guideline:	Range-finding study; no specific guideline applies. Study protocol appears to be close to OECD method 407.
Deviation:	No functional observation performed. Restricted gross pathology and only histopathology of livers. Limited description of protocol and reporting
Acceptability:	Acceptable as range finding study

Methods

Six groups of mice (10/sex/group) were administered napropamide racemate (the batch was used in an acute toxicity test, where the purity was reported to be 94.3 %, total D- + L-isomer) in the diet for 6 weeks at constant concentrations of 0, 2500, 3750, 5000, 7000, and 14 000 ppm.

Clinical signs were recorded daily, and body weights and food consumption were measured weekly.
Haematology and blood chemistry tests were performed on samples taken at study termination. Final body weights and organ weights (brain, liver, kidneys and gonads) were recorded for all animals. Livers from control and high dose (14 000 ppm) animals were examined microscopically.

Results

There were no deaths during the study. The average dose levels based on nominal concentrations were 386, 580, 737, 1123, and 2257 mg/kg bw/day for males and 513, 737, 1054, 1467 and 2937 mg/kg bw/day for females. Body weight means were comparable between the control and treated mice. Food consumption was not affected in either male or female mice.

Necropsy revealed enlarged livers in 4/5 females of the high dose group (14 000 ppm, corresponding to 2937 mg/kg bw/day). Analysis of organ weight revealed that napropamide administration resulted in statistically significant increases in absolute and/or relative liver weight in males at 5000 ppm (737 mg/kg bw/day) and above and in females at 7000 ppm (1467 mg/kg bw/day) and above. Livers from high dose (14 000 ppm: 2257 and 2937 mg/kg bw/day in males and females, respectively) and control animals were examined microscopically. There were no histopathological findings to explain the increased liver weights among animals fed napropamide in the diet. There were no other dose-related findings upon necropsy.

Conclusion

This range finding study with dietary administration of napropamide to mice up to 14 000 ppm was not conducted according to GLP and no guideline was stated. Protocol description and reporting are insufficient. However, the study protocol resembled OECD 407 but with restricted gross and microscopic evaluations. The study is acceptable as a dose-range finding study, bringing supporting evidence to the evaluation of the short-term toxicity of napropamide. The study showed statistically significantly increased liver weights in males at 5000 ppm (737 mg/kg bw/day) and in females at 7000 ppm (1467 mg/kg bw/day) and above. However, there were no histopathological changes in the liver to indicate an adverse effect. Therefore, these increased liver weights were most likely attributable to adaptive rather than adverse changes. The study has not been used to set a NOAEL.

B.6.3.1.4. Oral 28 -day range finding study in dogs

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] 1987
Study title:	An oral dose range finding study of Devrinol in the Beagle dog
Test substance:	Napropamide racemate
Purity:	94.7 % (total D- + L-isomer)
Batch no.:	WRC 4921-27-24
Test animals:	Beagle dogs
Groups:	2/sex/group
Dose:	0, 30*, 60, 125, 250, 500 and 1000* mg/kg bw/day *: From week 3, the animals initially dosed 30 mg/kg bw were dosed 1000 mg/kg bw, due to lack of signs of toxicity at the high dose level.)
Vehicle/solvent:	Test article was administered in gelatine capsules
Route:	Oral by gavage
Statistics/ measurements:	Too small groups for statistical evaluation. Group means and standard deviation were calculated.
GLP:	Yes (40 CFR part 160)
Guideline:	No guideline applies, as the study was a range-finding study conducted for selecting dose levels for a subsequent one-year study in dogs.
Deviation:	Not relevant.
Acceptability:	Acceptable as dose-range finding study. It is noted that only limited number of animals was used and histopathological examination was not performed.

Methods

Five groups of Beagle dogs (2/sex/group) were administered napropamide (Devrinol; purity 94.7 % total D- + L-isomer) daily for 28/29 days. The test substance was administered in gelatine capsules at dose levels of 30, 60, 125, 250 and 500 mg/kg bw/day. Because of the lack of any apparent effects after 2 weeks in any of the starting dose levels (starting on study day 16), the 30 mg/kg bw/day dose level was increased to 1000 mg/kg bw/day.

All animals were examined twice daily. Daily and weekly food consumption and terminal body weights were recorded. Ophthalmoscopic examinations were conducted on all dogs at study initiation and during week 4. Haematology and clinical chemistry parameters were evaluated for all dogs at pre-test and during week 4. At

necropsy, a complete gross pathological examination was conducted on all dogs. Organs were weighed and tissues/organs were preserved. No histopathological examination was performed.

Results

There were no deaths and no signs of severe adverse effects. Clinical signs occurred in all animals but with a higher frequency in treated animals than controls and included emesis and soft/unformed/mucoid or liquid faeces at 4-6 hours after dosing. The effects were not dose-related and therefore they were not considered to be treatment-related. Green staining of the fur of the paws was noted for dogs treated at 500 or 1000 mg/kg bw/day during weeks 3 to 4. At 1000 mg/kg bw/day, decreases in food consumption and loss in body weight were noted in males and females from week 3 to week 4, when the increased dose was employed. No treatment-related effects were noted in haematological or clinical chemistry parameters or at ophthalmological examination. No treatment-related effects were seen on organ weights and no treatment-related changes were detected at gross necropsy.

Conclusion

This dose-range finding study on the administration of napropamide racemate to Beagle dogs for 28 days at doses up to 1000 mg/kg bw/day is conducted in accordance with an US-EPA guideline (83-3) and GLP. No OECD guideline applies for range-finding studies. The study is acceptable as an indication of the dose to be used in the subsequent 1 year day dog study. However, it cannot be used to set an NOAEL, owing to the low number of animals per group and the absence of histopathological assessment. Reductions in food consumption and body weights at 1000 mg/kg bw/day were observed. No significant treatment related adverse effect was seen at any lower dose. Therefore, it was considered that the top dose for the proposed 1-year dog study should not exceed 500 mg/kg bw/day.

B.6.3.2. Oral 90-day study

The sub-chronic toxicity of napropamide-M has been investigated in a new 90-day dietary toxicity study in rats, which is evaluated here for the first time. A 90-day study in rats on napropamide racemate was previously evaluated by Denmark for the approval of napropamide and has been re-presented below.

B.6.3.2.1. Oral 90-day dietary toxicity study in rats

Introduction

The short term toxicity of napropamide-M was evaluated in this 90-day dietary toxicity study in the rat. Napropamide racemate was included at the same high dose level as napropamide-M in the 90-day study to facilitate a direct comparison of its toxic effects for the purposes of bridging to the napropamide racemate toxicology database. This is a new study; it was conducted to support the application for approval of napropamide-M and has been evaluated for the first time, here.

Author(s):	[redacted] 2014
Study title:	90-day dietary toxicity study of napropamide-M in Wistar rats
Test substance:	Napropamide and napropamide-M
Purity:	Napropamide: 97.2 % w/w (total D- + L-isomer) Napropamide-M: Total D+L: 97.2 %, D-isomer: 96.71 %, L-isomer: 0.49 %
Batch no.:	Napropamide: UPH-08/NE-261/Tech/20121228 Napropamide-M: UPH-08/DNE-263/Tech/20121226
Test animals:	RccHan: WIST strain rats
Groups:	Main group: 10 animals/sex Satellite group (toxicokinetic investigation): 4 animals/sex Recovery group: 10 animals/sex
Dose:	Napropamide-M: 0, 600, 2500 or 10 000 ppm Napropamide: 10 000 ppm
Vehicle/solvent:	None. Test article was admixed the diet
Route:	Oral by diet
Statistics/ measurements:	Yes. Statistical significance at 90 % and 95 % confidence level

GLP: Yes (certified laboratory)
Guideline: OECD N° 408, "Repeated Dose 90-Day Oral Toxicity Study in Rodents" (September 1998); US EPA Health Effects Test Guidelines, OPPTS 870.3100, 90 Day Oral Toxicity in Rodents (August 1998)
Deviation: None
Acceptability: Acceptable

Methods

Groups of 10 (main study) male and female RccHan: WIST strain rats were fed diets containing 0, 600, 2500 or 10 000 ppm napropamide-M for up to 90 consecutive days. A satellite group (for toxicokinetic investigation) consisted of 4 animals/sex and each recovery group consisted of 10 animals/sex. In addition, one group of rats was dosed with napropamide racemate at the high dose of 10 000 ppm (only).

Table 6.3.3: Study design and dose received

Test group	Concentration in diet (ppm)	Mean dose (mg/kg bw/day)	
		♂	♀
Main groups			
G1 (Control)	0	-	-
G2	600	46	50
G3	2500	185	203
G4	10 000	778	872
G5	10 000 (nap rac)	745	843
Satellite groups			
G1SG (Control)	0	-	-
G2SG	600	45	52
G3SG	2500	194	221
G4SG	10 000	856	889
G5SG	10 000 (nap rac)	831	890
Recovery groups			
G6 (Control)	0	-	-
G7	10 000	766	908
G8	10 000 (nap rac)	733	879

nap rac: napropamide (racemate) technical (reference test item)

Each rat was observed twice daily for clinical signs, morbidity and mortality during the study period. Body weight was recorded on day 1 (prior to treatment) and at weekly intervals thereafter during the treatment and recovery periods. Food consumption was calculated at weekly intervals until terminal and recovery sacrifice. Neurobehavioral observations (NBO) were conducted on each rat once prior to treatment initiation and at weekly intervals thereafter. Ophthalmological examination was performed on all rats prior to treatment initiation and prior to terminal and recovery sacrifices. A functional observational battery of tests was performed on all animals (G1 to G5) during the 12th week of treatment and 4th week of the recovery period (G6 and G8). Haematological and clinical chemistry analyses were performed at the end of the treatment (G1 to G5) and recovery periods (G6 to G8). Urine samples were collected from all animals at the end of the treatment (G1 to G5) and recovery periods (G6 to G8) for urine analysis.

All the rats were subjected to gross pathological examination at the end of the treatment (G1 to G5) and recovery (G6 to G8) periods. Absolute organ weights were recorded and organ weights relative to terminal body weight were calculated for the organs viz., adrenals, brain, ovaries/testes, uterus/epididymides, heart, kidneys, liver, prostate, seminal vesicles, spleen and thymus in all the rats. Detailed histopathological examination was carried out in the control and high dose groups while the examination was extended to lower groups (G2 and G3) and recovery groups (G6 to G8) for treatment-related effects in the liver, kidneys, spleen and mesenteric lymph nodes.

Results

Mortalities and clinical observations:

No treatment related signs of clinical toxicity were observed. All animals survived up to the scheduled necropsy.

Food consumption and bodyweights

Body weights of animals administered napropamide-M or napropamide at 10 000 ppm were lower than those of controls, occasionally achieving statistical significance. This was associated with a notably lower body weight gain, mainly in females. The effects were similar for napropamide-M and napropamide. There were no treatment-related effects on body weight in the low and intermediate dose groups (Table 6.3.4).

There were no treatment-related effects on food consumption with the possible exception of slightly lower food consumption in the female napropamide racemate group (10 000 ppm dose group), attaining statistical significance during weeks 5 and 11 to 13.

Table 6.3.4: Intergroup comparison of body weights (g) at selected time points – main groups (G1-G5)

	Male					Female				
Dose level (ppm)	0	600	2500	10000	10 000 NT	0	600	2500	10000	10 000 NT
Week 0 (PT)	180	180	180	177	182	154	153	157	153	155
Week 2	265	260	264	245*	254	198	195	200	185	184*
Week 4	314	308	318	298	307	219	223	223	205	199**
Week 8	387	376	392	361	368	252	253	254	226**	222**
Week 13	438	429	442	409	412	261	267	268	236*	236*
% difference vs control	-	-2.3	+0.7	-6.8	-6.2	-	+2.1	+2.6	-9.5	-9.5
Body weight gain (weeks 0-13)[1]	259	249	262	231	230	107	114	111	83	82
% difference vs control gain	-	-3.8	+1.2	-11	-11	-	+5.8	+3.5	-23	-24

PT: Pre-treatment
NT: Napropamide (racemate) technical
[1]: Not analysed statistically
*: Significantly different from control; p <0.05
**: Significantly different from control; p <0.01

After 4 weeks of recovery, body weights of both male and female rats of the high-dose napropamide-M-treated group were comparable with the control group while in the high-dose napropamide racemate treated group the reduction in body weight was not fully recovered in females (Table 6.3.4a).

Table 6.3.4a: Intergroup comparison of body weights (g) at selected time points – recovery groups (G1SG, G4SG and G5SG)

	Male			Female		
Dose level (ppm)	0	10000	10000 NT	0	10000	10000 NT
Week 0 (PT)	180	182	181	152	152	152
Week 13	428	413	421	253	234	224*
Week 17	454	456	469	261	256	239*
% difference vs control	-	+0.4	+3.3	-	-1.6	-8.4
Body weight gain (weeks 13-17)[1]	26	43	48	7	22	15
% difference vs control gai]	-	+67	+87	-	+209	+109

PT: pre-treatment
NT: napropamide (racemate) technical
[1]: Not analysed statistically
*: Significantly different from control; p <0.05

Toxicokinetic investigation (satellite group)

At the end of the treatment period the estimated mean concentrations of napropamide-M technical in rat plasma samples were 0.82, 2.78, 8.46 and 8.37 ng/mL in male rats of the control, low, mid and high dose groups, respectively, while in female rats the mean plasma concentrations were 3.41, 1.17, 5.64, 14.98 ng/mL in the control, low, mid and high dose groups, respectively. The estimated mean plasma concentrations of napropamide technical were 12.06 and 13.62 ng/mL in male and female rats, respectively. The isomeric analysis of plasma from male and female rats confirmed the presence of D (napropamide-M) and L -napropamide isomers in the expected ratio in napropamide-M technical and napropamide technical groups.

The napropamide content and isomer ratio determination was carried out using the method given in JRF Study Number 228-2-14-7333 (Sriram, 2014).

The results indicate a dose relationship in plasma concentrations although the level of variability and inconsistency is probably a reflection of the very low concentrations measured below the validated LOQ (50 ng/ML). The correlation co-efficient was ≥ 0.99 for linearity of the extended calibration curve below the validated LOQ and this provides some support for the reported concentrations.

The fact that all of the reported concentrations are below the validated LOQ may explain why low levels of napropamide-M technical were seen in controls.

Ophthalmoscopic examination

Ophthalmological examination did not reveal any abnormality in any of the groups.

Haematology

Lower erythrocyte counts, haemoglobin (male) and haematocrit levels accompanied by changes in MCV and MCH in the high dose napropamide-M and napropamide groups were indicative of mild anaemia, possibly related to red-cell destruction (haemolysis) or reduced synthesis. Some erythrocyte parameters were slightly lower than controls in the intermediate dose napropamide-M group and in females of the low dose group. The values for these parameters in the treated groups were generally within the historical control ranges (same laboratory and strain, and within 5 years of the date this study was performed). The parameters that were decreased at 600 ppm in females and at 2500 ppm in males were only marginally changed (approximately 5 %) and were, moreover, within the historical control range; therefore they are not considered by the RMS to be adverse at this dose. The changes at 2500 ppm in females were also marginal however for MCH and MCHC the change was outside or equal to the maximum of the historical control range and this is considered to be adverse. The changes were shown to be reversible in the high-dose napropamide-M and napropamide groups.

A dose-related reduction in prothrombin time among male treated groups is of uncertain toxicological significance. Although the values were outside the historical control range the concurrent control value was also below the minimum historical control mean. The changes were fully reversible at the end of the recovery period for the high dose napropamide-M and napropamide groups. The increased platelet count may be a related effect but no treatment-related effects on APTT or clotting time were observed. Overall, it is thought unlikely that the changes in PT were adverse. There were no changes in PT among treated female groups.

The increased platelet count (thrombocytosis) and increased proportion of neutrophils were probably secondary to the mild anaemia.

The proportion of lymphocytes was decreased in the high-dose napropamide-M and napropamide groups with a concomitant decrease in the proportion of neutrophils.

Refer to Tables 6.3.5 to Table 6.3.7

Table 6.3.5: Selected haematology parameters at week 13 (main groups, **males**)

Parameter	Mean values – male					HC range	
Dose level (ppm)	0	600	2500	10 000	10 000 NT	Min	Max
RBC (mi/µL)	8.74	8.77	8.40	7.28**	7.90**	8.39	9.46
HGB (g/dL)	16.94	16.79	16.13**	14.71**	16.23**	15.06	18.49
HCT (%)	44.56	44.62	43.49	38.87**	42.13*	40.30	49.33
MCV (fL)	51.10	50.93	51.81	53.50*	53.33*	48.53	55.46
MCH (pg)	19.44	19.17	19.20	20.23	20.55**	17.55	20.72
Platelet ($\times 10^3$/µL)	661.70	796.20*	723.60	860.50*	854.40**	668.90	999.00
PT (seconds)	10.66	9.06**	8.43**	8.22**	8.21**	10.76	12.17
WBC ($\times 10^3$/µL)	7.39	6.57	6.67	10.50*	8.93	6.16	8.30
Lymphocyte (%)	81.00	78.90	82.40	62.50**	59.70**	73.80	94.00
Neutrophil (%)	18.80	20.80	17.40	37.10**	40.10**	6.00	24.90

NT: Napropamide (racemate) technical
* Significantly different from control; p <0.05
** Significantly different from control; p <0.01

Table 6.3.6: Selected haematology parameters at week 13 (main groups, **females**)

Parameter	Mean values – female					HC range	
Dose level (ppm)	0	600	2500	10 000	10 000 NT	Min	Max
RBC (mi/µL)	8.02	7.72	7.48**	7.30**	6.92**	7.74	8.77
HGB (g/dL)	15.86	15.98	16.09	15.80	15.42	-	-
HCT (%)	43.41	41.04*	40.68**	40.02**	37.92**	40.36	46.78
MCH (pg)	19.81	20.71*	21.55**	21.68**	22.28**	18.35	21.08
MCHC (g/dL)	36.56	38.99**	39.56**	39.50**	40.68**	34.29	39.56
Platelet ($\times 10^3$/µL)	668.40	713.40**	728.80*	872.60**	892.60**	666.90	950.80
PT (seconds)	10.10	10.00	10.25	10.18	9.97	-	-
WBC ($\times 10^3$/µL)	4.97	4.96	5.33	5.61	5.28	-	-
Lymphocyte (%)	79.40	76.90	78.20	65.90**	64.60**	77.00	90.20
Neutrophil (%)	20.10	22.30	20.80	33.40**	34.40**	9.80	22.90

NT: Napropamide (racemate) technical
* Significantly different from control; p <0.05
** Significantly different from control; p <0.01

Table 6.3.7: Selected haematology parameters at week 17 (recovery groups)

Parameter	Mean values – male			Mean values – female		
Dose level (ppm)	0	10 000	10 000 NT	0	10 000	10 000 NT
RBC (mi/µL)	8.39	8.82	8.41	8.04	7.82	7.58
HGB (g/dL)	16.76	17.11	17.03	16.60	16.34	16.33
HCT (%)	43.24	45.40*	44.52	43.80	42.76	41.56
MCV (fL)	51.55	51.58	52.93	54.44	54.68	54.83
MCH (pg)	19.99	19.46	20.26	20.63	20.92	21.56**
Platelet (x10^3/µL)	692.10	717.60	738.20	668.70	715.10	773.80*
PT (seconds)	11.56	10.87	11.01	11.46	11.52	10.98
Lymphocyte (%)	79.70	71.10**	74.30**	77.40	77.50	77.50
Neutrophil (%)	20.10	29.50**	25.30*	21.80	21.80	21.90

NT: Napropamide (racemate) technical
* Significantly different from control; p <0.05
** Significantly different from control; p <0.01

Clinical Chemistry

Slight changes in serum ALT (male) and ALP and GGT (male and female) in high dose napropamide-M and napropamide groups were probably treatment related and were indicative of a minor perturbation in liver function (Tables 6.3.8 to Table 6.3.10). There was evidence of recovery 4 weeks after the withdrawal of treatment. There were no other treatment-related changes.

Urinalysis

Urine analysis revealed increased incidence of dark yellow to yellow brown discoloration in high dose napropamide-M and napropamide groups in both males and females when compared with the vehicle control group. All other urine analysis parameters were comparable in both treated and concurrent vehicle control groups. The urinary discoloration was not observed after the recovery period.

Table 6.3.8: Selected clinical chemistry parameters at week 13 (main groups, **males**)

Parameter	Mean values – male					HC range	
Dose level (ppm)	0	600	2500	10 000	10 000 NT	Min	Max
ALT (IU/L)	38.09	39.25	42.06	33.59	29.94*	35.98	56.73
ALP (IU/L)	87.41	98.20	111.12	109.90	118.05	70.29	185.52
GGT (IU/L)	0.38	0.03	0.09	1.52*	2.52**	0.00	1.56

NT: Napropamide (racemate) technical
* Significantly different from control; p <0.05
** Significantly different from control; p <0.01

Table 6.3.9: Selected clinical chemistry parameters at week 13 (main groups, **females**)

Parameter	Mean values – female					HC range	
Dose level (ppm)	0	600	2500	10 000	10 000 NT	Min	Max
ALT (IU/L)	28.80	27.83	30.27	24.79	24.42	-	-
ALP (IU/L)	40.30	40.36	38.15	57.54*	45.75	28.84	84.02
GGT (IU/L)	1.26	1.23	1.14	2.14	3.13**	0.00	1.48

NT: Napropamide (racemate) technical
* Significantly different from control; p <0.05
** Significantly different from control; p <0.01

Table 6.3.10: Selected clinical chemistry parameters at week 17 (recovery groups)

Parameter	Mean values – male			Mean values – female		
Dose level (ppm)	0	10 000	10 000 NT	0	10 000	10 000 NT
ALT (IU/L)	38.84	41.69	39.36	30.32	30.19	29.56
ALP (IU/L)	90.20	86.71	87.00	40.76	46.82	45.22
GGT (IU/L)	0.00	0.23	0.25*	1.57	1.11	0.72

NT: Napropamide (racemate) technical
* Significantly different from control; p <0.05

Gross pathology

No treatment-related findings were observed.

Organ weights

The absolute and relative weights of liver, kidneys and spleen were statistically significantly higher in males at 10 000 ppm napropamide-M and napropamide, with the magnitudes of the changes being comparable for the two compounds. In females a similar pattern was seen but for relative weight only. These findings are thought to be treatment related and correlated with histopathological changes in males but not in females. The increase in relative liver weight in both sexes was minor (< 5 % in all groups) and taken together with the histopathology findings (hepatocellular hypertrophy only in males; no indications of toxicity) is probably indicative of an adaptive rather than adverse change. Partial recovery was seen for liver and kidney weights in males and liver weights in females after the 4-week recovery period. Full recovery of spleen weights occurred in both sexes and full recovery of kidney weights was noted in females.

Higher relative heart weights in males at 10 000 ppm napropamide-M and napropamide, and higher relative ovary weights in females at 10 000 ppm napropamide-M, were not accompanied by any histopathological correlate; they probably reflected the decreased terminal body weight of the animals and were thus not an indication of direct activity against those organs. There were no significant weight differences from controls for these organs after the 4-week recovery period.

A summary of selected organ weights is given in Tables 6.3.11 to 6.3.13.

Table 6.3.11: Absolute and relative weights of selected organs (main groups, **males**)

Parameter	Mean values – male									
Dose level (ppm)	**0**		**600**		**2500**		**10 000**		**10 000 NT**	
	G	**%**	**g**	**%**	**G**	**%**	**G**	**%**	**g**	**%**
Terminal b.w.	421	-	414	**-1.7**	428	**1.7**	390	**-7.4**	394	**-6.4**
Liver (abs)	10.97	-	10.79	**-1.6**	11.91	**8.6**	13.11**	**19.5**	13.58**	**23.8**
Liver (rel)	-	**2.6**	-	**2.6**	-	**2.8**	-	**3.4****	-	**3.5****
Kidney (abs) g	2.45	-	2.33	**-4.9**	2.63	**7.3**	3.03**	**23.7**	3.10**	**26.5**
Kidney (rel)	-	**0.6**	-	**0.6**	-	**0.6**	-	**0.8****	-	**0.8****
Spleen (abs) g	0.68	-	0.66	**-2.9**	0.71	**4.4**	0.90**	**32.4**	0.79*	**16.2**
Spleen (rel)	-	**0.2**	-	**0.2**	-	**0.2**	-	**0.2****	-	**0.2****
Heart (abs) g	1.20	-	1.12	**-6.7**	1.20	**0.0**	1.24	**3.3**	1.22	**1.7**
Heart (rel)	-	**0.3**	-	**0.3**		**0.3**	-	**0.3****	-	**0.3***

abs: Absolute weight
rel: Organ weight relative to body weight (%)
NT: Napropamide (racemate) technical
†: units for organ weights releative to body weight: percentage
*: Significantly different from control; p <0.05
**: Significantly different from control; p <0.01

Table 6.3.12: Absolute and relative weights of selected organs (main groups, **females**)

Parameter	Mean values – female									
Dose level (ppm)	0		600		2500		10 000		10 000 NT	
	G	**%**	**g**	**%**	**G**	**%**	**G**	**%**	**g**	**%**
Terminal b.w. g	247	-	255	**3.2**	257	**4.0**	224.5*	**-9.1**	225*	**-8.9**
Liver (abs) g	6.95	-	7.07	**1.7**	7.27	**4.6**	7.39	**6.3**	7.95	**14.4**
Liver (rel)	-	**2.81**	-	**2.78**	-	**2.84**	-	**3.30****	-	**3.54***
Kidney (abs) g	1.58	-	1.61	**1.9**	1.71	**8.2**	1.66	**5.1**	1.65	**4.4**
Kidney (rel)	-	**0.64**	-	**0.63**	-	**0.67**	-	**0.74****	-	**0.73****
Spleen (abs) g	0.45	-	0.47	**4.4**	0.49	**8.9**	0.46	**2.2**	0.48	**6.7**
Spleen (rel)	-	**0.18**	-	**0.18**	-	**0.19**	-	**0.20**	-	**0.21***
Heart (abs) g	0.84	-	0.84	**0.0**	0.85	**1.2**	0.77	**-8.3**	0.78	**-7.1**
Heart (rel)	-	**0.34**	-	**0.33**	-	**0.33**	-	**0.35**	-	**0.35**
Ovaries (abs) g	0.11	-	0.11	**0.0**	0.12	**9.1**	0.12	**9.1**	0.10	**-9.1**
Ovaries (rel)	-	**0.05**	-	**0.04**	-	**0.05**	-	**0.06***	-	**0.05**

abs: absolute weight
rel: organ weight relative to body weight (%)

NT: Napropamide (racemate) technical
* Significantly different from control; p <0.05
** Significantly different from control; p <0.01

Table 6.3.13: Absolute and relative organ weights (recovery groups)

Parameter	Mean values – male						Mean values – female					
Dose level (ppm)	0		10 000		10 000 NT		0		10 000		10 000 NT	
	G	**%**	**G**	**%**	**G**	**%**	**g**	**%**	**g**	**%**	**g**	**%**
Terminal b.w.	436	-	441	**1.1**	453	**3.9**	248	-	247	**-0.4**	229*	**-7.7**
Liver (abs)	10.7	-	11.72*	**9.5**	12.28**	**14.8**	6.85	-	7.01	**2.3**	7.04	**2.8**
Liver (rel)	-	**2.5**	-	**2.7***	-	**2.7****	-	**2.8**	-	**2.8**	-	**3.1****
Kidney (abs)	2.38	-	2.56	**7.6**	2.74*	**15.1**	1.64	-	1.57	**-4.3**	1.49	**-9.1**
Kidney (rel)	-	**0.5**	-	**0.6**	-	**0.6***	-	**0.7**	-	**0.6**	-	**0.7**
Spleen (abs)	0.69	-	0.68	**-1.4**	0.72	**4.3**	0.50	-	0.47	**-6.0**	0.47	**-6.0**
Spleen (rel)	-	**0.2**	-	**0.2**	-	**0.2**	-	**0.2**	-	**0.2**	-	**0.2**
Heart (abs)	1.19	-	1.28	**7.6**	1.27	**6.7**	0.89	-	0.85	**-4.5**	0.81	**-9.0**
Heart (rel)		**0.3**	-	**0.3**	-	**0.3**	-	**0.4**	-	**0.3**	-	**0.4**
Ovaries (abs)	-	-	-	-	-	-	0.12	-	0.11	**-8.3**	0.11	**-8.3**
Ovaries (rel)	-	-	-	-	-	-	-	**0.05**	-	**0.04**	-	**0.05**

abs: absolute weight
rel: Organ weight relative to body weight (%)
NT: napropamide (racemate) technical
* Significantly different from control; p <0.05
** Significantly different from control; p <0.01

Histopathology

Treatment-related changes in the liver consisted of an increased incidence of centrilobular hypertrophy in 8/10 male rats at 10 000 ppm napropamide-M and 7/10 males at 10 000 ppm napropamide. One male at 2500 ppm napropamide-M was also affected. This finding was not observed in the male control and low dose groups or in any female groups. The single incidence of one male in the mid-dose group with minimal severity is considered not to be toxicologically significant in the absence of any effect on liver weight at this dose level and the absence of any other histopathological changes in the liver.

Histopathological findings in the kidneys of males and the spleen of both sexes are summarised in Tables 6.3.14 and Table 6.3.15.

The kidney findings comprised regenerative/ basophilic tubules in the cortex (multifocal or focal) often associated with granular casts in the cortico-medullary junctions and increased cytoplasmic eosinophilic granularity of proximal tubules. These changes were seen in the high-dose napropamide-M and napropamide groups but were limited to males only. The study authors noted that these histopathological characteristics have similarities to those of hydrocarbon or hyaline droplet nephropathy which is a male-rat-specific phenomenon. The presence of eosinophilic granularity in the cytoplasm of proximal tubules and the presence of granular casts were possibly indicative of this phenomenon. In addition, a few kidney samples from the high-dose groups and controls were re-cut and stained with chromotrope aniline blue. The representative samples from the high-dose group confirmed the presence of intracytoplasmic eosinophilic granules/inclusions. There was no analysis for alpha-2 urinary microglobulin and therefore the effects on the kidney cannot be attributed definitively to the presence of this protein.

Two male rats each from low-dose and mid-dose napropamide-M groups were also observed with minimal focal basophilic tubules, but these spontaneous lesions unaccompanied by any other changes were within the historical control range and were considered as non-adverse findings.

The increased incidence of extra medullary haematopoiesis (EMH) in the spleen of high-dose napropamide-M (males only) and napropamide racemate groups was considered to be treatment related and probably associated with the anaemia. However, the changes were minor (the majority of animals were scored as ‘minimal’, two animals as ‘mild’ and one as ‘moderate’) and had fully recovered by the end of the 4-week recovery period in the majority of animals. The incidences of EMH in female groups treated with napropamide-M were not dose related and were within the historical control range, and thus were not clearly associated with treatment. In males EMH was not observed at the low dose (600 ppm) and the incidence was very low at the mid-dose (2500 ppm – only seen in one animal and scored as ‘minimal’); however, at 10 000 ppm EMH was observed in all 10 male animals (napropamide-M) and 9 animals (napropamide racemate). It is therefore concluded that the incidence of EMH is treatment related in males, but is probably secondary to the anaemia; furthermore, in all cases the changes were minor (for napropamide-M only one animal was scored as ‘moderate’, the others were minimal (7) or mild (2) and for napropamide racemate no animals were scored as ‘moderate’, the others were minimal (3) or mild (6)).

There were no other treatment-related lesions.

Table 6.3.14: **Histopathological findings in kidney and spleen at week 13 (main groups)**

Microscopic finding	Dose level (ppm)					HC range[1]	
	0	600	2500	10 000	10 000 NT	Min	Max
Male (10 animals per group examined)							
Kidneys: Regenerative/basophilic tubules, cortex	0	2	2	8	6	0	20
Spleen: Extramedullary haematopoiesis (EMH)	0	0	1	10	9	0	20
Female (10 animals per group examined)							
Spleen: Extramedullary haematopoiesis (EMH)	0	3	1	3	5	0	30

NT: Napropamide (racemate) technical
1: percentage incidence

Table 6.3.15: Histopathological findings in kidney and spleen at week 17 (recovery groups)

Microscopic finding	Dose level (ppm)			HC range[1]	
	0	10 000	10 000 NT	Min	Max
Male (10 animals per group examined)					
Kidneys: Regenerative/basophilic tubules, cortex	0	4	5	0	20
Spleen: Extramedullary haematopoiesis (EMH)	3	0	0	0	20
Female (10 animals per group examined)					
Spleen: Extramedullary haematopoiesis (EMH)	2	3	0	0	30

NT: Napropamide (racemate) technical
1. % incidence

Conclusion

A guideline-compliant 90-day dietary toxicity study has been conducted in rats at doses of up to 10 000 ppm of napropamide-M and napropamide racemate.

Body weights of animals administered napropamide-M or napropamide at 10 000 ppm were lower than those of controls, occasionally achieving statistical significance. This was associated with a notably lower body weight gain, mainly in females. After 4 weeks of recovery, body weights of both male and female rats of the high-dose napropamide-M-treated group were comparable with the control group although in the high-dose napropamide racemate treated group the reduction in body weight was not fully recovered in females.

There were no treatment-related effects on food consumption with the possible exception of slightly lower food consumption in the female napropamide racemate group (10 000 ppm dose group), attaining statistical significance during weeks 5 and 11 to 13.

In females (2500 ppm and 10 000 ppm dose groups) and males (10 000 ppm dose group) the erythrocyte count was statistically significantly lower and below the range of the historical control data. The reduced erythrocyte count appears to be associated with changes in derived red cell parameters; the primary effect therefore appears to be on red blood cells which leads to effects on the spleen.

The absolute and relative weights of liver were statistically significantly higher in males at 10 000 ppm napropamide-M and napropamide, with the magnitudes of the changes being comparable for the two compounds. In females a similar pattern was seen but for relative weight only. There is a significant increase in GGT (male and female) in the top dose (10 000 ppm) napropamide-M and napropamide groups which is probably treatment related and indicative of a minor perturbation in liver function. There was evidence of recovery 4 weeks after the withdrawal of treatment and no pathological changes were reported for the liver. The liver effects were therefore not considered to be adverse.

The absolute and relative weights of spleen were statistically significantly higher in males at 10 000 ppm napropamide-M and napropamide, with the magnitudes of the changes being comparable for the two compounds. In females a similar pattern was seen but for relative weight only. This is associated with an increased incidence of extra medullary haematopoiesis (EMH) in the spleen of high-dose napropamide-M (males only) and napropamide racemate groups was considered to be treatment related in males and probably associated (secondary) with the anaemia.

The findings in kidney (absolute and relative weights statistically significantly higher in males at 10 000 ppm napropamide-M and napropamide) are thought to be adverse owing to the clear dose response and correlation with histopathological changes in males (regenerative/basophilic tubules, cortex). The kidney histopathology findings were only seen in males; however, a male rat-specific phenomenon was not definitively demonstrated.

The liver, kidneys and blood system were identified as the target organs following dietary administration of napropamide-M and napropamide racemate at 10 000 ppm for 90 days. There was no clear evidence of an effect on these organs at 2,500 ppm.

The toxicological profile of high dose napropamide-M was very similar to that of the equivalent high dose of napropamide racemate.

Based on the treatment-related toxicologically significant findings observed at the high dose level (10 000 ppm) and changes in haematology at 2500 ppm in females it is concluded that the NOAEL of napropamide-M is 600 ppm (equivalent to 46 mg/kg bw/day for males and 50 mg/kg bw/day for females) after dietary administration in Wistar rats under the procedure and conditions followed in the present study.

B.6.3.2.2. Oral 90-day dietary toxicity study in rats

Introduction

This study was conducted on napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

AUTHOR(S):	[redacted] 1970
Study title:	R-7465: safety evaluation by dietary feeding to rats for 13 weeks
Test substance:	Napropamide racemate
Purity:	89.1 %; 100 % (total D- + L-isomer)
Batch no.:	WRC 569-38-1; WRC 1120-32-1
Test animals:	Sprague-Dawley rats
Groups:	15/sex/group
Dose:	0, 13, 25, 50 mg/kg bw/day (doses adjusted for purity of batch, concentration in food adjusted in relation to food consumption)
Vehicle/solvent:	None. Test article was admixed in the diet
Route:	Oral by gavage
Statistics/ measurements:	None. For uterus weights, computerised analysis of variance analysis and student's T-test was performed
GLP:	No. Study performed prior to GLP
Guideline:	None stated. The study was conducted before official guidelines were implemented. Study design is close to the OECD Guideline No. 408
Deviation:	Deviations: Only limited number of parameters for clinical chemistry evaluated (Na, cholesterol, urea, BUN, creatinine, total protein and albumin and liver enzyme activity).

No urinalysis performed. Ophthalmoscopic and sensory reactivity examinations not performed. The study was conducted with too-low doses.

Acceptability: Acceptable, although too few parameters studied

Methods

Three groups of Sprague-Dawley rats (15/sex/group) were administered napropamide racemate (purity 89.1 % and 100 %, total D- + L-isomer), in the diet for 13 weeks at nominal concentrations of 13, 25 and 50 mg/kg bw/day. A control group of 15 male and 15 female rats received untreated basal diet. Through the first 8 weeks, dosages were adjusted for 100 % purity for the 89.1 % pure test material. Animals were observed daily for appearance and behaviour. Body weight and food consumption were measured weekly.

Haematology and clinical chemistry parameters were measured on 5 animals/sex from the control and 50 mg/kg bw/day groups at 4 and 8 weeks and on 5 animals/sex from each group at study termination. In addition, plasma and red blood cell cholinesterase activities were measured for 5 animals/sex/group at study termination. At study termination, organs were weighed, and gross and microscopic examinations of tissues/organs were performed on all animals.
Statistical evaluation (student's T-test and computerised analysis of variance) is only reported for uterus weights.

Results

Mortality, body weight and general observations
No mortality occurred. No treatment-related clinical signs of toxicity were noted. There was no treatment-related effect on food consumption or on body weights. No treatment-related changes in haematological or in clinical chemistry parameters were noted

Haematology and clinical chemistry

No treatment-related changes in haematological or in clinical chemistry parameters were noted.

Gross pathology
No treatment-related changes were detected during gross necropsy.

Organ weight
Absolute and relative organ weights were comparable in treated and control groups, except for uteri weights of females of the high dose group (50 mg/kg bw/day) that were lower than those of the control group females. This effect was statistically significant in a Student's T-test, but not in a computerized analysis of variance at p=0.05-level. In the absence of any adverse histopathology this is not considered to be treatment related.

Histopathology
No treatment-related changes were detected during histopathology examination.

Conclusion

This oral study of dietary administration of up to 50 mg/kg bw/day napropamide racemate to rats for 90 days was conducted before the implementation of GLP and OECD guideline. The study protocol was close to OECD guideline 408, although some deficiencies in the clinical chemistry parameters assessed were observed and no ophthalmological examination was performed. In principle this study was done with too low doses since no adverse effects were seen in the highest tested dose and the doses tested were low. However, the study is considered to be acceptable for the evaluation of the short-term toxicity of napropamide. No significant effect of the treatment was seen in any dose group. On this basis, the NOAEL is considered to be 50 mg/kg bw/day, the highest dose tested.

B.6.3.2.3. Oral 90-day dietary toxicity study in dogs

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] 1970
Study title:	Safety evaluation by repeated dietary administration to dogs for 13 weeks.
Test substance:	Napropamide racemate
Purity:	89.1 %; 100 % total D- + L-isomer (doses adjusted for purity of batch, concentration in food adjusted in relation to food consumption)
Batch no.:	WRC 569-38-1; WRC 1120-32-1
Test animals:	Beagle dogs
Groups:	4/sex/dose group.
Dose:	0, 16, 40, 100 mg/kg bw/day
Vehicle/solvent:	Acetone
Route:	Oral via diet
Statistics/ measurements:	No. However, student's t-test was performed on absolute and relative organ weight data of male dogs
GLP:	No. The study was performed prior to GLP requirements
Guideline:	None cited. This study was conducted before official guidelines were implemented. The study design is similar to the OECD Guideline No. 409.
Deviation:	Deviations: Only a limited number of clinical chemistry parameters was evaluated
Acceptability:	Acceptable

Methods

Three groups of Beagle dogs (4/sex/group) were administered napropamide racemate, purity 89.1 % and 100 %, in the diet, at nominal concentrations of 16, 40 and 100 mg/kg bw/day for 13 weeks. A control group of 4 male and 4 female dogs received untreated basal diet. Through the first 15 days of the study, dosages were adjusted for 100 % purity for the 89.1 % pure test material.

Daily observations were made for clinical signs, food consumption, behavioural changes, stool consistency, urinary excretions and indications of emesis. Body weight measurements and detailed physical examinations including body temperature, heart rate by auscultation, respiratory rate, appearance of the visible mucous membranes, skin and hair-coat and locomotor activity were made weekly. Blood pressure and electrocardiograms were taken for each dog at pre-test and at study termination.

Ophthalmoscopic examinations were conducted at pre-test, and at 4, 8 and 13 weeks of the study.

Haematological evaluations included all parameters required in OECD guideline 409 and were determined initially twice on all animals and at 4, 8 and 13 weeks.

Clinical chemistry was performed at the same time points as haematology including plasma urea nitrogen, plasma glucose, serum alkaline phosphatase, serum glutamic pyruvic transaminase, and serum glutamic oxalacetic transaminase. Electrolytes and some of the enzymes listed in OECD guideline 409 were not investigated.

Plasma and red blood cell cholinesterase were determined at termination of the study.

Urinalysis, which was conducted initially and at 8 and 13 weeks, included all OECD guideline 409 required parameters.

Gross pathology was performed at study termination. All tissues were preserved and histopathology was performed on all tissue of all animals of the high dose as well as the control animals and on selected tissues of all animals of the mid- and low dose groups.

Results

Mortality, bodyweight and general observations
No mortality and no treatment-related signs of toxicity were seen. No treatment-related effects on food consumption were observed.

At termination of the study mean body weights of males showed decreases of 0.6 kg (5.7 %) in the 100 mg/kg bw/day group (when compared to the control group). It is noted that the male control animals also lost weight; at termination the control animals had lost 0.3 kg (2.7 %) compared to their weight at week 0. Mean

body weights of females showed decreases of 0.7 kg (7.6 %) in the 100 mg/kg bw/day group (when compared to the control group). The decrease in the male control group was mainly due to one male that lost 1.3 kg from week 0 to termination. No other remarkable effects on body weights were seen. No statistical evaluation of the body weight data was performed and changes in body weight gains were not reported. There were no treatment-related changes in heart rates, body temperature, blood pressure, and electro-cardiograms.

Haematology, clinical chemistry and urinalysis

Haematological examinations showed lowered haemoglobin and haematocrit values from week 4 in 7 out of 8 dogs of the high dose group. The changes in haemoglobin during the treatment period varied from -6.9 % to -21 % in males and from –20.0 % to +20.0 % in females compared to week 0 for the same animal. Haematocrit values were reduced by 9.1 to 23 % in males and 0 to 22 % in females. No statistical evaluation or standard deviation was calculated. Statistical significance of the effect on blood parameters compared to controls was not calculated.
Clinical chemistry parameters were affected as follows: serum glutamate-oxaloacetate transaminase (SGOT) was markedly elevated in one female of the 100 mg/kg bw/day group at 4 and 8 weeks; serum glutamate pyruvate transaminase (SGPT) was elevated in the same female at the 8-week interval.
A trend toward increased serum alkaline phosphatase was observed in females of the high dose at 4 weeks. No increase was observed at subsequent intervals. Statistical significance of the effects on clinical chemistry compared to controls was not calculated.

The affected parameters are shown below in Table 6.3.16.

Table 6.3.16: Mean of clinical chemistry parameters with notable changes

Sampling	Parameter	Test group (mg/kg bw/day)			
		0	**16**	**40**	**100**
Males					
week 0	SGPT[1] (Sigma-Frankel units)	21	13	13	14
week 4		25	20	**45**[4]	26
week 8		22	20	22	20
week 13		27	19	21	15
Females					
week 0	SGPT[1] (Sigma-Frankel units)	13	16	15	14
week 4		17	22	20	**42**
week 8		14	17	24	**57**
week 13		17	20	19	14
Males					
week 0	SGOT[2] (Sigma-Frankel units)	18	16	18	15
week 4		21	13	12	15
week 8		24	15	20	17
week 13		22	20	17	18
Females					
week 0	SGOT[2] (Sigma-Frankel units)	19	19	18	19
week 4		14	18	14	12
week 8		23	21	16	**25**
week 13		16	17	20	18
Males					
week 0	AP[3] (King-Amtstron g units)	5.1	5.2	5.3	4.4
week 4		6.4	5.9	8.5	**9.5**
week 8		4.8	5.6	7.2	6.7
week 13		6.0	5.6	7.4	8.9
Females					
week 0	AP[3] (King-Amtstron g units)	4.7	4.7	4.3	5.0
week 4		7.5	8.7	8.1	**13.2**
week 8		6.6	7.2	8,3	10.9
week 13		7.5	5.8	8.0	11.4

[1] Serum glutamic pyruvic transaminase

[2] Serum glutamic oxalacetic transaminase
[3] Alkaline phosphatase
[4]: One male of the 40 mg/kg bw/day group had markedly elevated SGPT at 4 weeks only
Figures in bold are marked different from controls. However, statistical significance was not calculated in this study.

Ophthalmology
No abnormalities were found in any dog.

Gross pathology
No noteworthy gross lesions were reported in any dog upon necropsy.

Organ weight
Absolute and relative liver weight of males in the 100 mg/kg bw/day group (Student's t-test, $p<0.01$) were significantly increased. The findings are shown in Table 6.3.17.

Table 6.3.17: Mean liver weights of dogs treated 3 months with napropamide

	Test group (mg/kg bw/day)							
	0		**16**		**40**		**100**	
	Males							
Final body weight (kg)[a]	10.4	-	11.0	-5.8 %	10.9	4.8 %	9.9	-4.8 %
Absolute liver weights (g)	309.0	-	359.7	16.4 %	357.3	15.6 %	**397.8***	**28.7 %**
Relative liver weights (%)	2.96	-	3.27	10.5 %	3.28	10.8 %	**4.08***	**37.8 %**
	Females							
Final body weight (kg)[a]	9.1	-	9.4	3.3 %	8.4	-7.7 %	8.6	-5.5 %
Absolute liver weights (g)	329.5	-	324.0	-1.7 %	307.6	-6.6 %	324.5	-1.5 %
Relative liver weights (%)	3.66	-	3.46	-5.5 %	3.73	1.9 %	3.75	2.5 %

[a] no statistical evaluation of body weights was performed in the study
* = statistically significant different from controls ($p < 0.01$)

Histopathology
There were no adverse findings in any dogs.

Conclusion

This 90-day study in dogs with napropamide racemate was conducted prior to OECD guidelines and GLP. Statistical evaluation of the data is very limited. However, the study is close to OECD guideline 409. The study is considered to be acceptable for the evaluation of the short-term toxicity of napropamide to the dog. Under the conditions of this 90-day oral study in Beagle dogs with dietary administration of 0, 16, 40, 100 mg/kg bw/day of napropamide, effects were seen in the high dose group animals. These effects included body weight loss of 5.7 % in males and 7.6 % in females during the treatment period. Information on statistical significance of this effect, or compilation of data on body weight gain was not performed in the study. Statistically significant increase in the absolute and relative liver weights of males, trends of lowered liver enzyme values in females and also trends of lowered haemoglobin and haematocrit values were observed in males and females. Based on these findings, the liver is considered to be a target organ. However, the effects were mild, as there were no adverse findings at gross pathology or at histopathological examination. The NOAEL is 40 mg/kg bw/day based on treatment-related effects in the high dose group.

B.6.3.3. Oral 1 year study

Introduction

There are two 1-year studies in the dog. Both of the studies were performed on napropamide racemate and were previously evaluated by Denmark for the approval of napropamide. The summaries of these studies presented below have not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

B.6.3.3.1. One year dietary toxicity study in the dog

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide.

Author(s):	[redacted] 1988
Study title:	A 52-week toxicity study of Devrinol in the Beagle dog.
Test substance:	Napropamide racemate
Purity:	Not specified.
Batch no.:	4921-27-24
Test animals:	Beagle dogs
Groups:	5/sex/dose group
Dose:	0, 10, 70 and 500 mg/kg bw/day
Vehicle/solvent:	Gelatine in capsule
Route:	Oral by capsule
Statistics/ measurements:	Yes. Group means with standard variation and student's t-test
GLP:	Yes, US FDA
Guideline:	None cited, but complies with OECD guideline 452
Deviation:	None
Acceptability:	Acceptable

Methods

Three groups of 5 male and 5 female Beagle dogs received napropamide racemate (Devrinol; purity not specified) daily in gelatine capsules at 10, 70 and 500 mg/kg bw/day for 52 weeks. A control group of 4 male and 4 female dogs received empty gelatine capsules. The following observations/evaluations were made during the study: twice a day check for mortality and clinical signs of toxicity, daily food consumption over the 1-hour daily feeding period, once a week body weight measurements. Ophthalmoscopic examination was performed at pre-test and during weeks 12, 24 and 51. Laboratory investigations included haematology, clinical chemistry and urinalysis assessments conducted on all animals at pre-test and during weeks 12, 25, 37 and 50. At necropsy, a full macroscopic examination was performed on all animals. Organs were weighed and histopathological examination was conducted on tissues/organs for all animals.

Results

Mortality, general observations and *body weights*

No mortality occurred. Clinical signs included incidental findings; loose faeces, occasional vomiting and areas of alopecia. No treatment-related clinical signs were noted.
Food consumption was reduced at 500 mg/kg bw/day in males at week 17 to 18, 22 to 25, 27 to 30, 35, 37 to 39, 44, 49 to 50 and 52 and in females at week 1, 9 through 12, 16 to 19, week 25, 41 to 44 and 48 to 49. Food consumption was generally lower in all treatment groups than in controls but statistical significance was only achieved at intermittent occasions. Table 6.3.18 shows food consumption at selected time-points.

In the high dose group (500 mg/kg bw/day), a decreased body weight gain during the first week of the study was observed both in males and females. Females continued to gain weight at a slower rate than the controls; weight gains for males were comparable to controls. The weight gain in low and intermediate dosed animals was unaffected by treatment. Body weights of treated animals were not statistically significantly different from controls.

Haematology, clinical chemistry, urinalysis and ophthalmoscopy

At haematological examination, a significant depression of red blood cells (RBC), haemoglobin (HGB), haematocrit and lymphocytes was seen in females of the high dose group at 25 weeks of treatment. This effect was not seen at week 37 and 50. Males showed a significant increase in RBC and HGB at 50 weeks. At 12 weeks, prothrombin time (PT) and activated partial thromboplastin time (APTT) were significantly decreased in females of the high dose group. At 37 weeks, APTT was significantly decreased in females at 70 and at

500 mg/kg bw/day. At 50 weeks, PT was significantly higher in female treated with 70 mg/kg bw/day than in the controls, and significantly lower in the females treated with 500 mg/kg bw/day than in the controls. The findings on haematology, although indicating the blood as a possible target organ, were contradictory and showed no dose-response nor time relationship and therefore were not considered treatment related. See Table 6.3.19.

Table 6.3.18: Food consumption in dogs treated orally with napropamide.

	Dose (mg/kg bw/day)			
	0	**10**	**70**	**500**
Males				
Week 0	296.0	**254.8***	290.0	286.3
Week 1	333.0	317.7	338.3	282.9
Week 12	350.3	350.3	372.7	345.2
Week 20	374,5	330.1	362.2	340.3
Week 28	383.7	335.5	361.1	**339.3****
Week 37	382.0	**324.1****	351.0	**326.8****
Week 45	393.9	**346.9***	353.5	352.1
Week 52	400.0	**380.4***	380.7	**367.0***
Females				
Week 0	253.0	238.2	230.3	246.1
Week 1	289.8	277.3	266.1	**233.3***
Week 12	341.0	**301.3***	317.2	**284.7***
Week 20	335.9	**288.9*****	311.9	281.3
Week 28	303.0	297.6	286.9	278.1
Week 37	285.9	270.9	278.6	297.7
Week 45	339.2	291.4	337.1	301.1
Week 52	360.5	**303.3***	332.6	315.7

* statistically significantly ($0.01 < p < 0.05$) different from control
** statistically significantly ($0.001 < p < 0.01$) different from control
*** statistically significantly ($p < 0.001$) different from control

Table 6.3.19: Mean haematological parameters in dogs treated orally with napropamide for 1 year.

		RBC (x 10^6)	HGB (g/dl)	HTC (%)	PT (sec.)	APPT (sec.)	Lymph. (%)
Week -3	**Males**						
	0 mg/kg bw/ day	6.02 ± 0.346	14.0 ± 0.90	40.2 ± 2.61	7.3 ± 0.08	9.4 ± 0.32	32.6 ± 6.43
	10 mg/kg bw/day	5.86 ± 0.358	13.3 ± 0.71	38.1 ± 2.04	7.3 ± 0.25	9.6 ± 0.29	34.8 ± 14.13
	70 mg/kg bw/day	5.51 ± 0.728	12.7 ± 1.19	36.5 ± 3.29	7.7 ± 0.41	**10.1 ± 0.36***	35.4 ± 8.47
	500 mg/kg bw/day	6.02 ± 0.401	13.6 ± 0.92	39.0 ± 2.38	7.6 ± 0.26	9.7 ± 0.30	36.6 ± 2.41
	Females						
	0 mg/kg bw/ day	6.03 ± 0.363	14.3 ± 0.94	40.8 ± 2.57	7.1 ± 0.08	9.9 ± 0.50	35.2 ± 6.98
	10 mg/kg bw/day	5.62 ± 0.471	13.1 ± 1.16	37.7 ± 3.34	7.4 ± 0.29	10.4 ± 0.72	40.6 ± 13.79
	70 mg/kg bw/day	5.93 ± 0.553	13.5 ± 1.29	38.8 ± 3.68	7.5 ± 0.22	10.2 ± 0.58	35.6 ± 9.29
	500 mg/kg bw/day	5.82 ± 0.621	13.4 ± 1.59	38.4 ± 4.35	7.2 ± 0.15	9.6 ± 0.70	32.6 ± 9.18
Week 12	**Males**						
	0 mg/kg bw/ day	6.65 ± 0.433	15.4 ± 0.57	43.7 ± 2.07	7.1 ± 0.23	9.7 ± 0.45	37.2 ± 0.84
	10 mg/kg bw/day	6.88 ± 0.597	15.7 ± 1.57	44.1 ± 4.22	7.1 ± 0.30	9.6 ± 0.25	29.4 ± 4.22
	70 mg/kg bw/day	6.38 ± 0.642	14.7 ± 1.64	41.6 ± 4.31	7.2 ± 0.43	9.7 ± 0.51	37.0 ± 8.12
	500 mg/kg bw/day	6.68 ± 0.638	15.2 ± 1.39	43.3 ± 4.06	7.3 ±0.28	9.3 ± 0.30	35.6 ± 10.14
	Females						
	0 mg/kg bw/ day	6.73 ± 0.490	16.0 ± 1.06	45.6 ± 2.97	7.0 ± 0.18	10.4 ± 0.79	29.2 ± 8.98
	10 mg/kg bw/day	6.98 ± 0.345	16.3 ± 1.16	45.9 ± 3.14	7.2 ± 0.36	10.6 ± 0.63	31.6 ± 8.66
	70 mg/kg bw/day	6.93 ± 0.592	16.0 ± 1.49	45.2 ± 4.13	7.0 ± 0.30	10.7 ± 0.53	32.0 ± 8.66
	500 mg/kg bw/day	6.27 ± 0.498	14.6 ± 1.03	41.5 ± 2.84	**6.6 ± 0.16***	**9.4 ± 0.51***	22.2 ± 4.55
Week 25	**Males**						
	0 mg/kg bw/ day	6.70 ± 0.234	15.5 ± 0.51	45.1 ± 1.75	7.3 ± 0.10	9.4 ± 0.40	24.8 ± 11.69
	10 mg/kg bw/day	7.24 ± 0.433	16.7 ± 1.25	47.9 ± 3.39	7.4 ± 0.30	9.5 ± 0.29	24.2 ± 5.02
	70 mg/kg bw/day	6.67 ± 0.732	15.6 ± 1.56	45.3 ± 4.07	7.5 ± .29	9.5 ± 0.86	24.8 ± 5.54
	500 mg/kg bw/day	7.28 ± 0.549	16.8 ± 1.22	48.4 ± 3.47	7.6 ± 0.21	8.9 ± 0.19	24.2 ± 5.72
	Females						
	0 mg/kg bw/ day	6.63 ± 0.498	15.8 ± 1.12	45.7 ± 3.28	7.5 ± 0.16	9.6 ± 0.56	30.8 ± 6.72
	10 mg/kg bw/day	6.86 ± 0.585	16.5 ± 1.28	47.2 ± 3.75	7.6 ± 0.36	10.3 ± 1.08	34.2 ± 5.31
	70 mg/kg bw/day	6.79 ± 0.481	15.9 ± 1.39	45.6 ± 3.72	7.7 ± 0.13	9.8 ± 0.62	29.4 ± 6.35
	500 mg/kg bw/day	**5.88 ± 0.363***	**14.0 ± 0.74***	**39.7 ± 2.29****	7.3 ± 0.10	9.0 ± 0.63	**18.0 ± 3.81****
Week 37	**Males**						
	0 mg/kg bw/ day	6.97 ± 0.267	16.4 ± 0.85	46.7 ± 2.00	7.5 ± 0.11	9.8 ± 0.48	28.0 ± 11.0
	10 mg/kg bw/day	7.26 ± 0.429	17.1 ± 1.28	48.0 ± 3.84	7.7 ± 0.21	9.7 ± 0.32	23.4 ± 8.59
	70 mg/kg bw/day	6.75 ± 0.765	16.3 ± 1.82	45.9 ± 4.81	7.7 ± 0.35	9.7 ± 0.56	25.8 ± 11.21
	500 mg/kg bw/day	7.31 ± 0.399	17.4 ± 1.19	48.9 ± 3.00	7.7 ± 0.23	9.2 ± 0.43	34.2 ± 5.59
	Females						
	0 mg/kg bw/ day	7.01 ± 0.423	17.3 ± 1.12	48.0 ± 2.76	7.4 ± 0.19	10.1 ± 0.39	35.6 ± 8.85
	10 mg/kg bw/day	7.14 ± 0.407	17.4 ± 1.27	48.4 ± 3.23	7.5 ± 0.35	10.3 ± 0.58	36.0 ± 6.71
	70 mg/kg bw/day	6.92 ± 0.498	16.7 ± 1.54	46.6 ± 4.01	7.7 ± 0.21	**9.5 ± 0.34***	30.2 ± 8.98
	500 mg/kg bw/day	6.45 ± 0.487	15.5 ± 0.92	43.3 ± 2.73	7.3 ± 0.11	**9.1 ± 0.44****	33.4 ± 8.91
Week 50	**Males**						
	0 mg/kg bw/ day	6.81 ± 0.451	16.0 ± 1.11	45.2 ± 3.30	7.4 ± 0.18	9.6 ± 0.55	28.2 ± 5.97
	10 mg/kg bw/day	7.34 ± .394	17.4 ± 1.21	48.5 ± 3.58	7.6 ± 0.31	9.3 ± 0.38	26.0 ± 5.00
	70 mg/kg bw/day	6.74 ± 0.661	16.3 ± 1.51	45.3 ± 3.67	7.6 ± 0.40	9.9 ± 0.70	29.0 ± 6.78
	500 mg/kg bw/day	**7.58 ± 0.343***	**18.0 ± 0.78****	50.0 ± 1.92	7.6 ± 0.38	9.1 ± 0.30	32.0 ± 5.15
	Females						
	0 mg/kg bw/ day	6.97 ± 0.397	17.1 ± 0.86	47.7 ± 2.55	7.3 ± 0.09	9.8 ± 0.74	27.6 ± 10.67
	10 mg/kg bw/day	6.58 ± 0.810	16.2 ± 2.17	45.1 ± 5.98	7.3 ± 0.42	9.8 ± 0.97	34.6 ±4.28
	70 mg/kg bw/day	6.72 ± 0.548	16.2 ± 1.60	45.0 ± 4.04	**7.5 ± 0.23***	9.4 ± 0.33	28.8 ± 6.53
	500 mg/kg bw/day	6.22 ± 0.458	15.2 ± 1.14	42.1 ± 3.19	**7.0 ± 0.19***	9.3 ± 0.65	27.2 ± 6.83

± standard deviation
RBC: red blood cells; HGB: haemoglobin; HTC: haematocrit; PT: prothrombin time; APPT: activated partial thromboplastin time
* statistically significantly ($p<0.05$) different from controls
** statistically significantly ($p<0.01$) different from controls

Some clinical chemistry parameters showed statistically significant differences from controls. However, with no time or dose relationship, the findings are not considered to be treatment related. Urinalysis parameters were not affected by treatment. No ophthalmological abnormalities were seen at any assessment.

Organ weights
There were no significant differences between treated and untreated animals with respect to absolute or relative organ weights.

Gross pathology
No treatment related findings.

Histopathology
No treatment related findings.

Conclusion

This 52-week oral toxicity study of napropamide in dogs was conducted in compliance with GLP requirements and OECD guidelines. The study is acceptable for the evaluation of the short-term toxicity of napropamide. Under the conditions of the test, with the dose levels of 0, 10, 70 and 500 mg/kg bw/day in capsules, the dogs of the highest dose level showed a statistically significant reduction in food consumption and body weight gains at the beginning of the study. However, the findings were not consistent and no clear dose-relationship was shown. Sporadic haematological findings were not considered toxicologically significant. No other significant findings could be attributed to treatment. The NOAEL for dogs following long-term exposure was thus 500 mg/kg bw/day, the highest dose tested.

B.6.3.3.2. One year dietary toxicity study in the dog

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

AUTHOR(S):	[redacted] 1995
Study title:	Toxicity to dogs by repeated oral administration for 52 weeks.
Test substance:	Napropamide racemate
Purity:	94.9 % (total D- + L-isomer)
Batch no.:	Not reported
Test animals:	Beagle dogs
Groups:	4/sex/dose group
Dose:	0, 50, 250 or 1000 mg/kg bw/day
Vehicle/solvent:	Gelatine in capsule
Route:	Oral by capsule
Statistics/ measurements:	Yes. Variance analysis tests (Bartlett; Kruskall-Wallis; Student's t-test, Williams test)
GLP:	Yes. UK Dept. Health, EU, OECD US EPA and Japan MAFF
Guideline:	FIFRA 83-1 and OECD guideline 452
Deviation:	None
Acceptability:	Acceptable

Methods

Three groups of 4 male and 4 female Beagle dogs received napropamide racemate (purity 94.9 %, total D- + L-isomer) daily in gelatine capsules at 50, 250 and 1000 mg/kg bw/day for 52 weeks. A control group of 4 male and 4 female dogs received empty gelatine capsules. The following observations/evaluations were made during the study: daily check for mortality and clinical signs of toxicity, daily food consumption measurement; once a week body weight measurements; and ophthalmoscopic examination at pre-test and week 52. Laboratory investigations included haematology, clinical chemistry and urinalysis assessments conducted on all animals at pre-test, and at 13, 26 and 52 weeks of treatment. Prior to necropsy, bone marrow was obtained from all animals

and was examined. At necropsy a full macroscopic examination was performed and organs were weighed. Histopathological examination was conducted on tissues/organs from all animals.

Results

Mortality, general observations, food consumption and body weights

One female dog treated with 50 mg/kg bw/day was sacrificed for humane reasons (herniated small intestine) during week 13 of the study. No other mortality was seen. Vomiting occurred in some of the animals, usually within 2 hours from dosing, incidences being higher in females. The effect appears to be dose-response related from 250 mg/kg bw/day. This effect declined during the latter stages of the study and was very low after week 13 in males and week 39 in females. Table 6.3.20 shows the incidence of vomiting.

Table 6.3.20 Incidences of liquid vomiting

	Males		**Females**		**Combined**	
Dose in mg/kg bw/day	Total incidence (max incidence)	% incidence	Total incidence (max incidence)	% incidence	Total incidence (max incidence)	% incidence
0	19 (1456)	1.3	11 (1456)	0.8	30 (2912)	1.0
50	42 (1456)	2.9	24 (1179)	2.0	66 (2635)	2.5
250	369 (1456)	25.3	222 (1456)	15.2	591 (2912)	20.3
1000	653 (1456)	44.8	517 (1456)	35.5	1170 (2912)	40.2

An increased incidence of diarrhoea in males and females was seen at 250 and 1000 mg/kg bw/day. Also at 50 mg /kg bw/day, the incidence of diarrhoea was higher than that of the concurrent control group. However, the finding at 50 mg/kg bw/day was within the historical control range† for this finding at the testing facility. No other clinical signs were seen. Table 6.3.21 shows the incidences of liquid faeces.
†: according to the evaluation of this study by the RMS (DK) for napropamide racemate.

Table 6.3.21: Incidences of liquid faeces

	Males		Females		Combined	
Dose in mg/kg bw/day	Total incidence (max incidence)	% incidence	Total incidence (max incidence)	% incidence	Total incidence (max incidence)	% incidence
0	19 (1456)	1.3	11 (1456)	0.8	30 (2912)	1.0
50	42 (1456)	2.9	24 (1179)	2.0	66 (2635)	2.5
250	369 (1456)	25.3	222 (1456)	15.2	591 (2912)	20.3
1000	653 (1456)	44.8	517 (1456)	35.5	1170 (2912)	40.2

Mean body weight gain was reduced for males and females at 1000 mg/kg bw/day when compared with controls. The reductions were statistically significant in females and for combined sexes. No treatment-related effect on body weight gain was observed at 250 mg/kg bw/day. At 50 mg/kg bw/day, the mean body weight gain for females was slightly lower than that of the controls. From week 25, the weight gain curves were comparable with that of the controls. The mean body weight gain for males at 50 mg/kg bw/day was lower because of lower gain by a single animal. Because of the lack of dose response and because of the influence of data from single animals (2 females were heavy at study start and one male was diagnosed to suffer from kidney damage), the effects on mean body weight gain at 50 mg/kg bw/day were not considered to be treatment-related.

Table 6.3.22: Group mean bodyweight changes (kg) in dogs after 52 weeks of treatment

Dose (mg/kg bw/day)	Males	Females	Combined
Control	5.1	5.4	5.3
50	4.1 (5.1)[a]	3.8	4.0 (4.4)[a]
250	5.3	4.7	5.0
1000	4.0	3.9*	3.9*

[a] The figure in parentheses excludes the male dog with kidney damage.
* Statistically significant at $p < 0.05$.

There was a slightly, non-significant decreased food consumption in females of the high dose group.

Haematology, clinical chemistry, urinalysis and ophthalmoscopy

No treatment-related effects were noted on haematological parameters.

In clinical chemistry, there was a slight but statistically significant decrease in group mean albumin values for males and/or females of the 1000 mg/kg bw/day group, at weeks 13, 26, and 52. Also, combined group mean total protein values at week 13 were statistically significantly decreased at this dose level. Males of the high dose group showed statistically significant increases in the mean value for alkaline phosphatase (AP) at weeks 13, 26, and 52, whereas females of that group showed elevated AP values at week 52. Combined values attained statistical significance at weeks 26 and 52. Group mean cholesterol values were slightly decreased for males and females at week 13. At week 52, this parameter was only decreased in females.

Table 6.3.23: Selected clinical chemistry parameters in dogs treated with napropamide for 1 year

		Total protein	Albumin	AP	Cholesterol
Week -2	**Males**				
	0 mg/kg bw/ day	4.6	2.4	312	100
	50 mg/kg bw/day	4.9	2.6	303	116
	250 mg/kg bw/day	**5.0+**	**2.7++**	300	115
	1000 mg/kg bw/day	4.8	**2.6+**	336	120
	Females				
	0 mg/kg bw/ day	4.9	2.6	307	104
	50 mg/kg bw/day	5.2	2.7	**241+**	124
	250 mg/kg bw/day	4.8	2.5	267	121
	1000 mg/kg bw/day	4.8	2.6	248	114
	Combined				
	0 mg/kg bw/ day	4.7	2.5	309	102
	50 mg/kg bw/day	**5.0++**	2.6	272	**120+**
	250 mg/kg bw/day	4.9	2.6	284	118
	1000 mg/kg bw/day	4.8	2.6	292	117
Week 13	**Males**				
	0 mg/kg bw/ day	4.9	2.7	195	103
	50 mg/kg bw/day	4.9	2.6	191	117
	250 mg/kg bw/day	5.0	2.7	209	100
	1000 mg/kg bw/day	4.7	**2.5****	**281***	96
	Females				
	0 mg/kg bw/ day	5.0	2.7	182	115
	50 mg/kg bw/day	5.0	2.7	141	118
	250 mg/kg bw/day	4.8	2.5	190	117
	1000 mg/kg bw/day	4.7	**2.4****	180	**90***
	Combined				
	0 mg/kg bw/ day	4.9	2.7	189	109
	50 mg/kg bw/day	4.9	2.6	170	117
	250 mg/kg bw/day	4.9	2.6	200	108
	1000 mg/kg bw/day	**4.7***	**2.4****	230	93
Week 26	**Males**				
	0 mg/kg bw/ day	5.3	2.7	132	111
	50 mg/kg bw/day	5.3	2.7	153	118
	250 mg/kg bw/day	5.4	2.7	151	106
	1000 mg/kg bw/day	5.1	2.5	**255****	108
	Females				
	0 mg/kg bw/ day	5.3	2.8	116	127
	50 mg/kg bw/day	5.4	2.8	144	128
	250 mg/kg bw/day	5.1	2.6	144	128
	1000 mg/kg bw/day	4.9	**2.5***	194	100
	Combined				
	0 mg/kg bw/ day	5.3	2.8	124	119
	50 mg/kg bw/day	5.3	2.7	149	122
	250 mg/kg bw/day	5.3	2.7	147	117
	1000 mg/kg bw/day	5.0	**2.5****	**225****	104
		Total protein	Albumin	AP	Cholesterol
Week 52	**Males**				
	0 mg/kg bw/ day	5.4	2.8	127	105
	50 mg/kg bw/day	5.3	2.7	140	119
	250 mg/kg bw/day	5.4	2.8	132	101
	1000 mg/kg bw/day	5.3	**2.4****	**318****	104
	Females				
	0 mg/kg bw/ day	5.5	2.8	110	144
	50 mg/kg bw/day	5.4	2.7	134	129
	250 mg/kg bw/day	5.4	2.6	149	137
	1000 mg/kg bw/day	5.3	2.6	**168***	**96***
	Combined				

	0 mg/kg bw/ day	5.4	2.8	118	125
	50 mg/kg bw/day	5.3	2.7	137	123
	250 mg/kg bw/day	5.4	2.7	140	119
	1000 mg/kg bw/day	5.3	**2.5****	**243****	100

\+ P <0.05; ++ P< 0.01 Student's 't'test
* P < 0.05 ; ** P< 0.01 Williams test

At weeks 13, and/or 52, group mean urinary specific gravity values (SG) were elevated for males and combined groups at 250 and 1000 mg/kg bw/day. Occasionally statistical significance was achieved. There was no clear dose-response or time relationship. The raised mean SGs were predominantly due to one animal at 250 mg/kg bw/day and 2 animals at 1000 mg/kg bw/day and therefore were not considered to be treatment related. The group mean specific gravity values (SG) in this study and the background data from the same laboratory (number and type of studies and year-span not reported) for males and females are shown in Table 6.3.24.

Table 6.3.24: Specific urinary gravity in dogs treated with napropamide for 1 year.

	week -2	week 13	week 26	week 52
Males				
laboratory background values	1032-1056	1033-1057	1029-1065	1027-1062
0 mg/kg bw/ day	1041	1043	1049	1047
50 mg/kg bw/day	1040	1042	1046	1043
250 mg/kg bw/day	1045	**1062***	1065	1064
1000 mg/kg bw/day	1041	**1050***	1057	1053
Females				
laboratory background values	1031-1058	1033-1060	1034-1067	1028-1058
0 mg/kg bw/ day	1043	1046	1050	1044
50 mg/kg bw/day	1043	1048	1052	1046
250 mg/kg bw/day	1042	1048	1059	1048
1000 mg/kg bw/day	1044	1056	1056	1055
Combined				
0 mg/kg bw/ day	1042	1044	1049	1045
50 mg/kg bw/day	1042	1045	1049	1044
250 mg/kg bw/day	1044	**1055***	1062	**1056***
1000 mg/kg bw/day	1042	**1053***	1057	**1054***

* Statistically significant at p<0.05 level

No treatment-related effects were observed during ophthalmological examinations.

Bone marrow
All smears were considered normal for cellularity, cell distribution and morphology.

Organ weights
Group mean absolute and liver weights were increased for both sexes receiving 1000 mg/kg bw/day, with combined mean values achieving statistical significance. Group liver weights are shown in Table 6.3.25.
No other treatment related findings were observed on organ weights.

Gross pathology
No treatment-related effects were noted during gross necropsy.

Histopathology
No treatment-related effects were observed.
There were no changes associated with the increased group mean liver weights noted for animals receiving 1000 mg/kg bw/day.

Table 6.3.25: Group mean liver weights of dogs treated one year with napropamide

	Test group (mg/kg bw/day)			
	0	**50**	**250**	**1000**
	Males			
Absolute liver weights (g)	426.9	451.6	439.2	496.2
Relative liver weights (%)	3.10	3.61	3.21	3.82
	Females			
Absolute liver weights (g)	412.1	420.5	427.5	438.3
Relative liver weights (%)	3.29	3.44	3.48	3.79
	Combined			
Absolute liver weights[a] (g)	413.5	441.1	429.9	**474.2***
Relative liver weights (%)	3.20	3.53	3.35	**3.81***

bw = body weight
[a] adjusted means: terminal bw used as covariate because within-group relationship was significant at 10 % level.
* statistically significant different from controls ($p < 0.05$)

Conclusion

The study is conducted in accordance with GLP and follows OECD 452. It is acceptable for the evaluation of the end-point of short-term toxicity of napropamide. A NOAEL of 50 mg/kg bw/day is set on the basis of the findings of the study. Effects included a dose-response related increase in the incidence of vomiting and liquid faeces from 250 mg/kg bw/day as well as reduced body weight gains at 1000 mg/kg bw/day. The target organ is considered to be the liver, based on the statistically significant increase in the combined group means for absolute and relative liver weights, the significantly reduced albumin levels from week 13 and the significantly increased alkaline phosphatase from week 13 in the high dose group. However, the liver effect was marginal, as no adverse findings were noted at histopathology.

B.6.3.4. Other routes

B.6.3.4.1. 28-day dermal toxicity – in rat

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] 1991
Study title:	21-day dermal toxicity study in the rat
Test substance:	Napropamide racemate
Purity:	95.2 % (total D- + L-isomer)
Batch no.:	WRC 12485-03
Test animals:	Wistar derived [redacted] (WI) BR rats
Groups:	5/sex/dose group
Dose:	0, 10, 100 and 1000 mg/kg bw/day; 6-hour exposure periods, 5 days/week.
Vehicle/solvent:	Water at 100 and 1000 mg/kg, olive oil at 10 mg/kg. 2 groups of control, one only bandaged, the other treated with olive oil
Route:	Dermal under occlusive bandage
Statistics/ measurements:	Yes. VAR (analysis of variance). Toxstat system. Welch Trend Test. Neurostat system. Statistically significant at 95 % confidence level
GLP:	Yes: US EPA, FIFRA and TSCA; UK Dept. Health; OECD; Japan MAFF
Guideline:	None cited. The study design is similar to OECD Guideline No. 410 (1981)
Deviation:	None. The study runs over 28 days (30, actually) despite its title
Acceptability:	Acceptable

Methods

A preliminary study using 2 animals/sex/dose exposed for 5 days to 10, 100, 500 and 1000 mg/kg bw/day was performed as a basis for the selection of the dose-levels to be used for the main study.

In the main study, twenty-one dermal applications (6-hour exposure periods, 5 days/week) of napropamide (purity 95.2 %, total D- + L-isomer) were made over a period of 30 days to three groups of five male and five female Wistar rats per group at 10, 100 and 1000 mg/kg bw/day. Two control groups (5/sex/group) consisting of a group treated with olive oil and a bandaged-only control group (untreated) were included in the study design. For dose levels of 100 and 1000 mg/kg bw/day, a paste of the test material was prepared in a small amount of water. The 10 mg/kg bw/day dose level was prepared in olive oil. The test material was spread evenly onto the 10 cm x 5 cm areas (clipped free of hair approximately 16-24 hours prior to applications) on the dorso-lumbar region of each animal. Each application site (approximately 6 cm x 4 cm) was occluded during the exposure period and was cleaned free of any residual test material after completion of the exposure period. During an 18-hour 'rest' period following each application animals were fitted with plastic collars to prevent oral ingestion of the test substance.

Animals were observed for signs of systemic toxicity and skin irritation prior to dosing on each day, after removing the test material and at least daily during the 'rest' period. Body weight and food consumption were recorded daily. Clinical chemistry and haematology parameters were evaluated at study termination. At study termination, organs were weighed and gross observations were made for each animal. Histopathological evaluation (treated and untreated skin, liver, and kidney) was made on animals from the control group (olive oil) and those treated at the 1000 mg/kg bw/day level.

Results

Preliminary study
No signs of toxicity were seen that can be attributed to administration of the test item. Slight to moderate irritation was observed in males and females from all napropamide-exposed groups during the application period.

Main study
Mortality, body weight and general observations

No mortality occurred. Stains around the nose, urinary incontinence and discharge from the eyes were seen in all groups. These effects were not dose-related, but common in dermal toxicity studies as a result of bandaging. Erythema and oedema were seen in all groups, but the incidence was not dose-related.

No significant effect on food consumption was observed. Body weights were significantly lower at 100 and 1000 mg/kg bw/day in males and in all dose groups in females at various time points. However, no dose-relationship could be established.

Haematology, clinical chemistry
Differences in haematological and clinical chemistry parameters were minor and sporadic. No dose-relationship was established.

Organ weights
The absolute adrenal, kidney and liver weights of females dosed with 100 mg/ kg bw/day were lower than the vehicle control group, but the relative weights were not affected. No other effects on organ weights were noted.

Gross pathology
No differences in the incidences of gross lesions between treated and control animals were noted at necropsy.

Histopathology
Unilateral hydronephrosis was seen in treated as well as control animals. The degree of this effect was slightly greater in males and females of the 1000 mg/kg bw/day. However, this was not regarded as toxicologically significant.

Conclusion

This 4 week repeat dose dermal toxicity study in Wistar derived [redacted]: (WI) BR rats with occluded daily applications of 0, 10, 100 or 1000 mg/kg bw/day napropamide undiluted or in olive oil was conducted in

accordance with GLP and OECD guidelines. The study is acceptable for the assessment of short-term dermal toxicity of napropamide. The study showed no treatment-related effects at any dose level. A NOAEL of 1000 mg/kg bw/day, the highest dose tested, can therefore be set for systemic as well as local effects.

B.6.3.5. Overall summary on short-term toxicity (Annex II A 5.3)

Summary of the available studies

The following short term studies are available on napropamide racemate and napropamide-M.

28-day studies
Rat
28-day dose-range-finding study on napropamide-M
28-day range finding study
Mouse
6-week dietary range-finding study
Dog
28 day oral dose range finding study

90-day
Rat
90-day dietary toxicity study on napropamide-M
Oral ninety days dietary toxicity study in rats: safety evaluation by dietary feeding to rats for 13 weeks

1 year
There are two 1-year studies in the dog.

There is also a 21-day dermal toxicity study in the rat.

All but two of the studies were done using napropamide racemate and were previously evaluated by Denmark for the approval of napropamide. These were of varying quality and most are now old or dose-range finding studies with only limited information. Others were well conducted, more recent and guideline studies. The other two studies (28-day dose-range-finding study and a 90-day dietary toxicity study, both in the rat) are new and were conducted on napropamide-M. They have been evaluated here for the first time. The latter study also incorporated a group that was exposed to a high dose of napropamide racemate, to enable a direct comparison of its toxicity with that of napropamide-M and thus provide support for the bridging to the napropamide racemate toxicology database.

Summary of the findings

In the new 90-day dietary toxicity study doses of up to 872 mg/kg bw/day of napropamide-M and 843 mg/kg bw/day napropamide racemate were delivered daily in the diet for 90 consecutive days.

In the new 28-day range finding study doses of up to 849 or 971 mg/kg bw/d for males and females respectively were delivered daily in the diet for 28 consecutive days. The findings of this study were consistent with those of the new 90-day study although it must be noted that the study gave limited information (no clinical chemistry or histopathological examination).

The kidneys and blood system (with compensatory responses in the spleen) were identified as the target organs following dietary administration of napropamide-M and napropamide racemate at doses up to 10 000 ppm for 90 days. Absolute (males only) and relative weights (males and females) of kidneys and spleen were significantly higher. The effects on organ weights for the kidney and spleen were associated with histopathological findings (kidney in males: regenerative/basophilic tubules, cortex and spleen in males: extra medullary haematopoiesis, EMH).

In females (2500 ppm and 10 000 ppm dose groups) and males (10 000 ppm dose group) the erythrocyte count (and associated changes in derived red cell parameters) are statistically significantly lower and outside the range of the historical control data. The reduced erythrocyte count appears to be associated with changes in derived red cell parameters; the primary effect therefore appears to be on red blood cells which leads to effects on the spleen.

For the new 90-day study, based on the treatment-related toxicologically significant findings observed at the high dose level (10 000 ppm) and changes in haematology at 2500 ppm in females it is concluded that the NOAEL of napropamide-M is 600 ppm (equivalent to 46 mg/kg bw/day for males and 50 mg/kg bw/day for females) after dietary administration in Wistar rats.

In the studies on napropamide racemate using dosing periods of 4 to 6 weeks, effects were seen from doses of approximately 500 mg/kg bw/day and included decreased body weight and increased relative and/or liver weights. In rats, mild anaemia was seen, while dogs had decreased food consumptions at the highest doses. In the 90-day rat study on napropamide racemate the doses used were too low since no adverse effects were seen in the highest dose (50 mg/kg bw/day).

The picture of decreased food consumption, decreased body weights and increased liver weights, with occasional changes in liver enzymes, was also predominant in the dog in the 90-day study and in the two 1-year studies with napropamide racemate. No adverse findings were seen at gross pathology or at histopathology. The NOAELs in these studies varied from 40 to 70 mg/kg bw/day across the studies.

No inhalation studies on the short-term toxicity of napropamide racemate or napropamide-M were available.

The dermal short-term toxicity of napropamide racemate was investigated in a 4-week dermal study in rats conducted in accordance with GLP and OECD guidance. The study showed no treatment-related effects at any dose level. A NOAEL of 1000 mg/kg bw/day, the highest dose tested, was therefore set for systemic as well as local effects following dermal application of napropamide racemate.

Given the similarity of the adverse effects and the doses at which they occurred, with NOAELS in the range of 40 to 70 mg/kg bw/d for both substances in 90-day studies, the RMS concludes that the toxicological profile of napropamide-M is similar to that of napropamide racemate and it is therefore acceptable to bridge to the napropamide racemate toxicology database. The conclusions drawn on the basis of all of the short term studies on napropamide racemate, as described in the EFSA Conclusion (EFSA, 2010), can thus be applied to napropamide-M.

The results of these short-term studies indicate that there are no severe or significant toxic effects at doses below the guidance cut-off values given in Regulation 1272/2008 and therefore napropamide-M does not meet the criteria for classification for repeated dose toxicity. Furthermore, in the chronic / carcinogenicity studies, the only adverse effects observed (reductions in body weights and food consumption) occurred at doses that were far in excess of the adjusted guidance cut-off values for classification in STOT-RE category 2. There is therefore consistent evidence from all the repeated-dose toxicity studies that napropamide M does not meet the criteria for classification for STOT-RE.

Type of study	Species	Dose levels tested mg/kg bw/day	NOAEL	LOAEL	Findings	Reference
Range finding oral, dietary, 28 day	RccHan: WIST strain rats	**Napropamide-M** 0, 82.9/100, 410/484, 849/971 mg/kg bw/day in males and females, respectively.	None set as range-finding study.	Effects seen from 410/484 mg/kg bw/day in males and females, respectively.	Decreased bw. Increased relative kidney weights (males in the mid- and top-dose groups and females in the top dose group). Statistically significant increases in relative spleen weights (males only in the top-dose group), liver and ovaries (both only in females in the top-dose group).	[redacted] 2013
Range finding oral, dietary, 28 day	[redacted]: CD SD rats	Napropamide 0, 181/197, 303/320, 502/530, 861/873 and 1577/1604.	None set as range-finding study.	Effects seen from about 300 mg/kg bw/day	Decreased bw gain, mild anaemia, liver enzyme effects and increased liver weights from 303/320 mg/kg bw/day.	[redacted] (1988)
Range finding oral, dietary, 6 weeks	[redacted]:CD-1 (ICR) BR mice	Napropamide 0, 386/513; 580/737, 737/1054, 1123/1467 and 2257/2937.	None set as range-finding study.	Effects seen from 737 mg/kg bw/day in males and 1467 mg/kg bw/day in females.	Increased liver weight at 737 mg/kg bw/day in males and from 1467 mg/kg bw/day in females.	[redacted] (1988)
Range-finding, oral, gavage, 4 weeks	Beagle dogs	Napropamide 30/1000, 60, 125, 250, 500 mg/kg bw/day	None set as range-finding study.	Effects at 1000 mg/kg bw/day	Decreased food consumption and body weights at 1000 mg/kg bw/day.	[redacted] (1987)
Oral, dietary 90-day	RccHan: WIST strain rats	**Napropamide-M** 0, 46/50, 185/203, 778/872 mg/kg bw/day Napropamide racemate (top dose only): 745/843 mg/kg bw/day	46 mg/kg bw/day for males and 50 mg/kg bw/day for females	Effects seen from 185/203 mg/kg bw/day in males and females, respectively.	Reduced erythrocyte count and other related secondary effects at 185/203 mg/kg bw/day, below range of historical control data.	[redacted] 2014
Oral, dietary 13 weeks	Sprague-Dawley rats	Napropamide 13, 25, 50 mg/kg bw/day	50 mg/kg bw/day	No effects seen at the highest dose tested, of 50 mg/kg bw/day.	No significant effects seen.	[redacted] (1970)
Oral, dietary, 13 weeks	Beagle dogs	Napropamide 16, 40, 100 mg/kg bw/day	40 mg/kg bw/day	100 mg/kg bw/day	Body weight loss, increased absolute and relative liver weights in males, increased alkaline phosphatase in females, decreased haemoglobin and haematocrit values in both sexes at 100 mg/kg bw/day.	[redacted] [redacted] (1970)
Oral, 52 weeks	Beagle dog	Napropamide 10, 70, or 500 mg/kg bw/day	500 mg/kg bw/day	>500 mg/kg bw/day	No adverse effects.	[redacted] [redacted] (1988)
Oral, 52 weeks	Beagle dog	Napropamide 50, 250, or 1000 mg/kg bw/day	50 mg/kg bw/day	250 mg/kg bw/day	Vomiting and liquid faeces at 250 and 1000 mg/kg bw/day, reduced body weight gains and increased absolute and relative liver weights at 1000 mg/kg bw/day. Albumin and	[redacted] (1995)

Type of study	Species	Dose levels tested mg/kg bw/day	NOAEL	LOAEL	Findings	Reference
					alkaline phosphatase were decreased at 1000 mg/kg bw/day	
Dermal, 30 days	Wistar rat	Napropamide 10, 100, 1000 mg/kg bw/day	1000 mg/kg bw/day	>1000 mg/kg bw/day	No effects seen.	[redacted] (1991)

B.6.4. GENOTOXICITY

Introduction

The *in vitro* genotoxic potential of napropamide-M has been investigated in four studies:

- *In vitro* reverse mutation assay in bacteria (Ames test)
- Two *in vitro* forward mutation assays in mammalian cells (mouse lymphoma assay)
- *In vitro* mammalian chromosome aberration test

Two *in vitro* studies (forward mutation assay in mammalian cells) were also conducted on napropamide racemate. These studies were not submitted for the approval of napropamide-M but they have been included in this DAR as they provide useful supplementary information.

The *in vivo* genotoxic potential of napropamide-M has been investigated in a comet assay in rats. Napropamide racemate was investigated previously in two *in vivo* micronucleus tests and these were submitted for the approval of napropamide-M.

Table 6.4.1: Summary of *in vitro* and *in vivo* genotoxicity studies

Study type	Test system	Dose / concentration range (batch / purity)	Result
In vitro reverse mutation assay in bacteria (Ames test) OECD 471 (1997); GLP CA 5.4.1.1/01 Sokolowski (2010, 2011) No.: 1365602	*S. typhimurium* strains TA 1535, TA 1537, TA 98, TA 100; *E. coli* strain WP2 uvrA; plate incorporation and pre-incubation assay With/without S9-mix	Napropamide-M 0 - 5000 µg/plate in DMSO Tested in triplicate **Purity** Total D+L: 97.2 %, D-isomer: 96.71 %, L-isomer: 0.49 %	Negative
In vitro forward mutation assay in mammalian cells (mouse lymphoma assay) OECD 476 (1997); GLP CA 5.4.1.2/01 Wollny, 2011 No.: 1365603	Mouse lymphoma L5178Y cells With/without S9-mix Thymidine kinase ($tk^{+/-}$) locus	Napropamide-M 0 to 112 µg/ml[(a)] (4 h -S9) 0 to 56 µg/ml[(b)] and 0 to 28 µg/mL[(b)] (4 h +S9) 0 to 112 µg/ml[(b)] (24 h -S9) Dissolved in acetone Tested in duplicate in two independent experiments **Purity** Total D+L: 97.2 %, D-isomer: 96.71 %, L-isomer: 0.49 %	Equivocal +S9 Negative -S9
In vitro L5178Y Gene Mutation Assay at the tk locus OECD 490 (2016); GLP CA 5.4.1.2/02 Ballantyne, M., 2017 No.: 8357643	Mouse lymphoma L5178Y cells With/without S9-mix Thymidine kinase ($tk^{+/-}$) locus	Napropamide-M Short term treatment ***3 hours, -S9*** 50 to 250 µg/mL ***3 hours, +S9*** 0.5 to 10 µg/mL Continuous treatment **24 hours, -S9** 15 to 80 µg/mL Purity 97.98%	Positive +S9 Negative -S9
In vitro forward mutation assay	Mouse lymphoma L5178Y	Napropamide racemate	Positive

in mammalian cells (mouse lymphoma assay) OECD 476 *Not submitted in napropamide-M dossier.* Majeska, 1984a	cells.	0.012 – 0.024 mg/ml -S9 0.010 – 0.080 mg/ml +S9 Purity 94.6 % (total D- + L-isomer) (WRC 4921-27-24)	(+/-S9)
In vitro forward mutation assay in mammalian cells OECD 476 *Not submitted in napropamide-M dossier.* Pirovano R. (1986a)	Chinese hamster V79 lung cells	Napropamide racemate 10, 50, 100 and 150 µg/ml -S9 mix, 5, 10, 50 and 100 µg/ml +S9 mix Purity 92 % (total D- + L-isomer) (BDH 1003)	Positive (+S9) Negative (-S9)
In vitro Mammalian Chromosome Aberration Test OECD 473 (1997); GLP CA 5.4.1.3/01 Bohnenberger (2011) No.: 1365604	Human lymphocytes With/without S9-mix	Napropamide-M 109.7 to 1800 µg/ml[(c)] (4 h -S9) 0.7 to 2.2 µg/ml[(a)] (4 h +S9) 35.8 to 109.7 µg/ml[(d)] (22 h -S9) Dissolved in acetone **Purity** Total D+L: 97.2 %, D-isomer: 96.71 %, L-isomer: 0.49 %	Negative
In vivo Rat Alkaline Comet Assay OECD 489 (2016); GLP [redacted] (2017) No.: 8361879	Male Han Wistar rats	Napropamide-M Two doses of 0, 500, 1000 or 2000 mg/kg bw/d separated by 21 h (6 male rats/group) **Purity** 97.98% (total D- + L-isomer)	Negative
In vivo micronucleus test Comparable to OECD 474 (1984); Conducted prior to GLP CA 5.4.2/01 [redacted] (1984b) No.: T-11822	Male and female CD-1 mice, 5/sex/dose group/time point of sacrifice; two consecutive oral (gavage) doses, approximately 24 hours apart.	Napropamide racemate 0, 556, 1667, 5000, 5000 mg/kg bw in 10 % ethanol/corn oil 1000 polychromatic erythrocytes evaluated per animal Purity 94.6 % (total D- + L-isomer)	Negative
In vivo micronucleus test Comparable to OECD 474 (1984); Conducted prior to GLP [redacted] (1986) No.: T-12813	Female CD-1 mice, 5/dose group; single oral (gavage) dose.	Napropamide racemate 0, 556, 1667, 5000, 5000 mg/kg bw in 10 % ethanol/corn oil 1000 polychromatic erythrocytes evaluated per animal Purity 94.6 % (total D- + L-isomer)	Negative

(a): Maximum dose was limited by solubility in the test system; precipitation observed by eye at the end of treatment
(b): Maximum dose was limited by toxicity in the test system, with RTG reduced to between 10-20 %
(c): Precipitation observed by eye at the end of treatment at all doses.
(d): Maximum dose was limited by toxicity in the test system, with an MI of 52 %

Photomutagenicity

The molar extinction coefficient (or molar attenuation coefficient) at 291 nm (UV) of napropamide-M is 2.271 × 10^3 L × mol^{-1} × cm^{-1} (see Volume 3, CA B.2, Bates, G. (2014)) which exceeds the threshold of 1000 L × mol^{-1} × cm^{-1} specified in Regulation 283/2013. Nevertheless the active substance does not absorb electromagnetic radiation in the range 290-700 nm (the λ max (nm) values were ≤290 nm) and the levels of radioactivity in the eyes and skin (light exposed tissues) were negligible in terms of concentration and duration of exposure. Thus overall, the potential for any photomutagenicity associated with napropamide-M is considered to be very low.

The applicant has also pointed out that there is currently no OECD test guideline available for photomutagenicity testing and Regulation 283/2013 does not provide any guidance on suitable test methods. It is furthermore noted that the ICH Guideline on Photosafety Evaluation of Pharmaceuticals S10 (2013) explicitly discourages photogenotoxicity testing: *"Testing for photo-genotoxicity is not recommended as a part of the standard photosafety testing program. … experience … has indicated that these tests are substantially over-sensitive and even incidences of pseudo-photoclastogenicity have been reported (Ref. 8). Furthermore, the interpretation of photogenotoxicity data regarding its meaning for clinically-relevant enhancement of UV-mediated skin cancer is unclear."*

The RMS's view is that photomutagenicity testing of napropamide-M is not required.

B.6.4.1. *In vitro* studies

B.6.4.1.1. *In vitro* reverse mutation assays in bacteria (Ames tests)

Napropamide-M (batch: UPV/714-181/DEV/014; purity: Total D+L: 97.2 %, D-isomer: 96.71 %, L-isomer: 0.49 %) was tested in an Ames test (Sokolowski, 2010, 2011) with concentrations up to 5000 µg/plate and a standard series of test strains. Metabolic activation was provided by a hepatic S9-mix from phenobarbital/ß-naphthoflavone-induced rats.

Plate incorporation

Precipitate was observed in all strains in +/-S9 at 1000 µg/plate and above. Toxic effects were observed (reduction in the number of revertants) at 5000 µg/plate without S9 in strain TA 1537 and with S9 in strain TA 1535.

Pre-incubation

Precipitate was observed in all strains at 2500 and 1000 µg/plate and without and with S9, respectively.

There was no relevant increase in the number of revertant colonies in any test strain either with or without metabolic activation in several experiments that were performed independently of each other. The negative and positive controls gave the expected results and verified the validity of the study. See Table 6.4.2 and Table 6.4.3.

Napropamide-M showed no potential to induce gene mutations in bacteria in a standard study that was compliant with the relevant OECD guideline.

Table 6.4.2: Ames test (Experiment I, plate incorporation) - mean number of revertants (Sokolowski, 2010 and 2011)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	DMSO		14 ± 4	12 ± 3	30 ± 5	128 ± 6	51 ± 6
	Untreated		13 ± 6	9 ± 2	34 ± 9	146 ± 12	51 ± 7
	d-napropamide	3 µg	13 ± 4	11 ± 3	35 ± 9	144 ± 15	51 ± 5
		10 µg	12 ± 4	11 ± 4	29 ± 8	132 ± 20	50 ± 6
		33 µg	15 ± 5	10 ± 3	27 ± 3	139 ± 9	55 ± 6
		100 µg	16 ± 3	11 ± 4	32 ± 1	135 ± 10	52 ± 0
		333 µg	14 ± 1	13 ± 3	34 ± 3	126 ± 7	51 ± 1
		1000 µg	14 ± 4 P	13 ± 3 P	28 ± 2 P	121 ± 7 P	47 ± 4 P
		2500 µg	6 ± 2 P M	7 ± 1 P M	21 ± 2 P M	123 ± 4 P M	51 ± 10 P M
		5000 µg	9 ± 3 P M	5 ± 3 P M	20 ± 6 P M	113 ± 5 P M	43 ± 6 P M
	NaN3	10 µg	1880 ± 38			1855 ± 100	
	4-NOPD	10 µg			312 ± 25		
	4-NOPD	50 µg		74 ± 2			
	MMS	3.0 µL					1236 ± 87
With Activation	DMSO		19 ± 3	11 ± 3	43 ± 9	141 ± 7	64 ± 9
	Untreated		19 ± 5	8 ± 1	44 ± 7	148 ± 18	61 ± 3
	d-napropamide	3 µg	19 ± 3	12 ± 3	46 ± 9	133 ± 16	64 ± 10
		10 µg	17 ± 2	10 ± 2	40 ± 7	139 ± 20	63 ± 4
		33 µg	17 ± 3	10 ± 2	42 ± 7	133 ± 22	62 ± 5
		100 µg	17 ± 6	12 ± 3	43 ± 15	138 ± 3	73 ± 6
		333 µg	14 ± 3	12 ± 3	42 ± 3	126 ± 8	57 ± 9
		1000 µg	14 ± 1 P	5 ± 2 P	36 ± 4 P	95 ± 20 P	68 ± 8 P
		2500 µg	9 ± 1 P M	4 ± 1 P M	24 ± 6 P M	85 ± 6 P M	41 ± 2 P M
		5000 µg	8 ± 4 P M	4 ± 1 P M	21 ± 3 P M	92 ± 8 P M	45 ± 4 P M
	2-AA	2.5 µg	459 ± 34	340 ± 4	2169 ± 167	2544 ± 125	
	2-AA	10.0 µg					258 ± 30

Key to Positive Controls	
NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes	
P	Precipitate
M	Manual count

Table 6.4.3: Ames test (Experiment II, pre-incubation) - mean number of revertants (Sokolowski, 2010 and 2011)

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	DMSO		13 ± 2	8 ± 1	30 ± 5	146 ± 4	47 ± 8
	Untreated		15 ± 5	9 ± 4	28 ± 6	183 ± 11	46 ± 10
	d-napropamide	3 µg	11 ± 1	10 ± 3	30 ± 4	144 ± 4	38 ± 4
		10 µg	14 ± 3	8 ± 2	30 ± 3	135 ± 2	40 ± 11
		33 µg	11 ± 5	10 ± 2	28 ± 5	144 ± 9	38 ± 3
		100 µg	12 ± 2	9 ± 4	36 ± 6	147 ± 14	43 ± 8
		333 µg	12 ± 4	9 ± 3	26 ± 4	129 ± 9	38 ± 6
		1000 µg	13 ± 6	4 ± 2	29 ± 1	133 ± 10	38 ± 4
		2500 µg	12 ± 2 P M	4 ± 1 P M	18 ± 3 P M	104 ± 14 P M	34 ± 8 P M
		5000 µg	10 ± 3 P M	4 ± 2 P M	15 ± 2 P M	92 ± 6 P M	31 ± 5 P M
	NaN3	10 µg	1647 ± 30			1748 ± 37	
	4-NOPD	10 µg			436 ± 45		
	4-NOPD	50 µg		87 ± 4			
	MMS	3.0 µL					574 ± 61
With Activation	DMSO		13 ± 3	14 ± 7	40 ± 6	163 ± 9	53 ± 4
	Untreated		18 ± 5	18 ± 7	43 ± 10	169 ± 10	46 ± 11
	d-napropamide	3 µg	13 ± 4	14 ± 0	42 ± 8	140 ± 9	54 ± 14
		10 µg	14 ± 3	15 ± 4	41 ± 3	158 ± 7	47 ± 6
		33 µg	12 ± 5	12 ± 4	43 ± 7	163 ± 18	54 ± 7
		100 µg	15 ± 3	17 ± 2	35 ± 7	142 ± 14	59 ± 8
		333 µg	11 ± 6	14 ± 1	42 ± 3	114 ± 14	49 ± 10
		1000 µg	10 ± 4 P	14 ± 2 P	29 ± 2 P	75 ± 5 P	49 ± 2 P
		2500 µg	9 ± 3 P M	6 ± 1 P M	25 ± 2 P M	90 ± 12 P M	34 ± 4 P M
		5000 µg	10 ± 2 P M	6 ± 2 P M	27 ± 3 P M	91 ± 6 P M	32 ± 6 P M
	2-AA	2.5 µg	290 ± 16	225 ± 30	1718 ± 32	2098 ± 82	
	2-AA	10.0 µg					477 ± 45

Key to Positive Controls	
NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes	
P	Precipitate
M	Manual count

B.6.4.1.2. *In vitro* genotoxicity testing (mammalian assay for gene mutation)

Methods

Napropamide-M (batch: UPV/714-181/DEV/014; purity: Total D+L: 97.2 %, D-isomer: 96.71 %, L-isomer: 0.49 %) was tested in an *in vitro* assay for its ability to induce forward mutations in mammalian cells by assessing the mutation of the TK locus in mouse lymphoma L5178Y cells. Metabolic activation was provided by a hepatic S9-mix from phenobarbital/ß-naphthoflavone-induced rats.

In the study (Wollny, 2011), concentrations up to 112 µg/ml were used in the main experiment, based on the results of a preliminary cytotoxicity assay in which concentrations in the range of 14.1 to 1800 µg/ml were tested. Excessive cytotoxicity was observed from 112.5 µg/ml without metabolic activation and 14.1 µg/ml with metabolic activation, after 4-hours of incubation. Following 24 hours of treatment toxic effects were observed at 28.1 µg/ml and above.

Precipitation was observed with the unaided eye from 225.0 µg/ml following 4 and 24 hours of treatment in the absence of metabolic activation. In the presence of metabolic activation precipitation was determined at 450 µg/ml and above.

In the main test, two independent experiments (experiment I and II) were performed in two parallel cultures. The treatment interval for both experiments in the presence and absence of metabolic activation was generally four hours, except in experiment II (in the absence of metabolic activation) where a treatment interval of 24 hours was used. Methylmethanesulfonate (MMS) and cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.

The experiments were begun with more than four concentrations, but following the expression phase of 48 hours the cultures at the highest concentration in experiment I, culture I (- S9) and in experiment II (+S9) were discontinued because of excessive cytotoxicity.

In experiment I, cultures I and II, the cultures at the two lowest concentrations with metabolic activation were not continued because the test-guideline condition of at least four analysable test concentrations was met without that concentration being included; i.e. cytotoxicity was not excessive at the highest concentration, and so the cultures at that concentration were continued. This was also the case for the lowest concentration without metabolic activation for experiment I, culture II and experiment II, cultures I and II.

Relevant cytotoxic effects, indicated by a relative total growth (RTG) of less than 50 % of survival in both parallel cultures, were observed in both experiments; thus it is concluded that adequate concentrations were tested. It is noted that in Experiment I, Culture I (-S9) RTG was 55.6 % at the highest concentration and in Experiment I, Culture II (-S9) RTG was 51.9 %; these levels are close to 50 % and are not considered to invalidate the study. Since the negative and positive control cultures met the acceptance criteria, the study is considered by the RMS to be valid.

The study was performed in 2011, prior to the introduction of the new OECD 490 TG (2016). The new TG improves the interpretation of these studies and it is therefore appropriate to use the more modern guideline to establish if the result is positive.

In the OECD 490 TG (2016) an approach for defining positive and negative responses is recommended to assure that the increased mutant frequency (MF) is biologically relevant. The approach employs the Global Evaluation Factor (GEF). For the microwell version of the MLA the GEF is 126×10^{-6}.

A test chemical is considered to be clearly positive if, in any of the experimental conditions examined, the increase in MF above the concurrent background exceeds the GEF and the increase is concentration related (e.g. using a trend test, as stated in OECD 490 TG (2016)).

Results

The results of Experiment I and Experiment II (individual cultures) are presented in Table 6.4.4 and Table 6.4.5, respectively. OECD 490 TG states that the results of replicate cultures can be pooled for analysis and therefore the means of Cultures I and II, for each experiment, have also been presented in these tables.

There was no increase in mutant frequency in any culture without metabolic activation.

With metabolic activation, the mutant frequency exceeded the sum of the solvent control mutant frequency (MF) plus the Global Evaluation Factor (GEF) at one or more concentrations in all four cultures (across Experiment I and II). In Experiment II, Culture II, there was an increase (exceeding the GEF + control) at the highest tested concentration (28 µg/mL). The relevant MF (209) is exactly at the upper range of the solvent control in the historical control data (46-209) – see Table 6.4.6. The results for the lower concentrations appear to follow an approximately concentration-related response. In Experiment II, Culture I there was an increase (exceeding the GEF + control) at the second highest concentration (14 µg/mL) but not at the highest concentration. The MF (231) at 14 µg/mL was above the upper range of the solvent control in the historical control data (46-209) – see Table 6.4.6.

The historical control data relevant to the laboratory and for a period of two years prior to the date that the study was performed are presented in Table 6.4.6. The results for the positive and negative controls are consistent with the historical control data.

Conclusion

A clear negative result was not obtained in this study. The mutation frequency exceeded the value for a positive increase above background levels in at least one concentration in all cultures with metabolic activation. However, especially when the mean values of each experiment were considered, a clear concentration-related increase was not evident in either of the experiments.

The study was done before the introduction of the new OECD 490 TG (2016) and consequently it is not fully compliant with the recommendations of the revised mouse lymphoma OECD 490 TG. To address these deficiencies and provide a robust conclusion regarding the gene mutation potential of napropamide-M at the tk locus of L5178Y cells, a new OECD 490 TG compliant study was carried out by the applicant (Ballantyne, M., 2017).

Table 6.4.4: Results of mouse lymphoma assay – Experiment I (Wollny, 2011)

		MF / 10^6 cells	s. mutant colonies / 10^6 cells	l. mutant colonies / 10^6 cells	RTG	GEF + bck-grnd[(d)]	MF / 10^6 cells	s. mutant colonies / 10^6 cells	l. mutant colonies / 10^6 cells	RTG	GEF + bck-grnd[(d)]	MF / 10^6 cells	s. mutant colonies / 10^6 cells	l. mutant colonies / 10^6 cells	RTG	GEF + bck-grnd[(d)]
Without metabolic activation 4-hour exposure period																
Test item	Conc. [µg /mL]	**Culture I**					**Culture II**					**Cultures I & II (mean)**				
-ve ctrl. acetone		87	16	71	100.0	213	122	19	104	100.0	248	104.5	17.5	87.5	**100**	230.5
Naprop-amide-M	7.0	95	18	77	84.9	213	Culture was not continued[(b)]					95	18	77	**85**	230.5
	14.0	99	15	84	85.9	213	114	18	97	112.0	248	106.5	16.5	90.5	**99**	230.5
	28.0	102	21	81	82.7	213	95	13	82	87.6	248	98.5	17	81.5	**85**	230.5
	56.0	95	21	74	70.7	213	94	20	73	106.6	248	94.5	20.5	73.5	**89**	230.5
	84.0	102	28	74	55.6	213	90	20	70	69.9	248	96	24	72	**63**	230.5
	112[(c)]	Culture was not continued[(a)]					80	24	56	51.9	248	80	24	56	**52**	230.5
+ve ctrl. MMS	19.5	409	160	249	19.7	213	356	227	129	38.5	248	382.5	193.5	189	**29**	230.5
With metabolic activation 4-hour exposure period																
-ve ctrl. acetone		45	9	35	100.0	171	130	52	77	100.0	256	87.5	30.5	56	**100**	213.5
Naprop-amide-M	1.8	Culture was not continued[(b)]					Culture was not continued[(b)]					Culture was not continued[(b)]				
	3.5	Culture was not continued[(b)]					Culture was not continued[(b)]					Culture was not continued[(b)]				
	7.0	82	17	65	40.3	171	148	59	89	62.4	256	115	38	77	**51**	213.5
	14.0	**172***	60	112	17.2	171	175	90	86	14.9	256	173.5	75	99	**16**	213.5
	28.0	**186***	65	120	14.2	171	**285***	169	116	12.5	256	**235.5***	117	118	**13**	213.5
	42.0	**185***	88	97	10.6	171	199	110	88	12.1	256	192	99	92.5	**11**	213.5
	56.0	131	45	86	9.1	171	201	111	89	9.3	256	166	78	87.5	**9**	213.5
+ve ctrl. CPA	4.5	240	155	86	15.1	171	405	251	154	23.4	256	322.5	203	120	**19**	213.5

(a): culture was not continued owing to excessively strong cytotoxic effects
(b): culture was not continued since only a minimum of four concentrations is required by the guidelines
(c): precipitate observed by eye at the end of treatment
(d): sum of the solvent control mutant frequency (MF) plus the Global Evaluation Factor (GEF) of 126 x 10^{-6}
*: increase in mutant frequency of treated cultures exceeds the sum of the GEF + solvent control mutant frequency (MF)

Table 6.4.5: Results of mouse lymphoma assay – Experiment II (Wollny, 2011)

		MF / 10^6 cells	s. mutant colonies / 10^6 cells	l. mutant colonies / 10^6 cells	RTG	GEF + bck-grnd[(c)]	MF / 10^6 cells	s. mutant colonies / 10^6 cells	l. mutant colonies / 10^6 cells	RTG	GEF + bck-grnd[(c)]	MF / 10^6 cells	s. mutant colonies / 10^6 cells	l. mutant colonies / 10^6 cells	RTG	GEF + bck-grnd[(c)]
Without metabolic activation 24-hour exposure period																
Test item	Conc. [µg /mL]	**Culture I**					**Culture II**					**Cultures I & II (mean)**				
-ve ctrl. acetone		96	27	69	100.0	222	79	55	24	100.0	205	87.5	41	46.5	**100**	213.5
Naprop-amide-M	7.0	Culture was not continued[(b)]					Culture was not continued[(b)]					Culture was not continued[(b)]				
	14.0	76	12	64	40.9	222	83	73	9	38.9	205	79.5	42.5	36.5	**40**	213.5
	28.0	74	16	58	27.2	222	61	53	8	34.7	205	67.5	34.5	33	**31**	213.5
	56.0	76	9	67	31.0	222	50	39	11	24.9	205	63	24	39	**28**	213.5
	84.0	76	13	63	16.7	222	86	63	23	14.8	205	81	38	43	**16**	213.5
	112[(c)]	130	17	113	8.7	222	76	60	16	8.0	205	103	38.5	64.5	**8**	213.5
+ve ctrl. MMS	13.0	565	304	261	20.2	222	431	338	93	19.3	205	498	321	177	**20**	213.5
With metabolic activation 4-hour exposure period																
-ve ctrl. acetone		63	13	51	100.0	189	69	17	52	100.0	195	66	15	51.5	**100**	192
Naprop-amide-M	1.8	71	18	53	135.2	189	81	15	65	85.7	195	76	16.5	59	**111**	192
	3.5	49	7	43	211.5	189	70	11	59	94.3	195	59.5	9	51	**153**	192
	7.0	86	34	52	111.8	189	135	31	104	52.5	195	110.5	32.5	78	**82**	192
	14.0	**231***	171	60	12.5	189	153	62	92	13.6	195	**192***	116.5	76	**13**	192
	28.0	171	148	23	12.3	189	**209***	92	117	9.0	195	190	120	70	**11**	192
	42.0	Culture was not continued[(a)]					Culture was not continued[(a)]					Culture was not continued[(a)]				
	56.0	Culture was not continued[(a)]					Culture was not continued[(a)]					Culture was not continued[(a)]				
+ve ctrl. CPA	4.5	333	228	105	59.0	189	324	199	125	35.3	195	328.5	213.5	115	**47**	192

(a): culture was not continued owing to excessively strong cytotoxic effects
(b): culture was not continued since only a minimum of four concentrations is required by the guidelines
(c): sum of the solvent control mutant frequency (MF) plus the Global Evaluation Factor (GEF) of 126 x 10^{-6}
*: increase in mutant frequency of treated cultures exceeds (or, in one case, equals) the sum of the GEF + solvent control mutant frequency (MF)

Table 6.4.6: Historical control data relating to the mouse lymphoma assay – Experiment I and II (Wollny, 2011)

Historical control data for 2008 to 2009:

Number of mutant colonies per 10^6 cells		
4 h treatment / without metabolic activation		
	Solvent control (medium, DMSO, water, ethanol, acetone, THF)	Positive control (MMS)
range:	39 – 211	226 – 1318
Mean value:	114	378
Standard deviation:	37	135
Number of studies:	128	128
4 h treatment / with metabolic activation		
	Solvent control (medium, DMSO, water, ethanol, acetone, THF)	Positive control (CPA)
range:	46 – 209	377 – 6886
Mean value:	123	483
Standard deviation:	38	446
Number of studies:	129	129
24 h treatment / without metabolic activation		
	Solvent control (medium, DMSO, water, ethanol, acetone, THF)	Positive control (MMS)
range:	50 – 224	240 – 1484
Mean value:	122	537
Standard deviation:	38	212
Number of studies:	103	103

B.6.4.1.3. *In vitro* L5178Y gene mutation assay at the *tk* locus

Introduction

The mammalian assay for gene mutation (Wollny, 2011) was carried out before the introduction of the new OECD test guideline (OECD 490) and consequently was not fully compliant with it. To address this, and to provide a robust conclusion regarding the gene mutation potential of napropamide-M at the *tk* locus of L5178Y cells, the following *in vitro* gene mutation assay was carried out by the applicant.

Author(s):	Ballantyne, M., 2017
Study title:	Napropamide-M: *In vitro* L5178Y gene mutation assay at the *tk* locus. UPL Europe Ltd, Unpublished report No.: 8357643
Test substance:	Napropamide-M
Purity:	97.98% (total D- + L-isomer)
Batch no.:	UPH-08 / DNE-263 / Tech / 20160615
Test cells:	L5178Y $tk^{+/-}$ mouse lymphoma cells
Dose:	Preliminary cytotoxicity assay 3 h +/-S9: 0, 16, 31, 63, 1250, 250, 500 µg/mL 24 h –S9: 0, 2, 4, 8, 16, 31, 63, 1250, 250, 500 µg/mL (maximum dose [in all treatments] limited by solubility) Mutation assays **Short term treatment** 3h –S9: 0, 50, 75, 100, 125, 150, 200, 225, 250 µg/mL 3h +S9: 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 µg/mL **Continuous treatment:** 24h -S9: 0, 15, 30, 45, 60, 70, 80, 90, 100, 110, 125, 150 µg/mL
Vehicle/solvent:	Dimethyl sulphoxide (DMSO)
Statistics/ measurements:	Linear trend analysis (required if the mutant frequency of any test article concentration exceeded the sum of the vehicle control mutant frequency + GEF)
GLP:	Yes (certified)
Guideline:	OECD 490 (2016)
Deviation:	None
Acceptability:	Acceptable

Methods

Preliminary cytotoxicity assay

Cells were exposed to test article formulations or solvent controls for either 3 or 24 hours in the absence of S9 or 3 hours in the presence of S9. Single cultures were used. There was no positive control. At the end of treatment cultures were washed, cell culture counted, adjusted (where necessary to 2 x 10^5 cells/mL), transferred to cell culture flasks and incubated for 2 days, with cell adjustment made every 24 hours. At this time cultures were assessed for cytotoxicity. As the cytotoxicity range-finder experiment was restricted to 2 days post treatment, toxicity was expressed in terms of relative suspension growth (RSG). Solubility of the test article in culture medium was assessed by eye at the beginning and end of treatment.

Mutation assay

The same procedures were followed as in the preliminary cytotoxicity experiment (detailed above), with the following exceptions.

Each treatment, in the absence or presence of S9, was in duplicate (vehicle controls and test article formulation cultures). It is noted that exposure durations were 3 hours with and without S9 (short-term assays) and 24 hours without S9 (continuous exposure).

Suitable positive control cultures were included (single cultures only). At the end of the 2 days, cells were sub-cultured to assess cytotoxicity and to initiate the phenotypic expression.

Cloning efficiency was determined by plating approximately 10^6 cells/well into two 96 well plates. Plates were incubated for 10-14 days. After this period the number of wells without growth of cells was determined.

Mutation frequency was determined by plating approximately 2000 cells/well in cell culture medium containing 4 µg/ TFT/mL. Plates were incubated for 10-14 days. After this period the number of wells without growth was

determined to provide cloning efficiency in TFT. Wells with growth indicated evidence of TFT-resistance mutants. Colony sizing was performed on negative and positive controls.

Results

Preliminary cytotoxicity assay

In the cytotoxicity range-finder experiment (3-hour treatment), six concentrations were tested in the absence and presence of S9 ranging from 15.63 to 500 µg/mL (limited by solubility limit in culture medium). The highest concentration to provide >10% RSG was 125 µg/mL in the absence of S9, which gave 40 % RSG. Severe toxicity (≤5% RSG) was observed at all concentrations in the presence of S9.
In the 24-hour treatment, nine concentrations were tested in the absence of S9 ranging from 1.953 to 500 µg/mL. The highest concentration tested that provided >10 % RSG was 62.5 µg/mL, which gave 27 % RSG.

Mutation assay
Vehicle and positive controls

Vehicle controls
The mutant frequency (MF) in vehicle control cultures was within acceptable ranges. The number of wells containing small colonies and the number containing large colonies were scored. The proportion of small colony mutants in the absence and presence of S-9 ranged from 17% to 35%.

Positive controls
Clear increases in mutation were induced by the positive control chemicals. The positive control chemicals methyl methane sulphonate (MMS) and benzo[a]pyrene (B[a]P) resulted in the expected increases in the numbers of small and large colony mutants.. These results support the validity of the study.

Short term treatment
3 hours, -S9
Concentrations in the range 50 to 250 µg/mL were tested. Precipitation was observed at the time of treatment at concentrations of ≥ 125 µg/mL. No evidence of precipitation was observed at the end of treatment at any concentration. Concentrations ≥200 µg/mL were considered too toxic for selection to determine viability and TFT resistance and so were not further assessed or data for them presented in the report.

The MF of the concentrations plated were all less than the sum of the mean control MF plus the global evaluation factor (GEF, 126 mutants/10^6 viable cells) and there was no concentration-related increase, indicating a clearly negative result (refer to Table 6.4.7).

3 hours, +S9
Concentrations in the range 0.5 to 10 µg/mL were tested. No evidence of precipitation was observed at any concentration during any part of the exposure period. Concentrations ≥12 µg/mL were considered too toxic for selection to determine viability and TFT resistance and so were not further assessed or data for them presented in the report.

Increases in MF were observed at the top two concentrations (8 and 10 µg/mL). These concentrations were cytotoxic, but not excessively so (29% and 11% RTG, respectively, in accordance with the test guideline for selection of a maximum concentration). The increases in MF were greater than the sum of the mean control MF plus the GEF, with a concentration-related increase (and statistically significant linear trend test), indicating a positive result. Increases in both small and large colonies was observed, although the proportion of small colonies was increased compared with the negative control.

Continuous treatment
24 hours, -S9
Since the short-term test without metabolic activation was negative, an experiment with continuous exposure for 24 hours was also conducted (-S9). Concentrations in the range 15 to 80 µg/mL were tested. Precipitation was observed at the time of treatment at concentrations of ≥ 110 µg/mL but not at the end of treatment. Concentrations ≥90 µg/mL were considered too toxic for selection to determine viability and TFT resistance and so were not further assessed or data for them presented in the report.

The MF of the concentrations plated were all less than the sum of the mean control MF plus the GEF, indicating a negative result (refer to Table 6.4.8).

24 hours, +S9
No concentrations were tested.

Table 6.4.7: Mutant frequency data from L5178Y TK$^{+/-}$ cells treated with napropamide-M – short term treatment (Ballantyne, M., 2017), mean are of duplicates

3h –S9						3h +S9					
Conc (µg/mL)	SG	CE (%)	RTG (%)	MF (x10^{-6})	P.S.C.M.	Conc (µg/mL)	SG	CE (%)	RTG (%)[c]	MF (x10^{-6})	P.S.C.M.
0	21	84	100	134.06	0.35	0	19	99	100	124.06	0.33
50	15	100	80	142.56	-	0.5	16	95	83	139.68	-
75	14	90	70	149.53	-	1	16	100	87	119.60	
100	7	100	42	176.78	-	2	17	94	85	134.66	-
125 P	5	119	36	154.59	-	4	15	103	80	142.07	-
150 P	3	112	19	168.99	-	6	9	116	58	195.43	-
175 P	3	131	19	150.73	-	8	5	108	29	286.68[a]	0.49
MMS 15	16	64	58	1006.23	0.59	10	2	83	11	469.03[a]	0.54
MMS 20	6	84	27	1038.12	0.63	B[a]P 2	10	76	42	1033.31	0.49
						B[a]P 3	7	68	23	1186.83	0.53

%RTG Percent Relative Total Growth
MF Mutant Frequency
SG Suspension Growth
CE Cloning Efficiency (given as %V in the report which is defined as '%V is given as '% Day 2 viability')
P.S.C.M. Proportion of Small Colony Mutants (only scored for vehicle, positive controls + conc. that exceed GEF
P Precipitation noted at time of treatment
[mean control MF + GEF = 260.06]
Linear trend test on mutant frequency: *p*-value = 0.0469
a MF exceeds the sum of the vehicle control MF plus GEF [mean control MF + GEF = 250.06]
Linear trend test on mutant frequency: *p*-value = 0.0019

Table 6.4.8: Mutant frequency data from L5178Y TK+/- cells treated with Napropamide-M – continuous treatment – without metabolic activation (Ballantyne, M., 2017), mean are of duplicates

Conc (µg/mL)	SG	CE (%)	RTG (%)	MF ($x10^{-6}$)	P.S.C.M.
0	74	105	100	98.47	0.17
15	58	90	67	93.55	-
30	36	79	37	112.85	-
45	29	89	33	106.40	-
60	16	85	20	90.24	-
70	13	79	13	128.93	-
80	9	95	11	129.06	-
MMS 5	52	56	37	935.07	0.59
MMS 7.5	47	55	33	1193.65	0.59

%RTG Percent Relative Total Growth
MF Mutant Frequency
SG Suspension Growth
CE Cloning Efficiency (given as %V in the report which is defined as '%V is given as '% Day 2 viability')
P.S.C.M. Proportion of Small Colony Mutants (only scored for vehicle, positive controls + conc. that exceed GEF [mean control MF + GEF = 260.06]
Linear trend test on mutant frequency: *p*-value = 0.0901

Conclusions

Napropamide-M did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested up to the limit of toxicity in the absence of a rat liver metabolic activation system (S9) and incubated for 3 and 24 hours. In the presence of S9, a 3-hour incubation with napropamide-M induced an increase in mutant frequency (large and small colonies, although with an increased proportion of small colonies) at cytotoxic concentrations (11-29% RTG).

B.6.4.1.4. *In vitro* genotoxicity testing (mammalian assay for gene mutation)

Introduction

This is one of two *in vitro* mammalian cell gene mutation tests, done using napropamide racemate, that have been included in the napropamide-M DAR (though the studies were not submitted by the applicant in their dossier).

This study, and the other, was previously evaluated by Denmark for the approval of napropamide racemate. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	Majeska J.B. (1984a)
Study title:	Mutagenicity evaluation in mouse lymphoma multiple endpoint test – forward mutation assay.
Test substance:	Napropamide racemate
Purity:	94.6 % (total D- + L-isomer)
Batch no.:	WRC 4921-27-24
Test system:	Mouse lymphoma L5178Y cells, gene mutation in the thymidine kinase (TK) locus
Dose:	Concentrations ranging from 0.010 to 0.080 mg/ml in the absence of S9 metabolic activation and 0.012 to 0.040 mg/ml in the presence of metabolic activation
Vehicle/solvent:	Dimethylsulphoxide (DMSO)
Metabolic activation system:	S9 fraction of a liver homogenate obtained from Aroclor-induced rats
Statistics/ measurements:	No statistics
GLP:	No GLP statement but a QA statement is available
Guideline:	None cited (study followed international testing guidelines such as OECD 476 (1997) and US EPA)
Deviation:	Colony sizing has not been performed
Acceptability:	Acceptable

Methods

Mouse lymphoma L5178Y cells were treated with napropamide racemate (purity 94.6 %, total D- + L-isomer) in dimethylsulphoxide (DMSO) at concentrations ranging from 0.010 to 0.080 mg/ml in the absence of S9 metabolic activation and 0.012 to 0.040 mg/ml in the presence of metabolic activation. Negative (vehicle and medium) and positive controls were also tested with and without metabolic activation. After 4 hours of treatment at 37°C, cells were washed and incubated for another 48 hours to allow for expression of the mutant phenotype; cell viability and mutant frequency were assessed following a 9-11-day incubation period. Duplicate cultures were tested for each dose level. Two independent assays were performed.

Results

Napropamide racemate was cytotoxic at doses ≥ 0.006 mg/ml. In the presence of a metabolic activating system, napropamide racemate was more toxic over a narrower concentration range.

There was a reproducible increase in mutant frequency at concentration levels that reduced total relative growth to less than 20 % of the solvent controls in the absence of metabolic activation. There was a concentration-related increase in mutant frequency at all concentrations tested in the presence of metabolic activation. At these concentrations, survival was reduced to 3 to 78 % of concurrent controls.

Conclusion

Napropamide racemate induced an increase in mutation frequency in a concentration dependent manner in mouse lymphoma cells at the TK locus. An increase in the mutation frequency was observed in a concentration dependent manner with and without activation, when tested at concentrations from 0.012 to 0.020 mg/ml and 0.01 to 0.08 mg/ml, respectively. Excess cytotoxicity was observed at concentrations over 0.020 mg/ml and at about 0.080 mg/ml with and without activation, respectively.

The sensitivity of the assay system has been proven by the observed and expected response to known mutagens. There was a slight deviation from OECD 476 (1997 – the guideline that was current at the time the study was evaluated); colony sizing has not been performed, but this deviation does not compromise the result of the study. Napropamide racemate is considered positive under the conditions of this test.

B.6.4.1.5. *In vitro* genotoxicity testing (mammalian assay for gene mutation)

Introduction

This is one of two *in vitro* mammalian cell gene mutation tests, done using napropamide racemate, that have been included in the napropamide-M DAR (though the studies were not submitted by the applicant in their dossier).

This study, and the other, was previously evaluated by Denmark for the approval of napropamide racemate. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	Pirovano R. (1986a)
Study title:	Study of the capacity of the test article napropamide racemate to induce gene mutation in V79 Chinese hamster lung cells
Test substance:	Napropamide racemate
Purity:	92 % (total D- + L-isomer)
Batch no.:	BDH 1003
Test system:	Chinese hamster V79 lung cells, screening for point mutation of the HPRT gene
Dose:	10, 50, 100 and 150 µg/ml without metabolic activation and 5, 10, 50 and 100 µg/ml with metabolic activation
Vehicle/solvent:	Dimethylsulphoxide (DMSO)
Metabolic activation system:	S9 fraction of a liver homogenate obtained from Aroclor-induced rats
Statistics/ measurements:	Linear regression test was performed for mean mutant frequency
GLP:	No GLP statement but a QA statement is available
Guideline:	None cited (study followed international testing guidelines such as OECD (476) and US EPA)
Deviation:	No significant deviations. The exposure concentrations could have been higher in the main study, but the preliminary toxicity test indicated an unacceptable high cytotoxicity at the next concentration level.
Acceptability:	Acceptable

Methods

Chinese hamster V79 lung cells were treated with napropamide racemate (purity 92 %, total D- + L-isomer) in dimethylsulphoxide (DMSO) at concentrations ranging from 10 to 150 µg/ml without metabolic activation and from 5 to 100 µg/ml with metabolic activation. The chosen concentrations were based on a preliminary toxicity test with 5, 10, 50, 100 and 200 µg/ml, and in this study the relative cloning efficiency was less than 10 % at the highest concentration. Negative (solvent) and positive controls were also tested in the main study. Cells were treated with the test substance or negative and positive controls and incubated for 2 hours at 37ºC with or without metabolic activation (the S9 fraction of a liver homogenate obtained from Arochlor induced rats). To assess cytotoxicity, cells from each dose group were sub-cultured and incubated in non-selective medium. Additional cells were sub-cultured and incubated in selective medium for 6 or 9 days to allow for the expression of the mutant phenotype to detect mutant frequency and to determine the cloning efficiency. Three or four cultures were tested at each dose level. Two independent assays were performed with and without metabolic activation.

Results

The positive controls showed an increase in mutant frequency compared to solvent controls. A dose-related increase in mutant frequency was noted at concentrations ≥ 10 µg/ml in the presence of metabolic activation in

both experiments. An increase in mutant frequency was observed at 10 µg/ml without metabolic activation in the first experiment; however, this increase was not dose-related nor was it confirmed in the second experiment. Evidence of cytotoxicity was observed on day 0 at the highest concentration level (44 and 49 % relative cloning efficiency without metabolic activation and 64 and 62 % relative cloning efficiency with metabolic activation).

Conclusion

Napropamide racemate induced an increase in mutation frequency in a concentration dependent manner in Chinese hamster V79 lung cells at the HPRT locus. An increase in the mutation frequency was observed in a concentration dependent manner when tested at concentrations of 5, 10, 50 and 100 µg/ml with metabolic activation. Evidence of marked cytotoxicity was observed at a concentration of 200 µg/ml. The sensitivity of the assay system has been proven by the observed and expected response to known mutagens. Under the conditions of this test, napropamide racemate is considered positive in the presence of metabolic activation and negative without metabolic activation.

B.6.4.1.6. *In vitro* mammalian chromosome aberration test

A mammalian chromosome aberration test in human lymphocytes *in vitro* with napropamide-M (batch UPV/714-181/DEV/014; purity: Total D+L: 97.2 %, D-isomer: 96.71 %, L-isomer: 0.49 %) has been conducted in accordance with OECD test guideline 473 and to GLP (Bohnenberger, 2011).

Human lymphocytes were exposed to napropamide-M using acetone as the solvent in the presence of metabolic activation (+S9, 4 hours) and absence of metabolic activation (-S9, 4 and 22 hours).

For each treatment cells were examined for structural and numerical aberrations.

In the 4 h -S9 treatment concentrations of 109.7, 1028.6 and 1800 µg/mL were tested; precipitation at the end of treatment was observed in all concentration groups, with the mitotic index reduced to 55.6 % of control at the maximum concentration. In the 4 h +S9 treatment, concentrations of 0.7, 1.2 and 2.2 µg/mL were tested; precipitation at the end of treatment was observed in the maximum concentration group.

In the 22 h -S9 treatment, concentrations of 35.8, 62.7 and 109.7 µg/mL were tested; toxicity, as evidenced by a mitotic index of 52 % of control, was observed at the maximum concentration tested.

In both experiments, in the absence and presence of S9 mix, no statistically significant or dose related increase in the number of cells carrying structural chromosome aberrations was observed.

Table 6.4.9: Summary of results of the chromosomal aberration study with napropamide-M (Bohnenberger, 2011)

Exp.	Preparation interval	Test item concentration in µg/mL	Mitotic indices in % of control	Aberrant cells in % incl. gaps*	excl. gaps*	carrying exchanges
Exposure period 4 hrs without S9 mix						
I	22 hrs	Solvent control[1]	100.0	1.5	1.5	0.0
		Positive control[2]	95.6	10.0	**10.0**[S]	1.5
		62.7	103.1	0.5	0.5	0.0
		109.7[P]	88.0	1.0	1.0	0.0
		1028.6[P]	72.9	0.5	0.5	0.0
		1800.0[P]	55.6	1.0	0.5	0.0
Exposure period 22 hrs without S9 mix						
II	22 hrs	Solvent control[1]	100.0	1.5	1.0	0.0
		Positive control[3]	58.2	10.0	**9.5**[S]	2.0
		35.8	81.5	1.5	1.0	0.0
		62.7	67.7	1.5	1.5	0.0
		109.7	51.6	1.5	1.5	0.5
		335.9[P]	49.2	1.0	0.5	0.0
Exposure period 4 hrs with S9 mix						
I	22 hrs	Solvent control[1]	100.0	2.0	2.0	0.0
		Positive control[4]	50.8	17.0	**16.0**[S]	2.0
		0.4	88.6	2.0	1.5	0.0
		0.7[P]	86.6	0.5	0.5	0.0
		1.3[P]	103.1	3.5	2.5	0.5
II	22 hrs	Solvent control[1]	100.0	0.0	0.0	0.0
		Positive control[4]	64.9	9.0	**8.5**[S]	2.0
		0.7	87.6	1.0	1.0	0.0
		1.2	108.1	2.0	1.0	0.0
		2.2[P]	94.4	1.0	1.0	0.0

* Including cells carrying exchanges
P Precipitation occurred at the end of treatment
S Aberration frequency statistically significant higher than corresponding control values
1 Acetone 0.5 % (v/v)
2 EMS 825.0 µg/mL
3 EMS 660.0 µg/mL
4 CPA 7.5 µg/mL

It is concluded that napropamide-M did not show an increase in the incidence of chromosomal aberrations in isolated human lymphocytes.

B.6.4.2. *In vivo* studies in somatic cells

B.6.4.2.1. Alkaline *in vivo* Comet Assay

Introduction

Mouse lymphoma *tk* data ([redacted] 2017) confirmed clear concentration-related increases in MF that exceeded the sum of the mean control MF + GEF in the absence of excessive toxicity (RTG 11% to 29% at the concentrations that gave positive responses). These increases in MF were limited to a short-term exposure condition in the presence of a rat liver metabolic activation system (S9). There were increases in both small and large colony mutants, with a greater proportion of small colony mutants seen. The rat liver enzyme fraction S9

appears to have an effect on the test material, as seen by both the marked increase in toxicity in the presence of S9, and the increase in MF. It was therefore concluded that a positive result for napropamide-M in the presence of S9 in this assay system was obtained. To determine if this *in vitro* effect translated to *in vivo* mutagenicity, the applicant conducted an *in vivo* comet assay in rats.

Author(s):	[redacted] (2017)
Study title:	Napropamide-M: Rat Alkaline Comet Assay, [redacted] Unpublished report No.: 8361879
Test substance:	Napropamide-M
Purity:	97.98% (total D- + L-isomer)
Batch no.:	UPH-08 / DNE-263 / Tech / 20160615
Test animals:	Han Wistar rat, 49-56 days of age (weights: 237-260g in the range-finding study and 202-224g in the main experiment).
Dose:	Range-finding test: Animals received two doses at 2000 mg/kg bw/d, separated by 21 h (3 rats/sex) at a dose volume of 10 mL/kg bw Comet assay: Animals received two doses 0, 500, 1000 or 2000 mg/kg bw/d separated by 21 h (6 male rats/group) at a dose volume of 10 mL/kg bw
Vehicle/solvent:	1% methylcellulose in water
Statistics/ measurements:	Tail intensity data were used for statistical analysis.
GLP:	Yes (certified)
Guideline:	OECD 489 (2016)
Deviation:	None
Acceptability:	Acceptable

Methods

Animals were dosed orally *via* gavage with two doses of 0, 500, 1000 and 2000 mg/kg bw/d, administered 21 hours apart.

As no gender differences in the above factors or gender specific exposure have been previously identified testing solely in male animals was conducted.

Animals were killed at 27 hour (6 hours after the final administration, equivalent to the reported T_{max} [6 h], rather than the usual 3 h post the final dose). A positive control was dosed with EMS (150 mg/kg) on day 2, with sacrifice 6 hours after dosing.

Liver tissue was harvested and blood collected *via* the abdominal aorta for clinical chemistry analysis, within 1 hour of sacrifice. Single cell suspensions were prepared by mechanical dissociation. Cell suspensions were held on ice until slide preparation. A range of parameters were measured for liver function (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, total cholesterol, albumin, globulin, albumin:globulin ratio, total bilirubin) and for kidney function (sodium, potassium, calcium, chloride, inorganic phosphorus, creatinine, urea).

Stomach tissue was not harvested. The RMS considers this to be acceptable on the basis that a positive result for napropamide-M in the mammalian assay for gene mutation (Ballantyne, M., 2017) was only seen in the presence of S9.

Scoring
Slides were coded and scored blind using software linked to a fluorescence microscope. 150 cells/animal/tissue were scored for comet analysis, evenly split over three slides. The comet parameters reported were the % tail intensity (i.e. %DNA in the tail) and the Olive tail moment.

Concurrent target organ (liver) exposure was not considered necessary as although the liver was not a first site of contact following dosing via oral gavage, existing ADME data and a 90-day oral (via diet) repeated-dose toxicity study on napropamide-M confirmed the liver as a target organ.

Results

There were no clinical signs of toxicity, effects on body weight or clinical chemistry that could be related to the test article. The occurrence of hedgehogs on comet slides indicated there was no toxicity of the test article and that excessive mechanical disruption had not occurred during single sell preparation. There were no notable macroscopic or microscopic observations.

The median value/slide was calculated and the mean of the slide medians was calculated to give the mean animal value. The mean of the animal means and standard error of the mean was calculated for each group.

Table 6.4.10: Summary of group mean and individual animal liver data, (Tail Intensity and Tail Moment)

Dose level (mg/kg bw/d)	No. of animals	No .of cells scored	Mean tail intensity[a] (% ±SD)	SEM	Mean tail moment[a] (% ±SD)	SEM	Mean % hedgehogs
0	6	900	0.07 ±0.02	0.01	0.01 ±0.00	0.00	0.82
500	6	900	0.12 ±0.06	0.02	0.01 ±0.01	0.00	0.84
1000	6	900	0.07 ±0.05	0.02	0.01 ±0.01	0.00	0.50
2000	6	900	0.10 ±0.06	0.02	0.01 ±0.00	0.00	0.41
EMS, 150	3	450	13.18 ±7.20	0.75	1.54 ±0.85	0.10	1.33

Historical control data ranges for rat liver comet assay Data generated from 62 studies dosed between November 2009 to September 2016				
Vehicle	Mean tail intensity (% ±SD) [No. of animals: 436]		Mean % hedgehogs (±SD) [No. of animals: 412]	
	Mean:	1.20 ±1.59	Mean:	4.66 ±4.00
	Observed range:	0.01 – 12.36	Observed range:	0.00 – 29.08
	95% reference range:	0.04 - 5.50	95% reference range:	0.00 - 14.53
Positive	Mean tail intensity (% ±SD) [No. of animals: 343]		Mean % hedgehogs (±SD) [No. of animals: 331]	
	Mean:	36.41 ±14.72	Mean:	9.10 ±8.09
	Observed range:	8.47 – 77.20	Observed range:	0.00 – 35.90
	95% reference range:	11.98 - 65.88	95% reference range:	0.13 – 28.06

+ve control: EMS – ethyl methylsulphonate
SEM =- standard error mean
a median values of each slide calculated. The mean of the slide medians were calculated to give the individual mean animal value. The individual mean animal values were averaged to provide group means

Conclusions

It is concluded that napropamide-M did not induce DNA damage in the liver of male rats following oral gavage dosing at 0 and 21 hours, with harvesting of tissues 6 hours later (T_{max}). The maximum dose administered was 2000 mg/kg bw/day, which is the recommended maximum dose in the test guideline.

The RMS considers the *in vivo* comet assay to be an appropriate and sensitive follow up *in vivo* assay to assess the biological relevance of positive *in vitro* gene mutation data. This is in accordance with the Scientific Opinion of EFSA (EFSA, 2011[1]) and technical report (EFSA, 2016[2]).

[1] EFSA (2011). European Food Safety Authority. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379

[2] EFSA (2016). European Food Safety Authority. Technical report on the outcome of the pesticide peer review meeting on general recurring issues in mammalian toxicology. EFSA supporting publication 2016:EN-1074

B.6.4.2.2. Mammalian Erythrocyte Micronucleus Test

Introduction

Two *in vivo* mammalian-cell genotoxicity tests with napropamide racemate are available that provide information on the genotoxicity of napropamide-M.

Both studies were previously evaluated by Denmark for the approval of napropamide. Neither the summaries (other than minor amendments to improve readability) nor the conclusions presented below have changed from the previous assessment.

Author(s):	[redacted] (1984b)
Study title:	Mutagenicity evaluation in bone marrow micronucleus.
Test substance:	Napropamide
Purity:	94.6 % (total D- + L-isomer)
Batch no.:	4921-27-24
Test system:	Structural chromosome aberrations in bone marrow cells (RMS notes this is a micronucleus study not a formal chromosome aberration assay)
Test animals:	Male and female CD-1 mice, 6-7 weeks old and weighing 24 – 30 g at study start
Groups:	5/sex/dose group/time point of sacrifice.
Dose:	556, 1667 and 5000 mg/kg bw, two consecutive oral doses, approximately 24 hours apart
Vehicle/solvent:	10 % ethanol/corn oil
Statistics/ measurements:	Kastenbaum-Bowman test for testing no. of micronuclei/animal in test group against solvent control
GLP:	No GLP statement but a QA statement is available
Guideline:	None cited (study followed international testing guidelines such as OECD 474 and US EPA)
Deviation:	Only 1000 PCE per animal were scored for the incidence of micronucleated PCE; the OECD guideline No. 474 recommends a minimum of 2000 PCE/animal The number of micronucleated immature erythrocytes (PCE) was not given separately for each animal. The standard deviation of micronucleated immature erythrocytes was not given for each group mean
Acceptability:	Acceptable

Methods

Groups of CD-1 mice (5/sex/group) received two consecutive oral doses, approximately 24 hours apart, of napropamide (purity 94.6 %, total D- + L-isomer) as a suspension in 10 % ethanol/corn oil at dose levels of 556, 1667 and 5000 mg/kg bw. In an initial range-finding study there was no sign of toxicity at dose up to 5000 mg/kg bw. This level was therefore chosen as the highest dose level.

Negative (vehicle) and positive (cyclophosphamide) control substances were also tested. Bone marrow samples were taken from 5 mice/sex/dose at 24, 48 and 72 hours after administration. The number of micronucleated cells in 1000 polychromatic erythrocytes (PCEs) was scored for each animal and the ratio of PCEs to 1000 erythrocytes was determined.

Results

The positive control, cyclophosphamide, gave a statistically significant increase in micronuclei at the 48 harvest time ($p<0.01$) in both females and males, compared to the concurrent solvent control.

There was no significant increase in micronuclei in males at any dose level at any time point. An increase in micronuclei at the 24-hour harvest time in females was noted compared with solvent controls (not statistically significant). The solvent control value at 24 hours was significantly lower than the control values at the other harvest times and was also lower than historical control values. In addition, there was no evidence of a dose response. Consequently, when compared with pooled solvent controls, there was no significant increase in micronuclei. No increase in micronuclei was observed in females at the 48- and 72-hour harvest times.

Conclusion

Napropamide (94.6 %) was administered to CD-1 mice (5/sex/dose) by gavage in a single dose at 556, 1667 or 5000 mg/kg bw, and cells were harvested at 24, 48 and 72 hours. The study does not fully comply with present guidelines. Only 1000 PCE/animal were scored instead of 2000 PCE/animal. The number of micronucleated immature erythrocytes (PCE) was not given separately for each animal, and the standard deviation of micronucleated immature erythrocytes was not provided. In this case, the deviations were not considered detrimental for the study results, and the study was found acceptable. No toxic effects were reported.

Under the experimental conditions of this study napropamide racemate did not induce micronuclei in bone marrow cells of CD-1 mice *in vivo.*

Although no concurrent bone marrow exposure to napropamide was demonstrated, ADME data presented (see Section B.6.1.1) confirms that, following oral administration, > 90 % of an administered dose of napropamide is absorbed from the GI tract. .The concentration of the administered radioactivity was highest in blood and tissues 6 hours after dosing (the earliest time-point investigated), indicating that oral absorption is rapid; quantifiable radioactivity was also measured in bone marrow, as would be expected, given the highly-perfused nature of this tissue.

The terminal half-life of napropamide-M is greater than that of the positive control used in the two bone marrow assays. Furthermore, the maximum dose tested in this test (5000 mg/kg bw) far exceeded the guideline maximum-recommended dose (2000 mg/kg bw). It is therefore reasonable to conclude that the bone marrow would have been extensively exposed to napropamide.

The RMS (UK) concludes that a valid negative result was obtained in this study.

B.6.4.2.3. Mammalian Erythrocyte Micronucleus Test

This study was previously evaluated by Denmark for the approval of napropamide.

Author(s):	[redacted] (1986)
Study title:	Mutagenicity evaluation in bone marrow micronucleus
Test substance:	Napropamide
Purity:	94.6 % (total D- + L-isomer)
Batch no.:	4921-27-24
Test system:	Structural chromosome aberrations in bone marrow cells (RMS notes this is a micronucleus study not a formal chromosome aberration assay)
Test animals:	Female CD-1 mice, 6 weeks old and weighing about 22 g at study start
Groups:	5/dose group
Dose:	556, 1667 and 5000 mg/kg bw, one single oral dose
Vehicle/solvent:	10 % ethanol/corn oil
Statistics/ measurements:	Kastenbaum-Bowman test for testing no. of micronuclei/animal in test group against solvent control
GLP:	No GLP statement but a QA statement is available
Guideline:	None cited (study followed international testing guidelines such as OECD 474 and US EPA)
Deviation:	Only 1000 PCE per animal were scored for the incidence of micronucleated PCE; the OECD guideline No. 474 recommends a minimum of 2000 PCE/animal. The number of micronucleated immature erythrocytes (PCE) was not given separately for each animal. The standard deviation of micronucleated immature erythrocytes was not given for each group mean.
Acceptability:	Acceptable

Methods

This study was conducted to confirm the interpretation of the results of the original study ([redacted], 1984b) reported above. Groups of female CD-1 mice (5/group) received a single oral dose of napropamide (purity

94.6 %, total D- + L-isomer), as a suspension in 10 % ethanol/corn oil at dose levels of 556, 1667 and 5000 mg/kg bw. This time, the animals were fasted 16-20 hours before dosing.

Negative (vehicle) and positive (cyclophosphamide) control substances were also tested. Animals were sacrificed approx. 24 hours after the administration, and bone marrow was harvested and evaluated. The number of micronucleated cells in 1000 polychromatic erythrocytes (PCEs) was scored for each animal and the ratio of PCEs to 1000 erythrocytes was determined.

Results

Two females at 5000 mg/kg bw died. There was no significant increase in micronuclei in females at any dose level at any time point compared with controls. One control animal had a higher incidence of micronuclei, thus inflating the control levels. With this animal excluded from the mean levels, the control levels are more within historical limits. The positive control, cyclophosphamide, gave a statistically significant increase in micronuclei ($p<0.01$) in both females and males, compared with the concurrent solvent control.

Conclusion

The study was found acceptable as a supplement to the aforementioned study.

Under the experimental conditions of this study napropamide racemate did not induce micronuclei in bone marrow cells of female CD-1 mice *in vivo*. The highest dose administered, 5000 mg/kg bw, far exceeded the guideline recommended maximum of 2000 mg/kg bw. Moreover, two animals at this dose died, indicating that systemic exposure occurred. The ADME data (section B.6.1.1.) demonstrate that napropamide is extensively (> 90 %) and rapidly absorbed following oral administration, with distribution to the blood / plasma and bone marrow.

The RMS (UK) concludes that a valid negative result was obtained in this study.

B.6.4.3. *In vivo* studies in germ cells

Studies to detect the potential of napropamide-M to cause chromosome damage in mammalian germ cells have not been conducted. Because the *in vivo* studies in somatic cells are negative, it is not necessary to conduct *in vivo* studies in germ cells.

B.6.4.4. Overall summary of genotoxicity

Four *in vitro* and one *in vivo* studies conducted on napropamide-M were submitted for the purpose of this approval. Supplementary information was provided by *in vitro* and *in vivo* studies conducted on napropamide racemate and previously evaluated for the approval of that active substance.

The *in vitro* bacterial gene mutation study on napropamide-M confirmed a lack of any gene mutation potential in bacteria when using the plate incorporation methodology (when tested up to a suitable maximum concentration). A recent mammalian gene mutation study on napropamide-M revealed an equivocal result in mouse lymphoma cells in the presence of metabolic activation, whilst a clearly negative result was obtained without metabolic activation. A repeat of this test, conducted in accordance with the current (2016) OECD 490 guideline, confirmed that napropamide-M induced mutation at the tk locus in the presence of a metabolic activation. Both large (indicating point mutations) and small (indicating chromosomal damage) colonies were increased, although the proportion of small colonies was increased compared with the solvent control cultures. Two mammalian gene mutation studies performed with napropamide (and evaluated for the approval of napropamide) both also gave positive results with metabolic activation (information on colony size not available). It is therefore concluded that napropamide-M is genotoxic in the *in vitro* mammalian cell gene mutation test.

Napropamide-M was negative in an *in vitro* chromosome aberration assay when tested up to an appropriate maximum concentration.

No increase in bone marrow micronucleus frequency was observed in two independent *in vivo* mouse bone marrow micronucleus studies conducted on napropamide technical (racemate), following oral administration of doses well in excess of the limit dose. ADME data (see Section B.6.1.1) confirms that, following oral administration, napropamide-M is rapidly absorbed from the GI tract; the concentration of the administered

radioactivity was highest in blood and tissues 6 hours after dosing. Quantifiable radioactivity was measured in bone marrow in rats dosed with napropamide racemate. It is therefore reasonable to conclude that the bone marrow would have been extensively exposed to napropamide. The RMS considers these two studies to give valid negative results.

The biological relevance of the increases in mutant frequency observed in the *in vitro* mammalian gene mutation assay was investigated in an *in vivo* liver comet assay conducted with napropamide-M. Under the conditions of this comet assay, two oral administrations of napropamide-M up to the recommended maximum dose did not induce DNA damage in the liver of male rats.

In summary, napropamide-M was positive in an *in vitro* mammalian cell gene mutation test in the presence of S9. The proportion of small to large colonies was approximately 50 %, which was an increase in small colonies compared with the solvent control. Reassurance that napropamide-M was not clastogenic was provided by a negative *in vitro* chromosome aberration test conducted with this test material, and by two negative *in vivo* micronucleus tests conducted with the racemate. A new *in vivo* comet assay conducted with napropamide-M demonstrated that the active substance was not mutagenic *in vivo* when tested at doses up to the maximum recommended.

Both the mutagenic and clastogenic potential of napropamide-M (supplemented with information on the racemate) have been adequately investigated. The overall conclusion is that napropamide-M is not genotoxic *in vivo*.

According to the criteria of Regulation 1272/2008, no classification is warranted with respect to germ cell mutagenicity.

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

Introduction

The following long-term and carcinogenicity studies are available on napropamide racemate.

Rat:
Two 2-year chronic toxicity studies

Mouse:
2-year chronic toxicity study
18-month dietary mouse oncogenicity study

The studies were previously evaluated by Denmark for the approval of napropamide. The summaries of these studies presented below have not been changed (other than minor amendments to improve readability) and the conclusions have not changed from those described in the EFSA Conclusion (EFSA, 2010).

B.6.5.1. 24 month chronic toxicity and oncogenicity dietary study in rats

Introduction

There are two chronic toxicity studies on the rat conducted on napropamide racemate, both of which were previously evaluated by Denmark for the approval of napropamide. The summary presented below has been amended to improve readability, include additional data (tables) and discuss some of the findings. The conclusion has not changed.

Author(s):	[redacted], 1991a
Study title:	Two-year chronic toxicity/oncogenicity study with R-7465 in rats.
Test substance:	Napropamide racemate
Purity:	94.1 % (total D- + L-isomer)
Batch no.:	Lot no. EHC-0952-17/WRC-4921-27-27
Test animals:	Male and female [redacted]: CD® (SD) BR rats
Groups:	60-70/sex/dose group. Satellite group 20/sex.
Dose:	0, 250, 1100 and 5000 ppm + satellite group 10,000 ppm.
Vehicle/solvent:	None. Test article was admixed with diet.
Route:	Oral via diet
Statistics/ measurements:	Yes. One way analysis of variance and post-hoc test (Dunnett's test) on quantitative continuous variables. Statistical significance of differences in incidence of clinical observations or necropsy findings at 95 % or 99 % confidence level by non-parametric statistics (Fisher's exact test).
GLP:	Yes. US EPA FIFRA (40 CFR, part 160)
Guideline:	OECD 453 "combined chronic toxicity/carcinogenicity studies" (1981); method B.33 (88/302/EEC) and US FIFRA guideline 83-5
Deviation:	None
Acceptability:	Acceptable

Methods

Three groups of Sprague-Dawley rats (60/sex/group) were administered napropamide (purity 94.1 %, total D- + L-isomer) in the diet for up to 24 months at constant concentrations of 250, 1100 and 5000 ppm. A control group consisting of 70 animals per sex received basal laboratory diet. In addition, a high-dose satellite group of 20 animals/sex was administered napropamide racemate at a dietary concentration of 10,000 ppm for 12 months. After 12 months of exposure, 20 animals from the control and all animals in the 10,000 ppm groups and 10 animals from each of the remaining 3 groups were sacrificed for interim evaluation. The dose levels were selected from a 4-week range-finding study.

All animals were observed twice daily for general appearance, behaviour, signs of toxicity and mortality. A detailed physical examination was conducted on a weekly basis. Individual body weights and food consumption

were measured weekly for the first 13/14 weeks, and at least every 4 weeks thereafter until study termination. Body weights were also recorded immediately prior to 12- and 24-month scheduled sacrifices. Ophthalmological examinations were conducted on all animals from all groups at study initiation and on all animals scheduled for the 12-month sacrifice and all surviving control and 5000 ppm animals.

Haematology tests were performed at 3, 6, 12, 18 and 24 months on 20 rats/sex/dose level. Clinical chemistry and urinalysis were performed at the same time points on 10 rats/sex/dose level. Gross pathological examination was performed on all animals that died or were sacrificed in extremis, on 10 animals/sex/group during the 12-month sacrifice and all remaining surviving animals at study termination. Organs were weighed and tissues were preserved for histopathological examination. Histopathological examination was performed on tissues/organs collected from all animals sacrificed at 12 months, at study termination and those sacrificed moribundly.

Analyses for homogeneity, stability and confirmation of dose concentration indicated that the achieved concentrations were within acceptable limits and that the stability of napropamide in the diet was satisfactory.

Results

The high-dose satellite group (10,000 ppm) was terminated at week 49. This dose-group was specifically designated as a satellite group for the evaluation of pathology other than neoplasia and the study protocol specified that the group would be terminated after 12 months.

The compound consumption was calculated on the basis of food consumption and body weight data. The corresponding compound consumption was 10.48/12.28, 47.56/55.31, 221.44/260.81 and 521.57/583.82 mg/kg bw/day for 250, 1100, 5000 and 10,000 ppm groups, males/females, respectively.

Mortality, body weight and general observations
Exposure to napropamide did not adversely affect survival of male or female rats in any dose group. Median survival times associated with unscheduled deaths for 0, 250, 1100 and 5000 ppm males were 621, 616, 568 and 603 days, respectively. Corresponding median survival times in the female dose groups were 620, 628, 642 and 653 days. Survival percentages at term were 24 %, 20 %, 34 % and 32 % for males and 40 %, 44 %, 48 % and 58 % in females. It is noted that, in some groups, survival at the end of the study was below the normally accepted level of 50 %; this was due to a high level of mortality during the final 12 weeks of the study. Survival up to week 90 was approximately in excess of 50 % in all treated groups and therefore it can be concluded that the study is adequate to investigate carcinogenic potential.

In females, an increase in the incidence of generalised pallor (3/70 at 0 ppm, 7/60 at 250 ppm, 9/60 at 1100 ppm and 6/60 ppm at 5000 ppm) was reported during the last trimester of the study (no observations at this time period for the 10,000 ppm dose group). No further clinical observations suggestive of a relationship to treatment with napropamide were found. Given this was an isolated clinical observation, only seen in females and there was no clear dose response, the RMS (UK) does not consider this to be treatment related.

Body weights (BW, see Table 6.5.1) and body weight gains (BWG, see Table 6.5.2) were statistically significantly decreased at 1100, 5000 and 10,000 ppm during most of the study period. The effects were more severe in females.

It is noted that, at week 0, the mean body weights of males in the 1100 and 5000 ppm dose groups were marginally (≤ 4 %) lower than the control group (statistically significant at the $p < 0.05$ and $p < 0.01$ confidence interval for the 1100 and 5000 ppm dose groups, respectively). For the 1100 ppm dose group, by week 13 the means weights of animals were consistently close to the weights of the animals in the control group (no differences of statistical significance in any of the results from week 13 to termination). The differences at week 0 (for both the 1100 and 5000 ppm dose groups) appear to be a statistical anomaly that does not impact on the validity of the results.

Reductions of BW in the 10,000 ppm group were statistically significant in both sexes throughout the dosing period. At 5000 ppm, BW was statistically significantly reduced through the whole study period in females and in males in all weeks except weeks 49 to 61 and 97 to 101. In the 1100 ppm group, the effect on BW was statistically significant in males until week 12 and in females until week 57, and again at week 97. The reductions in BW in females at 1100 ppm were between 5 % and 13 %, at the end of the study.

Body weight gains (BWG) were statistically significantly reduced during the whole dosing period at 10,000 ppm in both sexes and at 5000 ppm in females, while the reduction in males of the 5000 ppm group was statistically significant until week 45. In the 1100 ppm group, BWG of males were reduced during the first trimester, with

values sporadically reaching statistical significance, while the BWG in females were statistically significantly reduced until week 53 and at week 97. The reductions in BWG in females at 1100 ppm were generally 4 - 10 %, but reached 15 % at the end of the study.

Table 6.5.1: Mean animal body weights (g) - males and females
Dose (units): ppm

	Week		Dose				
			0	250	1100	5000	10000
Males	**0**	mean	194	191	188 *	187 **	188
		s.d.	10.6	11.6	12.5	11.2	10.3
		change (%)	-	-2%	-3%	-4%	-3%
	8	mean	502	496	480 **	462 **	442 **
		s.d.	42.5	34.9	44.9	35.0	24.0
		change (%)	-	-1%	-4%	-8%	-12%
	13	mean	568	573	549	535 **	494 **
		s.d.	53.0	41.1	51.9	40.7	27.5
		change (%)	-	1%	-3%	-6%	-13%
	49	mean	758	774	746	729	678 **
		s.d.	80.0	72.8	98.7	71.2	50.3
		change (%)	-	2%	-2%	-4%	-11%
	101	mean	798	703	810	710	n.d.
		s.d.	126.4	136.6	167.7	78.5	-
		change (%)	-	-12%	2%	-11%	-
Females	**0**	mean	166	167	164	161	162
		s.d.	11.8	13.4	12.0	11.6	10.1
		change (%)	-	1%	-1%	-3%	-2%
	8	mean	286	287	273 **	247 **	243 **
		s.d.	26.4	25.3	23.5	17.3	19.5
		change (%)	-	0%	-5%	-14%	-15%
	13	mean	311	306	294 **	265 **	259 **
		s.d.	29.7	26.1	27.8	18.1	20.7
		change (%)	-	-2%	-5%	-15%	-17%
	49	mean	448	442	425	341 **	322 **
		s.d.	62.1	54.4	69.6	40.0	33.6
		change (%)	-	-1%	-5%	-24%	-28%
	105	mean	534	546	463	406 **	n.d.
		s.d.	130.4	145.0	114.4	90.3	-
		change (%)	-	2%	-13%	-24%	-

n.d.: not determined - 10,000 ppm dose group study terminated at week 49

*: significantly different from control using Dunnett's test, $p < 0.05$

[**: significantly different from control using Dunnett's test, $p < 0.01$

Table 6.5.2: Group mean cumulative body weight gain (g) - males and females
Dose (units): ppm

	Week		Dose				
			0	250	1100	5000	10000
Males	**1**	mean	61	61	60	58 *	44 **
		s.d.	5.6	6.8	7.7	6.5	8.1
		change (%)	-	0%	-2%	-5%	-28%
	8	mean	307	305	291 *	275 **	254 **
		s.d.	38.1	32.1	39.3	30.5	19.3
		change (%)	-	-1%	-5%	-10%	-17%
	13	mean	374	382	360	348 **	309 **
		s.d.	49.2	39.1	46.3	36.8	20.4
		change (%)	-	2%	-4%	-7%	-17%
	49	mean	564	584	558	542	491 **
		s.d.	78.4	69.6	93.6	69.3	48.9
		change (%)	-	4%	-1%	-4%	-13%
	101	mean	604	514	622	525	n.d.
		s.d.	122.7	138.2	157.0	77.1	-
		change (%)	-	-15%	3%	-13%	-
Females	**1**	mean	25	26	24	18 **	17 **
		s.d.	6.3	4.9	5.6	4.2	4.8
		change (%)	-	4%	-4%	-28%	-32%
	8	mean	120	120	110 **	86 **	82 **
		s.d.	20.1	17.6	16.0	11.4	14.1
		change (%)	-	0%	-8%	-28%	-32%
	13	mean	145	139	130 **	104 **	97 **
		s.d.	24.4	20.1	20.5	13.0	14.8
		change (%)	-	-4%	-10%	-28%	-33%
	49	mean	282	274	261	180 **	160 **
		s.d.	56.5	50.2	63.5	36.5	28.4
		change (%)	-	-3%	-7%	-36%	-43%
	105	mean	369	383	312	244 **	n.d.
		s.d.	126.4	138.3	104.4	89.5	-
		change (%)	-	4%	-15%	-34%	-

n.d.: not determined - 10,000 ppm dose group study terminated at week 49

*: significantly different from control using Dunnett's test, $p < 0.05$

**: significantly different from control using Dunnett's test, $p < 0.01$

Table 6.5.3: Mean animal food consumption (g/day) - males and females
Dose (units): ppm

	Week		Dose				
			0	250	1100	5000	10000
Males	**1**	mean	23	23	23	22	22
		s.d.	1.9	1.7	2.2	2.1	3.9
		change (%)	-	0%	0%	-4%	-4%
	8	mean	27	26	26	25 **	24 **
		s.d.	2.9	2.6	3.0	2.5	1.6
		change (%)	-	-4%	0%	-7%	-11%
	13	mean	26	26	26	26	23 **
		s.d.	2.6	2.0	2.3	2.2	2.9
		change (%)	-	0%	0%	0%	-12%
	49	mean	27	26	26	26 *	24 **
		s.d.	3.6	3.0	3.3	2.8	1.9
		change (%)	-	-4%	-4%	-4%	-11%
	101	mean	23	19	26	24	n.d.
		s.d.	8.9	9.2	3.8	7.2	-
		change (%)	-	-17%	13%	4%	-
Females	**1**	mean	17	17	16	15 **	15 **
		s.d.	1.8	1.9	2.7	2.7	2.9
		change (%)	-	0%	-6%	-12%	-12%
	8	mean	18	18	17	16 **	15 **
		s.d.	2.2	1.8	1.9	1.9	1.6
		change (%)	-	0%	-6%	-11%	-17%
	13	mean	17	17	18	16 **	15 **
		s.d.	2.1	1.8	2.5	2.2	1.3
		change (%)	-	0%	6%	-6%	-12%
	49	mean	18	18	18	16 **	16 **
		s.d.	2.5	1.8	2.4	1.8	2.5
		change (%)	-	0%	0%	-11%	-11%
	105	mean	18	18	18	16	n.d.
		s.d.	4.7	7.3	4.3	6.4	-
		change (%)	-	0%	0%	-11%	-

n.d.: not determined - 10,000 ppm dose group study terminated at week 49
*: significantly different from control using Dunnett's test, $p < 0.05$
**: significantly different from control using Dunnett's test, $p < 0.01$

Food consumption was significantly reduced in males fed diets containing 5000 ppm and in males fed 10,000 ppm throughout the first 21 weeks of the study and sporadically thereafter, see Table 6.5.3.

Haematology, clinical chemistry and urinalysis
Statistically significant decreases in haemoglobin and haematocrit as well as increases in platelet counts were noted at various intervals in males and females of the 5000 and/or 10,000 ppm groups. In males mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations were affected at a few, sporadic time points at the 5000 and 10,000 ppm levels. Selected haematological parameters are shown in Table 6.5.4.

Table 6.5.4 Haematological parameters in 2-year rat study with napropamide

		Doses (ppm)									
		Males					Females				
	Month	0	250	1100	5000	10000	0	250	1100	5000	10000
Haemoglobin (g/dl)	03	16.7	16.9	16.6	16.4	16.0	16.7	16.7	16.5	**16.0****	**15.5****
	06	16.0	16.0	15.8	15.8	**15.1***	15.9	15.8	15.6	**15.4***	**14.8****
	12[a]	15.5	15.9	15.8	15.1	14.9	15.4	15.3	15.0	**14.4***	**13.9****
	12[b]	14.9	14.1	14.5	14.2	13.8	14.4	14.3	14.0	13.8	**13.2****
	18	15.3	15.6	15.1	**14,4***		15.0	14.8	15.0	**14.3***	
	24	14.1	13.0	13.4	13.2		13.2	13.0	13.0	12.2	
Haematocrit %	03	50.3	50.4	49.6	48.8	48.3	49.4	49.6	48.8	**47.9***	**46.4****
	06	48.7	48.6	48.1	48.1	47.4	46.0	46.0	45.3	44.8	**43.6****
	12[a]	47.5	48.5	47.9	46.2	45.7	46.6	46.4	45.2	43.7	**42.7****
	12[b]	44.1	42.1	43.2	41.7	40.8	42.2	42.1	41.8	40.8	**38.4****
	18	48.3	49.3	47.9	46.4		45.8	45.1	46.5	44.5	
	24	41.6	38.7	39.7	38.7		37.9	36.7	36.7	34.6	
Platelet (x1000/µl)	03	1102	1045	1057	1132	1164	965	1022	1058	**1172****	**1216****
	06	1120	1079	1081	1107	1209	916	961	968	**1042****	**1125****
	12[a]	1129	1041	1004	1271	1107	814	833	**970***	**956***	**1049****
	12[b]	1022	1136	1049	1049	1123	856	878	861	944	948
	18	1123	1116	1089	1171		862	909	**977****	**1020****	
	24	1076	1357	1195	1185		883	898	883	990	

[a] Blood obtained via the orbital sinus
[b] Blood obtained via the abdominal aorta
* = statistically significant difference ($p < 0.05$)
** = statistically significant difference ($p < 0.01$)

Statistically significant increases in gamma-glutamyl transferase activity were noted in males and females in the 5000 and/or 10,000 ppm groups at 6, 12, 18 and 24 months. No other changes in clinical chemistry parameters were considered treatment related as they were small, and no dose-relationship was seen.

Statistically significant increases were observed in urine volume in the 10,000 ppm males at 6 and 12 months, in the 250 ppm males at 24 months and in the 10,000 ppm females at 12 months. Specific gravity decreased significantly in females at 5000 and 10,000 ppm at the 12 months' interim sacrifice. These data are suggestive of a mild diuretic effect of napropamide.

Ophthalmology
There were no treatment-related ophthalmological findings.

Organ weight

12-month sacrifice:
Absolute and relative liver weights were increased (statistically significant) in males of the 5000 and 10,000 ppm dose groups. Relative liver weights were increased significantly in females at those doses. Liver to brain weight ratios were increased in females at 10,000 ppm.
Relative kidney weights were increased significantly in males at 10,000 ppm and in females at 5000 and 10,000 ppm.
In females, the relative brain weights were increased significantly at 5000 and at 10,000 ppm, while the relative adrenal weights were also increased at 10,000 ppm.

24-month sacrifice:
Relative liver weights were increased statistically significantly in females at 5000 ppm.
Statistically significant increases in relative kidney weight were observed in males at 250 and at 5000 ppm and in females at 5000 ppm.
The relative adrenal weight and relative brain weight were also increased statistically in females at 5000 ppm.

Gross pathology

At the interim sacrifice at 12 months, discoloured foci were observed in the liver of males treated with 1100, 5000 and 10,000 ppm, with the incidence reaching statistical significance in the high dose group.
At terminal sacrifice, the liver foci were observed in males of all dose groups and controls, the increased incidence reaching statistical significance at 5000 ppm. Kidney cysts were also observed in males of all groups including controls, the finding reaching significance at 5000 ppm but not at 10,000 ppm.
In females, emaciation was observed in all dose groups and controls in a dose-related way, with statistical significance being reached at 5000 ppm. No other statistically significant macroscopic lesions were observed in either males or females.

Histopathology and neoplastic changes
Histopathological examination is reported separately, in the report by Hodge, 1993 (see Section B.6.5.1.1).

Conclusion

This 2 year chronic toxicity/carcinogenicity study of napropamide following dietary administration in rats was conducted according to GLP and to OECD guidelines, and is thus acceptable to cover this end-point. However, the separate report ([redacted], 1993) on histopathological findings lacks reporting of statistics (see Section B.6.5.1.1).

Animals were administered napropamide racemate at 0, 50, 250, 1,100, 5,000 and 10,000 ppm. The 10,000 ppm dose group study was terminated at week 49.

The mortality of control and treated animals was high and survival was below the normally accepted level of 50 %; this was due to a high a level of mortality during the final 12 weeks of the study. Survival up to week 90 was approximately in excess of 50 % in all treated groups and therefore it can be concluded that the study is adequate to investigate carcinogenic potential. The study shows statistically significant depression of body weights and body weight gains at 1100, 5000 and 10,000 ppm in both sexes, with reductions in the 1100 ppm group females of 5-13 % for BW and 4-15 % for BWG.

Food consumption was significantly decreased at 5000 and 10,000 ppm. At 5000 and 10,000 ppm, statistically significant changes in haematological and clinical chemistry parameters showed slight anaemia, small changes in the liver enzyme gamma-glutamyl transferase activity and mild diuretic effect of napropamide. Although there were statistically significant changes in relative organ weights (liver, kidney and brain), these were attributed to significant decreases in body weights of these animals. Findings at gross necropsy included foci of discoloration of the liver and kidney cysts at 10,000 ppm. A NOAEL of 250 ppm (10.48/12.28 mg/kg bw/day in males and females, respectively) is set based on the effects on body weights and food consumption from 1100 ppm.
The histopathological findings are discussed under Section B.6.5.1.1.

B.6.5.1.1. Histopathological report on 24 month chronic toxicity/ oncogenicity dietary study in rats

Introduction

This study is a supplement to [redacted] 1991a.

Author(s):	[redacted] 1993
Study title:	Two-year chronic toxicity/oncogenicity study with R-7465 in rats. Supplement to T-13276, histopathology report and overall study discussion
Test substance:	Napropamide racemate
Purity:	94.1 % (total D- + L-isomer)
Batch no.:	Lot no. EHC-0952-17/WRC-4921-27-27
Test animals:	Male and female [redacted]: CD® (SD) BR rats
Groups:	60-70/sex/dose group. Satellite group 20/sex.
Dose:	0, 250, 1100 and 5000 ppm + satellite group 10,000 ppm.
Vehicle/solvent:	None. Test article was admixed with diet.
Route:	Oral via diet
Statistics/ measurements:	The author of the report states that intergroup comparison of tumour incidence by Fisher's exact test and test for trend using the Cochran-Armitage Test described by Gart et al, 1986 were performed but statistical significance of the findings is not clearly reported in the body of the report and not at all reported in the tables. Level of statistical significance provided in the review process.
GLP:	Yes. US EPA FIFRA (40 CFR, part 160)
Guideline:	OECD 453 "combined chronic toxicity/carcinogenicity studies" (1981); method B. 33 (88/302/EEC) and US FIFRA guideline 83-5
Deviation:	None
Acceptability:	Acceptable

Methods

This report only contains the results of the histopathological examination performed in the study by Pettersen and Walberg, 1991a. The overall method is described in Section B.6.5.1. Histopathological examination of tissues/organs was performed on tissues/organs collected from all animals sacrificed at 12 months and at study termination and those sacrificed moribundly. The following tissues/organs collected and preserved from the above-referenced study were examined microscopically: adrenal gland, bone (femur including knee), bone marrow (femur and sternum), brain, caecum, cervix, colon, duodenum, epididymis, eye, Harderian gland, heart, ileum, jejunum, kidney, liver, lung, lymph node-mesenteric, mammary gland (females), nasal passages, oesophagus, ovary, pancreas, pituitary gland, prostate with coagulation gland, rectum, salivary gland, sciatic nerve, seminal vesicle, skin, spinal cord, spleen, sternum, stomach, testis, thymus, thyroid/parathyroid gland, trachea, urinary bladder, uterus, vagina, voluntary muscle, and any macroscopically-observed abnormal tissue.

Results

Only histopathological results are presented in this summary. The main reporting can be found under Section B.6.5.1.

The study report, although stating that statistical analyses were performed, does not indicate the statistical significance level of the findings in the reporting or discussion of the results or in the tables. Additional data on statistical evaluation of tumour finding was provided by the applicant to the RMS.

12-month sacrifice

Non-neoplastic findings: In females, the incidence of vascular ectasia of the adrenal gland was higher in the treated groups than in the controls (1/20, severity defined as 'slight') but the statistical significance level was not stated. The effect was predominantly of 'minimal' severity (9/20) or 'slight' (2/20) in the 10 000 ppm dose group. Given that the effect was also seen, though in a smaller proportion of animals, in the other dose groups: 'slight' or 'moderate' (4/10) in the 250 ppm group, 'slight' (3/10) in the 1100 ppm group and 'minimal' (3/10) in the 5000 ppm dose group, no clear dose-response was established. Increased incidences of liver effects including cholangitis, hepatocyte fat vacuolation and spongiosis hepatis were observed (statistical significance levels not stated) in males of the treated groups (all dose groups) compared to controls.

Neoplastic findings: The distribution of small numbers of neoplasms between control and the 10,000 ppm group did not indicate compound-related effects.

24-month sacrifice

Non-neoplastic findings: The incidence of chronic progressive glomerulonephropathy was high in all treated and control animals, and was more severe in males than in females. The severity was increased in 5000 ppm males compared to controls although the incidence in the 5000 ppm males was slightly lower than in the controls (statistical significance levels not stated). In the liver spongiosis hepatis was observed at dose levels of ≥1,100 ppm; this occurred without a clear dose response and was of low incidence (statistical significance level not stated) and is not considered by the RMS (UK) to be treatment related.

All other changes were considered typical of aging rats of the [redacted]:CD (SD) BR strain and there was no evidence that the incidence and severity of these changes were treatment related.

Neoplastic findings:

Only benign pheochromocytomas of the adrenals in females (at 5000 ppm only) achieved statistical significance compared with the control group. The incidence of this tumour type in females was 0/70, 1/60, 1/60 and 4/60, respectively, for the control, 250, 1100 and 5000 ppm group at termination of the study; at all dose levels at 12 months the findings were not of note. The incidence of benign pheochromocytomas in females of the 5000 ppm group was significant with p=0.021. The incidence of this tumour type was within the laboratory historical control range (12 studies conducted from 1980 to 1988, so concurrent with present study) of 0-17.5 %.

The incidences of mammary adenocarcinomas in females and squamous papillomas of skin in males were slightly higher in treated groups than in the control group. Mammary adenocarcinomas were seen in females with the following frequencies: 0, 1, 2, 3 at 0, 250, 1100 and 5000 ppm, respectively. Squamous papillomas (benign) of skin were seen in males with the following frequencies: 0, 4, 4, 2 at 0, 250, 1100 and 5000 ppm, respectively, and so without a dose-response relationship. Squamous carcinoma (malignant) of skin was also observed in 0, 0, 1, 3 males at 0, 250, 1100 and 5000 ppm, respectively. None of these findings were of statistical significance.

Prostate carcinoma and brain astrocytoma were seen in males with the following frequencies: 0, 0, 0, 2 and 0, 0, 1, 2 in 0, 250, 1100 and 5000 ppm, respectively. These finding were not of statistical significance.

As stated above the only historical control data presented in the study report are for benign pheochromocytomas of the adrenals in females

Conclusion

This study report on the histopathological findings in the 2-year study in rats by [redacted], 1991a was acceptable after additional information on statistical significance of the incidences was submitted.

As was noted in the evaluation of the main study (see section B.6.5.1), in some groups, survival at the end of the study was below the normally accepted level of 50 %. Survival up to week 90 was approximately in excess of 50 % in all treated groups and therefore it was concluded that the study was adequate to investigate the carcinogenic potential of napropamide.

The histopathological report showed increased severity of chronic progressive glomerulonephritis in males treated with 5000 ppm compared to controls. In the liver, the incidence of spongiosis hepatitis was higher in the 5000 ppm males than in the controls.

When considering the histopathological findings separately, the target organ is considered to be the kidney with a NOAEL of 1100 ppm (corresponding to 47.56/55.31 mg/kg bw/day in males and females, respectively). However, the overall NOAEL following chronic exposure of rats was established to be 250 ppm (corresponding to 10.48/12.28 mg/kg bw/day in males and females, respectively), based on effects reported in the main study on body weights and food consumption.

The only neoplastic finding that reached statistical significance was benign pheochromocytomas of the adrenals in females in the high-dose group. This finding showed no clear dose-response relationship and occurred at low incidences that were well within the relevant historical control range. The RMS also notes that the maximum tolerated dose was exceeded at 5000 ppm (583.82 mg/kg bw/d in females), as indicated by decreases in body-weight in excess of 10 % at the termination of the study (-24 % in females); females of the treatment groups were reported to be emaciated, a finding that was especially pronounced at 5000 ppm. Toxicity in this group was thus excessive. Overall, the RMS concludes that napropamide racemate was not carcinogenic in this two-year oral rat study. No concerns for carcinogenicity were identified from this study during the review of napropamide for approval.

B.6.5.2. 24 month chronic toxicity and oncogenicity dietary study in rats

Introduction

This study on napropamide racemate was previously evaluated by Denmark for the approval of napropamide. The summary and the conclusion have not changed, other than minor amendments to improve readability.

Author(s):	[redacted] 1978
Study title:	24-month chronic feeding study in rats - Devrinol.
Test substance:	Napropamide racemate
Purity:	94.6 % (total D- + L-isomer)
Batch no.:	WRC-4059-17-1
Test animals:	Male and female Sprague-Dawley rats
Groups:	60/sex/dose group.
Dose:	0, 10, 30 and 100 mg/kg bw/day
Vehicle/solvent:	Premixed with acetone (2 %) then admixed with diet.
Route:	Oral via diet
Statistics/ measurements:	Yes. Difference between control and treated groups were evaluated per sex at the 5 % probability level.
GLP:	No. The study was performed prior to GLP
Guideline:	None cited (the study design followed guidelines similar to US EPA Guideline 83-5, OECD 453 "combined chronic toxicity/carcinogenicity studies" (1981); method B. 33 (88/302/EEC))
Deviation:	None
Acceptability:	Acceptable

Methods

Three groups of Sprague Dawley rats (60/sex/group) were administered napropamide (purity 94.6 %, total D- + L-isomer) in the diet for 24 months at 10, 30 and 100 mg/kg bw/day. A fourth group of 60 rats/sex received basal laboratory diet and served as a control group. Ten rats/sex/group were sacrificed during week 53.

All animals were observed daily for mortality and signs of morbidity. Individual body weights and feed consumption were recorded weekly for the first 13 weeks, every 4 weeks through week 101, and at week 104. Observations for gross signs of toxicity and pharmacological effects were recorded at the same intervals. The incidence, size, and location of palpable nodules and masses were recorded weekly.

Clinical chemistry, haematology and urinalysis parameters were evaluated for 10 animals/sex/group at weeks 13, 26, 53, 78 and at study termination. In addition, erythrocyte and plasma cholinesterase activities were measured at weeks 2, 4, 13, 26, 53, 78 and at study termination for 10 animals/sex/group. Brain cholinesterase activity was measured for 10 animals/sex/group at week 53 and at study termination.

Gross pathological examination was performed on all animals that died or were sacrificed *in extremis*, on 10 animals/sex/group during week 53, and on all remaining surviving animals at study termination. Organ weights were recorded and tissues were preserved for histopathological examination. Histopathological examination of tissues/organs was performed for all control and high-dose animals sacrificed at 53 weeks and at study termination and those that were sacrificed moribundly. Kidney, liver and gross lesions were examined for the low- and mid-dose group animals.

Results

Dose concentration analyses indicated mean group analytical values were minimally to moderately lower than the respective calculated values. The fluctuations between the calculated and analytical results were attributed to variability in analytical and mixing procedures and not to the test material instability in the diet.

Mortality, body weight and general observations

There were no statistically significant differences in the mortality rates in any of the treated groups when compared to the controls. Mortality data were the following: 17, 10, 18 and 19 in males and 18, 12, 17 and 21 in females for the 0, 10, 30 and 100 mg/kg bw/day groups, respectively. The corresponding percentages were 34, 17, 34 and 32 in males and 34, 20, 32 and 36 in females. The high mortality in all groups is probably related to genetic drift of this rat strain resulting in overweight animals and early death. Clinical signs were observed with generally comparable frequency between control and treated groups and included the following: body sores, reddish crusts around the eyes and nose, lacrimation, stains on fur, soft faeces, rough hair coat, urine stains, thin and/or hunched appearance.
Mean body weights for females receiving 100 mg/kg bw/day were statistically significantly lower than that of the controls at interim as well as at terminal sacrifice. No treatment-related effects on body weights were seen in males or in females receiving lower doses. Total food consumption for females treated with 100 mg/kg bw/day was statistically significantly lower than the controls through weeks 25 and 49. The food consumption of treated males and lower group females was decreased but did not achieve statistical significance. No notable differences in feed consumption were noted during the second year of the study.

Haematology, clinical chemistry and urinalysis
There were no treatment-related effects.

Table 6.5.5 Percentage difference from controls in erythrocyte cholinesterase values

		Dose (mg/kg bw/day)			
		0	**10**	**30**	**100**
Males	**Week 2**	0.0 %	**17.6 %***	+11.8	+14.4
	Week 4	0.0 %	-12.9 %	- 11.8 %	-9.2 %
	Week 13	0.0 %	-2.4 %	-14.6 %	**-23.5 %***
	Week 26	0.0 %	**+15.5 %** *	**+21.6 %***	**23.4 %***
	Week 53	0.0 %	-11.1 %	-10.4 %	**-12.7 %***
	Week 78	0.0 %	-2-2 %	-9.0 %	+9.8 %
	Week 104/105	0.0 %	+0.3 %	+7.2 %	+1.7 %
Females	**Week 2**	0.0 %	+5.2 %	-12.9 %	-9.6 %
	Week 4	0.0 %	-3.8 %	-14.6 %	-7.7 %
	Week 13	0.0 %	+3.7 %	-0.6 %	+11.6 *
	Week 26	0.0 %	**+16.8 %** *	-5.2 %	**+23.4 %** *
	Week 53	0.0 %	**-26.4 %** *	-3.1 %	**-11.9 %** *
	Week 78	0.0 %	-0.8 %	-5.3 %	- 4.6 %
	Week 104/105	0.0 %	+0.3 %	+1.0 %	+1.6 %

*** Statistically significant difference from controls (p < 0.05)**

Organ weight
A statistically significant increase in relative liver and kidney weights was noted in the high-dose females at the interim sacrifice at week 53.

Table 6.5.6 Statistically significant effects on mean body and organ weights at 24 months sacrifice

	Dose level mg/kg bw/day							
	0	**10**	**30**	**100**	**0**	**10**	**30**	**100**
	Males				**Females**			
Absolute liver weights (g) ± SD	15.12 ± 2.76	17.04 ± 3.53	16.60 ± 3.66	**17.40* ± 2.07**	11.45 ± 2.03	11.37 ± 2.18	11.11 ± 1.68	10.78 ± 2.36
Relative liver weights (%) ± SD	2.82 ± 0.46	3.08 ± 0.83	3.09 ± 0.94	3.18 ± 0.55	2.85 ± 0.64	2.87 ± 0.59	2.81 ± 0.52	3.87 ± 0.50
Absolute kidney weights (g) ± SD	4.05 ± 0.80	**4.92* ± 1.49**	4.57 ± 1.60	4.52 ± 0.84	2.77 ± 0.70	2.83 ± 0.51	2.59 ± 0.35	2.60 ± 0.42
Relative kidney weights (%) ± SD	0.76 ± 0.18	0.90 ± 0.36	0.85 ± 0.35	0.83 ± 0.24	0.69 ± 0.22	0.73 ± 0.10	0.66 ± 0.15	0.76 ± 0.18

bw = body weight
SD = standard deviation
* = statistically significant difference ($p < 0.05$)

Gross pathology

No differences were noted in the frequency of pathological findings between treated and control animal groups.

Histopathology and neoplastic changes
An increased incidence of hepatic neoplastic nodules (5/60) was observed in males of the 100 mg/kg bw/day group sacrificed at study termination compared with controls (1/60). The applicant was asked by the RMS (UK) to provide historical control data to assist with the interpretation of these results. The applicant was not able to provide such data. Nevertheless, the increase in the incidence of hepatic neoplastic nodules in this group (compared with the controls, 1/60) is slight and is likely to reflect normal biological variation. This assertion is supported by the increased incidence of hepatic neoplastic nodules in female controls (6/60) compared with the high-dose group (1/60). When this study was previously considered by EFSA this finding does not appear to have been of concern, probably on the basis that it was reflective of normal biological variation. It is therefore concluded that these results do not indicate that the test substance is carcinogenic.

An increase in the incidence of pituitary hyperplasia was also observed in males of this group (20/55; controls 15/52); in females the incidences of these findings were 23/56 and 33/54 in high dose and controls, respectively. No dose-response was seen for any of these findings. No statistical evaluation of histopathological findings was reported.

Conclusion

This 2 year dietary study on napropamide racemate was conducted before GLP requirements existed. The protocol used is similar to OECD guideline 453. The study is found acceptable even though no statistical evaluation of the findings at gross pathology and at histopathology was reported.

The study results show a statistically significant but transient depression of food consumption in females with a subsequent statistically significant reduction of body weights at 100 mg/kg bw/day both at interim and terminal sacrifice. After 24 months the relative brain weights of females and absolute liver weights of males were increased in the high dose group. The increase in the relative brain weight is a reflection of the significant reduced body weight in females in the high-dose group and is not considered of toxicological relevance. The statistically significant increase in the absolute liver weights in male rats was accompanied with a slight increase in the relative liver weight; however statistical significance was not reached. Based on these findings, the NOAEL in this 2 year study in rats was considered to be 30 mg/kg bw/day. The findings of increased incidences of hepatic nodules and pituitary hyperplasia in males of the high dose group were likely to be due to biological variation. The findings are thus not regarded to be related to treatment with napropamide. Napropamide racemate is concluded not to be oncogenic in this 2 year study in rats with up to 100 mg/kg bw/day.

This study was assessed for the evaluation of napropamide. The original summary of this study that was agreed for napropamide has been supplemented for clarity; however, the conclusion has not changed.

B.6.5.3. Dietary chronic/carcinogenicity study – 24 months in mice

Introduction

This is one of two chronic toxicity studies in the mouse. It was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide.

Author(s):	[redacted] 1978a
Study title:	Lifetime oral study in mice
Test substance:	Napropamide racemate
Purity:	94 % and 94.6 % (total D- + L-isomer)
Batch no.:	Lots #3905-41-12 and #4059-17-1
Test animals:	Male and female CD-1 mice
Groups:	60/sex/dose group.
Dose:	0, 10, 30 and 100 mg/kg bw/day
Vehicle/solvent:	Acetone. The test article/acetone mix was admixed with basal diet
Route:	Oral via diet
Statistics/ measurements:	Yes: Variance analysis and group comparisons to controls. Survival values and tumour incidences in Chi-square test and/or Fisher's exact test
GLP:	No, the test was conducted prior to GLP requirements
Guideline:	None stated. Close to OECD 453
Deviation:	Macroscopic examination only on 10 mice/sex/group
Acceptability:	**Not acceptable**. Reporting too scarce and data not reliable. In the raw data pages were missing and mixed with studies made on rats.

Three groups of CD-1 mice (60/sex/group) were administered napropamide racemate (purity 94.6 %, total D- + L-isomer) in the diet at 10, 30 and 100 mg/kg bw/day for approximately 24 months. A control group of 60 mice/sex received untreated basal laboratory diet.

The study was not to GLP and the data were not considered to be reliable; reporting was scarce and in the raw data pages were missing and mixed with studies on rats. The study is therefore not acceptable. No further information from the study is provided as it would be of no value.

B.6.5.4. Dietary carcinogenicity study – 18 months in mice

Introduction

This is one of two chronic toxicity studies in the mouse. It was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusions have not changed.

Author(s):	[redacted] 1991b
Study title:	18-Month dietary mouse oncogenicity study with R-7465
Test substance:	Napropamide racemate
Purity:	94.3 % (total D- + L-isomer)
Batch no.:	EHC-0952-17/WRC-4921-27-27
Test animals:	Male and female [redacted]: CD®-1 mice
Groups:	50/sex/dose group
Dose:	0, 60, 450, 3500 and 7000 ppm
Vehicle/solvent:	None. Test article was admixed with basal diet
Route:	Oral via diet
Statistics/ measurements:	Yes. One way analysis of variance and post-hoc test (Dunnett's test) on quantitative continuous variables. Statistical significance of differences in incidence of clinical observations or necropsy findings at 95 % or 99 % confidence level by non-parametric statistics (Fisher's exact test).
GLP:	Yes, US EPA: 40 CFR part 160
Guideline:	US EPA §82-283-2 and OECD 451

Deviation: None
Acceptability: Acceptable

Methods

Four groups of CD-1 mice (50/sex/group) were administered napropamide (purity 94.3 %, total D- + L-isomer) at dietary concentrations of 60, 450, 3500 and 7000 ppm for 18 months. A control group of 50 mice/sex received untreated basal laboratory diet.

The following observations/examinations were made during the study: twice daily mortality, signs of overt toxicity and behaviour; weekly detailed physical examination by palpation for the presence of nodules or tissue masses; individual body weight and feed consumption - weekly for the first 13 weeks and once every 4 weeks thereafter until study termination. Body weights were also recorded at necropsy.

Ophthalmological examinations were conducted on all animals at pre-test and on surviving control and high-dose groups at 18 months.
Haematology parameters (erythrocyte count, haematocrit, haemoglobin, total and differential leukocyte count and platelet count) were measured on 10 mice/sex/group at 12 and 18 months.
Macroscopic observations were performed on all surviving mice at study termination and on those dying prematurely. Organ weights (adrenals, brain, kidneys, liver, testes, and ovaries) were recorded for 10 animals/sex/dose level. Histopathological examination of tissues/organs was performed for all mice.

Results

The mean daily doses, based on nominal concentrations of napropamide, were 7.4/9.4, 55/70, 455/568 and 931/1216 mg/kg bw/day for males/females, respectively.

Mortality, body weight and general observations

Survival was statistically significantly reduced for males at 60 and 450 ppm and for females at 60, 450 and 3500 ppm when compared with the controls. However, since there was no clear difference from control in the high-dose (7000 ppm) group for either sex, there was not a treatment-related effect. Survival rates for both sexes in the high-dose group were ≥50 %.

There were no treatment-related clinical signs of toxicity.

Body weights of male mice of the 3500 and the 7000 ppm groups and those of female mice of the 7000 ppm group were consistently statistically significantly lower than controls throughout the study. Mean body weight gains were significantly reduced compared to controls at several time points in the 3500 ppm and the 7000 ppm dosed males and females.
Feed consumption was similar in treated and control groups with the exemption of a few sporadic significant increased or decreased values.

Haematology
Statistically significant decreases in mean platelet counts and mean white cell counts were observed at the 12 months interim sacrifice in males of all dose levels, however they were not dose related. No significant changes in haematological parameters were seen in females.

Ophthalmology
No treatment-related effects on the eye were observed.

Gross pathology
No statistically significant observations were noted in treated animals as compared to controls.

Organ weight
Statistically significant increases in absolute and relative (to body weight) liver weights and relative (to body weight) kidney weight were observed in males at 3500 and 7000 ppm. The increases, as a percentage of control, are given below:

Kidney:
Relative to body weight
3500 ppm: 127 % of control
7000 ppm: 121 % of control
Absolute weight
Not statistically significant

Liver:
Relative to body weight
3500 ppm: 144 % of control
7000 ppm: 141 % of control

Absolute
3500 ppm: 128 % of control
7000 ppm: 123 % of control

No significant changes in either absolute or relative organ weights were noted in females.

Histopathology and neoplastic changes
Non-neoplastic findings
There was a statistically significant increase in the incidence of hepatocellular hypertrophy in males at 7000 ppm. The incidences were 0/50, 3/50, 0/50, 1/50 and 11/50 in the 0, 60, 450, 3500 and 7000 ppm groups, respectively. No treatment related or toxicologically relevant non-neoplastic findings were noted for females.

Neoplastic findings
There were no relevant neoplastic findings.

Conclusion

The study was conducted in accordance with GLP and OECD guidelines. Significant reductions in survival in the mid-dose groups were not considered treatment-related as no effect was seen at the highest dose. Treatment-related, statistically significant findings included reduced body weights in males at 3500 and 7000 ppm and in females at 7000 ppm, decreased body weight gains in males and females at 3500 and 7000 ppm, decreased platelet counts at 12 months in males at 7000 ppm, increased absolute and relative liver and kidney weights at 3500 and 7000 ppm. These findings indicated a slightly higher sensitivity of male mice for certain end-points compared to female mice. On the basis of this information, a NOAEL following long-term oral exposure of mice to napropamide was 450 ppm for both sexes (55 mg/kg bw/day for males and 70 mg/kg bw/day for female mice). Napropamide racemate is not oncogenic to CD-1 mice when tested at up to 7000 ppm (931/1216 mg/kg bw/day for males/females, respectively).

B.6.5.5. Overall summary of long-term toxicity and carcinogenicity (Annex IIA 5.5)

The following long-term and carcinogenicity studies are available on napropamide racemate. There are no studies on napropamide-M.

Rat:
Two 2-year chronic toxicity studies

Mouse:
2-year chronic toxicity study
18-Month dietary mouse oncogenicity study

All of the studies were previously evaluated by Denmark for the approval of napropamide.

Type of study/ Species/ Purity	Dose levels	NOAEL Males/females mg/kg bw/day	LOAEL Males/females mg/kg bw/day	Findings	Reference
Oral, 24 months Sprague Dawley Rat 94.1 % (total D- + L-isomer)	0, 250, 1100, 5000, 10,000 ppm corresponding to 0, 10.48/12.28, 47.56/55.31, 221.44/260.81, 521.57/583.82 mg/kg bw/day for males/females	250 ppm (10.48/12.28 mg/kg bw/day)	1100 ppm (47.56/55.31 mg/kg bw/day)	Decreased body weights and feed consumption from 1100 ppm. Haematological, clinical parameter changes from 5000 ppm Not oncogenic	[redacted] (1991a) [redacted] (1993)
Oral, 24 months Sprague Dawley Rat 94.6 % (total D- + L-isomer)	0, 10, 30, 100 mg/kg bw/day	30 mg/kg bw/day	100 mg/kg bw/day.	Decreased feed consumption and body weights; increased liver weights. Not oncogenic	[redacted] (1978)
Oral, lifetime, mouse CD-1 mice 94 % and 94.6 % **Study unacceptable**	0, 10, 30, 100 mg/kg bw/day	30 mg/kg bw/day	100 mg/kg bw/day	Body weight loss and decreases in liver and kidney weights. Not oncogenic	[redacted] (1978a)
Oral, 18-months, mouse CD-1 mice 94 3 % (total D- + L-isomer)	0, 60, 450, 3500, 7000 ppm corresponding to 0, 7.4/9.4, 55/70, 455/568, 931/1216 mg/kg bw/day for male/females	450 ppm (55/70 mg/kg bw/day)	3500 ppm: 455/568 mg/kg bw/day in males and females	Reduced body weights and body weight gains. Increased relative liver weights. Not oncogenic	[redacted] (1991b)

The long term and carcinogenic potentials of napropamide racemate were tested in two acceptable rat studies and one acceptable mouse study. An additional mouse study was available ([redacted], 1978a), but because of reporting deficiencies and inconsistencies was not acceptable.

The earlier rat study ([redacted], 1978) was conducted before OECD guidelines and GLP requirements were implemented; however, it is still considered to be acceptable, as is the other rat study ([redacted] 1991a), which is relatively modern and appears to be well conducted. The mouse study ([redacted] 1991b) appears to be well conducted and is acceptable for these two end-points.

Although there are no studies on napropamide-M, given the similarity between the racemate and napropamide-M, already established on the basis of the acute toxicity, short term and genotoxicity studies, it is considered appropriate to read-across to the database on napropamide racemate.

In a 24-month toxicity/oncogenicity study in rats by [redacted] (1991a), a number of adverse effects were seen in animals dosed at the high dose levels of 5000 and 10,000 ppm (satellite group, terminated at 12 months in accordance with the study protocol). These effects included decreased haematological parameters indicative of mild anaemia, small increases in gamma-glutamyl transferase activity and increased absolute and relative liver and kidney weights. In addition, decreased body weight and body weight gains and decreased food consumption occurred from 1100 ppm (47.56/55.31 mg/kg bw/day). Macroscopically, cysts in the kidney and foci of discoloration in the liver in males and emaciation in females were seen at 5000 ppm, which was the highest dose examined. A NOAEL of 250 ppm (10.48/12.28 mg/kg bw/day in males and females, respectively) was set based on the effects on body weights and food consumption from 1100 ppm.

The associated histopathological report ([redacted], 1993) showed increased severity of chronic progressive glomerulonephritis in males treated with 5000 ppm (the 10,000 ppm group was, intentionally, not analysed) compared to controls. In the liver, the incidence of spongiosis hepatis was higher in the 5000 ppm males (the satellite group was, intentionally, not analysed) than in controls.

Overall, there was no increase in neoplasm incidence associated with napropamide exposure.

In an earlier rat study by [redacted] (1978), a statistically significant decrease was observed in food consumption and body weights at the high dose level of 100 mg/kg bw /day. Based on these findings, the NOAEL in this 2 year study in rats was considered to be 30 mg/kg bw/day. Napropamide was evaluated not to be oncogenic in this 2-year study in rats at doses up to 100 mg/kg bw/day.
In the 18-month mouse oncogenicity study by [redacted], 1991b, treatment-related, statistically significant findings included decreased body weights and body weight gains and increased liver and kidney weights from 3500 ppm (455/568 mg/kg bw/day in males and females, respectively). Based on these results, a NOAEL following long-term oral exposure of mice to napropamide racemate was 450 ppm (55 mg/kg bw/day for male and 70 mg/kg bw/day for females). Napropamide racemate was not oncogenic to CD-1 mice when tested up to 7000 ppm (equivalent to 931/1216 mg/kg bw/day for males/females, respectively).

Overall, the RMS concludes that napropamide was not carcinogenic in acceptable studies in rats and mice. This is in agreement with the EFSA Conclusion of the peer-review of napropamide that *"no carcinogenic potential was observed in either rats or mice upon long-term exposure to napropamide"* and in the Appendix A (List of End Points) that *"napropamide is unlikely to pose a carcinogenic risk to humans".*

Effect levels in these chronic studies in mice and rats range from 47.56 mg/kg bw/day in the 2 year rat study to 455 mg/kg bw/day in an 18 month mouse study. These are well above the adjusted guidance value cut-off values for classification for STOT-RE (12.5 mg/kg bw/d for a two-year rat study) Thus, no classification for repeated dose toxicity is warranted. As no carcinogenic effect of napropamide racemate was seen in any of the studies, no classification for carcinogenicity is warranted for napropamide-M.

B.6.6. REPRODUCTIVE TOXICITY

Introduction

The following reproductive toxicity studies are available on napropamide racemate.

Multi-generational study
Rat

Developmental toxicity
Rat
One range-finding study
Four main studies

Rabbit
One range-finding study
Two main studies

All of the studies were conducted on napropamide racemate and were previously evaluated by Denmark for the approval of napropamide. The summaries of these studies presented below have not been changed (other than minor amendments to improve readability). The individual study conclusions and final conclusions drawn on the basis of all of the reproductive toxicity studies on napropamide racemate have not changed and are as described in the EFSA Conclusion (EFSA, 2010).

B.6.6.1. Generational studies

B.6.6.1.1. Three-generation dietary study in rats

Introduction

This study was performed on napropamide racemate and was previously evaluated by Denmark for the approval of napropamide.

Author(s):	[redacted] 2007
Study title:	Three Generation Reproduction Study in Rats and Devrinol: Three Generation Reproduction Study in Rats Statistical Assessment of Parental Body Weight Data
Test substance:	Napropamide racemate (Devrinol technical).
Purity:	94.6 % (total D- + L-isomer)
Batch no.:	Lot 4059-17-1
Test animals:	Male and female [redacted] CD (Sprague Dawley) rats
Groups:	15 male and 30 female in each dose group in all generations.
Dose:	0, 10, 30, and 100 mg/kg bw/day
Vehicle/solvent:	None; test article was admixed with basal diet.
Route:	Oral via diet
Statistics/ measurements:	Chi-square test and/or Fisher's exact probability test, Mann-Whitney U-test, one-way analysis of variance, Bartlett's test for homogeneity of variances and appropriate t-test. Statistically significant at 95 % and 99 % confidence level.
GLP:	The study was conducted prior to current GLP
Guideline:	The study was conducted prior to OECD guidelines, but the protocol used is comparable with OECD 416.
Deviation:	From OECD 416: F_2 and F_3 pups were not weighed individually at days 4, 7, and 14. No examination of sperm parameters. Histopathology was restricted to the F_2 parental generation.
Acceptability:	Acceptable

Method

Groups of 15 male and 30 female rats were administered napropamide racemate in the diet in order to achieve dosage levels of 0, 10, 30 and 100 mg/kg bw/day from pre-mating (about 8 weeks in parents of F0 generation, from weaning in subsequent generations). The study period was 85 weeks, including three generations with two sequential litters at each generation. Treatment was performed weeks 0-26 weeks for the F0 generation (week 26 first week of F1), weeks 26-55 for the F1 generation and weeks 55-85 for the F2 generation. In every generation the rats were continuously exposed from weaning though to termination. Every generation included pre-mating treatment up to 100 days of age, 15 days of mating, 21-22 days of pregnancy, 21 days of lactation (of litter 'a'), 10 days of rest, 15 days of mating 21-22 days of pregnancy and 21 days of lactation (of litter 'b'). Litter size was reduced to 10 pups of equal sex ratio on day 4 of lactation, if necessary. Two-thirds of the pups of all the litters were sacrificed post weaning and used for teratological examinations, while the remaining one-third of pups from the first litter were discarded. The remaining one-third of pups from the second litters was used for continuous breeding. Parental animals were sacrificed and necropsied after weaning of their second litters. Histopathology was only performed on F_2 parents.

Clinical signs of toxicity, general behaviour and mortality were recorded daily in all rats. All rats were weighed weekly; females were also weighed on days 0, 7, 14 and 21 of gestation and on days 1, 7, 14 and 21 of lactation. Organ weights were not measured. Food consumption was determined weekly for all rats, food consumption of pregnant rats was also recorded every 2 days from gestation day 10 to lactation day 1 of the first generation. Macroscopic and microscopic examination of tissues from 10 male and 10 female F_2 parental rats was performed.

Specific reproductive observations included fertility, litter size, numbers of male and female pups at weaning and the viability, growth and survival of the pups through weaning. F_1 pups were weighed individually on days 1, 4, 14 and 21 of lactation. For F_2 and F_3 pups, mean pup weights for each litter were recorded on days 1, 4, 7 and 14 of lactation. On day 21 of lactation individual body weights of the pups were recorded.

Results

Mortality and general behaviour
No treatment related changes were seen for parental rats or pups with respect to general behaviour, appearance or survival. Incidental findings included reddish nasal discharge and soft stools in treated as well as control rats.

Body weight and food consumption
The original report and dossier did not give information on statistical evaluation of body weights and body weight gains. A separate document was prepared by the applicant to address this, and reporting of body weight information for the parental generations is based on this document ([redacted] 2007).

Lower body weights (BW) were seen at 100 mg/kg bw/day during gestation and lactation in the F0 (up to 5 % reduction) and F1 generations (up to 6 % reduction) (see Table 6.6.1) but no adverse effects were seen on body weight gain (BWG) of animals during these periods. Lower body weights were reported at the beginning (pre-mating) of the F1 (8 % reduction) and F2 generations (13 % reduction). These initial differences recovered over the first few weeks (see Table 6.6.2). Occasionally lower body weights were seen subsequently (F2 males and F1 females) but these did not show a consistent pattern. No effects on body weights were seen in the 30 mg/kg bw/day in the F0 or F2 generations (see Table 6.6.2). No treatment related effects were seen at 10 mg/kg bw/day.

Table 6.6.1: Mean body weight (g) of dams on Day 21 of gestation and lactation

Parental generation	Litter group	Mean body weight (g) of dams on Day 21 of gestation / lactation			
		Controls	**10 mg/kg bw/day**	**30 mg/kg bw/day**	**100 mg/kg bw/day**
F0 dams	**F1a**	378 / 307 -	376 / 301 -1 % / -2 %	382 / 305 1 % / -1 %	363 / 292 -4 % / **-5 %**
	F1b	427 / 322 -	431 / 339 1 % / 5 %	427 / 334 0 % / 4 %	416 / 324 -3 % / +1 %
F1 dams	**F2a**	357 / 303 -	367 / 315 3 % / -4 %	359 / 312 1 % / 3 %	350 / 298 -2 % / -2 %
	F2b	406 / 339 -	400 / 341 -1 % / 1 %	404 / 334 0 % / -1 %	383 / 327 **-6 %** / -4 %
F2 dams	**F3a**	336 / 305 -	360 / 311 7 % / 2 %	363 / 313 8 % / 3 %	363 / 316 8 % / 4 %
	F3b	399 / 329 -	412 / 339 3 % / 3 %	398 / 337 0 % / 2 %	390 / 335 -2 % / 2 %

Table 6.6.2: Group mean body weights (g) at initiation, mid-point and termination for F0, F1 and F2 parents

	Mean body weight (g)							
	Controls		**10 mg/kg bw/day**		**30 mg/kg bw/day**		**100 mg/kg bw/day**	
	Male	Female	Male	Female	Male	Female	Male	Female
F0 parents								
Week 0:	229	166	229	166	229	166	229	166
Week 13:	493	325	473	323	479	320	472	314
Week 26:	565	293	537	297	555	296	545	293
F1 parents								
Week 26:	156	130	152	125	137**	123	147	120**
Week39:	437	291	447	284	440	273	437	267
Week 55:	545	307	544	301	537	296	523	279*
F2 parents								
Week 55:	131	107	128	103	124	103	115**	93*
Week 70:	478	288	474	301	472	298	458	286
Week 85:	565	304	560	312	565	312	539	301

*: significantly lower than control group mean, $p<0.01$
**: significantly lower than control group mean, $p<0.05$

There were no changes in body weights of pups at the 10 and 30 mg/kg bw/day treatment levels except for a statistically significant reduction in the F_{3a} pup body weights in the 30 mg/kg bw/day treatment level at lactation day 21.

Pups at the 100 mg/kg bw/day treatment level showed statistically reduced body weights on lactation day 7 (F_{2a}) and lactation day 14 (F_{1b} and F_{3a}). On lactation day 21, body weights of the pups of this treatment group were consistently lower in all generations compared to the control litters as presented in Table 6.6.2.

Food consumption values of the treated groups were comparable to the control values. An amendment (Goldenthal, 1981) was prepared to clarify the reported data on napropamide racemate consumption, giving the formula for its calculation:

$$\text{Napropamide consumption in mg/kg bw/day} = \frac{\text{mg napropamide admixed to the diet}^{(1)}}{\text{g BLD}^{(2)}} \times \text{food consumption (g feed/kg bw/day)}$$

(1): The concentrations of napropamide used for mixing the diet for a particular week were based on food consumption and body weights of two weeks prior

(2): BLD: Basal laboratory diet

It was stated in the amendment that Appendices I and II (of the amendment) include the analytical data and theoretical levels of napropamide racemate in the diet for several weeks during the study. However, no legend is given and they therefore did not bring any further clarification.

Thus it was concluded that the reported food consumption values were based on the theoretical level of napropamide racemate in the diet and not based on analytical data.

Table 6.6.3. Mean weight of pups (g) in the lactation period

Age of pups Treatment group	Day 1 Mean weight (g)	Day 4[a] Mean weight (g)	Day 7 Mean weight (g)	Day 14 Mean weight (g).	Day 21 (male) Mean of individual weights (g)	Day 21 (female) Mean of individual weights (g).
F_{1a} (control)	5.8	8.5	-	28.3	44.4	43.2
F_{1a} (100 mg/kg bw/day)	6.0	8.4	-	27.4	39.7^{++}	37.7
F_{1b} (control)	6.3	10.2	-	29.7	45.9	44.0
F_{1b} (100 mg/kg bw/day)	6.1	9.4	-	27.4^{*}	41.1^{++}	39.1^{++}
F_{2a} (control)	6.7	9.7	15	28.4	45.7	43.6
F_{2a} (100 mg/kg bw/day)	6.4	9.1	13.8^{*}	27.3^{+}	41.9^{+}	40.6^{+}
F_{2b} (control)	6.5	9.2	14.2	28.0	45.6	43.9
F_{2b} (100 mg/kg bw/day)	6.4	9.6	14.3	27.2	41.4	40.2
F_{3a} (control)	6.6	9.6	14.8	27.8	42.8	40.9
F_{3a} (100 mg/kg bw/day)	6.4	9.3	14.0	25.1^{**}	38.4^{++}	36.6^{++}
F_{3b} (control)	6.9	9.8	15.5	28.7	44.0	42.1
F_{3b} (100 mg/kg bw/day)	6.9	9.3	15.2	27.8	41.8	40.5

[a] Mean weight of pups (g) after reduction of litter size.
* statistically significant difference from controls ($p<0.05$)
** statistically significant difference from controls ($p<0.01$)
+ Mean of individual weights statistically significant lower than the control group, $p<0.05$
++ Mean of individual weights statistically significant lower than the control group, $p<0.01$
- Not applicable

Litter data, pup survival and pup development
There were no significant differences in the calculated reproductive parameters (fertility index, gestation survival and lactation days survival index) in the treated groups when compared to the control groups in any generation.

Reproductive performance
There were no treatment-related effects on mating, fertility, and gestational length in any of the treated rats.
In the F_1 males at the mid-dose level of 30 mg/kg bw/day, a statistically lower number of males were fertile when mating for the F_{2a} generation. This was not found when the males were mated for the F_{2b} generation or in any other males at any treatment level and therefore was not treatment related.

Gross pathology and histopathology of parental animals
No dose-related effects at any treatment level were observed in the F_2 male and female parental rats sacrificed at the termination of the study or in any pups necropsied during the study.

Conclusion

This 3-generation study in rats was conducted prior to GLP and OECD guidelines. However, it only deviates from OECD guideline 416 in relation to a few parameters and is considered acceptable for the evaluation of the effects of napropamide on fertility.

Under the conditions of this study, napropamide racemate administered in daily doses of 0, 10, 30 or 100 mg/kg bw/day for approximately 80 days pre-mating, during mating, gestation and lactation throughout 3 generations, including two litters per generation, showed significantly decreased body weights in the first weeks of F1 and F2 parental generations and during gestation and lactation in parents of F1 and F2 generations at 100 mg/kg bw/day. Slight effects on body weights at 30 mg/kg bw/day were transient and therefore not deemed toxicologically significant.

No significant effects were seen on reproductive parameters; a lowered fertility at 30 mg/kg bw/day of males of the F_{2a} generation was not seen in other cohorts. In the pups, there were significantly reduced body weights at 100 mg/kg bw/day in all generations. No other effect on the pups was seen. The NOAEL for parental and pup toxicity was 30 mg/kg bw/day, based on decreased body weights in parents and pups at 100 mg/kg bw/day (LOAEL) and the NOAEL for reproductive toxicity was 100 mg/kg bw/day, the highest dose tested.

B.6.6.2. Developmental toxicity studies

Introduction

These studies were all conducted with napropamide racemate and were previously evaluated by Denmark for the approval of napropamide. The summaries (other than amendments to improve readability and clarity) and the conclusions have not changed. The final conclusions drawn on the basis of all of the developmental studies on napropamide racemate have not changed and are as described in the EFSA Conclusion (EFSA, 2010).

B.6.6.2.1. Range finding developmental toxicity study in rabbits

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide.

Author(s):	[redacted], 1984
Study title:	A range finding teratology study in New Zealand White rabbits with Devrinol.
Test substance:	Napropamide racemate
Purity:	Purity not provided
Batch no.:	Not given
Test animals:	New Zealand White rabbits
Groups:	5 females/dose group
Dose:	0, 100, 300, and 500 mg/kg bw/day
Vehicle/solvent:	Corn oil
Route:	Oral (by gavage)
Statistics/ measurements:	Mean, standard deviation and relative standard deviation
GLP:	The study was conducted prior to current GLP
Guideline:	Not applicable (range finding study)
Deviation:	Not applicable (range finding study)
Acceptability:	Acceptable as a range-finding study, although scarce reporting.

Methods

Groups of non-pregnant female New Zealand White rabbits (5/group) each received napropamide racemate as a suspension in corn oil at concentrations of 0, 100, 300 and 500 mg/kg bw/day orally by gavage daily for 13 consecutive days; the dosing volume was 2 ml/kg bw. Animals were observed for 21 days. Clinical observations, body weights and food consumption were recorded. Liver, kidney and adrenal weights were recorded. A gross necropsy was performed in all animals.

Results

Owing to clogging of the dosing needle at 500 mg/kg bw/day, dosing at this level was not initiated until day 5. Occasional difficulties were also experienced at the 300 mg/kg bw/day level, but dosing was not delayed. Because of dosing accidents, one rabbit of the 100 mg/kg bw/day was killed on day 11, and one animal at 500 mg/kg bw/day was killed on day 1.
Two rabbits at 300 mg/kg bw/day died on day 7 and one rabbit at 500 mg/kg bw/day died on day 9. Clinical signs including reduced activity, stained fur, alopecia, anorexia, blood in stool, loose stools, diarrhoea were recorded in 1/5 controls, 3/4 animals at 100 mg/kg bw/day, 4/5 animals at 300 mg/kg bw/day and 2/4 animals at 500 mg/kg bw/day. The effects in the controls and at 100 mg/kg bw/day only included slight effects as alopecia (extent not specified), stained fur and loose stools. There were no treatment-related effects on body weights and food consumption. No treatment-related changes in liver, kidney and adrenal weights were observed.

Conclusion

In this range finding study increased clinical signs of toxicity and mortality were observed at doses of 300 and 500 mg/kg bw/day. In the 100 mg/kg bw/day treatment group, no clear treatment related signs of toxicity and no mortality were observed. Based on mortality and toxicity in the 300 and 500 mg/kg bw/day treatment groups,

and because of difficulties in dose preparation at 300 and 500 mg/kg bw/day it was determined that the high dose should be less than 300 mg/kg bw/day in the main developmental toxicity study.

B.6.6.2.2. Developmental toxicity study in rabbits

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] (1984)
Study title:	A teratology study in New Zealand White rabbits with Devrinol
Test substance:	Napropamide racemate
Purity:	94.6 % (total D- + L-isomer)
Batch no.:	ECH-0586-02
Test animals:	New Zealand White rabbits
Groups:	16-25/dose group
Dose:	0, 10, 50, and 200 mg/kg bw/day. The dosing volume was 1 ml/kg bw.
Vehicle/solvent:	Corn oil
Route:	Oral (by gavage)
Statistics/ measurements:	Fisher's exact probability test with Bonferroni's correction for multiple comparisons to a control value, and Mann-Whitney U-test, Statistically significant at 95 %, two tailed
GLP:	The study was conducted prior to current GLP
Guideline:	The study was conducted prior to current guidelines. Close to EU method B31
Deviation:	Too few animals/group. Treatment from day 7 to19 instead of 6-18 of gestation. Scarce and unclear reporting.
Acceptability:	Not acceptable

Methods

Eight-two females were mated with two different bucks on the same day to obtain the 79 successfully mated females assigned to the study. Mating was considered successful when visually observed. The day of the successful mating was designated day 0 of gestation. 18, 16, 25, and 20 mated females were assigned to the 0, 10, 50 and 200 mg/kg bw/day treatment groups. The females received napropamide racemate daily as a suspension in corn oil at concentrations of 0, 10, 50 and 200 mg/kg bw/day by oral gavage from days 7 to 19 of gestation.

Animals were observed daily for clinical signs of toxicity; individual body weights were recorded on days 0, 7, 9, 14, 21 and 29 of gestation; individual food consumption was recorded daily for gestation day interval of 0-7, 7-14, 14-21 and 21-29. On day 29 of gestation, female rabbits were sacrificed, the fetuses were taken by caesarean section and the does were examined for visceral gross pathology. The liver, kidneys, adrenals, spleen, ovaries and uterine muscle were weighed. The reproductive tract, ovaries, uteri with contents and cervices were removed, weighed and examined. The number of corpora lutea, number and distribution of fetuses and early and late resorptions were determined. Obvious malformations in late resorptions were described when present but not examined further or included in statistical analyses of structural anomalies.
Each fetus was weighed and examined for external malformations. Live pups were sacrificed. The heads of all fetuses were removed for examination. Visceral, and skeletal malformations and variations and the sex of each fetus were also determined.

Results

The reporting of the study is very scarce, with little summary information and few explanations.

Maternal toxicity
Eighteen pregnant females and 1 non-pregnant female were terminated before scheduled sacrifice; 11 were found dead or died as a result of dosing accidents and 8 were sacrificed for humane reasons or after aborting.
The necropsy of the 11 does on study that died indicated that these deaths were due to difficulties in administering the dose and not directly related to the test material. The mortality in the treated pregnant does was

not statistically different from the controls. There were no significant differences in clinical signs, food consumption or on maternal body weights in treated animals compared to controls. Laboured breathing was observed in most animals probably due to aspiration of the dosing suspensions which appeared as an oily substance in the lungs at necropsy. The abortions occurred only in the 50 and 200 mg/kg bw/day treatment groups. The reduction in the proportion of pregnant does with live fetuses was statistically significant at 50 mg/kg bw/day. There were no treatment related mortality or abortions in the 10 mg/kg bw/day treatment group.
The absolute and relative spleen weights for the 200 mg/kg bw/day group were significantly reduced ($p<0.05$) compared to the control group. No other organ weights were significantly affected by treatment.
Two of the does which aborted at 50 mg/kg bw/day and one at 200 mg/kg bw/day had changes in the liver (pale livers with depressed areas, caseous nodules) and ketonuria. These factors were described by the authors as signs of toxaemia of pregnancy. The incidence and character of necropsy observations were similar in the treated and control groups.
A summary of effects on reproduction and mortality are presented in Table 6.6.4.

Table 6.6.4. Summary of fertility and mortality of does treated with napropamide

	Dose level (mg/kg bw/day)			
Parameter	0	10	50	200
Females assigned	18	16	25	20
Not pregnant (%)	1	2	5	1
Surviving (%)	1 (100)	2 (100)	4 (80)	1 (100)
Pregnant (%)	17 (94)	14 (88)	20 (80)	19 (95)
Surviving (%)	15 (88)	13 (93)	15 (75)	15 (79)
With live fetuses at term (%)	15 (88)	12 (86)	**9[*] (45)**	13 (67)
Aborted (%)	0	0	4 (27)	2 (13)
Resorbed (%)	0	1 (8)	2[a] (13)	0

[a] Includes one female with dead fetuses at term.
[*] Statistically significant reduction ($p<0.05$).

Developmental toxicity
The reproductive parameters on day 29 are presented in Table 6.6.5. The only statistically significant difference between the treatment groups and the control group was smaller ratios of implants to corpora lutea in the 50 and 200 mg/kg bw/day groups. However, the number of live fetuses was similar for all groups. No treatment-related effects on reproductive parameters (sex ratio, pregnancy rate, reproductive tract weights, incidence of total resorptions, incidence of early deliveries, corpora lutea, number of live or dead fetuses, or the number of stunted fetuses) were observed.
No treatment related external, visceral or skeletal variations or malformations were observed. The only statistically significant change in the incidence of anatomical changes was the incidence of extra full-sized ribs, which was expressed less frequently at 200 mg/kg bw/day than in the control group. The incidence of this variant fluctuates widely in control groups and is not an indication of a teratogenic effect.

Table 6.6.5. Reproductive parameters of does with live fetuses on gestation day 29

Parameter	Dose levels (mg/kg bw/day)			
	0	10	50	200
	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
Does with implants	15	13	11	13
Corpora lutea/doe	11.3 (1.9)	11.2 (3.2)	10.7 (3.3)	10.8 (1.8)
Implants/doe	11.1 (1.8)	9.8 (3.0)	8.7 (2.8)	8.9 (1.6)
Live fetuses	8.9 (2.7)	8.0 (3.4)	6.8 (3.6)	8.2 (1.7)
Does with live fetuses at term	15	12	**9***	13
Corpora lutea/doe	11.3 (1.9)	11.8 (2.7)	11.8 (2.1)	10.8 (1.8)
Implants/doe	11.1 (1.8)	9.9 (3.0)	9.7 (1.1)	8.9 (1.6)
Live fetuses	8.9 (2.7)	8.7 (2.6)	8.3 (1.3)	8.2 (1.7)
Dead fetuses				
Resorptions – early	1.0 (2.0)	0.3 (0.9)	0.6 (0.7)	0.4 (0.5)
- mid	0.3 (0.8)	0.2 (0.4)	0.3 (0.5)	0.2 (0.4)
- late	0.7 (1.0)	0.8 (1.4)	0.4 (0.9)	0.2 (0.4)
Implants/corpora lutea (%)	99 (13)	89 (30)	**83* (12)**	**84* (16)**
Live Fetuses/implants (%)	81 (23)	88 (15)	86 (9)	91 (10)
Mean number of female pups	4.1 (2.0)	4.0 (1.8)	3.9 (1.3)	4.5 (2.1)
Female pups/live pups (%)	43 (18)	47 (16)	46 (12)	55 (24)
Mean placenta weight (live fetuses)	6.3 (0.8 (14))	7.0 (1.4)	5.8 (1.3)	7.0 (1.5)
Mean fetus weight at birth	35 (6)	38 (8)	36 (7)	39 (7)
Mean number of ossified caudal vertebrae	14.9 (0.4)	14.9 (0.3)	14.8 (0.5)	14.9 (0.3)

*Statistically significant reduction ($p<0.05$).

Conclusion

In this study performed prior to the implementation of GLP and current guidelines but following a procedure close to OECD 414 (EU B31), napropamide racemate was administrated in daily doses of 0, 10, 50, and 200 mg/kg bw/day by gavage on gestation day 7-19 to pregnant rabbits. The study has too few animals/group to secure enough pregnancies at term. Accidents in dosing and aspirations appeared to be responsible for the death of 11 animals. Reporting is too brief (overly summarised) and some of the tabulated results are not clear. The study is not acceptable for evaluating the developmental toxicity of napropamide to rabbits.

Maternal toxicity (increased incidences of abortions, liver changes) was observed in the 50 and 200 mg/kg bw/day treatment groups. In the highest dose group, a significantly decreased spleen weight was also observed. No treatment related effects were observed at gross pathology or in any other organ weights. No treatment related effects on reproductive parameters nor any external, visceral or skeletal variations or malformations were observed. The study conduct, especially difficulties in dosing, indicates that the observed maternal toxicity may very well be caused by stress from the dosing rather than an effect of treatment with napropamide racemate. The laboured breathing observed was probably due to aspiration of the dosing suspensions, which appeared as an oily substance in the lungs at necropsy in most animals independent of treatment level. Reliable NOAEL values for maternal and developmental toxicity could not be established from this limited study in rabbits.

B.6.6.2.3. Developmental toxicity study in rabbits (addendum to [redacted], 1984)

Introduction

The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s): [redacted] (1985).
Study title: Addendum to T-11898: a teratology study in New Zealand White rabbits with Devrinol.

Aim

The purpose of this addendum was to answer the comments and questions raised by the Environmental Health Directorate, Health and Welfare Canada, during its review of the study by [redacted], 1984 "T-11898: a teratology study in New Zealand White rabbits with Devrinol".

Maternal toxicity

The applicant explains the difficulties in dosing by the poor solubility and suspensibility of napropamide in corn oil and the inability of the rabbits to handle repeated dosing with larger volumes of corn oil. The problems were already encountered in the range-finding study (see section B 6.6.2.1) at 300 and 500 mg/kg bw/day, and the highest dose in the main study therefore set at 200 mg/kg bw/day.

The increased mortality and the increased abortions at 50 and 200 mg/kg bw/day and the effect on the spleen weight at 200 mg/kg bw/day were explained by stress induced by administering the thick corn oil dosing suspensions.

Animal health

The comments ascertain that animal health was good, as the animals used in the study were from a pasteurella free stock and deemed to be suitable for study assignment by the veterinarian. It is argued that the clinical signs of these animals during the study were those normally observed in rabbits of this age group except for the laboured breathing and the numerous discharges from the mouth. The laboured breathing could be due to aspiration of the dosing suspensions, which appeared as an oily substance in the lungs at necropsy. The reduction in body weights in all dose groups including the controls during the treatment period was presumably due to the stress caused by aspiration of the corn oil dosing suspensions into the lungs. In three other rabbit teratology studies, conducted by the laboratory during 1983 to 1985, the controls gained weight normally. In these studies, a dosing volume of 0.5 ml/kg was used compared to 1 ml/kg used in the [redacted] (1984) study. A higher dosing volume was used in this study because of the difficulty in adequately suspending napropamide in smaller quantities of corn oil.

Protocol deviations

Several parameters to be calculated as listed in the protocol were not reported in the final report. These parameters were corrected body weight, actual body weight change, corrected body weight change, organ weight relative to corrected body weight and dead implants. Also, all fetuses were weighed but only the weights of the live fetuses were reported. These data were collected but were inadvertently left out of the final report. This was due to a change from an older computer system to a new one, which did not calculate the same parameters from the raw data. According to the applicant, these data and calculated parameters are not essential to the integrity of the study or the interpretation of the study results. In the absence of an effect on either final body weight or reproductive tract weight, corrected weights offer no interpretative advantages. The doe body weight changes and fetal body weights reported were adequate for the interpretation of maternal and fetal toxicity. An explanation for all other protocol deviations occurring during the conduct of the study was issued.

Additional animals

Apparently some animals were added to the study at a later date. These are explained to be from the same shipment as the earlier group. They were assigned to the 0, 50 and 200 mg/kg bw/day groups, because of the low number of animals (<12) with live fetuses at the terminal sacrifice in the 50 and 200 mg/kg bw/day dose groups. Thus, for the later treatment period, 2, 5 and 2 animals were used in the 0, 50 and 200 mg/kg/bw/day dose groups, respectively. The interpretation of the study was not changed by the addition of the animals from the later treatment period.

Conclusion

This amendment confirms the conclusion made under Section B.6.6.2.2 that the observed maternal toxicity, probably is caused by stress from the dosing of napropamide racemate. It also confirms that the study quality is not sufficient for the evaluation of the developmental toxicity of napropamide racemate in rabbits.

B.6.6.2.4. Developmental toxicity study in rabbits

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] (1990)
Study title:	A teratology study in rabbits with R-7465
Test substance:	Napropamide racemate
Purity:	94.6 % (total D- + L-isomer)
Batch no.:	ECH-0952-17
Test animals:	New Zealand White rabbits
Groups:	17-19/dose group
Dose:	0, 100, 300, and 1000 mg/kg bw/day. The dosing volume was 5 ml/kg bw
Vehicle/solvent:	Aqueous 0.5 % Tween 80 vehicle
Route:	Oral (by gavage)
Statistics/ measurements:	Fisher's exact probability test with Bonferroni's correction for multiple comparisons to a control value, and Mann-Whitney U-test, one way analysis of variance and Dunnett's t-test. The level of significance is $p<0.05$
GLP:	Yes (40 CFR part 160)
Guideline:	OECD 414
Deviation:	Dosing days 7-19 instead of 6-18
Acceptability:	Acceptable

Method

Female rabbits were mated with a buck of proven fertility. Mating was considered successful when observed visually. The day of the successful mating was designated day 0 of gestation. Groups of mated rabbits (17-19 rabbits/dose group) each received napropamide racemate daily at concentrations of 0, 100, 300 and 1000 mg/kg bw/day by oral gavage from days 7 to 19 of gestation.

Animals were observed for clinical signs of toxicity daily; individual body weights were recorded on days 0, 3, 7, 10, 13, 19, 24 and 29 of gestation; and individual food consumption was recorded daily for days 0 to 29 of gestation. On day 29 of gestation, surviving female rabbits were sacrificed, the fetuses were delivered by caesarean section, and the does were examined for visceral gross pathology. The reproductive tract was removed, weighed and examined. The number of corpora lutea, number and distribution of fetuses and early and late resorptions were observed. Each fetus was weighed and examined for external, visceral and skeletal malformations and variations. The heads of all fetuses were sectioned for examination.

Results

Maternal toxicity
In the control group one female died on gestation day 11 and one female aborted on gestation day 18.
In the 1000 mg/kg bw/day treatment group one female died on gestation day 29, one was sacrificed for humane reasons (inanition) on gestation day 28 and three aborted between gestation days 18 and 26. At scheduled sacrifice 13, 14, 14 and 10 females were pregnant at the 0, 100, 300 and 1000 mg/kg bw/day dose levels, respectively. A summary of the reproductive parameters are presented in Table 6.6.6 with more detail given in Table 6.6.7.

No treatment-related effects on body weights, body weight gain or food consumption were observed for rabbits at 100 and 300 mg/kg bw/day. In the 1000 mg/kg bw/day treatment group, statistically significant decreases in mean body weight gain were observed during gestation days 0-3 and days 13-19 which was associated with rabbits that aborted. When considering the body weight values for the 13 females with live fetuses on gestation day 29, no statistical significance was achieved. Furthermore statistically significant decreases in food consumption were observed during gestation days 7-10 and 10-13. The food consumption decreases were also associated with rabbits that aborted. When recalculating the food consumption values for the 13 females with live fetuses on gestation day 29, no statistical significance was achieved.

Absolute or relative organ weights were not significantly affected by treatment with napropamide racemate. There were no treatment-related effects on the reproductive tract, the placenta, the ovaries or uterus weights. There were no treatment related findings at necropsy.

Table 6.6.6. Summary of reproductive parameters of rabbits treated with napropamide racemate

Parameter	Dose level (mg/kg bw/day)			
	0	100	300	1000
Females assigned	18	17	17	19
Treated	18	17	17	19[a]
Not pregnant (%)	3 (17)	3 (18)	3 (18)	6 (32)
Pregnant (%)	15 (83)	14 (82)	14 (82)	13 (68)
Surviving (%)	13 (87)	14 (100)	14 (100)	10 (77)
Totally resorbed (%)	0 (0)	0 (0)	0 (0)	0 (0)
Does with live fetuses (%)	13 (87)	14 (100)	14 (100)	10 (77)
Unscheduled sacrifice	1	0	0	4
Aborted	1[b]	0	0	3
Humane sacrifice	0	0	0	1[c]
Found dead	1	0	0	1
Pregnant	1	0	0	0
Not pregnant	0	0	0	1

[a] One doe received a partial dose on the first day of treatment.
[b] One doe had fetal tissue on the cage pad on gestation day 18, and no live fetuses when sacrificed day 29.
[c] Not pregnant.

Developmental toxicity
No treatment-related effects on reproductive parameters (sex ratio, reproductive tract weights, incidence of total resorptions, pre- or post-implantations, incidence of early deliveries, corpora lutea, number of live or dead fetuses, or the number of stunted fetuses) were observed. The statistically significant decrease in affected implants (dead fetuses, resorptions and malformed live fetuses; see footnote (g) in Table 6.6.7) at 300 mg/kg bw/day was not a finding of toxicological relevance. No treatment-related external, visceral, or skeletal variations or malformations were observed. Incomplete ossification of the 5th sternebra observed at 1000 mg/kg bw/day was not considered toxicologically significant when compared with historical data from the laboratory showing higher significances in 3 of 4 other studies. The reproductive parameters for does with live fetuses are presented in Table 6.6.7.

Table 6.6.7. Reproductive parameters of does with life fetuses on gestation 29

Parameter	Dose level (mg/kg bw/day)			
	0	100	300	1000
Does with implants	15	14	14	13
Does with live fetuses	13	14	14	10
Does with resorptions	6	2	2	4
Does with affected implants[a]	9	3	3	5
	Mean[b] (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
Corpora lutea/doe	9.6 (1.6)	9.8 (1.5)	9.6 (1.6)	9.3 (1.5)
Implants/doe	8.5 (2.2)	7.9 (1.7)	7.9 (2.3)	8.6 (1.8)
Implantation index (%)[c]	87.2 (13.8)	81.8 (20.7)	81.9 (22.0)	91.9 (8.2)
Implant viability index (%)[d]	90.2 (10.2)	97.0 (7.7)	97.2 (7.1)	94.4 (7.9)
Post-implantation loss (%)[e]	9.8 (10.2)	3.0 (7.7)	2.8 (7.1)	5.6 (7.9)
Live fetuses/doe	7.6 (2.1)	7.6 (1.5)	7.6 (2.4)	8.1 (1.8)
Dead fetuses/doe	0.1 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Resorptions/doe – early	0.1 (0.3)	0.0 (0.0)	0.1 (0.3)	0.1 (0.3)
--mid	0.2 (0.4)	0.2 (0.6)	0.0 (0.0)	0.1 (0.3)
-- late	0.5 (0.9)	0.1 (0.3)	0.1 (0.5)	0.3 (0.7)
Affected implants (%)[f]	11.3 (9.3)	4.0 (8.2)	**3.6[*] (7.4)**	6.7 (7.9)
Malformed live fetuses (%)[g]	1.5 (3.7)	1.0 (3.8)	0.8 (3.0)	1.1 (3.6)
Mean body weight (g)/live fetus	42.7 (8.2)	45.5 (6.1)	44.4 (5.4)	44.1 (3.9)
Placenta (g)/live fetus	7.5 (1.8)	7.6 (1.1)	7.6 (1.3)	7.8 (1.0)
Sex ratio[h]	45.6 (21.4)	54.9 (24.7)	52.8 (21.0)	45.1 (18.2)

[a] Affected implants includes dead fetuses, resorptions, and malformed live fetuses
[b] Values determined on a per litter basis
[c] (Implant/corpora lutea) x 100
[d] (Viable implants/total implants) x 100
[e] 100 – Implant viability index
[f] [(Dead fetuses + resorptions + malformed live fetuses)/total implants] x 100
[g] (Fetuses with anomalies classified as malformations/total live fetuses) x 100
[h] (Live female fetuses/total live fetuses) x 100
[*] Statistically significant ($p < 0.05$)

Conclusion

In this study in accordance with OECD guideline 414, napropamide racemate was administered to pregnant rabbits in daily doses of 0, 100, 300, and 1000 mg/kg bw/day by gavage on gestation day 7-19. At the highest dose level, statistically significant decreases in food consumption and body weight gain of pregnant does during treatment with napropamide racemate were observed. These effects were associated with the three aborting animals; no treatment related effects on mortality, body weights, food consumption or organ weights were observed in any treatment group, when considering females with live fetuses only.

The smaller number of does giving birth to live fetuses appears to be due to a lower pregnancy rate, which is determined before dosing started, and maternal toxicity, leading to reduced survival of fetuses and, in three females, abortion. The abortions in the 1000 mg/kg bw/day treatment group were probably caused by the reduced food consumption and hence decreased body-weight gain in the affected animals and were thus a secondary effect of the severe maternal toxicity in these animals. In conclusion; there is no clear evidence of specific developmental effects.

There were no clinical signs of toxicity or any treatment related necropsy findings. Based on reduced body weight gain and reduced food consumption at 1000 mg/kg bw/day the NOAEL of maternal toxicity is 300 mg/kg bw/day.

No signs of developmental toxicity were observed at any dose level and no reproductive or toxic effects could be attributed to the administration of napropamide racemate. Based on these results, the NOAEL for developmental toxicity is 1000 mg/kg bw/day. As 1000 mg/kg bw/day is the limit dose recommended by OECD for teratology studies, it can be concluded that napropamide racemate is not teratogenic or feto-toxic in rabbits.

B.6.6.2.5. Range finding developmental toxicity study in rats

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted]. 1989
Study title:	A range finding teratology probe in rats with R-7465
Test substance:	Napropamide racemate
Purity:	94.3 % (total D- + L-isomer)
Batch no.:	ECH-0952-17
Test animals:	[redacted] CD (Sprague Dawley) rats
Groups:	11/dose group
Dose:	0, 500, 750, and 1000 mg/kg bw/day. The dosing volume was 10 ml/kg bw.
Vehicle/solvent:	Aqueous, 0.5 % Tween 80
Route:	Oral (by gavage)
Statistics/ measurements:	Fisher's exact probability test with Bonferroni's correction for multiple comparisons to a control value, and Mann-Whitney U-test, Dunnett's t-test. The level of significance is $p<0.05$
GLP:	No, study only intended to be a range finding study
Guideline:	Not applicable (range finding study)
Deviation:	Not relevant)
Acceptability:	Acceptable as a range-finding study

Methods

The purpose of this study was to provide an assessment of maternal and embryofetal toxicity and teratogenic effects of napropamide racemate and to determine dose levels for a definitive teratology study in rats. Groups of time-mated Sprague Dawley rats each received napropamide racemate daily at concentrations of 0, 500, 750 and 1000 mg/kg bw/day by oral gavage during gestation days 8 through 20.

Clinical observations were made daily; body weights were recorded on gestational days 7, 8, 9, 12, 16, and 20. Food consumption was measured for the following intervals 8-12, 12-16, and 16-20. Females were allowed to deliver their litters and the dam and litter were examined soon after delivery and on postpartum day 4. The following parameters were evaluated: dam body weight, total litter size, number of live and dead pups, gross external anomalies and total litter weight. Food consumption was measured for the postpartum day 0-4. Pups were sacrificed on postpartum day 4. Gross necropsy was performed for all dams on postpartum day 4-5 and the reproductive tract was examined.

Results

Maternal toxicity
Maternal toxicity was observed at 1000 mg/kg bw/day and consisted of statistically significantly decreased food consumption during gestation days 8-12. There were no treatment-related effects on mortality, body weight, body weight change or fertility. There were no treatment-related clinical signs of toxicity or necropsy findings. No statistically significant differences from control group were observed for any of the treated groups with respect to duration of gestation, total born, total implants, average implants minus total born, average percent implant sites seen as offspring, number of live pups/litter, number of dead pups/litter, litter weights, mean pup weights or pup weight gains, and pup viability.

Conclusion

Under the condition of this range finding study, some maternal toxicity was observed in the highest dose group in the form of reduced food consumption in the initial treatment period. However, the reduced food consumption did not cause any reduction in mean body weights or mean body weight changes of pregnant females. No treatment-related embryofetal or teratogenic effects were observed at any dose level. The recommended high dose for a definitive rat teratology study was 1000 mg/kg bw/day.

B.6.6.2.6. Developmental toxicity study in rats

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] (1971)
Study title:	Safety evaluation by a teratological study in rats.
Test substance:	Purified napropamide racemate, napropamide (consisting of napropamide racemate plus 3 % furan), or aspirin.
Purity:	94.3 % (total D- + L-isomer)
Batch no.:	ECH-0952-17
Test animals:	[redacted] CD (Sprague Dawley) rats
Groups:	20-21/dose group
Dose:	25 or 75 mg/kg bw/day of purified napropamide racemate and 77 mg/kg bw/day of napropamide (consisting of napropamide racemate plus 3 % furan). A control group received untreated diet only. A positive control group was also tested with 250 mg/kg bw/day of aspirin
Vehicle/solvent:	None, test article was admixed with basal diet
Route:	Oral via diet. The actual diet levels were 250, 750, 770, and 2500 ppm
Statistics/ measurements:	No statistical tests were performed
GLP:	The study was conducted prior to current GLP
Guideline:	Protocol roughly in accordance with to OECD guideline 414
Deviation:	Too few and too low dose levels. No record of food consumption No information on clinical signs. Scarce reporting, lacking individual data
Acceptability:	Not acceptable

Method

Female rats were paired for mating during a 10-day period with young adult males. The day of copulation was confirmed by the presence of a vaginal plug and designated as day 0 of gestation. Successfully mated females were assigned in rotation to one of the treatment groups. Test compounds (25 or 75 mg/kg bw/day of purified napropamide racemate, 77 mg/kg bw/day of napropamide (napropamide racemate with 3 % furan), 250 mg/kg bw/day of aspirin†) were administered in the diet from day 6 through day 15 of gestation. Food consumption was not reported. Body weights were recorded on days 0, 6, 15 and 20 of gestation. Females were sacrificed on day 20 of gestation after Caesarean section. The uterus of each dam was examined and the number of implantation and resorption sites was recorded. Ovaries were examined for corpora lutea. Number of viable fetuses and any external signs of fetal abnormality were recorded. Approximately one-third of the fetuses from each litter were examined viscerally and the head of each fetus was sectioned and examined. The remaining fetuses of each litter were examined for skeletal malformations.
†: According to the study report aspirin is used as a positive control. No further information is provided.

Results

Maternal toxicity
All mated female rats survived until sacrifice. There were no differences in maternal body weights during pregnancy. Uterine implantation site and corpora lutea counts were comparable in all groups. Fetal resorption was uniformly low in incidence for the five groups.

Developmental toxicity
The reproduction data are presented in Table 6.6.8. The data show no significant difference between the various groups. No gross abnormalities were seen in the fetuses examined at delivery with the possible exception of incomplete ossification of the centra (not specified further – assumed to be the ossification centres of the tubular ones) in both napropamide-treated and aspirin-treated groups. The incidences of this finding were reported in the comments to be 8/19;17/21, 13/18 14/19 and 15/20 for the controls, 25 and 75 mg/kg bw/day purified napropamide racemate, 77 mg/kg bw/day napropamide racemate groups and 250 mg/kg bw/day aspirin groups, respectively, evaluated on a litter basis. In the original report percentages "of specimens" examined with

incomplete ossification of centra were 6.2, 15.9 %, 21.5, 27.8 and 20.7 % for the controls, 25 and 75 mg/kg bw/day purified napropamide racemate, 77 mg/kg bw/day napropamide racemate groups and 250 mg/kg bw/day aspirin groups, respectively. Historical control data from 3 recent studies (not specified if same laboratory) were 4.7, 5.1 and 7.0 %.

Table 6.6.8. Summary of reproductive parameters

	Dose level (mg/kg bw/day)				
	Control	Purified napropamide		Napropamide racemate	Aspirin
Parameter	0	25	75	77	250
Females pregnant per group	19/20	21/21	18/20	19/20	20/20
Total live fetuses	218	255	216	237	246
Total dead fetuses	1	0	1	0	2
Live fetuses per litter	11.5	12.1	12.0	12.5	12.3
Total fetal body wt. (g)	812	936	792	848	896
Mean fetal body wt. (g)	3.56*	3.67	3.67	3.58	-†
Total male fetuses	112	136	105	125	122
Total female fetuses	106	119	111	112	124
Total implantation sites	231	266	229	245	261
Implantation sites, left horn	109	124	100	122	122
Implantation sites, right horn	122	142	129	123	139
Total resorption sites	12	11	12	8	13
Percent resorption	5.2	4.1	5.2	3.3	5.0
Corpora lutea of pregnancy	227	268	227	246	263

*: Pups delivered naturally are excluded.
†: This value appears to be 3.52 however this is not certain as the page of the report has been poorly reproduced and the value is obscured.

Conclusions

In this developmental toxicity study in rats, conducted before OECD guidelines were implemented, too few and very low levels of napropamide were used. Furthermore, a number of parameters were not measured or not reported. Food consumption was not measured: the actual levels of napropamide are uncertain. The study is not acceptable.

The study results showed no effects on the dams in any napropamide treatment groups. No fetotoxicity was seen in any group. With respect to developmental toxicity, the groups were comparable to one another with the exception of incomplete ossification of the centra (not specified – assumed to be ossification centres of the tubular bones) in all napropamide-treated groups. No NOAEL for developmental toxicity can therefore be established. The NOAELs for maternal toxicity were 75 mg/kg bw/day for purified napropamide racemate and 77 mg/kg bw/day for napropamide racemate, the highest dose tested, although it is not sufficiently reliable to inform on the potential developmental toxicity of the test item or the setting of developmental NOAELs, owing to deficiencies in the study

B.6.6.2.7. Developmental toxicity study in rats

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s): [redacted] (1982)
Study title: A teratology study in rats with Devrinol
Test substance: Napropamide racemate
Purity: Purity not specified; known from other study to be 94.6 % pure, total D- + L-isomer)
Batch no.: 4921-27-24
Test animals: [redacted] CD (Sprague Dawley) rats
Groups: 25/dose group
Dose: 0, 30, 110, 400 mg/kg bw/day on gestation day 6-15. The dosing volume was

	10 ml/kg bw
Vehicle/solvent:	Mazola corn oil
Route:	Oral (by gavage)
Statistics/ measurements:	Chi-square test with Yates correction factor, Fisher's exact probability test, and Mann-Whitney U-test and Dunnett's t-test. The minimum level of significance is $p<0.05$
GLP:	The study was conducted prior to current GLP
Guideline:	US EPA Guidelines for Teratologic Evaluations. Appear to resemble OECD 414
Deviation:	Order of filing information in report confusing.
Acceptability:	Acceptable

Methods

Female rats were mated and consecutively assigned to the treatment groups. The day of copulation was confirmed (by the presence of a vaginal plug) and designated as day 0 of gestation. During pregnancy, the animals were dosed orally by gavage daily from days 6 to 15 of gestation. The females were observed daily for clinical signs of toxicity. Body weights and food consumption were recorded on days 0, 6, 9, 12, 16 and 20 of gestation. The females were sacrificed on day 20 of gestation and were examined for gross lesions. The reproductive tract was removed, weighed and examined. A detailed external examination of each fetus was conducted. Approximately one-half of the fetuses were examined viscerally and skeletal variations were observed for the remaining fetuses were examined.

Results

Maternal toxicity
One female in the 400 mg/kg bw/day group died immediately following dosing on gestation day 10. Prior to death, there were no abnormal clinical signs. A cause of death could not be determined at necropsy. All remaining females survived to the scheduled termination except for 2 animals at the 110 mg/kg bw/day that were sacrificed after delivering externally normal term pups.

No treatment-related effects on body weight, body weight gain or food consumption were observed at 30 or 110 mg/kg bw/day. In the 400 mg/kg bw/day group, body weight loss (not statistically significant) was observed during the first 3 days (gestation days 6-9) of treatment; a statistically significant increase in mean body weight gain during gestation days 9-16 compensated for the loss during initial treatment. This resulted in slightly reduced (not statistically significant) body weight gain for the entire treatment period. Statistically significant decreases in food consumption were observed during gestation day intervals 6-9 and 12-16. A slight increase in food consumption was observed from gestation days 9-12.

There were no significant treatment-related clinical signs of toxicity at 30 or 110 mg/kg bw/day. In the 400 mg/kg bw/day group there was a slight increase in the number of females with staining and matting of the urogenital haircoat and hair loss. No other clinical symptoms were observed that could be related to treatment.

Table 6.6.9. Summary of maternal survival and pregnancy status

Parameter	Dose level (mg/kg bw/day)			
	0	30	110	400
	No. (%)	No. (%)	No. (%)	No. (%)
Females on study	25	25	25	25
Females that aborted/delivered	0 (0)	0 (0)	2 (8)	0 (0)
Females that died	0 (0)	0 (0)	0 (0)	1 (4)
Non gravid	0 (0)	0 (0)	0 (0)	0 (0)
Gravid	0 (0)	0 (0)	0 (0)	1 (100)
Females examined at laparotomy	25 (100)	25 (100)	23 (92)	24 (96)
Non gravid	2 (8)	5 (20)	2 (8.7)	8 (12.5)
Gravid	23 (92)	20 (80)	21 (91.3)	21 (87.5)
Resorptions only	0 (0)	0 (0)	0 (0)	0 (0)
Dams with viable fetuses	23 (100)	20 (100)	21 (100)	21 (100)
Females that were gravid	23 (92)	20 (80)	23 (92)	22 (88)

Developmental toxicity

No treatment-related effects on reproductive parameters (number of viable or non-viable fetuses, early or late resorptions, post-implantation loss, implantation sites, fetal body weight, sex ratio, or uterine weight) were observed. A statistically significant increase in the mean number of viable fetuses at 110 mg/kg bw/day was observed as well as a significant increase in the mean number of corpora lutea at 400 mg/kg bw/day; both increases were attributed to spontaneous occurrence as ovulation and implantation occur prior to treatment. No treatment-related external, visceral, or skeletal variations or malformations were observed.

Table 6.6.10. Summary of mean fetal parameters

Parameter	Dose level (mg/kg bw/day)			
	0	30	110	400
Sex (m/f)	6.3/6.6	7.3/6.2	6.8/7.8	6.5/7.1
Viable fetuses	12.9	13.5	**14.6***	13.6
Dead fetuses	0	0	0	0
Early resorptions	0.7	0.6	0.3	0.9
Late resorptions	0	0	0	0
Postimplantation loss	0.8	0.6	0.3	0.9
Implantation sites	13.7	14.1	15.0	14.5
Corpora lutea	16.0	16.3	17.0	**19.2***
Foetal weights (g)	3.3	3.3	3.3	3.3

*Statistically significant ($p<0.05$).

Conclusion

In this study conducted according to OECD guideline 414, daily doses of 0, 30, 110 and 400 mg/kg bw/day napropamide racemate were administrated orally to pregnant rats on gestation day 6-15. The study was acceptable. Some reduction of body weight, although not statistically significant, was observed in the 400 mg/kg bw/day treatment group during the initial period of the treatment. This was probably caused by a statistically significant decrease in food consumption. In the same group, a slight increase in the number of females with staining and matting of the urogenital haircoat and hair loss was observed. No treatment related effects were found in any other groups. There was no treatment related effect on reproductive parameters and no effects on external, visceral or skeletal variations or malformations were observed. Based on these results the NOAEL for maternal toxicity is 110 mg/kg bw/day and the NOAEL for fetal and developmental toxicity 400 mg/kg bw/day, the highest dose tested.

B.6.6.2.8. Developmental toxicity study in rats

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] (1990a).
Study title:	T-13274: A teratology study in CD rats with R-7465 (T-13274)
Test substance:	Napropamide racemate
Purity:	94.3 % (total D- + L-isomer)
Batch no.:	0952-17 (WRC 4921-27-27)
Test animals:	[redacted] CD (Sprague Dawley) [redacted]: Cd (SD) BRVAF/Plus™
Groups:	26 female rats/group resulted in 19, 23, 20 and 16 pregnant females at 0, 100, 300, and 1000 mg/kg bw/day, respectively
Dose:	0, 100, 300, and 1000 mg/kg bw/day on gestation day 6-15. The dosing volume was 10 ml/kg bw
Vehicle/solvent:	Water and 0.5 % Tween 80
Route:	Oral (by gavage)
Statistics/ measurements:	Fisher's exact probability test with Bonferroni's correction, Mann-Whitney U-test and Dunnett's t-test. The minimum level of significance was $p<0.05$
GLP:	Yes (40 CFR part 160)
Guideline:	OECD 414
Deviation:	The OECD 414 guideline recommendation of 20 pregnant females per dose group was not met for the 0 and 1000 mg/kg bw/day groups, as only 19/26 and 16/26 females became pregnant. No explanation can be given for the decreased fertility seen at these dose levels. The reduced fertility at 1000 mg/kg bw/day is not considered to be a treatment-related reproductive effect. This deviation did not have an impact on interpretation of the study results
Acceptability:	Acceptable

Methods

Females were mated with proven male breeders. The day copulation was confirmed (by the presence of a vaginal plug or the presence of sperm in a vaginal smear) was designated as day 0 of gestation. Groups of 26 mated female Sprague Dawley rats each received napropamide racemate daily at concentrations of 0, 100, 300 and 1000 mg/kg bw/day by oral gavage from days 6 to 15 of gestation. The females were observed daily for clinical signs of toxicity. Body weights were recorded on days 0, 6, 7, 9, 12, 16 and 21 of gestation. Food consumption was measured on the following intervals: days 0-6, 6-9, 9-12, 12-16 and 16-21 of gestation. Females were sacrificed on day 21 of gestation and were examined for gross lesions. The reproductive tract was removed, weighed and examined. The number of corpora lutea, number and distribution of fetuses and early and late resorptions were observed. All fetuses were examined externally and one-half of the fetuses were examined viscerally and the remaining fetuses were examined for skeletal malformations.

Results

Maternal toxicity

There were no significant treatment-related clinical signs of toxicity or treatment related necropsy findings. In the 1000 mg/kg bw/day treatment group the mean body weight gain was significantly decreased for dams during gestation day interval 6-9. During the same interval a statistically significant decrease in food consumption was observed at the high dose. No treatment-related effects on body weight, body weight gain or food consumption were observed at 100 or 300 mg/kg bw/day. The body weight and food consumption data in gestation day interval 6-9 are presented in Table 6.6.11.

Table 6.6.11. Body weight gain and food consumption on gestation day 6-9

Treatment group	Mean body weight changes (g)	Mean food consumption (g/day)
Control	16 (±11.8)	24 (±3.3)
100 mg/kg bw/day	15 (±12.2)	23 (±2.4)
300 mg/kg bw/day	12 (±15.4)	22 (±2.5)
1000 mg/kg bw/day	**2.6 (±5.7)** **	**19 (± 2.1)****

** Significantly different from the 0 mg/kg bw/day treatment group, p>0.01, two-tailed.

Developmental toxicity

The reproductive and mortality data are presented in Table 6.6.12. No treatment-related effects on reproductive parameters (sex ratio, reproductive tract weights, incidence of total resorptions, post-implantations, incidence of early deliveries, number of live or dead fetuses or the number of stunted fetuses) were observed.

Table 6.6.12. Summary of fertility and mortality

Parameter	Dose level (mg/kg bw/day)			
	0	100	300	1000
Females assigned	26	26	26	26
Females treated	26	26	26	26
Not pregnant (%)	7 (27)	3 (12)	6 (23)	10 (38)
Surviving (%)	7 (100)	3 (100)	6 (100)	10 (100)
Pregnant (%)	19 (73)	23 (88)	20 (77)	16 (62)
Surviving (%)	19 (100)	23 (100)	20 (100)	16 (100)
Totally resorbed (%)	0 (0)	0 (0)	0 (0)	0 (0)
Delivered early (%)	0 (0)	0 (0)	0 (0)	0 (0)
Aborted (%)	0 (0)	0 (0)	0 (0)	0 (0)
Dams with live fetuses on GD 21	19	23	20	16

No treatment-related external, visceral or skeletal variations or malformations were observed. In the 300 mg/kg bw/day treatment group, statistically significant decreases in the mean litter percent of fetuses with normal skeletons were observed. This decrease was due to a statistically significant increase in the collective incidence of skeletal variations; however, no single skeletal variation was significantly increased. No decreases in the incidences of normal skeletons or increases in skeletal variations were observed at other dose levels.

Conclusion

This developmental study in rats was conducted in accordance with OECD guideline 414. Napropamide racemate was administrated in daily doses of 0, 100, 300, and 1000 mg/kg bw/day by gavage to pregnant rats at gestation days 6-15. The treatment elicited maternal toxicity at 1000 mg/kg bw/day consisting of significantly reduced food consumption and body weight gain on gestation day 6-9. No treatment related effects were observed at lower dose levels. No effects of napropamide treatment on intrauterine parameters, fetal body weights or the incidence of fetal malformations were observed at any dose level. 19, 23, 20 and 16 females of 26 were pregnant at the 0, 100, 300, and 1000 mg/kg bw/day dose level, respectively. No explanation can be given for the decreased fertility seen at the 0 and 1000 mg/kg bw/day dose levels. The reduced fertility at 1000 mg/kg bw/day is not considered to be a treatment-related reproductive effect, because there were no concomitant decreases in litter size or fetal body weight and no increases in implantation loss, totally absorbed litters or malformed or affected implants. Though the OECD 414 guideline recommends 20 pregnant females per dose group, this deviation (there were only 16 pregnant females at the 1000 mg/kg bw/day dose level) is regarded not to have had an impact on interpretation of the study results. Based on these findings the NOAEL of napropamide racemate for maternal toxicity is 300 mg/kg bw/day and the NOAEL of fetal and developmental toxicity is 1000 mg/kg bw/day.

B.6.6.2.9. Developmental toxicity study in rats

Introduction

This study was done using napropamide racemate and was previously evaluated by Denmark for the approval of napropamide. The summary presented below has not been changed (other than minor amendments to improve readability) and the conclusion has not changed.

Author(s):	[redacted] (1990b).
Study title:	T-13589: A teratology study in CD rats with R-7465
Test substance:	Napropamide racemate (Devrinol)
Purity:	94.3 % (total D- + L-isomer)
Batch no.:	0952-17 (WRC 4921-27-27)
Test animals:	[redacted] CD (Sprague Dawley) [redacted]: Cd (SD) BRVAF/Plus™
Groups:	30/dosing group
Dose:	0 and 1000 mg/kg bw/day on gestation day 6-15. The dosing volume was 10 ml/kg bw
Vehicle/solvent:	Water and 0.5 % Tween 80
Route:	Oral (by gavage)
Statistics/ measurements:	Fisher's exact probability test, Mann-Whitney U-test, one way analysis of variance and Dunnett's t-test. The minimum level of significance was $p<0.05$
GLP:	Yes (40 CFR part 160)
Guideline:	OECD 414
Deviation:	None
Acceptability:	Acceptable

Methods

This study is a follow-up on the previous study (see Section B.6.6.2.8, T-13274) where dose levels of 0 and 1000 mg/kg bw/day were used to supplement the reduced number of litters at these dose levels in the previous study.

Females were mated with proven male breeders. The day of copulation was confirmed (by the presence of a vaginal plug or the presence of sperm in a vaginal smear) and designated as day 0 of gestation. Groups of 30 mated female Sprague Dawley rats each received napropamide racemate at concentrations of 0 or 1000 mg/kg bw/day by oral gavage from days 6 to 15 of gestation. The females were observed daily for clinical signs of toxicity. Body weights were recorded on days 0, 6, 7, 9, 12, 16 and 21 of gestation. Food consumption was measured for the following intervals: 0-6, 6-9, 9-12, 12-16 and 16-21 of gestation. Females were sacrificed on day 21 of gestation and were examined for gross lesions. The reproductive tract was removed, weighed and examined. The number of corpora lutea, number and distribution of fetuses and early and late resorptions were evaluated. All fetuses were examined externally and one-half of the fetuses were examined viscerally and the remaining fetuses were examined for skeletal malformations.

Results

Maternal toxicity
All females survived until terminal sacrifice on day 21. No female delivered early or had totally resorbed litter or other treatment related clinical signs of toxicity. In the 1000 mg/kg bw/day treatment group mean body weight gain was significantly decreased for dams during gestation day intervals 6-9, 7-9, and 6-16. In the gestation day interval 6-9, statistically significant decreases in food consumption were observed. The body weight and food consumption data in gestation day interval 6-9 are presented in Table 6.6.13.

Table 6.6.13. Body weight gain and food consumption on gestation day 6-9

Treatment group	Mean body weight changes (g)	Mean food consumption (g/day)
Control	16 (±7.1)	26 (±3.1)
1000 mg/kg bw/day	**9.1 (±7.6)** **	**22 (± 2.3)** **

*Significantly different from the 0 mg/kg bw/day treatment group, $p>0.01$, two-tailed.

Developmental toxicity
No treatment-related effects on reproductive parameters (sex ratio, reproductive tract weights, incidence of total resorptions, post-implantations, incidence of early deliveries, number of live or dead fetuses, or the number of stunted fetuses) were observed. No treatment-related external, visceral, or skeletal variations or malformations were observed.

Conclusion

This study is conducted as a follow-up on the previous study under Section B.6.6.2.8 (T-13274) to comply with the OECD guideline No. 414. Dose levels of 0 and 1000 mg/kg bw/day were used to supplement the reduced number of litters at these dose levels in the previous study. Maternal toxicity was observed at 1000 mg/kg bw/day and consisted of significantly reduced food consumption and body weight gain during initial treatment interval on gestation day 6-9. The effect on body weight was observed beyond the initial treatment interval. Significantly reduced body weight gain was seen in the gestation intervals 7-9 and 6-16. No treatment-related effects of napropamide on intrauterine parameters were observed. Napropamide racemate did not increase the incidences of external, soft-tissue or skeletal variations or malfunctions in the 1000 mg/kg bw/day treatment group. Based on these findings, the NOAEL of napropamide racemate for maternal toxicity is < 1000 mg/kg bw/day and the NOAEL for developmental toxicity is 1000 mg/kg bw/day.

B.6.6.2.10. Overall summary of reproductive studies (Annex IIA 5.6)

Type of study (purity)	Species	Dose range tested	NOAEL	LOAEL and effects	Reference
Three-generation reproduction (94.6 %, total D- + L-isomer)	Sprague Dawley Rat	0, 10, 30, or 100 mg/kg bw/day	Parental: 30 mg/kg bw/day Pups: 30 mg/kg bw/day Fertility effects: 100 mg/kg bw/day	Parental: 100 mg/kg bw/day, based on reduced body weights Pups: 100 mg/kg bw/day, based on reduced body weights Fertility: >100 mg/kg bw/day, no effects at high dose	[redacted] (1978b), [redacted] (2007) and [redacted], 1981
Teratology-range-finding (21 day repeated dose study) Purity not stated	NZW Rabbit	0, 100, 300, 500 mg/kg bw/day	Range-finding study purity and batch number not given	300 mg/kg bw/day. Increased mortality and clinical signs	[redacted] (1984)
Teratology (94.6 %) **Study unacceptable**	NZW Rabbit	0, 10, 50, 200 mg/kg bw/day	The study was evaluated not to be acceptable because of high mortality due to dosing difficulties	Maternal toxicity: Abortions and liver changes from 50 mg/kg bw/day, decreased spleen weight at 200 mg/kg bw/day. Developmental tox: None	[redacted] (1984, 1985)
Teratology (94.6 %, total D- + L-isomer)	NZW Rabbit	0, 100, 300, 1000 mg/kg bw/day	Maternal: 300 mg/kg bw/day Developmental: 1000 mg/kg bw/day	Maternal tox: 1000 mg/kg bw/day. Decreased body weights and food consumption Fetotox/developmental tox: >1000 mg/kg bw/day – no effects seen	[redacted] (1990)
Teratology-range-finding (94.3 %, total D- + L-isomer)	Sprague Dawley Rat	0, 500, 750, 1000 mg/kg bw/day	Not relevant as range-finding study	1000 mg/kg bw/day: Reduced food consumption No effects on fetus/development	[redacted] (1989)
Teratology (94.3 %, total D- + L-isomer) **Study unacceptable**	Sprague Dawley Rat	0, 25 or 75 (purified racemate), 77 (napropamide racemate) mg/kg bw/day	The study is not acceptable because of study conduct and reporting shortcomings.	Maternal tox >77 mg/kg bw/day – no effects seen. Pups: Incomplete ossification of centra at all dose-levels	[redacted] (1971)
Teratology (94.6 %, total D- + L-isomer)	Sprague Dawley Rat	0, 30, 110, 400 mg/kg bw/day	Maternal: 110 mg/kg bw/day Developmental: 400 mg/kg bw/day	Maternal: 400 mg/kg bw/day: decreased food consumption and clinical signs (stained or matted fur)	[redacted] (1982)
Teratology (94.3 %, total D- + L-isomer)	Sprague Dawley Rat	0, 100, 300, 1000 mg/kg bw/day	Maternal: 300 mg/kg bw/day Developmental: 1000 mg/kg bw/day	Maternal: 1000 mg/kg bw/day: reduced food consumption and body weight gain – decreased reproductive performance (high non-pregnant rate), which also occurred in controls. Fetal and developmental >1000 mg/kg bw/day as no effects were seen	[redacted] (1990a)

Type of study (purity)	Species	Dose range tested	NOAEL	LOAEL and effects	Reference
Teratology (94.3 %, total D- + L-isomer)	Sprague Dawley Rat	0 and 1000 mg/kg bw/day	Maternal: <1000 mg/kg bw/day Developmental: 1000 mg/kg bw/day	Maternal: 1000 mg/kg bw/day: reduced food consumption and body weight gain. Fetal/Developmental: >1000 mg/kg bw/day, as no effects were seen.	[redacted] (1990b)

All of the available reproductive toxicity studies were done using napropamide racemate and were previously evaluated by Denmark for the approval of napropamide.

A three-generation rat reproduction study with two litters per generation did not reveal evidence of reproduction toxicity in doses up to 100 mg/kg bw/day given for approximately 100 days. In the high-dose, parental toxicity consisted of decreased body weight in the F1 females at the beginning and termination of the generation, and in F2 males and females at the beginning of the generation. No treatment-related reproductive effects were observed; however, pup toxicity (decreased body weight) occurred at the same dose level (100 mg/kg bw/day) causing decreased body weight in the parental generation.

Two teratology studies were performed in rabbits. In one study maternal toxicity consisted of increased mortality and abortions, significantly and decreased spleen weights at dose levels of 50 and 200 mg/kg bw/day. These effects were probably due to the stress induced by difficulties in administering the thick corn oil dosing suspensions and not related to napropamide administration. This study was not acceptable to be used in the evaluation of maternal or developmental toxicity of napropamide.

In a second rabbit developmental toxicity study, no evidence of maternal or fetal toxicity was observed in rabbits treated with napropamide (as a suspension in aqueous 0.5 % Tween 80 in water) at dose levels as high as 1000 mg/kg bw/day.

Four teratology toxicity studies have been conducted in rats. One of the studies was not acceptable as very low doses were used and the actual dose was uncertain because of not-calculated food consumption.

A second study showed evidence of maternal toxicity comprising clinical signs of toxicity, decreased body weight gain during gestation and decreased food consumption in rats given the highest dose of 400 mg/kg bw/day. However, neither fetal nor developmental toxicity was apparent at that dose.

In two other rat developmental toxicity studies, maternal toxicity consisted of decreased body weight gain and food consumption at a dose level of 1000 mg/kg bw/day. Again, no treatment-related fetal effects were observed at the highest dose tested.

In conclusion, no reproductive toxicity due to napropamide racemate was found in male or female rats at doses up to 100 mg/kg bw/day. No developmental effects of napropamide racemate were observed in the rat or rabbit after prenatal doses up to 1000 mg/kg bw/day. Maternal toxicity consisting of reduced body weight gain and decreased food consumption was observed in rats and in rabbits. Based on these conclusions, the NOAEL for maternal toxicity is 110 mg/kg bw/day in rats and 300 mg/kg bw/day in rabbits.

Although there are no studies on napropamide-M, given the similarity between the racemate and napropamide-M, already established on the basis of the acute toxicity, short term and genotoxicity studies, it is considered appropriate to read-across to the database on napropamide racemate to conclude on the reproductive toxicity of napropamide-M.

According to the criteria of Regulation 1272/2008, no classification is warranted with respect to effects on fertility, as no effects on reproductive parameters were observed in the available 3-generation study in the rat. No specific developmental effects were seen up to 1000 mg/kg bw/day in rats or rabbits. Therefore, no classification is warranted with respect to effects on development.

B.6.7. NEUROTOXICITY

Napropamide-M does not have a structure similar or related to those capable of inducing neurotoxicity, and the 90-day toxicity study on napropamide-M in the rat did not show specific indications of potential neurotoxicity. Furthermore, the toxicology studies submitted on napropamide racemate revealed no evidence of specific neurotoxicity. Therefore, neurotoxicity studies on rodents are not required for napropamide-M.

B.6.8. OTHER TOXICOLOGICAL STUDIES

B.6.8.1. Toxicity studies on metabolites and relevant impurities

For napropamide-M there were no relevant metabolites in groundwater, surface water, soil or air observed in environmental fate and behaviour studies that needed to be considered from a toxicological point of view.

It is noted, for completeness, that α-naphthoxy propionic acid (NOPA) was an important groundwater metabolite for napropamide racemate and it was concluded that it does not give rise to toxicological concern.

B.6.8.2. Supplementary studies on the active substance

According to the applicant, no supplementary studies on the active substance have been carried out.

B.6.8.3. Studies on endocrine disruption

According to the new data requirements (Commission Regulation (EU) No 283/2013) if there is evidence that the active substance may have endocrine disrupting properties, additional information or specific studies shall be required. There was no evidence of endocrine effects in the toxicity studies on napropamide-M or napropamide racemate and therefore, endocrine specific studies have not been conducted.

Napropamide-M is not, or has not, to be classified in accordance with the provisions of Regulation (EC) No. 1272/2008 as carcinogen category 2 or toxic for reproduction category 2 and therefore does not fulfil the interim criteria in Annex II of Regulation 1107/2009 for determining substances with endocrine disrupting properties.

B.6.9. MEDICAL DATA AND INFORMATION

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

The applicant provided the following information on occupational health surveillance at the Sandbach and Gujarat production sites.

B.6.9.1.1. Report on the medical surveillance procedures for personnel involved in the manufacture of napropamide-M technical, Gujarat site

Report:	Desia, A., 2015 Medical surveillance of manufacturing personnel napropamide-M technical material. UPL Europe Ltd, Unpublished report No.: 2015/01
Guidelines:	Not applicable Deviations: not applicable
GLP:	Not applicable
Test System:	The occupational health of personnel engaged in the manufacture of napropamide-M was monitored during the production period 15th December 2012 to 31st December 2013 and 1st November 2013 to 30th January 2014, at the UPL Limited manufacturing plant in Gujurat, India. The surveillance program consisted of an annual medical examination consisting of body weight and height, blood pressure, acuity, haematology (RBC, haemoglobin, haematocrit, WBC), blood biochemistry (fasting blood glucose, serum urea, serum uric acid, serum protein, serum albumin, serum globulin), urine analysis (pH, glucose, protein, occult blood, urobilinogen, specific gravity) and a medical interview. Napropamide has been manufactured by UPL at the plant (Ankleshwar Unit I) since 2012. The report states that a total of 14 workers were involved in the manufacture

of napropamide-M.

Results

No abnormal findings.

Conclusions

No abnormalities were found during medical examinations.

B.6.9.1.2. Report on the medical surveillance procedures for personnel involved in the manufacture of napropamide (racemate) technical, [redacted] site

Report:	P. Patel (2008) Report on the medical surveillance procedures for personnel involved in the manufacture of napropamide technical. United Phosphorus Ltd., Unpublished report No.: not stated
Guidelines:	Not applicable Deviations: not applicable
GLP:	No
Test System:	The report contains information on the medical surveillance procedures carried out by United Phosphorus Limited to monitor the health of employees involved in the manufacture of napropamide (racemate) technical. As of June 2009 napropamide racemate was manufactured at the following manufacturing site, owned by United Phosphorus Limited: United Phosphorus Limited [redacted] At that time napropamide had been manufactured at the plant for 5 years. There were 19 employees involved in the manufacture of napropamide (racemate). Napropamide racemate is only produced in plants where workers are trained in the normal safety measures (helmet, gloves, mask, safety glasses and boots), which are monitored and enforced by management. Medical surveillance was performed by a qualified doctor and comprised a pre-employment and an annual medical check for employees. The pre-employment checks and the annual medical checks are summarised below.

Pre-employment checks

Sr.No.	Pre-employment
1	Eye vision checking
2	Cardiovascular system, Abdomen
3	Central Nervous System, Respiratory
4	Haemoglobin (HB) / Total counts (TC) / Differential counts (DC-P), Lymphocyte (L), Eosinophil (E), Monocyte (M) / Erythrocyte sedimentation rate (ESR)
5	Urine - Sugar/ Fasting blood sugar (FBS) / Post pandial blood sugar (PP2 BS) / Post Glucose 1 hour blood sugar (PG1 BS), 2 hours (2/S). Creatinine / Syrum Glutamate Oxaloacetate trans aminase (SG OT/S), Cholesterol
6	X-ray chest

7	Audiometry
8	Spirometry
9	General check-up - Height / Blood Pressure / Skin / Pulse

Annual medical check

Sr.No.	**Periodic**
1	Physical Examination - Weight / Blood Pressure / Skin / Pulse
2	Blood
3	Sugar
4	Urine - Sugar/ Fasting blood sugar (FBS) / Post pandial blood sugar (PP2 BS) / Post Glucose 1 hour blood sugar (PG1 BS), 2 hours (2/S). Creatinine / Syrum Glutamate Oxaloacetate trans aminase (SG OT/S), Cholesterol
5	ECG
6	Lung test
7	Ear
8	Serum Glutamate Oxaloacetate trans aminase (S.G.O.T.)
9	Eye
10	Hemoglobin

Results

No abnormal findings.

Conclusions

No abnormalities were found during medical examinations.

B.6.9.1.3. Report on the medical surveillance procedures for personnel previously involved in the manufacture of napropamide (racemate) technical, [redacted] site

Report:	P.T. Rowlands (2008) Report on the medical surveillance procedures for personnel previously involved in the manufacture of napropamide technical. United Phosphorus Ltd., Unpublished report No.: not stated
Guidelines:	Not applicable Deviations: not applicable
GLP:	No
Test System:	The report contains information on the medical surveillance procedures carried out by United Phosphorus Limited to monitor the health of employees previously involved in the manufacture of napropamide (racemate) technical. Napropamide was manufactured at the following manufacturing site owned by United Phosphorus Limited, production commenced in October 1997 and ceased in December 2001. United Phosphorus Limited, [redacted]

[redacted]

A consultant from Leighton Hospital gave employees a pre-employment and an annual medical check. Eight employees were involved in the manufacture of napropamide (racemate) technical at this plant. The pre-employment checks and the annual medical checks are summarised below.

Findings: There were no abnormal medical findings during the study.

Pre-employment checks

Sr.No.	Pre-employment
1	Eye vision checking
2	Central Nervous System, Respiratory
3	General check-up - Height / Blood Pressure / Skin / Pulse

Annual medical check

Sr.No.	Periodic
1	Physical Examination - Weight / Blood Pressure / Skin / Pulse
2	Urine - Blood Sugar
3	Lung test

Results

No abnormal findings.

Conclusions

No abnormalities were found during medical examinations.

B.6.9.2. Data collected on humans

Based on information from the manufacturing plant, as well as a review of published literature, it is concluded that there have been no reported incidents of napropamide-M poisoning in humans.

B.6.9.3. Direct observation

No direct observations of clinical cases or poisoning incidents with napropamide were submitted.

B.6.9.4. Epidemiological studies

No information was submitted. Apparently, no database search was performed.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

According to the applicant, no cases of poisoning have been reported to date. Symptoms of poisoning have thus not been described. No specific clinical tests are reported to be available for napropamide racemate or napropamide-M.

Due to the generally low toxicity of napropamide-M and its rapid absorption and excretion from the body, effects of poisoning are expected to be acute only and should be treated on a symptomatic basis. Treatment could also be done by controlled removal of exposure, followed by symptomatic and supportive care.

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

First aid measures:

Upon inhalation:	Remove the person from the danger area. Supply person with fresh air, keeping warm and consult doctor according to symptoms.
Following skin contact:	Remove contaminated clothes immediately. Wash thoroughly using soap and copious water. If skin irritation occurs, consult doctor.
Following eye contact:	Wash thoroughly for 15 minutes using copious water, holding eyelids apart. If applicable, remove contact lenses whilst rinsing. If irritation persists, contact doctor.
Upon swallowing:	Rinse mouth with water. Allow the injured party to drink plenty if conscious. Do not induce vomiting. Get medical attention immediately. Keep Material Safety Data Sheet available.

Information for physicians:

Antidotes:	No antidote is available. The treatment must be symptomatic.

B.6.10. REFERENCES RELIED ON

Literature Review

A literature search is required for all new active substances under Regulation (EC) 1107/2009 Article 8(5) and the applicants for napropamide-M provided a report. The document addressed all specialisms and identified relevant publications accordingly.

Study	Napropamide-M Literature Review Report Submission of Scientific Peer-Reviewed Open Literature under Regulation (EC) No 1107/2009
Reference	Wilkinson, D., Tucker, K., 2016
Date performed	11.08.2016
Test facility	JSC International Limited
Report reference	UPL/16/01-LRR3
Guideline(s)	None mentioned however it appears to be in accordance with EFSA guidance document EFSA Journal 2011;9(2):2092
Deviations from the guideline	Not relevant
GLP	Not relevant
Study acceptable	Yes

The report (Wilkinson, D., Tucker, K., 2016) summarises the search for scientific peer-reviewed open literature on the active substance napropamide-M and its relevant metabolite(s). Health, the environment and non-target species were all included and the search was focused on the 10 years before the date of submission of the dossier.

In summary the report collates and summarises relevant published information on the pre-emergence herbicide active substance napropamide-M and any relevant metabolites. The search used separate focused strategies for individual or grouped data requirements for the active substance and its synonyms or relevant metabolites and their synonyms combined with one or more other concepts related to the data requirements.

Following this initial search an assessment of the relevance of these search results was conducted by review of the summary record titles and abstracts to exclude any studies that were clearly irrelevant. The records that appeared to be relevant and those of unclear relevance were then assessed in detail.

The detailed assessment comprised of a review of the full text of the documents. Those studies that had not been excluded during the detailed assessment were assigned a reliability score according to the Klimisch Score definitions.

Searches were performed using Pubmed, Wiley online library, Science Direct and STN databases.

STN is an online database service that provides global access to published research, journal literature, patents, structures, sequences, properties, and other data. In this case the following STN databases were searched:

- Anabstr - Analytical abstracts
- Biosis
- Caplus - chemical abstracts plus
- Chemlist
- Embase - The Excerpta Medica database
- Scisearch
- Toxcenter
- Medline
- Rtecs- Registry of Toxic Effects of Chemical Substances

The overview of substances searched is given below.

Napropamide-M

Pubmed, Wiley online library, Science Direct and the STN databases the search terms used were: “napropamide-M”, “D-Devrinol”, “Dnapropamide”, “HBW07”, “napropamide”, “Devrinol”, “R-7465”, “R65728”, “R-007465”, “N,Ndiethyl- 2-(1-naphthalenyloxy)propanamide”, and CAS numbers “41643-35-0” and “15299-99-7”. The search terms “(R)-(-)-N,N-diethyl-2-(1-naphthyloxy)propionamide”, “(-)-N,N-diethyl-2-(1-naphthalenyloxy)propanamide” and “(RS)-N,N-diethyl-2-(1-naphthyloxy)propionamide” were expanded and not found in the databases.

Potentially relevant metabolites

Pubmed, Wiley online library, Science Direct and the STN databases the search terms used were: metabolite name and its synonyms and the CAS number, if available. The metabolite name and synonyms, CAS number and structure searched are presented in the table below for each respective relevant metabolite.

Code number/name	**Metabolite names and CAS number**
NOPA R7465/15 Compound 15 Compound VIII U12	“2-naphthoxypropionic acid”, “naphthoxypropionic acid”, "1-naphthoxypropionic acid", "2-(naphthalen-1-yloxy) propanoic acid", "2-(1-napthyloxy) propionic acid", "2-(α-naphthoxy)-propionic acid), “13949-67-2”
Isomer I	“N,N-diethyl-2-(4-hydroxy-1-naphthyl)propanamide”, " N,N-diethyl-2-(4-hydroxy naphthalen-1-yl) propanamide”, the fragment "1-Naphthaleneacetamide", 131933-40-9”
Isomer II	“N,N-diethyl-2-(1-hydroxy-2-naphthyl)propanamide”, “N,N-diethyl-` 2-(1-hydroxy naphthalen-2-yl) propanamide”, the fragment “2-Naphthaleneacetamide”, “131933-41-0”
1-Naphthol Compound XI	“1-Naphthol”, “naphthalene-1-ol”, “1-Naphthalenol”, “90-15-3”
PA R7465/11 Compound 11 Compound XII	“o-Phthalic acid”,” phthalic acid”, “88-99-3”
2-OH-NQ Compound XIII	“2-hydroxy-1,4-Napthoquinone”, “2-hydroxynaphthalene-1,4-dione”, "83-72-7”

Napropamide-M and its synonyms or relevant metabolites and their synonyms were searched in combination, using the Boolean operator “AND”, with sets of search terms e.g. mutagen, oral and toxicant etc. An exhaustive list of these search terms can be seen in table below.

Data requirement Search terms – Toxicology
(MUTAG? OR CANCER? OR TERATO? OR GENETOX? OR CARCIN? OR PHOTOMUTAGEN?) (TUMOUR? OR TUMOR? OR CYTOTOX? OR GENOTOX? OR MELANOM? OR GENTOX?) (NEUROTOXI? OR LD50 OR LC50 OR IC50 OR ((LD OR IC OR LC)(W)50)) (((LONG OR SHORT)(W)TERM?)(L)(EFFECT? OR STUD? OR TOXIC?)) (ENDOCRIN? OR INHALAT? OR IRRITAT? OR REPROTOX? OR FERTILI? OR IMMUNOTOX?) (PERCUTANEOU? OR DERMAL? OR ORAL? OR INTOXICAT? OR INGEST?) (((REPRODUCT? OR EMBRYO? OR FOET? OR DEVELOP?)(5A)TOXI?)) ((ACUTE? OR CHRONIC?)(5A)(EFFECT? OR TOXIC? OR TOXIN#) OR PHOTOTOX?) (GIRL# OR CHILD OR CHILDREN OR PATIENT# OR HUMAN# OR MAN) (MEN OR WOM!N OR BOY# OR WORKER# OR OPERATOR# OR FARMER#) (APPLICATOR# OR PERSONNEL? OR WORKFORCE OR EMPLOYEE#) (MAMMAL? OR RODENT# OR RAT OR RATS OR MOUSE OR MICE)

(ACCIDENT? OR INCIDENCE? OR INCIDENT? OR POISON? OR ALLERG? OR EXPOSURE? OR EXPOSE#) (OCCUPAT? OR EPIDEMIOL? OR SENSITIZ? OR SENSITIS?) ((HEALTH OR ADVERSE)(5A)(EFFECT# OR RISK#)) (MEDICAL OR (FIRST(W)AID) OR (TOXIC?(3A)STUD?) OR THERAPE?) (TOXICOKINETIC# OR EXTRACTAB? OR ABSORPTION OR ABSORB? OR DISTRIBUT? OR EXCRET? OR METABOL? OR TRANSFORM? OR BIOTRANSFORM? OR (RADIO(W)LABEL?)) (DOG# OR (GUINEA(W)PIG#) OR RABBIT# OR SKIN? OR EYE#) (HAND# OR DERMAL? OR BYSTANDER# OR RESIDENT#) (PK? OR TK? OR PHARMACOKINETIC# OR AUC? OR TMAX? OR CMAX? OR BIOAVAIL?) (HEPATOCYTE? OR S9? OR MICROSOME?)
Search terms Legend
• # represents one or zero characters at the designated position • ? represents any number of characters to the right of the term • ! represents only one character at the designated position • W represents terms must be adjacent and in the order specified • (nw) represents terms must be adjacent and in the order specified with n or fewer intervening terms • (nA) represents terms must be adjacent but in any order with n or fewer intervening terms • L represents terms must occur in the same information unit

The additional key words combined with napropamide-M and its relevant metabolites were designed to cover all endpoints, species, exposure routes/scenarios and risk for all specialisms.

The list of metabolites provided in the DAR by the applicant (document N3) has more metabolites than have been included in the literature search by the applicant. This is justified in the literature review summary report – extract:

"Although the metabolites of napropamide-M were detected in regulatory plant metabolism studies they are below trigger levels and are not in the residue definitions for monitoring or risk assessment. Therefore, publicly available literature documents on the metabolites are not relevant with respect to residues and consumer risk assessment arising from uses of napropamide-M.

N,N-diethyl-2-(4-hydroxy-1-naphthyl)propanamide and N,N-diethyl-2-(1-hydroxy-2-naphthyl)propanamide were identified as aqueous photolysis metabolites and were potentially relevant for environmental risk assessment. Therefore, publicly available literature documents on these isomers are not relevant with respect to human health risk assessment.

2-hydroxy-1,4-Napthoquinone and 2-naphthoxypropionic acid are not found in environmental fate or ecotoxicological studies and o-Phthalic acid is not considered a significant metabolite in either area of study. Therefore, publicly available literature documents on these isomers are not relevant with respect to environmental risk assessment."

The databases searched cover a wide range of scientific areas from botany and agriculture to toxicology but with an emphasis on medical and biological sciences. A summary of the results for toxicology only is given in the table below.

Results of the search process for napropamide-M / CAS no. 41643-35-0

Data requirement(s) captured in the search	Number
Total number of summary records retrieved*:	1080
Total number of summary records retrieved after limiting date span, removal of patent documents and removal of duplicates:	326
Number of summary records excluded from the search results after rapid assessment for relevance	325
Total number of full-text documents assessed in detail	1
Number of studies excluded from further consideration after detailed assessment for relevance	1
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and	0

studies of unclear relevance)	

* Bibliographic details of the results of the searches

Results of the search process for napropamide-M potentially relevant metabolites

Data requirement(s) captured in the search	Number
Total number of summary records retrieved*:	16897
Total number of summary records retrieved after limiting date span, removal of patent documents and removal of duplicates:	2105
Number of summary records excluded from the search results after rapid assessment for relevance	2103
Total number of full-text documents assessed in detail	2
Number of studies excluded from further consideration after detailed assessment for relevance	1
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	1 (addressed in previously submitted literature review report)

* Bibliographic details of the results of the searches

Results of the search process for napropamide-M metabolite 2-hydroxy-1,4-napthoquinone

Data requirement(s) captured in the search	Number
Total number of summary records retrieved*:	2411
Total number of summary records retrieved after limiting date span, removal of patent documents and removal of duplicates:	281
Number of summary records excluded from the search results after rapid assessment for relevance	281
Total number of full-text documents assessed in detail	0
Number of studies excluded from further consideration after detailed assessment for relevance	0
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0

* Bibliographic details of the results of the searches

Results of the search process for napropamide-M metabolite 2-naphthoxypropionic acid

Data requirement(s) captured in the search	Number
Total number of summary records retrieved*:	24
Total number of summary records retrieved after limiting date span, removal of patent documents and removal of duplicates:	4
Number of summary records excluded from the search results after rapid assessment for relevance	4
Total number of full-text documents assessed in detail	0
Number of studies excluded from further consideration after detailed assessment for relevance	0
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0

* Bibliographic details of the results of the searches

Results of the search process for o-phthalic acid

Data requirement(s) captured in the search	Number
Total number of summary records retrieved*:	25051

Total number of summary records retrieved after limiting date span, removal of patent documents and removal of duplicates:	2253
Number of summary records excluded from the search results after rapid assessment for relevance	2251
Total number of full-text documents assessed in detail	2
Number of studies excluded from further consideration after detailed assessment for relevance	1
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	1

[*] Bibliographic details of the results of the searches

The first tier relevance assessment was based on the study titles and abstracts alone and the second tier screen for relevance was completed following an assessment of the full article.

The criteria for relevance applied by the applicant were as follows:
"Publications meeting the relevance criteria are those showing new/unknown effects or information potentially contradictory to the regulatory data package for the active substance, its relevant metabolites and/or the plant protection products on human health, animal health and/or the environment, which could impact the endpoints or the risk assessment parameters. The table below provides specific criteria used for assessment of relevance for each of the main categories of data requirements given in Regulation (EC) No 1107/2009."

Relevance Criteria

Data requirements (corresponding data points)	Criteria for relevance
Toxicological and metabolism literature (CA 5.1 – 5.8 and CP 7.1 – 7.4)	1. Appropriate and well defined test material (including its purity and impurity profile) 2. Relevant test species (to the mammalian toxicological assessment - preferred species are rodents - rats and mice, the dog is the preferred non-rodent species) 3. Number of animals per group sufficient to establish a statistical significance 4. Several dose levels tested (at least 3), preferably including a negative control, to establish a dose-response 5. Relevant route of administration in terms of risk assessment (oral, dermal or by inhalation) 6. Description of the observations, examinations, analysis performed, or necropsy 7. In addition: studies which may be helpful for the interpretation of other studies present in the dossier, but do not fit under a specific toxicological endpoint

Two of the publications, seen in the table below, were found to be relevant to toxicology or non-dietary exposure. The RMS agrees with the allocated Klimisch scores for these two studies.

Author	Data requirements	Year	Title	Source	Reliability (Klimisch Score)
Bang, Du Yeon; Lee, In Kyung; Lee, Byung-Mu	Toxicological and metabolism literature (CA 5.8.1-06)	2011	Toxicological characterization of phthalic acid	Toxicological Research (Seoul, Republic of Korea) (2011), 27(4), 191-203	4
Kapuci, Mete; Ulker, Zeynep; Gurkan, Sezin; Alpsoy, Lokman (addressed in previously submitted literature review report)	Toxicological and metabolism literature (CA 5.8.1-06)	2014	Determination of cytotoxic and genotoxic effects of naphthalene, 1-naphthol and 2-naphthol on human lymphocyte culture	Toxicology and Industrial Health (2014), 30(1), 82-89, 8 pp.	2

These studies have not been considered in this document as these metabolites (phthalic acid, naphthalene, 1-naphthol and 2-naphthol) are not relevant to the assessment of napropamide-M.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
B.6.1. ADME								
B.6.1.	EFSA	2010	European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance napropamide. EFSA Journal 2010; 8(4):1565. [73 pp.]. doi:10.2903/j.efsa.2010.1565.	N	N/A	n/a	EFSA	No
B.6.1.	[redacted]	2015	Napropamide-M: the metabolism of [^{14}C] napropamide-M following oral administration in the rat. [redacted] Company Report No.: 34510 GLP: yes Unpublished	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No

B.6.1.	[redacted]	1970	Metabolism of R-7465-^{14}C[2-α-naphthoxy) N, N-diethylpropionamide]: balance and tissue residue elimination studies in the rat [redacted] Company report No.: ARC-B-27 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.1.	[redacted]	1988	Napropamide: tissue distribution in animal (plus report supplement) [redacted] Company report No.: [redacted]/C/2689 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.1.	[redacted]	1991	Napropamide: repeat dose study in the rat (30 mg/kg) [redacted] Company report No.: [redacted]/P/3403 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.1.	[redacted]	1991	Napropamide: biotransformation study in the rat [redacted] Company report No.: [redacted]/P/3404 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.2. Acute tox								

B.6.2.	[redacted]	2010a	D-napropamide: acute oral toxicity study in rats Company Report No. D03381 [redacted] GLP, Unpublished	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.2.	[redacted]	2010b	D-napropamide: acute dermal toxicity study in rats Company Report No. D03392 [redacted] GLP, Unpublished	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.2.	[redacted]	2011	D-napropamide: 4 hour acute inhalation toxicity study in the rat Company Report No. D03403 [redacted] Not GLP, Unpublished	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.2.	[redacted]	1989	Napropamide: 4-hour acute inhalation toxicity study in the rat [redacted] Company report No.: [redacted]/P/2418 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)

B.6.2.	[redacted]	2010c	D-napropamide: Primary skin irritation study in rabbits (4-hour semi-occlusive application) Company Report No. D03414 [redacted] GLP, Unpublished	Y	Y	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.2.	[redacted]	2011	D-napropamide: primary eye irritation study in rabbits Company Report No. D03425 [redacted] GLP, Unpublished	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.2.	[redacted]	2011	Local lymph node assay (LLNA) in mice with d-napropamide Company Report No. 1365601 [redacted] GLP, Unpublished	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.3. Short-term tox								

B.6.3.	[redacted]	2013	28-day dose range finding dietary toxicity study of napropamide-M technical in Wistar rats Company Report No. 410-1-02-6144 [redacted] GLP, Unpublished	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.3.	[redacted]	1988a	Memorandum report for T-13275: 4-week dietary range-finding study with R-7465 in rats [redacted] Company report No.: T-13275 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.3.	[redacted]	1988b	Memorandum report for T-13271: 6-week dietary range-finding study with R-7465 in mice [redacted] Company report No.: T-13271 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.3.	[redacted]	1987	An oral dose range finding study of Devrinol technical in the Beagle dog [redacted] Company report No.: T-12921 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)

B.6.3.	[redacted]	2014	90-day dietary toxicity study of napropamide-M technical in Wistar rats Company Report No. 443-1-03-6145 [redacted] GLP, Unpublished	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.3.	[redacted]	1970	R-7465: Safety evaluation by dietary feeding to rats for 13 weeks [redacted] Company report No.: T-2203 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.3.	[redacted]	1970	R-7465: Safety evaluation by repeated dietary administration to dogs for 13 weeks [redacted] Company report No.: T-2166 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.3.	[redacted]	1988	A 52-week toxicity study of Devrinol technical in the Beagle dog [redacted] Company report No.: T-12924 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.3.	[redacted]	1995	Napropamide: toxicity to dogs by repeated oral administration for 52 weeks [redacted] Company report No.: CT/C/2860 GLP: Yes, Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)

B.6.3.	[redacted]	1991	Napropamide: 21-day dermal toxicity to the rat [redacted] Company report No.: [redacted]/P/3397 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.4. Genotox								
B.6.4.	Sokolowski	2010, 2011	*Salmonella typhimurium* and *Escherichia coli* reverse mutation assay with d-napropamide (report amendment 1) Company Report No. 1365602 Harlan, Cytotest Cell Research GmbH (Harlan CCR), Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.4.	Wollny, H-E.	2011	Cell mutation assay at the thymidine kinase locus ($TK^{+/-}$) in mouse lymphoma L5178Y cells with d-napropamide Company Report No. 1365603 Harlan, Cytotest Cell Research GmbH (Harlan CCR), Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No

B.6.4.	Ballantyne, M.	2017	Napropamide-M: *In vitro* L5178Y gene mutation assay at the *tk* locus. UPL Europe Ltd, Unpublished report No.: 8357643	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.4.	EFSA	2011	EFSA Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA, 2011	N	N/A	n/a	EFSA	No
B.6.4.	Majeska, J.B.	1984a	Mutagenicity evaluation in mouse lymphoma multiple endpoint test – forward mutation assay Stauffer Chemical Company Company report No.: T-11912 GLP: No Published: No	N	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.4.	Pirovano, R.	1986a	Study of the capacity of the test article technical napropamide to induce gene mutation in V79 Chinese hamster lung cells Instituto di Ricerche Biomediche (RBM) Company report No.: RIC0011 GLP: No Published: No	N	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)

B.6.4.	Bohnenberger, S.	2011	Chromosome aberration test in human lymphocytes *in vitro* with d-napropamide Company Report No. 1365604 Harlan, Cytotest Cell Research GmbH (Harlan CCR), Germany GLP, Unpublished	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.4.	[redacted]	2017	Napropamide-M: Rat Alkaline Comet Assay, [redacted] Unpublished report No.: 8361879	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	No
B.6.4.	[redacted]	1984b	Mutagenicity evaluation in bone marrow micronucleus [redacted] Company report No.: T-11822 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.4.	[redacted]	1986	Mutagenicity evaluation in bone marrow micronucleus [redacted] Company report No.: T-12813 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.5. Long term and car								

B.6.5.	[redacted]	1991a	Two-year chronic toxicity/oncogenicity study with R-7465 in rats [redacted] Company report No.: T-13276 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.5.	[redacted]	1993	Two-year chronic toxicity/oncogenicity study with R-7465 (Napropamide) in rats. Supplement to T-13276, histopathology report and overall study discussion [redacted] Company report No.: [redacted]/P/4137 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.5.	[redacted]	1978	24-month chronic feeding study in rats – Devrinol technical [redacted]. Company report No.: T-6158 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.5.	[redacted]	1978a	Three-generation reproduction study in rats Company Report No. T-6334 [redacted] Not GLP, Unpublished	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)

B.6.5.	[redacted]	1991b	18-month dietary mouse oncogenicity study with R-7465 [redacted] Company report No.: T-13272 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
Reproductive Tox B.6.6.								
B.6.6.	[redacted]	1978b	Three generation reproduction study in rats [redacted] Company report No.: T-6334 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.6.	[redacted]	2007	Devrinol tech: three generation reproduction study in rats, statistical assessment of parental body weight data Company Report No. not stated Not GLP, Unpublished	N	N	n/a	UPL	No
B.6.6.	[redacted]	1981	Three generation reproduction study in rats – amendment to the final report [redacted] Company report No.: T-6334* GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)

B.6.6.	[redacted]	1984	A range finding teratology study in New Zealand white rabbits with Devrinol [redacted] Company report No.: T-11851 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.6.	[redacted]	1984	A teratology study in New Zealand White rabbits with Devrinol Company Report No. T-11898 [redacted] Not GLP, Unpublished	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.6.	[redacted]	1985	Addendum to T-11898 [redacted] 1984): a teratology study in New Zealand White rabbits with Devrinol	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.6.	[redacted]	1990	T-13270: a teratology study in rabbits with R-7465 technical [redacted] Company report No.: T-13270 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.6.	[redacted]	1989	A range finding teratology probe in rats with R-7465 technical [redacted] Company report No.: T-13273 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)

B.6.6.	[redacted]	1971	R-7465: safety evaluation by a teratological study in rats Company Report No. T-6392 [redacted] Not GLP, Unpublished	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.6.	[redacted]	1982	A teratology study in rats with Devrinol [redacted] Company report No.: T-11038 GLP: No Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.6.	[redacted]	1990a	A teratology study in CD rats with R-7465 technical [redacted] Company report No.: T-13274 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.6.	[redacted]	1990b	T-13589: A teratology study in CD rats with R-7465 technical [redacted] Company report No.: T-13589 GLP: Yes Published: No	Y	N	n/a	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)
B.6.10.	Wilkinson, D., Tucker, K., 2016	2016	Napropamide-M Literature Review Report Submission of Scientific Peer-Reviewed Open Literature under Regulation (EC) No 1107/2009	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No. 1107/2009.	UPL	Evaluated in the DAR for napropamide (racemate). RMS = Demark, September 2005)