

European Commission



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

Mecoprop-P **Volume 3 – B.6 (AS)**

Rapporteur Member State : United Kingdom
Co-Rapporteur Member State : Ireland

Version History

When	What
31/03/2016	Initial Renewal Assessment Report (RAR)

Table of contents

B.6. TOXICOLOGY AND METABOLISM DATA	5
B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS	6
B.6.1.1. Absorption, distribution, metabolism and excretion by oral route	6
B.6.1.1.1 Absorption, distribution, metabolism and excretion by oral route in the rat	6
B.6.1.1.2 Absorption, distribution, metabolism and excretion by oral route - interspecies comparison	11
Relevance of the dog for human risk assessment of mecoprop-P	12
B.6.1.2. Absorption, distribution, metabolism and excretion by other routes	15
B.6.2. ACUTE TOXICITY	16
B.6.2.1. Oral	16
B.6.2.2. Dermal	22
B.6.2.3. Inhalation	24
B.6.2.4. Skin irritation	27
B.6.2.5. Eye irritation	30
B.6.2.6. Skin sensitization	33
B.6.2.7. Phototoxicity	37
B.6.3. SHORT-TERM TOXICITY	40
B.6.3.1. Oral 28-day studies	41
B.6.3.2. Oral 90-day study	43
B.6.3.3. Other routes	58
B.6.4. GENOTOXICITY	64
B.6.4.1. In vitro studies	64
B.6.4.2. <i>In vivo</i> studies in somatic cells	73
B.6.4.3. <i>In vivo</i> studies in germ cells	75
B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS	77
B.6.6. REPRODUCTIVE TOXICITY	98
B.6.6.1. Generational studies	99
B.6.6.1.1 Two-generation reproductive toxicity study in the rat	99
B.6.6.1.2 Preliminary one-generation reproductive toxicity study in the rat	103
B.6.6.2. Developmental toxicity studies	107
B.6.7. NEUROTOXICITY	118
B.6.7.1. Neurotoxicity studies in rodents	118
B.6.7.2. Delayed polyneuropathy studies	120
B.6.8. OTHER TOXICOLOGICAL STUDIES	121
B.6.8.1. Toxicity studies on metabolites and relevant impurities	121
B.6.8.2. Supplementary studies on the active substance	122
B.6.8.3. Studies on endocrine disruption	127
B.6.9. MEDICAL DATA AND INFORMATION	127
B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies	127
B.6.9.2. Data collected on humans	129
B.6.9.3. Direct observation	129
B.6.9.4. Epidemiological studies	132

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test.....	135
B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment	135
B.6.9.3 Expected effects of poisoning.....	136
B.6.10. REFERENCES RELIED ON.....	136

B.6. TOXICOLOGY AND METABOLISM DATA

Introduction

Mecoprop-P was evaluated by Denmark as rapporteur Member State (RMS) for the first review of approval and included into Annex I of Directive 91/414/EC under the Inclusion Directive 2003/70/EC of 17 July 2003.

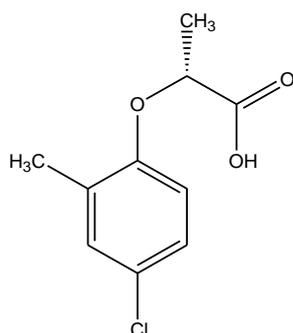
Since the 91/414/EC Review, a number of new toxicology studies have been conducted and are submitted in support of this review. Some studies were generated in support of other regulatory requirements or became available to the notifier due to mergers and acquisitions and are submitted here for completeness. Others are submitted to support the new guidelines to be followed and reflect the new data requirements under Regulation (EC) No.1107/2009 (as set out in Regulation (EC) No. 283/2013).

This toxicology evaluation is a copy of the text of the original 1998 DAR and 2002 addendum together with evaluation of studies not included in the previous renewal but added by the UK as the current RMS and including new information/new interpretation of the data where this has been taken into account.

Mecoprop-P is a systemic herbicide which belongs to the group of auxin-type herbicides. Its mode of action is to mimic auxin, a natural plant growth hormone, but unlike endogenous auxin mecoprop-P is metabolically stable. It is toxic to plants in high concentrations. It is thought to effect plasmalemma ATPases and proton gradient development influencing cell wall plasticity, induction of ethylene biosynthesis, and aberrant nucleic acid metabolism induced by hormonal imbalance in treated tissues. Its effects include a decrease in root and shoot growth, severe chloroplast damage, reduced water consumption, tissue collapse and decay. Mecoprop-P degrades rapidly (geometric mean soil $DT_{50} = 6.0$ days).

There are two isomers of mecoprop, but it only the P isomer has herbicidal activity. Most of the studies in this submission have been conducted on mecoprop-P. However studies on the racemate (mixture of both isomers) are also included where there is limited information on the P isomer. Overall the studies on the racemate are considered also to be applicable to mecoprop-P as the toxicity and target organs are very similar.

The chemical structure of mecoprop-P is shown below.



B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS**B.6.1.1. Absorption, distribution, metabolism and excretion by oral route****B.6.1.1.1 Absorption, distribution, metabolism and excretion by oral route in the rat**

A metabolism study on the racemic mecoprop b [REDACTED] (1978) was submitted in the 1998 DAR but is not included in this renewal as a newer study b [REDACTED] (1997) conducted on mecoprop-P and submitted in the addendum in 2002 is more relevant.

Intravenous administration or biliary excretion studies are required under the data requirements of (EC) 283/2013 in order to determine bioavailability, but were not submitted. Biliary excretion of racemic mecoprop was determined in the study b [REDACTED] (1978) submitted in the 1998 DAR and confirmed a high level of biliary excretion for racemic mecoprop, but as a low level of test substance was excreted in the urine it was concluded enterohepatic recirculation was occurring. In the study on mecoprop-P for this current renewal, high levels of unmetabolised mecoprop-P were excreted in the urine and provide evidence that bioavailability of mecoprop-P is high, therefore biliary or intravenous studies are considered by the RMS to be unnecessary.

Previous evaluation:	In Addendum to DAR for first review (2002).
----------------------	---

Study	(¹⁴ C)-Mecoprop-P: Absorption, distribution, metabolism and excretion in the rat
Reference	[REDACTED] (1997)
Date performed	15 March 1994 – 16 August 1996
Test facility	[REDACTED]
Report reference	1149/3-1007
Guideline(s)	Broadly follows OECD 417
Deviations from the guideline	No
GLP	Yes
Test material	(¹⁴ C)-Mecoprop-P, Batch 469-05, radiochemical purity 99.5%, chemical purity 98.6%
Study acceptable	Yes

Study design and quality:

The study was conducted in accordance with UK Principles of Good Laboratory Practice (1989) and EPA Good Laboratory Practice Regulations (1989) and according to a Hazleton Europe protocol, referring to the guideline of FIFRA Office of Pesticides Programmes, Agrochemical Assessment Guidelines, Subdivision F, 85-1 (EPA, November 1984) and Safety Evaluation of Agricultural Chemicals (59, Nohsan No. 4200, January 28, 1985, Japan). Five oral studies were performed each with groups of 5 male and 5 female Wistar Crl:(WI)BR strain rats and the 6th study (also oral) – on tissue distribution – was performed using 3 groups of 4 animals of each sex of the same strain (see below). Doses of 5 mg or 100 mg (¹⁴C)-mecoprop-P (uniformly labelled in the ring) (nominal radioactive dose 15 µCi (555kBq) per animal) of a chemical purity of 98.6 % and a radiochemical purity of 99.5 % (specific activity 142.4 µCi/mg (5.27 MBq/mg)) were administered as a suspension in 1 w/v % carboxymethyl cellulose in water by gavage to overnight fasted animals. Feed was made available 4 hours after dosing. Radioactivity was determined by liquid scintillation counting (LSC) (determination limit twice the background disintegration rate). Solubilisation or combustion was performed if required before counting.

Examination of radiolabelled metabolites in urine and faeces was performed by thin layer chromatography (TLC) or high performance liquid chromatography (HPLC) and LC-mass spectrometric (LC-MS) techniques to profile and identify the ^{14}C -labelled metabolites.

Diagram of the structure of (^{14}C)-Mecoprop-P

* = position of the carbon-14 label, arrow indicates chiral centre

Study design details:	Dose level (mg/kg bw)	Number of animals		Study duration
		Males	Females	
Excretion balance Single dose	5	5	5	7 days
Excretion balance Repeated dose*	5	5	5	7 days
Excretion balance Single dose	100	5	5	7 days
Pharmacokinetic	5	5	5	7 days
Pharmacokinetic	100	5	5	7 days
Tissue distribution	5	4	4	0.5 hours
	5	4	4	3 hours
	5	4	4	6 hours

*Administered 24 hours after the last of daily doses of 5 mg/kg body weight/day of non-radiolabelled mecoprop-P (unlabelled substance had chemical purity 99.8 %) given as single oral doses for 14 days.

Excretion balance studies

A pilot study was performed using two animals to examine expiration with expired air (100 mg ^{14}C -mecoprop-P/kg body weight). Expired air was trapped at 12 hours, 24 hours and then again every 24 hours till termination of the study.

In the main study urine was collected at 6 hours, 12 hours, 24 hours and then with 24 hours intervals at day 3, 4, 5, 6 and 7 after administration of the dose/last dose, respectively. Faeces was collected every 24 hours post-dose. Cages were rinsed after each collection of excreta, and cage debris and rinsing water were collected as well. After termination of the study the animals were killed by exsanguination and the following tissues were removed:

Bone, brain, blood, fat (abdominal), gonads, heart, kidney, adrenals, thyroid, stomach, liver, lung, muscle (quadriceps), plasma, spleen, uterus, residual carcass, skin and stomach contents. Radioactivity was determined in urine, faeces, expired air traps, cage debris, cage washing, tissues and residual carcasses.

Radiolabelled metabolites were characterised after enzyme and chemical hydrolyses by HPLC and TLC as was the non-radiolabelled mecoprop-P. Further identification of the major urinary metabolite was performed by ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

Toxicokinetic studies

Blood was sampled from the animals at 1.5, 3, 6, 9, 12, and 24 hours, and on day 2, 3, 5 and 7 post-dosing.

Plasma was prepared and radioactivity in plasma was determined by LSC.

Tissue distribution studies

Animals were killed by exsanguination at hours shown in the above scheme and tissues as listed under the excretion studies were removed or sampled and assayed for radioactivity by LSC.

Results:

No overt toxic signs were observed in any animal during the study.

Excretion balance studies: Rapid absorption and extensive (complete) elimination within 24 hours of administration was seen after single and repeat oral doses of 5 mg (¹⁴C)-mecoprop-P/kg body weight whereas after the single oral dose of 100 mg ¹⁴C-mecoprop-P/kg body weight the time course was extended so that 48 hours post dosing male animals had excreted more than 97 % of the administered radioactive dose and female rats more than 85 % (corresponding to 93 % of the totally recovered radioactivity dose for the female rats). No trace of radioactivity was detected in expired air.

Table B.6. 1 Excretion of radioactivity in rats following oral administration of mecoprop-P

Comparison of excretion relative to dosing		Excretion hours 0-24 % of administered dose		Excretion hours 24-48 % of administered dose		Total excretion by 168 h % of administered dose	
		Males	Females	Males	Females	Males	Females
Single dose	Urine*	95.29	92.3	2.6	1.0	100.08	94.03
5 mg (¹⁴ C)-mecoprop-P/kg	Faeces	7.37	3.05	0.64	0.31	8.23	3.56
Repeat dose	Urine*	89.97	86.46	3.37	3.75	94.32	91.16
5 mg (¹⁴ C)-mecoprop-P/kg	Faeces	4.19	3.77	0.8	0.93	5.27	4.9
Single dose	Urine*	61.31	56.78	24.27	20.01	89.41	79.73
100 mg (¹⁴ C)-mecoprop-P/kg	Faeces	8.66	6.89	3.35	1.93	12.53	9.09

* Combined values of actually collected urine and cage wash as proposed by the study team

Repeat administration had a negligible effect on the rate and route of excretion.

Tissue distribution studies:

The single oral dose of 5 mg (¹⁴C)-mecoprop-P/kg body weight gave the following results in tabular form (except stomach and stomach contents) measured up to 6 hours post dosing (see Table B.6. 2). Only concentrations of more than 5 µg equivalents (¹⁴C)-mecoprop-P/g tissue are included in the table.

Table B.6. 2 Tissue distribution of radioactivity in rats following oral administration of 5 mg/kg bw mecoprop-P

Distribution of radioactivity in tissues	Concentration of radioactivity ½ hour post dosing µg equivalents (¹⁴ C)-mecoprop-P/g		Concentration of radioactivity 3 hours post dosing µg equivalents (¹⁴ C)-mecoprop-P/g		Concentration of radioactivity 6 hours post dosing µg equivalents (¹⁴ C)-mecoprop-P/g	
	Male	Female	Male	Female	Male	Female
	Skin	< 5	< 5	< 5	5.049	< 5
Plasma*	16.60	24.37	27.46	25.25	20.09	22.23
Blood*	18.86	19.66	16.49	17.08	13.41	14.72
Heart	7.983	8.468	7.308	9.111	6.593	6.853
Lung	7.717	8.801	6.849	8.304	6.883	7.127
Liver	9.444	8.436	8.799	7.370	7.689	6.539
Kidney	23.62	19.08	23.43	20.23	13.79	15.86
Thyroid	30.17	42.16	23.02	19.77	12.26	32.01

Distribution of radioactivity in tissues	Concentration of radioactivity ½ hour post dosing µg equivalents (¹⁴ C)-mecoprop-P/g		Concentration of radioactivity 3 hours post dosing µg equivalents (¹⁴ C)-mecoprop-P/g		Concentration of radioactivity 6 hours post dosing µg equivalents (¹⁴ C)-mecoprop-P/g	
	Male	Female	Male	Female	Male	Female
	Gonads	< 5	11.16	< 5	11.58	< 5
Uterus	-	9.451	-	12.10	-	6.923
Adrenals	9.477	9.298	10.74	10.78	9.761	7.746
Bone	1.743	1.331	1.576	1.784	1.450	0.710

* µg equivalents (¹⁴C)-mecoprop-P/ml

High concentrations are found only in thyroid gland, the kidneys and in blood/plasma. Levels in the brain are in both male and female rats more than one order of magnitude lower than the levels in other tissues.

Seven days after dose administration, totals of 0.39 % /0.594 % (males/females, single dose) and 0.26 % /0.911 % (males/females, repeat dose) residual radioactivity were found in the tissues in the low dose studies. In the high dose study 2.93 % (males) and 3.19 % (females) of the radioactivity was left in the tissues at the end of the study. This was predominantly located in fat, and to a lesser extent in skin (see Table B.6. 3).

Table B.6. 3 Tissue concentration of radioactivity in rats 7 days following oral administration of mecoprop-P

Contents in tissues 7 days post dosing	Radioactivity in tissues after single oral dose of 5 mg (¹⁴ C)-mecoprop-P µg equivalents (¹⁴ C)-mecoprop-P/g		Radioactivity in tissues after repeated oral dose of 5 mg (¹⁴ C)-mecoprop-P µg equivalents (¹⁴ C)-mecoprop-P/g		Radioactivity in tissues after single oral dose of 100 mg (¹⁴ C)-mecoprop-P µg equivalents (¹⁴ C)-mecoprop-P/g	
	Male	Female	Male	Female	Male	Female
	Carcass	0.019	0.031	0.012	0.050	2.397
Skin	0.048	0.078	0.023	0.082	7.401	7.328
Plasma*	0.010	0.004	0.056	0.012	0.540	0.335
Fat	0.167	0.137	0.057	0.186	23.03	20.38
Spleen	n.d.	n.d.	n.d.	0.008	0.318	0.075
Kidney	0.020	0.012	0.027	0.029	1.183	1.030
Liver	0.013	n.d.	0.018	n.d.	1.205	0.447
Ovaries	-	0.028	-	0.076	-	5.854
Uterus	-	n.d.	-	0.005	-	4.576
Adrenals	n.d.	0.026	0.023	0.066	6.698	5.441
Bone	ND	ND	ND	ND	0.065	ND

* µg equivalents (¹⁴C)-mecoprop-P/ml. No other organs contained detectable amounts of radioactivity.

ND = not detected

Toxicokinetic studies:

In the high dose group there was an increase in time taken to reach maximum concentrations in the plasma levels (see Table B.6. 4). This was mirrored in the elimination in the urine with significant levels of radioactivity being present up to 48 hours in the high dose group.

Table B.6. 4 Toxicokinetic parameters in rats following administration of 5mg/kg bw or 100 mg/kg bw mecoprop-P

Plasma toxicokinetic parameters	Single oral dose of 5 mg/kg bw (¹⁴ C)-mecoprop-P		Single oral dose of 100 mg/kg bw (¹⁴ C)-mecoprop-P	
	Male	Female	Male	Female
C _{max} (µg equivalents/g)	27.77	31.54	384.0	394.1
T _{max} (hours = h)	1.8	2.7	4.2	4.2
T _{1/2} elim (hours = h)	6.354	4.234	7.886	7.787
Area under the curve (AUC) (µg equivalents x h/g)	252.0	182.7	8449	7884

Metabolites

Urine and faeces from the low, high and repeat dose groups were analysed to identify metabolites. The metabolite profiles of urine and faeces indicate that mecoprop-P was excreted predominantly as parent (43% - 56% of administered dose in males and 55% - 69% in females). Hydroxymethyl-mecoprop-P was also present (4.2% to 34.2% of administered dose). There were at least six additional minor components but none of these metabolites accounted for more than 2% of the administered radioactivity. There was a quantitative sex difference in metabolism with males excreting a greater proportion of the dose as hydroxymethyl-mecoprop-P (23.4% to 34.2%) compared to females (4.2% to 7.3%), and levels approximately 10 to 20% higher in both sexes in the repeat dose studies compared to a single dose.

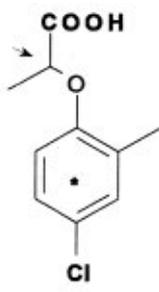
Metabolites in urine: All urine samples from both males and females were qualitatively similar. Upon enzymatic digest with β-glucosidases of various origin and with sulphatase the profile of the contents of the urine was unchanged, and the same happened after alkalic hydrolysis, whereas acidic hydrolysis caused degradation of the parent compound.

The only compounds in the urine accounting for more than 2 % of the administered radioactivity in a pool representing about 70 % all radioactivity administered were the parent compound – approximately 66 % of the excreted radioactivity in males and 83 % in females (after a single oral dose of 5 mg/kg bw) - and one major metabolite - up to 32.6 % of the radioactivity in male urine, considerably less in urine from females. This metabolite was identified by mass spectrometry as a hydroxylated mecoprop-P, and the ¹³C-NMR spectrum confirmed hydroxylation of the 2-methyl moiety of mecoprop-P.

One more metabolite was identified in female urine-pools by HPLC and MS - at a level of 0.05 % in low dose animals and 0.07 % in high dose animals - the carboxy-mecoprop-P which is a known metabolite of mecoprop-P in plants.

Metabolites in faeces: In faeces approximately 60 % of the radioactivity pertained to the parent compound. Hydroxymethyl-mecoprop-P was also detected in faeces with the highest concentration found in excreta from male animals of the single dose group (although the study director speculated that this metabolite could be the result of contamination with urine during collection).

Table B.6. 5 Diagram showing structure of mecoprop-P (left) Hydroxymethyl-mecoprop-P (centre) and Carboxy-mecoprop-P (right)

Mecoprop-P	Hydroxymethyl-mecoprop-P (HMCPP)	Carboxy-mecoprop-P (CCPP)
		
Parent compound	Main metabolite	Minor metabolite in females
Levels in urine: approximately 66 % of the excreted dose in males and 83 % in females	Levels in urine: up to 32.6 % of the excreted dose in males, considerably less in females	Levels in urine: up to 0.07% of excreted dose in females (not detected in males)

Discussion and conclusion:

The oral studies in rats are well reported and performed in compliance with UK Principles of Good Laboratory Practise and EPA Good Laboratory Practise Regulations and with a protocol following FIFRA (EPA 85-1) guidelines and the following results were obtained and seem reliable:

- No toxicity of the compound was observed in any animal during the study.
- Single and repeat oral doses of 5 mg (¹⁴C)-mecoprop-P/kg body weight were rapidly and extensively absorbed and distributed and were eliminated within 24 hours of administration - around 90 % with the urine and 3-7.5 % with faeces - whereas after a single oral dose of 100 mg ¹⁴C-mecoprop-P/kg body weight the time course was extended to about 48 hours for the same excretion - 77 % and 85 % with urine for female and male rats, respectively, and 9-12 % with the faeces for female and male rats respectively.
- No radioactivity was found in expired air.
- Little radioactivity remained in the tissues 7 days after dosing - only about 3 % of the high dose (mainly in the fat) and less than 1 % of the low dose, whether single or repeat.
- Peak plasma concentrations (C_{max}) of approximately 30 to 400 µg /gram ¹⁴C-mecoprop-P were reached around 2 to 4 hours (T_{max}) after dosing for rats receiving single oral doses of 5 or 100 mg ¹⁴C-mecoprop-P/kg bw respectively.
- Elimination half-life (T_{½ elim}) increased from around 4 hours (females) and 6 hours (males) to about 8 hours (in both sexes) when doses increased from 5 mg to 100 mg mecoprop-P per kg body weight.
- There was only one major metabolite – 2-hydroxy-mecoprop-P. More than 30 % of the excreted radioactivity pertained to this metabolite in repeat dose males. Females metabolised far less.
- Major part of radioactivity in urine (> 60 % and > 85 % from males and females, respectively) pertained to unchanged - and unconjugated - parent compound.
- A metabolite known to occur in plants - carboxy-mecoprop-P - was identified in pooled female urine at low concentration - 0.05-0.07 %.

B.6.1.1.2 Absorption, distribution, metabolism and excretion by oral route - interspecies comparison

Data requirements: under (EU) 283/2013, comparative *in vitro* metabolism studies are required (microsomes or intact cell system) to compare metabolism in animal species with humans. No comparative *in vitro* metabolism

studies have been submitted although the applicant has indicated that a study has been commissioned but is not yet completed. A published paper has been submitted and is summarised below which compares *in vivo* studies on rats, dogs and humans and is considered by the RMS to be adequate to address this data requirement.

Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012 New Study from the open literature
----------------------	--

Study	Comparative inter-species pharmacokinetics of phenoxyacetic acid herbicides and related organic acids. Evidence that the dog is not a relevant species for evaluation of human health risk
Reference	Timchalk C (2004)
Date performed	Not relevant
Test facility	Not relevant – Scientific review paper from the open literature
Report reference	Toxicology 200 (2004), p 1-19
Guideline(s)	Not relevant
Deviations from the guideline	Not relevant
GLP	Not relevant
Test material	Investigates phenoxyacetic acids including Mecoprop and 2,4-D.
Study acceptable	Yes

Relevance of the dog for human risk assessment of mecoprop-P

Since the evaluation of mecoprop-P according to 91/414/EEC, a published article on the comparative inter-species pharmacokinetics of phenoxyacetic acid herbicides and related organic acids provides evidence that the dog is not a relevant species for the evaluation of human health risk (Timchalk, 2004).

Abstract

Phenoxyacetic acids including 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) are widely utilized organic acid herbicides that have undergone extensive toxicity and pharmacokinetic analyses. The dog is particularly susceptible to the toxicity of phenoxyacetic acids and related organic acids relative to other species. Active renal clearance mechanisms for organic acids are ubiquitous in mammalian species, and thus a likely mechanism responsible for the increased sensitivity of the dog to these agents is linked to a lower capacity to secrete organic acids from the kidney.

Using published data describing the pharmacokinetics of phenoxyacetic and structurally-related organic acids in a variety of species, including humans, inter-species comparative pharmacokinetics were evaluated using allometric parameter scaling. For both 2,4-D and MCPA, the dog plasma half-life ($t_{1/2}$) and renal clearance (Cl_r; mL/h) rates did not scale as a function of body weight across species; whereas for all other species evaluated, including humans, these pharmacokinetic parameters reasonably scaled.

This exceptional response in the dog is clearly illustrated by comparing the plasma $t_{1/2}$ at comparable doses of 2,4-D and MCPA, across several species. At a dosage of 5 mg/kg, in dogs, the plasma $t_{1/2}$ for 2,4-D and MCPA were ~92–106 and 63 h, respectively, which is substantially longer than in the rat (~1 and 6 h, respectively) or in humans (12 and 11 h, respectively). This longer $t_{1/2}$, and slower elimination in the dog, results in substantially higher body burdens of these organic acids, at comparable doses, relative to other species. Although these results indicate the important role of renal transport clearance mechanisms as determinants of the clearance and potential toxicity outcomes of phenoxyacetic acid herbicides across several species, other contributing mechanisms such as reabsorption from the renal tubules is highly likely.

These findings suggest that for new structurally similar organic acids, a limited comparative species (rat versus dog) pharmacokinetic analysis early in the toxicology evaluation process may provide important insight into the relevance of the dog. In summary, the substantial difference between the pharmacokinetics of phenoxyacetic acids and related organic acids in dogs relative to other species, including humans, questions the relevance of using dog toxicity data for the extrapolation of human health risk.

Comment by Applicant:

The position taken for 2,4-D during the recent EU renewal of approval was to consider that the rat and mouse were the preferred species for establishing reference doses:

“Toxicokinetic and metabolism data in dogs were distinct from other species; dogs were found to have a reduced capacity for urinary excretion of weak organic acids, such as 2,4-D, that lead to a higher plasma half-life and higher sensitivity of dogs to the toxic effects of 2,4-D in comparison with other species, including humans (European Commission, 2001 (Scientific Committee on Plants, 2001)). This conclusion was confirmed in more recent pharmacokinetic investigations and therefore the dog is not considered the most relevant species to extrapolate 2,4-D toxicity to humans.” Source: Conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-D, EFSA Journal 2014;12(9):3812

RMS comment and summary:

This is a review paper that looked at the pharmacokinetic parameters of the phenoxy herbicide 2,4-D, which bears a close structural similarity to mecoprop. The paper then compared these findings to data on other phenoxy herbicides such as mecoprop.

Protein binding

The review asserts that there is extensive evidence in the literature that low molecular weight organic acids (including mecoprop and other phenoxy herbicides) bind extensively to plasma proteins (90-99%) especially at low concentrations in numerous species including rats, dogs, goats and humans.

Only free mecoprop can be excreted in the kidney (by glomerular filtration or active secretion). Only the unbound fraction of mecoprop-P will be free to enter the tissues, with the bound fraction in the blood acting as a reservoir. The ADME study on mecoprop-P (██████ 1997) indicates that mecoprop-P is rapidly eliminated in the urine in rats with an elimination half-life of approximately 4 to 8 hours and suggests protein binding is not markedly hindering urinary excretion after a single oral dose. Elimination half-life after repeat dose studies was not investigated in the ADME studies for mecoprop-P, though tissue distribution after 7 days of dosing (██████ 1997) did not suggest any accumulation. This would agree with the review paper which asserts that active renal tubular secretion is a major route of excretion of organic acids, via high-affinity transporters.

Renal clearance

Renal clearance is the net result of glomerular filtration, and active secretion and reabsorption in the kidney tubules. Clearance is influenced by a number of factors including protein binding, affinity to export transporters, and dose.

Studies *in vitro* of renal clearance of phenoxy-acetic acid herbicides including mecoprop demonstrate dose-dependent non-linear pharmacokinetic properties which the review paper considers to be due to saturation of renal transporters involved in tubular secretion as plasma concentrations increase. However, at higher doses plasma protein binding can also become saturated, resulting in a high percentage of free mecoprop in the plasma that is then available for excretion via glomerular filtration. The studies in the rat on mecoprop-P (Lappin 1997) do not show any saturation of elimination at the high dose group compared to the low dose.

Species differences

The review paper refers to the NOAELs of several studies to show the dog is slightly more sensitive to the toxicity of 2,4-D and mecoprop in repeat dose studies (but not enough evidence presented to exclude the possibility that this effect is an artefact of dose spacing). The paper proposes that the apparent sensitivity of the dog to these compounds is due to slower renal clearance, resulting in higher body burden. Evidence

includes *in vitro* studies on a similar compound, 2,4,5-T that found saturation of renal tubular secretion of 2,4-D occurred more readily in the dog compared to the rat. Studies on 2,4-D found that like mecoprop-P it is extensively absorbed and excreted in the urine largely as parent compound in the rat, mouse and humans. However, a study in the dog following a dose of 5 mg/kg bw found extensive conjugation was occurring with unchanged parent accounting for less than 1% of the administered dose.

Table B.6. 6 Pharmacokinetic parameters of mecoprop in rat, dog and human

Parameters	Rat ^a	Dog ^a	Human ^b
Weight kg	0.25	13	70
Dose mg/kg bw	5	5	0.015
Renal clearance mL/hr	3.7	12	132
Volume distribution L	0.04	1.81	5.25
Plasma half-life hr	5.8	46	11

^a From ██████████ (2002) Xenobiotical 32 (2), p153-163

^b From ██████████. (1983) Arch Toxicol. 54, p297-301.

Allometric scaling

Allometric scaling was used to compare pharmacokinetic parameters between species following low dosed administration of phenoxy herbicides. This approach overcomes some species differences in blood volumes and body size, allowing a more accurate comparison between species. Most of the investigations focused on effects at a dose of 5 mg/kg bw. This dose is relevant to the risk assessment as it is close to the NOAELs seen in the short term repeat dose studies.

Volume distribution

The volume distribution of phenoxy herbicides including mecoprop is low in rats, dogs and humans and is equivalent to the volume of the blood and extracellular water.

Renal clearance and plasma half-life

Plasma half-life and renal clearance of 2,4-D increased in proportion to body weight in mice, rats, dogs, pigs, calves and humans. The dog was the exception as it had lower clearance and a correspondingly higher plasma half-life than would be expected based on body weight. The data on mecoprop was limited to rats, dogs and humans but a similar trend was seen in dogs administered mecoprop when compared to rats and humans, demonstrating the decreased capacity of the dog to clear mecoprop compared with rats and humans. The reduced excretory capacity of the dog compared with the rat or human means that the steady state body burden is likely to be higher and therefore may cause increased toxicity in the dog compared with rats and humans. Increased body burden was demonstrated in a study where plasma concentrations and AUC were higher in the dog compared with the rat following administration of 2,4-D.

Conclusion

In conclusion this review paper provides evidence that plasma half-life and renal clearance of mecoprop are prolonged in the dog. It should be remembered that toxicokinetics are not the only drivers of toxicity and that other differences between species may affect relative sensitivity to mecoprop. However, taking toxicokinetic parameters into consideration suggests that the rat is a better model than the dog for the determination of the effects of mecoprop in humans.

This view was adopted during the recent renewal of approval for 2,4-D (see applicant comment above) where the dog was considered to be not the most relevant species for extrapolation to humans. The evidence for a similar effect in mecoprop is less robust but the RMS considers it is sufficient for these findings to also apply to mecoprop.

The studies in the review paper only considered the mecoprop racemate. This renewal is for the isomer mecoprop-P. Repeat dose studies (see section B.6.3) indicate that the toxicity of mecoprop-P is similar to mecoprop. Toxicokinetic studies on mecoprop that were included in the first review of mecoprop (in the 1998 DAR but not reported in this renewal) do not report any pharmacokinetic parameters, although the data

available suggest the mecoprop racemate probably has similar pharmacokinetic properties to mecoprop-P isomer. Therefore it is considered that the findings in the dog on mecoprop can also be applied to mecoprop-P

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

Not considered necessary.

B.6.1.3 Summary of toxicokinetics studies

The toxicokinetic properties of mecoprop-P have been investigated in the rat in acceptable GLP studies.

Absorption and excretion

Mecoprop-P is rapidly and extensively absorbed reaching peak blood levels at 2 hours at the low dose (5 mg/kg bw) or 4 hours at the high dose (100 mg/kg bw). Based on urinary excretion, absorption is between 90 to 100% in males at the low and high dose, including after repeated dosing. In females absorption was slightly lower, being between 80 and 95% depending on dose or repeated exposure.

Following oral administration mecoprop-P is rapidly excreted predominantly via the urine. The elimination half-life was under 8 hours in both the low and high dose.

Biliary excretion was not investigated, but studies on the mecoprop racemate included in the 1998 DAR indicated extensive biliary excretion and evidence of significant enterohepatic recirculation which may be presumed to also occur in mecoprop-P.

Metabolism

Mecoprop-P is largely excreted as parent material.

The only metabolite of any significance was hydroxymethyl-mecoprop-P (HMCPP), which has an OH group attached to a methyl group, and accounted for approximately one third of the urinary excretion in males but considerably less in females. Carboxy-mecoprop-P (CCPP) was identified as a minor metabolite in females (up to 0.07% in urine).

Tissue distribution

The thyroid, kidney, blood and plasma were the main organs with the highest exposure to mecoprop-P. The decline of the levels in fat and skin during the elimination phase is remarkably slower than for other tissues.

Comparison of rat metabolism with animal, plant and environmental metabolism

Evidence from the open literature suggests that rat and mouse studies are more relevant to humans than the studies in the dog. The dog appears to have reduced capacity for renal clearance of mecoprop which may make it more sensitive to toxic effects at equivalent doses in rats and humans. Therefore the dog is not the most relevant species for determining the effects of mecoprop in humans. Mecoprop-P is a phenoxy herbicide and this finding is believed to apply to all phenoxy herbicides (eg. 2,4-D). Despite this evidence of reduced renal capacity in dogs, in repeat dose studies rodents were more sensitive to mecoprop-P than the dog.

When radiolabelled mecoprop-P was administered to goats, 96% and 93% of the radioactivity in urine at the low and high dose respectively was identified as the parent compound. A similar profile was observed in faeces. Further identification of minor metabolites was considered unnecessary as it is apparent that mecoprop-P is excreted largely unmetabolised.

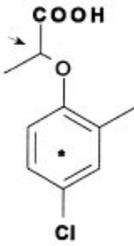
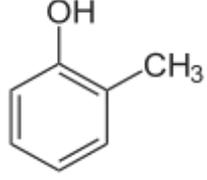
Plant metabolism

The metabolites 2-hydroxymethyl-4-chloro-phenoxypropionic acid (HMCPP) and 2-carboxy-4-chloro-phenoxypropionic acid (CCPP) have been detected in grain and straw. The absolute levels of the metabolites in grain are low but they occur at significant levels in straw (12% and 14% of the administered dose for HMCPP and CCPP respectively). Carboxy-mecoprop-P (CCPP) is a minor urinary metabolite in female rats. Due to the low levels of this metabolite, the toxicity studies on mecoprop-P are not sufficient to determine the toxicity of carboxy-mecoprop-P. As HMCPP is a major rat metabolite in male rats the toxicity of HMCPP has been adequately investigated in studies conducted on mecoprop-P.

Environmental metabolism

The environmental metabolite *o*-Cresol (also known as 2-methylphenol) was observed only in aqueous photolysis studies (Mecoprop-P in pH buffered solutions exposed to artificial sunlight) and was reported at a maximum of 30.4% of the parent dose at pH 7. It is only likely to occur in surface waters. The structure of *o*-Cresol is shown below. This metabolite was not detected in rats so its toxicity to mammals has not been investigated.

Table B.6. 7 Diagram showing structure of mecoprop-P, and its identified metabolites

Mecoprop-P	Hydroxymethyl-mecoprop-P (HMCPP)	Carboxy-mecoprop-P (CCPP)	2-methylphenol (<i>o</i> -Cresol)
			
Parent compound	Main metabolite in rats	Minor metabolite in female rats	Environmental metabolite
Levels in rat urine: approximately 66 % of the excreted dose in males and 83 % in females.	Levels in rat urine: up to 32.6 % of the excreted dose in males, considerably less in females. Detected in straw at 12% administered dose. Trace levels in grain.	Levels in rat urine: up to 0.07% of excreted dose in females (not detected in males). Detected in straw at 14% administered dose. Trace levels in grain.	Levels: Up to 30.4% of parent dose at pH7 in surface waters. Not found in rats.

B.6.2. ACUTE TOXICITY

The data requirement Regulation (EU) 283/2013 stipulates that the acute toxicity studies should be sufficient for classifying the active substance in accordance with the Regulation (EC) No 1272/2008.

B.6.2.1. Oral

Regulation (EU) 283/2013 stipulates that the acute oral toxicity of the active substance is always required.

Three acute oral studies are available on mecoprop-P which were previously evaluated in the 1998 DAR. They are all to OECD 401 which has now been superseded for animal welfare reasons, but this does not affect the validity of the studies.

Two studies on racemic mecoprop [REDACTED] 1983b, e) were included in the 1998 DAR but are not presented here as adequate studies are available on mecoprop-P.

In addition a new acute oral study (██████████2009) conducted in mice, with a dietary route of admomition has been submitted for the purposes of refining the ecotoxicity risk assessment

B.6.2.1.1 Acute oral toxicity in the rat

B.6.2.1.1/01

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Report on the study of the acute oral toxicity in rats of CMPP (Mecoprop) (D-Form)
Reference	██████████ (1983a)
Date performed	4 November to 8 December 1982
Test facility	██████████ ██████████ ██████████ ██████████ ██████████
Report reference	Report no. 84/028
Guideline(s)	Not specified but broadly similar to OECD 401 (1981)
Deviations from the guideline	No significant deviations
GLP	No
Test material	Mecoprop-P, Code 82/302. Purity not stated
Study acceptable	Yes

Study report:

██████████ 1983a): Report on the study of the acute oral toxicity in rats of CMPP (Mecoprop) (D-Form). ██████████ Report no. 84/028, 29 December 1983. Unpublished report. (Dossier ref. 5.3).

Study design and quality:

Five male and five female Wistar rats in each dose group were after a fasting period of 16 h by gavage administered mecoprop-P in doses of 681, 1000, 1470, and 2150 mg/kg bw. Mecoprop-P (not further specified) was suspended in 0.5% aqueous carboxymethyl cellulose and a volume of 10 ml was administered by gavage.

The conduct of the study complies to a great extent with the requirements of the EU test method B1. However, the documentation is not according to GLP. There is lack of details in the reporting of the study. However, these deficiencies are considered of limited importance and overall the study should be considered as acceptable.

Results:

After 14 days of observation the following results with respect to dead animals were obtained:

Table B.6. 8 LD₅₀ value for mecoprop-P in rats, oral administration

Doses (mg/kg bw)	Number of dead male rats	Number of dead female rats
681	0/5	0/5
1000	0/5	5/5
1470	4/5	5/5
2150	5/5	5/5
LD ₅₀ , mg/ kg bw	1327 (interpolation)	681 < LD ₅₀ < 1000
LD ₅₀ (combined),	1050	

mg/ kg bw	(lower and upper 95% confidence interval: 890 and 1020)
-----------	---

All deaths occurred during the first two days after dosing. The following signs of toxicity were observed (not quantified): dyspnoea, apathy, abnormal position, staggering, atonia, paresis, absence of pain reflex and corneal reflex, narcotic like state, tremors, twitching, spastic gait, piloerection, exsiccosis, lacrimation, blood in urine, and poor general state. At 681 mg/kg bw blood in urine was observed. Macroscopic findings at necropsy: general congestive hyperaemia; bloody ulcerations in the glandular stomach was noted in 2 animals from the 1000 mg/kg bw group; intestine was found slightly atonic in some cases, and the urinary bladder was strikingly full in a number of cases. In the surviving animals sacrificed after day 14 no abnormalities were detected.

Discussion and conclusion:

Mecoprop-P is moderately toxic after oral administration to Wistar rats with a LD₅₀ value of 1050 mg/kg bw.

B.6.2.1.1/02

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P: Acute Oral LD ₅₀ in the rat
Reference	██████████ 1994a)
Date performed	29 March to 20 April 1994
Test facility	██████████
Report reference	████████████████████
Guideline(s)	OECD 401 (1987)
Deviations from the guideline	None
GLP	Yes
Test material	Mecoprop-P Batch DA928, purity 94.4%
Study acceptable	Yes

Study report:

Dange M (1994a): Mecoprop-P acute oral LD₅₀ in the rat. ██████████
 ██████████ Report ██████████. 2 September 1994. ██████████. Unpublished report.
 (Dossier ref. 5.4).

Study design and quality:

Five male and five female nine week old Sprague Dawley rats were dosed with 100, 180, 320, and 580 mg/ kg bw mecoprop-P. The animals were fasted overnight before start of the study. Mecoprop-P (purity: 897 g/kg) was suspended in 0.5% aqueous carboxymethyl cellulose and a single oral administration of 10 ml/kg bw was given. The study was conducted and reported according to OECD guideline 401 and to GLP principles.

Results:

Number of death animals during the observation period is given in Table B.6. 9.

Table B.6. 9 LD₅₀ value for mecoprop-P in rats, oral administration

Doses (mg/kg bw)	Number of dead male rats	Number of dead female rats
100	0/5	0/5
180	0/5	0/5
320	0/5	0/5
580	5/5	5/5

LD ₅₀ , mg/ kg bw	431*	431*
LD ₅₀ (combined), mg/ kg bw	431*	

Calculated according to the Dragstedt-Lang method.

No treatment related changes were found at necropsy. The body weight of surviving animals was unaffected by the treatment. At 100 mg/ kg bw no clinical signs were noted. At 180 and 320 mg/kg bw piloerection, reduced motor activity and hunched posture were observed on day 1 and day 2. At 580 mg/kg bw piloerection, dyspnoea, prostration, absence of traction and grasping reflex, muscular atony, reduced motor activity and unconsciousness were observed.

Discussion and conclusion:

Mecoprop-P is moderately toxic after oral administration to Sprague Dawley rats with a LD₅₀ value of 431 mg/kg bw.

B.6.2.1.1/03

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P: acute oral toxicity study in the rat
Reference	██████████ (1990a)
Date performed	20 February to 29 March 1990
Test facility	██
Report reference	Report no. ██████████
Guideline(s)	OECD 401 (1987)
Deviations from the guideline	None
GLP	Yes
Test material	Mecoprop-P, Batch 4235, purity not specified
Study acceptable	Yes

Study report:

██████████ (1990a): Mecoprop-P: acute oral toxicity study in the rat. ██████████
 ██████████ Report no. ██████████. 6 august 1990. Unpublished report. (Dossier ref. 5.5).

Study design and quality:

Groups of five male and five female CD rats were given single oral doses of 450, 567, 714, and 900 mg/ kg bw. The animals were fasted overnight prior to dosing. Mecoprop-P, technical grade (not further specified) was suspended in 0.5% aqueous carboxymethyl cellulose and a volume of 20 ml/kg was administered by gavage. The study design was comparable to OECD 401 and EU method B1 and compliance to GLP was stated.

Results:

The following clinical signs were observed: lethargy, unconsciousness, decreased motor activity, prone posture, ataxia, clonic convulsion, muscle tremor, breathing irregularities, ungroomed appearance, pigmented orbital secretion and hunched posture. In surviving animals unconsciousness and clonic convulsions were not observed. Animals at 450 mg/kg bw were less severely affected. After 14 days of observation the results with respect to dead animals given in Table B.6. 10 were obtained.

Table B.6. 10 LD₅₀ value for mecoprop-P in rats, oral administration

Doses (mg/kg bw)	Number of dead m rats	Number of dead f rats
450	0/5	0/4
567	0/5	1/5
714	1/5	0/5
900	4/5	5/5
LD ₅₀ (mg/ kg bw) 95% confidence limits	803 710-896	756 651-861
LD ₅₀ combined (mg/ kg bw) 95% confidence limits	775 666-885	

In the animals that died from the treatment the findings at necropsy were: altered stomach and jejunum content, dark thymic lymph nodes and pale perineal staining. No abnormal macroscopic findings were found in the surviving animals.

Discussion and conclusion:

The LD₅₀ for oral toxicity of mecoprop-P in rats was found to 775 mg/kg bw.

B.6.2.1.2 Acute oral toxicity in the mouse – dietary administration

This study was conducted to OECD 425 (2001) as a limit test. The current version of OECD 425 was published in 2008. The limit test is the same in both versions of the guideline.

Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012. New Study
----------------------	--

Study	Acute dietary toxicity of MCPP-p in mice Report No. 25405
Reference	██████████ (2009)
Date performed	17 June to 3 July 2008
Test facility	██
Report reference	Study No. ██████████
Guideline(s)	OECD 425 (2001) Limit test
Deviations from the guideline	Dose administered in the diet over a period of 24 hours to provide information on acute effects on small mammals for ecotoxicity assessment. The animals were dosed all at once and not sequentially.
GLP	Yes
Test material	Mecoprop-P potassium salt, sample HXP/1233, code Q121A, purity 595.6g/IOAI
Study acceptable	Yes, but study design is not suitable for acute oral toxicity classification purposes.

Executive Summary

An acute dietary toxicity study was conducted on mice to determine the toxicity via the dietary route.

The background food intake of five healthy female mice was established two days prior to commencement of the study. On day 0 the mice were given diet containing 20000 ppm Mecoprop-P. The treated diet was offered for 24 hours and consumption was tracked at 2, 4, 6, 8 and 24 hours. Mice were then fed a standard diet

throughout the 14-day observation period. Food consumption was measured daily on Days 1-7 and then on Day 14 (termination).

There were no deaths. All animals appeared active and healthy during the study. There were no clinical signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour.

Consumption of the treated diet was about 50% less than the standard food consumption.

All animals exhibited a slight reduction in body weight from Day 0 to Day 1, probably due to reduced food consumption of the treated diet. All animals gained weight from Day 0 to Day 7. One animal lost weight between Day 7 and Day 14, but all other animals gained weight.

No macroscopic abnormalities were noted for any of the animals at necropsy.

Based on the above results, the median lethal dietary dose (LDD₅₀) to female mice of Mecoprop-P K 600 after a single dietary dose is >3393 mg Mecoprop-P/kg.

A. MATERIALS AND METHODS

1. Test materials: Mecoprop-P K 600
Description: Clear brown liquid
Lot/Batch #: HXP/1233
Purity: 595.6 g Mecoprop-P/L
CAS #: 66423-05-0
Stability of test compound: Stable
2. Vehicle and/or positive control: Diet
3. Test animals
Species: Mouse
Strain: CD-1 mouse (Swiss derived) albino
Age: 9 weeks at dosing
Weight at dosing: 22-25 g

B. STUDY DESIGN AND METHODS

1. In life dates:

17 June – 03 July 2008

2. Animal assignment and treatment

Five female mice were selected for the study. Animals were fasted for 4 hours prior to the study start, to establish a baseline dietary assessment. Feed consumption was then monitored for 24 hours. Mice were then fasted for a further 4 hours before offering treated diet. Treated diets were prepared fresh on the day of administration to contain 20000 ppm Mecoprop-P.

Mice were observed several times on Day 0 and Day 1 and then daily during the 14-day observation period for clinical signs, morbidity and mortality. Individual body weights were recorded on days 0, 7 and 14.

Following the 14-day observation period, rats were sacrificed by carbon dioxide asphyxiation and subjected to gross pathological examination, consisting of external examination and opening of the abdominal and thoracic cavities.

3. Statistics

The data did not warrant statistical analysis.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed in any of the mice.

B. CLINICAL OBSERVATIONS

No clinical signs were observed in any of the mice.

C. BODYWEIGHT

All animals exhibited a slight reduction in body weight from Day 0 to Day 1, probably due to reduced food consumption of the treated diet. All animals gained weight from Day 0 to Day 7. One animal lost weight between Day 7 and Day 14, but all other animals gained weight.

D. NECROPSY

External examination of all mice did not reveal any significant abnormalities.

E. DEFICIENCIES

None

III. CONCLUSIONS

Based on the above results, the median lethal dietary dose (LDD₅₀) to female mice of Mecoprop-P K 600 after a single dietary dose is >3393 mg mecoprop-P/kg.

B.6.2.2. Dermal

Regulation (EU) 283/2013 stipulates that the acute dermal toxicity of the active substance is required unless waiving is scientifically justified. Both local and systemic effects shall be investigated.

Three studies on mecoprop-P are available which were done to the current guideline OECD 402 (1987).

A study on racemic Mecoprop () was included in the 1998 DAR but is not presented here as adequate studies are available on Mecoprop-P.

B.6.2.2.1/01

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Report on the study of the acute dermal toxicity in rats of CMPP (Mecoprop) - D-form
Reference	(1984)
Date performed	15 February to 9 March 1983
Test facility	partment of Toxicology, Ludwigshafen/ Rhein, Germany
Report reference	Report no. 84/152. 25
Guideline(s)	Equivalent to OECD 402 (1987)
Deviations from the guideline	No
GLP	No
Test material	Mecoprop-P, no batch or purity specified
Study acceptable	Yes

Study report:

(1984): Report on the study of the acute dermal toxicity in rats of CMPP (Mecoprop) - D-form. Report no. 84/152. 25 May 1984. Unpublished report. (Dossier ref. 5.6)

Study design and quality:

Five male and five female Wistar rats were by skin application exposed to mecoprop-P at doses of 2000 and 4000 mg/kg bw. Mecoprop-P (not further specified) was moistened with 0.5% aqueous carboxymethyl cellulose and was applied on a shaved area of 50 cm² on the dorsal part of the animals. A semi occlusive dressing was applied for 24 hours.

Although not stated, the study seems to comply to the OECD 402 guideline and EU test method B3. No statement according to GLP. Overall the study should be considered as acceptable.

Results:

No deaths and no abnormal findings were observed during clinical observation or at necropsy. Local erythema of the skin was observed day 1 at 4000 mg/kg bw.

Discussion and conclusion:

Mecoprop-P showed no acute toxicity after dermal application.

B.6.2.2.1/02

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P, acute dermal LD ₅₀ in the rat
Reference	██████████ (1994b)
Date performed	29 March to 12 April 1994
Test facility	██ ████████████████████
Report reference	██
Guideline(s)	OECD 402 (1987)
Deviations from the guideline	No significant deviations
GLP	Yes
Test material	Mecoprop-P, batch DA928, purity 897 g/kg
Study acceptable	Yes

Study report:

██████████ (1994b): Mecoprop-P, acute dermal LD₅₀ in the rat. ██████████
██ Report no. ██████████, 26 July 1994. Unpublished report. ██████████
94/11743. (Dossier ref. 5.7).

Study design and quality:

Five male and five female Sprague Dawley rats was dermally exposed to 2000 mg/ kg bw mecoprop-P (purity 897 g/kg). The test substance was moistened with 1 ml of 0.9% saline and applied to the clipped dorsal surface of the animals. The substance was covered with a gaze dressing for 24 hours. The study was stated to follow OECD 402 and GLP.

Results:

No treatment related abnormal finding was noted at the clinical observations. The body weight gain was not affected. One animal died, however, the death was judged to be due to a too tight dressing. At gross pathology general congestion of the organs of the abdominal cavity was found. In four animals a mild and diffuse pale colouring of the kidneys were observed, however, this was not considered treatment related.

Discussion and conclusion:

The LD₅₀ value for dermal application of mecoprop-P to the rat is above 2000 mg/kg bw.

B.6.2.2.1/03

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P: Acute percutaneous toxicity study in the rat
Reference	██████████ (1990b)
Date performed	1 March to 15 March 1990
Test facility	██
Report reference	Report no ██████████
Guideline(s)	OECD 402 (1987)
Deviations from the guideline	No significant deviations
GLP	Yes
Test material	Mecoprop-P, batch and purity not specified
Study acceptable	Yes

Study report:

██████████ (1990b): Mecoprop-P: Acute percutaneous toxicity study in the rat. ██████████
 ██████████ Report no. ██████████ 6 august 1990, unpublished report. (Dossier ref. 5.8).

Study design and quality:

Five male and five female CD rats were dosed with 2000 mg/kg bw mecoprop-P (technical grade, not further specified) on the clipped dorsum of each animal. The test substance was moistened with 0.2 ml of water and an occlusive dressing was applied for 24 hours.

The study was stated to comply to OECD 402 and GLP.

Results:

No animal died. No effect was observed with respect to body weight gain, clinical signs and reactions, or macroscopic lesions. The LD₅₀ by percutaneous administration was concluded to be greater than 2000 mg/kg bw.

Discussion and conclusion:

No signs of toxicity were observed after dermal exposure to 2000 mg/kg bw of technical mecoprop-P (purity not specified).

B.6.2.3. Inhalation

Regulation (EU) 283/2013 stipulates that the acute inhalation toxicity of the active substance required if the active substance meets criteria that indicate humans may be exposed via the inhalation route. The acute inhalation toxicity of mecoprop-P should be determined as it is included in products that are applied by spraying.

The Regulation specifies that head/nose only exposure shall be used unless whole body exposure can be justified. Three studies have been submitted which were reviewed in the 1998 DAR. Two used nose only exposure, but one of these are considered of limited reliability as adequate respirable particle size was not achieved. One study used whole body exposure and achieved a more acceptable particle size. In the interests of minimising vertebrate testing the whole body study is considered to be acceptable.

A study on racemic mecoprop ██████████ (1986b) was included in the 1998 DAR but is not presented here as adequate studies are available on mecoprop-P.

Study	Acute inhalation toxicity in rats 4 hour exposure to the dust of mecoprop (MCP) and mecoprop, isomer D
Reference	██████████ (1977)
Date performed	21 to 22 September 1977
Test facility	██████████
Report reference	BASF doc 94/11741
Guideline(s)	Broadly similar to OECD 403 (1981)
Deviations from the guideline	No significant deviations
GLP	No
Test material	Mecoprop-P, lot GD6720, purity 100% and mecoprop racemate lot GD6849 purity 93.0%
Study acceptable	Yes

Study report:

██████████ (1977): Acute inhalation toxicity in rats 4 hour exposure to the dust of mecoprop (MCP) and mecoprop, isomer D. ██████████ report no. not indicated (BASF doc 94/11741), report not dated (the study was conducted September-October 1977), unpublished report. (Dossier ref. 5.10).

Study design and quality:

Seven male and seven female Sprague-Dawley rats were exposed (whole body exposure) for 4 hours to either pure air, 0.012 mg/l mecoprop (purity: 93%), or 2.13 mg/l mecoprop-P (purity: 100%). Due to the wax-like nature of the quality of racemic mecoprop it was not possible to generate higher dust aerosol concentration than 0.012 mg/l. The dust aerosols were produced by a Wright Dust generator and air samples were analysed for particle size distribution using an Andersen mini-sampler. For mecoprop 73 wt. % and for mecoprop-P 61 wt.% of the particles had a diameter less than 5.5 µm.

In addition to the observation for clinical signs and post mortem macroscopic examinations, lung/body weight ratios were calculated.

Overall, the study is comparable to an OECD 403 study. The study was performed before implementation of GLP.

Results:

During mecoprop 0.012 mg/l exposure indications of irritation resulting in avoidance behaviour and peripheral vasodilation were observed. Ten minutes after exposure all animals appeared normal. During mecoprop-P 2.13 mg/l exposure laboured breathing and gasping occurred and at the end of the exposure one animal was found dead. The next day all animals appeared normal.

In both exposure groups some animals exhibited a slight decrease in body weight during the first three days of the observation period. No macroscopic changes or altered lung/body weight ratios were observed in the surviving animals. Haemorrhage and oedema was observed in the lungs from the animal that died during exposure.

Discussion and conclusion:

The LC₅₀ value for acute inhalation exposure to mecoprop-P dust in Sprague-Dawley rats is above 2.13 mg/L. The mass mean aerodynamic diameter was not calculated but 73% of particles were below 5.5 µm and therefore within the respirable range. This study is considered to be acceptable.

B.6.2.3/03

This study on mecoprop-P was done to OECD 403 (1981) which precedes the current guideline OECD 403 (2009). The guideline is very similar to the current version except that in the 1981 guideline the monitoring of the test atmosphere is less prescriptive.

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P: Acute inhalation toxicity study in the rat
Reference	██████████ (1990)
Date performed	1 to 15 February 1990
Test facility	██
Report reference	Report no. 90/0278
Guideline(s)	OECD 403 (1981)
Deviations from the guideline	No significant deviations
GLP	Yes
Test material	Mecoprop-P Batch 4235, purity not stated
Study acceptable	No – adequate respirable particle size was not achieved

Study report:

██████████ (1990): Mecoprop-P: Acute inhalation toxicity study in the rat. ██████████
 ██████████ report no. 90/0278, 15 June 1990, unpublished report. (Dossier ref. 5.11).
 First amendment to test report: no. 90/AMS022/1264, 9 January 1991.
 Second amendment to test report: no. 95/AMS022/0965, 25 September 1995.

Study design and quality:

Five male and five female CD rats were exposed (nose only exposure) to dust aerosols of 0.87 mg/l mecoprop-P (purity not specified) for 4 hours. A Wright dust feed mechanism generated the test atmosphere and the exposure level used was the maximal obtainable level. The chamber atmosphere was analysed five times during the study and a mean level of 0.87 mg/l was determined. The aerosol was characterized using a cascade impactor and the mass median equivalent aerodynamic diameter (MMEAD) was determined to 6.31 µm. 42.6% of particles were <6.0 µm and therefore within the respirable range. The relatively low exposure level and the relatively large particle size that was obtained was explained by the nature of the test substance.

The study is considered comparable to OECD 403. The report includes a statement concerning GLP standard.

Results:

No animal died during the study. During exposure the animals had reduced respiratory rate and brown staining around the snout. Body weight gain and body weight-relative organ weights were unaffected by the treatment.

Discussion and conclusion:

Inhalation exposure to 0.87 mg/l of mecoprop-P (dust exposure with a MMEAD of 6.3 mm) did not result in any acute lethality in rats. Some respiratory irritation occurred. Current guideline OECD 403 (2009) specifies the MMEAD should be 1 to 4 µm. Only 42.5% of particles were <6.0 µm and therefore within the respirable range. This test is considered of limited reliability as adequate respirable particle size was not achieved.

B.6.2.4. Skin irritation

Regulation (EU) 283/2013 stipulates that the skin irritancy of the active substance, and where relevant, the potential reversibility, is required.

Three *in vivo* studies are available on mecoprop-P. These are all conducted to (or similar to) OECD 1981, which is similar in protocol to the current guideline OECD 404 (2015) except that the new guideline proposes that *in vivo* testing should not be undertaken if classification can be determined by alternative methods.

A study on racemic mecoprop (██████████ 1983g) was included in the 91/414/EC Review but is not required as adequate studies are available on mecoprop-P.

B.6.2.4/01

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Report on the study of the irritation to the intact and abraded dorsal skin of the white rabbit based on Draize
Reference	██████████ (1983f)
Date performed	Experiment start date 21 Feb 1983
Test facility	████ ██████████ ██████████ █ ██████████ ████████████████████
Report reference	Report no. 84/030
Guideline(s)	Broadly similar to OECD 404 (1981)
Deviations from the guideline	Exposure was for 24 hours instead of 4 hours.
GLP	No
Test material	Mecoprop-P, batch and purity not stated
Study acceptable	Yes

Study report:

██████████ (1983f): Report on the study of the irritation to the intact and abraded dorsal skin of the white rabbit based on Draize. ██████████
Report no. 84/030, 29 December 1983, unpublished report. (Dossier ref. 5.13)

Study design and quality:

Four male and two female White Vienna rabbits were each exposed on two skin areas of 2.5 x 2.5 cm² (intact and abraded) to 0.5 g mecoprop-P (not further specified) suspended 1:1 in water. Exposure duration was 24 hours under an occlusive dressing. Readings according to the Draize system were done 30-60 min, 48 h, 72h, 8 d and 15 d after removal of the test substance.

Deviations from OECD guideline 404 and EU test method B4. Exposure period is 24 h instead of 4 h. Abraded skin exposure conducted as well. No statement concerning compliance to GLP. The study is considered to be of acceptable quality with respect to study conduct and reporting, however, the longer exposure duration should be noticed.

Results:

One animal died but this was not considered to be due to the treatment (no further explanation).

Intact skin: After 24 hours of exposure five animals developed well-defined erythema and one animal moderate to severe erythema. All animals developed very slight edema. The erythema persisted at the 48 h reading but at day 8 the erythema was barely perceptible. Haemorrhagic appearance and scaling was noted in one animal.

Abraded skin: After 24 hours of exposure three animals had developed well-defined and other three animals moderate to severe erythema. Two animals developed slight edema and four animals barely perceptible edema. At day 8 no edema was present and erythema was scored as barely perceptible in all animals. The erythema persisted at the 48 hours reading but at day 8 the erythema was barely perceptible. Haemorrhagic appearance (three animals) and scaling (all animals) were observed.

The mean score (intact skin area) for erythema and eschar formation can from the score table in the study report be calculated to 2.1 for the 24 h, 48h and 72h readings. For oedema the mean score can be calculated to 0.6.

Table B.6. 11 Skin irritation scores after application of mecoprop-P to the rabbit for 24 hours

Animal no.	Erythema						Oedema					
	1	2	3	4	5	6	1	2	3	4	5	6
24 hr	2	2	2	2	3	2	1	1	1	1	1	1
48hr	2	2	2	2	2	2	1	0	0	0	1	0
72 hr	2	2	2	2	2	2	0	0	0	0	1	0
Mean score per rabbit 24 – 72 hr	2	2	2	2	2	2	0.7	0.3	0.3	0.3	1	0.3
Group mean score 24-72hr	2.1						0.6					

Discussion and conclusion:

The response does not meet the criteria for classification as a skin irritant under Regulation (EC) 1272/2008. Under the conditions of this study mecoprop-P is not a skin irritant in the rabbit.

B.6.2.4/02

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Acute dermal irritation test in rabbit
Reference	██████████ (1994c)
Date performed	121 April to 15 April 1994
Test facility	██
Report reference	██
Guideline(s)	OECD 404 (1981)
Deviations from the guideline	No significant deviations
GLP	Yes
Test material	Mecoprop-P, purity 897 g/kg (94.4%), Batch No 6
Study acceptable	Yes

Study report:

██████████ (1994c): Acute dermal irritation test in rabbit ██████████
Report ██████████, 16 August 1994, unpublished report. (Dossier ref. 5.14)

Study design and quality:

Three female New-Zealand rabbits were on a clipped skin area of 6 cm² exposed to 500 mg of mecoprop-P (purity: 897 g/kg) moistened with 1 ml 0.9% saline. The area was covered with a semi-occlusive dressing for the exposure period of 4 hours. Cutaneous reactions according to the Draize scheme were evaluated 1, 24, 48, and 72 hours after removal of the dressing. The study was stated to be in accordance with GLP and OECD guideline 404.

Results:

At the 1h, 24h, 48h, and 72h readings the score "0" was obtained in all instances in all the animals for the evaluation for erythema and edema. The substance was concluded to be a non-irritant.

Discussion and conclusion:

According to this OECD 404 study mecoprop-P is not irritating.

B.6.2.4/03

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P: Acute dermal irritation/corrosion test in the rabbit
Reference	██████████ (1990a)
Date performed	27 February to 11 March 1990
Test facility	██
Report reference	Report no. 90/0499
Guideline(s)	OECD 404 (1981)
Deviations from the guideline	No significant deviations. Six animals used instead of three.
GLP	Yes
Test material	Mecoprop P, batch and purity not stated
Study acceptable	Yes

Study report:

██████████ (1990a): Mecoprop-P: Acute dermal irritation/corrosion test in the rabbit. ██████████
 ██████████ report no. 90/0499, 6 August 1990, unpublished report. (Dossier ref. 5.15).
 First amendment to test report: no. 90/AMS017/0499, 17 December 1990.

Study design and quality:

Six albino New Zealand male rabbits were exposed to 0.5 g mecoprop-P (technical grade, not further specified) on the closely clipped dorsa. The test site (6 cm²) was moistened with 0.2 ml of water before the test substance and a semi-occlusive dressing was applied for a duration of 4 hours. Dermal reactions were assessed at 1, 24, 48, and 72 hours and on day 7, 10 and 13.

The study is considered comparable with OECD 404 and was carried out according to GLP.

Results:

Very slight, occasionally, slight erythema was observed at the test site of five animals from the 1 h reading to the reading on day 10. Three animals showed exfoliation on day 7 and/or day 10. Normal appearance of the skin on day 13. The mean score for erythema (for the 24 h, 48h and 72 h readings) was calculated to 0.8. The mean score for oedema was calculated to 0.0. The substance was concluded to be a "non-irritant".

Discussion and conclusion:

In this study only very slight to slight irritative responses were noted after 4 h of dermal contact to mecoprop-P. The test results do not meet the criteria for classification.

B.6.2.5. Eye irritation

Regulation (EU) 283/2013 stipulates that the potential of eye irritancy of the active substance, and where relevant, the potential reversibility, is required.

Two *in vivo* eye irritation studies on mecoprop-P have been submitted. Both were conducted to previous versions of the OECD guideline 404, but are broadly similar to the current guideline OECD 404 (2012). The new guideline puts more emphasis on animal welfare and proposes that *in vivo* testing should not be undertaken if classification can be determined by alternative methods.

A study on racemic mecoprop (██████████ 1983i) was included in the 91/414/EC Review but is not required as adequate studies are available on mecoprop-P.

B.6.2.5/01

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Report on the study of the irritation to the eye of the white rabbit based on Draize of CMPP (Mecoprop) (D-form)
Reference	██████████ (1983h)
Date performed	Not stated but presumed to be in 1983
Test facility	██████████ ██████████ ██████████ ██████████ ██████████
Report reference	Report no. 84/031
Guideline(s)	Equivalent to OECD 405 (1981)
Deviations from the guideline	No significant deviations
GLP	No
Test material	Mecoprop-P, no batch or purity specified
Study acceptable	Yes

Study report:

██████████ (1983h): Report on the study of the irritation to the eye of the white rabbit based on Draize of CMPP (Mecoprop) (D-form). ██████████
 ██████████ Report no. 84/031, 29 December 1983, unpublished report. (Dossier ref. 5.16).

Study design and quality:

Three male and three female White Vienna rabbits were by application in the conjunctival sac exposed to 0.1 ml (39 mg) mecoprop-P (not further specified). Signs of irritation were evaluated 1 h, 24 h, 48 h, and 72 h after application. No statement with respect to compliance to GLP. The study seems very much comparable to OECD 405 and thus is considered as acceptable.

Results:

At 72 hours the study was discontinued because of severe irritation in all animals. Signs consisting of suppuration, pupil contraction, marginal vascularization of the cornea, detachment of the cornea, and small retractions of the eyelids were observed in the animals after 72 hours.

The following scoring was obtained (based on Draize):

Table B.6. 12 Corneal and iris score after administration of mecoprop-P to the rabbit eye

	Number of animals with						iris score			
	cornea score (opacity)						iris score			
	0	1	2	3	4	*	0	1	2	*
24 h reading	-	-	5/6	-	-	1/6	-	1/6	4/6	1/6
48 h reading	-	-	4/6	-	-	2/6	-	-	4/6	2/6
72 h reading	-	-	-	3/6	-	3/6	-	-	3/6	3/6
Mean score	2.3						1.6			

Cornea score 2: easily discernible translucent areas, details of iris slightly obscured.

Cornea score 3: opalescent areas, no details of iris visible, size of pupil barely discernible

Iris score 1: Folds above normal, congestion, swelling, circumcorneal injection. Iris still reacting to light.

Iris score 2 : no reaction to light, haemorrhage, gross destruction (any or all of these)

* Animals could not be scored because of suppuration (are not included in mean score calculation)

For the conjunctivae the following mean scores could be calculated:

Conjunctivae mean score (redness): 2.6 (mean score from a total of 12 scorings, six scorings could not be undertaken due to supuration).

Conjunctivae mean score (chemosis): 2.7 (mean score for all animals)

Discussion and conclusion:

The study determines mecoprop-P to be a severe eye irritant. The scoring shown in the table B.6.12 meets the Classification under (EC) 1272/2008 as a Category 1 for eye irritation/corrosion with the associated hazard statement: H318 causes serious eye damage.

B.6.2.5/02

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P: Acute eye irritation/corrosion test in the rabbit. Life Science Research Limited, Suffolk
Reference	██████████ (1990b)
Date performed	19 to 22 March 1990
Test facility	██
Report reference	90/AMS018/0500
Guideline(s)	OECD 405 (1987)
Deviations from the guideline	No significant deviations
GLP	Yes
Test material	Mecoprop-P, batch and purity not stated.
Study acceptable	Yes

Study report:

██████████ (1990b): Mecoprop-P: Acute eye irritation/ corrosion test in the rabbit ██████████

██████████ Report no. 90/0500 (BASF 90/10932), unpublished report.

First amendment to study report: no. 90/AMS018/1135, 17 December 1990. (Dossier ref. 5.17).

Study design and quality:

One albino New Zealand rabbit was exposed by application of 0.1 g mecoprop-P (not further specified) in the conjunctival sac. Ocular reactions were examined 1, 24, 48 and 72 hours after the treatment. The study complies with OECD 405 and is stated to follow GLP.

Results:

Cornea: slight opacity (covering the entire cornea) was observed one hour after installation and was accompanied by areas of severe opacity (score "4") at the 24, 48 and 72 hour observations.

Conjunctiva: Injection of the conjunctival blood vessels, conjunctival redness (diffuse and beefy-red appearance), slight chemosis and moderate discharge with mucous were apparent during the 72 hour observation period. A mean value of 2 for redness and of 1.3 could be calculated for the 24h , 48h, and 72h readings.

Iris: congestion was apparent at the 72 hour examination (score "1"). Mean score for the 24h, 48h, and 72h readings could be calculated to 0.3.

Due to the severity of the reactions the animal was killed after the 72 hour examination. Mecoprop-P was considered to be "severely irritant".

Discussion and conclusion:

Mecoprop-P was in this study found to be a severe eye irritant that due to the scoring for corneal opacity meets the criteria in Regulation (EC) 1272/2008 as a Category 1 for eye irritation/corrosion with the associated hazard

statement: H318 causes serious eye damage.

B.6.2.6. Skin sensitization

Regulation (EU) 283/2013 stipulates that the potential of the active substance to provoke skin sensitisation reactions is required (except where the active substance is a known sensitiser). The local lymph node assay (LLNA) shall be used. Where a guinea pig assay (Maximisation or Buehler), meeting OECD guidelines and providing a clear result, is available, further testing shall not be carried out for welfare reasons.

Three skin sensitisation studies conducted on mecoprop-P have been submitted: two are guinea pig maximisation tests and one is a Buehler test. Two of the tests were conducted to the current OECD 406 test guideline (1992) and are acceptable studies. A third study conducted to the superseded guideline OECD 406 (1981) has some deficiencies.

A maximisation and an open epicutaneous test on racemic mecoprop [REDACTED], 1984a + b) were included in the 1998 DAR but are not required as adequate studies are available on mecoprop-P.

B.6.2.6/01

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Report on the maximization test for the sensitizing potential of CMPP (mecoprop) - D-form in guinea pigs
Reference	[REDACTED] (1985)
Date performed	10 July to 9 August 1984
Test facility	[REDACTED]
Report reference	Report no. 85/392 (project no. 30H20/83-1)
Guideline(s)	OECD 406 (1981)
Deviations from the guideline	No justifications were given with regard to choice of vehicle in the study and with regard to solubility of the test substance in the vehicle used.
GLP	No but subjected to quality assurance inspection
Test material	Mecoprop-P, purity 98.5%. Batch 154 241 N1
Study acceptable	No, the aqueous vehicle was considered inappropriate

Study report:

[REDACTED] (1985): Report on the maximization test for the sensitizing potential of CMPP (mecoprop) - D-form in guinea pigs. [REDACTED] Report no. 85/392 (project no. 30H20/83-1), 29 November 1985, unpublished report. (Dossier ref. 5.18).

Study design and quality:

A guinea-pig maximization test was performed according to OECD 406 with twenty female Pirbright White guinea pigs. Two control groups of ten animals were used.

The following formulations were used (mecoprop-P was of a purity of 98.5%):

Intradermal induction (day 0).

Injection site 1 (test group and control groups) - 0.1 ml Freund's adjuvant/ aqua dist. (1:1)-
 Injection site 2 (test group) - 0.1 ml 10% mecoprop-P in paraffin oil/ aqua dist. (1:1)-
 Injection site 2 (control groups) - 0.1 ml paraffin oil/ aqua dist. (1:1)-
 Injection site 3 (test group) -0.1 ml 10% mecoprop-P in Freund's adjuvant/ aqua dist. (1:1)-
 Injection site 3 (control groups) -0.1 ml Freund's adjuvant/ aqua dist.

Percutaneous induction (day 7-9).

Test group: 50% mecoprop-P in aqua dist., administered on paper strips under occlusion.

Control groups: No treatment.

First challenge (day 20).

Test group: 10% mecoprop-P in aqua dist., administered on paper strips under occlusion.

Control group 1: 10% mecoprop-P in aqua dist., administered on paper strips under occlusion.

Control group 2: no treatment

Second challenge (day 27).

Test group: 10% mecoprop-P in aqua dist., administered on paper strips under occlusion.

Control group 1: 10% mecoprop-P in aqua dist., administered on paper strips under occlusion.

Control group 2: 10% mecoprop-P in aqua dist.

In a preliminary test (skin application of paper strips soaked with test formulation under occlusive dressing for 24 h) minimum irritant concentrations of mecoprop-P was found to be a 50% aqueous formulation and the maximum non-irritant concentration was found to 25% aqueous formulation.

No justifications were given with regard to choice of vehicle in the study and with regard to solubility of the test substance in the vehicle used.

The report is stated to follow OECD guideline 406.

Results:

The number of animals with skin findings is shown in Table B.6. 13. The response in most of the animals (controls and test animals) consisted of a thin scab like layer. The total response represents number of animals with a reaction of scab-like layer and/or erythema and/or oedema. Furthermore, the animals reacting solely with erythema or oedema are also indicated in the table. The highest score obtained for erythema and oedema was a score of "1" indicating slight erythema or slight oedema.

Table B.6. 13 Challenge response in guinea pig maximization test with mecoprop-P

	1st challenge 10% mecoprop-P in aqua dist.		2nd challenge 10% mecoprop-P in aqua dist.	
	48 h after application	72 h after application	48 h after application	72 h after application
control group 1* total response erythema or oedema	0/9 0/9	8/9 0/9	1/9 0/9	6/9 0/9
control group 2* total response erythema or oedema	no application of test solution		0/9 0/9	4/9 1/9
test group total response erythema or oedema	2/20 1/20	20/20 0/20	13/20 4/20	19/20 6/20

* The reduced number of control animals was due to sacrifice of animals because of suspicion of pneumonia.

The percentage of reacting animals in the control groups at 72 h was judged to be comparable to the percentage of reacting animals in the test group. From this finding it was suggested that the pre-treatment with Freund's adjuvant had lowered the irritation threshold. Further the time course of the skin reactions was taken into account and it was concluded that mecoprop-P should not be considered as sensitizer.

Discussion and conclusion:

The total response at 72 h after second challenge in the test group is higher (19/20) than the response from the two

control groups (10/18). It is difficult to assess the relevance of the observation of the so-called scab-like layer. If it is considered as sign of irritation then a too high mecoprop-P concentration was used for challenge as reaction was observed in control animals as well. If only the observation of erythema or edema is considered the response at 72 h after the second challenge (6/20) is borderline with respect to EU classification for R43 (may cause sensitization by skin contact).

Further, mecoprop-P is only slightly soluble in distilled water (about 860 mg/l) and thus the 50% and 10% suspensions possess a low and variable degree of bioavailability for the dermal tissue. Accordingly it is preferable to use solutions of mecoprop-P (ethanol/water or acetone/water solutions) to obtain a higher and more uniform bioavailability. Compared to the Buehler test described below in section B.6.2.6/02 a much lower irritation threshold was found for dissolved mecoprop-P in acetone/water (irritation threshold in the range of 0.3-5% solution) than in this study (irritation threshold in the range of 25-50% for aqueous suspensions).

Thus the conduct of another guinea pig maximization test using more suitable solvents to ensure bioavailability is recommended for establishing a better basis for assessment of sensitizing potential.

B.6.2.6/02

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P delayed contact hypersensitivity study in the guinea pig
Reference	██████████ (1994)
Date performed	19 April to 1 June 1994
Test facility	██
Report reference	Report no. 94/0517 (BASF Doc 94/11740)
Guideline(s)	OECD 406 (1992)
Deviations from the guideline	No significant deviations
GLP	Yes
Test material	Mecoprop-P, Batch DA 928, purity 897 g/kg (94.4%)
Study acceptable	Yes

Study report:

██████████ (1994): Mecoprop-P delayed contact hypersensitivity study in the guinea pig. ██████████ report no. 94/0517, 1 August 1994, unpublished report. (Dossier ref. 5.19).

Study design and quality:

A modified Buehler test was performed with ten male and ten female albino guinea pigs in the test group and with five male and five female guinea pigs in the control group. The study was conducted according to OECD 406 with the exception that induction exposure was performed on day 0, 2, 4, 7, 9, 11, 14, 16, and 18 instead of day 0, 7, and 14 only.

Induction exposures were performed at the left flank of the animals using a patch moistened with 0.5 ml of a 5% w/v mecoprop-P (purity: 897g/kg) solution in 80% ethanol/water. The application site was covered with an occlusive dressing for the exposure period of six hours. For challenge exposure at day 27 patches moistened with 0.5 ml of pure acetone, 0.5 ml of 0.5% w/v mecoprop-P in acetone and 0.5 ml of 3% w/v mecoprop-P in acetone were applied on three parts of shaved skin on the opposite site of the induction site areas and covered with occlusive dressing for six hours. (Due to equivocal results in a primary irritation screen with acetone as vehicle both 0.5% and 3% mecoprop-P solutions were used).

Statement of compliance to GLP. The deviations made from the original Buehler test is not considered to weaken the test. Thus, the study is considered acceptable.

Body weight: Individual body weights for all animals were recorded at test commencement and at test completion.

The induction procedure consisted of intradermal injection of a 0.1 ml dose of Freund's adjuvant emulsified in saline 1:1, test material alone and Freund's adjuvant and test material in saline 1:1. Percutaneous induction was carried out one week after the intradermal application by applying paper strips soaked in the test substance for 48 hr.

The animals were challenged three weeks after induction with dermal application of 1 % test solution as mentioned above but only half the amount was applied.

Results:

After challenge none of the animals had positive reactions.

Conclusion:

Mecoprop-P was considered a non-sensitiser in this study.

Comments:

The study is well documented. The dose and vehicle selections are properly made and the study seems well performed.

B.6.2.7. Phototoxicity

Regulation (EU) 283/2013 stipulates that the potential phototoxicity of the active substance should be investigated in an *in vitro* study where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light exposed areas of the skin, either by direct contact or through systemic distribution (unless the ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than $10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$).

For mecoprop-P the maxima (peaks) for absorbance are all <290 nm, in the range 227 – 286 nm and the coefficients (for the peaks) are much greater than 10 L/mol/cm. However, there is absorbance (but no maxima) at 290 nm and the coefficient is likely to be > 10 L/mol/cm.

In conclusion absorption > 10 L/mol/cm in the range 290-700 nm is likely, therefore the need for a phototoxicity study is triggered.

Previous evaluation:	None: Submitted for the purposes of renewal New Study. Submitted to meet the new data requirements under (EU) 283/2013.
----------------------	--

Study	Mecoprop-P TGAI: cytotoxicity assay in vitro with BALB/c 3T3 cells: Neutral Red (NR) testing during simultaneous irradiation with artificial sunlight
Reference	██████████ (2014)
Date performed	15 to 17 July 2014
Test facility	██
Report reference	1643404
Guideline(s)	OECD 432 (2004)
Deviations from the guideline	No
GLP	Yes
Test material	Mecoprop-P, purity 92.63%, batch 3860
Study acceptable	Yes

Method:

96 well plates were seeded with 2×10^4 cells per well of BALB/c3T3 cells clone 31. The cells were treated with Eagle's Balanced Salt Solution (EBSS) containing mecoprop-P (dissolved in DMSO). The final concentration of DMSO in the culture medium was 1%. Chlorpromazine was used as the positive control. The solvent control was EBSS containing 1% DMSO. Eight different concentrations of mecoprop-P were tested.

The light source was a Dr Hönle Sol 500 solar simulator. The wavelength produced was >320 nm. The irradiated groups were treated with artificial sunlight for 50 minutes with 1.65 mW/cm² UVA, resulting in an irradiation dose of 5 J/cm² UVA. The unirradiated groups were kept in the dark for 50 minutes. The cells were then treated with Neutral Red in accordance with the OECD test guideline, and absorbance at 540 nm was determined as a measure of cell viability.

Results:

The optical density was measured at 540 nm and compared to the solvent control. No cytotoxic effects were observed after treatment with mecoprop-P neither in the presence or absence of irradiation. The positive control induced phototoxicity in a range that was within the historical positive control range.

Table B.6. 14 Phototoxicity test conducted on mecoprop-P and chlorpromazine (positive control). % OD₅₄₀ nm compared to solvent control

Chlorpromazine Without irradiation		Chlorpromazine With irradiation		Mecoprop-P without irradiation		Mecoprop-P with irradiation	
µg/mL	% of solvent control	µg/mL	% of solvent control	µg/mL	% of solvent control	µg/mL	% of solvent control
6.25	98.72	0.125	103.53	7.81	98.77	7.81	101.18
12.5	78.05	0.25	96.67	15.6	99.50	15.6	101.00
25	49.18	0.5	78.05	31.3	96.88	31.3	99.38
37.5	45.17	0.75	58.70	62.5	98.49	62.5	102.20
50	44.69	1.0	49.32	125	94.70	125	98.21
75	44.02	1.5	54.68	250	99.22	250	98.00
100	48.42	2.0	49.32	500	96.79	500	94.04
2000	45.31	4.0	56.33	1000	97.82	1000	92.67

Conclusion:

Under these experimental conditions mecoprop-P did not have any phototoxic effects on BALB/c3T3 cells.

B.6.2.8 Summary of acute toxicity studies

Most of the acute toxicity studies were submitted for the previous renewal review in the 1998 DAR. New studies are highlighted in grey. The most relevant and reliable studies for classification purposes are highlighted in **bold**.

Table B.6. 15 Summary of acute toxicity studies on Mecoprop-P

Study	Species	Test Substance: Racemate or D-Isomer	LD ₅₀ (mg/kg bw)	Reference
Acute oral	Rat	D-Isomer (Mecoprop-P)	1050 (m/f: 1327/ 681-1000)	██████████ (1983a)
Acute oral	Rat	D-Isomer (Mecoprop-P)	431 (for both m and f)	██████████ (1994a)

Study	Species	Test Substance: Racemate or D-Isomer	LD ₅₀ (mg/kg bw)	Reference
Acute oral	Rat	D-Isomer (Mecoprop-P)	775 (m/f: 803/ 756)	██████████ (1990a)
Acute dietary	Rat	D-Isomer (Mecoprop-P)	3393	██████████ 2009)
Acute dermal	Rat	D-Isomer (Mecoprop-P)	>4000	██████████ (1984)
Acute dermal	Rat	D-Isomer (Mecoprop-P)	>2000	██████████ (1994b)
Acute dermal	Rat	D-Isomer (Mecoprop-P)	>2000	██████████ (1990b)
Acute inhalation	Rat	D-Isomer (Mecoprop-P)	> 5.6 mg/L*	██████████ (1986a)
Acute inhalation	Rat	D-Isomer (Mecoprop-P)	> 2.13mg/L	██████████ 1977
Acute inhalation	Rat	D-Isomer (Mecoprop-P)	> 0.87mg/L*	██████████ (1990)
Skin irritation	Rabbit	D-Isomer (Mecoprop-P)	Non-irritant**	██████████ (1983f)
Skin irritation	Rabbit	D-Isomer (Mecoprop-P)	Non-irritant	██████████ (1994c)
Skin irritation	Rabbit	D-Isomer (Mecoprop-P)	Non-irritant	██████████(1990a)
Eye Irritation	Rabbit	D-Isomer (Mecoprop-P)	Category 1 eye irritant	██████████ (1990b)
Eye Irritation	Rabbit	D-Isomer (Mecoprop-P)	Category 1 eye irritant	██████████ (1983)
Skin sensitisation (M&K)	Guinea pig	D-Isomer (Mecoprop-P)	Not a skin sensitiser***	██████████ (1985)
Skin sensitisation (Buehler)	Guinea pig	D-Isomer (Mecoprop-P)	Not a skin sensitiser	██████████ (1994)
Skin sensitisation (M&K)	Guinea pig	D-Isomer (Mecoprop-P)	Not a skin sensitiser	██████████ (1995)
Phototoxicity	<i>In vitro</i> BALB/c 3T3 cell line	D-Isomer (Mecoprop-P)	Not phototoxic	██████████ (2014)

* Study of limited reliability because of limit on the percentage of respirable particles generated

** Study had 24 hour exposure period instead of 4 hour exposure period normally required for classification

*** Study of limited reliability because of inappropriate vehicle

Summary and classification for acute toxicity under (EC) 1272/2008

Three acute oral toxicity studies have been conducted on mecoprop-P. The LD₅₀ ranged from 431 to 1050 mg/kg bw. The study by ██████████ (1994a) with LD₅₀ of 431 mg/kg bw is considered the most reliable as a basis for classification. In conclusion mecoprop-P should be classified Category 4 for acute oral toxicity with the hazard statement H302 Harmful if swallowed. This is in agreement with the current harmonised classification for mecoprop-P.

There are three suitable acute dermal studies which indicate that mecoprop-P is of low toxicity via the dermal

route so does not require classification for dermal toxicity. The most reliable study is considered to be the one by Dange (1994b) with a LD₅₀ > 2000 mg/kg bw.

Three acute inhalation studies have been conducted on mecoprop-P report LC₅₀ values of >0.87 mg/L, >2.13 mg/L, and >5.6 mg/L. In all three studies a dust aerosol was generated but in all the studies there were some technical difficulties in achieving particles in the respirable range. There was no evidence that mecoprop-P is toxic by the inhalation route. The most reliable study is the one by [REDACTED] (1977) with LC₅₀ of >2.13 mg/L. It is concluded that mecoprop-P does not require classification with respect to acute inhalation toxicity.

Three skin irritation studies have been conducted on mecoprop-P. In all three studies mecoprop-P was not classified as a skin irritant. The most reliable study is considered to be the one by [REDACTED] (1994c). Mecoprop-P does not require classification as a skin irritant.

There are two eye irritation studies conducted on mecoprop-P. In both studies mecoprop-P was severely irritating to the eyes. In conclusion mecoprop-P should be classified Category 1 for eye irritancy/corrosion with the hazard statement H318 causes serious eye damage. This is in agreement with the current harmonised classification for mecoprop-P.

Three skin sensitisation studies have been conducted on mecoprop-P, a Buehler test and two guinea pig maximisation tests. Mecoprop-P was not found to be a skin sensitiser in any of the studies. The most reliable study was the one by [REDACTED] (1995) submitted in the 2002 DAR addendum. In conclusion mecoprop-P does not require classification with regards to skin sensitisation.

A new acute dietary mouse study using dietary administration has been submitted for this renewal. In the study, mice were given diet containing 20,000 ppm mecoprop-P (3,393 mg/kg bw) over the duration of 1 day, rather than receiving a single gavage dose. Under the conditions of the study there were no mortalities. The median lethal dietary dose (LDD₅₀) to female mice of Mecoprop-P after a single dietary dose is >3,393 mg/kg bw. The study reflects a more typical exposure for wild mammals feeding on food contaminated with mecoprop-P, and therefore this endpoint should be used in the mammals' risk assessment (refer to CP Section 10).

Mecoprop-P triggers the need for a phototoxicity study. There were no indications of phototoxicity in a new guideline compliant study.

Consideration of STOT SE classification

Specific target organ toxicity – single exposure (STOT SE) is defined as specific, non lethal target organ toxicity arising from a single exposure. STOT SE classification is relevant to effects caused after a single exposure that are not covered more appropriately by another hazard class. Mecoprop-P already has a harmonised classification for acute oral toxicity H302 due to lethal effects via oral exposure. Clinical signs observed prior to death were generalised indicators of toxicity and distress, and did not indicate any particular type of target organ toxicity (such as neurotoxicity or narcotic effects) nor was any target organ toxicity identified during the pathology examination. It is concluded that mecoprop-P does not require STOT SE classification.

B.6.3. SHORT-TERM TOXICITY

Studies on both racemic mecoprop and mecoprop-P were considered in the 91/414/EC review and were considered to be acceptable. It is considered that seven studies on mecoprop-P are most relevant to this submission to support mecoprop-P.

A gavage study on racemic mecoprop potassium salt [REDACTED], 1989) was included in the 91/414/EC Review but is not included in this renewal as adequate studies are available on mecoprop-P.

Study design and quality:

Five groups of ten male and ten female Wistar rats were for 7 weeks fed a diet containing either 0, 50, and 400 ppm mecoprop (racemic form; 92.7% purity) equivalent to 0, 4.4/4.8, 35.1/37.5 mg/kg bw/day in males/females or mecoprop-P (D-form; 99.4% purity) equivalent to 0, 4.4/4.8, 35.2/38.0 mg/kg bw/day in males/females. The aim of study was to determine differences in the toxic response between racemic mecoprop and mecoprop-P (D-form). The duration of the 28 days study was extended by 21 days of dosing with the purpose to follow an observed increase in creatinine level found in the 400 ppm mecoprop-P group at day 23.

The study was conducted according to OECD 407 (except study duration). The study is not conducted according to GLP, but there has been Quality Assurance. The study is considered acceptable. The liver, kidneys, testes and adrenals were weighed. Histopathology was conducted on the liver and kidney in all dose groups, and all gross lesions and the spleen, adrenals and heart in the control and 400 ppm dose groups.

Results:

No changes in food consumption and body weight were seen between the dosed groups and the control group. In none of the dosed groups clinical toxic signs were observed. No animals died during the study period. The following clinicochemical changes were found:

- significantly reduced cholesterol level in females at 400 ppm mecoprop racemate and at 400 ppm mecoprop-P in both blood samples (day 23 and day 43) and in male rats at 400 ppm mecoprop-P only in the first blood sample.
- significantly reduced calcium concentration in female animals at 400 ppm racemate in blood sample from day 43.
- significantly increased creatinine and urea values in females at 400 ppm mecoprop-P (blood sample at day 23 only) and increased urea concentration in males at 400 ppm mecoprop racemate (day 43 only). Due to these findings a marginal renal insufficiency was suggested.
- significantly increased glutamic-pyruvic transaminase (alanine aminotransferase) in males at 400 ppm mecoprop and mecoprop-P.

Table B.6. 16 Kidney weight changes in rats administered mecoprop (racemate) or mecoprop-P (D form) in the diet for seven weeks (mean values 10 animals per dose/sex)

Dose ppm	Males					Females				
	0	50 ppm racemate	50 ppm D form	400 ppm racemate	400 ppm D form	0	50 ppm racemate	50 ppm D form	400 ppm racemate	400 ppm D form
Dose Mg/kg bw/day	0	4.4	4.4	35.1	35.2	0	4.8	4.8	37.5	38.0
Terminal body weight (g)	401.80	409.90	401.80	402.50	396.00	236.90	234.40	233.90	227.10	233.10
Kidney weight (g)	2.668	2.834	2.806	2.868	2.892*	1.736	1.720	1.703	1.765	1.882*
Kidney weight compared to control		6%↑	5%↑	7%↑	8%↑		1%↓	2%↓	2%↑	8%↑
Relative kidney weight (%)	0.664	0.691	0.699	0.713	0.730	0.733	0.734	0.728	0.777	0.807
Relative kidney weight compared to control		4%↑	5%↑	7%↑	10%↑		0%	1%↓	6%↑	10%↑

*Statistically different to control

No statistical tests conducted on relative weight changes.

The only significant pathological finding was a slight increase in absolute kidney weight which occurred both sexes treated with mecoprop and mecoprop-P, but only attained statistical significance in females dosed with 400 ppm mecoprop-P (relative kidney weight showed a similar trend). The change was not accompanied by any adverse kidney histopathology, but some clinical chemistry changes suggested a marginal renal insufficiency.

Due to these findings it was concluded that there were no indications of any significant differences in toxicity from racemic mecoprop compared to mecoprop-P (D-form).

Discussion and conclusion:

No significant differences in toxicity between mecoprop in racemic form and D-form were found in this study.

The kidney was found to be the target organ for toxicity. The NOAEL in the study was 50 ppm for both mecoprop and mecoprop-P (corresponding to 4.4/4.8 mg/kg bw/day in males/females respectively) based on increased kidney weight and marginal changes in clinical chemistry at 400 ppm.

B.6.3.2. Oral 90-day study

The data requirements under (EU) 283/2013 stipulate that a 90 day study in rodents and non-rodents shall be provided. 90 day studies in rat and dog have been submitted and meet this data requirement. In addition there is a 90 day mouse study.

B.6.3.2.1 Oral 90-day study in the rat

B.6.3.2.1/01

This study on mecoprop and mecoprop-P was conducted in 1977 which is prior to GLP and OECD test guidelines the study is similar to OECD 408 (1998) except relative organ weights were not recorded in the original study report.

During this renewal review the RMS has reviewed this study and reduced the LOAEL from 400 ppm to 200 ppm because historical control data critical to support the LOAEL is not to current standards.

The applicant considers the NOAEL in this study should remain at 200 ppm and that this is a critical study for setting the AOEL.

Previous evaluation:	In DAR for first review (1998).
----------------------	---------------------------------

Study	Mecoprop, 3-month oral toxicity study in the rat (racemate, D-isomer)
Reference	██████████ (1979)
Date performed	12 April to 13 July 1977
Test facility	██
Report reference	Rep no. ██████████ (BASF77/10592)
Guideline(s)	Comparable to OECD 408 (1998)
Deviations from the guideline	Relative organ weights were not calculated
GLP	No
Test material	Mecoprop racemate, batch GD 6849, 93% purity, and mecoprop D-isomer, batch GD 6720, 100% purity
Study acceptable	Yes

Study report:

██████████ 979): Mecoprop, 3-month oral toxicity study in the rat (racemate, D-isomer). ██████████ (not further specified), France. Rep no. ██████████ (BASF77/10592), 14 November 1977 (translated 17 April 1979), unpublished report. (Dossier ref. 5.21).

Study design and quality:

Fifteen male and fifteen female Sprague Dawley rats in each dose group were for 3 months given a diet containing 0, 200, 800, and 3200 ppm racemic mecoprop (93% purity) equivalent to 0, 16.5/18.2, 67.9/75.9,

390.8/398.7 mg/kg bw/day in males/females respectively, and 0, 200, 400, 800, 1600, and 3200 ppm mecoprop-P (D-form, 100% purity) equivalent to 0, 15.6/18.4, 31.9/37.8, 67.6/75.8, 146.4/170.1, 403.2/403.5 mg/kg bw/day in males/females respectively. The design and conduct of the study was very comparable to OECD 408. No satellite group for follow-up observation was included and there were some minor deviations with respect to the examination of clinical chemical parameters. Clinical chemical and haematological examinations were conducted week 4, 8, and 12. Due to technical problems no histopathological examination of the eyes were made. Organs that would provide an indication of potential immunotoxicity, neurotoxicity and hormonal effects were also subject to histological examination, such as the spleen, thymus, mesenteric lymph node, thyroid, adrenals, bone marrow, pituitary, testicles, seminal vesicles, ovaries, uterus, mammary gland, prostate, and nervous tissue. Haematological parameters such as leucocyte count, and differential leucocyte count would provide further indications of potential immunotoxicity. The following organs were weighed: heart, liver, spleen, kidneys, adrenals, uterus, gonads (not further specified), brain, thyroid, pituitary, prostate, thymus.

No statement with regard to GLP. The study is considered acceptable.

Results:

Table B.6. 17 Body and organ weight changes following dietary administration of mecoprop or mecoprop-P to rats for 13 weeks (mean values, 15 animals per dose/sex)

Parameter	Males						Females					
	0 ^A	200	400	800	1600	3200	0 ^A	200	400	800	1600	3200
Mecoprop mg/kg bw/day	0	16.5	-	67.9	-	390.8	0	18.2	-	75.9	-	398.7
Mecoprop-P mg/kg bw/day	0	15.6	31.9	67.6	146.4	403.2	0	18.4	37.8	75.8	170.1	403.5
Mecoprop Body weight at 13 weeks	376	377	-	373	-	<u>242</u> ***	258	251	-	242*	-	<u>186</u> ***
Mecoprop-P Body weight at 13 weeks	384	382	388	378	<u>348</u> **	<u>241</u> ***	251	251	244 *	240 *	<u>221</u> ***	<u>192</u> ***
Mecoprop Liver weight (g)	12.91	12.78	-	12.72	-	12.80	8.13	7.81	-	8.47	-	<u>9.45</u> **
Mecoprop-P Liver weight (g)	13.22	12.86	13.06	12.50	13.87	12.90	7.57	7.90	7.98	8.39	<u>9.12</u> **	<u>9.94</u> ***
Mecoprop Kidney weight (g)	2.28	<u>2.48</u> **	-	<u>2.52</u> **	-	<u>1.63</u> ***	1.51	<u>1.64</u> **	-	1.57	-	<u>1.27</u> ***
Mecoprop Kidney weight change to control group A		9%↑	-	11%↑	-	29%↓		9%↑	-	4%↑	-	16%↓
Mecoprop-P Kidney weight (g)	2.22	<u>2.47</u> ***	<u>2.60</u> ***	<u>2.49</u> ***	2.28	<u>1.63</u> ***	1.48	1.56	1.58	1.57	1.49	1.35 **
Mecoprop-P Kidney weight versus group A		8%↑	14%↑	9%↑	3%↓	29%↓		3%↑	5%↑	4%↑	1%↓	11%↓
Mecoprop Relative kidney weight (%) ^B	0.606	0.658	-	0.676	-	0.674	0.585	0.653	-	0.649	-	0.683
Mecoprop Relative kidney weight change		9%↑	-	12%↑	-	11%↑		12%↑	-	11%↑	-	17%↑
Mecoprop-P Relative kidney weight (%) ^B	0.578	0.647	0.670	0.659	0.655	0.676	0.590	0.653	0.648	0.654	0.674	0.703
Mecoprop-P Relative kidney weight change		7%↑	11%↑	9%↑	8%↑	12%↑		12%↑	11%↑	12%↑	15%↑	20%↑

Statistical analysis was conducted using the student 't' test

Note that the RMS considers that the student 't' test is not an appropriate test when making multiple comparisons.

^AThere were two control groups, A and B which are equally relevant to either mecoprop or mecoprop-P.

Statistical tests were conducted against control group A for mecoprop and mecoprop-P.

Tests against control group A: *significant difference at 95%, ** significant difference at 99%,*** significant difference at 99.9%.

Statistical tests were conducted against control group B only if a significant difference was found between control group A.

Underlined values are those significantly different from both control groups A and B at 99% and over.

^B Statistical tests not conducted on relative organ weights.

No adverse symptoms or behavioural changes were observed during the study. No treatment related increase in mortality occurred. At 3200 ppm for both substances significantly ($p < 0.01$, Students t-test) reduced body weights (30% and 35% less than control animals in females and males, respectively) were registered compared to the control animals. The magnitude of body weight decrease compared to control animals in the 3200 ppm group clearly exceeds the OECD definition of a Maximum Tolerated Dose. Significantly reduced body weight (16 and 7% in females and males, respectively) was also seen in both sexes at 1600 ppm mecoprop-P and in females at 800 ppm mecoprop racemate.

Significant decreases in white blood cell counts, haemoglobin concentrations and red blood cell counts were sporadically observed throughout the study, however, most consistently at the higher dose levels for both substances. It is concluded that administration of mecoprop and mecoprop-P induced a slight normochromic anaemia at the highest dose tested (3200 ppm) which was probably related to a general toxic effect.

For both male and female rats increases (with respect to frequency and degree) of blood urea nitrogen, serum alkaline phosphatase and alanine aminotransferase were observed from 800 ppm and above for mecoprop racemate and from 400 ppm and above for mecoprop-P (D-form). Decreases in cholesterol were noted most clearly for mecoprop-P at 3200 and 1600 ppm. Increased blood urea nitrogen may be an indicator of kidney effects.

Increased kidney weights compared to both control groups were observed in males at 200, 800 ppm mecoprop racemate ($p < 0.01$ Students t-test) and at 200, 400, 800 ppm mecoprop-P ($p < 0.001$). At 3200 ppm for both substances a significant decrease in kidney weight and other organs weights occurred. This was suggested to be due to the reduced body weight from the general toxicity at high dose levels. Relative kidney weight was increased in all dose groups. Liver weights were increased in females and relatively increased in males at 1600 and 3200 ppm mecoprop-P and 3200 ppm mecoprop racemate.

No treatment related histopathological changes were found in any group.

The liver was considered to be the main organ for the toxicity of mecoprop racemate and mecoprop-P in this study, as the increased kidney weight was noted to remain within the laboratory historical control range. A NOAEL of 200 ppm (equal to daily doses of 16 mg/kg bw/day) was suggested due to the effects on liver (increased organ weight in combination with altered biochemical parameters) for both substances.

The historical control data to demonstrate the findings in the kidney are within the historical control range were not provided in the original study report and the applicant has been unable to provide it. Effects on the kidney have been seen in other studies (eg the seven week rat study) therefore the RMS for this renewal review considers that the findings in the kidney are likely to be treatment related even if they fall within the historical control range. Relative kidney weight was more than 10% higher than controls in females treated with mecoprop or mecoprop-P in all dose groups although increasing dose did not produce a proportionate increase in severity. In males the effect on relative kidney weight was less severe, and there was no evidence of a dose-response so the finding is not considered adverse. The sex difference in the effect on kidney weight also corresponds to blood urea nitrogen, which was consistently higher in females compared to males at equivalent doses. Female rats were also more susceptible to increased alkaline phosphatase levels which can be an indicator of tissue damage which increased in both sexes.

Discussion and conclusion:

The liver and the kidneys were the target organs with respect to the toxicity of racemic mecoprop and mecoprop-P in this 3 month study with rats. As the increase in absolute and relative kidney weights in the 200 ppm groups of male rats is slight and as there are no histopathological findings in the kidneys the effect is considered treatment-related but not considered adverse in males. In females the increase in relative kidney weight was more marked than in the males and exceeded 10% of the control level, but in the absence of any other findings it is questionable whether this is adaptive or adverse.

There was no evidence of any immunotoxicity, neurotoxicity, or hormonal system changes.

Mecoprop-P

In males the NOAEL is 200 ppm (equivalent to 15.6 mg/kg bw/day) based on a 14% increase in absolute kidney weight, a 11% increase in relative kidney weight, and clinical chemistry changes (increased blood urea nitrogen and increased alkaline phosphatase) at 400 ppm (31.9 mg/kg bw/day).

The LOAEL in females is 200 ppm (18.4 mg/kg bw/day) based on a 12% increase in relative kidney weight. 200 ppm was the lowest dose tested therefore the NOAEL in females is < 200 ppm.

Mecoprop

In males the NOAEL is 200 ppm (equivalent to 16.5 mg/kg bw/day) based on a 11% increase in absolute kidney weight, and a 12% increase relative kidney weight, and clinical chemistry changes (increased blood urea nitrogen and increased alkaline phosphatase) at 800 ppm (67.9 mg/kg bw/day).

The LOAEL in females is 200 ppm (18.2 mg/kg bw/day) based on a 12% increase in relative kidney weight. 200 ppm was the lowest dose tested therefore the NOAEL in females is < 200 ppm.

The overall NOAEL for Mecoprop-P in this study is <200 ppm (<18.2 mg/kg bw/day).

Applicant: Proposes that the NOAEL is 200 ppm and that the kidney weight changes at 200 ppm are not adverse.

B.6.3.2.1/02

Previous evaluation:	In DAR for first review (1998). This was a supplementary study to the 3 month rat study by ██████████ (1979) – see B.6.3.2.1/01 to examine ocular effects
----------------------	---

Study	Mecoprop supplementary 3-month oral toxicity study in rats
Reference	██████████ (1979)
Date performed	6 April to 3 July 1978
Test facility	██████████ (not further specified).
Report reference	██████████ report 811222,
Guideline(s)	No
Deviations from the guideline	Not applicable
GLP	No
Test material	Mecoprop racemate, batch GD 6849, 93% purity, and mecoprop D-isomer, batch GD 6970, 99.9% purity.
Study acceptable	Yes

Study report:

██████████ (1979): Mecoprop supplementary 3-month oral toxicity study in rats. Laboratory ██████████ (not further specified). ██████████ report 811222, 18 December 1978 (translated 6 March 1979), unpublished report. (Dossier ref. 5.22).

Study design and quality:

As histopathological examination of the eyes due to technical problems were not conducted in the study described in section B.6.3.2.1/01, this study was undertaken. Ten male and ten female Sprague-Dawley rats were for 13 weeks given a diet containing 0, 800 and 3200 ppm racemic mecoprop (93% purity) equivalent to 0, 81.7/121.1, 452.5/537.1 mg/kg bw/day in males/females or 800, 1600, and 3200 ppm mecoprop-P (D-isomer, 99.9% purity) equivalent to 84.1/117.8, 178.1/239.9, 429.5/539.0 mg/kg bw/day in males/females. The following parameters were registered: body weight (weekly), food consumption (weekly), clinical observations (daily), ophthalmoscopic examinations (week 0 and 13), and histopathological examinations of the eyes.

Concerning clinicochemical parameters increased creatine value was observed in females at 450 ppm. Reduced glucose concentration was found in plasma from males at 450 ppm. Significantly increased albumin levels and significantly reduced levels of platelets at all dose levels in female rats were explained to be due to incidental abnormal control values. At gross examination increased kidney and relative kidney weight was found according to the values given in Table B.6. 18. At 150 ppm relative kidney weight was 14% higher in males and 9% higher in females. At 450 ppm relative kidney weight was 17% higher in males and 8% higher in females.

Table B.6. 18 Treatment related changes in organ weights

	0 ppm m/f	50 ppm m/f	150 ppm m/f	450 ppm m/f
Average dose, mg/ kg bw/day	0/0	3.8/4.4	11.4/ 13.4	34.0 / 39.3
Kidney weight, g	2.60/ 1.81	2.68/ 1.80	2.94**/ 1.91	2.99**/ 1.94*
Kidney weight % compared to control		3%↑ / 1%↓	13%↑ / 6%↑	17%↑ / 8%↑
Relative kidney weight, g/ 100 g bw	0.66/ 0.79	0.69 / 0.79	0.75**/ 0.86**	0.77**/ 0.85**
Relative kidney weight % compared to control		5%↑ / 0%	14%↑ / 9% ↑	17% ↑ / 8%↑
Plasma creatinine (sampled in final week of study)	46.91/ 50.93	53.04/ 54.20	No data/ 53.63	46.10/ 59.18*

* and **: 5% and 1% significance level, t-test (adapted for multiple comparisons)

No treatment related changes were found at histopathological examination.

Discussion and conclusion:

In this study on mecoprop the kidney was found to be the target organ for racemic mecoprop toxicity (increased creatinine level and increased absolute and relative kidney weight). It is considered that the NOAEL is 50 ppm (equal to 3.8/4.4 mg/kg bw/day) based on a significant increase in kidney weight at 150 ppm.

Note that the applicant agrees that the NOAEL for this study is 50 ppm.

B.6.3.2.2 Oral 90-day study in the mouse

Under the data requirement Regulation (EU) 283/2013 a 90 day mouse study is not necessary as a 90 day rat study is available. Therefore this study is considered to be a supplementary study. It was conducted to the previous version of the OECD guideline 408 (1981), which is similar to the current guideline 1998 guideline except that only the organ weights of the liver, kidneys, adrenals and testes were weighed.

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Report on the study of the oral toxicity of Mecoprop-p acid in B6C3F1 mice, administration in diet for 3 months
Reference	████████████████████ (1993)
Date performed	22 March to 25 June 1991
Test facility	████ ██████████ ██████████ ██████████ ██████████

Report reference	Report no. [REDACTED]
Guideline(s)	OECD 408 (1981)
Deviations from the guideline	No significant deviations
GLP	Yes
Test material	Mecoprop-P, batch 91-1, 96.5%
Study acceptable	Yes

Study report:

[REDACTED] (1993): Report on the study of the oral toxicity of Mecoprop-p acid in B6C3F1 mice, administration in diet for 3 months. [REDACTED], 7 September 1993, unpublished report. (Dossier ref 5.23 & 5.3.2/01).

Study design and quality:

Mecoprop-P (96.5% purity) was administered to 10 male and 10 female B6C3F1 mice per group in the diet for 3 months at dose levels of 0, 100, 1000, and 2500 ppm (equivalent to 0, 20/30, 220/330, 740/930 mg/kg bw/day in males/females). The study was stated to follow GLP and the conduct of the study complied to OECD 408 and EU guideline B26. The study included a full histopathological examination of organs in the control and top dose group that would provide an indication of potential immunotoxicity, neurotoxicity and hormonal effects such as the brain and spinal cord, testes, ovaries, uterus, virgina, mammary gland, adreanals, pancreas, pituitary, thyroid, thymus, spleen, mesentic lymph nodes.

Results:

No treatment related death or abnormal behaviour occurred during the test period.

Table B.6. 19 presents the overall significant responses in the mecoprop-P treated groups.

Table B.6. 19 Observations after 3 months dietary administration of mecoprop-P to mice.

Dose, ppm sex	0 m/f	100 m/f	1000 m/f	2500 m/f
mean dose, mg/kg bw/day	-/-	20/30	220/330	740/930
body weight, g (3 mo)	33.6/ 28.4	32.2/ 26.1	30.9*/ 25.8*	30.4**/ 25.8**
Organ weights:				
liver weight, g	1.21/ 1.11	1.15/ 0.99	1.21/ 1.13	1.63** /1.66**
kidneys weight, g	0.51/ 0.37	0.50/ 0.36	0.48/ 0.40	0.42**/ 0.35
adrenal glands weight, g	6.2/ 11	7.1/ 10.2	5.8/ 10.1	6.4/ 8.8*
clinical chemistry/ haematology:				
alkaline phosphatase	-/-	-/-	-/-	↑/↑
cyan.in.pal.-CoA-ox. ¹	-/-	n.d.	n.d.	↑/↑
urea	-/-	-/↑	↑/↑	↑/↑
cholesterol	-/-	-/-	-/-	↑/↑
triglycerides	-/-	-/↓	↓/↓	↓/↓
creatinine	-/-	-/-	↑/-	↑/-
glucose	-/-	-/-	-/-	↑/-

alanine aminotransferase	-/-	-/-	-/-	-/↑
haemoglobin	-/-	-/-	-/-	↓/-
mean corpuscular haemogl.	-/-	-/-	-/-	↓/-
globulins	-/-	-/-	-/-	↓/-

* p < 0.05 ** p < 0.01 Dunnet-test (two sided) n.d.: not determined

1) cyanide-insensitive palmitoyl-CoA-oxidation in liver homogenate

Pathology:

At 2500 ppm macroscopically diagnosed dark-brown discoloration of the liver and decrease of lipid storage was observed in both sexes. Eosinophilic cytoplasm of hepatocytes was found in both sexes. In the kidney eosinophilic cytoplasm of tubular epithelial cells was found in both sexes. At 1000 ppm decrease of lipid storage in the liver was observed in both sexes.

The liver and the kidneys were pointed out to be the target organs for mecoprop-P toxicity in this study. A 10-15 fold increase in the cyanide-insensitive palmitoyl-CoA-oxidation in liver homogenate was an indication of peroxisomal proliferation. With respect to the findings in clinical chemistry a NOAEL was found to 100 ppm for males and was suggested to be slightly below 100 ppm for females.

Discussion and conclusion:

The liver and kidneys were found to be the target organs for mecoprop-P toxicity. A NOAEL of 100 ppm (equal to 20 mg/kg bw/day) was found for male mice, whereas for female mice the NOAEL could not be established. There was no evidence of any immunotoxicity, neurotoxicity, or hormonal system changes.

B.6.3.2.3 Oral 90-day study in the dog

The data requirements Regulation (EU) 283/2013 stipulates that a 90 day study in non-rodents should be submitted. This study was conducted prior to the adoption of GLP and OECD test guidelines but is consistent with the current OECD guideline 409 (1998) with the exception that for each dose group the results from each sex were combined.

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Sub chronic (13-week) oral toxicity study with Mecoprop (MCP) in beagle dogs
Reference	██████████ (1979)
Date performed	21 September to 20 December 1977
Test facility	██████████ ██████████
Report reference	Report no. R6105 (BASF doc. 79/0100)
Guideline(s)	Similar to OECD 409 (1998)
Deviations from the guideline	No significant deviations. During reporting for each dose group the results from males and females were combined.
GLP	No
Test material	Mecoprop, purity 93.3% (calculated as 99.0% anhydrous dry material) code TPH.G. (analysis of the batch is provided on page 30 of the study report)
Study acceptable	Yes

Study report:

██████████ (1979): Subchronic (13-week) oral toxicity study with Mecoprop (MCP) in beagle dogs. ██████████ report no. R6105 (BASF doc. 79/0100), May 1979, unpublished report. (Dossier 5.24 & 5.3.1/01).

Study design and quality:

Four groups of 4 male and 4 female beagle dogs (4-5 months old) were during a period of 90 days fed a diet containing racemic mecoprop (93.3% purity) at levels which provided daily intakes of 0, 4, 16 and 64 mg/kg bw/day.

The study was not conducted according to any study guideline or GLP. The study was comparable to an OECD 409 study with regard to: clinical observations for toxicity during the study, body weight registration, haematology and biochemical parameters, necropsy and histopathology. Further, a liver function test (bromosulphophthalein method) and a kidney function test (phenolsulphophthaleine method) were performed at the end of the study. In addition, organs that can provide an indication of potential immunotoxicity, neurotoxicity or endocrine effects were also subjected to a histological examination, such as the spleen, thymus, bone marrow, mesenteric lymph node, thyroid, adrenals, pituitary, testicles, ovaries, uterus, mammary gland, prostate, brain, and nervous tissue. Haematological parameters such as leucocyte count, and differential leucocyte count were also conducted.

Overall, the study should be considered as acceptable.

Results:

No abnormal observations with respect to behaviour occurred in any group. Two dogs at the highest dose group developed transitory inflammatory response of the gingivae. In a male dog at top dose ulcers in the buccal mucosa resulted in withdrawal from mecoprop dosing and treatment with antibiotics. Another male dog at top dose developed a corneal ulcer which was suggested to be treatment related.

Body weight gain and relative organ weights were affected according to Table B.6. 20:

Table B.6. 20 90 day dietary administration of mecoprop to dogs: Body weight gain, organ weight and relative organ weight

Observation, week 13	Sex	Dose level, mg mecoprop/kg bw/day			
		0	4	16	64
body weight (weight gain), kg	m	12.9	11.8(4.	11.8(4.	9.9 (1.9)
	f	(4.8) 9.2 (2.8)	2) 8.9 (2.6)	0) 8.3 (2.0)	8.1 (1.7)
organ weights,					
kidney, g	m + f	55	50	52	53
liver, g	m + f	387	365	374	392
heart, g	m + f	88	85	81	81
thymus, g	m + f	19	16	13	10
lungs, g	m + f	96	88	94	91
brain, g	m + f	74	76	75	76
relative organ weights,					
kidney, g/ 100 g body weight	m + f	0.49	0.48	0.53	0.59**
liver, g/ 100 g body weight	m + f	3.52	3.52	3.72	4.40***
heart, g/ 100 g body weight	m + f	0.80	0.82	0.81	0.94*
thymus, g/100 g body weight	m + f	0.18	0.15	0.14	0.12
lungs, g/100 g body weight	m + f	0.87	0.85	0.95	1.01*
brain, g/100 g body weight	m + f	0.69	0.67	0.78	0.87*

* P<0.05; ** P<0.01; *** P<0.001 Wilcoxon test

None of the absolute organ weights are in the report indicated to be significantly affected by the treatment although a dose-related reduction (down to about 50% at high dose) of the thymus weight was recorded.

Significant changes in organ weight are seen at the top dose of 64 mg/kg bw/day where relative kidney weight is increased by 20%, relative liver weight is increased by 25%. There are also increases in the relative heart, lungs and brain weight although these are likely to be secondary to reduced body weight gain.

The haematological and biochemical examinations reveal changes at the top dose of 64 mg/kg bw/day, and to a lesser extent at 16 mg/kg bw/day include haematological changes indicative of anaemia, and biochemical changes include increased blood urea nitrogen at the top dose which may be an indicator of kidney damage.

Table B.6. 21 90 day dietary administration of mecoprop to dogs: Haematological and biochemical parameters (both sexes analysed together)

Haematological and biochemical parameters
(Numerical values are in the table immediately following this one)

Observation, week 13	Dose level, mg mecoprop/kg bw/day			
	0	4	16	64
Haematology				
haemoglobin	-	-	-	↓
packed cell volume	-	-	(↓)	↓
red blood cell count	-	-	(↓)	↓
lymphocytes	-	-	-	(↓)
neutrophils	-	-	-	(↑)
Biochemistry				
urea	-	-	-	↑
total protein	-	-	-	(↓)
albumin	-	-	-	(↓)
alkaline phosphatase	-	-	-	(↓)
bilirubin	-	-	(↓)	-

(↓) only significantly affected after 6 weeks

Table B.6. 22 90 day dietary administration of mecoprop to dogs: Haematological parameters (both sexes analysed together)

mg/kg bw/day	clotting time sec.	Hb m mol/ 1	packed cell volume 1/1	RBC x10 ¹² /1	WBC x 10 ⁹ /1	thrombo- cytes x 10 ⁹ /1	Differential count %				
							lymph	neutr	mono	eos	baso
week 0											
0	26	8.1	0.411	5.0	13.2	335	44	53	1	2	0
4	26	8.1	0.406	5.1	14.8	281	40	54	2	5	0
16	26	8.3	0.411	5.1	15.1	323	41	55	1	3	0
64	26	8.0	0.397	5.0	13.1	415	41	56	1	2	0
week 6											
0	30	8.7	0.436	5.6	12.4	274	37	53	2	8	0
4	28	8.6	0.424	5.5	12.7	323	38	55	2	5	0
16	27	8.0	0.404*	5.1*	12.8	368*	33	60	7	1	0
64	29	6.7***	0.330***	4.2***	14.7	324	25*	66*	1	9	0

week 13											
0	25	9.3	0.456	6.2	13.9	270	39	55	1	4	0
4	25	9.3	0.444	6.2	14.0	258	40	55	1	4	0
16	24	9.0	0.436	5.9	16.1	292	41	56	0	3	0
64	24	7.8**	0.371***	5.1**	14.6	329	32				

Hb = haemoglobin content

RBC = red blood cell count

WBC = white blood cell count

*P<0.05; **P<0.01; ***P<0.001 according to Wilcoxon test

Table B.6. 23 90 day dietary administration of mecoprop to dogs: Biochemistry parameters (both sexes analysed together)

mg/kg bw /day	glucose m mol/l	urea m mol/l	G O T U/l	G P T U/l	AP U/l	T P g/l	albumin g/l
week 0							
0	7.0	2.2	3.9	4.0	59	60	32
4	6.9	2.6	4.1	4.6	57	57**	31
16	6.8	2.8	4.0	4.1	54	59	32
64	7.0	2.6	3.6	3.3	53	59	32
week 6							
0	6.2	3.4	6.4	8.5	46	54	29
4	6.5	4.0	6.1	8.0	46	54	27
16	6.4	3.8	5.6	7.7	39	53	26
64	5.9	5.4**	5.0	6.6	33***	49**	23**
week 13							
0	6.3	4.5	6.9	8.1	43	60	32
4	6.5	4.5	6.5	7.8	42	62	33
16	6.4	4.5	5.3	6.2	39	60	32
64	5.0	5.6*	5.5	8.8	35	56	31

Urea = urea nitrogen

GOT = glutamic-oxalacetic transaminase

GPT = glutamic-pyruvic transaminase

AP = alkaline phosphatase

TP = total protein

*P<0.05; **P<0.01; ***P<0.001 according to Wilcoxon test

Table B.6. 24 90 day dietary administration of mecoprop to dogs: Biochemistry parameters (continued)

mg/kg bw/day	Na in plasma mg/100 ml	K in plasma mg/100 ml	Bilirubin Umol/l	OCT U/L	Creatinine Umol/L
week 0					
0	350	16.3	1.6	6.7	59
4	351	16.2	1.7	6.9	56
16	350	15.7	1.8	6.4	57
64	352	16.3	1.8	6.5	57
week 6					
0	351	16.9	1.6	4.8	64
4	327	16.2	1.4	4.8	69
16	337	16.3	1.2*	4.6	60
64	328	16.0	1.6	4.4	59
week 13					
0	372	18.0	1.3	7.9	71
4	371	17.3	1.2	8.2	69
16	378	18.0	1.3	7.5	64
64	386	18.8	1.5	8.0	67

OCT: Ornithine Carbamoyl Transferase

*P<0.05 according to Wilcoxon test

Increased retention of phenol red and bromosulphophthalein observed in the tests for kidney and liver function at the top-dose animals indicated impaired function of these organs.

At necropsy a brown discolouration of adipose tissue in the mesentery was noted in three dogs in the highest dose group. At the microscopic examination no findings were considered treatment related.

At the highest dose there was reduced weight gain although it was not statistically significant. Some of the haematological and biochemical parameters were affected in the highest dose group. E.g. there was a decrease in haemoglobin, packed cell volume and red blood cell count. At 16 mg/kg bw/day packed cell volume and red blood cell count were only significantly decreased after 6 weeks.

Discussion and conclusion

The above findings indicate that the main effects are the haematological effects and effects on kidney and liver. A NOAEL of 4 mg/kg bw/day is found from the study based on changes in haematological parameters indicative of anaemia. Note that this study was conducted on mecoprop racemate. There was no evidence of any immunotoxicity, neurotoxicity, or hormonal system changes.

Results:

No animals died during the study. Except for a few and incidentally occurring cases of diarrhoea and vomiting no clinical observations were noted throughout the study. There were no pathological findings in the eyes of any animal neither before commencement of feeding nor at the end of the study. Body weight and body weight gain were decreased in high dose males but not in females. However total body weight gain over the 12 month period of the study was not affected by treatment (see Table B.6.25). Neither food consumption nor food efficiency were affected of dosing in any group. Haematology parameters only showed decreases in haemoglobin concentration and in haematocrit values in the high dose males on day 87 (see Table B.6.26) and in haemoglobin concentration on day 185. One case of a clotting time in high dose males which was significantly elevated over the clotting time measured for control males seems to be due to decreased clotting time in controls and thus incidental. The only significant changes in blood chemistry between high dose and control animals occurred in females with a decrease in inorganic phosphate and calcium on day 90. This finding was not repeated later on in the study but was by the performing laboratory considered to be related to dosing. Intergroup differences in clinical chemistry parameters (deviations in sodium and magnesium concentrations) in males and females of lower dose groups either marginal or inconsistent. No deviations were found in urinalysis at any time of the study in neither males nor females. Decreases in absolute and relative liver weight in low dose males and decrease in absolute brain weight in mid-dose females did not reveal dose response relationships and were regarded as unrelated to the treatment. Most of the gross and histopathological findings noted were either single observations in controls or low dose animals or they were seen in all or almost all groups at a comparable incidence, lacking a dose response relationship. Exceptions from this are: One case of slight focal tubular degeneration of the kidneys (unilateral) of one male high dose dog, and slight focal atrophy of the prostate gland in one male of each dose group, but not in controls. Three females of the high dose group had cystic corpora lutea (not a normal term/diagnosis, maybe cystic follicle) and oedema occurred in the interstitium of the mammary gland of one high dose female; these findings are considered to reflect the stage in the sexual cycle.

Table B.6. 25 1 year dietary administration of mecoprop-P to dogs test substance intake and body weight gain

Parameter	Males				Females			
	0	60	180	600	0	60	180	600
Dose ppm	0	60	180	600	0	60	180	600
Dose mg/kg bw/day	0	1.8	5.2	18.3	0	2.0	5.7	19.0
Body weight gain over 12 months (kg)	2.0	1.9	2.6	1.7	1.7	2.1	2.2	2.7

Table B.6. 26 1 year dietary administration of mecoprop-P to dogs: day 87 Haematological parameters, males.

Males		Day 87 WBC GIGA/L	Day 87 RBC TERA/L	Day 87 HGB MMOL/L	Day 87 HCT L/L	Day 87 MCV FL	Day 87 MCH FMOL	Day 87 MCHC MMOL/L	Day 87 PLT GIGA/L
Group 0 0 ppm	M	10.44	6.73	9.9	0.456	67.9	1.47	21.73	297
	SD	2.22	0.19	0.2	0.007	2.3	0.05	0.38	46
	N	5	5	5	5	5	5	5	5
Group 1 60 ppm	M	11.41	7.05	10.2	0.470	66.7	1.45	21.76	295
	SD	3.10	0.22	0.4	0.013	1.1	0.02	0.53	11

Males		Day 87 WBC GIGA/L	Day 87 RBC TERA/L	Day 87 HGB MMOL/L	Day 87 HCT L/L	Day 87 MCV FL	Day 87 MCH FMOL	Day 87 MCHC MMOL/L	Day 87 PLT GIGA/L
	N	5	5	5	5	5	5	5	5
Group 2 180 ppm	M	9.10	6.84	9.9	0.445	65.3	1.44	22.14	277
	SD	1.69	0.53	0.6	0.020	3.1	0.05	0.36	29
	N	5	5	5	5	5	5	5	5
Group 3 600 ppm	M	10.02	6.45	9.4*	0.430**	66.8	1.46	21.91	301
	SD	1.68	0.37	0.3	0.014	3.0	0.06	0.21	34
	N	5	5	5	5	5	5	5	5

WBC GIGA/L: White Blood Cells; RBC TERA/L: Red Blood Cells; HGB MMOL/L: Haemoglobin; HCT L/L: haematocrit; MCV FL: Mean cell volume; MCH FMOL : mean corpuscular haemoglobin MCHC MMOL/L: mean corpuscular haemoglobin concentration PLT GIGA/L: Platelets
Kruskal-Wallis + Mann-Whitney u-tests (two-sided): * P<4.05; ** P<4.02; *** P<.0.002 (Statistical unit - Animal)

Discussion and conclusion:

The study was well performed in accordance with the guidelines for testing of chemicals from EEC, OECD, EPA and the Japanese MAFF and in accordance with GLP rules and the results seem reliable. Male and female pure bred Beagle dogs were given dietary concentrations of 0, 60, 180 or 600 ppm mecoprop-P for 12 months. The dietary concentrations corresponded to daily doses of 0, 2, 5, 19 mg mecoprop-P/kg body weight which were all tolerated without affecting the clinical or ophthalmological status of the animals, the food consumption or the final body weights to a significant degree.

The following findings were obtained and assessed as being substance-related:

600 ppm (approx. 19 mg/kg body weight/day) :

The mean body weight (and the mean body weight gain) of the high dose male dogs in the beginning of the administration period (up to day 49 of the study) was significantly lower than for control males and the mean body weight stayed slightly reduced throughout the study. A marginal adverse effect on red blood cells of dosing with 19 mg mecoprop-P/kg bw/day was also indicated by the decreased haemoglobin concentrations on day 87 and day 185 and the decreased haematocrit value on day 87 in male dogs. Likewise the slight decrease in inorganic phosphate and calcium in high dose females was considered related to dosing.

The observed macro- and microscopic pathological changes in males as well as in females were found to be unrelated to the administration of mecoprop-P. They were either single incidental findings, reflecting stage of sexual cycle, or evenly distributed in all four groups and never interfered with the general status of the animals or were of a high severity grade. All lesions were regarded as spontaneous findings in Beagle dogs.

180 ppm (approx. 5 mg/kg body weight/day): No substance-induced changes

60 ppm (approx. 2 mg/kg body weight/day): No substance-induced changes

The NOAEL under the conditions of this chronic study in Beagle dogs was 5 mg mecoprop-P/kg body weight/day based on retarded body weight change and to slight decreases in haemoglobin concentrations and haematocrit values in the peripheral blood in the males and to slight decreases in inorganic phosphate and calcium in the females at 18 mg/kg bw/day. There was no evidence of any immunotoxicity, neurotoxicity, or hormonal system changes.

Applicant commentary: It should be noted that the dog is generally more sensitive to the effects of phenoxy herbicides than are humans and other mammals. This is because the half-life of elimination of phenoxy compounds for dogs is significantly longer than for other species because of a decreased capacity of the dog to eliminate organic acids. Dog studies with other phenoxyacetic herbicides have therefore not been used to select endpoints.

The following skin reactions were observed:

10 mg/kg bw/day: from day 3 to day 20 reactions consisting of slight erythema were observed in seven animals.

100 mg/kg bw/day: after day 8 well-defined erythema and slight or well defined oedema had developed in seven animals. By microscopic examination diffuse acanthosis was observed in two animals.

1000 mg/kg/day: slight erythema was observed at day 2 progressing to day 8 to well-defined erythema with slight or well defined oedema in eight animals. Well-defined erythema and moderate oedema in one animal. Desquamation (sloughing) of the stratum corneum were recorded in the majority of the rabbits. By microscopic examination diffuse acanthosis was observed in six animals.

In female rabbits the blood level of urea was significantly decreased at all dose levels and the level of cholesterol was decreased at the two highest dose levels. The reduction in blood urea nitrogen is in contrast to increased blood urea nitrogen seen in oral repeat dose studies, and is considered to be incidental, the study director also considered that decreased cholesterol was likely to be incidental as wide variation is common in laboratory rabbits. Thus no treatment related changes were found to occur in haematology, blood biochemistry, urinalysis, macroscopic or microscopic pathology.

Table B.6. 27 21 day dermal application of mecoprop-P to rabbits: clinical chemistry findings

Parameter	Males				Females			
	0	10	100	1000	0	10	100	1000
Dose mg/kg bw/day								
Blood urea nitrogen mg/dL	22	22	21	23	27	21*	23*	21**
Cholesterol mg/dL	44	40	37	45	59	43	32*	35*

* P<0.05

** P<0.01

A NOAEL of 1000 mg/kg bw/day was concluded for systemic effects, whereas a no effect level could not be established for dermal irritation.

Discussion and conclusion:

Dermal irritation occurred after dermal application in all exposure groups, however, most pronounced at the two highest dose levels. The NOAEL of 1000 mg/kg bw/day seems reliable as the decrease in spleen weight for mecoprop-P dosed female animals most probably is due to high spleen weight value of the control group and thus the finding should not be considered treatment related.

B.6.3.4 Summary of Short-term toxicity

Rat, oral

Two oral studies (repeated dose studies with a duration of 7 weeks and 3 months, respectively) were conducted both with mecoprop-P (purity: 99-100%) and with racemic mecoprop (purity of 93%) and comparison was made between the toxicity of the two substances.

In the 7 weeks study (██████████, 1985) rats were fed a diet containing 0, 50 and 400 ppm mecoprop-P. At 400 ppm the following was observed: increased kidney weight (females), increased blood level of urea (females) and creatinine (females), reduced blood level of cholesterol (males and females). Thus the NOAEL in this study was 50 ppm (equal to 4.4(m)-4.8(f) mg/kg bw/day). In two groups of rats receiving racemic mecoprop at the same dose levels identical effects were observed and no distinction between the toxicity of mecoprop-P and racemic mecoprop could be made.

In the three months study (██████████ 1979) rats were fed 0, 200, 400, 800, 1600, and 3200 ppm mecoprop-P in the diet. Further groups received a diet containing 0, 200, 800, and 3200 ppm racemic mecoprop. At 1600 ppm mecoprop-P (females) and 3200 ppm mecoprop-P (both males and females) reduced body weight was observed. At the highest dose levels for both substances decreases in white blood cell counts, haemoglobin concentration, and red blood cell count were observed during the study. At 400 ppm mecoprop-P and at 800 ppm mecoprop racemate and above increases in urea, alkaline phosphatase and alanine aminotransferase were determined in the blood samples. Increased kidney weight was most prominently found in males, at 200 (equal to 16 mg/kg bw/day), 400 and 800 ppm mecoprop-P and at 200 and 800 ppm mecoprop. Increased liver weights (in females) and relative liver weights (in males) were observed at 1600 and 3200 ppm mecoprop-P and at 3200 ppm mecoprop. Also in this study no distinction in the toxic effects could be made between racemic mecoprop and mecoprop-P. The increase of the kidney weight in the 200 ppm groups of male rats is slight (less than 10%) and as there are no histopathological findings in the kidneys the effect is not considered adverse. However in females relative kidney weight was 12% higher than controls in the 200 ppm mecoprop and mecoprop-P dose groups, and was also increased at all doses above this. There were no other adverse findings at this dose in females so it is questionable whether this finding is adaptive. The RMS has taken a precautionary approach and considers this finding to be adverse so the LOAEL is considered to be 200 ppm (ADME studies indicate the kidney is highly exposed to the test substance, the kidney is a target organ at higher doses in this study, and findings in the kidney are confirmed in other studies). There was no evidence of any immunotoxicity, neurotoxicity, or hormonal system changes.

Further, a 3 month study (██████████ 1985) in which rats were dosed with a diet containing 0, 50, 150 and 450 ppm racemic mecoprop (purity of 92.7%) was submitted with the dossier. Increases in the organ weight of the kidney and the relative kidney weight were found at both 150 and 450 ppm. Increase in creatinine value was found in females at 450 ppm. Thus a NOAEL of 150 ppm (equal to 11.4 (m) and 13.4 (f) mg/kg bw/day) was found in this study, as the effect in 150 ppm is not considered adverse. The RMS considers this study is of limited relevance as it was conducted on racemic mecoprop and there are adequate studies available on mecoprop-P.

A scientific publication (██████████ 1989) was submitted considering short term studies with oral (gavage) dosing of racemic mecoprop (potassium salt with a purity of 97%) to rats. Both in a 14 day study (dose levels 0, 100, 320, and 800 mg/kg bw/ day) and in a 90 day study (dose levels: 0, 0.8, 8, 80 and 320 mg/kg bw/day) reduced organ weight of the thymus was seen. At microscopy degenerative processes in the cortex of the organ was observed at and above 320 mg/kg bw/day. In the 90 day study the LOAEL with respect to organ weight of the thymus was a dose level of 8 mg/kg bw/day for males (NOAEL: 0.8 mg/kg bw/day) and 320 mg/kg bw/day for females (NOAEL: 80 mg/kg bw/day). Reduced organ weight of the spleen was found at 800 mg/kg bw/day in the 14 day study. At microscopic examination of the spleen, reduction of the white pulp tissue and enlargement of the haematopoietic tissue was observed. Morphometry of the tissue confirmed these findings also at the 320 mg/kg bw/day dose level. Further dose-dependent changes in differential leucocyte counts were reported (decrease in lymphocytes and increase in neutrophilic granulocytes). The findings in the thymus and spleen were not seen in other studies at much higher dose level therefore the relationship to treatment with mecoprop-P is doubtful. The RMS considers this study is of limited relevance as it was conducted on racemic mecoprop and there are adequate studies available on mecoprop-P.

Mouse, oral

In a three months study with mice (██████████ 1993) concerning oral administration of 0, 100, 1000, and 2500 ppm mecoprop-P (purity: 96.5%) in the diet, haematological effects were found at the dose level of 2500 ppm. Clinicochemical findings included increased urea and decreased triglyceride values at all dose levels except in males at 100 ppm. At the top dose increased liver weight (males and females) and decreased kidney weight (males) were observed. The NOAEL in this study was 100 ppm for males (equal to 20 mg/kg bw/day) but could not be established for females owing to the increased level of urea in blood. There was no evidence of any immunotoxicity, neurotoxicity, or hormonal system changes in mice.

Dog, oral

In a three months study (██████████ 1979) beagle dogs were gavaged with either 0, 4, 16 or 64 mg/kg bw/day mecoprop racemate (purity: 93.3%). There were increased relative liver and kidney weights and effects on

some of the haematological and biochemical parameters in the highest dose group, as e.g. decreased haemoglobin, packed cell volume and red blood cell count and increased urea. At 16 mg/kg bw/day packed cell volume and red blood cell count were only significantly decreased after 6 weeks. Therefore it is concluded that the NOEL is 4 mg/kg bw/day while the NOAEL is 16 mg/kg bw/day.

In a one year dog study on mecoprop-P (██████████ 1997) the NOAEL was 5 mg/kg bw/day based on decreased body weight and body weight gain and minor effects on blood cells (decreased haemoglobin and haematocrit) and decreased phosphate and calcium in the highest dose group 19 mg/kg bw/day.

There was no evidence of any immunotoxicity, neurotoxicity, or hormonal system changes in either of the studies in the dog.

Evidence from the literature (see Volume 3 Section B.6.1.1.2) shows that plasma half-life and renal clearance of mecoprop are prolonged in the dog compared to rats and humans therefore the dog is not the most relevant species for determining the effects of mecoprop in humans.

Rabbit, dermal

In a twenty-one day study with dermal exposure to rabbits at dose levels of 0, 10, 100, and 1000 mg/kg bw/day (██████████ 1993) signs of dermal irritation were recorded with increasing severity at increasing dose levels. The spleen weight was reduced at all dose levels in females (at the two highest dose levels to a significant degree), however, this finding is thought to be due to rather high organ weights in control females. In females, the blood level of urea was significantly decreased at all dose levels and the level of cholesterol was decreased at the two highest dose levels, although they were within the range of normal values. The NOAEL is 1000 mg/kg bw/day.

Overall appraisal

The RMS considers that the studies submitted have sufficiently investigated the repeat dose toxicity of mecoprop-P, including neurotoxic, immunotoxic or endocrine system effects. Toxicokinetic data (blood concentration) and micronuclei were not measured as these were old studies that were conducted before (EU) 283/2013 applied. It is considered that in the interests of minimising vertebrate testing it is not necessary to meet these data requirements.

The studies provide convincing evidence to conclude that there was no difference in toxicity between mecoprop (racemic form, purity 93-97%) and mecoprop-P (D-form, purity > 99%). The most common findings from the studies were haematological effects in the dog and effects on liver and kidney in rats, mice and dogs. One study with the racemate reported reduced organ weight of the thymus and the spleen, and toxicity towards these organs was verified by histopathological findings. However such findings were not seen in other studies at much higher doses.

An overview of the NOAEL's and LOAEL's is given in Table B.6.28.

Table B.6. 28 Summary of short term studies with mecoprop-P and mecoprop

(no new studies have been submitted since the previous review, studies considered superfluous are highlighted in grey)

Study	Dosing	Effects at LOAEL	LOAEL/ NOAEL	Reference
Rat; 7 weeks; oral; 10m+10f/ group	0, 50, 400 ppm mecoprop in diet Equivalent to 0, 4.4/4.8, 35.1/37.5 mg/kg bw/day in m/f 0, 50, 400 ppm mecoprop-P in diet Equivalent to 0, 4.4/4.8, 35.2/38.0 mg/kg bw/day in m/f	Mecoprop: In males 7% ↑ abs. and rel. kidney weight, ↑ blood urea nitrogen, ↓ cholesterol. In females ↓ calcium, ↓ cholesterol . Mecoprop-P: In males 8% ↑ abs. kidney weight, 10% ↑ rel. kidney weight, ↓ cholesterol. In females 8% ↑ abs. kidney weight, 10% ↑ rel. kidney weight, ↓ cholesterol. ↑ blood urea nitrogen, ↑creatinine.	Mecoprop: LOAEL 400 ppm NOAEL 50 ppm (4.4/4.8 mg/kg bw/day in m/f) Mecoprop-P: LOAEL 400 ppm NOAEL 50 ppm (4.4/4.8 mg/kg bw/day in m/f)	█ (1986)
Rat; 3 months; oral; 15m+15f/group	0, 200, 800, 3200 ppm mecoprop in diet Equivalent to 0, 16.5/18.2, 67.9/75.9, 390.8/398.7 mg/kg bw/day in m/f 0, 200, 400, 800, 1600, 3200 ppm mecoprop-P in diet Equivalent to 0, 15.6/18.4, 31.9/37.8, 67.6/75.8, 146.4/170.1, 403.2/403.5 mg/kg bw/day in m/f	Mecoprop: 12% ↑kidney weight in females. Mecoprop-P: In females 12% ↑ rel. kidney weight.	Mecoprop: LOAEL 200 ppm (18.2 mg/kg bw/day in f) NOAEL < 18.2 mg/kg bw/day in females Mecoprop-P: LOAEL 200 ppm (18.4 mg/kg bw/day in f) NOAEL < 18.4 mg/kg bw/day in females	█ (1979)
Rat; 3 months; oral; 15m+15f/group	0, 800 and 3200 ppm racemic Mecoprop (93% purity) in diet Equivalent to 0, 81.7/121.1, 452.5/537.1 mg/kg bw/day in m/f 0. 800, 1600, and 3200 ppm Mecoprop-P (D- isomer, 99.9% purity) in diet	Only ocular effects were examined; this study is a supplementary study to the Reinert (1979) study.	Mecoprop and mecoprop-P : NOAEL > 3200 ppm in diet (equal to 430- 539 mg/kg bw/day) for ocular effects	█ (1979)

Study	Dosing	Effects at LOAEL	LOAEL/ NOAEL	Reference
	Equivalent to 84.1/117.8, 178.1/239.9, 429.5/539.0 mg/kg bw/day in m/f			
Rat; 3 months; oral; 15m+15f/ group	0, 50, 150, 450 ppm mecoprop in diet Equivalent to 0, 3.8/4.4, 11.4/13.4, 34.0/39.3 mg/kg bw/day in m/f	In males 13%↑ kidney weight, 14%↑ rel. kidney weight. In females 9% ↑ rel. kidney weight.	Mecoprop: LOAEL 150 ppm NOAEL 50 ppm (3.8/4.4 mg/kg bw/day in m/f)	(1985) ¹
Rat; 3 months; oral; 20m+20f/ group	0, 0.8, 8, 80, 320 mg/kg bw/day mecoprop potassium salt solution, gavage	↓ thymus weight	Mecoprop: LOAEL: 8 mg/kg bw/day NOAEL: 0.8 mg/kg bw/day	(1989) ¹
Mouse; 3 months; oral; 10m+10f/ group	0, 100, 1000, 2500 ppm mecoprop-P in diet Equivalent to 20/30, 220/330, 740/930 mg/kg bw/day in m/f	In females at 100 ppm: ↑ blood urea nitrogen, ↓ triglycerides.	Mecoprop-P: LOAEL females: 100 ppm (30 mg/kg bw/day in f) NOAEL < 30 mg/kg bw/day in females	(1993)
Dog; 3 months; oral; 4m+4f/ group	0, 4, 16, 64 mg/kg bw/day mecoprop in diet	Haematological changes: ↓ PCV, ↓RBC, ↓ bilirubin (both sexes analysed together).	Mecoprop: LOAEL: 16 mg/kg bw/day NOAEL: 4 mg/kg bw/day	CFM (1979)
Dog; 12 months; diet; 5m+5f/ group	0, 2, 5, 19 mg/kg bw/day Mecoprop-P in diet	Males: Slightly reduced body weight, and haematological changes: ↓Hb, ↓HCT Females : Slight clinical chemistry changes : ↓ phosphate, ↓calcium	Mecoprop-P : LOAEL : 19 mg/kg bw/day NOAEL : 5 mg/kg bw/day	(1997)
Rabbit; 3 month; dermal; 5m+5f/ group	0, 10, 100, 1000 mg/kg bw/d mecoprop-P dermal	Local effects: Dermal irritation at all dose groups. Systemic effects: no adverse findings at any dose group.	Mecoprop-P: NOAEL (systemic effects): 1000 mg/kg /day LOAEL (local effects): 10 mg/kg bw/day	(1993)

¹ Study on mecoprop included in DAR for first review (1998) and included here for completeness but not relevant for mecoprop-P as adequate studies are available on mecoprop-P
m/f = males/females

Consideration of specific target organ toxicity repeat exposure (STOT RE) classification

STOT RE is defined as specific target organ toxicity following repeated exposure. The classification is relevant to effects caused by repeated exposure that are not covered more appropriately by another hazard class. Classification is appropriate where substances cause significant or severe toxic effects in animals. Significant effects are defined as changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant and impair function, both reversible and irreversible.

The kidneys are the most sensitive target organs in the repeat dose studies on rats, mice and dogs. The LOAEL for kidney effects in the 90 day studies are between 10 and 100 mg/kg bw/day which is within the guidance value for STOT RE Category 2 classification. The findings at these doses are characterised in particular by a significant increase in relative kidney weight, and increased blood urea nitrogen. Although increased blood urea nitrogen might indicate kidney damage, in the absence of any effects on other biochemical parameters indicative of kidney damage and in the absence of any histopathological findings up to the highest dose tested, it is concluded that the effects in the kidney observed below the guidance values for classification with STOT-RE are minor and do not warrant classification.

In the carcinogenicity study in rats there was a slight increase in histopathological findings in the kidneys but only at doses higher than the guidance trigger values for STOT RE classification, and there was no increase in chronic nephropathy. In the carcinogenicity studies in mice there was an increase in chronic nephropathy but only at doses higher than the guidance trigger value for STOT RE classification.

It is concluded that though the kidney is a target organ, the findings are not of sufficient magnitude to be considered to be significant or severe at doses relevant to STOT RE classification. Classification for STOT RE is therefore not triggered.

In the 90 day and 1 year dog studies the LOAELs were 16 and 19mg/kg bw/day respectively which are within the guidance value for STOT RE classification. The findings at the LOAEL were primarily confined to haematological changes indicative of anaemia. In the 90 day study there was reduced packed cell volume (8% reduction), reduced red blood cells (9% reduction). In the 1 year dog study there was a reduction in haemoglobin (5% reduction), and a reduced haematocrit (6% reduction) but both findings were only seen in males. These changes are small in magnitude, and there was no increase in severity in the 1 year study compared to the 90 day study. They are considered to be minor effects therefore classification for STOT RE is not triggered.

B.6.4. GENOTOXICITY

The data requirement Regulation (EU) 283/2013 stipulates the need for the following *in vitro* tests: bacterial assay for gene mutation, a combined test for structural and numerical chromosome aberrations in mammalian cells, and a test for gene mutation in mammalian. Depending on the results of the *in vitro* tests further studies *in vivo* are triggered.

According (EU) 283/2013 it may be necessary to consider photo-genotoxicity if the uv/vis molar absorption coefficient is $>1000 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$. Mecoprop-P meets this criterion. No photomutagenicity study has been submitted. There was no OECD test guideline available at the time of submission for photo-genotoxicity and no photogenotoxicity assays are currently recommended by Regulatory Agencies, therefore it is not clear how this data requirement should be met. The phototoxicity test (see Section B.6.2.7) may be able to give a preliminary indication of whether mecoprop-P is likely to be photoreactive.

B.6.4.1. In vitro studies

No additional studies have been conducted since the last review.

B.6.4.1.1 Gene mutation in bacterial cells

The three Ames tests on mecoprop-P were conducted to OECD 471 (1983) which precedes the current guideline OECD 471 (1987). The superseded guideline only requires the plate incorporation method, uses 4 strains instead of 5, and does not require historical positive and negative control data. In other respects the superseded protocol is broadly similar to the current guideline but less prescriptive.

Three studies on racemic mecoprop (██████████ *et al.*, 1988; ██████████ 1983 and ██████████ 1989) were submitted in the 1998 DAR. They are not required as adequate are available on mecoprop-P.

The 1998 DAR also evaluated several papers from the open literature which included Ames tests. None of these studies were considered to be reliable in the 1998 evaluation so are not considered further.

B.6.4.1.1/01

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Report on the study of MCPP (D-form), Ames test
Reference	██████████ (1984)
Date performed	February to April 1983
Test facility	██████████ ██████████
Report reference	Report no. 84/199
Guideline(s)	Not specified but similar to OECD 471 (1983)
Deviations from the guideline	No
GLP	Yes
Test material	Mecoprop-P, batch and purity not specified
Study acceptable	Yes

Study report:

██████████ (1984): Report on the study of MCPP (D-form), Ames test. ██████████
██████████ Report no. 84/199, 31 July 1984. Unpublished report. (Dossier ref. 5.26).

Study design, reporting and quality:

Mecoprop-P was tested in the Ames standard plate test using the strains TA 1535, TA 100, TA 1537, TA 1538, TA 98 both with and without S-9 mix (S-9 mix prepared according to the method described by Ames). Mecoprop-P (quality not further specified) was dissolved in DMSO and added at doses of 0, 20, 100, 500, 2500, and 5000 mg/plate. Positive control substances (2-aminoanthracene, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, 4-nitro-*o*-phenyldiamine, 9-aminoacridine chloride) were used in all assays. Two series of assays were conducted, the first using 4 test plates and the second using 2 test plates per dose level. Both tests used the plate incorporation method. Compliance to GLP stated in the report. The study is considered comparable to the test methods described in OECD 471 and EU test method B14.

Results:

No increase in revertants per plate was observed neither without nor with S-9 mix. A weakly bacteriostatic effect was observed at 5000 mg/plate with S-9 mix. Mecoprop-P was concluded not to be mutagenic in the Ames test.

Discussion and conclusion:

Mecoprop-P was not mutagenic in this assay. However, it is noted that no specifications are given with respect to the quality (purity and impurities) of the test substance.

B.6.4.1.1/02

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Ames <i>Salmonella typhimurium</i> bacterial reverse mutation assay on MCPPP-P acid
Reference	██████████ (1993)
Date performed	19 May 1993
Test facility	██
Report reference	Report no. ██████████
Guideline(s)	OECD 471 (1983)
Deviations from the guideline	No
GLP	Yes
Test material	Mecoprop-P, purity 92.6%, batch 91-1
Study acceptable	Yes

Study report:

██████████ (1993): Ames *Salmonella typhimurium* bacterial reverse mutation assay on MCPPP-P acid. ██████████ Report no. ██████████ 19 May 1993. Unpublished report. (Dossier ref. 5.27).

Study design and quality:

Mecoprop-P was tested in the Ames test using *Salmonella typhimurium* strain TA 1535, TA 100, TA 1537, and TA 98 both with and without S-9 mix. Mecoprop-P (purity of 92.6%) was dissolved in ethanol and added at doses of 50, 150, 500, 1500, and 5000 mg/plate. Positive controls were used in all assays. Two series of plate incorporation assays were conducted using 3 test plates per dose and per positive and negative control plates. As positive control substances *N*-ethyl-*N*-nitro-*N*-nitrosoguanidine, 9-aminoacridine, 2-nitrofluorene, and 2-aminoanthracene were used. Compliance to GLP stated in the report. The study was stated to be conducted according to OECD 471 and the EU test method B14.

Results:

No bacteriostatic effect was observed. With the exception of the second assay with TA 98 at 150 mg/ plate (with S-9 mix) no increase in revertants was observed. As the increase was low (+29% compared to the solvent control) and as no other positive results were obtained it was assumed that the finding occurred by change and thus, mecoprop-P was concluded not to be mutagenic in this Ames test.

Discussion and conclusion:

Mecoprop-P (purity 92.6%) was not found to be mutagenic in this assay.

B.6.4.1.1/03

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P: Assessment of mutagenic potential in histidine auxotrophs of <i>Salmonella typhimurium</i> (the Ames test)
Reference	██████████ (1990)
Date performed	23 February 1990
Test facility	██
Report reference	Report no. ██████████
Guideline(s)	OECD 471 (1983)

GLP	Yes
Test material	Mecoprop-P, purity 92.6%, batch 91-1
Study acceptable	Yes

Study report:

██████████ (1993): Chinese hamster ovary/ HGPRT locus assay MCPP-P acid ██████████. Report no. ██████████ 9 December 1993. Unpublished report. BASF doc. 93/11410. (Dossier ref. 5.35).

Study design and quality:

Mecoprop-P (purity of 92.6%) dissolved in ethanol was tested in a Chinese hamster ovary/ HGPRT locus assay both with and without metabolic activation (S9 mix). A pretest for toxicity was conducted on media without 6-thioguanine for the selection of dose levels for the main test. The pretest and the main test were performed twice with small deviations in concentration levels, (the main test dose levels are presented in table B.6.29).

Table B.6. 29 Dose levels of mecoprop-P in CHO cells/ HGPRT locus assay

Test 1	- S-9 mix	mecoprop-P µg/ml: 0 23.2 46.3 92.6 185.2 370.4 463 578.8 694.5
	+ S-9 mix	mecoprop-P µg/ml: 0 46.3 92.6 185.2 370.4 694.5 810.3 926 1041.8
Test 2	- S-9 mix	mecoprop-P µg/ml: 0 50 100 200 400 500 625 750 800 850
	+ S-9 mix	mecoprop-P µg/ml: 0 25 50 100 200 400 750 875 925 1000

positive control without S-9 mix: ethyl methansulphonate with S-9 mix: 20-methylcholanthrene

In each test duplicate cultures was used for each 4 h treatment at 37 °C at each concentration level. Following treatment and a 7 day incubation period for mutagen expression three plates were seeded with cells in non-selective medium to determine plate efficiency and five plates in selective medium (+ 6-thioguanine) to determine the number of mutant colonies (at each dose level). The plates were incubated at 37°C for 7 days whereafter the colonies were fixed, stained and counted.

The study was stated to follow OECD 476 and to comply to GLP.

Results:

In the *absence of S9 mix* cell survival compared to control was found to 116-51% in toxicity test 1 and 129-8% in toxicity test 2 at the lowest and highest dose tested. No increase in mutant frequency was found at any dose level in any of the main tests.

In the *presence of S9 mix* cell survival compared to control was found to 122-1% in toxicity test 1 and 165-33% in toxicity test 2 at the lowest and highest dose tested. In main test 1 at 92.6 µg/ml a mutant frequency of 10 per 10⁶ was obtained, which was significantly higher than in the vehicle control (1 per 10⁶). At the other dose levels no significant increase was found, and the result was not reproduced in main test 2, where the mutant frequency in control was 10 per 10⁶. (The mutant frequency in historical controls was stated to be 15 per 10⁶ cells). The test system was verified by positive outcome by the use of positive control substances (ethyl methanesulphonate and 20-methylcholanthrene).

It was concluded that mecoprop-P did not demonstrate mutagenic potential in the test.

Discussion and conclusion:

Mecoprop-P with a purity of 92.6% was not found to be mutagenic in this study.

B.6.4.1.2 /02 Chinese hamster ovary

This test used very high maximum doses (the maximum recommended in the current guideline OECD 476 (2015) is 2000 µg/mL).

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P: Investigation of mutagenic activity at the HGPRT locus in a Chinese hamster V79 cell mutation system
Reference	██████████ (1990)
Date performed	13 July to 11 September 1990
Test facility	██
Report reference	Report no. ██████████
Guideline(s)	Similar to OECD 476 (1984)
Deviations from the guideline	No significant deviations
GLP	Yes
Test material	Mecoprop-P, batch 4235, purity 88.4%
Study acceptable	Yes

Study report:

██████████ 990): Mecoprop-P: Investigation of mutagenic activity at the HGPRT locus in a Chinese hamster V79 cell mutation system. ██████████ Report no. ██████████ 26 October 1990. Unpublished report. (Dossier ref. 5.36).

Study design and quality:

Mecoprop-P (quality not specified) was tested in a Chinese hamster V79/ HGPRT locus assay both with and without metabolic activation (S9 mix). Test for toxicity was conducted on media without 6-thioguanine for the selection of dose levels for the main test. The main test consisted of two independent tests. Mecoprop-P was dissolved in DMSO and the following dose levels were used:

Table B.6. 30 Dose levels of mecoprop-P in Chinese Hamster V79 cells/HGPRT locus

Test 1	- S-9 mix	mecoprop-P µg/ml: 0 6.4 32 160 800 4000
	+ S-9 mix	mecoprop-P µg/ml: 0 6.4 32 160 800 4000
Test 2	- S-9 mix	mecoprop-P µg/ml: 0 7.2 36 180 900 4500
	+ S-9 mix	mecoprop-P µg/ml: 0 6 30 150 750 3750

positive control without S-9 mix: ethyl methanesulphonate and dimethylbenzanthracene
positive control with S-9 mix: dimethylbenzanthracene

In each test, duplicate cultures were used for each 3 h treatment at 37°C at all dose levels. Following treatment and a 7 day incubation period for mutagen expression three plates were seeded with cells in non-selective medium to determine plate efficiency and three plates in selective medium (+ 6-thioguanine) to determine the number of mutant colonies at each dose level. The plates were incubated at 37°C for 6 days whereafter the colonies were fixed, stained, and counted.

Although not stated, the study fulfils the requirements of OECD 476 and the EU test method B17. Compliance to GLP was stated in the test report.

Results:

In the *absence of S-9 mix* the dose levels were altered because no cytotoxicity was noted in test one. In test 2 a cytotoxic concentration was reached at 4500 µg/ml (plate efficiency 10% of control plates).

In the *presence of S-9 mix* the dose levels were altered due to cytotoxic response at 4000 where the plate efficiency was 14% compared to control. At 3750 µg/ml in the second test a plate efficiency of 37% was found.

No increases in mutation frequencies were observed at any of the dose levels in either of the tests both with and without S-9 mix. The sensitivity of the test system was verified by positive outcome from the use of the positive control substances.

Discussion and conclusion:

Mecoprop-P (quality not specified) was not found to be mutagenic in this study.

B.6.4.1.3 Chromosome aberration in mammalian cells

Two *in vitro* mammalian chromosome aberration tests have been conducted on mecoprop-P. Both have been conducted using human lymphocytes to OECD 473 (1983) which precedes the current guideline OECD 473 (2014). The superseded guideline is similar to the current guideline but is far less prescriptive on the experiment conditions, for example it does not specify exposure times or harvest times although it recommends multiple harvest times. It does not specify maximum concentrations, nor the number of metaphase cells that should be counted (current guidance stipulates 300 cells per dose).

B.6.4.1.3 /01 Human Lymphocytes

This was a well-conducted study which largely meets the requirements of the current guidance OECD 473 (2014) with the exception that only 200 metaphase cells were counted per dose, and the donor in experiment 1 was older than recommended age of 18-35).

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Chromosome aberration assay in human lymphocytes in vitro with mecoprop-P acid
Reference	██████████ (1994)
Date performed	4 August to 6 October 1993
Test facility	██
Report reference	Project no. 429401, BASF doc. 94/10089 and 94/10368 (1 st amendment)
Guideline(s)	OECD 473 (1983)
Deviations from the guideline	No
GLP	Yes
Test material	Mecoprop-P, purity 92.2%
Study acceptable	Yes

Study report:

██████████ (1994): Chromosome aberration assay in human lymphocytes in vitro with mecoprop-P acid. ██████████ Project no. 429401, 2 February 1994. BASF doc. 94/10089. Unpublished report. (Dossier ref. 5.37.).

Study design and quality:

Mecoprop-P (purity of 92.2%) dissolved in ethanol was in two independent experiments tested for the induction of chromosome aberrations in human lymphocytes (both with and without the addition of S-9 mix). For each experimental group duplicate cultures were used. In experiment 1 cells were collected from a female donor aged 44. In experiment 2 cells were collected from a female donor aged 26.

In the two experiments without S9 mix the cultures were exposed to media containing mecoprop-P concentrations at 0, 100, 300, and 600 µg/ml for 20 h before harvesting (and at the 300 µg/ml level also for 44 h before harvesting). In the two experiments with S-9 mix added to the media, the cultures were exposed to mecoprop-P concentrations of 300, 1000, and 2000 µg/ml for 4 h. These cultures were harvested 20 h after treatment start (at 2000 µg/ml also 44 h after treatment start). The cell cultures were treated with Colcemide three hours before harvesting. As positive controls ethylmethanesulfonate (in test without S9 mix) and cyclophosphamide (in test with S-9 mix) were used. There were two negative controls with and without solvent. The dose levels were selected on basis of a dose selection study where mecoprop-P levels in the range of 3-2000 µg/ml was used.

At least 100 well spread metaphases per culture were scored for cytogenetic damage. The evaluation was performed only for cells carrying aberrations, gaps were recorded but not included in the calculation of aberration rates.

The study was stated to comply to GLP and to the guideline described in OECD 473 and EU test method B10.

Results:

In the tests without S9 mix reduction in mitotic index were found at 300 and 600 µg/ml (reduced 34% at 300 µg/ml and 57% at 600 µg/ml compared to solvent control). Aberration rates are shown in Table B.6. 31.

Table B.6. 31 Mecoprop-P induced chromosome aberration in human lymphocytes

- S-9 mix	Aberration rate (%) at 20h (44h)			
	0 µg/ml (vehicle)	300 µg/ml	600 µg/ml	Positive control (EMS) 330 µg/ml
experiment 1	3.0% (2.5% at 44h)	8%	8.5% (7.0% at 44 h)	11.0%
experiment 2	2.0% (1.5% at 44h)	2.5%	3.5% (4.0% at 44h)	18%

In the test with S9 mix only marginal changes in the mitotic index occurred. However, the dose level used was limited because of precipitation of the test substance at higher levels.

No increases in aberration rate were found in experiment 1. In experiment 2 increased aberration rates were noted at 1000 µg/ml and 2000 µg/ml at 20 h (3.0% at both levels) compared to the solvent control (0.0%). However, the value for the solvent control was considered extremely low and the negative control showed an aberration rate of 2.5%.

It was concluded that in the presence of S9 mix mecoprop-P was not a clastogenic substance. In absence of S9 mix a final assessment was found difficult due to conflicting results.

Discussion and conclusion:

No clastogenic effect was found in the test using S9 mix. In the absence of S9 increased aberration rates (characterised by increased breaks and multiple aberrations, gaps were also increased) were found in one of two experiments. No firm conclusion with respect to clastogenic potential can be drawn from this study alone.

B.6.4.1.3 /02 Human Lymphocytes

This was a well-conducted study which largely meets the requirements of the current guidance OECD 473 (2014) with the exception that only 200 metaphase cells were counted per dose, and top doses used exceeded the maximum recommended level of 2000 µg/mL.

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	<i>In vitro</i> assessment of the clastogenic activity of mecoprop-P in cultured human lymphocytes
Reference	██████████ (1990)
Date performed	6 February to 17 March 1990
Test facility	██
Report reference	Project no. ██████████
Guideline(s)	OECD 473 (1983)
Deviations from the guideline	No significant deviations
GLP	Yes
Test material	Mecoprop-P, batch 4235, purity 88.4% mecoprop-P, 93.2% mecoprop
Study acceptable	Yes

Study report:

██████████ (1990): *In vitro* assessment of the clastogenic activity of mecoprop-P in cultured human lymphocytes. ██████████ Project no. ██████████ 5 July 1990. Unpublished report. (Dossier ref. 5.38).

Study design and quality:

Mecoprop-P (quality not specified) dissolved in DMSO was in a single experiment tested for the induction of chromosome aberrations in human lymphocytes (both with and without the presence of S9 mix). For each experimental group triplicate cultures were used.

In the experiment without S9 mix the cultures were exposed to media containing mecoprop-P concentrations at 0, 100, 200, 400 and 800 µg/ml g/ml for 24 h before harvesting. In the experiment with S9 mix the cultures were exposed to mecoprop-P concentrations of 0, 400, 800, 1600, and 3200 µg/ml g/ml for 3 h. These cultures were harvested 20 h after treatment start. The cell cultures were treated with Colcemide three hours before harvesting. Chlorambucil and cyclophosphamide were used as positive controls in the respective tests without and with S9 mix. The dose levels were selected on basis of a dose selection study, where mecoprop-P levels in the range of 100-3200 µg/ml was used.

One hundred metaphases from at least two slides from each culture and dose level were scored. The evaluation was performed both for aberrations including gaps and for aberrations excluding gaps.

The study was stated to comply to GLP and to OECD guideline 473.

Results:

In the test without S9 mix a reduced mitotic index compared to solvent control was found at 19, 22, 47, and 94% at 100, 200, 400 and 800 µg/ml, respectively. Due to this the culture at 800 µg/l was not scored for chromosome aberrations. At the other levels no treatment related increase in chromosome aberrations was found.

In the test with S9 mix at 1600 µg/ml a 76% reduction of the mitotic index compared to solvent control occurred (OECD guideline 473 recommends that cytotoxicity levels of up to approximately 50% reduction should be included for the evaluation). At 3200 µg/ml the mitotic activity was almost completely inhibited and therefore this culture was not scored for chromosomal aberrations. At 1600 µg/ml a statistically significant increase of aberrant

metaphases was observed at a level of 5.3% (range: 2-9%) for aberrations including gaps and 4% (range: 2-6%) for aberrations excluding gaps compared to solvent control (0.7% including, and 0.3% excluding gaps). At lower mecoprop-P concentrations no significant increases were found.

It was concluded that with the use of S9 mix mecoprop-P showed clastogenic activity at a cytotoxic dose level.

Discussion and conclusion:

No clastogenic effect was found in the test without S9 mix. In the presence of S9 increased aberration rate was found at a clearly cytotoxic level (where mitotic index was 23% of solvent control, which is a higher cytotoxic level than prescribed by OECD 473). No firm conclusion with respect to the clastogenic potential of mecoprop-P can be drawn from this study alone.

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

In the *in vitro* genotoxicity tests conducted on mecoprop-P there were equivocal findings of clastogenicity in the chromosome aberration tests in mammalian cells. The data requirement Regulation (EU) 283/2013 stipulates that if there is a positive *in vitro* test for clastogenicity an *in vivo* test for clastogenicity using somatic cells such as metaphase analysis in rodent bone marrow or micronucleus test is triggered.

Two *in vivo* genotoxicity studies have been conducted on mecoprop-P, an *in vivo* metaphase analysis in rodent bone marrow and an *in vivo* micronucleus test.

No additional studies have been conducted since the last review.

Two *in vivo* genotoxicity studies on racemic mecoprop (██████████, 1985b + c) were included in the 1998 DAR. They are not required since adequate studies are available on mecoprop-P.

B.6.4.2.1 *In vivo* mammalian bone-marrow cytogenetic test

B.6.4.2.1/01

This study on mecoprop-P has been conducted to OECD 475 (1984) which precedes the current guideline OECD 475 (2014). The current study protocol is broadly similar to the 2014 guideline, the main difference being that historical control data were not submitted, the sampling times differ slightly from those recommended, only 100 metaphase cells were counted (the current guideline recommends 200 per animal), and the mitotic index (an indicator of bone marrow toxicity) was not measured. Plasma levels of mecoprop-P were not measured in this study, however the tissue distribution study (██████████, 1997) in Section B.6.1.1.1 shows that mecoprop-P does reach the bone marrow although the level of exposure is relatively low. Overall this test is considered by the RMS to be valid.

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Report on the cytogenetic investigations in Chinese hamsters after a single oral administration of MCPP; D-form- bone marrow chromosome analysis
Reference	██████████ (1985a)
Date performed	January 1984
Test facility	██████████ ██████████ ██████████ ██████████ ██████████
Report reference	Report no. 85/225 (project no. 10M0020/8346)

Guideline(s)	Similar to OECD 475 (1984)
Deviations from the guideline	No
GLP	No
Test material	Mecoprop-P, purity >99%, batch 154 241 N1
Study acceptable	Yes

Study report:

██████████ (1985a): Report on the cytogenetic investigations in Chinese hamsters after a single oral administration of MCPP; D-form- bone marrow chromosome analysis. ██████████

██████████ Report no. 85/225 (project no. 10M0020/8346), 17 July 1985. Unpublished report. (Dossier ref. 5.42).

Study design and quality:

Seven groups of five male and five female Chinese hamsters (age: 7-13 weeks) were by gavage administered a single dose of mecoprop-P at 0, 60, 650, 1300, 2600, 2600 (A), and 2600 (B) mg/kg bw. Mecoprop-P (purity > 99%) was given as an aqueous 0.5 % carboxymethylcellulose formulation (probably suspension but not indicated) at a volume of 10 ml/kg (at the highest dose level 20 ml/kg bw was given). Twenty-four hours after dosing the animals were killed and bone marrow of the two femora was prepared (the subgroups A and B were killed 6 h and 48 h after dosing). Three hours before sacrifice the animals were given i.p. injections of Colcemid to arrest mitosis in metaphase. After preparation 100 metaphases were analysed per animal. A further group serving as positive control was given a dose of 60 mg/kg bw of cyclophosphamide.

No evaluation of the mitotic index was done. No GLP statement in the test report. Although not stated the study is considered to comply to OECD 475 and the EU test method B11.

Results:

The doses of 1300 and 2600 mg/kg bw led to irregular respiration, squatting posture, apathy and poor general state. Two animals at 2600 mg/kg bw (subgroup B) died 2 days after the treatment. In chromosome analysis no significant differences in the type and frequency of aberrations between the dose groups and the solvent control groups were found.

Thus, it was concluded that mecoprop-P did not have any chromosome damaging effect in the study.

Discussion and conclusion:

Cytotoxic levels in the bone marrow were not verified by the determination of mitotic index, although the highest recommended dose level according to the guidelines was reached. The conclusion in the test report is considered reliable. It should be noted that the mecoprop-P used was of high purity (>99%).

B.6.4.2.1/02

This study on mecoprop-P has been conducted to OECD 474 (1983) which precedes the current guideline OECD 474 (2014). The current study appears to be well-conducted and the protocol is broadly similar to the 2014 guideline, the main difference being that historical control data were not submitted and fewer erythrocytes were counted (the current guideline recommends 4000 per sample), and exposure of bone marrow to mecoprop-P was not demonstrated. The tissue distribution study (██████████ 1997) in Section B.6.1.1.1 shows that mecoprop-P does reach the bone marrow although the level of exposure is relatively low.

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P: Assessment of clastogenic action on bone marrow erythrocytes in the micronucleus test
Reference	██████████ (1991)

Date performed	8 October to 1 November 1990
Test facility	██
Report reference	Report no. ██
Guideline(s)	OECD 474 (1983)
Deviations from the guideline	No
GLP	Yes
Test material	Mecoprop-P, sample 4235, 88.4% mecoprop-P, 93.2% mecoprop
Study acceptable	Yes

Study report:

██████████ (1991): Mecoprop-P: Assessment of clastogenic action on bone marrow erythrocytes in the micronucleus test. ██ Report no. ██ 11 February 1991. Unpublished report. (Dossier ref. 5.43).

Study design and quality:

Nine groups of five male and five female CD-1 mice (4-5 weeks old) were gavaged with single doses of mecoprop-P at either 0, 0 (A), 0 (B), 20, 100, 500, 500 (A), and 500 (B) mg/ kg bw. (The dose levels were chosen on the basis of a preliminary toxicity test using mecoprop-P doses in the range of 62.5 - 500 mg/kg bw). Mecoprop-P (88.4% mecoprop-P, 93.2% mecoprop) was suspended in 0.5% methylcellulose and a dose volume of 10 ml/kg bw suspension was used for gavage administration. Further, a positive control group treated with chlorambucil, 30 mg/kg bw was included.

The animals were killed 24 h after treatment. Subgroups A and B were killed 48 h and 72 h after treatment, respectively. From the animals samples from the femoral bone marrow were taken and after preparation at least 2000 erythrocytes were examined for micronuclei (the number of micronucleated cells per 1000 erythrocytes was calculated) and the ratio of polychromatic to mature erythrocytes determined.

The study was stated to be conducted according to GLP and according to OECD 474.

Results:

At 500 mg/kg bw signs of toxicity including hypoactivity, hunched posture and rales were observed. Two animals were killed because of poor condition. No differences were observed between the ratios of polychromatic to mature cells, compared with the controls indicating a lack of cytotoxic response in the bone marrow. Frequencies of micronucleated polychromatic erythrocytes in animals killed 24, 48 and 72 h after administration of mecoprop-P were similar to those in concurrent controls.

Thus, mecoprop-P was concluded not to induce micronucleus formation in erythrocytes of mice.

Discussion and conclusion:

It should be noted that no specification for the test substance is available.

For the test substance used, this study does not indicate any chromosome damaging effects with regard to micronucleus formation in erythrocytes.

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

In vivo genotoxicity in germ cells are not required, since the *in vivo* study in somatic cells demonstrated that mecoprop-P is not genotoxic.

B.6.4.4 Summary of Genotoxicity studies

The following Table B.6. 32 presents those genotoxicity studies submitted with the dossier from the notifier that are considered acceptable with respect to purpose and quality.

Table B.6. 32 Summary of genotoxicity testing of mecoprop and mecoprop-P

Test system	Dose range, mecoprop-P	Response -S9 / +S9	Reference
Bacterial assays			
<i>Salmonella typhimurium</i> strain TA98, TA100, TA1535, TA1537, TA1538	20-5000 mg/plate	negative/ negative	██████████ (1984)
<i>Salmonella typhimurium</i> strain TA98, TA100, TA1535, TA1537	50-5000 mg/plate	negative/ negative	██████████ (1993)
<i>Salmonella typhimurium</i> strain TA98, TA100, TA1535, TA1537	10-1000 mg/plate	negative/ negative	██████ (1990)
<i>Salmonella typhimurium</i> strain TA97, TA98, TA100, TA102	0.001-1000 mg/plate (racemic mecoprop)	negative/ negative	██████████ (1988)
<i>Salmonella typhimurium</i> strain TA98, TA100, TA1535, TA1537	20-5000 mg/plate (racemic mecoprop)	negative/ negative	██████████ (1983)
<i>Escherichia coli</i> strain PQ37	0.1-100 000 mg/ml (racemic mecoprop)	negative/ negative	██████████ (1989)
Mammalian cell <i>in vitro</i> assays			
Chinese hamster ovary cells/ HGPRT locus	23-1040 µg/ml	negative/ negative	██████████ (1993)
Chinese hamster V79 cells/ HGPRT locus	6-4500 µg/ml	negative/ negative	██████ (1990)
Human lymphocytes cytogenetic test	100-2000 µg/ml	positive*/ negative	██████████ (1994)
Human lymphocytes cytogenetic test	100-3200 µg/ml	negative/ positive*	██████████ (1990)
Mammalian <i>in vivo</i> assays			
Chinese hamster, bone-marrow cytogenetic test	60-2600 mg/kg bw	negative	██████████ (1985a)
Chinese hamster, bone-marrow cytogenetic test	60-3800 mg/kg bw (racemic technical mecoprop)	positive at 3800 mg/kg bw*	██████████ (1985b)
Chinese hamster, bone marrow sister chromatid exchange	60-3800 mg/kg bw (racemic technical mecoprop)	positive dose related*	██████████ (1985c)
Mouse, micronucleus test	20-500 mg/kg bw	negative	██████████ (1991)

* only positive results at cytotoxic/ toxic levels.

Tests highlighted in grey were included in the 1998 DAR but are not considered further in this renewal as they were conducted on the racemic mix.

In bacterial assays and in mammalian cell *in vitro* assays no mutagenic potential was found for mecoprop-P. Conflicting results were obtained in two *in vitro* assays with human lymphocytes at cytotoxic levels. One test

showed positive clastogenic response with S9 mix and the other test showed positive effect without S9 mix, however, only at clearly cytotoxic levels. *In vivo* no genotoxic potential was found at any time in a bone-marrow cytogenetic test and in a micronucleus test with mecoprop-P.

Further as the *in vivo* tests with mecoprop-P are negative there is no clear evidence for genotoxicity *in vivo* and thus the test results do not meet the criteria under (EC) 1272/2008 for classification as a mutagen.

In the review report previous review it was concluded that taking a weight-of-evidence approach there was no genotoxic concern for mecoprop-P.

The RMS for current renewal agrees with the conclusion of the previous evaluation for Annex I inclusion. Mecoprop-P had equivocal evidence of clastogenicity in mammalian cells *in vitro* at doses where cytotoxicity was evident, but two *in vivo* studies for clastogenicity were clearly negative. Most of the studies submitted were conducted to older versions of the OECD test guidelines, but overall this is not considered to invalidate the test results. It is concluded that from the evidence provided, mecoprop-P does not require classification for mutagenicity under (EC) 1272/2008.

Applicant:

The mutagenic potential of Mecoprop-P was studied in two human lymphocytes studies. In the first study (██████████, 1994), no clastogenic effect was found in the test using S-9 mix. In the absence of S-9 increased aberration rates were found in one of two experiments. In the second study (Edwards, 1990), no clastogenic effect was found in the test without S-9 mix. In the presence of S-9 increased aberration rate was found at a clearly cytotoxic level (at higher cytotoxic level than prescribed by OECD 473). On balance it was considered that Mecoprop-P is not of genotoxic concern.

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

A new 2 year carcinogenicity study in the rat conducted on mecoprop-P was submitted for this renewal. All other studies have been previously evaluated.

The data requirements in Regulation (EU) 283/2013 state that a long-term study and a long-term carcinogenicity study in the rat is required in addition a second carcinogenicity in in the mouse is normally also expected. Carcinogenicity studies conducted in the rat and mouse are available for mecoprop-P. No long term (1 year) study was conducted on mecoprop-P in the rat. This is potentially a data gap. A long term cohort was included in the carcinogenicity study conducted on racemic mecoprop that was submitted in the 1998 DAR (██████████, 1988) so is also included in the current submission. In addition a one-year dog study conducted on mecoprop-P has been submitted (summarised in the short term studies in Section B.6.3.3.1) and may also be considered to be a chronic study.

Regulation (EU) 283/2013 has specific guidance for the acceptability of historical control data. The historical control data for carcinogenicity studies submitted for the previous renewal review have been reappraised to current data requirements.

B.6.5.1 Chronic toxicity and carcinogenicity in the rat

This study on racemic mecoprop was done to OECD 453 (1981) which precedes the current guideline OECD 453 (2009). The main difference between this guideline and the current one is that in the 1981 guideline historical control data requirements are less prescriptive, organ weights are not required and there is no requirement for immunotoxicity and neurotoxicity investigations to be carried out if triggered by effects in previous studies. In the current study no historical control data was provided. The organ weights of the heart, liver, kidneys, testes, brain and adrenals, and ovaries were determined. It is concluded that this study largely meets the current OECD data requirements the main deficiencies being that the spleen, epididymides, uterus, brain and thyroid were not weighed.

Previous evaluation:	In DAR for first review (1998). This study on racemic mecoprop was considered applicable also to mecoprop-P in the previous renewal. No historical control data supplied with this study
----------------------	--

Study	Study on the chronic toxicity and oncogenic potential of MCPP in rats. Administration in the diet over 24 months
Reference	██████████ (1988): Study on the chronic toxicity and oncogenic potential of MCPP in rats
Date performed	2 May 1984 to 11 June 1986
Test facility	██████████ ██████████ ██████████ ██████████
Report reference	Report no. 71S0047/8352 (BASF doc 88/0386)
Guideline(s)	OECD 453 (1981)
Deviations from the guideline	No significant deviations. Clinical chemistry and urine analysis were not measured at 3 months (haematology was evaluated in the 90 day study by Reinert 1979, and urinalysis in the 90 day study by Kirsch 1985).
GLP	Yes
Test material	Mecoprop racemate, purity 92.7%, Batch TPH
Study acceptable	Yes

Study report:

██████████ (1988): Study on the chronic toxicity and oncogenic potential of MCPP in rats. Administration in the diet over 24 months. ██████████
 ██████████ Report no. 71S0047/8352 (BASF doc 88/0386), 23 August 1988, unpublished report. (Dossier ref. 5.44 & 5.5/01).

Study design and quality:

Four groups of 50 male and 50 female 6 week-old Wistar rats were through the diet given 0, 20, 100, and 400 ppm racemic mecoprop (purity. 92.7%) for 24 months. The dose levels were selected on the basis of the results in the 3 months study described in section B.5.3.1.4. A *satellite group I* with ten male and ten female rats at each dose level was dosed for 12 months, whereas *satellite group II* with 15 male and 15 female rats at each dose level was dosed for 24 months. In satellite group I determination of body weight, feed consumption, urinalyses, and hormone analyses (T3/T4) were performed. In satellite group II clinicochemical and haematological examinations were performed with intervals of 6 months and the animals were subjected to necropsy after 24 months. Absolute and relative organ weights of the heart, liver, kidneys, testes, brain and adrenals, and ovaries were determined for all groups. The study was stated to follow OECD 453 and GLP. The use of two satellite groups is a minor deviation from OECD 453 and the EU test method B33 as these guidelines prescribe one satellite group of 20 animals per sex at each dose level. This deviation, however, is not considered to make any great difference with respect to the quality of the study.

Results:

No influence on mortality was noted in the dosed groups. Body weight was significantly reduced in males at 20 and 100 ppm in satellite group I. However, as no such finding was observed in the main group and in satellite group II, this was not considered treatment related. No treatment related findings in feed consumption were noted. No treatment related clinical findings were observed. In the clinicochemical and the haematological examinations of blood samples collected with intervals of six months only isolated parameters were significantly altered compared to control values. These changes were by the authors not considered treatment related as no consistent patterns of the changes were observed (such as persistent changes over several blood sampling periods or dose-response relationship). However, significantly increased levels of urea were found after 18 and 24 months in males at the 400 ppm level. Significantly increased levels of triglycerides were found at all dose levels in males after 24 months of dosing, while no such finding was noted in females.

The results from the determination of organ weights and relative organ weights were presented as pooled results from the main group and the satellite group II. The significant findings are presented in Table B.6. 33:

Table B.6. 33 Absolute and relative organ weights after 12 and 24 months administration of mecoprop in the diet to rats

	0 ppm m/f	20 ppm m/f	100 ppm m/f	400 ppm m/f
Dose in mg/kg bw/day *	-/-	1.1/ 1.4	5.5/ 6.9	22.2/ 27.9
	Findings at 12 months			
Kidney weight, g	3.13/2.16	3.08/2.07	3.21/2.15	3.47/2.21
Kidney weight compared to control		2% ↓ / 4% ↓	2% ↑ / 0%	11% ↑ / 2% ↑
Relative kidney weight, g	0.50/0.69	0.55/0.68	0.58 [#] /0.70	0.59 ^{##} /0.73
Relative kidney weight compared to control		10% ↑ / 1% ↓	16% ↑ / 1% ↑	18% ↑ / 7% ↑
	Findings at 24 months			
Kidney weight, g	3.80/ 2.69	3.83/ 2.77	4.07 [#] / 1.97	4.31 ^{##} / 2.82
Kidney weight compared to control		0% / 3% ↑	7% ↑ / 27% ↓	13% ↑ / 5% ↑
Relative kidney weight, g	0.60/ 0.76	0.61/ 0.74	0.62/ 0.76	0.66 ^{##} / 0.77
Relative kidney weight compared to control		2% ↑ / 3% ↓	3% ↑ / 0%	10% ↑ / 1% ↑

*: calculated for the main group # and ##: 5% and 1% significance level (Dunnett's test)

Histopathologically no treatment related changes were found in any organ in the dosed groups. No differences were observed with regard to type and occurrence of tumours, and with regard to the overall occurrence of benign and malignant tumours.

Discussion and conclusion:

Mecoprop (racemic form) was not tumorigenic in this study. The increase of kidney weight in male rats at 100 and 400 ppm was the most prominent observation. The NOEL is 20 ppm (corresponding to 1.1 mg/kg bw/day). At 24 months 100 ppm the absolute kidney weight only is slightly increased in males and not the relative weight. Furthermore there are no other effects on the kidneys in this dose as e.g. changes in clinical chemical parameters or histopathological changes. Increased levels of blood urea were found at 400 ppm. No further consistent effects were found on the haematological and clinicochemical parameters. Similar findings were also seen in the 12 month cohort except that the increase in relative kidney weight was more marked being 16% and 18% in the 100 and 400 ppm dose groups respectively therefore the NOAEL in the 12 month cohort is considered to be 20 ppm.

This study was discussed in the ECCO93 discussion for the 1998 DAR

The minutes of the meeting report: 'A long discussion took place as to which would be the appropriate value. In the end the experts agreed to take the lower figure of 1.1 mg/kg bw/day (20 ppm; 2-year rat) to be on the safe side. The proposed NOAEL of 5 mg/kg bw/d (100 ppm) was not taken because an increase of relative or absolute kidney weight in males has been observed at this dose level. Some of the experts were however not really convinced that the kidney weight increase in male rats at 100 ppm was considered a real adverse effect.'

The RMS for this current renewal upholds the original decision that the NOAEL at 24 months is 1.1 mg/kg bw/day. Though it might appear to be conservative it is supported by a clear NOAEL of 1.1 mg/kg bw/day in the 12 month cohort.

B.6.5.1.1 Carcinogenicity study in the rat

This study on mecoprop-P was done to OECD 451 (1981) which precedes the current guideline OECD 451 (2009). The main difference between the old guideline and the current one is that the criteria for acceptable historical control data are different. Therefore the historical control data in this study have been re-checked for this submission to ensure they meet current standards.

No validation data were supplied for this study, so the method of analysis used cannot be validated in accordance with SANCO/3029/99/rev.4.

This new study is a carcinogenicity study conducted on mecoprop-P – there was no 12 month cohort in this study. A chronic study in the rat is not available for mecoprop-P. A combined chronic and carcinogenicity study in the rat conducted on racemic mecoprop was evaluated in the 1998 DAR (see Section B.6.5.1).

A supplementary study (██████████ 007) was combined with this carcinogenicity study (██████████ 008) in order to elucidate a mechanism for effects seen in the liver (summarised in B.6.5.1.2). Liver samples were analysed for peroxisomal β -oxidation activity in order to estimate the degree of peroxisome proliferation.

Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012
----------------------	--

Study	Mecoprop-P dietary two year carcinogenicity study in the rat
Reference	██████████ (2008)
Date performed	8 March 2005 to 17 October 2007
Test facility	██
Report reference	██████████
Guideline(s)	OECD 451 (1981)
Deviations from the guideline	None
GLP	Yes
Test material	Mecoprop-P, purity w/w 92.0%, batch MC/05/01
Study acceptable	Yes

Method:

1. Animal assignment and treatment

Fifty two rats per sex/dose, strain HsdRCCHan:WIST, were approximately 21 to 26 days old at the start of the study. The animals were fed diets containing 0, 100, 600 or 1200 ppm mecoprop-P (equivalent to 0, 5.3/6.6, 32.0/39.9, 64.6/81.7 mg/kg bw/day in males/females) for a period of at least 104 weeks. 2% amorphous silica was added as an anti-caking agent added to all dose groups including control.

2. Statistics

The data did not warrant statistical analysis.

3. Haematology & clinical chemistry

At weeks 53 and again at week 79 bloods was collected from all rats to produce a blood smear. These were not examined. At scheduled termination of the study blood was collected and a differential white cell count was performed. A blood film was prepared for all animals at scheduled termination and examined where the automated analysis results suggest this was necessary.

4. Urinalysis

Urinalysis was not performed.

5. Sacrifice and pathology

At necropsy the following organs were weighed for all surviving animals: the adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes and uterus – including cervix.

Samples for electron microscopy were taken and processed but were not examined further.

Samples for liver biochemistry were taken from 12 animals per sex and group and were analysed in the study by ██████████ (2007).

All animals were examined *post mortem*. A complete tissue inventory was collected from each animal, and processed for routine histopathological examination. Tissues in the control and top dose group, tissues from animals that died before the end of the study, and any tissues with gross lesions were examined for histopathological changes.

Results:

1. Clinical signs of toxicity

There were more males in the 1200 ppm group with dermal or subcutaneous masses than in the control or intermediate dose groups. Other findings are of a type and incidence commonly seen in rats and are considered to be unrelated to administration of Mecoprop-P.

2. Mortality

At least 80 % of males and 70 % of females survived to scheduled termination in the 0, 600 and 1200 ppm groups. Survival was lower in both males and females in the 100 ppm group, 69 % in males and 58 % in females. The survival is within the acceptance criteria for a reliable study.

There were no treatment related alterations in the factors which were considered contributory to death.

3. Bodyweights and food consumption

Body weights of females in the 1200 ppm group were up to 18% lower than controls throughout the study. Body weights of males in the 1200 ppm group were slightly reduced during the first year of the study. The maximum difference was 4% below group mean control weight.

Body weights of females in the 600 ppm group were reduced by up to 7 % compared to the control group for most of the study. There was no evidence of a reduction in body weight in males in the 100 or 600 ppm groups or females in the 100 ppm group.

Food consumption of females in the 1200 ppm group was reduced throughout the study. The maximum difference was approximately 10% below the control group. Food consumption of males in the 1200 ppm group was statistically significantly below the control group on some occasions during the first 13 weeks of the study, thereafter it was similar to the control. Food utilisation was less efficient in the 1200 ppm group overall (weeks 1-13) and for all intervals in females and for weeks 5-8 and 9-13 in males.

For females in the 600 ppm group there was a trend towards lower food consumption in the first year of the study but the differences from control are small and achieved statistical significance on only a few occasions. There was no consistent effect on food consumption in males in the 600 ppm group. Food utilisation was lower in females in the 600 ppm group for weeks 9-13 and overall. There was no effect on food consumption or food utilisation in males or females in the 100 ppm group.

Dose rates (based on nominal dietary levels of Mecoprop-P) were calculated in terms of mg Mecoprop-P/kg bodyweight. Mean values are shown below Mecoprop-P/kg/day.

Table B.6. 34 Mean dose received mg Mecoprop-P/kg/day

	Dietary concentration (ppm)		
	100	600	1200
Males	5.3	32.0	64.6
Females	6.6	39.9	81.7

4. Haematological findings

Two males in the 100 ppm group had abnormally high white cell counts. Both animals had pathology findings to account for the abnormal white cell values; one had a tubular adenocarcinoma affecting the lymph glands and the other had lymphoid proliferation in the spleen. There was no evidence of a treatment-related effect on the differential white cell count of treated rats.

5. Organ weight

Organ weight findings are summarised in Table B.6. 35. In females relative liver weights were 14% higher than controls in the 1200 ppm group.

In females relative kidney weights show a clear dose related increase compared to controls of 51%, 65% and 73% in the 100, 600 and 1200 ppm dose groups respectively which is considered to be treatment related and adverse. In males relative kidney weights were 21% higher in the 1200 ppm dose group and are considered to be treatment related and adverse.

Weights of adrenals, brain, epididymides, heart, ovaries, spleen, testes and uterus including cervix were similar in treated and control animals.

Table B.6. 35 Organ weight findings in 2 year carcinogenicity study in rats conducted on mecoprop-P

Dose ppm	Males				Females			
	0	100	600	1200	0	100	600	1200
Dose Mg/kg bw/day	0	5.3	32.0	64.6	0	6.6	39.9	81.7
Body weight gain (g)	417.9	428.7	422.3	410.3	259.8	252.9	247.0	199.7**
Terminal body weight (g)	544.1	557.8	545.2	538.0	368.8	361.5	358.3	308.0**
Liver weight (g)	15.0	15.2	14.3	14.1	10.3	10.2	10.6	9.9
Relative liver weight (%) ¹	2.8	2.7	2.6	2.6	2.8	2.8	3.0	3.2
Relative liver weight compared to control		4%↓	7%↓	7%↓		0%	7%↑	14%↑
Kidney weight (g) ¹	2.62	2.91**	3.08**	3.07**	2.00	2.02	2.18	1.96
Kidney weight compared to control		11%↑	18%↑	17%↑		1%↑	9%↑	2%↓
Relative kidney weight (%) ¹	0.48	0.53	0.57	0.58	0.37	0.56	0.61	0.64
Relative kidney weight compared to control		10%↑	1%↑	21%↑		51%↑	65%↑	73%↑

** Statistically significant difference from the pooled control group mean at the 1% level (Student's t-test, two-sided).

* Statistically significant difference from the pooled control group mean at the 5% level (Student's t-test, two-sided).

¹ No statistical analysis conducted on relative organ weights

6. Gross and histopathology

Histopathological findings are summarised in Table B.6. 36. Significant treatment related effects were recorded in the liver of males and females in the 600 and 1200 ppm groups. The changes were more pronounced in females. Significant changes were also seen in the kidney in the 1200 ppm group.

Liver

Changes in the liver consisted of the presence of hyalinization (defined by a ground glass eosinophilic appearance of hepatocytes), predominantly in centri-lobular regions, which is characteristic of peroxisome proliferators. It was observed in 37 out of 52 females in the 1200 ppm group but only in three males in this dose group. This change was not seen at lower doses. In addition, an increase in pigment deposition (within macrophages and centri-lobular hepatocytes) was observed in similar numbers of animals in the 1200 ppm

group but also in a small number of females in the 600 ppm group. The incidence of fat recorded within the liver was decreased in both males and females in the 600 and 1200 ppm groups. Hypertrophy of liver cells was recorded in approximately 25% of females in the 1200 ppm group.

Dose dependent increases in hepatic peroxisomal β -oxidation (see study by ██████████ (2007) in Section B.6.5.1.2 below) as determined by measurement of cyanide-insensitive palmitoyl CoA (PCO) oxidation was observed. Although this response was significantly different from control animals at 600 ppm in female rats and 1200 ppm for both sexes, mecoprop-P increased PCO in both sexes by less than two-fold, and was thus a minimal increase in peroxisomal enzyme activity.

Kidney

There was a decreased incidence of chronic progressive nephropathy in the kidneys of males in the 1200 ppm group compared to control. This finding is considered not to be adverse. There was a slight increase in pelvic urolithiasis, mononuclear cell infiltration and vascular ectasis of the pelvis in males in the top dose group, but these findings were all of minimal severity. In females in the 1200 ppm group there was a higher incidence of a number of findings in the kidneys compared to control (intratubular microlithiasis, tubular basophilia/dilatation and vascular ectasia of the renal pelvis). These changes were minimal in extent and were not seen as part of a pattern in the same animals. However overall it is considered there was a slight increase in findings in the kidneys at the top dose group that is considered to be treatment-related.

Other findings are shown in Table B.6. 36 below. At the top dose group in both sexes there was a slight increase in mononuclear cell infiltration in the Harderian glands and increased pituitary cysts – both of these findings were minimal in severity. In males in the top dose there was a very slight increase in vascular ectasia of the adrenal gland (graded minimal). In females in the top dose group there was a slight increase in haemosiderin in the spleen, increased dilation of the glandular stomach and increased glandular dilation in the uterus all of these were graded minimal in severity. As these findings occur are only graded slight and only slightly higher than background levels it is possible that these are incidental findings, although currently no historical control data are available to confirm this.

Table B.6. 36 Non-neoplastic findings in 2 year carcinogenicity study in rats administered mecoprop-P in the diet (all animals in terminal kill and that died during course of study)

Dose ppm	Males				Females			
	0	100	600	1200	0	100	600	1200
Dose Mg/kg bw/day	0	5.3	32.0	64.6	0	6.6	39.9	81.7
Liver								
Liver centrilobular hepatocyte hyalinisation	0/52	0/52	0/52	3/52	0/52	0/52	0/52	37/52
Liver increased pigmentation	0/52	0/52	0/52	3/52	8/52	8/52	12/52	42/52
Hepatocyte fat vacuolation	23/52	26/52	11/52	5/52	22/52	23/52	11/52	13/52
Hepatocyte hypertrophy	0/52	0/52	0/52	0/52	1/52	3/52	3/52	14/52
Kidney								
Chronic progressive nephropathy	10/52	4/18	1/11	1/52	5/52	3/23	1/13	4/52
Kidney pelvic urolithiasis	10/52	6/18	2/11	17/52	37/52	9/23	10/13	40/52
Kidney tubular basophilia	1/52	1/18	0/11	1/52	0/52	1/23	1/13	5/52
Kidney tubular dilation	1/52	0/18	0/11	1/52	0/52	1/23	1/13	6/52

Dose ppm	Males				Females			
	0	100	600	1200	0	100	600	1200
Dose Mg/kg bw/day	0	5.3	32.0	64.6	0	6.6	39.9	81.7
Kidney interstitial mononuclear cell infiltration	11/52	1/18	2/11	19/52	8/52	3/23	0/13	5/52
Kidney pelvis vascular ectasia	0/52	0/18	0/11	1/52	1/52	0/23	0/13	13/52
Findings in other organs								
Adrenal gland vascular ectasia	2/34	5/19	4/13	9/52	35/52	17/24	12/17	26/52
Harderian gland mononuclear cell infiltration	8/52	2/15	1/10	14/52	9/52	5/23	2/10	14/52
Pituitary cysts	8/52	1/20	3/17	13/52	3/52	4/33	3/31	9/52
Spleen increased haemosiderin	2/52	1/20	0/10	2/52	4/52	5/23	6/14	9/52
Stomach glandular dilation	0/52	0/18	0/10	3/52	4/52	1/22	0/11	7/52
Thymus epithelial cysts	9/43	1/11	2/9	10/42	3/49	3/22	2/14	6/46
Uterus glandular dilation	-	-	-	-	12/52	9/24	4/15	19/52
Uterus stromal polyp (Benign)	-	-	-	-	1/52	0/24	2/15	4/52

7. Neoplastic findings

There was a slightly higher incidence of lipoma (benign fatty tumour) of the sub cutis in males in the 1200 ppm group. The incidence was 0, 0, 1 and 4 in the control, 100, 600 and 1200 ppm groups respectively. This was statistically significant when analysed by the Peto trend test for groups 1 to 4, but not for groups 1 to 3. It should be noted that in males very few samples of sub-cutaneous tissue were examined (for animals that died during the study or displayed gross lesions).

Historical control data for the incidence of benign lipoma in males from two studies conducted within 5 years of the current study are:-

PR1248 start August 2002 incidence 2/52 males

PR1321 start January 2005 incidence 0/52 males

The lab and strain used in these historical control studies has not been confirmed. The incidence of lipoma in males at the top dose group exceeds the historical control. In the metabolism studies rats administered a high dose of mecoprop-P showed a highest levels of radioactivity in fat (see Table B.6. 37) providing evidence that fat is highly exposed to mecoprop-P.

There were no differences in the intergroup comparison of overall tumour incidences in any group.

Table B.6. 37 Subcutaneous findings in 2 year carcinogenicity study in rats conducted on mecoprop-P

Dose ppm	Males				Females			
	0	100	600	1200	0	100	600	1200
Dose Mg/kg bw/day	0	5.3	32.0	64.6	0	6.6	39.9	81.7
Subcutaneous tissue lipoma (Benign)	0/4	0/4	1/7	4/10	0/16	2/13	0/19	0/7

There were 52 animals per sex/dose group. Only animals with gross subcutaneous lesions or animals that died during the study were examined histologically for lipoma

Numbers of findings are expressed as number of animals with lesions/numbers of animals examined

Conclusion:

In this 2 year carcinogenicity study the findings were:

1200 ppm :

Males: Slightly reduced bodyweight gain during first year of study, slightly reduced food consumption during the first 3 months of the study. In the liver there was decreased fat vacuolation. In the kidney there was a 21% increase in relative kidney weight, slightly increased incidence of pelvic urolithiasis, and increased interstitial cell mononuclear cell infiltration. However there was also a reduction in chronic progressive nephropathy. The main findings in other organs were slight increases in vascular ectasia in the adrenal gland, increased mononuclear cell infiltration in the Harderian gland, a margin increase in pituitary cysts and glandular dilution of the stomach. The only neoplastic finding was a slight increase in benign lipoma in subcutaneous tissue (4/52 at 1200 ppm versus 0/52 in controls) which exceeds the historical control incidence (2/52 but only two historical control studies were available). Adipose tissue was found to be highly exposed to the test substance in the metabolism studies. However taking a weight of evidence approach these tumours are most likely to be incidental since lipoma only occurred in single sex, and only marginally exceed the historical control incidence, and was not seen in the previous carcinogenicity study conducted on racemic mecoprop. This tumour type is unlikely to progress to malignancy.

Females: Reduced bodyweight gain and food consumption throughout the study. In the liver there was a 14% increase in relative liver weight, increased hepatocyte hypertrophy and hepatocyte hyalinization in the centrilobular area, reduced fat vacuolation and increased pigmentation. In the kidney there was a 73% increase in relative kidney weight, and increased incidence of tubular basophilia, increased tubular dilation and increased vascular ectasia in the kidney pelvis. The main findings in other organs were increased mononuclear cell infiltration in the Harderian gland, a marginal increase in pituitary cysts and glandular dilation of the stomach, increased haemosiderin in the spleen, increased epithelial cysts in the thymus and increased glandular dilation of the uterus, and a slight increase in benign stromal polyps of the uterus, which is probably incidental.

600 ppm:

Males: No adverse findings.

Females: slightly reduced bodyweight gain for most of the study, slightly reduced food consumption in the first year of the study.

In the kidney there was a 65% increase in relative kidney weight.

100 ppm:

Males: No adverse findings.

Females: In the kidney there was a 51% increase in relative kidney weight.

The NOAEL for non-neoplastic findings is 600 ppm in males (32.0 mg/kg bw/day), but no NOAEL could be determined in females as relative kidney weight was increased even at the lowest dose. The NOAEL in females is below 100 ppm (equivalent to 6.6 mg/kg bw/day).

The NOAEL for neoplastic findings is > (equivalent to 39.9 mg/kg bw/day in males) based on increased benign subcutaneous lipoma in males.

The applicant was requested to provide further historical control data form subcutaneous lipoma from the same lab and strain and conducted within 5 years of the current study but was unable to do so.

Applicant conclusion: Mecoprop-P is concluded not to be carcinogenic under the conditions of this test.

The NOAEL was 100 ppm (5.3 mg/kg bw for males and 6.6 mg/kg bw for females). The target organs were the liver and kidneys.

B.6.5.1.2 Carcinogenicity study in the rat: enzyme activity assay

Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012
----------------------	--

Study	Ex-vivo enzyme analysis of liver samples taken at termination of a dietary 2 year carcinogenicity study of Mecoprop-P in the rat
Reference	██████████ (2007)
Date performed	29 May 2007
Test facility	██
Report reference	██████████
Guideline(s)	Not applicable
Deviations from the guideline	Not applicable
GLP	No – but the principles of GLP were followed
Test material	Mecoprop-P, purity w/w 92.0%, batch MC/05/01

Liver samples were taken from rats treated for 104 weeks with mecoprop-P in the diet. Six livers from each sex and dose were analysed for peroxisomal β -oxidation by analysing for cyanide insensitive acyl CoA oxidation activity, using palmitoyl CoA as a substrate.

Results

There was a dose dependent increase in hepatic peroxisomal β -oxidation. The increase was less than 2-fold higher than control, indicating a minimal increase in peroxisomal enzyme activity.

Table B.6. 38 Hepatic cyanide-insensitive palmitoyl CoA oxidation in rats treated for 2 years with mecoprop-P in the diet (n = 6)

Dose ppm	Males				Females			
	0	100	600	1200	0	100	600	1200
Dose Mg/kg bw/day	0	5.3	32.0	64.6	0	6.6	39.9	81.7
Palmitoyl CoA Oxidation Nmol NAD ⁺ reduced/min/mg protein	11.49	11.11	13.46	21.02**	12.07	13.54	17.14**	22.52***
% of control	100	96.73	117.18	182.99	100	112.17	142.01	186.54

Conclusion

NOAEL was 100 ppm in females (equivalent to 6.6 mg/kg bw/day) based on a significant increase in palmitoyl CoA oxidation in females at doses of 600 ppm and 1200 ppm. In males the NOAEL for increased palmitoyl CoA oxidation was 600 ppm (equivalent to 32.0 mg/kg bw/day). These findings are evidence that mecoprop-P induces peroxisomal proliferation.

B.6.5.2 Chronic toxicity and carcinogenicity in the mouse

B.6.5.2/01

This study on mecoprop-P was done to OECD 451 (1981) which precedes the current guideline OECD 451 (2009). The main difference between the old guideline and the current one is that the criteria for acceptable historical control data are different.

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Mecoprop-P - Carcinogenicity study in B6C3F1/CrIBR mice. Administration in the diet for 18 months
Reference	██ (1996)
Date performed	30 December 1992 to 26 July 1994
Test facility	██████████ ███ ██████████ ██████████ ██████████ ██
Report reference	Report no. 76S0002/91102
Guideline(s)	OECD 451 (1981)
Deviations from the guideline	Animals in the top dose group were sacrificed after 12 months due to safely reduced body weight gain.
GLP	Yes
Test material	Mecoprop-P, batch 91-1, purity 92.7%

Study report:

██ (1996): Mecoprop-P - Carcinogenicity study in B6C3F1/CrIBR mice. Administration in the diet for 18 months. ██████████ ███ ██████████ ██████████
██. Report no. 76S0002/91102, June 21, 1996, unpublished report.

Study design and quality:

Four groups of 50 male and 50 female B6C3F1/CrIBR mice 50 days old were through the diet given 0, 25, 250, and 2500 ppm mecoprop-P of 92.7% purity for 18 months. The dose levels were selected on the basis of two 4-week and two 3-months feeding studies in mice with racemic mecoprop.

The study is stated to have been carried out according to GLP and to follow OECD test guideline 451 and EU test method B32. Investigations included a differential blood cell count, and organ weights of liver, kidneys, adrenal glands, testes, ovaries, and brain, histopathological examination of all gross lesions, all organs in the control and mid dose group, and the lungs, liver and kidneys of the low dose group (the top dose group was not subject to histopathology or organ weights as the animals displayed extreme bodyweight effects and were sacrificed early).

Results:

In the 2,500 ppm group one male died on day 280, and body weight gain was severely affected, being statistically significant from day 7 (males) or day 21 (females) though food consumption was equal in all four groups.

On day 343 the body weight of the 2,500 ppm animals were 27 and 37% below controls for males and females, respectively. On the same day the body weight gain was 58 and 71% below that of controls for males and females, respectively.

The animals of the 2,500 ppm group were killed after about 12 months without further examination due to the severely affected body weight gain.

The mean daily intakes of mecoprop-P in the three dosed groups were as given in Table B.6. 39

Table B.6. 39 Mean daily intake of mecoprop-P for mice in the 18-months carcinogenicity study.

Concentration in diet (ppm)	Intake male animals (mg/kg bw/day)	Intake female animals (mg/kg bw/day)	Intake all animals (mg/kg bw/day)
25	4	4	4
250	40	46	43
2,500*	592	732	662

*Note only up to day 315.

The mortality in the three groups, which were allowed to live for 18 months, is given in Table B.6. 40 Mortality is within acceptable levels (except for the top dose group 25000 ppm).

Table B.6. 40 Mortality in the 18-months carcinogenicity study with mecoprop-P in mice.

Test group	% mortality males	% mortality females
0 ppm	2	2
25 ppm	0	4
250 ppm	0	6

There were no treatment related effects on haematological parameters.

The organ weights are given in Table B.6. 41

Table B.6. 41 Organ and relative organ weights from carcinogenicity testing in mice

	0 ppm m/f	25 ppm m/f	250 ppm m/f	2500 ppm m/f
Dose in mg/kg bw/day	-/-	4/ 4	40/46	592/732
liver weight, g	1.64/1.56	1.51/1.44#	1.59/1.51	n.i.
relative liver weight, %	4.44/3.62	3.94/3.57	4.16/3.75	n.i.
Relative liver weight compared to control		11%↓/1%↓	6%↓/3%↑	n.i.
kidney weight, g	0.79/ 0.52	0.86/ 0.51	0.91/0.59##	n.i.
relative kidney weight, %	2.10/ 1.22	2.32/ 1.27	2.48/1.47##	n.i.
Relative kidney weight compared to control		10%↑/4%↑	18%↑/20%↑	n.i.
adrenal glands weight, mg	7.10/9.51	5.76##/8.79	6.04##/9.09	n.i.
relative adrenal glands weight, %	0.019/0.022	0.015##/0.022	0.016##/0.022	n.i.
Relative adrenal weight compared to control		21%↓/0%	16%↓/0%	n.i.

n.i.: not investigated # and ##: 5% and 1% significance level (Dunnett's test)

The mean adrenal weight was significantly decreased in male mice at both 25 and 250 ppm with no dose response relation. The mean kidney weight was significantly increased in the 250 ppm females and the mean liver weight was significantly decreased in 25 ppm females. The relative organ weights of adrenals in male mice were significantly decreased in the 25 ppm group (-21.05%) and in the 250 ppm group (- 15.79%). However no dose-response relationship could be established. In female mice of the 250 ppm group the mean relative kidney weight was significantly increased (+20.74%).

There were no treatment related gross lesions.

In histopathology no treatment related increase of tumours was observed. In kidneys of 250 ppm females chronic nephropathy was increased to 25 animals compared to 13 females in the control group and 7 females in the 25 ppm group. The severity of the nephropathy was in most of the cases minimal. Small foci of amorphous basophilic or black foci, diagnosed as calcification, were observed in 13 females of the control group, 9 females of the 25 ppm group, and 26 females of the 250 ppm group. This observation is taken as a dose-response relation. In males the number of animals with calcification of the kidney was 36, 35, and 40 for the 0, 25 and 250 ppm groups, respectively. In the liver there were no treatment-related non-neoplastic lesions. There was a slight increase in hepatocellular adenoma and carcinoma in both sexes, the study director considered that these findings were incidental. In females hepatocellular carcinoma exceeded the historical control range, although incidence in the female controls was also higher than the historical control range.

Table B.6. 42 Neoplastic and non-neoplastic findings in 18 month carcinogenicity study in mice administered mecoprop-P in the diet (50 animals examined per dose/sex)

Dose ppm	Males			Females			Historical control incidence from B63F1 mice in studies conducted within 5 years of the current study (50 animals per sex group, 7 studies)
	0	25	250	0	25	250	
Dose mg/kg bw/day	0	4	40	0	4	46	
Liver							
Hepatocellular adenoma	3	4	5	1	1	3	Range: males: 1 - 7 (2 - 14%) Females: 0 - 5 (0 - 10%)
Hepatocellular carcinoma	7	6	8	3	2	5	Range: males: 2 - 10 (4 - 20%) Females range: 0 - 1 (0 - 2%)
Kidney							
Calcification	45	37	47	13	9	26	
Lipid vacuoles	43	44	0	0	0	0	
Chronic nephropathy	46	45	47	13	7	27	
Tubular hyperplasia	0	0	1	0	0	1	
Carcinoma	0	1	1	0	0	0	

Discussion and conclusion:

The design of the study is not optimal as the dose levels each differ by a factor of ten instead of the recommended total difference of a factor 10 from lowest to highest dose level. Furthermore, the animals were 50 days old at the start of dosing instead of the recommended start as soon as possible after weaning. However, the OECD guideline states the animals should be less than 8 weeks old at the start of dosing; since these animals were 7 weeks old the age of the animals is acceptable. The killing of the 2,500 ppm group at approximately 12 months is a deviation from the guideline requirements.

If the high dose level animals had been monitored closer, the body weight changes of the high dose animals could have been improved by decreasing the dose level to eg 1,000 ppm. This might have made it possible for this group to survive to the end of the experiment.

Mecoprop-P was not carcinogenic in this study in doses up to 250 ppm in the diet corresponding to 43 mg/kg bw/day. At 250 ppm significant increases in absolute and relative kidney weights were observed in females. In addition, this group showed an increase in the number of chronic nephropathies. At 25 ppm no dose related chronic toxicity was observed. The effects on the adrenals in males are not considered substance related as there is no clear dose response relationship in the decreased weight and as there are no other effects on the adrenals.

In all, the NOAEL for non-neoplastic findings in this study can be set to 25 ppm corresponding to 4 mg/kg bw/day for females based on 20% increase in relative kidney weight, increased chronic nephropathy. In males there were no adverse findings therefore the NOAEL in males is > 250 ppm corresponding to 40 mg/kg bw/day.

Strictly speaking the study is not sufficient as only two dose levels have been investigated and there are no substance related toxic effects in the males at the highest dose. However, for the purpose of elucidating the carcinogenic effects of mecoprop-P it is judged by the rapporteur that a new study will be unnecessary because:

- 1) The effects seen at the 250 ppm dose level show that mecoprop-P is toxic at this level for females.
- 2) Seen in conjunction with the rat carcinogenicity study using racemic mecoprop, which is negative, and the mutagenicity studies carried out with mecoprop-P, all of which, including 2 *in vivo* studies, are negative, it seems much unlikely that mecoprop-P is carcinogenic.

There was a slight increase in hepatocellular carcinoma in females at 250 ppm (46 mg/kg bw/day) which slightly exceeds the historical control incidence. However incidence in the concurrent controls was also higher than the historical control incidence, therefore the historical control data are not relevant. This finding was dismissed as incidental by the study director and was not reported in the 1998 DAR, however a marginal increase in these tumours was also seen in the supplementary study (see [REDACTED] 1999).

During the peer review of the 1998 DAR it was concluded that this study was insufficient to evaluate the carcinogenicity of mecoprop-P in the mouse. Therefore a second study was conducted with a more appropriate high-dose group, as a supplement to this study, and is summarised below.

B.6.5.2/02 Chronic toxicity and carcinogenicity in the mouse – supplementary study

During the ECCO93 discussion for the 1998 DAR it was agreed to require the submission of the new mouse study conducted on mecoprop-P with a control and a "high" dose group to provide for the deficiencies in the mouse carcinogenicity study by [REDACTED] 1996). The study was submitted in the 2002 DAR addendum.

This study on mecoprop-P was done to OECD 451 (1981) which precedes the current guideline OECD 451 (2009). The main difference between the old guideline and the current one is that the criteria for acceptable historical control data different. Therefore the historical control data in this study have been re-checked for this submission to ensure they meet current standards.

Previous evaluation:	In Addendum to DAR for first review (2002).
----------------------	---

Study	Mecoprop-P - Carcinogenicity study in B6C3F1/CrIBR mice. Administration in the diet for 18 months (Supplementary study)
Reference	[REDACTED] (1999)
Date performed	15 November 1995 to 28 May 1997
Test facility	[REDACTED]
Report reference	Report Project No: [REDACTED]
Guideline(s)	OECD 451 (1981)
Deviations from the guideline	Only one treated dose group as this is a supplementary study to the original mouse carcinogenicity study.
GLP	Yes
Test material	Mecoprop-P, batch 91-1, purity 92.7%
Study acceptable	Yes

Study report:

██████████ Mecoprop-P - Carcinogenicity study in B6C3F1/CrLBR mice. Administration in the diet for 18 months (Supplementary study). ██████████
 ██████████ Report Project No: 76S0002/91142 (supplementary to 76S0002/91102). January 20 1999.

Study design and quality:

The study was performed in accordance with OECD Principles of Good Laboratory Practice (Paris, 1981) and test guidelines of the EC Commission Directive 87/302/EEC (18 November 1987), the OECD Guidelines for Testing of Chemicals, Carcinogenicity Study (451) (12 May 1981) and U.S. EPA, Pesticide Assessment Guidelines (F §83-2) (November 1984). The study was a supplementary study to the previously conducted carcinogenicity study (██████████) to test the oncogenicity of mecoprop-P at a dose level fulfilling the criteria of a maximum tolerated dose. Groups of 50 male and 50 female B6C3F/CrLBR mice (49 days old at start of dosing) were for 18 months given feed *ad libitum* containing either 0 ppm or 700 ppm (males) or 800 ppm (females) mecoprop-P of 92.7 % purity. These concentrations correspond to daily doses of mecoprop-P of 0 mg/kg body weight in both control groups and to 112 mg/kg body weight for male mice and 188 mg/kg body weight for female mice in the dose groups. All animals were examined for clinical signs every day, food consumption was recorded initially every week and later on at 4 weeks intervals, body weights were recorded at the start of the study and then weekly for 13 weeks and then every 4 weeks. Differential blood counting was performed one year after study initiation and at study termination. Surviving animals were killed at day 546 and full necropsy was performed, including organ weights, gross pathology and histo-pathology.

Results:

The daily clinical examinations of all animals did not show any differences between dosed animals and control groups. Mortality was not increased in animals exposed to the test substance.

Table B.6. 43 mortalities in the 18 month carcinogenicity study in mice administered mecoprop-P in the diet

Mortalities in the two parts of the carcinogenicity study in mice		% mortality in males	% mortality in females
76S0002/91102 study	0 ppm	2	2
	25 ppm	0	4
	250 ppm	0	6
76S0002/91142 study	0 ppm	2	2
	700 ppm	2	
	800 ppm		6

There was no consistent effect on food consumption of including mecoprop-P in the diet (it is remarked, that spilling occurred in all groups, irrespective of dose, and that the measured food consumption is not representative for exact food consumption, i.e. that the real figures for food consumption and consequently the doses are lower than those presented in the report). Body weights and body weight gains were statistically significantly impaired from 11 months and 7 months onwards for dosed male and female mice, respectively. Body weights and body weight changes impairments were as tabled below:

Table B.6. 44 body weight findings in the 18 month carcinogenicity study in mice administered mecoprop-P in the diet

	Final body weight male mice (mean)		Final body weight female mice (mean)	
	0 mg mecoprop-P/kg/day	112 mg mecoprop-P/kg/day	0 mg mecoprop-P/kg/day	188 mg mecoprop-P/kg/day
Weight g	37.282 g ± 4.066 g	35.457 g* ± 3.806 g	31.52 g ± 5.76 g	28.332 g** ± 4.01 g

Weight %	100 %	95.1 %	100 %	89.8 %
Change g	18.9 g ± 4.0 g	16.4 g** ± 4.0 g	16.5 g ± 5.4 g	13.4 g** ± 3.8 g
Change %	100 %	86.8 %	100 %	81.2 %

p= 0.05; ** p= 0.01 (Student's t-test (two sided))

The food efficiencies were sometimes significantly different between dosed animals and controls, but changes were not consistent, thus they were most probably incidental. Haematology did not reveal any differences between dosed animals and controls at 12 months or 18 months with respect to white blood cells (neither counts nor morphology) or red blood cells (morphology).

Absolute organ weights: Significantly increased testis weight in dosed males and significantly increased kidney weight in dosed females.

Table B.6. 45 Organ weight findings in the 18 month carcinogenicity study in mice administered mecoprop-P in the diet

Significantly increased absolute and relative organ weights	Male mice - organ weight as % of terminal body weight		Female mice - organ weight as % of terminal body weight	
	Controls	700 ppm	Controls	800 ppm
Absolute liver weight (g)	1.394	1.487	1.277	1.319
Relative liver weight (%)	3.768	4.228** (12% ↑)	4.13	4.698** (14% ↑)
Absolute kidney weight (g)	0.719	0.717	0.485	0.559** (15% ↑)
Relative kidney weight (%)	1.941	2.032* (5% ↑)	1.575	1.991** (26% ↑)
Absolute testes weight (g)	0.222	0.232* (5% ↑)	-	-
Relative testes weight (%)	0.601	0.661** (10% ↑)		
Absolute brain weight (g)	0.490	0.490	0.507	0.507
Relative brain weight (%)	1.327	1.398* (5% ↑)	1.659	1.818** (10% ↑)

* p= 0.05; ** p= 0.01 (Wilcoxon-Test (two sided))

No other relative organ weights were significantly different between control animals and dosed animals. Macroscopically observable masses occurred in the liver of 8 males and 0 females of the control groups and in 15 males and 9 females of the dosed groups.

Histopathology.

Table B.6. 46 Number of animals with non-neoplastic histopathological lesions in the liver and kidney in the 18 month carcinogenicity study in mice administered mecoprop-P in the diet

50 animals per sex and dose examined	Males		Females		Historical control incidence from B63F1 mice in studies conducted within 5 years of the current study (50 animals per sex group, 6 studies)
	0	700	0	800	
Dose (ppm)	0	700	0	800	
Dose (mg/kg bw/day)	0	112	0	188	
Chronic nephropathy	38	50	8	38	
Calcification in kidney	25	48	0	13	
Kidney: Lipid vacuoles	48	0	0	0	

Liver: Basophilic foci of cellular alteration	4	11	0	4	Range: males: 4 – 10 (8 – 20%) Females: 0 – 7 (0 – 14%)
---	---	----	---	---	--

Kidney

The major non-neoplastic lesion found was chronic nephropathy characterised by areas of tubular atrophy, regeneration and dilatation, proteinaceous casts inside the tubules and/or interstitial fibrosis. These features occurred in 38 (males) and 8 (females) control animals and in 50 (males) and 38 (females) of the dosed animals. Also the severity of the chronic nephropathy was slightly increased in dosed animals as compared to controls. Minimal or slight focal calcification of the renal tubules, the arterial wall, the interstitial tissue and/or along the tubular basement membranes occurred in 48 males and 13 females of the dosed groups compared to 25 males and no females from the control group. Lipid vacuoles in the proximal tubular epithelial cells were absent in substance treated males but present in almost all control males. Other findings in the kidneys did not occur to a greater extent in dosed animals compared to controls or were only observed in single cases.

Liver

Some of the macroscopically diagnosed masses in the livers were foci of cellular alterations by histological examination. Basophilic foci of the cellular alteration occurred in eleven males and four females of the treated animals and only in 4 males and no females of the untreated group.

All other non-neoplastic findings recorded were single observations or occurred evenly distributed between treated and untreated groups of animals.

Table B.6. 47 Neoplastic findings in 18 month carcinogenicity study in mice administered mecoprop-P in the diet

	Tumour incidence in male mice		Tumour incidence female mice		Historical control incidence from B63F1 mice in studies conducted within 5 years of the current study (50 animals per sex group, 6 studies)
Dose (ppm)	0	700	0	800	
Dose (mg/kg bw/day)	0	112	0	188	
Number of animals per group	50	50	50	50	
Number of tumour-bearing animals					
Neoplasms	13	14	10	14	
1 primary neoplasm	13	13	9	11	
2 or > primary neoplasms	0	1	1	3	
Benign neoplasms	9	7	5	10	
Benign neoplasms only	9	7	4	7	
Malignant neoplasms	4	7	6	7	
Malignant neoplasms only	4	7	5	4	
Systemic neoplasms	1	2	5	2	
Total numbers of tumours	Total number of tumours in male mice		Total number of tumours in female mice		
Primary neoplasms	13	15	11	17	
Benign neoplasms	9	7	5	10	
Malignant neoplasms	4	7	6	7	
Systemic neoplasms	1	2	5	2	
Liver tumours					
Hepatocellular adenomas	4	4	0	5 (6 tumours)	Range: males: 1 - 7 (2 – 14%) Females: 1 – 5 (2 – 10%)
Hepatocellular carcinomas	2	4	0	4 (5 tumours)	Range: males: 4 – 10 (8 – 20%) Females range: 0 – 3 (0 – 6%)

Hepatocellular adenomas occurred in 10% of dosed females and not at all in control females. Hepatocellular carcinomas were found in 8 % dosed males and females each and in 4 % of control males and none in control females. The occurrence of 0 hepatocellular tumours in control females could be incidental.

Discussion:

This carcinogenicity study was performed in accordance with U.S. EPA (83-2), EC Commission Directive 87/302/EEC, and OECD (451) guidelines and OECD (Paris, 1981) and German (1994) Principles of Good Laboratory Practice. The study was a well conducted and reported feeding study with daily doses 92.7 % pure (R)-2-(4-chloro-2-methylphenoxy) propionic acid (mecoprop-P) of 112 mg/kg body weight to male (700 ppm) and 188 mg/kg body weight to female (800 ppm) B6C3F1/Cr1BR mice for 18 months. Deviations from the guidelines have been found in the late start of the dosing of the animals - day 49 instead of just after weaning as recommended (day 35 to 42) and the fact that only one dose level (different for males and females) was tested besides the control groups. However the OECD guideline states the animals should be less than 8 weeks old at the start of dosing, since these animals were 7 weeks old the age of the animals is acceptable. Significantly decreased body weights and body weight changes were found in both sexes and there were increases in nephropathy in both sexes and significantly increased absolute and relative kidney weight in female mice and increased relative kidney weight in male mice, which were all found to be related to feeding with the substance, indicating that the maximum tolerated dose was achieved in both sexes. The significantly increased testes weights (absolute and relative to body weight) are considered by the study director to be incidental (there are no histological findings in testes from dosed males, but cases of macroscopically identified cases of reduced size testes in control animals). The increase in absolute testes weight is slight (only 5%) and too small to be of toxicological relevance. The increase in relative testes weight is 10% but is considered to be secondary to the 5% reduction in body weight compared to the controls, as the brain and testes weight tends to be conserved when there is a slight decrease in body weight.

The increased incidences of basophilic foci of cellular alterations in the liver (22 %) over the historic control of male B6C3F1 mice in the laboratory (8-20 %) should not just be accepted as incidental. There was a high incidence - 8 % - of hepatocellular carcinomas in dosed female mice. Historic controls from the same laboratory show incidences from 0 to 6 % of these neoplasms, only, whereas the 10 % hepatocellular adenomas are just covered by these historic controls (0-10 %). It should be noted though, that a very high number of females are tumour bearing since the adenomas and the carcinomas do not occur in the same individuals (4 animals bear 5 carcinomas, and 5 other animals bear 6 adenomas). The incidences for male mice do not differ significantly between treated and untreated groups. The authors of the study report interpret the results as incidental. The data, however, does not exclude that the findings in this study show a potential weak tumourigenic effect in female mice.

Conclusion:

Since the tumourigenic effect is only seen in one sex, is not seen in rats and the overall results from the studies for genotoxicity were negative, mecoprop-P is not considered a carcinogen.

The adverse effects seen in this study at 112/188 mg/kg bw/day in males/females are a reduction in bodyweight gain (of 13% to 19% in males/females), and an increase in relative liver weight (of 12%/14% in males/females) accompanied by increased basophilic foci of cellular alteration in both sexes although this was within the historical control range. Relative kidney weight was increased in females (by 26%) and in both sexes there was increased chronic nephropathy and calcification in the kidney.

There were increased hepatocellular adenomas in females and increased hepatocellular carcinomas in both sexes. In males these tumours were within the historical control range so can be dismissed as incidental. In females the incidence of malignant hepatocellular tumours (4/50 versus 0/50 in controls) marginally exceeds the historical control incidence (of 3/50) but only by one animal. This marginal increase is not considered to be sufficient evidence of a carcinogenic effect. It is noted that there was also a slight increase in hepatocellular carcinoma in females (5/50 at 46 mg/kg bw/day versus 3/50 in controls) in the previous study, but again this very slight increase is not clearly treatment-related.

During the first review it was concluded that although there were increased liver tumours in mice in this study, overall it was concluded there was no carcinogenic potential relevant for humans.

The applicant has confirmed the lab from which the historical control data was obtained and provided some additional historical control data on hepatocellular tumours since the previous review.

Applicant conclusion: Although slightly elevated (8 to 10% incidence), the occurrence of hepatocellular tumours in female mice is considered to be incidental. The absence of liver tumours in female control animals in this study is highly unusual for this strain of mice. In an oncogenicity study with Dichlorprop-p (BASF, 1998) which was carried out at the same time and in the same laboratory, the incidence of liver tumours in untreated female controls was 10%. In addition, the strain of mouse used in this study, B6C3F1, is known for its very high incidence of spontaneously-occurring liver tumours (Reference: Toxicol Pathol. 1998 May-Jun;26(3):428-41, where a 26.3% incidence of spontaneously-occurring liver adenoma/carcinomas is reported by the National Toxicology Program in its carcinogenicity studies).

B.6.5.3 Summary of long-term and carcinogenicity studies

Table B.6. 48 Summary of long-term and carcinogenicity studies

(new study submitted for current evaluation highlighted in bold)

Study	Test Substance	Dosing	Effects at LOAEL	NOAEL	Reference
Rat; 2 years dietary 50m/f, per dose Included 1 year chronic cohort 10m/f per dose	Mecoprop racemate	0, 20, 100, and 400 ppm Equivalent to 0, 1.1/1.4, 5.5/6.9, 22.2/27.9 mg/kg bw/day in m/f	<u>Non-neoplastic:</u> In males at 2 years 7% ↑abs. kidney weight. In males at 1 year 16% ↑abs. kidney weight. No adverse findings in females <u>Neoplastic:</u> No tumours identified	<u>Non-neoplastic:</u> Males: 20 ppm (1.1 mg/kg bw/day) Females: >27.9 mg/kg bw/day <u>Neoplastic:</u> > 22.2/27.9 mg/kg bw/day in m/f	██████████ (1988) ¹
Rat; 2 years; dietary 52m/f per dose	D-Isomer (Mecoprop-P)	0, 100, 600, 1200 ppm Equivalent to 0, 5.3/6.6, 32.0/39.9, 64.6/81.7 mg/kg bw/day in m/f	<u>Non-neoplastic:</u> Males at 1200 ppm: ↓ bw gain, 21%↑ rel. kidney weight, ↑ histopathological kidney findings. Peroxisome proliferation in liver. Females: at 100 ppm: 51%↑ rel. kidney weight <u>Neoplastic:</u> No neoplastic findings	<u>Non-neoplastic</u> Males: 600 ppm (32 mg/kg bw/day) Females: below 100 ppm (<6.6 mg/kg bw/day) <u>Neoplastic:</u> > 64.6/81.7 mg/kg bw/day	██████████ (2008)

Study	Test Substance	Dosing	Effects at LOAEL	NOAEL	Reference
Mouse; 18 months; dietary 50m/f per dose	D-Isomer (Mecoprop-P)	0, 25, 250, (2500) ² ppm in the diet Equivalent to 0, 4/4, 40/46, 592/732 mg/kg bw/day in m/f	<u>Non-neoplastic findings:</u> At 2500 ppm all animals sacrificed at one year due to severe body weight loss. Males: At 250 ppm: no adverse findings Females: at 25 ppm: 20% ↑ rel. kidney weight ↑ chronic nephropathy. <u>Neoplastic:</u> At 250 ppm: Males: not carcinogenic Females: ↑ hepatocellular carcinoma (4 animals versus 3 in controls)	<u>Non-neoplastic:</u> Males: > 250 ppm (40 mg/kg bw/day) Females: 25 ppm (4 mg/kg bw/day) <u>Neoplastic:</u> 25 ppm (4 mg/kg bw/day)	█ (1996)
Mouse; 18 months; dietary 50 m/f per dose	D-Isomer (Mecoprop-P)	0, 700 (males), 800 (females) ppm Mecoprop-P in the diet Equivalent to 112/188 mg/kg bw/day in m/f	<u>Non-neoplastic:</u> Males: 13% ↓ bw gain, 12% ↑ rel. liver weight, ↑ chronic nephropathy. Females: 19% ↓ bw gain, 14% ↑ rel. liver weight, ↑ chronic nephropathy, 26% ↑ rel. kidney weight <u>Neoplastic:</u> Males: not carcinogenic Females: ↑ hepatocellular carcinomas at 800 ppm (5 animals versus 0 in controls)	<u>Non-neoplastic:</u> Males: < 700 ppm (112 mg/kg bw/day) Females: < 800 ppm (188 mg/kg bw/day) <u>Neoplastic:</u> Males: > 700 ppm (112 mg/kg bw/day) Females: < 800 ppm (188 mg/kg bw/day)	█ (1999)
Dog; 12 months; dietary; 5m/f per dose	Mecoprop racemate	0, 2, 5, 19 mg/kg bw/day Mecoprop-P in diet	Males: Slightly reduced body weight, and haematological changes: ↓Hb, ↓HCT Females: Slight clinical chemistry changes: ↓ phosphate, ↓calcium	Mecoprop-P : LOAEL : 19 mg/kg bw/day NOAEL : 5 mg/kg bw/day	█ (1997)

¹ Study on mecoprop racemate included in DAR for first review (1998) and included here for completeness but not relevant for mecoprop-P

²: This dose group was terminated after 12 months and not investigated further
m/f = males/females

Rat, chronic and carcinogenicity studies

One combined chronic toxicity/carcinogenicity study with mecoprop racemate (█ 1988) conducted with rats was originally included in the 1998 dossier from the notifiers. Rats were during 24 months fed with diet containing 0, 20, 100, and 400 ppm racemic mecoprop equivalent to 0, 1.1/1.4, 5.5/6.9, 22.2/27.9 mg/kg bw/day in males/females (purity of 92.7%). No histopathological and neoplastic changes were found. Increased kidney weight was found in male rats at 100 and 400 ppm and significantly increased level of blood urea nitrogen was found in males at 400 ppm. The NOAEL is considered to be 20 ppm (corresponding to 1.1 mg/kg bw/day for males) based on 7% increase in absolute kidney weight after 2 years administration and a 17% increase in relative kidney weight after 1 year in the 100 ppm dose group.

Since the original 1998 DAR a new rat carcinogenicity study (█ 2008) has been conducted on mecoprop-P. Rats were fed mecoprop-P in the diet at a dose of 0, 100, 600, 1200 ppm equivalent to 0, 5.3/6.6, 32.0/39.9, 64.6/81.7 mg/kg bw/day. Reduced body weight gain and food consumption were evident in both sexes at the top dose, but also in females at 600 ppm. Both sexes had a marked increase in relative kidney weight and slight

changes in kidney histopathology at 1200 ppm. In females the liver was also a target organ as evidenced by increased relative liver weight, hepatocyte hypertrophy and other histopathological changes at 1200 ppm. Further investigations revealed significantly increased hepatic cyanide-insensitive palmitoyl CoA oxidation in both sexes at 1200 ppm and in females at 600 ppm, which is a typical marker of peroxisome proliferation. There were no adverse findings in males at 600 ppm or 100 ppm, whereas in females a 51% increase in relative kidney weight was seen at 100 ppm and due to the magnitude of the effect is considered to be an adverse finding although it was not accompanied by any other indicators of toxicity.

There was no increase in malignant tumours. The only finding of note was an increase in subcutaneous lipoma (benign) at 1200 ppm in males (4/52 at 1200 ppm versus 0/52 in controls) which exceeded the historical control incidence (2/52 but only two historical control studies were available). Adipose tissue was found to be highly exposed to the test substance in the metabolism studies. However these tumours are probably incidental since lipoma only occurred in a single sex, and only marginally exceeded the historical control incidence. This tumour type is not thought to progress to malignancy. The NOAEL for neoplastic findings in males is therefore > 64.6 mg/kg bw/day. There were no neoplastic findings in females therefore the neoplastic NOAEL in females is > 81.7 mg/kg bw/day.

Mouse carcinogenicity study

Mice were fed for 18 months with diets containing 0, 25, 250 or 2500 ppm with mecoprop-P (purity of 92.7%) equivalent to 0, 4/4, 40/46, 592/732 mg/kg bw/day in males/females (1996). However the highest dose group was killed after 12 months because of severe reduction in bodyweight gain that indicated the MTD was exceeded and not investigated further. A NOAEL for systemic effects was found to be 25 ppm (corresponding to 4 mg/kg bw/day) for the females and 250 ppm (corresponding to 40 mg/kg bw/day) for the males. At 250 ppm increased kidney weight was seen in females and they had chronic nephropathy. In males there were decreased absolute and relative adrenal weights in both the 25 and 250 ppm dose groups. This effect was not clearly dose-related and there were no other effects on the adrenals. Therefore this effect is not considered substance related. There was a slight increase in hepatocellular carcinoma (5/50 versus 3/50 in controls) in females at 250 ppm (46 mg/kg bw/day). This slightly exceeds the maximum historical control incidence (of 1/50 (2%)) from seven concurrent studies. As tumour incidence in the concurrent controls was also higher than the historical controls the historical control data are not relevant. The very slight increase in tumours is not clearly treatment-related so is not considered evidence of a carcinogenic effect.

A supplementary study in mice (1999) was conducted because the MTD was exceeded in the former study. Mice were fed for 18 months with diets containing 0, 700 ppm (males) or 800 ppm (females) corresponding to 0, 112/188 mg/kg bw/day in males/females. Both sexes had decreased bodyweight gain (13%/19% in males/females). The target organs were the liver and kidney. Findings in the liver were increased relative liver weight (12/14% in males/females) but the only histopathological finding in the liver was had increased incidence of basophilic foci of cellular alteration (in 11/50 males versus 4/50 in controls, and in 4/50 females versus 0/50 in controls) but this incidence was within the historical control range so is of limited toxicological relevance. In the kidney there was an increase in relative kidney weight in females (26%) and increased chronic nephropathy in both sexes (30% increase in males, 5 fold increase in females). There was an increased incidence of hepatocellular carcinomas (4/50 versus 0/50 in controls) in females that slightly exceeded the historical control incidence (of 3/50) but only by one animal. This marginal increase is not considered to be sufficient evidence of a carcinogenic effect.

The overall NOAEL for neoplastic findings in female mice is 188 mg/kg bw/day based on a slight increase in hepatocellular carcinoma. There were no treatment-related neoplastic findings in males so the NOAEL in males is > 112 mg/kg bw/day.

One year dog study

In a one year dog study (1997) summarised in Section B.6.3.3.1 the NOAEL was 5 mg/kg bw/day based on decreased body weight and body weight gain and minor effects on blood cells (decreased haemoglobin and haematocrit) and decreased phosphate and calcium in the highest dose group 19 mg/kg bw/day.

Classification and labelling for carcinogenicity

Mecoprop-P does not currently have a harmonised classification for carcinogenicity.

In the previous review it was concluded that increased liver tumour incidence occurred in female mice at the highest dose tested, but that overall there was no carcinogenic potential relevant to humans.

Hazard categories for carcinogenicity according to EC 1272/2008

Category	Criteria
1A	Category 1A are known human carcinogens largely based on evidence from humans.
1B	Category 1B are presumed human carcinogens largely based on animal studies where there is clear evidence of carcinogenicity in animals.
2	Category 2 are suspected human carcinogens where there is some evidence of carcinogenicity from humans or animal studies, but where the evidence is not sufficiently convincing to place the substance in Category 1. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

In the new 2 year rat study on mecoprop-P the only finding was an increased incidence of benign lipoma in male rats; however, these are not considered to be evidence of carcinogenic potential as they only marginally exceeded the historical control levels. Furthermore, this tumour type is a common finding and is not thought to progress to malignancy. It is concluded that mecoprop-P shows no carcinogenic potential in the rat.

In mice, hepatocellular carcinoma in females exceeded the concurrent control incidence and marginally exceeded historical control levels in both studies conducted in mice. The increase is very slight and not clearly treatment-related. In the historical control data provided by the lab the incidence of hepatocellular carcinoma in females was low. The strain of mice used in these two studies was B6C3F/Cr1BR, which are reported to have a high spontaneous incidence of liver tumours (as reported in Guidance on the Application of the CLP Criteria version 4.1, June 2015 section 3.6.2.3.2); therefore the significance of this slight increase in tumours in females only is considered to be of limited toxicological relevance and not sufficient to warrant classification.

Overall the RMS considers that classification for carcinogenicity is not warranted.

B.6.6. REPRODUCTIVE TOXICITY

A new one-generation reproduction dose-range-finding study in rats conducted on mecoprop-P was submitted for this renewal. All other studies have been previously evaluated.

The data requirement Regulation (EU) 283/2013 stipulates the need for a two-generation reproduction toxicity study (or extended one-generation study) and developmental toxicity studies in the rat and rabbit.

Potential neurotoxic, immunotoxic effects and effects potentially related to changes in the hormonal system also need to be addressed and reported.

Reproductive functions that should be investigated include impairment of male and female reproductive capacity, such as effects on oestrus cycle, sexual behaviours, spermatogenesis, oogenesis, hormonal activity, that interfere with the capacity to fertilise ova up to and including implantation.

	B (1992)
Date performed	6 June 1989 to 17 April 1990
Test facility	██████████ ███ ██████████ ██████████ ██████████ ██████████
Report reference	Project No.: ██████████ 8 (BASF Doc 92/10869)
Guideline(s)	OECD 416 (1983)
Deviations from the guideline	The P generation produced two litters and blood and urine were analysed. This does not affect the validity of the study
GLP	Yes
Test material	Mecoprop, purity 92.7%, TPH batch
Study acceptable	Yes

Study report:

██████████ B (1992): Reproduction study with MCPP in rats. Continuous dietary administration over 2 generations (2 litters in the first and 1 litter in the second generation). ██████████ ██████████ Project No.: ██████████ (BASF Doc 92/10869) 7 August 1992, unpublished report. (Dossier ref. 5.45 and 5.6.1/01).

Study design and quality:

Four groups of 25 male and 25 female Wistar rats 35 days old were given 0, 20, 100, or 500 ppm mecoprop (racemic form, 92.7% pure) in the diet for their whole lifetime (F_0 generation), during which two matings and rearings of offspring took place. At least 70 days after the beginning of treatment the animals were allowed to mate in a 1:1 ratio. Females were allowed to litter and rear their pups (F_{1a} generation pups) until either day 4, when the pups were culled to 8 pups/litter preferably with 4 males and four females/litter or day 21 after parturition. At least 10 days after the last weaning of the F_{1a} generation pups, the F_0 parental animals were mated again in a ratio of 1:1 for the F_{1b} generation and the females were allowed to rear their pups as the F_{1a} generation. After the F_{1b} generation had been weaned, the F_0 generation was fasted for 16 hours before sacrifice.

After weaning the F_{1a} pups were used to establish the F_2 generation by choosing 25 males and 25 females from each dose group with all litters being represented if possible. The F_{1a} generation received the test substance at the same concentration as their parents, and at least 98 days after formation of the F_1 generation parental animals, the males and females were mated at a ratio of 1:1 avoiding mating of siblings. Females were allowed to litter (F_2 pups) and at day 4 after parturition culling to 8 pups/female was carried out. After the F_2 pups had been weaned the F_1 generation was fasted for 16 hours before sacrifice.

All pups, which were not used for establishing the next generation, were sacrificed after weaning.

If an animal of the F_0 or F_1 generation parental animals had not produced any offspring these animals were mated again with control animals to assess their fertility.

Food consumption and body weight were determined at the requested intervals with few minor exceptions. Clinical observations were carried out daily. Male and female reproduction data and pup development data were calculated using relevant data and formulas. The following developmental and behavioural tests were carried out: 1) pinna unfolding, 2) opening of the auditory canal, 3) opening of the eyes, 4) gripping reflex on day 13, hearing test on day 21, and pupillary reflex on day 29.

Before sacrifice of parental animals urine and blood were collected and analysed.

Parental animals of the F_0 and F_1 generations were subjected to gross pathology after weighing of animals, liver, kidneys, and testes, and after fixation of 13 organs these were all subjected to a histopathological examination in all animals from the control and 500 ppm groups. In addition livers, kidneys, and gross lesions in the 20 and 100 ppm groups were studied likewise.

All in all the study appears to be of good quality, carried out according to GLP and OECD Guideline 416 and EU guideline B35, with additional developmental and behavioural observations done during lactation.

Results:

There were no substance-related differences in food consumption and body weight. The intake of test substance in the different generations is given in Table B.6. 49.

Table B.6. 49 Intake in mg mecoprop/kg bw/day for the 2 generation study.

	20 ppm	100 ppm	500 ppm
F ₀ males	2.0	9.8	49.0
F ₀ females (prematuring)	2.1	10.6	52.5
F ₀ females (F _{1a} litter)			
- gestation period	1.7	8.7	42.8
- lactation period*	2.9	14.4	72.6
F ₀ females (F _{1b} litter)			
- gestation period	1.6	8.0	40.0
- lactation period*	2.6	13.2	67.3
F ₁ males	1.8	9.3	47.3
F ₁ females (prematuring)	2.0	10.3	50.7
F ₁ females (F ₂ litter)			
- gestation period	1.6	8.5	41.6
- lactation period*	2.5	13.3	67.5

* days 0 - 14 post-partum only

In the F₀ males and females there were no treatment-related differences in mating and fertility indices for the F_{1a} and F_{1b} generations. There were no treatment-related differences in F_{1a} and F_{1b} pup numbers and status at delivery. The number of pups, which died or were cannibalized from day 1 to day 4 post-partum (before culling), was statistically increased in the F_{1a} 500 ppm group (p<0.01). Also in the F₂ pups the number of dead pups on day 1 was significantly increased (p<0.01) in the 500 ppm group. A summary of these findings is given in Table B.6. 50.

Table B.6. 50 Number of dead pups day 0-4 in the generation study with mecoprop.

Litter type	Sum dead pups day 4 (dead day 0 + dead day 1-4)			
	0 ppm	20 ppm	100 ppm	500 ppm
F _{1a}	8(1+7)	6(0+6)	15(2+13)	23(3+20 ^{##})
F _{1b}	5(1+4)	8(1+7)	13(4+9)	17(6+11)
F ₂	17(1+16)	22(2+20)	22(0+22)	32(13 ^{##} +19)
	Mean number of pups delivered per litter			
F _{1a}	14.5	13.1	14.6	14.9
F _{1b}	15.8	15.0	15.3	15.7
F ₂	13.0	11.6	13.3	14.9

significance level 0.05, ## significance level 0.01.

As a consequence of the increased pup death from day 1 to 4 post-partum in the F_{1a} pups of the 500 ppm group the viability index of this group was significantly ($p < 0.01$) reduced. The lactation index, an indicator of pup survival from day 4 to 21 post-partum, was not influenced by administration of the test substance, nor was the sex ratio affected. The body weight gain of pups of the 500 ppm F_{1a} group was significantly ($p < 0.05$) reduced. In the F₂ 500 ppm pups body weight gain from day 4 to 7 and day 7 to 14 was significantly ($p < 0.05$) reduced in males and in males and females combined. This resulted in a significant ($p < 0.05$) reduction in body weight gain from day from day 4 to 21 in both males and females separately and in both sexes combined.

In the developmental and behavioural tests the F_{1b} pups had delayed pinna unfolding ($p < 0.01$) in the 100 and 500 ppm groups. In the F₂ pups pinna unfolding was delayed in the 20, 100, and 500 ppm groups ($p < 0.01$), and auditory canal opening was delayed ($p < 0.01$) in the 500 ppm group.

At pup necropsy no substance related findings were observed in F_{1a}, F_{1b}, and F₂ pups.

There were no substance related differences in food consumption and body weight gains in the F₁ males and females during the whole study period, including gestation and lactation periods of the dams of the F₂ pups.

In clinical chemistry, urinalyses, and pathology no substance related changes were observed in the F₀ and F₁ parental animals except for increased absolute and relative kidney weights of both sexes and generations in the 500 ppm group and in the F₀ males and both sexes of the F₁ generation. Relative kidney weight increases of 12%/9% occurred in F₀ and 10%/8% in F₁ parental males/females respectively. No treatment-related histopathological findings were identified in the reproductive organs or in the liver or kidneys of the parental animals.

Discussion and conclusion:

The study has been carried out well according to OECD guideline 416 and GLP. The test report considers a NOAEL of 500 ppm for adult “fertility”. However, the rapporteur for the 1998 renewal considers 100 ppm as the NOAEL for the overall reproductive function as significant decreases were seen at the 500 ppm dose level for pup viability day 0-4 post-partum, for pinna unfolding and for body weight gain during lactation in at least two of the three tested breeds. A general NOAEL for systemic toxicity is 100 ppm as absolute and relative kidney weights were affected in the 500 ppm groups. The increased pup death observed is not substance related, as the affected litters were large in number of pups, and the decrease in pup weight gain during lactation is most likely caused by maternal toxicity.

During the previous renewal of approval there was uncertainty as to whether the effects on pup death (day 0-4, significant at $P = 0.01$ in the 500 ppm group in both F_{1a} and F₂ generation) were substance related. The only other effect in pups was a slight but statistically significant ($P = 0.05$) adverse effect on pup weight gain (estimated at 6% reduction on days 7-14 in the F_{1a} generation, 8-11% reduction in the F₂ generation over days 4-14 post-partum). These reductions in body weight gain are not very severe, and do not occur at all time points, but were considered to be the critical effect in the pups during the previous review. There was also a delay in pinna unfolding in the F_{1b} and F₂ generation and delayed auditory canal opening in the F₂ generation. These developmental delays are believed to be secondary to retarded body weight gain.

The applicant considers the increased pup death to be caused by poor maternal care because of maternal toxicity. The only evidence of maternal toxicity is increased relative kidney weight, although based on the 90 day rat study parental toxicity would be expected at 500 ppm. It could be that the pups were sickly when born, or became sickly though the milk. The new study by ██████ (2003) did not replicate the increase in pup mortality even at doses higher than 500 ppm.

This study was discussed in the ECCO93 discussion for the 1998 DAR

It was concluded that there was an equivocal effect on pup mortality at 100 ppm dose level. The experts agreed to ask for further information on the study and proposed a data requirement for a statement on possible relationship between litter size and increased mortality in the pups as well as a statistical re-evaluation of this effect (██████████, 1992) (IIA 5.6).

UK RMS has not seen any resubmission or re-evaluation of the findings on pup deaths although pup death was not included as a critical effect in the list of endpoints. When considering the mean litter size (in Table B.6.50) there is no apparent increase in the number of pups born in the top dose group that would explain the increase in pup mortality.

Maternal/paternal NOAEL is 100 ppm (equivalent to 9.3/8.0 mg/kg bw/day in males/females) based on significant increase in absolute and relative kidney weight at 500 ppm.

Offspring NOAEL is 100 ppm (equivalent to 9.3/8.0 mg/kg bw/day in males/females) based on increased pup death in days 0 to 4 post-partum at 100 ppm and reduced pup weight gain.

Reproductive/fertility NOAEL is >500 ppm (> 47.3/40.0 mg/kg bw/day in m/f).

There was no evidence of any neurotoxic or immunotoxic effects or effects related to changes in the hormonal system.

Applicant conclusion: The NOAEL was set at 100 ppm based on a reduced survival rate of 1 to 4 day old pups which was significantly elevated at 500 ppm. This reflected poor maternal care. 100 ppm is equivalent to approximately 10 mg/kg bw/day in adult rats in this study. Maternal food consumption increases almost two fold in early lactation, with no increase in bodyweight. Thus, the effective dose-rate for lactating dams and neonatal pups is very much higher than that in non-lactating adults. This is the likely cause of the poor maternal care seen at the high dose. The achieved dose rate for young animals of the second generation in the first week post-weaning can be calculated to be in the region of 82 mg/kg/day at the high dose and about 16-17 mg/kg/day at the mid dose, regarded as the NOEL. Dose rates in late lactation will have been higher still. Thus the “true” NOEL is at least 16mg/kg/day and potentially much higher.

B.6.6.1.2 Preliminary one-generation reproductive toxicity study in the rat

The 2-generation study with mecoprop [REDACTED] 1992 – see Section B.6.6.1.1) at the top dose of 500 ppm showed minimal effects on parents (increased relative kidney weight) while the offspring showed significant perinatal growth retardation and increased pup death. This caused the study to be rejected by some regulatory authorities on the grounds that a parental MTD was not achieved during gestation, but caused increased offspring mortality during lactation. The achieved lactating dose in mg/kg bw/day is much higher than during gestation and might lead to a relatively high dose administered to the pups through lactation and post weaning. Reducing doses in the diet during lactation on the basis of assumed higher consumption levels for pups abolishes the “differential” effect seen at a constant dose in the diet and allows an MTD to be achieved with parent rats without unacceptably severe effects on the young pups.

The current study was designed as a dose-range-finding study for a 2-generation study to a modern protocol required by some regulatory authorities on the grounds that the study with the racemate applied a protocol which was deficient by modern standards, did not achieve an MTD and used the racemic mixture whereas modern usage is based on the single (herbicidal) isomer.

The method for determining mecoprop-P in ground diet in this study is not strictly validated in accordance with SANCO 3029/99/rev. 4 on the following grounds: there were no sample preparation details, and the linearity graph and specificity chromatograms were not provided (though a linear range is stated and the applicant claims no interference was observed). The LOQ is 0.02 ppm (200 µg/g).

Historical control data were provided in this submission. It is data for Wistar rats from [REDACTED] but is not acceptable under current data requirements for historical control data because no dates have been provided. The applicant has been unable to confirm the dates of the historical control data.

Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012
----------------------	--

Study	Mecoprop-P: oral (dietary administration) preliminary reproduction toxicity study in the rat
Reference	██████████ (2003)
Date performed	25 November 2002 to 29 April 2003
Test facility	██
Report reference	Report No. ██████████
Guideline(s)	OECD 415(1983)
Deviations from the guideline	Only 12 animals per dose group instead of 20. Gross pathology should be performed on the P generation, but in this study gross pathology was conducted only on P and F1 animals which had external abnormalities.
GLP	Yes
Test material	Mecoprop-P, batch 91-1, purity 92.8%
Study acceptable	Acceptable as a preliminary study, but low number of animals means the reliability of the study is reduced. This study is not sufficient to meet the fertility data requirements without the supporting evidence of the Hellwig study conducted on mecoprop.

Method:

1. Animals and treatment

Twelve rats per sex/dose, strain ██████████, 7 to 9 weeks old at the start of the study were fed diets containing 0, 500, 800 or 1200 ppm Mecoprop-P. Both sexes were treated for 10 weeks before pairing. The females continued with this treatment throughout gestation and then were allowed to litter and rear their offspring to weaning. During the lactation period, the females were given diets containing nominal concentrations of 0, 300, 530 and 790 ppm mecoprop-P. Males were treated until evaluation of the females had been completed. On day 4 post-partum, all litters were culled to a maximum of 8 pups with an equal sex distribution where possible. At weaning (day 21 post-partum), allocation of the F1 generation was by random selection of 10 males and 10 females from the available litters. The F1 generation received Mecoprop-P at the original concentrations for 4 weeks post-weaning.

2. Rationale on dose selection

The low dose of 500 ppm was the high dose in the previous 2-generation study with mecoprop (██████████ 1992). There were only minor effects on the parents at this dose. The higher doses were chosen by considering the findings in the 90 day studies.

3. Sacrifice and pathology

At necropsy all P and F1 animals were examined externally and discarded if found normal. Abnormal animals were examined for structural or pathological changes. The uterus of all F0 females was stained in 10% ammonium sulphide and the number of implantations recorded. All pups found dead were examined macroscopically for structural or pathological changes. Culled pups were examined externally and discarded if normal. Abnormal pups were examined for structural or pathological changes.

Results:

The following mean test substance intakes (mg/kg/day) of test article were achieved:

Table B.6. 51a Preliminary one-generation reproductive study in the rat: intake of mecoprop-P mg/kg bw/day

	Mean dose received mg mecoprop-P/kg/day for the F0 generation		
	500 ppm	800 ppm	1200 ppm
Males, pre-pairing	34.5	53.7	82.9
Females, pre-pairing	41.0	64.7	98.4
Females, gestation	38.2	60.6	88.8
Mean female (pre-birth)	39.6	62.7	93.6
	300 ppm	530 ppm	790 ppm
Females, lactation	48.1	85.8	130.2
Mean P gen female	42.4	70.4	105.8
Sexes combined	38.5	62.1	94.4
	Mean dose received mg mecoprop-P/kg/day for the F1 generation		
	500 ppm	800 ppm	1200 ppm
Males	59.6	98.0	148.4
Females	61.1	101.5	147.7
Sexes combined	60.4	99.8	148.1

Parental toxicity**1. 1. Mortality and Clinical signs of toxicity**

There were no deaths and no treatment-related clinical signs of toxicity in the P and F1 adults.

2. Bodyweight gain and food consumption

There was a reduction in body weight gain in the high dose group during gestation and pre-mating (by 16% in males days 0 to 18, 20%/26% in males/females days 0 to 10). Mean body weight gain in females was also reduced throughout gestation at the top dose, by 50% days 0-7, 30% days 7-14, 20% days 14-20. Group mean food intake in the high dose group for both F1 males and females was slightly lower than the controls during the 4 weeks of this generation, particularly the females during the last week.

3. Necropsy

No treatment-related external findings in the P generation at necropsy.

Reproductive toxicity:**1. 1. Mating**

Mating performance was unaffected by treatment.

2. 2. Litter data

The mean duration of gestation was unaffected by treatment.

There was a statistically significant, dose-related reduction in the mean numbers of implantation sites in all treated groups compared with control. However, the values for the control, low and intermediate dose groups were higher than expected when compared with the available historical control data from the same strain and laboratory, albeit with uncertainty about the dates they were conducted. Although the mean numbers of pups born were lower in all treated groups compared with the controls, again, the numbers in the control, low and intermediate dose groups were higher than expected when compared with the historical control data. Given that the group sizes were smaller than in a standard two-generation study and the reduction in implantation sites was $\leq 10\%$ in the low and intermediate dose groups, the biological significance of this finding and the decreased number of pups born in these groups is questionable. In the high dose group, the mean number of pups born was

statistically significantly lower than in the controls. There was no effect of treatment on post-implantation survival index.

One female in each of the low and intermediate dose groups showed total litter deaths but the viability of the offspring in the treated groups was generally similar to that of the controls.

Table B.6. 51b Reproductive findings in the one-generation study in the rat following dietary administration of mecoprop-P

Dose (ppm)	0	500	800	1200	Historical control incidence (from 5 studies) for Han Wistar rats at Covance (applicant has been unable to confirm the date of these studies)
Mg/kg bw/day (females)	0	38.2	60.6	88.8	
Mean implantation sites	13.8	12.5**	12.4**	10.9***	Range : 10.3 – 11.7
% implantation sites compared with control		9%↓	10%↓	21%↓	
Mean pups born	12.7	11.2*	11.7	10.0**	Range : 9.3 – 11.1
% pups born compared with control		12%↓	8%↓	21%↓	
Mean pups alive day 4	11.1	9.7	11.0	9.0	Range : 9.1 – 10.8

* statistically significant compared to control: * = P<0.05, ** = P<0.01, *** = P<0.001

The mean body weight of the treated pups was similar to the controls on day 1 post-partum. Mean body weight gain of the pups in the treatment groups was slightly lower than that of the controls, particularly in the high dose group between days 7 and 21 post-partum, but there was no dose-relationship or statistical significance.

Necropsy examination of the weanling offspring showed only one male pup from the high dose group with slight, unilateral, renal pelvic dilatation.

Conclusion:

Dietary administration of 1200 ppm mecoprop-P to adult male and female rats for 10 weeks prior to mating, 790 ppm to the female rats during the lactation period, and again giving 1200 ppm to the F1 generation for 4 weeks, produced some adult and pup toxicity in terms of lower body weight gains and food intakes. At these concentrations there were fewer implantation sites compared with controls.

At 500 and 800 ppm (reduced to 300 and 530 ppm), there were marginal effects on adult and pup weight gain and food intake.

In conclusion, it is considered that reduction of dietary concentration by approximately one third during the lactation period in a 2-generation study, would allow demonstration of a maternal MTD without inducing excessive effects in the offspring.

The preliminary study on mecoprop-P demonstrated comparable toxicity to that seen in the existing 2-generations study on racemic mecoprop (██████████ 1992). In the interests of preventing unnecessary testing on animals it was decided not to run the 2-generation study on mecoprop-P and instead refer to the racemic study which was previously accepted in the review for mecoprop-P.

Maternal/paternal NOAEL is 800 ppm (equivalent to 53.7/60.6 mg/kg bw/day in males/females) based on a significant reduction in bodyweight gain at 1200 ppm.

Reproductive NOAEL is 800 ppm (equivalent to 53.7/60.6 mg/kg bw/day) based on a significant reduction in the number of implantation sites at 1200 pm.

Offspring NOAEL is > 1200 ppm (equivalent to 82.9/88.8 mg/kg bw/day in m/f).

There was no evidence of any neurotoxic or immunotoxic effects or effects related to changes in the hormonal system.

B.6.6.2. Developmental toxicity studies

In the 1998 DAR development toxicity studies in the rat and rabbit conducted on mecoprop-P were submitted and are included here.

For developmental findings the International Federation of Teratology Societies terminology should be used if possible to describe any effects.

The 1998 DAR also included developmental studies on mecoprop racemate in the rat and rabbit, and a mouse developmental study conducted on mecoprop and mecoprop-P. These extra studies are considered superfluous to the current renewal review and are not considered further.

B.6.6.2.1 Developmental toxicity in the rat

This study was conducted to OECD 414 (1981) which precedes the current guideline OECD 414 (2001). The main difference compared with the current guideline was that the test substance was administered only during organogenesis, whereas the 2001 guideline stipulates administration should be from implantation to scheduled kill. This 1981 study protocol is considered sufficient to determine developmental toxicity.

Historical control data were provided for this study but there was insufficient information provided for it to be acceptable under current data requirements.

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Study of the prenatal toxicity of Mecoprop-P in rats after oral administration (gavage)
Reference	██████████ (1993a)
Date performed	9 to 31 July 1991
Test facility	██████████ ███ ██████████ ██████████ ██████████ ██████████
Report reference	Project No. ██████████ (BASF Doc 93/10160)
Guideline(s)	OECD 414 (1981)
Deviations from the guideline	No
GLP	Yes
Test material	Mecoprop-P, purity > 92.2%, Batch 91-1
Study acceptable	Yes

Study report:

██████████ (1993a): Study of the prenatal toxicity of Mecoprop-P in rats after oral administration (gavage). ██████████ ███ ██████████ ██████████ ██████████ ██████████ ██████████ Project No.: ██████████ (BASF Doc 93/10160) February 22, 1993, unpublished report. (Dossier ref 5.46).

Study design and quality.

Four groups of 25 time-mated female Wistar rats (Chbb:THOM (SPF)) received 0, 20, 50, or 100 mg mecoprop-P/kg/day by gavage from day 6 to 15 of gestation. The mecoprop-P was >92.2% pure and was given in a 0.5% carboxymethylcellulose suspension. The study was carried out according to GLP and OECD Guideline 414 (1981). Housing conditions were as required, and all requested analyses were carried out. At necropsy the dams were subjected to gross pathology examination, the uterus and ovaries removed. The uterus weight, number of corpora lutea, number of implantation sites, early and late resorptions, dead implantations and foetuses were recorded. Foetuses were examined for external, skeletal and visceral malformations and variations.

The study is regarded as being of excellent quality.

Results:

In the control group one animal was not pregnant. In the mecoprop-P dosed groups 5, 2, and 5 females were not pregnant in the 20, 50, and 100 mg/kg bw/day groups, respectively. The resulting conception rate varied between 96% (control group) and 80% (the 20 and 100 mg/kg bw/day groups). Only pregnant females were used for calculation of food consumption, body weight and body weight change.

The food consumption was significantly reduced (by about 9%) in the 50 mg/kg bw/day group from day 6-8 post coitus ($p<0.05$), and in the 100 mg/kg bw/day group from day 6-10 post coitus ($p<0.01$) (by about 22%). Both the body weight at day 8 ($p<0.05$) and body weight gain during days 6-15 ($p<0.01$), and corrected body weight gain ($p<0.01$) were impaired at 100 mg/kg bw/day. The overall body weight gain of the high dose group during the whole study was 18% less than the controls.

Clinical symptoms were not seen in any of the dams, and no mortalities occurred. At necropsy no substance related observations were done, and the uterus weight was not affected by treatment.

There were no effects of treatment on sex distribution of the foetuses, and the weight of the placentae. Weight of the foetuses was slightly ($p<0.05$) reduced (by 2%) in the 100 mg/kg bw/day group. The study director considered this finding was secondary to the increased number of live foetuses in this dose group, but a treatment-related effect cannot be excluded. At the external examination of the foetuses fused placenta was observed in one foetus of each of the 50 and 100 mg/kg bw/day groups. At the soft tissue examination of the foetuses no substance related changes were seen. Dilated renal pelvis and/or hydroureter were detected in all groups at levels within the range of biological variation.

In the skeletal examination the occurrence of rudimentary cervical ribs was significantly ($p<0.01$) increased in the 100 mg/kg bw/day group, and the number of foetuses with not ossified sternbrae was also significantly ($p<0.01$) increased (Table B.6. 52). These increases are clearly dose related. There was no indication of a substance related occurrence of malformations. Historical control data were provided on the incidence of all findings but the reporting of this data is insufficient to be acceptable. The applicant has been unable to provide relevant historical control data.

Table B.6. 52 Basic teratogenicity data for mecoprop-P after gavage administration to rats.

Dose (mg/kg bw/day)	0	20	50	100
No. of inseminated rats	25	25	25	25
No. of pregnant rats	24	20	23	20
No. of implantations/rat	13.8	14.9	13.8	14.9
No. of live foetuses/rat	12.8	13.8	13.1	13.9
Mean foetal weight (g)	4.0	4.0	4.0	3.9 [#]
Rudimentary cervical ribs (foetal incidence)	6	5	9	26 ^{##}
Sternebrae not ossified	6	12	8	24 ^{##}

[#] significant p<0.05, ^{##} significant p<0.01

Discussion and conclusion:

In the 100 mg/kg bw/day group dams the statistically significant reduced food consumption during days 6-10 post coitus and the statistically significant lower body weight gain day 6-15 are clearly effects related to dosing.

No adverse effects were seen in the dams at 50 mg/kg bw/day.

In the foetuses the decreased foetal weight, and the increases in skeletal variations (rudimentary cervical ribs) and in retardations (not ossified sternebrae) are clear indications that foetotoxicity occurred at 100 mg/kg bw/day.

No foetotoxicity was seen at 50 and 20 mg/kg bw/day.

The LOAEL for foetotoxicity is under the conditions of this study 100 mg/kg bw/day, and the NOAEL is 50 mg/kg bw/day.

For maternal toxicity the LOAEL is 100 mg/kg bw/day, and the NOAEL is 50 mg/kg bw/day.

There was no evidence of any neurotoxic or immunotoxic effects or effects related to changes in the hormonal system.

The UK RMS agrees with the conclusion of the previous review.

Applicant commentary:

USEPA 2007 evaluation summary:

In a developmental toxicity study, 25 presumed pregnant Wistar (Chbb:THOM (SPF)) rats per group were administered 0, 20, 50, or 100 mg/kg/day of Mecoprop-p (92.2% a.i.; Lot No. 91-2) in 0.5% carboxymethyl cellulose by gavage on gestation days (GD) 6-15, inclusive. On GD 20, all surviving dams were sacrificed, and all foetuses were weighed, sexed, and examined for external malformations/variations. Approximately one-half of the foetuses from each litter were examined for visceral anomalies and the remaining one-half processed for skeletal examination. All animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed during the study, and gross necropsy was unremarkable. Body weights, body weight gains, and food consumption by the low- and mid-dose groups were similar to those of the controls throughout the study. Absolute body weights of the high-dose dams were significantly (p # 0.05) less than the controls on GD 8 due to a mean body weight loss of 1 g during GD 6-8 (compared with a weight gain of 8 g for the control group). Body weight gain by the high-dose group was 82% (p # 0.01) of the control group level for the entire dosing interval. Food consumption by the high-dose group was significantly (p # 0.05 or 0.01) less than the controls during GDs 6-8 (77% of control) and 8-10 (88% of control). Therefore, the maternal toxicity LOAEL for Mecoprop-P is 100 mg/kg/day based on reduced body weights, body weight gains, and food consumption. The maternal toxicity NOAEL is 50 mg/kg/day. Gravid uterine weights, numbers of corpora lutea, implantation sites, resorptions, and pre- and post-implantation losses were similar between the treated and control groups. Foetal body weights were significantly (p # 0.05) reduced in the high-dose group as compared with the

During the experimental period determination of body weight, food consumption, and clinical observations were carried out. At sacrifice on day 29 post insemination the standard teratogenicity analyses were carried out, including number of corpora lutea, implantation sites, live and dead foetuses, early and late resorptions, and examination of foetuses for external, visceral and skeletal malformations and variations.

The study was carried out according to GLP and OECD guideline 414.

Results:

One doe in the 5 mg/kg bw/day group died on day 7 post insemination and two does in the 50 mg/kg bw/day group showed minor skin lesions in the laryngeal area. There were no substance related differences in food consumption, body weight or body weight gain.

There were no substance related differences in number of implantation sites, number of live foetuses, foetal weight, and in sex ratio. There was a slight, statistically significant ($p < 0.05$) increase in the number of late resorptions in the 50 mg/kg bw/day group. However, there was no increase in the number of early or total resorptions or in the post implantation loss.

No dose related increase in malformations, variations and retardations were seen.

Table B.6. 53 Teratogenicity data for mecoprop-P in the rabbit

Dose (mg/kg bw/day)	0	5	20	50
No. of inseminated rabbits	15	15	15	15
No. of pregnant rabbits	15	15	15	14
No. of implantations/rabbit	7.3	7.1	6.8	6.9
Mean pre implantation loss (%)	8.2	9.5	14.7	13.7
Mean post implantation loss (%)	13.4	7.2	5.2	13.1
Mean No. of early resorptions/rabbit (total)	0.7 (11)	0.6 (8)	0.3 (5)	0.6 (9)
Mean No. of late resorptions/rabbit (total)	0.1 (1)	0.0 (0)	0.1 (1)	0.4 [#] (5)
Mean No. of total resorptions/rabbit (total)	0.8 (12)	0.6 (8)	0.4 (6)	1.0 (14)
No. of live foetuses/rabbit	6.9	6.5	6.4	5.9
Mean foetal weight (g)	40.2	40.1	39.5	40.7
No. foetuses with incomplete ossification of sternebrae	28	30	23	30

[#] significant $p < 0.05$

Discussion and conclusion:

Gavaging pregnant Himalayan rabbits on days 7 to day 19 post insemination did not in this study result in clear signs of maternal or foetal toxicity in doses of 5, 20, and 50 mg/kg bw/day, except for an increase in the number of late resorptions at 50 mg/kg bw/day. The study director considered that this finding was not biologically relevant. Considering that the number of total resorptions is only marginally higher than the control group and with no dose response, the RMS agrees that this finding is not biologically relevant.

Additional information is available from a preliminary dose range-finding study conducted with 5 rabbits per dose. Mean late resorptions were 0.2, 2.4, 0.6, 2.5 per rabbit in doses of 0, 40, 80, 120 mg/kg bw/day

respectively. Overt signs of maternal toxicity (including increased kidney weight and increased creatinine, reduced food consumption and body weight) were seen at doses of 80 and 120 mg/kg bw/day without any teratogenic effects. At 40 mg/kg bw the signs of maternal toxicity included marginally reduced food consumption and body weight loss. The lack of a clear dose response in this study suggests late resorptions can be very variable. There was an increase in late resorptions in all treated groups compared with the controls, indicating that this was a treatment-related effect, but maternal toxicity was also evident.

It can be concluded that under these test conditions the NOAEL for maternal toxicity was >50 mg/kg bw/day and the NOAEL for foetal toxicity is >50 mg/kg bw/day.

There was no evidence of any neurotoxic or immunotoxic effects or effects related to changes in the hormonal system.

B.6.6.2.3 Developmental toxicity in the mouse

This extra study is considered superfluous to the current renewal review.

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Vergleichende Untersuchungen zur Embryotoxizität von 2-Methyl-4-chlorphenoxyessigsäure, Mecoprop und Dichlorprop bei NMRI-Mäusen
Reference	██████████ (1983)
Date performed	Not stated
Test facility	Not stated
Report reference	BASF Doc no. 83/10103. Published in: <i>Arzneim Forsch</i> , 33, 1479-1483 (1983).
Guideline(s)	Not stated
Deviations from the guideline	Not applicable
GLP	No
Test material	Mecoprop-P, purity and batch not stated
Study acceptable	Yes

Study report:

██████████ (1983): Vergleichende Untersuchungen zur Embryotoxizität von 2-Methyl-4-chlorphenoxyessigsäure, Mecoprop und Dichlorprop bei NMRI-Mäusen. Published in: *Arzneim Forsch*, 33, 1479-1483 (1983). Published scientific article. BASF Doc no.: 83/10103. (Dossier ref 5.49).

Study design and quality:

Groups of 22-59 NMRI mice received 0, 100, 200, 300, 400, 500, or 700 mg mecoprop or 0, 200, 300, 400, or 500 mg mecoprop-P/kg in arachidis oil by gavage from day 6 to day 15 of gestation. (Quality of test substances not indicated). The animals were sacrificed on day 18 of gestation and subjected to the standard teratology procedure. Body weights were determined daily.

The study report (a scientific article) does not contain information on GLP and guideline. The study seems to be of acceptable standard.

Results:

For mecoprop maternal toxicity was seen in the 700 mg/kg bw/day group, but not in the lower dose groups. For mecoprop-P no maternal toxicity was seen in the doses tested.

The results of the teratological examinations are given in Table B.6. 54 (mecoprop) and Table B.6. 55 (mecoprop-P).

Table B.6. 54 Teratogenicity data for mecoprop in NMRI mice

Dose (mg/kg bw)	0	100	200	300	400	500	700
No. females	24	37	34	30	27	34	22
Early resorptions (%)	10.7	8.1	9.5	8.5	8.5	5.7	25.4 ^{##}
Post implantation loss (%)	11.3	10.2	10.7	11.1	10.5	8.9	29.4 ^{##}
Foetal weight (g)	1.17	1.16	1.12	1.09 [#]	1.06 [#]	1.03 ^{##}	0.82 ^{##}
Cleft palate (%)	1.5	2.1	1.6	1.1	1.9	3.8 [#]	19.7 ^{##}
Extra ribs (%)	-	0.3	-	-	1.1	0.6	14.6

[#] significant p<0.009, ^{##} significant p<0.0027

Table B.6. 55 Teratogenicity data for mecoprop-P in NMRI mice

Dose (mg/kg bw)	0	200	300	400	500
No of animals	59	25	33	36	34
Early resorptions (%)	8.4	11.4	9.4	7.6	13.8 ^{##}
Post implantation loss (%)	10.6	12.3	11.8	10.6	17.1 [#]
Foetal weight (g)	1.17	1.12	1.11 [#]	1.04 ^{##}	1.00 ^{##}
Cleft palate (%)	1.6	1.1	0.8	2.5	3.4 [#]
Extra ribs (%)	-	-	1.1	4.5 [#]	8.7 ^{##}

[#] significant p<0.009, ^{##} significant p<0.0027

Discussion and conclusion:

Comparing data in the two tables show that foetal weight is the most sensitive parameter for teratogenicity in this study and for both mecoprop and mecoprop-P the LOAEL is 300 mg/kg bw/day. However, it is difficult to compare these data with those of the other studies, as this is a published scientific paper, which uses other levels for the statistical significance p<0.009, and p<0.0027. As it is a scientific paper recalculation of the data is not possible. There was no evidence of any neurotoxic or immunotoxic effects or effects related to changes in the hormonal system.

B.6.6.3 Summary of reproductive and developmental toxicity

The data requirement Regulation (EU) 283/2013 stipulates the need for a two-generation reproduction toxicity study (or extended one-generation study), and developmental toxicity studies in the rat and rabbit. These studies should investigate any neurotoxic, immunotoxic effects and effects related to changes in the hormonal system. Investigations should include impairment of male and female reproductive capacity, such as effects on oestrus cycle, sexual behaviours, spermatogenesis, oogenesis, hormonal activity, that interfere with the

capacity to fertilise ova up to and including implantation. Studies should investigate harmful developmental effects on the progeny both before and after birth including malformations such as anogenital distance, nipple retention, and functional disturbances such as reproductive and neurological effects.

No reproductive study is available for mecoprop-P but a two-generation reproductive toxicity study was conducted on racemic mecoprop and was evaluated in the previous renewal review (1998 DAR). For the current renewal a new one-generation reproductive toxicity dose-range finding preliminary study conducted on mecoprop-P has been submitted.

The developmental effects of mecoprop-P have been investigated in developmental studies with rats and rabbits (full study reports) and in mice (scientific article including both mecoprop-P and mecoprop). These studies were submitted and evaluated in the previous renewal review (1998 DAR) and are considered adequate.

Further studies with mecoprop racemic form that were submitted in the 1998 DAR included developmental studies in rats and rabbits (all full study reports) but are not included in the current renewal as sufficient information is available on mecoprop-P. Additional studies in the form of scientific articles were included in the 1998 dossier but were considered unreliable or irrelevant so were not included in this renewal review.

The studies submitted are considered adequate to investigate the reproductive and developmental toxicity of mecoprop-P.

Table B.6. 56 presents the overall results (NOAELs) from the studies for an evaluation of the reproduction and developmental toxicity of mecoprop-P.

Table B.6. 56 Overview of NOAELs for reproduction and developmental toxicity studies submitted with the mecoprop-P dossier.

(new study submitted for the current evaluation highlighted in **bold**)

Study	Test Substance	Dosing	Effects at LOAEL	NOAEL in mg/kg bw/day	Reference
Rat (Han Wistar); One-generation dietary (10 week dose ranger) Parental animals : 12m/12f per dose group Pups: 10 per litter	Mecoprop-P	0, 500, 800, 1200 ppm Equivalent to 0, 34.5/38.2, 53.7/60.6, 82.9/88.8 mg/kg bw/day in m/f Dose during lactation: 0, 300, 530, 790 ppm Equivalent to 0, 48.1, 85.8, 130.2 mg/kg bw/day	Parental: 20%/26% ↓ bw gain in m/f during pre mating, 50% ↓ bw gain in f on days 0-7 during gestation Offspring: No adverse effects Fertility: 21% ↓ implantation sites	Parental: 800 ppm (530 ppm during lactation) equivalent to 53.7/60.6 mg/kg bw/day in m/f (85.8 mg/kg bw/day in f during lactation) Offspring: > 1200 ppm (82.9/88.8 mg/kg bw/day in m/f) Fertility : 800 ppm equivalent to 53.7/60.6 mg/kg bw/day in m/f	█ (2003)

Study	Test Substance	Dosing	Effects at LOAEL	NOAEL in mg/kg bw/day	Reference
Rat (Wistar); Two-generation dietary Parental animals: 25m/f per dose group Pups: 8 per litter	Mecoprop	0, 20, 100, 500 ppm Equivalent to 0, 1.8/1.6, 9.3/8.0, 47.3/40.0 mg/kg bw/day in m/f (0, 2.5, 13.2, 67.3 mg/kg bw/day in lactating females)	Parental: ↑ relative kidney weight 12%/9% in m/f Offspring: ↑ pup mortality days 0 – 4, up to 11% ↓ pup body weight gain. Fertility: no affects	Parental: 100 ppm (9.3/8.0 mg/kg bw/day in m/f) Offspring: 100 ppm (9.3/8.0 mg/kg bw/day in m/f) Fertility: >500 ppm (> 47.3/40.0 mg/kg bw/day in m/f)	█ (1992)
Rat (CD); Developmental Gavage, 19 to 25 per dose group	Mecoprop	0, 20, 50, 125 mg/kg bw/day	Maternal: ↓ bodyweight gain Developmental: ↓ bodyweight, ↓ crown/rump length, ↑ delayed ossification	Maternal: 50 mg/kg bw/day Developmental: 50 mg/kg bw/day	█ 1980a)
Rat (Wistar); Developmental. Gavage, 25 per dose group	Mecoprop-P	0, 20, 50, 100 mg/kg bw/day	Maternal: 22% ↓ food consumption, 18% ↓ bodyweight gain Developmental: 2% ↓ foetal weight, four fold ↑ rudimentary cervical ribs, four fold ↑ sternbrae not ossified	Maternal: 50mg/kg bw/day Developmental: 50mg/kg bw/day	█ (1993a)
Rabbit (Himalayan); Developmental Gavage, 15 per dose group	Mecoprop-P	0, 5, 20, 50 mg/kg bw/day	Maternal: no adverse findings Developmental: no affects	Maternal: >50 mg/kg bw/day Developmental: >50 mg/kg bw/day	█ (1993b)
Mouse (NMRI); Developmental Gavage 22 to 59 per dose group	Mecoprop and Mecoprop-P	0, 200, 300, 400, 500 mg/kg bw/day mecoprop-P 0, 100, 200, 300, 400, 500, 700 mg/kg bw/day mecoprop racemate	Maternal: maternal effects not specified but only seen in mecoprop at 700 pmg/kg bw/day Developmental: ↓ foetal weight (both subst.)	Maternal: 500 mg/kg bw/day* Developmental: 200 mg/kg bw/day * * both substances	█ 1983)
Rabbit (Dutch belted); Developmental Gavage 15 per dose group	Mecoprop	0, 12, 30, 75 mg/kg bw/day	Maternal: No adverse findings Developmental: No adverse findings	Maternal: 75 mg/kg bw/day Developmental: 75 mg/kg bw/day	█ (1990b)

Studies shaded grey were included in the 1998 DAR but are not considered in the current renewal as they are supplementary to requirements.

Summary of reproductive and developmental toxicity

The studies submitted are considered adequate to investigate the reproductive and developmental toxicity of mecoprop-P. The only new study submitted for this renewal was a one generation reproductive study in the rat conducted on mecoprop-P. The new one generation study is considered to be less reliable as it is only a preliminary dose-ranging study. Therefore the original evaluation in the 1998 DAR is considered relevant for the current renewal of mecoprop-P.

In the two-generation reproductive study using mecoprop racemate there was no effect on fertility up to the highest dose tested. The main findings in the pups were increased pup mortality on days 0 to 4 post partum and a reduction in pup body weight gain at the top dose of 500 ppm (47.3/40.0 mg/kg bw/day in males/females). Delayed pinna unfolding in the F1a and F2 generation and delayed auditory canal opening in the F2 generation are probably secondary to body weight effects. In the parental generation the only treatment-related effect was an increase in relative kidney weight at 500 ppm. There were no effects on fertility. The increased pup mortality occurred in the absence of clear maternal toxicity. It is possible that the finding is due to systemic toxicity in young pups rather than reproductive toxicity (mecoprop-P is acutely toxic in adults with an acute oral toxicity of 431 mg/kg bw day, and pups are likely to be more susceptible as their food consumption relative to bodyweight is higher than in adults). The new one-generation study did not replicate the increase in pup mortality even at much higher doses.

In the new one-generation study using mecoprop-P the doses were increased to 500, 800 and 1200 ppm to ensure that a MTD was achieved in adults, but was reduced to 300, 530 and 790 ppm during lactation in an attempt to mitigate adverse effects on body weight and mortality in pups post partum seen in the two generation study. In this new study there was a significant reduction in bodyweight gain in parental animals at the top dose of 1200 ppm (82.9/88.8 mg/kg bw/day) demonstrating sufficient parental toxicity had been achieved. There were no adverse effects on pup body weight or survival. The only reproductive finding was a statistically significant reduction in implantation sites in all dosed groups, although the RMS concludes that some uncertainty surrounds the biological significance of the finding in the low- and mid-dose groups. This finding was accompanied by maternal toxicity in the form of reduced body weight gain in the high-dose group and is therefore possibly secondary to maternal toxicity. The two-generation study did not investigate all reproductive endpoints required in current OECD test guidelines; however, it is considered sufficient for the determination of effects on sexual maturation and fertility as parental males of both generations were exposed to the test substance for at least one sperm cycle prior to mating to determine effects on spermatogenesis, and for the second generation section of the study the parental animals were exposed from prior to conception through to full sexual maturity and mating, which includes oocyte development in females in the womb, and sexual development and maturity of male and female reproductive organs. The reproductive studies can be supplemented with the short term toxicity studies where histological examination and weights of reproductive organs were investigated, but no adverse effects were detected.

The developmental effects of mecoprop-P were investigated in the rat, rabbit and mouse. In rabbits administered doses of 0, 5, 20 and 50 mg/kg bw/day mecoprop-P the only finding was a statistically significant increase in late resorptions at 50 mg/kg bw/day, in the absence of any signs of maternal toxicity. The increase in late resorptions was not considered to be biologically relevant as total number of resorptions remained similar to the controls; therefore this finding is considered to be incidental. In rats administered 0, 20, 50 and 100 mg/kg bw mecoprop-P the only developmental finding was a slight retardation in foetus weight accompanied by unossified sternebrae and increased incidence of rudimentary cervical ribs at 100 mg/kg bw/day. At the same dose parental toxicity was evident by a significant retardation of body weight gain (18%) and reduced food consumption (22%). Therefore the developmental effects in the rat are considered to be secondary to maternal toxicity. In mice administered mecoprop-P at 0, 200, 300, 400 and 500 mg/kg bw mecoprop-P or 0, 100, 200, 300, 400, 500, 700 mg/kg bw/day mecoprop racemate the most sensitive endpoint was a reduction in foetal weight at 300 mg/kg bw/day (seen in both the racemate and P isomer). Maternal effects were only evident at a higher dose of 700 mg/kg bw/day. However, a reduction in foetal weight on its own is not considered sufficient for classification. The developmental studies were all conducted to previous OECD test guidelines with the main difference compared to the current guideline being the test substance was only administered during organogenesis. This protocol is considered sufficient to determine any developmental effects.

There was no evidence of any neurotoxic or immunotoxic effects or effects related to changes in the hormonal system.

The overall reproductive/fertility NOAEL was 53.7/60.6 mg/kg bw/day (in males/females) based on a 21% decrease in implantation sites in the one generation study on mecoprop-P (at a dose of 82.9/88.8 mg/kg bw/day in m/f). These findings occurred in the presence of significant parental toxicity (reduction in parental bodyweight gain).

In the two generation study on mecoprop the NOAEL for offspring toxicity was 9.3/8.0 mg/kg bw/day (in males/females) based on reduced pup body weight gain (accompanied by increased pup mortality and delayed pinna opening and auditory canal opening) at 47.3/40.0 mg/kg bw/day (in males/females). This finding was accompanied by increased kidney weight in the parental animals.

The overall NOAEL for developmental effects is 50 mg/kg bw/day based on delayed ossification, reduced pup weight and reduced crown/rump length in the developmental rat study at 100 mg/kg bw/day.

In the previous review it was concluded that there was no evidence of reproductive or developmental toxicity in the absence of maternal toxicity. Since then, new data has become available that provides further information on the reproductive toxicity of mecoprop-P to supplement the already-existing data.

Classification and labelling for reproductive toxicity

Mecoprop-P does not currently have any classification for reproductive toxicity.

Hazard categories for reproective toxicity according to EC 1272/2008

Category	Criteria
1A	Category 1A are known human reproductive toxicants largely based on evidence from humans.
1B	Category 1B are presumed human reproductive toxicants largely based on animal studies where there is clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.
2	Category 2 are suspected human reproductive toxicants where there is some evidence from humans or animal studies, of an adverse effect on sexual function and fertility, or on development and where the evidence is not sufficiently convincing to place the substance in Category 1. Such evidence shall have occurred in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Classification for reproductive effects: According to the CLP criteria adverse effects on sexual function and fertility that may warrant classification include any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

The only effect on fertility was a reduction in implantation sites (and consequently the mean number of pups born) in the one-generation study that occurred in all the treatment groups but was only considered by the RMS to be of biological significance in the high-dose group, in which maternal toxicity was also reported.

Classification for deveopmental effects: According to the CLP criteria adverse effects on development of the offspring that may warrant classification include in its widest sense, any effect which interferes with normal

development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency. Adverse effects on or via lactation are included under reproductive toxicity.

In the two generation study increased pup mortality on days 0 to 4 post partum as well as a reduction in pup body weight gain and signs of delayed development were seen in the absence of any significant maternal toxicity (maternal toxicity was limited to increased relative kidney weight). However increased pup mortality was not replicated in the one generation range-finding study, in which higher doses were administered.

Minor developmental effects in the rat developmental study (unossified sternebrae, and increased rudimentary ribs) are considered secondary to severe maternal toxicity so do not warrant classification. A reduction in foetal weight in the mouse developmental study is also not sufficient to warrant classification. Additionally an increase in late resorptions in the rabbit developmental study can be dismissed as incidental as the overall number of resorptions was not affected by treatment.

Overall, the RMS concludes that further consideration of this endpoint is warranted to reconcile the different findings in the two-generation and one-generation studies.

B.6.7. NEUROTOXICITY

The data requirements in Regulation (EU) 283/2013 state that neurotoxicity studies are required for active substances with structures that are similar or related to those capable of inducing neurotoxicity, and for active substances which induce specific indications of potential neurotoxicity at dose levels not associated with marked general toxicity, or for pesticides with a neurotoxic mode of action.

Mecoprop-P has no structural alerts for neurotoxicity (Derek Nexus 2.0) and there are no indications from the toxicity studies evaluated that mecoprop-P causes neurotoxicity. In addition the tissue distribution data from the metabolism studies (Section B.6.1.1.1) indicate that the brain is not highly exposed to mecoprop-P. It is concluded that the need for neurotoxicity studies is not triggered. An acute neurotoxicity study has been submitted and evaluated. This is considered to be a supplementary study.

B.6.7.1. Neurotoxicity studies in rodents

Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012
----------------------	--

Study	Mecoprop-P: acute oral neurotoxicity study in Wistar rats
Reference	██ (1995)
Date performed	25 April to 26 May 1994
Test facility	██████████ ███ ██████████ ██████████ ██████████ ██████████
Report reference	Report No. ██████████
Guideline(s)	US EPA 81-8 (1991), similar to OECD 424 (1997)
Deviations from the guideline	No
GLP	Yes
Test material	Mecoprop-P, Batch N31, purity 89.9%
Study acceptable	Yes

Mecoprop-P does not have a structure that is associated with neurotoxicity. Furthermore there are no indications of neurotoxicity in any of the existing toxicology studies. However, since the 91/414/EC review, the following study has become available to the notifier and is therefore submitted for completeness as a supplemental study.

Method:

Mecoprop-P was administered to groups of 10 male and 10 female Wistar rats (Chbb: THIM (SPF)), 42 days old at the start of the study, as a single oral gavage dose at levels of 0, 175, 350 and 700 mg/kg bw in 10 mL/kg 0.5 % carboxymethyl cellulose preparation. The control group received vehicle only. 700 mg/kg bw was selected as the high dose on the basis that it is close to the LD₅₀ value. 350 mg/kg bw was selected as an intermediate dose and 175 mg/kg bw as the low dose. It was ensured that all animals were dosed at the same age and examined at the same time after dosing.

The animals were observed for up to 2 weeks after dosing. Body weight was determined weekly. The general state of health of the rats was checked at least daily. Functional observational batteries and motor activity measurements were carried out before test substance administration, 2 – 8 hours, 7 and 14 days after administration. Tissues from 5 animals per sex and dose were fixed in situ perfusion and subjected to neuropathological examinations.

Functional observational battery

The FOB consisted of 4 parts, starting with passive observations without disturbing the animals. Posture, tremors, convulsions, behaviour, defecation, urination and general observations were noted. This was followed by removal from the cage, with observations in an open arena. Effects on fur, skin colour, posture, salivation, respiration, activity, arousal level, vocalisation, tremors, convulsions, bizarre behaviour, impairment of gait, lacrimations, exophthalmos and number of rearings within 2 minutes were recorded. Thereafter animals were removed from the arena and subjected to sensory-motor tests and reflex tests including hyperesthesia, abdominal tension, palpebral closure, winking reflex, pupil size, pupillary reflex, pinna reflex, startle response, olfaction, pain perception, righting response and vision. Quantitative measurements, including grip strength of forelimbs and hind-limbs and landing foot-splay were conducted.

Motor activity measurement

Motor activity was measured on the same day as FOB was performed using the Multi-Varimax-System with 4 infrared beams per cage. The number of beam-interrupts was counted over 18 intervals, each lasting 5 minutes, over a 1.5 hour period.

Necropsy

The five surviving animals per sex and dose that showed the most distinct neurological symptoms were deeply anaesthetised with 4 ml Nembutal/kg bw and sacrificed by perfusion fixation.

Results :

There were no deaths. Observations showed no treatment-related effects. Body weight gain was significantly lower in the high dose males on Day 7 (about 17% below controls). No statistically significant deviations were seen on other days or in other test groups.

Functional observational battery:

1. Home cage observations

On Day 0, half closure of eyelids was seen in the high dose (7 males, 3 females) and the mid dose (3 males, 1 female). Abnormal posture was observed in 4 high dose and 2 mid dose females. No other abnormalities were observed in all other groups or on other test days.

2. Open field observations

In the high dose group the following effects, which were considered to be substance related, were noted on Day 0 – abnormal posture (2 males, 3 females), impaired activity (5 males, 8 females), impaired gait (4 males, 6 females), slight ataxia (4 males, 5 females), severe ataxia with spreading of fore- and hind-limbs (1 female)

and hypothermia (1 male, 3 females). Decreased arousal (1 female) and a reddish area in the bulbus (2 females) were observed, but were not considered to be treatment related.

In the mid dose group the following effects, which were considered to be substance related, were noted on Day 0 – abnormal posture (2 males, 1 female), impaired activity (8 males, 2 females) and impairment of gait (4 males, 1 female).

In the low dose group the following effects, which were considered to be substance related, were noted on Day 0 – impaired activity (1 female) and impairment of gait (1 male).

On Day 7, 1 mid-dose male showed impairment of activity. This observation was considered to be incidental. On Day 14 no abnormal signs were detected in any of the treated animals.

3. Sensory-motor tests/ reflexes

On Day 0, in the high dose group half closure of the eyelids (2 males, 1 female) was seen during pupillary reflex testing. No other abnormalities were observed in all other groups or on other test days.

4. Quantitative observations

In the high and mid-dose females, the number of rearings was statistically significantly lower on Day 0.5. The values of the landing foot-splay test were statistically significantly increased in high and mid dose males on Day 0. Statistically significant reduces values in low dose males on Day 7 were considered to be incidental. Grip strength was not statistically significantly affected by treatment.

5. Motor activity measurement

On Day 0, overall motor activity values were statistically significantly decreased in the high and mid dose males and high dose females. Slight impairment was also seen in low dose males and mid dose females. No statistically significant deviations were seen in the other dose group or during the examinations on Days -7, 7 and 14.

6. Histopathology

Comprehensive neuropathological examinations of the central and peripheral nervous system did not reveal any substance related effects.

Conclusion :

Substance related clinical signs were observed in males and females at 350 and 700 mg/kg bw. At 175 mg/kg bw single animals still showed impairment of gait or activity and motor activity was slightly reduced in males. The NOAEL for this study was < 175 mg/kg bw.

Signs recorded in the study are considered due to acute systemic toxicity and not due to selective neurotoxicity. All clinical signs, except for the reduced bodyweight gain observed in males in the high dose group, were reversed by day 7. Mecoprop-P did not cause permanent damage to the nervous system when administered as a single gavage to Wistar rats up to a maximum dose of 700 mg/kg bw.

It is concluded that mecoprop-P is not neurotoxic.

B.6.7.2. Delayed polyneuropathy studies

Mecoprop-P does not have a structure that is associated with neurotoxicity. Furthermore there are no indications of neurotoxicity in any of the existing toxicology studies, including an acute neurotoxicity study (██████████ 1995). Therefore studies on delayed polyneuropathy are not required.

B.6.7.3 Summary of neurotoxicity

It is concluded that the need for neurotoxicity studies is not triggered. An acute neurotoxicity study has been submitted and evaluated. This is considered to be a supplementary study.

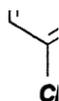
In an acute oral neurotoxicity study, mecoprop-P did not cause permanent damage to the nervous system of rats up to a maximum dose of 700 mg/kg bw. The NOAEL for this study was < 175 mg/kg bw based on acute systemic toxicity.

B.6.8. OTHER TOXICOLOGICAL STUDIES

B.6.8.1. Toxicity studies on metabolites and relevant impurities

No studies on the metabolites of mecoprop-P were submitted in the 1998 DAR. A new study conducted on the metabolite Hydroxymethyl-mecoprop-P (HMCPP) has been submitted. This is a rat metabolite (present at levels in urine of up to 32.6%).

Structure of hydroxymethyl-mecoprop-P (HMCPP) shown below:



Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012
----------------------	--

Study	Report on the study of the acute oral toxicity of Mecoprop-hydroxy-metabolite (M7/173) in the rat
Reference	██████████
Date performed	31 August 1979
Test facility	Not stated
Report reference	Original report by ██████████ translated by ██████████ (1980)
Guideline(s)	Not stated
Deviations from the guideline	Not applicable
GLP	No
Test material	Mecoprop-hydroxy-metabolite (M7/173), batch and purity not stated
Study acceptable	Appears acceptable but full study report needed to fully evaluate study.

2150 mg/kg bw mecoprop-hydroxy-metabolite was administered by oral gavage in an emulsion of 0.5% aqueous carboxymethylcellulose to 5 males and 5 female Sprague-Dawley rats. The rats were fasted overnight prior to dosing and observed for 14 days after dose administration.

There were no mortalities. There were no symptoms and no abnormalities at necropsy. The LD₅₀ was concluded to be > 2150 mg/kg bw.

This report was a translation and is a brief summary, and does not include full study report details including the name of the laboratory that conducted the study. The findings indicate that the metabolite mecoprop-hydroxy-metabolite is not expected to be more toxic than the parent compound mecoprop-P with respect to acute oral toxicity.

B.6.8.2. Supplementary studies on the active substance

B.6.8.2.1 Effects on metabolism – Liver enzyme induction in the mouse

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	The effect of structurally divergent herbicides on mouse liver xenobiotic-metabolizing enzymes (P-450-dependent mono-oxygenases, epoxide hydrolases and glutathione <i>S</i> -transferases) and carnitine acetyltransferase
Reference	Moody DE, Narloch BA, Shull LR, & Hammock BD (1991)
Date performed	Not stated
Test facility	Departments of Entymology and Environmental Toxicology, University of California.
Report reference	BASF doc. 91/10680. Published in: Toxicology Letters 59 , 175-185 (1991).
Guideline(s)	No
Deviations from the guideline	Not relevant
GLP	No
Test material	Mecoprop, batch and purity not stated
Study acceptable le	Yes

Study report:

Moody DE, Narloch BA, Shull LR, & Hammock BD (1991): The effect of structurally divergent herbicides on mouse liver xenobiotic-metabolizing enzymes (P-450-dependent mono-oxygenases, epoxide hydrolases and glutathione *S*-transferases) and carnitine acetyltransferase. Published in: Toxicology Letters **59**, 175-185 (1991). BASF doc. 91/10680. (Dossier ref. 5.56)

Study design and quality:

Three male Swiss Webster mice were dosed with daily i.p. injections of 100 mg/kg bw/day of racemic mecoprop (purity not specified) during three days. The test substance was suspended in corn oil and a volume of 1.0 ml/kg bw was used for the i.p. injection. Four control mice received i.p. injections with corn oil only. After the last injection the animals were killed and enzyme assays were performed on liver homogenates to assess any influence of the treatment on the metabolizing enzymes.

The study was not conducted according to any guidelines. No GLP statement present.

The study is considered appropriate for its purpose.

Results:

Originally a mecoprop dose of 250 mg/kg bw was used. However, this dose was lethal to all the test animals, whereafter a dose of 100 mg/kg bw was used.

Mecoprop treatment caused significantly increased activity of aminopyrine *N*-demethylase, cytosolic epoxide hydrolase and carnitine acetyltransferase. The latter enzyme is found to be a fairly sensitive indicator for peroxisome proliferation.

It was concluded that mecoprop (and other herbicides tested in the assay) significantly increased one or more of the liver enzymes tested.

Discussion and conclusion:

The activity of liver enzymes was increased by mecoprop in mice after acute exposure. Thus it was shown that mecoprop has the potential to alter the liver function.

B.6.8.2.2 Haematological effects - Human platelets *in vitro*

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Inhibition of human and rabbit platelet aggregation by chlorophenoxyacid herbicides
Reference	Elo HA, Luoma T, & Ylitalo P (1991)
Date performed	Not stated
Test facility	Department of Pharmaceutical Chemistry and Department of Pharmacology and Toxicology, University of Kuopio, Finland
Report reference	BASF doc. 91/11683 Published in: Archives of Toxicology 65 , 140-144 (1991)
Guideline(s)	Not stated
Deviations from the guideline	Not relevant
GLP	Not stated
Test material	Mecoprop purity >99%
Study acceptable	Acceptable as supporting evidence

Study report:

Elo HA, Luoma T, & Ylitalo P (1991): Inhibition of human and rabbit platelet aggregation by chlorophenoxyacid herbicides. Published in: Archives of Toxicology **65**, 140-144 (1991). BASF doc. 91/11683. (Dossier ref. 5.55).

Study design and quality:

Platelet aggregation in platelet-rich plasma was induced by adding different amounts of either adenosine diphosphate, adrenaline or collagen to a sample at 37 °C. The effects of mecoprop (purity above 99%) and other chlorophenoxy acids on the aggregation system was studied at different concentration levels.

The study was not conducted according to any guidelines. No GLP statement present.

The study is considered appropriate for its purpose.

Results:

At concentrations between 0.1 and 2 mg/ml, mecoprop and other chlorophenoxy acids caused a clear dose-dependent inhibition of human platelet aggregation in all the three aggregation inducing systems. No inhibition was seen at 0.05 mg/ml of any of the herbicides. The authors noted that cases of severe and lethal poisoning with chlorophenoxy herbicides had resulted in blood levels of 0.67-1.15 mg/ml of the herbicides corresponding to the effect levels used in this study.

Discussion and conclusion:

From this *in vitro* study mecoprop was shown to have a potential of inhibiting platelet aggregation in humans.

B.6.8.2.3 Immunotoxicity studies

The data requirement Regulation (EU) 283/2013 states that immunotoxicity studies are only required if immunotoxic effects have been observed in other studies on the active substance. There is no evidence of immunotoxicity in the studies conducted on mecoprop-P therefore the need for further specific immunotoxicity studies is not triggered. Two immunotoxicity studies were submitted in the 1998 DAR and are included here. They are considered to be supplementary studies.

B.6.8.2.3/01 Acute, 14-day and 90-day immunotoxicity in the rat

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Pathomorphologische und hämatologische Untersuchungen zur Immuntoxizität der Phenoxyalkansäure Mecoprop an Ratten
Reference	Moeller T & Solecki R (1989)
Date performed	Not stated
Test facility	Institut für Pflanzenschulzforschung der Akademie der Landwirtschaftswissenschaften der DDR
Report reference	BASF doc. 89/10807. Published in: Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete 35 (5), 258-260 (1989).
Guideline(s)	Not stated
Deviations from the guideline	Not applicable
GLP	Not stated
Test material	Mecoprop, purity 97%
Study acceptable	Yes as supporting evidence, insufficient information to establish a NOAEL

Study report:

Moeller T & Solecki R (1989): Pathomorphologische und hämatologische Untersuchungen zur Immuntoxizität der Phenoxyalkansäure Mecoprop an Ratten. Published in: Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete **35**(5), 258-260 (1989). BASF doc. 89/10807. (Dossier ref. 5.53).

Study design and quality:

An acute, a 14 day and a 90 day oral study was conducted with Wistar rats according to the table below.

Table B.6. 57 Study design of oral studies

Study	Dose levels, mg/kg bw/day					total no. of doses	no. of rats in each dose group, m/f
	0	320	800	1300	-		
acute	0	320	800	1300	-	1	12/-
14-day	0	100	320	800	-	10*	12/-
90-day	0	0.8	8	80	320	95	20/20

* At 800 mg/kg only 5 rats were used.

The animals were dosed by gavage. Racemic mecoprop (potassium salt, purity of 97%) was dissolved in water and the animals were dosed with a volume of 5 ml/kg bw.

Organ weights of thymus and spleen were determined. Histopathology was performed on thymus, spleen and mesenteric lymph nodes. The spleen was subjected to morphometry. Total and differential leucocyte counts were performed. Housing conditions only differed marginally from the conditions described in OECD 401, OECD 407 and 408.

From the reporting of the studies it can not be seen, if the studies were conducted according to any guidelines or GLP.

No information about body weight gain, organ weights of other organs (liver, kidney) etc. is available and some

details on e.g. blood chemistry are lacking. Therefore the report can only be used as supplementary to the other tests.

Results:

Thymus. The thymus weight was significantly ($p < 0.05$, t-test) decreased at 1300 mg/kg bw in the acute study, at 320 and 800 mg/kg bw in the 14-day study and at 8 mg/kg bw (males), 80 mg/kg bw (males) and 320 mg/kg bw (males + females) in the 90-day study. At the histological examination degenerative processes at and above 320 mg/kg bw/day were observed consisting of decreased density of lymphocytes and increased decay of leucocytes in the cortex of the organ. No significant findings were noted in the medulla.

Spleen. The organ weight was significantly reduced at 1300 mg/kg bw in the acute study and at 800 mg/kg bw in the 14-day study. At microscopy a reduction in white pulp tissue was noted in all of the studies, and in the 14-day and 90-day study at the same time enlargement of the haematopoietic tissue of the spleen was found. These findings were confirmed by morphometric examination of the spleen tissue from the animals in the 14-day study, where the changes occurred to a significant extent at 320 mg/kg bw.

No histological changes were found in the mesenteric lymph nodes.

The number of total leucocytes was not affected, however, in the 14-day and the 90-day study significant dose-dependent changes in differential leucocyte counts were noted (decrease in lymphocytes and increase in neutrophilic granulocytes).

The results from these studies were interpreted as well known immunotoxic signs of increased glucocorticoid response. A stress symptom with increased adrenal secretion of glucocorticoids was suggested.

Discussion and conclusion:

The consistent findings between the three experiments, strongly indicates mecoprop exposure to affect thymus and spleen function and cause haematological changes. The reporting of the haematological effects do not allow for establishing a NOAEL, but the description of these findings are very brief. With respect to decrease in thymus organ weight NOAEL in the 90-day study is 0.8 mg/kg bw/day for male rats and 80 mg/kg bw/day for females. As this was a gavage dose conducted using racemic mecoprop it has less relevance for the human risk assessment compared to dietary studies on mecoprop-P.

B.6.8.2.3/02 Immunotoxicity study in the rat

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Funktionelle Testung der Immuntoxizität der Phenoxyalkansäure Mecoprop an Ratten und Ausschaltung immuntoxischer Effekte durch Adrenalektomie
Reference	Moeller T (1990)
Date performed	Not stated
Test facility	Institut für Pflanzenschulzforschung der Akademie der Landwirtschaftswissenschaften der DDR
Report reference	BASF doc. 90/10924. Published in: Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete 36 (6), 314-316 (1990).
Guideline(s)	Not stated
Deviations from the guideline	Not applicable
GLP	Not stated
Test material	Mecoprop, purity 97%
Study acceptable	Yes

Study report:

Moeller T (1990): Funktionelle Testung der Immuntoxizität der Phenoxyalkansäure Mecoprop an Ratten und Ausschaltung immunotoxischer Effekte durch Adrenalectomie. Published in: Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete **36**(6), 314-316 (1990). BASF doc. 90/10924. (Dossier ref. 5.54).

Study design and quality:

Three substudies were conducted with Wistar rats to investigate the effect of mecoprop on humoral and cell immunity and to elucidate the possible mode of action by dosing adrenalectomized animals. The studies were conducted according to the following table.

Table B.6. 58 Study designs

Study no.	Mecoprop dose levels, mg/kg bw/day	no. of doses	no. of animals in humoral immunity testing	no. of animals in cellular immunity testing
1	0	3*	12	9
	150	3*	12	-
	500	3*	12	9
2	0	15	12	9
	320	15	12	9
3	0	10	adrenalectomized animals (n=?)	
	320	10	adrenalectomized animals (n=?)	
	0	10	controls (n=?)	
	80	10	controls (n=?)	
	320	10	controls (n=?)	

* the animals were dosed every second day

The animals were dosed by gavage. Mecoprop (racemic form with a purity of 97%) was suspended in water and the animals were dosed with a volume of 5 ml/kg bw.

Study 1. Humoral immunity testing: immunization with sheep erythrocytes on the same day as the administration of the second dose. Haemagglutination titre and serum IgG was determined 5 days after immunization.

Cellular immunity testing: An ear test for delayed sensitivity reaction was performed. On the day of the second mecoprop dosing a shaved area of the abdomen was exposed to 25 ml of a 3% dinitrofluorobenzene solution in acetone. Five days later 10 ml of the same solution was applied on the inner and outer skin surface of the ear. The thickness of the ear was measured just before and 24 h after this application.

Study 2. Humoral immunity testing: Immunization with sheep erythrocytes at the day of administration of the last dose. Haemagglutination titre and serum IgG was determined 5 days after immunization.

Cellular immunity testing: The test procedure as described above was started on day 10 of mecoprop dosing.

Study 3. After adrenalectomy was conducted on the animals an adaption period of five days followed before mecoprop dosing. After the last dosing the animals were killed and observations were made with respect to organ weight of the spleen, serum IgG and lymphocyte counts. Histological examinations were performed on thymus and spleen.

The studies were not conducted according to any guidelines. With regard to study conditions the article refers to the study described in section B.5.8.3.1, however, important information concerning number of animals in study 3 is missing. The study should be considered supportive to the studies described above in section B.5.8.3.1.

Results:

Study 1. Significantly decreased serum IgG was determined at 500 mg/kg bw compared to controls. Also at this level significantly decreased reaction was observed with respect to delayed sensitivity reactions in the ear test.

Study 2. At the dosed group (15 x 320 mg/kg bw) significantly reduced IgG and significantly reduced response in the ear test were determined.

Study 3. At the 320 mg/kg bw level in the control group significant findings compared to the 0 mg/kg bw group were recorded with respect to reduced spleen organ weight, reduced IgG-concentration, and decreased lymphocyte count. Furthermore identical histological findings were observed as mentioned in the prior study (described in section B.5.8.3.1). None of these findings were noted in the adrenalectomized animals at 80 and 320 mg/kg bw.

The author concludes that mecoprop does affect the cellular and the humoral immunity. The study furthermore supports the suggestion of an indirect action of mecoprop mediated by the activity of the adrenals.

Discussion and conclusion:

Mecoprop (racemic form) in acute and subacute dosing at and above 320 mg/kg bw was shown to induce immunotoxic response in rats. An indirect mechanism via increased adrenal (glucocorticoid) activity seems plausible. As this was a gavage dose conducted using racemic mecoprop it has less relevance for the human risk assessment compared to dietary studies on mecoprop-P

B.6.8.2.4 Conclusion on immunotoxicity studies

During the previous renewal review of mecoprop-P it was concluded that studies on immunotoxicity indicate indirect effects related to a stress-induced release of steroid hormones from adrenals. This is likely to be a secondary effect related to general toxicity.

B.6.8.3. Studies on endocrine disruption

The RMS considers that mecoprop-P does not meet interim criteria for endocrine disrupting properties under Regulation (EC) 1107/2009.

An evaluation of the regulatory toxicology studies and published literature revealed no significant evidence of endocrine disruption. Overall, the weight of evidence indicates that mecoprop-P is not an endocrine disrupting chemical.

The UK CRD has assessed the available data on mecoprop, which were considered as part of the EU Review. Their review¹ begins on page 96 and they conclude that mecoprop does not exhibit any evidence of an endocrine effect. Therefore additional studies on endocrine disruption are not required. A copy of their assessment is available in Document K, CA 5.8.3/01.

¹Extended impact assessment study of the human health and environmental criteria for endocrine disrupting substances proposed by HSE, CRD WRc Ref: Defra9088.01 January 2013

B.6.9. MEDICAL DATA AND INFORMATION**B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies**

A new report is provided which contains the procedures for monitoring the workforce from 2008- to present day (White, 2014). Nufarm UK performs annual medicals by the company occupational nurse and compares the results from these medicals against previous medicals to assess for areas of concern. A general practitioner is also available to attend the workforce on a weekly basis. By observing adverse effects through studying those

exposed to elevated concentrations of material on a frequent basis Nufarm have no indication of any adverse effects within the workforce.

There have been no medical incidences in the workforce at [REDACTED] (current manufacturing site) or [REDACTED] (previous alternative manufacturing site).

In the 1998 DAR a paper (Becher et al., 1992) was submitted on factory monitoring, but no useful information was available at the time of submission. The manufacturing facility in this paper is no longer involved with the production of mecoprop-P.

B.6.9.1/01

Previous evaluation:	None; Submitted for the purpose of renewal under Regulation 844/2012
----------------------	--

Study	Mecoprop-P Manufacturing Medical Surveillance Report
Reference	White, S. (2014)
Date performed	18 November 2014
Test facility	[REDACTED]
Report reference	
Guideline(s)	Not relevant
Deviations from the guideline	Not relevant
GLP	Not relevant
Test material	Mecoprop-P
Study acceptable	Yes

All Nufarm employees are offered a voluntary annual medical check. The annual medical is performed by the companies Occupational Health Nurse and includes the following questionnaires (copies provided in Appendix 1a to 1d):

Annual medical – the tests and questionnaire assess blood pressure and heart rate, mobility test, and voluntary blood test.

Spirometry / skin – the tests and questionnaire assess symptoms related to skin and respiratory problems, and a skin examination of the hands.

The results of which are then compared against previous medicals to assess if there are any areas of concern.

Mecoprop-p technical has been made in the UK at the [REDACTED] manufacturing site since the mid to late 1980's (1986-1987). There has not been continual health surveillance in this period, however since 2008 annual medicals have been in operation. Prior to this they were done on an ad hoc basis but were offered at least every 3 years. The qualified nurse has reviewed the medical files for the personnel currently working on the Mecoprop-p manufacturing plant, and from these records it shows that medicals were typically carried out in 2005, 2008, 2009, 2011, 2012 and 2013. In all cases the nurse states that the results are "consistent", in other words no adverse trends have been noted.

B.6.9.1/02

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	A cohort study on persons exposed to phenoxy acid herbicides and their contaminants in Germany -design and first results
Reference	Becher H, Wahrendorf J, & Angerer R (1992)
Date performed	Started in 1990.
Test facility	Research conducted by Department of Epidemiology, German

	Cancer Research Centre
Report reference	BASF doc. 92/12297. Published in: <i>Chemosphere</i> 25 , 1007-1014 (1992).
Guideline(s)	Not relevant
Deviations from the guideline	Not relevant
GLP	Not relevant
Test material	Mecoprop
Study acceptable	This paper has insufficient information for any evaluation. Follow-up papers likely to be more useful

Study report:

Becher H, Wahrendorf J, & Angerer R (1992): A cohort study on persons exposed to phenoxy acid herbicides and their contaminants in Germany -design and first results. Published in: *Chemosphere* **25**, 1007-1014 (1992). BASF doc. 92/12297. (Dossier ref. 5.57).

Study design, reporting and quality:

The cohort consists of 3000 workers from four herbicide producing companies in German factories employed with synthesis, formulation, packing or otherwise exposed to phenoxy acid herbicides (one of these mecoprop). Especially notice is made to TCDD exposure from TCDD contamination of the herbicides. Exposure conditions were assessed by company questionnaires and from an industrial hygienist. Mortality follow-up started in autumn 1990.

Results:

The article presents an on-going study and no specific details concerning mecoprop levels at the factories are given. The article does not contain any information concerning mortality or health effects in the cohort.

Discussion and conclusion:

The report at this stage does not contain any specific information for evaluation concerning health effects or concerning exposure to mecoprop.

B.6.9.2. Data collected on humans

No data available to the applicant.

B.6.9.3. Direct observation

No new data has been provided since the previous renewal review. In the 1998 DAR, two published papers (Meulenbelt *et al.*, 1988 and Prescott *et al.*, 1979) were submitted. The papers described the clinical findings from human poisoning with racemic mecoprop.

Summary of clinical cases and poisoning incidents

The clinical findings from acute human poisoning at plasma levels of about 300-750 mg/l were reported to be muscle cramps, muscle cell damage, metabolic acidosis, respiratory failure, arterial hypoxemia, renal failure, and coma. Supportive treatment and induction of increased renal clearance by alkaline diuresis is recommended in cases of poisoning.

B.6.9.3.1 Clinical cases and poisoning incidents

No new information has been submitted since the 1998 DAR. In the literature review for this renewal a few papers on clinical cases and poisoning were been dismissed by the applicant as irrelevant, but the RMS considers that further data on poisoning is useful because mecoprop-P is acutely toxic.

B.6.9.3.1/01

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Acute MCPP intoxication: report of two cases
Reference	Meulenbelt J, Zwaveling JH, van Zoonen P, & Notermans NC (1988)
Date performed	Not applicable
Test facility	National Poison Control Centre, The Netherlands
Report reference	BASF doc. 88/10595. Published in: Human Toxicology 7, 289-292 (1988)
Guideline(s)	Not relevant
Deviations from the guideline	Not relevant
GLP	Not relevant
Test material	Mecoprop (not further specified) and alcohol
Study acceptable	Yes for information on poisoning, but some symptoms may have been due to alcohol poisoning.

Study report:

Meulenbelt J, Zwaveling JH, van Zoonen P, & Notermans NC (1988): Acute MCPP intoxication: report of two cases. Published in: Human Toxicology 7, 289-292 (1988). BASF doc. 88/10595. (Dossier ref. 5.59).

Study design and quality:

The report describes two cases of acute mecoprop poisoning of two men (69 and 70 year-old). The patients had ingested unknown amounts of mecoprop (not further specified) one together with alcohol consumption.

The descriptions are thorough with many details and should be considered of value when assessing the risk by acute mecoprop poisoning.

Results:

In one of the patients a plasma level of mecoprop of 298 mg/l was determined 3-4 hours after ingestion. The plasma level decreased with a $t_{1/2}$ of about 17 h.

The major clinical findings were coma, muscle cramps, respiratory failure and arterial hypoxemia. Respiratory depression and hypoxemia were thought partly to be due to CNS depression and partly due to aspiration. Both patients developed serious renal failure, probably caused by rhabdomyolysis. Rhabdomyolysis (elevated serum myoglobin and serum aldose activity) was suggested to be secondary to muscle cramps. Both patients developed hyperkalemia as a result of a combination of renal failure, muscle cell damage and metabolic acidosis. (The hyperkalaemia and the acidosis were treated with infusions of glucose/insulin and sodium bicarbonate). Both patients exhibited serious decrease in arterial blood pressure and developed anaemia and thrombocytopenia (the latter resulting in multiple petechia in one of the patients). Caustic mucous lesions were not observed in any of the patients. Both patients survived.

Discussion and conclusion:

No comments regarding the findings of the poisoning cases.

B.6.9.3.1/02

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Treatment of severe 2,4-D and mecoprop intoxication with alkaline diuresis
Reference	Prescott LF, Park J, & Darrien I (1979)
Date performed	Not applicable
Test facility	Royal Infirmary, Edinburgh
Report reference	BASF doc. 79/10296. Published in: British Journal of Clinical Pharmacology 7 , 111-116 (1979).
Guideline(s)	Not relevant
Deviations from the guideline	Not relevant
GLP	Not relevant
Test material	20% mecoprop and 10% 2,4-D (not further specified)
Study acceptable	Yes for information on poisoning, but some symptoms may have been due to 2,4-D poisoning.

Study report:

Prescott LF, Park J, & Darrien I (1979): Treatment of severe 2,4-D and mecoprop intoxication with alkaline diuresis. Published in: British Journal of Clinical Pharmacology **7**, 111-116 (1979). BASF doc. 79/10296. (Dossier ref. 5.61).

Study design and quality:

The report describes a case of a 39 year old man that ingested half a lemonade bottle of a pesticide containing 10% 2,4-D and 20% mecoprop (not further specified). Although the case is a mixed exposure the description should be used in assessment of acute mecoprop poisoning, as great similarities between 2,4-D and mecoprop are to be expected.

Results:

The plasma concentrations at admission were 400 and 751 mg/ml of 2,4-D and mecoprop, respectively. The total amounts of 2,4-D and mecoprop recovered in urine were 6.66 and 7.64 g. From this it was judged that active substances from about 70 ml of the weedkiller was absorbed.

The toxic signs included deep prolonged coma, pyrexia, hyperventilation, hypoxemia, myotonia, skeletal muscle damage and electrocardiographic changes consistent with cardiomyopathy. Muscle damage was a major complication with a striking elevation of creatine phosphokinase.

The patient remained gravely ill with no signs of improvement for 2 days with supportive therapy. When alkaline diuresis was induced by the means of intravenous administration of sodium bicarbonate a dramatic fall in plasma 2,4-D followed. The fall for mecoprop was less dramatic with half-lives of 24, 11, and 28 h just before, during and after the urine became alkaline. The patient regained consciousness at plasma levels of about 100 mg/ml for each of the substances.

Discussion and conclusion:

No further comments to the report.

B.6.9.4. Epidemiological studies

No new epidemiological data has been provided since the previous renewal review. In the 1998 DAR, three published papers (Maroni & Fait, 1993; Wiklund *et al.*, 1987 and Bond & Rossbacher, 1993) were submitted. From available epidemiological studies no clear association between cancer development and exposure to phenoxy herbicides (including mecoprop) could be established.

In the 2003 Review Report for mecoprop-P it was concluded that available epidemiological data are inadequate for determining an association between exposure and cancer in humans.

In the literature review for this renewal a number of epidemiology studies have been dismissed that may show a lack of association between exposure to mecoprop and cancer. The RMS considers that any studies that show a lack of association between mecoprop and cancer are relevant and should not have been excluded. Another epidemiology study reports a significant association of mecoprop exposure with multiple myeloma. In particular the RMS questions the case for excluding the following papers as irrelevant:

1. International Journal of Cancer (2013) Vol. 133(8), pp. 1846-1858 (effects of pesticide exposure and risk of multiple myeloma).
2. Journal of Occupational and Environmental Medicine (2011) Vol. 53(11), pp. 1279-1286 (pesticide associations with soft-tissue sarcoma).
3. International Journal of Cancer (2012) Vol. 131(11), pp. 2650-2659 (pesticide use on asthma and lymphoma).
4. American Journal of Epidemiology (2011) Vol. 173 Suppl. 11, P S255 (herbicide exposure and childhood leukaemia).

In conclusion the applicant should provide further information on the findings from these studies and give further consideration of their relevance. Overall, however, the RMS considers that none of the toxicology papers dismissed are likely to add significant information to the toxicology dossier that would lead to a change in the overall conclusions.

B.6.9.4.1

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Health effects in man from long-term exposure to pesticides -A review of the 1975-1991 literature
Reference	Maroni M & Fait A (1993)
Date performed	Review of literature from 1975 to 1991
Test facility	International Centre for Pesticide Safety, Italy
Report reference	BASF doc. 93/11634 Published in: Toxicology 78 , 1-180 (1993)
Guideline(s)	Not relevant
Deviations from the guideline	Not relevant
GLP	Not relevant
Test material	Some information on phenoxy herbicides but none specifically on mecoprop
Study acceptable	Insufficient to draw conclusions on mecoprop-P

Study report:

Maroni M & Fait A (1993): Health effects in man from long-term exposure to pesticides -A review of the 1975-1991 literature. Published in: Toxicology **78**, 1-180 (1993). BASF doc. 93/11634. (Dossier ref. 5.58).

Study design and quality:

This extensive publication on pesticides in general reviews the 1975-1991 literature with respect to health effects from long term occupational or environmental exposure.

No specific data concerning mecoprop is by the notifier extracted from the review. However for phenoxy herbicides in general the authors conclude that evidence for chloracne is well established for impurities such as TCDD. Further, the authors call for additional data for confirmation as some data suggest teratogenic effects and increased cancer risk with regard to the following sites: soft-tissue, lymphatic and haematopoietic system, stomach, colon, and prostate gland.

Discussion and conclusion:

No conclusion can be made specifically on mecoprop from the data.

B.6.9.4.2

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Risk of malignant lymphoma in Swedish pesticide applicators
Reference	Wiklund K, Dich J, & Holm L-E (1987)
Date performed	Study investigates pesticide applicators who had licences issued between 1965 to 1976
Test facility	Department of Cancer Epidemiology and General Oncology, Radiumhemmet, Karolinska Hospital and Institute, Stockholm.
Report reference	BASF doc. 87/10823 Published in: British Journal of Cancer 56 , 505-508 (1987)
Guideline(s)	Not relevant
Deviations from the guideline	Not relevant
GLP	Not relevant
Test material	Pesticides
Study acceptable	No because it does not distinguish mecoprop exposure from other types of pesticides

Study report:

Wiklund K, Dich J, & Holm L-E (1987): Risk of malignant lymphoma in Swedish pesticide applicators. Published in: British Journal of Cancer **56**, 505-508 (1987). BASF doc. 87/10823. (Dossier ref. 5.61).

Study design and quality:

A follow-up study was conducted with a cohort of 20,245 Swedish pesticide applicators. Exposure was assessed from questionnaires sent to a random sample of 273 persons. The persons were followed-up in the Swedish Cancer Register with respect to diagnosis of Hodgkin's disease and non-Hodgkin lymphoma from date of licence until 31 December 1982 or until death, if prior to that date.

The study is considered adequate for its purpose. However, the relevance for mecoprop specifically, is limited.

Result:

Exposure information from 273 persons indicated that about 72% of the cohort had been exposed to phenoxy acid herbicides (no specific data concerning mecoprop exposure).

A total of 11 cases with Hodgkin's disease and 21 cases with non-Hodgkin lymphoma were observed compared to 9.1 and 20.8 expected (compared to age-specific incidences in the whole Swedish population), thus no

increased risk was found. The relative risk rose (not statistically significant) with increased duration having pesticide licence for both diagnoses.

Discussion and conclusion:

No conclusion concerning the carcinogenicity of mecoprop can be drawn from this study.

B.6.9.3.1/03

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	A review of potential human carcinogenicity of the chlorophenoxy herbicides MCPA, MCPP, and 2,4-D
Reference	Bond GG & Rossbacher R (1993)
Date performed	1993
Test facility	Review paper by Dow Chemical Company USA and BASF Germany
Report reference	BASF doc. 93/11582 Published in: British Journal of Industrial Medicine 50 , 340-348 (1993)
Guideline(s)	Not relevant
Deviations from the guideline	Not relevant
GLP	Not relevant
Test material	Chlorophenoxy herbicides including mecoprop
Study acceptable	Insufficient evidence to draw conclusions on mecoprop

Study report:

Bond GG & Rossbacher R (1993): A review of potential human carcinogenicity of the chlorophenoxy herbicides MCPA, MCPP, and 2,4-D. Published in: British Journal of Industrial Medicine **50**, 340-348 (1993). BASF doc. 93/11582. (Dossier ref. 5.63).

Study design and quality:

The authors review the epidemiological evidence for carcinogenic effects from chlorophenoxy herbicides exposure (mecoprop being a part of the total exposure).

Results:

It is emphasized that although there is substantial epidemiological evidence available on the chlorophenoxy compounds as a group, little of it is specific to MCPA, MCPP, and 2,4-D.

The *ecological (geographical correctional) epidemiological studies* suggests an increased risk for certain types of malignancies, particularly those of the haematological system. However, evidence from this kind of studies is considered weak and have to be further substantiated from other more analytical studies.

Some *case-referent studies* have shown increased risk for Hodgkin's disease and non-Hodgkin lymphoma. Overall, the pattern from these studies was found to be very inconsistent with variability in the risk estimates and with several studies without any relation to chlorophenoxy herbicide exposure.

Two cohort studies on workers from the phenoxy herbicide production did not find any clear relation between exposure and malignancies although increased risk for cancer at one or more organ sites were found in both studies. Potential confounders and chance were considered as viable explanations as well.

Overall, the evidence with respect to carcinogenic effects for the whole group of chlorophenoxy herbicides was

considered to be suggestive and far from conclusive.

Conclusion:

No specific human data is available concerning carcinogenic effects of mecoprop.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

No data submitted.

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

B.6.9.6.1 First Aid measures

Inhalation:	Remove casualty from exposure ensuring one's own safety whilst doing so. If conscious, ensure the casualty sits or lies down. If unconscious, check for breathing and apply artificial respiration if necessary. If unconscious and breathing is OK, place in the recovery position. Consult a doctor.
Skin contact:	Remove all contaminated clothes and footwear immediately unless stuck to skin. Drench the affected skin with running water for 10 minutes or longer if substance is still on skin. Consult a doctor.
Eye contact:	Bathe the eye with running water for 15 minutes. Consult a doctor.
Ingestion:	Wash out mouth with water. Do not induce vomiting. If conscious, give half a litre of water to drink immediately. If unconscious, check for breathing and apply artificial respiration if necessary. If unconscious and breathing is OK, place in the recovery position. Consult a doctor.
Antidote:	No antidote is available.
Medical treatment:	Alkaline diuresis may be used to treat acute poisoning in the presence of coma or other poor prognostic indicators, such as acidemia, or if total chlorophenoxy concentrations are 0.5 g/l or more. Alkaline diuresis increases renal clearance.

B.6.9.6.2 Medical treatment

Previous evaluation:	In DAR for first review (1998)
----------------------	--------------------------------

Study	Alkaline diuresis for acute poisoning with chlorophenoxy herbicides and ioxynil
Reference	Flanagan RJ, Meredith TJ, Ruprah M, Onyon LJ, & Liddle A (1990)
Date performed	Poisoning cases from 1984 to 1987
Test facility	Guy's Hospital London
Report reference	BASF doc. 90/10922. The Lancet 335, 454-458 (1990).
Guideline(s)	Not relevant
Deviations from the guideline	Not relevant
GLP	Not relevant

Test material	Study on phenoxy herbicide poisoning including mecoprop
Study acceptable	Yes as a report on treatment for poisoning cases

Study report:

Flanagan RJ, Meredith TJ, Ruprah M, Onyon LJ, & Liddle A (1990): Alkaline diuresis for acute poisoning with chlorophenoxy herbicides and ioxynil. Published in: The Lancet **335**, 454-458 (1990). BASF doc. 90/10922. (Dossier ref. 5.66).

Study design and quality:

The report describes cases with patients admitted to a poisoning unit of a hospital in London. In 38 cases phenoxy herbicides were involved. Most cases were cases of exposure to more than one substance, however poisoning with phenoxyherbicides as a group characterized 30 cases. Mecoprop was involved in 16 cases.

The general characteristics of the cases with respect to clinical findings, supportive treatment and medical treatment (especially alkaline diuresis) are described.

Results:

Four of 16 cases in which mecoprop was involved resulted in fatal outcome.

Of the 30 persons with chlorophenoxy herbicide poisoning, 3 were found dead. Eleven, of which two died, were given supportive treatment alone. Alkaline diuresis was used to treat 16 patients of which 15 survived. The alkaline diuresis increased renal clearance for 2,4-D, DCPD and MCPD to values similar to those obtained at lower non-toxic doses. It was concluded that alkaline diuresis should be used to treat acute poisoning with chlorophenoxy herbicides in the presence of coma or other poor prognostic indicators, such as acidemia, or if total chlorophenoxy concentrations are 0.5 g/l or more.

Discussion and conclusion:

The value of alkaline diuresis in cases of acute mecoprop poisoning (and other chlorophenoxy herbicides) is documented in the report.

B.6.9.3 Expected effects of poisoning

Effects of poisoning may include coma, muscle cramps, pyrexia, hyperventilation, respiratory failure, arterial hypoxemia, myotonia, skeletal muscle damage and electrocardiographic changes consistent with cardiomyopathy.

B.6.10. REFERENCES RELIED ON**Evaluation of literature review**Evaluation the Literature Review (with respect to mammalian toxicology)

A literature search was conducted by the applicant. No papers relevant to toxicology were identified by the applicant. The RMS considers that the case for dismissing all toxicology papers as irrelevant is not sufficiently robust with regards to studies on cancer epidemiology and poisonings.

Under Article 8(5) of (EU) 1107/2009 the applicant is required to submit peer reviewed studies published in the open literature in the past ten years that are relevant to the assessment of mecoprop-P. EFSA has provided guidance on conducting an acceptable literature search (EFSA Journal 2011; 9(2):2092).

1. Search terms

Acceptable. Included mecoprop and synonyms which produced 6864 hits. The date range was reduced to 2004 to 2014 and this reduced the hits down to 671. This is acceptable as only literature published within the last 10 years is required (note that MCPD and CMPP were excluded from the search terms due to the fact that they were retrieving a large number of hits totally unrelated to mecoprop).

2. Databases searched

Acceptable. 26 databases were searched. These included Medline, New England Journal of Medicine, Toxfile, Registry of Toxic Effects of Chemical Substances, Lancet Titles, HSEline, Embase.

3. Relevance criteria

The 671 papers were filtered initially by a manual review of the titles and obviously irrelevant titles were discarded, this left 142 papers.

The abstracts of these papers were retrieved and considered for their relevance, this left 44 papers either relevant or of unclear relevance.

The full text of these papers were considered to

- a) determine their relevance and
- b) if the study could impact on the endpoints and risk assessment of the active substance

A summary of relevance criteria for toxicology endpoints is given below:

Relevance criteria for toxicology were as follows:

1. Well-defined test material including purity and impurity profile
2. *In vivo* tests in relevant species – preferred species being rat, mouse and dog or *in vitro* tests
3. Endpoints specific to the data requirements
4. Relevant route of exposure
5. Sufficient numbers of animals per group to establish statistical significance
6. At least 3 doses tested
7. Epidemiological studies, poisonings and clinical cases also considered

The RMS agrees with the relevance criteria except for the stipulation of a well-defined test material including impurity profile which is considered too specific for epidemiology studies, poisonings and clinical cases. The RMS is concerned that this may have caused the exclusion of relevant or potentially relevant studies.

The RMS also considers that the relevance criteria should have included studies which may be helpful in the interpretation of other studies present in the dossier, but do not fit under specific toxicological endpoints (these could include non-standard studies).

According to the EFSA guidance on literature reviews the applicant should have provided copies of all papers that were relevant or potentially relevant (44 papers). The applicant only provided copies of the 5 papers that met the reliability score.

A total of 20 papers were considered to be relevant or of unclear relevance. These papers were scored for reliability.

4. Reliability criteria

Papers were assessed for reliability according to Klimisch et al. (1977). This is an acceptable method for reliability scoring. 5 papers were considered relevant and reliable after Klimisch scoring. None of these papers were for toxicology endpoints.

Potentially relevant studies excluded by the applicant

The RMS has reviewed the titles of the 142 papers of relevance or potential relevance and the comments made by the applicant. The RMS agrees that none of the toxicology papers dismissed are likely to add significant information to the toxicology dossier that would lead to a change in the overall conclusions.

However a number of epidemiology studies have been dismissed that may show a lack of association between mecoprop exposure and carcinogenicity endpoints. The RMS considers that any studies that confirm a lack of association between mecoprop and cancer are useful data to add weight of evidence that mecoprop is not carcinogenic in humans (especially as tumours were seen in animal studies). Another epidemiology study reports a significant association of mecoprop exposure with multiple myeloma. In particular the RMS questions the case for excluding the following papers as irrelevant :

1. International Journal of Cancer (2013) Vol. 133(8), pp. 1846-1858 (effects of pesticide exposure and risk of multiple myeloma).
2. Journal of Occupational and Environmental Medicine (2011) Vol. 53(11), pp. 1279-1286 (pesticide associations with soft-tissue sarcoma).

3. International Journal of Cancer (2012) Vol. 131(11), pp. 2650-2659 (pesticide use on asthma and lymphoma).
 4. American Journal of Epidemiology (2011) Vol. 173 Suppl. 11, P S255 (herbicide exposure and childhood leukaemia).

In addition a few papers on clinical cases and poisoning have also been dismissed as irrelevant, but the RMS considers that further data on poisoning is useful because mecoprop-P is acutely toxic so the exclusion of this information is questioned.

In conclusion the applicant should provide further information on the findings from potentially relevant studies and give further consideration of their relevance.

The references relied on list has been updated to include the newly submitted data relied on as well as those original submitted tests and studies (in *italics*) that are still considered relevant to support the application for renewal.

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
CA 5.1.	[REDACTED]	1997	<i>(¹⁴C)-Mecoprop-P: Absorption, distribution, metabolism and excretion in the rat</i> [REDACTED] [REDACTED] [REDACTED] GLP Not published ⇒Previously submitted, data relied upon	Y	Y (but expired)		MCPP-P Task Force	In DAR Addendum 2002
CA 5.1	Timchalk C	2004	Comparative inter-species pharmacokinetics of phenoxyacetic acid herbicides and related organic acids. Evidence that the dog is not a relevant species for evaluation of human health risk Toxicology 200, 1-19 Not GLP Published	N	N		Public	Submitted for the purposes of renewal
CA 5.2.1	[REDACTED]	1994a	<i>Mecoprop-P: Acute Oral LD₅₀ in the rat.</i> [REDACTED] [REDACTED] [REDACTED]	Y	N		MCPP-P Task Force	1998 DAR

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
			<i>GLP</i> <i>Not published</i> <i>⇒Previously submitted, data relied upon</i>					
CA 5.2.2	██████████ ██████████ ██████████	1984	<i>Report on the study of the acute dermal toxicity in rats of CMPP (Mecoprop) – D-Form RZ 84/152</i> ██████████ ██████████ <i>Not GLP</i> <i>Not published</i> <i>⇒Previously submitted, data relied upon</i>	Y	N		MCPP-P Task Force	1998 DAR
CA 5.2.3	██████████ ██████████	1986a	<i>Report on the Study of the Acute Inhalation Toxicity LC₅₀ 4 hours (Rat); dust aerosol of Mecoprop (CMPP) – D Form</i> ██████████ ██████████ ██████████ <i>Not GLP</i> <i>Not published</i> <i>⇒Previously submitted, data relied upon</i>	Y	N		MCPP-P Task Force	1998 DAR
CA 5.2.3	██████████ ██████████ ██████████	1977	<i>Acute inhalation toxicity in rats 4 hour exposure to the dust of mecoprop (MCP) and mecoprop, isomer D.</i> ██████████ ██████████ <i>No report no.</i> <i>GLP: N</i> <i>Published: N</i>	Y	N		MCPP-P Task Force	1998 DAR
CA 5.2.4	██████████	1994c	<i>Mecoprop-P: Acute Dermal Irritation Test in the Rabbit.</i> ██████████ ██████████ <i>GLP</i>	Y	N		MCPP-P Task Force	1998 DAR

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
			<i>Not Published</i> ⇒Previously submitted, data relied upon					
CA5.2.4	██████████	1990a	<i>Mecoprop-P: Acute Dermal irritation/Corrosion Test in the Rabbit & amendment 90/AMS017/0499 & 90/AMS017/1134</i> ██████████ ██████████ GLP <i>Not published</i> ⇒Previously submitted, data relied upon	Y	N		MCCP-P Task Force	1998 DAR
CA 5.2.5	██████████ ██████████	1983h	<i>Report on the study of the irritation to the eye of the white rabbit based on Draize of CMPP (Mecoprop) - D-form</i> ██████████ ██████████ ██████████ Not GLP <i>Not published</i> ⇒Previously submitted, data relied upon	Y	N		MCCP-P Task Force	1998 DAR
CA 5.2.5	██████████	(1990 b)	<i>Mecoprop-P: Acute Eye irritation/Corrosion Test in the Rabbit &Amendment 90/AMS018/0500 & 90/AMS018/1135</i> ██████████ ██████████ <i>Not published</i> ⇒Previously submitted, data relied upon	Y	N		MCCP-P Task Force	1998 DAR
CA 5.2.6	██████████	1985	<i>Report on the</i>	Y	N		MCCP-P	1998 DAR

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
	[REDACTED]		<i>maximization test for the sensitizing potential of CMPP (Mecoprop) - D-form in guinea pigs 85/392 (30H20/83-1)</i> [REDACTED] [REDACTED] [REDACTED] Not GLP Not published ⇒Previously submitted, data relied upon				P Task Force	
CA 5.2.6	[REDACTED]	1994	<i>Mecoprop-P delayed contact hypersensitivity study in the guinea pig 94/0517</i> [REDACTED] [REDACTED] GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCPP-P Task Force	1998 DAR
CA 5.2.6	[REDACTED]	1995	<i>Maximisation Test for the sensitising potential of Mecoprop-p in Guinea Pigs</i> [REDACTED] [REDACTED] [REDACTED] [REDACTED] GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCPP-P Task Force	In DAR Addendum July 2002
CA 5.2.7/01	[REDACTED]	2014	Mecoprop-P TGAI: Cytotoxicity Assay <i>in vitro</i> with BALB/c 3T3 Cells: Neutral Red (NR) Test during Simultaneous Irradiation with Artificial Sunlight 1643404	Y	Y	New data requirements	Nufarm	Submitted for the purposes of renewal

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 on claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			██████████ ██████████ GLP: Y Published: N					
CA 5.3.1	██████████ ██████████	1986	Report on the comparative study of the toxicity of the racemate and D-form of Mecoprop in rats after 7-week administration in the diet ██████████ ██████████ ██████████ Not GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCPP-P Task Force	1998 DAR
CA 5.3.2	██████████	1979	Mecoprop, 3-month oral toxicity study in the rat (racemate, D-isomer) ██████████ Not GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCPP-P Task Force	1998 DAR
CA 5.3.2	██████████ ██████████	1993	Report on the study of the Oral Toxicity in BG6C3F1 Mice Administration in the diet for 3 months ██████████ ██████████ GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCPP-P Task Force	1998 DAR
CA 5.3.2.3	██████████ ██████████	1979	Sub chronic (13-week) oral toxicity study with Mecoprop	Y	N		MCPP-P Task Force	1998 DAR

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
	██████		(MCP) in beagle dogs ██████ ██████ Not GLP; Published					
CA 5.3.3.1	██████ ██████	1997	Mecoprop-p - Chronic Oral Toxicity Study in Beagle Dogs Administration in the Diet for 12 months ██████ ██████ ██████ Not GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCP- P Task Force	In DAR Addendum July 2002
CA 5.3.3	██████ ██████	1993	Twenty-one day dermal toxicity study in the rabbit with MCP-p acid ██████ ██████ ██████ GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCP- P Task Force	1998 DAR
CA 5.4.1	██████ ██████	1993	Chinese hamster ovary/HGPRT locus MCP-P acid ██████ ██████ ██████ GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCP- P Task Force	1998 DAR
CA 5.4.1	██████ ██████	1990	In vitro assessment of the clastogenic activity of mecoprop- P in cultured human lymphocyte ██████	Y	N		MCP- P Task Force	1998 DAR

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
			<p>[REDACTED]</p> <p>GLP Not published ⇒Previously submitted, data relied upon</p>					
CA 5.4.1	[REDACTED]	1984	<p>Report on the study of MCPP (D-form), Ames test</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>Not GLP Not published ⇒Previously submitted, data relied upon</p>	Y	N		MCPP-P Task Force	1998 DAR
CA 5.4.1	[REDACTED]	1994	<p>Chromosome aberration assay in human lymphocytes in vitro with mecoprop-P acid 429401</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>GLP Not published ⇒Previously submitted, data relied upon</p>	Y	N		MCPP-P Task Force	1998 DAR
CA 5.4.1	[REDACTED]	1993	<p>Ames Salmonella typhimurium bacterial reverse mutation assay on MCPP-P acid</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>GLP Not published ⇒Previously submitted, data relied upon</p>	N	N		MCPP-P Task Force	1998 DAR
CA 5.4.1	[REDACTED]	1990	<p>Mecoprop-P: Investigation of mutagenic activity at the HGPRT locus in a</p>	Y	N		MCPP-P Task Force	1998 DAR

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
			<i>Chinese hamster V79 cell mutation system</i> [REDACTED] [REDACTED] GLP Not published ⇒Previously submitted, data relied upon					
CA 5.4.1	[REDACTED]	1990	<i>Mecoprop-P: Assessment of mutagenic potential in histidine auxotrophs of Salmonella typhimurium (the Ames test)</i> [REDACTED] [REDACTED] GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCPP-P Task Force	1998 DAR
CA 5.4.2	[REDACTED]	1991	<i>Mecoprop-P: Assessment of clastogenic action on bone marrow erythrocytes in the micronucleus test</i> [REDACTED] [REDACTED] GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCPP-P Task Force	1998 DAR
CA 5.4.2	[REDACTED]	1985a	<i>Report on the cytogenetic investigations in Chinese hamsters after a single oral administration of MCPP; D-form - bone marrow chromosome analysis</i> [REDACTED]	Y	N		MCPP-P Task Force	1998 DAR

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 on claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			<p>[REDACTED]</p> <p>[REDACTED]</p> <p>Not GLP Not published ⇒Previously submitted, data relied upon</p>					
CA 5.5.1	[REDACTED]	1988	<p>Study on the chronic toxicity and oncogenic potential of MCPP in rats Administration in the diet over 24 months.</p> <p>[REDACTED]</p> <p>GLP: Y Published: N</p>	Y	N		BAS NUF	1998 DAR
CA 5.5.1.1	[REDACTED]	2008	<p>Dietary two year carcinogenicity study in the rat</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>GLP Not published</p>	Y	Y	New data submission	MCPP-P Task Force	Submitted for the purposes of renewal
CA 5.5.1.2	[REDACTED]	2007	<p>Ex-vivo enzyme analysis of liver samples taken at termination of a dietary 2 year carcinogenicity study of Mecoprop-P in the rat</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>GLP Not published</p>	Y	Y	New data submission	MCPP-P Task Force	Submitted for the purposes of renewal
CA 5.5.2.1	[REDACTED]	1996	<p>Mecoprop-P - Carcinogenicity study in B6C3F1/Cr1BR mice. Administration in the diet for 18</p>	Y	N		MCPP-P Task Force	1998 DAR

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
			months [REDACTED] [REDACTED] [REDACTED] [REDACTED] Not GLP Not published ⇒Previously submitted, data relied upon					
CA 5.5.2.2	[REDACTED]	1999	Mecoprop-P - Carcinogenicity study in B6C3F1/CrlBR mice. Administration in the diet for 18 months, (supplementary study [REDACTED] [REDACTED] [REDACTED] [REDACTED] Not GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCCP-P Task Force	In DAR Addendum July 2002
CA 5.6.1.1	[REDACTED]	1992	Reproduction study with MCCP in rats. Continuous dietary administration over 2 generations (2 litters in the first and 1 litter in the second generation) [REDACTED] [REDACTED] [REDACTED] Not GLP Not published ⇒Previously submitted data	Y	N		MCCP-P Task Force	1998 DAR
CA 5.6.1.2	[REDACTED]	2003	Mecoprop-P: oral (dietary administration) preliminary reproduction toxicity study in the rat [REDACTED]	Y	N		Nufarm	Submitted for the purposes of renewal

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
			[REDACTED] GLP Not published					
CA 5.6.2.1	[REDACTED]	1993a	Study of the prenatal toxicity of Mecoprop-P in rats after oral administration (gavage) [REDACTED] [REDACTED] [REDACTED] Not GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCCP-P Task Force	1998 DAR
CA 5.6.2.2	[REDACTED]	1993b	Study of the prenatal toxicity of Mecoprop-P in rabbits after oral administration (gavage) [REDACTED] [REDACTED] [REDACTED] GLP Not published ⇒Previously submitted, data relied upon	Y	N		MCCP-P Task Force	1998 DAR
CA 5.6.2.3	Roll R & Matthiasch k G	1983	Vergleichende Untersuchungen zur Embryotoxizität von 2-Methyl-4-chlorphenoxyessigsäure, Mecoprop und Dichlorprop bei NMRI-Mäusen. Published in: <i>Arzneim Forsch</i> , 33, 1479-1483 Not GLP Published ⇒Previously submitted data	Y	N		Public	1998 DAR
CA 5.7.1	[REDACTED]	1995	Mecoprop-P: acute oral neurotoxicity	Y	Y	Study not previously	MCCP-P Task	Submitted for the purposes

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
			study in Wistar rats [REDACTED] [REDACTED] [REDACTED] GLP Not published			submitted in EU, conducted for US registration applications	Force	of renewal
CA 5.8.1	[REDACTED] [REDACTED] [REDACTED] [REDACTED] (1980)	1980	Report on the study of the acute oral toxicity of Mecoprop-hydroxy-metabolite (M7/173) in the rat [REDACTED] [REDACTED] [REDACTED] Not GLP; Not published.	Y	N		Nufarm	Submitted for the purposes of renewal
CA 5.8.2.1	Moody, D.E., Narloch, B.A., Shull, L.R., Hammock, B.D.	1991	<i>The effect of structurally divergent herbicides on mouse liver xenobiotic-metabolizing enzymes (P-450-dependent mono-oxygenases, epoxide hydrolases and glutathione S-transferases) and carnitine acetyltransferase</i> <i>Toxicology Letters</i> 59: 175-185 (1991). Not GLP Published	Y	N	N	Public	1998 DAR
CA 5.8.2.2	Elo HA, Luoma T, & Ylitalo P	1991	<i>Inhibition of human and rabbit platelet aggregation by chlorophenoxyacid herbicides</i> <i>Archives of Toxicology</i> 65: 140-144 Not GLP Published	Y	N		Public	1998 DAR
CA 5.8.2.3/01	Moeller T & Solecki R	1989	<i>Pathomorphologische und hämatologische Untersuchungen zur Immuntoxizität der Phenoxyalkansäure Mecoprop an Ratten</i> <i>Zeitschrift für die</i>	Y	N		Public	1998 DAR

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
			<i>gesamte Hygiene und ihre Grenzgebiete 35: 258-260</i> Not GLP Published					
CA 5.8.2.3/02	Moeller T	1990	<i>Funktionelle Testung der Immuntoxizität der Phenoxyalkansäure Mecoprop an Ratten und Ausschaltung immuntoxischer Effekte durch Adrenalektomie</i> <i>Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete 36: 314-316</i> Not GLP Published	Y	N		Public	1998 DAR
CA 5.9.1/01	White S	2014	Mecoprop-P Manufacturing Medical Surveillance Report ██████████ Nufarm UK Ltd Not GLP Not published	N	N	New data	Nufarm	Submitted for the purposes of renewal
CA 5.9.1/02	Becher H, Wahrendorf J, & Angerer R	1990	<i>A cohort study on persons exposed to phenoxy acid herbicides and their contaminants in Germany -design and first results</i> <i>Chemosphere 25: 1007-1014</i> Not GLP Published	N	N		Public	1998 DAR
CA 5.9.3.1/01	Meulenbelt J, Zwaveling JH, van Zoonen P, & Notermans NC	1988	<i>Acute MCPP intoxication: report of two cases</i> <i>Human Toxicology 7: 289-292</i> Not GLP Published	Y	N		Public	1998 DAR
CA 5.9.3.1/02	Prescott LF, Park J, & Darrien I	1979	<i>Treatment of severe 2.4-D and mecoprop intoxication with alkaline diuresis</i>	Y	N		Public	1998 DAR

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
			<i>British Journal of Clinical Pharmacology</i> 7: 111-116 Not GLP Published					
CA 5.9.6.2	Flanagan RJ, Meredith TJ, Ruprah M, Onyon LJ, & Liddle A	1990	<i>Alkaline diuresis for acute poisoning with chlorophenoxy herbicides and ioxynil</i> <i>The Lancet</i> 335: 454-458 Not GLP Published	Y	N		Public	1998 DAR