



# **Draft Assessment Report**

## **Evaluation of Active Substances**

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**  
as it applies in Great Britain (GB PPP)

### **Cinmethylin (BAS 684 H)**

### **Volume 3 – B.6 (AS) – Part I**

### **Toxicology & Metabolism Data**

Great Britain

November 2020

**Version History**

<b>When</b>	<b>What</b>
November 2020	Initial DAR

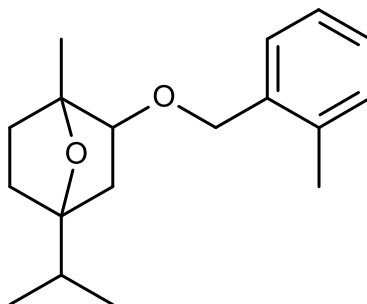
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## **B.6. TOXICOLOGY AND METABOLISM DATA**

Cinmethylin (CAS No. 87818-31-3, also known as BAS 684 H, structure shown below) is a new herbicidal active substance, developed by BASF.



Cinmethylin inhibits a unique and novel target enzyme in fatty acid (FA) biosynthesis for which no HRAC-classification has been assigned yet. Depleting plants of FAs has dramatic physiological impact: cell membranes are irreversibly disrupted, which has a detrimental effect on emerging plant tissue. In pre-emergence treatments, seedlings quickly become non-viable when FA storage is exhausted. In addition, transport and receptor functions, indispensable for photosynthetic activity can no longer be fulfilled. This results in a starvation of the plant, since absorbed sunlight can no longer be transformed into energy to sustain plant viability. Cinmethylin uniquely targets the fatty acid thioesterase (FAT) enzyme family and thus prevents the termination of fatty acid chain elongation.

The representative product is the EC (Emulsifiable Concentrate) formulation BAS 684 03 H, containing 750 g/L cinmethylin.

Cinmethylin was originally developed by Shell in the 1980's and marketed in some Asian countries mostly as rice herbicide. There is some information on its toxicity in publicly available literature (see section B.6.10 of document CA\_B6). The available regulatory studies were conducted for the purpose of this approval and have not been previously evaluated in the EU or by HSE; the new/modern studies are GLP-compliant and follow the respective OECD test guidelines.

This document uses the term 'cinmethylin' when referring to the active substance. However, other synonyms (development codes) may have been used by the applicant within the individual study reports: 'BAS 684 H', 'SD 95481', 'CINCH technical' and 'WL95481'.

The batches of cinmethylin used in the toxicology studies are considered representative of the technical specification (see Vol 4 for more details).

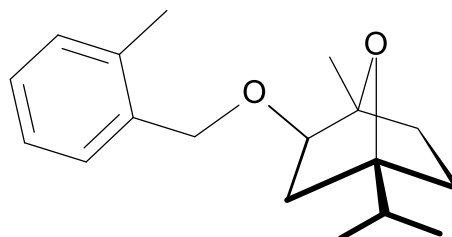
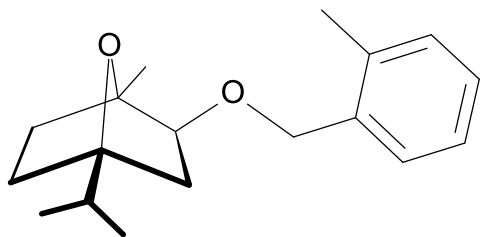
The majority of the methods of analysis for the active substance in different matrices (diet, air, gavage solutions) used in the *in vivo* toxicological studies are either validated or fit for purpose (see Volume 3 CA B5, section B.5.1.2).

The human health classification of cinmethylin has been addressed in an aligned Mandatory Classification and Labelling (MCL) dossier produced by HSE.

The data requirements of regulation (EC) 1107/2009 and Reg 213/2013 have been met and HSE concludes that there are no data gaps.

Cinmethylin is manufactured and placed on the market as a 50:50 racemic enantiomer mixture consisting of (-)-cinmethylin (Reg.No. 5925581) and (+)-cinmethylin (Reg.No. 5925632). All but one of the batches used for the new/modern toxicological studies had a racemic composition of approx. 50:50. The one exception was batch COD-001794 (used in the 28-day study in the rat – [REDACTED], 2015), which had an (-)/(+) enantiomer ratio of 70:30. No information on the isomeric ratio is provided for the old studies, but these are either unreliable, supplementary or used in a weight-of-evidence approach with the new/modern studies. Overall, the isomeric ratio of batches used in the toxicological studies supporting the risk assessment (new/modern studies) is representative of the active being placed on the market.

Structural Formula (Racemic mixture):



RegNo. 5925632	RegNo. 5925581
(1 <i>R</i> ,2 <i>S</i> ,4 <i>S</i> )-1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo[2.2.1]heptane	(1 <i>S</i> ,2 <i>R</i> ,4 <i>R</i> )-1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo[2.2.1]heptane
(+)-BAS 684 H	(-)-BAS 684 H

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

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

The ADME of cinmethylin have been largely investigated in rats via oral dosing only. There are two data sets available, a modern data set (3 studies) using both  $^{14}\text{C}$ -phenyl labelled cinmethylin and  $^{14}\text{C}$ -cyclohexyl labelled cinmethylin in rats only and an older set of studies conducted with  $^{14}\text{C}$ -phenyl labelled cinmethylin only, which includes some limited information on *in vivo* metabolism of cinmethylin in dogs. There is also an *in vitro* comparative metabolism study, employing primary hepatocytes from humans, rats, dogs and rabbits exposed to  $^{14}\text{C}$ -phenyl labelled cinmethylin and  $^{14}\text{C}$ -cyclohexyl labelled cinmethylin. Furthermore, there are a number of toxicokinetic investigations in the rat and mouse chronic toxicity studies.

The three modern studies followed OECD Test Guideline (TG) No. 417 and are GLP compliant. The earlier set of studies are available as brief summaries and the original test reports were not considered by HSE. These summaries are included for completeness and supportive information only. For these studies, we are unable to determine GLP status and which TG, if any, were followed.

HSE considers these modern standard studies to provide a thorough understanding of the ADME of cinmethylin in experimental animals following oral dosing.

As part of the modern dataset, three *in vivo* studies were conducted to investigate the ADME of cinmethylin. The first study comprises a series of experiments conducted to investigate the absorption, distribution, excretion and toxicokinetics of cinmethylin in rats. The second study was performed to investigate further the distribution of cinmethylin and collect tissue samples for analysis of metabolites. The third study was conducted to provide detailed information on the metabolism of cinmethylin. As part of this metabolism study, bile samples from the oral absorption experiment in the first study and tissue samples from the second study were also analysed to provide additional information on metabolites.

To facilitate accurate structural analysis of individual metabolites via HPLC-MS/MS,  $^{13}\text{C}$ -labelled cinmethylin was also added to the  $^{14}\text{C}$ -radiolabelled cinmethylin prior to dosing.

#### Study 1: ADME

Study	$^{14}\text{C}$ -BAS 684 H: Study on kinetics in Wistar rats after oral and intravenous administration
Reference	██████████, 2018
Date performed	April 2018
Test facility	██ ████████████████
Report reference	BASF ID no 2017/1145830
Guideline(s)	OECD TG 417
Deviations from the guideline	None
GLP	Yes
Test material	Table 6.1-1
Study acceptable	Yes

A series of experiments were included in a single study conducted to investigate the absorption, distribution excretion and toxicokinetics of cinmethylin in Wistar rats. In each experiment, test animals were administered radiolabelled cinmethylin ( $^{14}\text{C}$ -phenyl labelled and  $^{14}\text{C}$ -cyclohexyl labelled cinmethylin). Experiment one investigated the oral absorption of cinmethylin in bile-duct cannulated rats dosed with  $^{14}\text{C}$ -phenyl- and

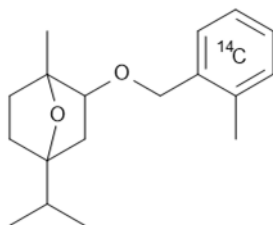
$^{14}\text{C}$ -cyclohexyl labelled cinmethylin. Experiment two investigated tissue distribution of  $^{14}\text{C}$ -phenyl- and  $^{14}\text{C}$ -cyclohexyl radiolabelled cinmethylin following oral dosing in rats. Experiment three investigated the excretion of  $^{14}\text{C}$ -phenyl- and  $^{14}\text{C}$ -cyclohexyl radiolabelled cinmethylin following oral dosing in rats. Experiment four was conducted to investigate the toxicokinetics of  $^{14}\text{C}$ -phenyl- and  $^{14}\text{C}$ -cyclohexyl radiolabelled cinmethylin following oral dosing and i.v dosing in rats.

The purity and radiochemical purity of the  $^{13}\text{C}$ - and  $^{14}\text{C}$ -radiolabelled cinmethylin used in this study are reported below (Table 6.1-1).

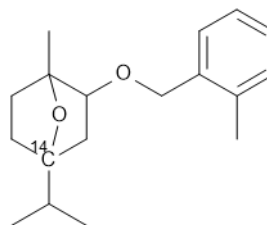
Table 6.1-1. Chemical and radiochemical purity of cinmethylin

Label	Batch no.	Radiochem. purity [%]	Chemical purity [%]	Specific radioactivity [MBq/mg]	Used in
Phenyl- $^{14}\text{C}$	1147-2001	98.9	97.0	17.1	Absorption, Distribution and Toxicokinetics
Cyclohexane-4- $^{14}\text{C}$	1146-2001	97.9	95.9	8.1	Distribution and Toxicokinetics
Cyclohexane-4- $^{14}\text{C}$	1146-1001	99.4	99.3	7.8	Absorption
Benzyl- $^{13}\text{C}$	1159-1012	-	99.6	-	phenyl label
Cyclohexane-4- $^{13}\text{C}$	1165-2001	-	98.1	-	cyclohexane label
Unlabelled cinmethylin	L87-84	-	99.0		All
Unlabelled cinmethylin	COD-001950	-	95.3		All

Position of  $^{14}\text{C}$  radiolabel



phenyl- $^{14}\text{C}$  cinmethylin



cyclohexane-4- $^{14}\text{C}$  cinmethylin

## Experiment 1 - Absorption

### Method

The oral absorption of cinmethylin has been investigated in groups of bile-duct cannulated rats (Wistar strain, 6 sex/dose) administered single gavage doses of  $^{14}\text{C}$ -cyclohexyl- or  $^{14}\text{C}$ -phenyl radiolabelled cinmethylin at 15 or 350 mg/kg bw in aqueous 1 % carboxymethylcellulose/1 % cremophor. Following dosing, animals were housed singly in metabowls and bile collected at 3 hourly intervals and faeces at 24 hour intervals up to 72 hours. After 72 hours, all animals were sacrificed and the following were collected for radioactivity analysis; stomach and stomach/gut contents, and carcass. The cage wash was also assayed for residual radioactivity. The total radioactivity of liquid samples was determined by direct liquid scintillation counting (LSC). Solid samples were homogenized and combusted; the  $^{14}\text{CO}_2$  formed was trapped and measured by LSC. Bile samples were also retained for identification of metabolites. These data are reported in a separate metabolism study (see below).



*Results*

The overall results of the bile-duct cannulation experiment are presented below (Tables 6.1-2 and 6.1-3). The mean total recoveries ranged from 90.99 % to 103.01 % of the administered radioactivity for animals administered phenyl-labelled cinmethylin and from 89.72 % to 101.91 % of the administered radioactivity for animals administered cyclohexane-labelled cinmethylin and were therefore acceptable.

Table 6.1-2. Excretion of radioactivity (% of administered radioactivity)

	Phenyl label				Cyclohexane label			
	350 mg/kg bw		15 mg/kg bw		350 mg/kg bw		15 mg/kg bw	
	male	female	male	female	male	female	male	female
Urine 0-24 h	29.53	26.49	39.83	32.01	44.41	13.93	27.32	46.98
Urine 24-48 h	0.94	3.95	0.71	0.73	1.89	3.90	0.59	0.72
Urine 48-72 h	0.43	2.66	0.49	0.26	0.33	0.52	0.22	0.25
<b>Total urine</b>	<b>30.83</b>	<b>33.10</b>	<b>40.79</b>	<b>32.94</b>	<b>46.63</b>	<b>18.36</b>	<b>28.13</b>	<b>47.95</b>
Faeces 0-24 h	7.41	3.02	3.13	1.61	4.73	1.18	2.17	0.52
Faeces 24-48 h	0.38	3.02	0.20	0.22	0.86	1.53	0.38	0.63
Faeces 48-72 h	0.04	1.34	0.06	0.06	0.06	0.45	0.06	0.11
<b>Total faeces</b>	<b>7.82</b>	<b>7.38</b>	<b>3.37</b>	<b>1.87</b>	<b>5.64</b>	<b>3.15</b>	<b>2.62</b>	<b>1.26</b>
Bile 0-24 h	58.38	32.29	56.28	54.30	42.13	65.15	69.21	39.19
Bile 24-48 h	0.82	7.39	0.76	0.71	2.08	7.37	0.87	0.19
Bile 48-72 h	0.14	2.12	0.06	0.07	0.11	0.64	0.17	0.11
<b>Total bile</b>	<b>59.32</b>	<b>41.81</b>	<b>57.07</b>	<b>55.09</b>	<b>44.32</b>	<b>73.15</b>	<b>70.25</b>	<b>39.48</b>
Cage wash	0.48	3.00	1.07	0.59	0.80	1.66	0.78	0.91
Stomach content	0.00	5.83	0.00	3.46	0.35	0.02	0.00	0.01
Stomach	0.00	0.15	0.09	0.01	0.02	0.00	0.00	0.00
Gut content	0.01	0.65	0.10	1.11	0.04	0.06	0.01	0.02
Gut	0.01	0.04	0.02	0.03	0.01	0.01	0.00	0.00
<b>Carcass</b>	<b>0.31</b>	<b>0.49</b>	<b>0.50</b>	<b>0.27</b>	<b>0.29</b>	<b>0.22</b>	<b>0.12</b>	<b>0.08</b>
<b>Total recovery</b>	<b>98.77</b>	<b>90.99</b>	<b>103.01</b>	<b>95.37</b>	<b>98.10</b>	<b>96.63</b>	<b>101.91</b>	<b>89.72</b>
<b>Absorption</b>	<b>90.46</b>	<b>75.4</b>	<b>98.36</b>	<b>88.30</b>	<b>91.24</b>	<b>91.73</b>	<b>98.5</b>	<b>87.51</b>

Absorption = total urine + total bile + carcass

Oral absorption appears to be rapid and almost complete within 24-hours, independent of sex, with  $T_{max}$  values of 1 and 4-hours for the single low and single high dose animals. Oral absorption has been calculated by summation of the recovered radioactivity in urine, bile, and carcass. The calculated values indicate that oral absorption is high, around 75.4 to 98.5 % of the administered dose, depending on dose level and sex. This is supported by information from the toxicokinetic experiment, which found the dose corrected plasma AUC ratios after oral (15 mg/kg bw) and i.v. dosing (1 mg/kg bw) were 66 % and 73 % for males and females.

For the high dose of 350 mg/kg bw absorption of the cyclohexyl label was nearly identical for both males and females (91.24/91.73 %). In the low dose group of the cyclohexyl label dosed animals, absorption was 87.51 % for females and 98.5 % for males. There were some differences apparent with the phenyl label, with 75.4 % absorbed for females compared to 90.46 % in males in high dose animals. For low-dose animals administered phenyl-labelled cinmethylin, absorption was 88.3 % in females and 98.36 % in males. Absorption appeared slightly lower in females and at the high dose. However, HSE considers that any apparent differences represent experimental variation and do not suggest any sex, positional or dose-related differences in oral absorption.

Table 6.1-3. Biliary excretion 0-72 hours (% of administered radioactivity)

Sampling Time (Hours)	Phenyl label				Cyclohexane label			
	350 mg/kg bw		15 mg/kg bw		350 mg/kg bw		15 mg/kg bw	
	male	female	male	female	male	female	male	female
0-3	16.45	4.59	34.18	30.65	7.15	9.80	39.93	20.46
3-6	18.91	7.97	12.74	15.68	10.86	15.29	16.79	13.31
6-9	13.21	5.86	5.51	5.01	9.80	13.65	7.79	4.82
9-12	5.40	5.31	1.87	2.28	5.98	9.81	2.38	1.83
12-15	2.33	3.72	0.97	1.02	4.09	5.52	1.15	0.77
15-18	1.17	2.04	0.58	0.58	2.49	4.65	0.61	0.39
18-21	0.56	1.52	0.24	0.40	1.46	4.15	0.34	0.17
21-24	0.36	1.28	0.2	0.55	0.79	3.10	0.23	0.09
24-27	0.28	1.06	0.22	0.17	0.75	2.98	0.21	0.05
27-30	0.18	1.04	0.18	0.14	0.47	2.52	0.21	0.05
30-33	0.10	1.03	0.14	0.11	0.32	1.37	0.12	0.01
33-36	0.10	0.71	0.10	0.08	0.19	1.19	0.09	0.02
36-39	0.06	0.73	0.09	0.08	0.15	0.51	0.08	0.02
39-42	0.04	0.85	0.08	0.07	0.10	0.33	0.06	0.02
42-45	0.04	0.93	0.05	0.07	0.06	0.14	0.05	0.01
45-48	0.03	1.04	0.03	0.04	0.04	0.26	0.04	0.01
48-51	0.03	0.25	0.02	0.03	0.03	0.22	0.04	0.02
51-54	0.02	0.47	0.02	0.01	0.03	0.17	0.03	0.03
54-57	0.02	0.47	0.02	0.01	0.01	0.12	0.02	0.01
57-60	0.02	0.44	0.01	0.00	0.01	0.11	0.02	0.04
60-63	0.03	0.51	0.01	0.01	0.01	0.07	0.02	0.04
63-66	0.02	0.32	0.01	0.01	0.01	0.05	0.01	0.01
66-69	0.01	0.13	0.01	0.01	0.01	0.03	0.02	0.01
69-72	0.01	0.12	0.01	0.04	0.02	0.03	0.01	0.02
<b>Total</b>	<b>59.32</b>	<b>41.81</b>	<b>57.07</b>	<b>55.09</b>	<b>44.32</b>	<b>73.15</b>	<b>70.25</b>	<b>39.48</b>

In bile duct cannulated rats, the percent of the administered dose recovered in bile after 0-3 hours was 4.59 – 39.93 % depending on sex and doses, rising to 32.29 – 68.59 % (cumulative) at 24 hours. These data indicate that biliary excretion of the single low and the single high dose of cinmethylin is rapid and essentially complete after 24 hours in rats.

As a significant amount of the administered dose is excreted in bile within the first 6 hours (33.77 to 56.72 % noted at the low dose and 12.56 to 35.36 % at the high dose) and might not be systemically available following oral dosing, a value for post-hepatic systemic availability has also been derived. Radiolabel detected in the bile from 6 hours post dose onwards (6 - 72 hours) is considered to be potentially systemically available and is included in the calculation of post-hepatic systemic bioavailability (Table 6.1-4).

Table 6.1-4. Post-hepatic systemic bioavailability

	Phenyl label				Cyclohexane label			
	350 mg/kg bw male	350 mg/kg bw female	15 mg/kg bw male	15 mg/kg bw female	350 mg/kg bw male	350 mg/kg bw female	15 mg/kg bw male	15 mg/kg bw female
<b>Urine 0-72 hours (%)</b>	<b>30.83</b>	<b>33.10</b>	<b>40.79</b>	<b>32.94</b>	<b>46.63</b>	<b>18.36</b>	<b>28.13</b>	<b>47.95</b>
Bile 0-6 hours	35.36	12.56	46.92	46.33	18.01	25.09	56.72	33.77
Bile 0-72 hours	59.32	41.81	57.07	55.09	44.32	73.15	70.25	39.48
<b>Late bile* (%)</b>	<b>23.96</b>	<b>29.25</b>	<b>10.15</b>	<b>8.76</b>	<b>26.31</b>	<b>48.06</b>	<b>13.53</b>	<b>5.71</b>
<b>Carcass</b>	<b>0.31</b>	<b>0.49</b>	<b>0.50</b>	<b>0.27</b>	<b>0.29</b>	<b>0.22</b>	<b>0.12</b>	<b>0.08</b>
<b>Bioavailability ** (%)</b>	<b>55.10</b>	<b>62.84</b>	<b>51.44</b>	<b>41.97</b>	<b>73.23</b>	<b>66.64</b>	<b>41.78</b>	<b>53.74</b>

\* Late bile = Bile 0-72 hours – Bile 0-6 hours

\*\* Post hepatic systemic bioavailability = late bile + urine (0-72 hours) + carcass

HSE notes that a significant proportion of the total radioactivity excreted into the bile, is detected within 6 hours of dosing, independent of sex, dose or position of radiolabel, with very little radiolabel excreted into the bile from 6 hours onwards.

Considering post-hepatic systemic bioavailability, HSE propose using information derived from low dose animals only, as this is more relevant to the NOAEL values used for the AOEL and AAOEL derivation. Given the range of values for post-hepatic systemic bioavailability (41.78 to 53.74 %) and the uncertainty surrounding these estimates, a value of 50 % can be derived using this approach.

However, there are relevant data available from a toxicokinetic experiment employing i.v. and orally dosed rats (██████████, 2018) which enable derivation of a more scientifically robust value for post-hepatic systemic bioavailability of cinmethylin based on the plasma AUC<sub>oral</sub>:AUC<sub>iv</sub> ratios. From this toxicokinetic experiment, dose corrected post-hepatic systemic bioavailability values of 66 % and 73 % for males and females respectively were obtained and an overall average value of 70 % can be calculated. This value is considered to be more robust and less uncertain than that derived from the bile-duct cannulation experiment. **Overall, HSE proposes a post-hepatic systemic bioavailability value of 70 %.**

## Experiment 2 - Distribution

### Method

In the tissue distribution experiment, groups of rats (Wistar strain 12/sex/dose) were administered single oral doses of <sup>14</sup>C-radiolabelled cyclohexyl- or phenyl- cinmethylin at 15 or 350 mg/kg bw by gavage in 1 % carboxymethylcellulose/1 % cremophor. These animals were sacrificed at four time points post-dosing corresponding to the time to maximal plasma concentration (MPC), 1/2 MPC, 1/4 MPC and 1/8 MPC, where the MPC was determined in the TK study at the low and high dose for males and females (Table 6.1-5).

Table 6.1-5. Sacrifice times for distribution experiment

	Cinmethylin (phenyl label)				Cinmethylin (cyclohexyl label)			
	15 mg/kg bw		350 mg/kg bw		15 mg/kg bw		350 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
MPC	1 hour	1 hour	4 hours	4 hours	1 hour	1 hour	1 hour	1 hour
½ MPC	4 hours	2 hours	14 hours	14 hours	2 hours	2 hours	8 hours	8 hours
¼ MPC	8 hours	6 hours	18 hours	18 hours	21 hours	8 hours	20 hours	21 hours
1/8 MPC	24 hours	18 hours	24 hours	24 hours	42 hours	18 hours	32 hours	34 hours

The total radioactive residue of liquid samples was determined by direct LSC for all dose groups. Solid tissue samples were homogenized and combusted using an automated sample oxidizer. The <sup>14</sup>CO<sub>2</sub> formed was trapped and the radioactivity was measured by LSC. Residual radioactivity was determined in the following tissues/organs at terminal sacrifice:

heart	bone	blood cells and plasma
liver	muscle	pancreas
spleen	kidney	thyroid gland
brain	carcass	adrenal glands
skin	adipose tissue	gut and gut contents
lung	testes	stomach and stomach contents
uterus	ovaries	bone marrow

### Results

For both radiolabels, both dose levels and all sampling time points, the highest tissue concentrations were observed in the GI tract including GI contents of male and female animals. The overall results are reported below (Tables 6.1-6 to 6.1-9). The mean total recoveries ranged from 90.99 to 103.01 % of the administered

radioactivity for animals administered phenyl-labelled cinmethylin and from 89.72 to 101.91 % of the administered radioactivity for animals administered cyclohexane-labelled cinmethylin and are therefore acceptable.

For male rats administered 15 mg/kg bw cinmethylin (phenyl label), the highest tissue concentrations 1 hour post-dose were observed in liver and kidney (32.59 µg Eq/g and 10.95 µg Eq/g, respectively) and the lowest levels were detected in brain and bone (<1.00 µg Eq/g). For female rats sacrificed after 1 hour, the highest tissue concentrations were observed in liver (19.64 µg Eq/g) and the lowest radioactivity levels were recovered in spleen, muscle, brain and bone (<1.00 µg Eq/g).

For male rats administered 350 mg/kg bw cinmethylin (phenyl label), the highest concentrations 4 hours post dose were observed in liver (94.94 µg Eq/g), kidney, carcass, plasma, adrenal glands, lung, pancreas, thyroid and adipose tissue (22.91-41.81 µg Eq/g) and the lowest levels of radioactivity were observed in bone marrow, testes, muscle, brain and bone (3.33-9.80 µg Eq/g). For the females sacrificed after 4 hours, the highest tissue concentrations were determined in thyroid, liver, adrenal glands, adipose tissue and ovaries (81.01-139.04 µg Eq/g) followed by kidney, skin, pancreas, plasma and lung (41.47-54.78 µg Eq/g). The lowest radioactivity levels were recovered in bone (5.47 µg Eq/g).

For female rats administered 15 mg/kg bw cinmethylin (cyclohexane label), the highest tissue concentrations 1 hour after treatment were observed in liver (35.75 µg Eq/g), thyroid, plasma and adrenal glands (5.01-8.19 µg Eq/g). For the respective female animals sacrificed after 1 hour, the highest tissue concentrations were detected in liver (41.01 µg Eq/g) kidney, thyroid, adrenal glands and plasma (8.25-12.46 µg Eq/g). The lowest radioactivity levels were detected in the bones (each <1.00 µg Eq/g).

For female rats administered 350 mg/kg bw cinmethylin (cyclohexane label), the highest tissue concentrations 1 hour after treatment were recovered in liver (135.02 µg Eq/g) followed by plasma, blood cells, adrenal glands, kidney, lung, thyroid and pancreas (41.60-66.93 µg Eq/g). For female animals after 1 hour, the highest tissue concentrations were measured in liver, thyroid, adrenal glands and pancreas (82.09-175.25 µg Eq/g). The lowest radioactivity levels were detected in bone samples from both male and female rats (6.32 µg Eq/g and 5.48 µg Eq/g, respectively).

Tissue radioactivity declined in parallel to plasma levels for all animals of the low dose groups. For adipose tissue from animals of the high dose groups, the radioactivity levels increased reaching the maximum between 8 and 32 hours for male rats and between 1 hour and 24 hours for female rats, but declined afterwards.

Overall, cinmethylin is widely distributed, principally to the liver, kidney, thyroid, adrenals and adipose tissue.

Table 6.1-6. Mean tissue concentration of radioactivity (in µg Eq/g tissue) after oral administration of <sup>14</sup>C-cinmethylin at 15 mg/kg bw (phenyl label)

Time after administration [h]	Males				Females			
	1	4	8	24	1	2	6	18
Blood cells	1.71	0.78	0.66	0.22	1.22	1.20	1.26	0.42
Plasma	6.55	1.89	1.47	0.45	3.12	2.75	2.14	0.43
Lung	3.62	1.10	0.84	0.29	2.44	1.78	1.31	0.33
Heart	2.45	0.80	0.57	0.19	1.36	1.13	0.85	0.18
Spleen	1.92	1.24	0.63	0.51	0.88	1.39	0.83	0.80
Kidney	10.95	3.66	3.06	0.88	4.60	4.34	3.41	1.05
Adrenal glands	3.74	1.39	0.90	0.51	2.32	2.17	1.73	0.37
Testes/ovaries	1.26	0.56	0.38	0.11	1.92	3.24	1.90	1.79
Uterus	---	---	---	---	1.45	2.54	2.21	2.26
Muscle	1.19	0.35	0.24	0.07	0.70	0.55	0.40	0.07
Brain	0.82	0.22	0.16	0.06	0.54	0.35	0.24	0.06
Adipose tissue	1.40	5.80	2.47	1.86	1.13	2.43	2.27	1.43
Bone	0.56	0.13	0.12	0.04	0.19	0.26	0.17	0.03
Bone marrow	1.60	0.61	0.39	0.12	1.00	0.96	0.67	0.17
Thyroid	5.49	1.40	1.14	0.61	2.70	3.00	3.46	0.60
Pancreas	3.53	3.07	1.86	1.98	1.71	3.13	2.35	2.54

Stomach content	321.86	54.71	20.82	0.42	387.90	185.75	36.89	1.49
Stomach	96.96	26.91	7.75	0.79	116.34	83.89	15.22	1.37
Gut content	122.38	213.86	287.63	103.16	60.23	156.91	308.78	86.11
Gut	52.49	53.18	37.85	24.75	23.00	68.98	46.69	23.19
Liver	32.59	19.66	17.93	8.40	19.64	24.11	22.11	9.71
Skin	2.08	0.64	0.51	0.15	1.54	1.29	1.00	0.22
Carcass	1.64	0.84	0.69	0.43	1.11	1.29	0.99	0.35

Table 6.1-7. Mean tissue concentration of radioactivity (in  $\mu\text{g Eq/g}$  tissue) after oral administration of  $^{14}\text{C}$ -cinmethylin at 350 mg/kg bw (phenyl label)

Time after administration [h]	Males				Females			
	4	14	18	24	4	14	24	38
Blood cells	16.68	16.09	20.72	13.59	28.04	18.15	14.01	11.40
Plasma	33.05	26.46	23.38	12.31	44.52	17.57	15.06	4.69
Lung	30.06	14.90	16.68	9.34	41.47	14.91	12.00	5.88
Heart	15.13	11.92	11.01	6.54	27.01	9.36	7.39	4.46
Spleen	14.85	12.07	51.36	11.46	23.67	21.85	15.74	13.13
Kidney	41.81	40.54	52.01	31.71	54.78	26.20	25.32	13.33
Adrenal glands	30.73	24.25	20.57	13.53	111.26	37.60	17.67	9.74
Testes/ovaries	9.20	5.98	6.33	3.28	81.01	78.63	62.84	30.38
Uterus	---	---	---	---	38.43	47.44	71.69	37.85
Muscle	7.16	5.40	6.76	2.68	28.37	10.84	17.52	7.35
Brain	5.49	3.41	3.55	1.70	14.99	3.81	2.59	1.48
Adipose tissue	22.91	26.11	135.68	72.45	98.82	114.97	85.98	70.44
Bone	3.33	2.94	2.48	2.16	5.47	1.77	1.54	0.52
Bone marrow	9.80	8.74	8.98	8.51	20.13	7.46	6.00	2.69
Thyroid	25.98	33.97	21.07	18.51	139.04	22.42	37.59	15.95
Pancreas	27.00	30.19	199.16	66.53	46.95	35.95	51.16	35.40
Stomach content	13677.59	4933.40	3220.71	99.93	8319.09	5841.46	599.15	537.97
Stomach	3249.96	1342.97	1762.18	116.57	3160.72	2801.63	490.63	206.43
Gut content	2038.69	1905.27	1766.22	1774.74	1756.02	1508.97	1665.19	667.14
Gut	466.12	464.55	831.31	648.93	500.96	465.40	668.88	259.46
Liver	94.94	92.40	109.72	89.26	112.83	80.34	89.06	59.80
Skin	15.72	14.72	11.49	8.37	53.43	23.51	12.06	6.29
Carcass	39.97	17.40	30.37	16.59	27.86	23.90	15.67	12.30

Table 6.1-8. Mean tissue concentration of radioactivity (in  $\mu\text{g Eq/g}$  tissue) after oral administration of  $^{14}\text{C}$ -cinmethylin at 15 mg/kg bw (cyclohexyl label)

Time after administration [h]	Males				Females			
	1	2	21	42	1	2	8	18
Blood cells	2.72	1.49	0.62	0.22	3.10	1.70	1.42	0.49
Plasma	6.65	2.89	0.92	0.17	8.25	2.86	1.88	0.38
Lung	3.54	1.98	0.58	0.13	4.89	2.16	1.20	0.33
Heart	3.08	1.59	0.45	0.10	3.38	1.42	0.90	0.23
Spleen	2.13	1.99	0.51	0.21	2.94	1.80	0.82	0.45
Kidney	2.15	1.82	0.65	0.10	12.46	4.71	3.37	0.95
Adrenal glands	5.01	2.79	0.77	0.16	9.57	3.31	1.39	0.39
Testes/ovaries	8.19	4.41	1.71	0.38	5.21	3.60	1.43	1.54
Uterus	---	---	---	---	4.32	5.33	1.24	2.19
Muscle	1.71	1.12	0.31	0.06	1.98	0.98	0.60	0.15
Brain	1.44	0.93	0.27	0.05	1.46	0.70	0.46	0.38
Adipose tissue	2.35	2.38	1.62	0.65	3.17	3.93	2.83	2.23
Bone	0.63	0.39	0.13	0.03	0.79	0.31	0.22	0.06
Bone marrow	2.12	1.30	0.42	0.11	2.62	1.31	0.82	0.23
Thyroid	7.53	4.77	1.40	0.28	11.62	3.53	1.83	0.89
Pancreas	3.62	5.92	1.73	0.77	5.69	3.40	1.34	2.29
Stomach content	340.29	166.64	2.28	0.28	191.81	138.29	28.76	4.72
Stomach	98.51	69.26	1.45	0.37	103.25	73.88	19.74	1.36
Gut content	158.61	184.22	124.85	29.51	237.35	233.96	266.57	68.97
Gut	43.88	116.88	21.59	8.01	53.62	125.22	29.00	18.11
Liver	35.75	23.64	11.38	3.72	41.01	27.50	21.93	6.73
Skin	2.30	1.45	0.35	0.12	2.90	1.40	0.74	0.20
Carcass	2.75	2.27	0.94	0.30	2.64	2.36	1.22	0.82

Table 6.1-9. Mean tissue concentration of radioactivity (in  $\mu\text{g Eq/g}$  tissue) after oral administration of  $^{14}\text{C}$ -cinmethylin at 350 mg/kg bw (cyclohexyl label)

Time after administration [h]	Males				Females			
	1	8	20	32	1	8	21	34
Blood cells	64.87	44.03	23.19	9.50	24.00	38.66	25.63	16.89
Plasma	66.93	86.41	33.27	10.52	50.68	52.12	31.05	18.98
Lung	56.52	48.52	23.83	6.94	46.55	39.53	25.10	14.36
Heart	35.42	41.45	20.88	5.82	40.44	35.22	21.38	11.73
Spleen	22.81	31.05	33.87	6.24	32.32	29.84	20.17	12.30
Kidney	59.20	80.72	59.99	42.11	59.03	84.36	38.95	32.11
Adrenal glands	61.57	57.12	27.37	7.51	102.36	75.64	32.12	17.69
Testes/ovaries	16.85	30.93	18.20	4.47	63.00	63.79	46.15	20.84
Uterus	---	---	---	---	34.77	48.15	38.01	18.98
Muscle	19.02	27.83	16.32	4.05	22.42	23.37	19.40	10.00
Brain	17.63	23.19	14.52	3.64	31.97	23.07	17.11	8.56
Adipose tissue	26.01	52.71	92.44	13.01	43.96	120.75	96.20	45.12
Bone	6.32	10.64	6.53	1.87	5.48	6.16	5.50	2.48
Bone marrow	27.67	29.80	20.68	5.84	33.14	29.89	17.06	13.85
Thyroid	55.47	70.74	37.08	14.72	105.12	57.77	47.66	28.34
Pancreas	41.60	53.30	88.12	10.28	82.09	57.44	33.23	17.05
Stomach content	10055.47	8946.26	1229.81	126.96	12121.82	12458.98	138.58	130.53
Stomach	3123.84	2589.20	385.47	31.18	2854.19	4235.37	106.84	67.92
Gut content	1175.62	5735.37	4229.62	2705.58	720.48	2955.22	2529.51	1974.25
Gut	258.27	653.61	781.35	237.30	310.21	647.64	540.46	276.96
Liver	135.02	156.52	118.28	73.75	175.25	139.81	108.74	102.84
Skin	30.39	43.63	18.15	5.77	30.77	44.36	29.75	12.77
Carcass	28.36	40.87	41.18	11.83	34.37	44.14	44.58	18.09

### Experiment 3 - Excretion

#### Method

In the excretion experiment, groups of rats (Wistar strain 4/sex/dose) were administered single oral doses of  $^{14}\text{C}$ -radiolabelled cyclohexyl- or phenyl- cinmethylin at 15 or 350 mg/kg bw by gavage. Further groups of rats (Wistar strain 4/sex) were administered daily gavage doses of unlabelled cinmethylin (350 mg/kg bw/day) for 14 days, followed by a single gavage dose (350 mg/kg bw) of either phenyl or cyclohexane radiolabelled cinmethylin on day 15. In both cases, the dosing vehicle was 1 % carboxymethylcellulose/1 % cremophor. All animals were housed individually in plastic metabowls, except for four male animals (low dose, two animals per radiolabel), where the exhaled air was also analysed. These animals were housed individually in all glass metabolism cages.

The total radioactive residue of liquid samples was determined by direct liquid scintillation counting (LSC) for all dose groups. Solid samples were homogenized and combusted using an automated sample oxidizer. The  $^{14}\text{CO}_2$  formed was trapped and the radioactivity was measured by LSC.

Urine was collected 6, 12- and 24-hours post-dose, then subsequently at 24 hourly intervals to 168 hours post dose. Faecal samples were collected over 24-hour intervals up to 168 hours post dose. For the single dose group only, two males were placed in metabowls to collect expired air for up to 48 hours.

Residual radioactivity was determined in the following tissues/organs at terminal sacrifice:

heart	bone	blood cells and plasma
liver	muscle	pancreas
spleen	kidney	thyroid gland
brain	carcass	adrenal glands
skin	adipose tissue	gut and gut contents
lung	testes	stomach and stomach contents
uterus	ovaries	bone marrow

### Results

The mean total recoveries of radioactivity were between 90 % and 110 % of the administered dose, and was therefore acceptable. The results are summarised below (Tables 6.1-10 and 6.1-11).

For the low dose group, mean total urinary excretion accounted for 58.10 % (male) and 59.72 % (female) of the administered dose for phenyl-labelled cinmethylin and 52.19 % (male) and 60.81 % (female) of the administered dose for cyclohexane-labelled cinmethylin. Mean faecal excretion was 38.47 % and 32.76 % of the administered dose in male and female rats, respectively, treated with  $^{14}\text{C}$ -phenyl labelled cinmethylin and 40.97 % and 33.26 % of the administered dose for male and female rats, respectively, treated with  $^{14}\text{C}$ -cyclohexane labelled cinmethylin.

For rats treated singly or repeatedly with high dose cinmethylin, mean urinary excretion amounted to 58.38 % (single dose) and 57.13 % (repeated dosing) of the administered dose for males and for 52.38 % (single dose) and 60.58 % (repeated dosing) of the administered dose for females treated with the phenyl-labelled test item. For rats treated with the  $^{14}\text{C}$ -cyclohexane-labelled cinmethylin, radioactivity recovered in urine was 50.95 % (single dosing) and 51.38 % (repeated dosing) of the administered dose for males and was 54.50 % (single dosing) and 56.96 % (repeated dosing) of the dose for females. Faecal excretion accounted for 35.22 % and 41.42 % of the administered dose for males treated once or repeatedly, and 41.13 % and 36.87 % of the administered dose for females treated once and repeatedly, respectively, with the  $^{14}\text{C}$ -phenyl-labelled cinmethylin. Faecal excretion accounted for 37.32 % (single dosing) and 42.79 % (repeated dosing) of the administered dose for males and 37.03 % (single dosing) and 32.09 % (repeated dosing) of the administered dose for females treated with the cyclohexane-labelled test item. From the limited investigations on elimination via exhaled air (two low males per radiolabel position) less than 2 % of the administered dose was recovered in expired air.

Table 6.1-10. Excretion balance (% of administered radioactivity, phenyl label)

Administration frequency Balance / excretion	Single 350 mg/kg bw		Single 15 mg/kg bw		15-days repeated 350 mg/kg bw/day	
	male	female	male	female	male	female
Urine 0-6h	12.30	5.58	18.40	17.73	17.56	19.48
Urine 6-12h	18.45	11.39	12.26	13.95	18.12	17.01
Urine 12-24h	15.25	19.11	13.40	13.74	8.92	12.54
Urine 24-48h	8.07	10.01	8.49	8.17	6.28	9.50
Urine 48-72h	2.19	3.18	2.35	2.92	2.88	2.46
Urine 72-96h	1.08	1.40	1.39	1.33	1.15	2.14
Urine 96-120h	0.62	0.92	0.91	0.61	0.86	1.10
Urine 120-144h	0.27	0.34	0.55	0.59	0.78	0.79
Urine 144-168h	0.16	0.44	0.35	0.68	0.59	0.43
<b>Subtotal urine</b>	<b>58.38</b>	<b>52.38</b>	<b>58.10</b>	<b>59.72</b>	<b>57.13</b>	<b>60.58</b>
Faeces 0-24h	14.26	18.17	21.34	19.87	22.43	16.44
Faeces 24-48h	14.11	18.08	9.93	8.99	12.35	15.25
Faeces 48-72h	4.13	2.92	3.62	2.38	3.87	3.67
Faeces 72-96h	1.60	1.23	1.15	0.83	1.55	0.85
Faeces 96-120h	0.61	0.44	1.31	0.41	0.70	0.36
Faeces 129-144h	0.35	0.21	0.90	0.15	0.34	0.18
Faeces 144-168h	0.15	0.08	0.21	0.12	0.18	0.12



<b>Subtotal faeces</b>	<b>35.22</b>	<b>41.13</b>	<b>38.47</b>	<b>32.76</b>	<b>41.42</b>	<b>36.87</b>
Cage wash	0.73	0.76	0.73	1.19	0.65	1.22
Blood cells	0.05	0.02	0.01	0.01	0.01	0.01
Plasma	0.00	0.00	0.00	0.00	0.00	0.00
Lung	0.00	0.00	0.00	0.00	0.00	0.00
Heart	0.00	0.00	0.00	0.00	0.00	0.00
Spleen	0.00	0.00	0.00	0.00	0.00	0.00
Kidney	0.00	0.00	0.00	0.00	0.00	0.00
Adrenals	0.00	0.00	0.00	0.00	0.00	0.00
Testes/ovaries	0.00	0.00	0.00	0.00	0.00	0.00
Uterus	---	0.00	---	0.00	---	0.00
Muscle	0.00	0.00	0.00	0.00	0.00	0.00
Brain	0.00	0.00	0.00	0.00	0.00	0.00
Adipose tissue	0.00	0.00	0.00	0.00	0.00	0.00
Bone	0.00	0.00	0.00	0.00	0.00	0.00
Bone marrow	0.00	0.00	0.00	0.00	0.00	0.00
Thyroid	0.00	0.00	0.00	0.00	0.00	0.00
Pancreas	0.00	0.00	0.00	0.00	0.00	0.00
Stomach content	0.00	0.00	0.00	0.00	0.00	0.00
Stomach	0.00	0.00	0.00	0.00	0.00	0.00
Gut content	0.13	0.09	0.10	0.08	0.12	0.08
Gut	0.02	0.02	0.01	0.01	0.02	0.02
Liver	0.11	0.06	0.06	0.05	0.07	0.05
Skin	0.04	0.03	0.03	0.02	0.02	0.04
Carcass	0.10	0.10	0.02	0.02	0.06	0.06
Exhaled air	---	---	0.06	---	---	---
<b>Total</b>	<b>94.80</b>	<b>94.59</b>	<b>97.59</b>	<b>93.88</b>	<b>99.52</b>	<b>98.94</b>

Table 6.1-11. Excretion balance (% of administered radioactivity, cyclohexane label)

Administration frequency Balance / excretion	Single 350 mg/kg bw		Single 15 mg/kg bw		15-days repeated 350 mg/kg bw/day	
	male	female <sup>1)</sup>	male	female	male	female
Urine 0-6h	4.28	3.31	8.98	16.28	10.20	10.34
Urine 6-12h	5.39	7.50	11.05	12.19	9.01	14.57
Urine 12-24h	14.10	20.06	11.55	15.98	15.03	15.26
Urine 24-48h	14.89	14.99	11.03	9.53	11.00	10.12
Urine 48-72h	6.54	4.66	4.62	3.43	2.96	3.59
Urine 72-96h	2.69	1.99	2.39	1.34	1.49	1.56
Urine 96-120h	1.50	1.12	1.33	0.85	0.91	0.75
Urine 120-144h	0.93	0.52	0.80	0.78	0.50	0.35
Urine 144-168h	0.63	0.33	0.44	0.42	0.28	0.40
<b>Subtotal urine</b>	<b>50.95</b>	<b>54.50</b>	<b>52.19</b>	<b>60.81</b>	<b>51.38</b>	<b>56.96</b>
Faeces 0-24h	8.49	8.66	19.71	13.82	15.00	11.67
Faeces 24-48h	15.63	22.25	14.00	13.63	17.88	14.12
Faeces 48-72h	7.19	3.54	3.90	4.17	5.76	3.92
Faeces 72-96h	3.08	1.53	1.92	0.95	2.19	1.51
Faeces 96-120h	1.51	0.60	0.87	0.31	1.15	0.53
Faeces 129-144h	0.97	0.31	0.37	0.22	0.50	0.21
Faeces 144-168h	0.44	0.13	0.22	0.16	0.32	0.13
<b>Subtotal faeces</b>	<b>37.32</b>	<b>37.03</b>	<b>40.97</b>	<b>33.26</b>	<b>42.79</b>	<b>32.09</b>
Cage wash	0.72	0.72	0.95	0.84	0.85	0.90
Blood cells	0.01	0.01	0.00	0.01	0.00	0.00
Plasma	0.00	0.00	0.00	0.00	0.00	0.00
Lung	0.00	0.00	0.00	0.00	0.00	0.00
Heart	0.00	0.00	0.00	0.00	0.00	0.00

Spleen	0.00	0.00	0.00	0.00	0.00	0.00
Kidney	0.00	0.00	0.00	0.00	0.00	0.00
Adrenals	0.00	0.00	0.00	0.00	0.00	0.00
Testes/ovaries	0.00	0.00	0.00	0.00	0.00	0.00
Uterus	---	0.00	---	0.00	---	0.00
Muscle	0.00	0.00	0.00	0.00	0.00	0.00
Brain	0.00	0.00	0.00	0.00	0.00	0.00
Adipose tissue	0.00	0.00	0.00	0.00	0.00	0.00
Bone	0.00	0.00	0.00	0.00	0.00	0.00
Bone marrow	0.00	0.00	0.00	0.00	0.00	0.00
Thyroid	0.00	0.00	0.00	0.00	0.00	0.00
Pancreas	0.00	0.00	0.00	0.00	0.00	0.00
Stomach content	0.00	0.00	0.00	0.00	0.00	0.00
Stomach	0.00	0.00	0.00	0.00	0.00	0.00
Gut content	0.70	0.14	0.26	0.14	0.30	0.14
Gut	0.10	0.03	0.03	0.03	0.04	0.03
Liver	0.11	0.01	0.06	0.04	0.04	0.02
Skin	0.05	0.04	0.05	0.01	0.02	0.01
Carcass	3.62	0.38	0.46	0.12	0.08	0.08
Exhaled air	---	---	0.05	---	---	---
<b>Total</b>	<b>93.59</b>	<b>92.87</b>	<b>95.02</b>	<b>95.26</b>	<b>95.51</b>	<b>90.25</b>

Considering the above urinary and faecal excretion profiles; excretion via both the urine and faeces is rapid, and essentially complete within 48-hours. There is no evidence for a preferential route although excretion via the urine was slightly higher, for all treatment groups than faecal excretion. The expired air is not a significant route of excretion for cinmethylin. There is no evidence for dose or sex-dependent differences in urinary or faecal excretion. Comparing urinary excretion in this experiment (50 – 60 %) with bile duct cannulated animals (18 – 48 %) suggests that there is some enterohepatic recirculation but is relatively minor for cinmethylin. Based on the urinary and faecal excretion profiles in the above experiment, there do not appear to be any clear differences between single high dose and repeated high dose animals for both  $^{14}\text{C}$  cyclohexy and  $^{14}\text{C}$ -phenyl labelled cinmethylin, in males or females.

#### Experiment 4 - Toxicokinetics

##### Method

The toxicokinetics of cinmethylin ( $^{14}\text{C}$ -phenyl and  $^{14}\text{C}$ -cyclohexane label) were investigated in rats (Wistar strain 4/sex/dose) employing single oral doses of 15 and 350 mg/kg bw in 1 % carboxymethylcellulose/1 % cremophor. Further groups of rats (Wistar strain - 6/sex/dose) were administered a single i.v dose of cinmethylin (1 mg/kg bw  $^{14}\text{C}$ -phenyl label only) using rat plasma as a dosing vehicle. Femoral blood samples were taken from all groups of animals at the following time points: directly after administration and 0.5 h (for i.v. only) and 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and by exsanguination at the last time point (168 h). The concentrations of the radioactive residues in plasma and blood cells were analysed by liquid scintillation counting (LSC). No information was provided in the test report to inform on how the toxicokinetic parameters were derived from the raw data.

##### Results

The toxicokinetic parameters are reported below (Tables 6.1-12 and 6.1-13). The maximum plasma concentrations occurred almost immediately after i.v. administration ( $^{14}\text{C}$ -phenyl label), and for oral administration, maximum plasma concentrations were achieved 1 hour after low dose administration (both radiolabels) and 4 - 8 hours after high dose administration (for the  $^{14}\text{C}$ -phenyl and  $^{14}\text{C}$ -cyclohexane radiolabels, respectively). For male rats administered 350 mg/kg bw cinmethylin ( $^{14}\text{C}$ -cyclohexane label), an earlier  $C_{\text{Max}}$ -value was also observed at 1-hour post dose. It is possible this indicates some biphasic absorption at the high dose.

Following oral dosing, the maximum plasma concentrations were sublinear (rather than proportional) for both radiolabels and for both sexes (Tables 6.1-12 and 6.1-13). However, the area under the curve (AUC) values were supralinear rather than proportional), for both radiolabels and for both sexes. There is no clear explanation

The dose corrected plasma AUC ratios after oral (15 mg/kg bw) and i.v. dosing (1 mg/kg bw) were 66 %<sup>1</sup> and 73 %<sup>2</sup> for males and females, respectively. Since the AUC values are based on radioactivity equivalents in plasma, and metabolic profiles are not taken into consideration, these ratios give a strong indication that bioavailability of cinmethylin is relatively high.

Gender	Dose [mg/kg bw]	C <sub>max</sub> [µg Eq/g]	T <sub>max</sub> [h]	half-life [h]	AUC <sub>0→∞</sub> [µg Eq* <sup>h</sup> /g]
Male	350, p.o.	118.0	4	22.6	2004
	15, p.o.	6.2	1	21.8	52
	1, i.v.	1.4	directly	51.3	5
Female	350, p.o.	78.2	4	12.9	1423
	15, p.o.	9.4	1	7.7	48
	1, i.v.	0.8	directly	26.2	4

2 (AUC/dose)<sub>16.4 mg/kg bw p.o.</sub> / (AUC/dose)<sub>10 mg/kg bw i.v.</sub> [%]:73

Gender	Dose [mg/kg bw]	C <sub>max</sub> [μg Eq/g]	T <sub>max</sub> [h]	half-life [h]	AUC <sub>0→∞</sub> [μg Eq* <sup>h</sup> /g]
Male	350, p.o.	70.5; 77.9	1; 8	22.1	1767
	15, p.o.	4.5	1	23.3	59
Female	350, p.o.	67.2	8	16.0	1705
	15, p.o.	9.0	1	15.1	54

Study	In-life phase of distribution and metabolism of <sup>14</sup> C-cinmethylin in tissues and plasma after oral single administration in male and female Wistar rats
Author	[REDACTED]
Date performed	October 2017
Test facility	[REDACTED] [REDACTED]
Report reference	[REDACTED], 2017; BASF Study ID: 2017/1158148 (Final report) and [REDACTED], 2018; BASF Study ID: 2018/1072281 (Final report amendment no. 1)
Guideline(s)	OECD TG 417
Deviations from the guideline	None
GLP	Yes
Test material	<sup>14</sup> C-cyclohexyl radiolabel, chemical purity 95.9%, radiochemical purity 97.9% <sup>14</sup> C-phenyl radiolabel, chemical purity 95.4%, radiochemical purity 99.6%
Study acceptable	Yes

*Method*

Wistar rats, 3-4 sex/dose were administered single oral gavage doses of cinmethylin at 15 or 350 mg/kg bw to generate tissue (liver and kidney) and plasma samples for isolation and identification of metabolites. Animals were administered radiolabelled cinmethylin ( $^{14}\text{C}$ -phenyl labelled and  $^{14}\text{C}$ -cyclohexyl labelled cinmethylin). To facilitate structure elucidation of metabolites the  $^{14}\text{C}$ -labelled cinmethylin ( $^{14}\text{C}$ -phenyl labelled and  $^{14}\text{C}$ -cyclohexyl) was supplemented with the respective  $^{13}\text{C}$ -labelled, benzyl- $^{13}\text{C}$ - or cyclohexane-4- $^{13}\text{C}$ -labelled cinmethylin. Animals were sacrificed and tissue and plasma samples were taken for metabolite identification at  $T_{\max}$ , corresponding to 1 hour post dose for low and high dose cyclohexyl radiolabel and 1 and 4 hours post dose for low and high dose phenyl radiolabel respectively. The levels of radioactive residues were also investigated in a limited range of tissues: liver, kidney, plasma, blood (remaining after separation of plasma), testes / ovaries abdominal fat, muscle and thyroid. The metabolism data are reported as part of the following study (■■■■■, 2018).

*Results – Distribution*

The distribution of radiolabel for this experiment is reported below (Tables 6.1-14 and 6.1-15). The highest radioactive residues were found at  $T_{\max}$  in the liver, ranging from 7.69 to 9.60 % of the administered dose for the low dose group and from 1.68 to 2.42 % of the administered dose for the high dose group. For all other organs/tissues of both dose groups and labels, the levels were each below 1 % of the administered dose. No significant sex, dose or label specific differences of the radioactive residue levels in tissues, plasma and blood were observed.

Table 6.1-14. Distribution of radioactivity in plasma and selected tissues at  $t_{\max}$  ( $^{14}\text{C}$ -phenyl label)

Tissue	Dose group (1-h post dose) 15 mg/kg bw				Dose group (4-hours post dose) 350 mg/kg bw			
	males		females		males		females	
	[% Dose]	[mg/kg]	[% Dose]	[mg/kg]	[% Dose]	[mg/kg]	[% Dose]	[mg/kg]
Liver	8.35	33.692	7.69	30.211	2.18	199.445	2.33	206.813
Kidney	0.93	19.033	0.62	12.552	0.44	203.585	0.26	117.943
Plasma	0.49	5.743	0.65	5.662	0.47	120.486	0.27	71.098
Blood	0.15	1.946	0.17	1.718	0.24	55.546	0.16	44.500
Testes / ovaries	0.09	1.392	0.02	4.503	0.10	33.603	0.02	94.512
Abdominal fat	0.10	2.012	0.12	2.973	0.18	75.752	0.56	263.468
Muscle	0.04	1.186	0.04	1.140	0.06	29.807	0.08	42.821
Thyroid	<0.01	3.876	<0.01	6.361	<0.01	136.413	<0.01	188.653

Table 6.1-15. Distribution of radioactivity in plasma and selected tissues at  $t_{\max}$  ( $^{14}\text{C}$ -cyclohexane label)

Matrix	Dose group (1-h post dose) 15 mg/kg bw				Dose group (4-hours post dose) 350 mg/kg bw			
	males		females		males		females	
	[% Dose]	[mg/kg]	[% Dose]	[mg/kg]	[% Dose]	[mg/kg]	[% Dose]	[mg/kg]
Liver	7.14	26.813	9.60	31.357	2.42	228.080	1.68	156.121
Kidney	0.42	8.312	0.57	12.225	0.30	148.892	0.16	74.793
Plasma	0.46	5.066	0.55	5.735	0.37	96.937	0.20	44.035
Blood	0.13	1.754	0.16	1.922	0.15	42.073	0.08	20.165
Testes / ovaries	0.08	1.123	0.02	3.981	0.08	29.237	0.01	58.485
Abdominal fat	0.05	1.427	0.09	1.958	0.09	43.421	0.18	78.152
Muscle	0.04	1.183	0.05	1.357	0.05	33.204	0.04	21.131
Thyroid	<0.01	4.612	<0.01	3.885	<0.01	116.286	<0.01	53.175

Overall, from the above study, no significant sex, dose or label specific differences of the radioactive residue levels in the investigated tissues, plasma and blood were observed.

### Study 3 - Metabolism

Study	Excretion and metabolism of <sup>14</sup> C-cinmethylin fter oral administration in rats
Reference	[REDACTED], 2018
Authors	[REDACTED]
Date performed	March 2018
Test facility	[REDACTED] [REDACTED]
Report reference	BASF ID no 2017/1078601
Guideline(s)	OECD TG 417
Deviations from the guideline	None
GLP	Yes
Test material	<sup>14</sup> C-phenyl radiolabel, chemical purity 95.4-97%, radiochemical purity 98.9-99.6% <sup>14</sup> C-cyclohexyl radiolabel, chemical purity 94.3-95.9%, radiochemical purity 97.9 – 99.4% <sup>13</sup> C-benzyl cinmethylin, chemical purity – 99.6% Cyclohexane-4- <sup>13</sup> C-cinmethylin – chemical purity 98.1%
Study acceptable	Yes

## Method

Cinnmethylin metabolites were identified from liver, kidney and plasma of rats following oral administration at doses of 15 mg/kg and 350 mg/kg bw (██████, 2018a) and in urine, faeces and bile samples from the bile duct cannulation experiment (██████, 2018). The methods for the ADE study (██████, 2018) have been described previously. Metabolites were identified by HPLC-MS and HPLC-MS/MS. The HPLC-MS data were also used for quantification of metabolites. The data from these studies were also used to construct a metabolic pathway for cinnmethylin in rats.

To determine whether there is any preferential metabolism of one enantiomer, a limited enantiomer specific analysis was performed using  $^{14}\text{C}$ -phenyl radiolabelled cinmethylin only from liver and faecal samples. There was insufficient unchanged cinmethylin from other matrices to make such analysis technically feasible.

### Results – Enantiomers

The ratio of the (-)/(+) enantiomers of unchanged cinnemethylin in representative methanol extracts of liver and faeces had shifted from approx. 50:50 (-)/(+) in the starting material towards higher relative amounts of the (-) enantiomer and ranged from approximately 70:30 to 76:24 (-)/(+) in faeces extracts and from approximately 63:37 to 69:31 (-)/(+) in liver extracts. These data indicate some preferential metabolism for the (+) enantiomer.

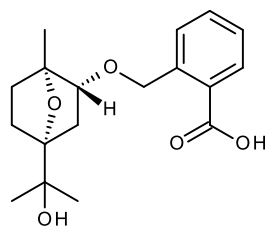
### Results – Metabolites

## Urine

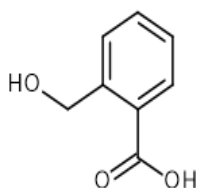
The identified urinary metabolites are shown below (Tables 6.1-16 and 6.1-17). In total, 27 biotransformation products were identified. For some metabolites a chemical name and structure have been assigned, for others only a chemical structure has been assigned.

For both the  $^{14}\text{C}$ -phenyl and  $^{14}\text{C}$ -cyclohexyl radiolabelled cinmethylin, the principal urinary metabolite was M684H011 (abbreviated to 2-hydroxypropyl cinmethylin benzoate) found in single low dose (24.39 to 29.87 % of administered dose) single high dose (15.44 to 18.82 % of administered dose) and repeated high dose animals (12.87 to 19.05 % of administered dose). It is possible the lower levels of this metabolite in high dose animals compared to the low dose animals may indicate saturation of a specific pathway at high doses. Another significant metabolite, M684H010 was identified in the urine of rats administered  $^{14}\text{C}$ -phenyl radiolabelled cinmethylin only (3.47 - 18.86 %). This difference is interpreted as a consequence of the position of radiolabel and does not reflect the existence of a unique metabolic pathway. Apart from the change in M684H011 levels from low to high dose, there were no other clearly sex or dose related, or positional differences in urinary metabolites. Overall, around 50 – 60 % of the administered dose was recovered as urinary metabolites, irrespective of sex, dose (single low and high or repeated high dose) or position of radiolabel. No unchanged parent was detected in the urine.

M684H011 (2-hydroxypropyl cinmethylin benzoate)



M684H010 (2- hydroxymethyl benzoate)

Table 6.1-16. Metabolites identified in rat urine (phenyl label) and their levels (expressed as % of administered dose)

Compound M Code	Chemical name	Percent of administered dose					
		Single low dose (15 mg/kg bw)		Single high dose (350 mg/kg bw)		Repeated high dose (14 + 1 x 350 mg/kg bw)	
		male (0-96 h)	female <sup>1)</sup> (0-96 h)	male (0-96 h)	females (0-96 h)	male (0-96 h)	female (0-96 h)
<b>M684H010</b>	2-(hydroxymethyl)benzoic acid	8.01	3.47	18.86	10.93	14.74	12.08
M684H009	N-(2-methylbenzoyl)glycine Or 2-methyl-hippuric acid	5.63	4.77	8.53	7.93	8.04	10.71
M684H020	Not assigned	0.93	0.43	1.43	0.96	1.61	1.50
M684H021	Not assigned	not detected	not detected	0.01	0.09	0.10	0.11
M684H058 + M684H021	1-O-(2-methylbenzoyl) hexopyranuronic acid	0.52	0.25	1.47	1.20	3.82	2.51
M684H058	1-O-(2-methylbenzoyl) hexopyranuronic acid	not detected	not detected	not detected	not detected	0.19	not detected
M684H022b	Not assigned	not detected	0.05	0.12	0.23	0.31	0.41
M684H022d	Not assigned	0.05	0.03	not detected	not detected	not detected	not detected
M684H031b	Not assigned	not detected	not detected	not detected	not detected	0.03	not detected
M684H022a + M684H031b	Not assigned	not detected	not detected	0.03	not detected	not detected	not detected
M684H022a	Not assigned	not detected	0.20	not detected	0.22	1.37	1.28
M684H022c	Not assigned	not detected	not detected	not detected	0.07	0.10	0.29
M684H027a	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)- 7-oxabicyclo[2.2.1]heptan-2-yl β-D- glucopyranosiduronic acid	1.40	1.27	0.98	0.70	0.83	0.80
M684H027b	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)- 7-oxabicyclo[2.2.1]heptan-2-yl β-D- glucopyranosiduronic acid	0.67	0.14	not detected	0.10	not detected	0.03
M684H027b + M684H031d	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)- 7-oxabicyclo[2.2.1]heptan-2-yl β-D- glucopyranosiduronic acid	0.65	1.22	0.99	0.54	0.82	0.65
M684H023	Not assigned	0.76	0.33	1.28	0.87	1.57	0.87
M684H023 + M684H042	Not assigned	not detected	not detected	not detected	not detected	not detected	0.07
M684H042	Not assigned	not	not	not	0.06	not	0.06

Compound M Code	Chemical name	Percent of administered dose					
		Single low dose (15 mg/kg bw)		Single high dose (350 mg/kg bw)		Repeated high dose (14 + 1 x 350 mg/kg bw)	
		male (0-96 h)	female <sup>1)</sup> (0-96 h)	male (0-96 h)	females (0-96 h)	male (0-96 h)	female (0-96 h)
		detected	detected	detected		detected	
M684H031e	Not assigned	0.02	not detected	0.01	not detected	0.05	0.06
M684H037a	Not assigned	1.23	1.04	1.16	0.72	0.49	0.85
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.26	3.49	1.11	1.37	0.41	1.19
M684H037b	Not assigned	0.62	0.76	0.46	0.42	0.44	0.59
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	6.15	6.23	2.46	2.29	0.93	1.65
<b>M684H011</b>	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	24.39	27.88	15.96	15.44	13.82	19.05
M684H025a	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	not detected	not detected	0.02	not detected	not detected
M684H025b	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	not detected	not detected	0.07	not detected	not detected
M684H025c	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	not detected	not detected	0.01	not detected	not detected
M684H025d	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	not detected	not detected	0.07	not detected	not detected
M684H012a	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenyl]methyl beta-D-glucopyranosiduronic acid	not detected	0.12	not detected	0.28	0.83	1.40
M684H012a + M684H012b	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenyl]methyl beta-D-glucopyranosiduronic acid	not detected	0.16	not detected	not detected	not detected	not detected
M684H012b	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenyl]methyl beta-D-glucopyranosiduronic acid	not detected	0.04	not detected	0.14	not detected	0.13
M684H025e	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	not detected	not detected	0.03	not detected	not detected
M684H025f	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	not detected	not detected	0.03	not detected	not detected

Compound M Code	Chemical name	Percent of administered dose					
		Single low dose (15 mg/kg bw)		Single high dose (350 mg/kg bw)		Repeated high dose (14 + 1 x 350 mg/kg bw)	
		male (0-96 h)	female <sup>1)</sup> (0-96 h)	male (0-96 h)	females (0-96 h)	male (0-96 h)	female (0-96 h)
M684H025g	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	0.97	0.01	0.71	0.06	0.65
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.37	2.38	0.17	0.97	0.46	2.05
M684H043b + M684H018	Not assigned	not detected	not detected	not detected	not detected	not detected	0.06
Total identified		53.66	55.25	55.05	46.46	51.01	59.03
Total characterised		2.63	4.63	2.29	4.21	3.90	4.10
Grand total		56.29	59.88	57.34	50.67	54.91	63.13

Table 6.1-17. Metabolites identified in rat urine (cyclohexane label) and their levels (expressed as % of administered dose)

Compound M Code	Chemical name	Percent of administered dose					
		Single low dose (15 mg/kg bw)		Single high dose (350 mg/kg bw)		Repeated high dose (14 + 1 x 350 mg/kg bw)	
		male (0-120 h)	female <sup>1)</sup> (0-120 h)	male (0-120 h)	females (0-120 h)	male (0-120 h)	female (0-120 h)
M684H029a	Not assigned	2.22	not detected	3.15	2.77	5.23	3.12
M684H036	Not assigned	0.31	0.03	0.62	0.93	1.02	0.45
M684H029b	Not assigned	0.41	not detected	0.74	1.19	0.40	0.54
M684H029c	Not assigned	0.45	not detected	0.97	1.27	1.61	1.16
M684H029e	Not assigned	0.10	0.02	not detected	0.08	not detected	not detected
	(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]heptan-2-ol	not detected	not detected	0.80	not detected	not detected	not detected
M684H026	(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]heptan-2-ol	4.09	2.50	5.91	9.88	5.90	6.62
M684H029f	Not assigned	0.06	not detected	0.06	not detected	0.65	0.68
M684H029d	Not assigned	0.14	not detected	0.75	0.90	1.29	1.18
M684H035b	Not assigned	not detected	not detected	0.01	not detected	1.31	0.24
M684H035b +	Not assigned	not	not	0.17	0.03	not	not



Compound M Code	Chemical name	Percent of administered dose					
		Single low dose (15 mg/kg bw)		Single high dose (350 mg/kg bw)		Repeated high dose (14 + 1 x 350 mg/kg bw)	
		male (0-120 h)	female <sup>1)</sup> (0-120 h)	male (0-120 h)	females (0-120 h)	male (0-120 h)	female (0-120 h)
M684H035a		detected	detected			detected	detected
M684H035a	Not assigned	not detected	0.02	0.01	0.14	0.08	0.23
M684H028c	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)- 7- xabicyclo[2.2.1]heptan-2-yl β-D- glucopyranosiduronic acid	not detected	not detected	0.68	1.01	0.28	0.51
M684H028a	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)- 7- xabicyclo[2.2.1]heptan-2-yl β-D- glucopyranosiduronic acid	0.79	0.56	0.42	0.23	0.14	0.35
M684H028a + M684H021	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)- 7-oxabicyclo[2.2.1]heptan-2-yl β-D- glucopyranosiduronic acid	not detected	not detected	0.15	0.37	0.40	0.44
M684H028b	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)- 7-oxabicyclo[2.2.1]heptan-2-yl β-D- glucopyranosiduronic acid	3.63	3.28	3.12	3.21	3.37	3.81
M684H022b	Not assigned	not detected	not detected	0.05	not detected	0.28	0.62
M684H040b	Not assigned	not detected	not detected	not detected	not detected	0.02	0.27
M684H040b + M684H039	Not assigned	not detected	not detected	not detected	not detected	not detected	0.09
M684H022a	Not assigned	not detected	0.15	0.15	0.21	1.17	1.72
M684H022a + M684H031b	Not assigned	not detected	not detected	0.02	not detected	not detected	not detected
M684H022c	Not assigned	not detected	0.02	not detected	0.11	0.04	0.39
M684H027a	Not assigned	1.39	1.27	1.09	0.73	0.71	0.98
M684H027b	Not assigned	not detected	0.39	0.06	not detected	not detected	not detected
M684H027b + M684H031d	Not assigned	1.20	0.89	0.72	0.57	0.53	0.72
M684H042	Not assigned	not detected	not detected	not detected	0.11	0.03	0.14
M684H031e	Not assigned	not detected	not detected	0.01	0.04	0.03	0.06
M684H037a	Not assigned	1.07	1.26	0.97	0.76	0.59	0.67
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan- 2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2- yl]oxy}methyl)benzoic acid	1.80	3.23	1.54	1.08	0.58	0.88
M684H037b	Not assigned	0.52	0.82	0.50	0.45	0.43	0.39
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan- 2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2- yl]oxy}methyl)benzoic acid	4.59	5.25	2.71	1.49	1.48	1.19
<b>M684H011</b>	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan- 2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2- yl]oxy}methyl)benzoic acid	22.62	29.87	18.82	15.44	12.87	15.98
M684H025a	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4- (propan-2-yl)-7-oxabicyclo[2.2.1]heptan-	not detected	not detected	0.01	0.09	not detected	not detected

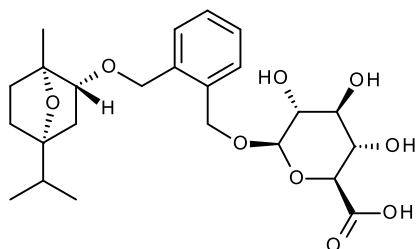
Compound M Code	Chemical name	Percent of administered dose					
		Single low dose (15 mg/kg bw)		Single high dose (350 mg/kg bw)		Repeated high dose (14 + 1 x 350 mg/kg bw)	
		male (0-120 h)	female (0-120 h)	male (0-120 h)	females (0-120 h)	male (0-120 h)	female (0-120 h)
	2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid						
M684H025b	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	not detected	not detected	0.01	not detected	0.04
M684H025c	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	not detected	not detected	0.20	not detected	not detected
M684H012a	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)phenyl]methyl beta-D-glucopyranosiduronic acid	0.04	0.69	0.10	0.19	0.77	2.40
M684H012a + M684H025d		not detected	not detected	not detected	0.07	not detected	not detected
M684H012b	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)phenyl]methyl beta-D-glucopyranosiduronic acid	not detected	not detected	not detected	0.12	not detected	0.17
M684H025e	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	0.64	0.03	0.30	0.01	0.34
M684H025f	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	0.07	not detected	0.03	not detected	0.04
M684H025g	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	0.91	0.06	1.13	0.07	1.22
M684H034b + M684H025g	Not assigned	not detected	not detected	not detected	not detected	not detected	0.22
M684H034c	Not assigned	not detected	not detected	not detected	0.02	not detected	0.15
M684H034d	Not assigned	not detected	not detected	not detected	0.01	not detected	0.03
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy} methyl)benzoic acid	0.25	2.75	0.43	0.76	0.33	1.20
M684H043b + M684H018 2)	Not assigned	not detected	not detected	not detected	not detected	not detected	0.04
BAS 684 H	Cinmethylin	not detected	not detected	not detected	0.05	not detected	0.02
Total identified		45.66	54.62	44.84	45.97	41.60	49.33
Total characterised		5.27	4.98	4.55	5.62	9.00	6.87
Grand total		50.93	59.60	49.39	51.59	50.60	56.19

Bile

The metabolites identified in bile of single low and high dose animals are shown below (Tables 6.1-18 and 6.1-19). In total, 31 biotransformation products were identified in bile samples, 16 of which were assigned a structure and chemical name. The remainder were assigned a chemical structure only.

The most abundant component in bile from rats of all dose groups and for both labels was M684H012 (M684H012a + M684H012b) abbreviated to cinmethylin benzyl alcohol glucuronide; see below. M684H012a and b are diastereoisomers of cinmethylin benzyl alcohol glucuronide

M684H012 (cinmethylin benzyl alcohol glucuronide)



M684H012 was present in bile at 14.96 to 19.96 % of the administered dose for <sup>14</sup>C-phenyl labelled cinmethylin and at 13.17 to 20.87 % of the administered dose for <sup>14</sup>C-cyclohexyl labelled cinmethylin, depending on dose and sex. None of the remaining metabolites with a chemical name were present above 2 % in the bile. It is noted that the prominent urinary metabolite, M684H011 was present in bile below 2 %. Overall, there were no clear differences in biliary excretion pattern between dose levels, sex and position of radiolabel. Unchanged cinmethylin was present at <1 % in the bile.

Table 6.1-18. Metabolites identified in rat bile (phenyl label) and their levels (expressed as % of administered dose)

Compound M Code	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw)		High dose (350 mg/kg bw)	
		male (0-18 h)	female (0-24 h)	male (0-18 h)	female (0-24 h)
M684H010	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	not detected	not detected	0.09	0.06
M684H038	Not assigned	0.35	not detected	1.19	0.23
M684H020	Not assigned	not detected	not detected	0.10	not detected
M684H022b	Not assigned	1.05	1.16	1.67	0.74
M684H040b	Not assigned	0.40	0.07	1.02	0.33
M684H040b + M684H039	Not assigned	not detected	not detected	0.02	not detected
M684H039	Not assigned	not detected	not detected	0.63	not detected
M684H031a	Not assigned	1.03	0.36	0.20	0.22
M684H031b	Not assigned	0.94	0.49	0.02	not detected
M684H022a	Not assigned	4.10	4.60	5.96	4.49
M684H022c	Not assigned	1.09	0.80	1.55	0.62
M684H022c + M684H031c	Not assigned	0.12	not detected	not detected	not detected
M684H022c + M684H032a	Not assigned	not detected	0.11	not detected	not detected
M684H031c	Not assigned	0.07	not detected	not detected	not detected
M684H032a	Not assigned	not detected	0.68	not detected	not detected

Compound M Code	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw)		High dose (350 mg/kg bw)	
M684H027a	Not assigned	not detected	0.07	1.25	0.69
M684H032c + M684H027a	Not assigned	1.76	1.02	not detected	0.09
M684H031d + M684H032a	Not assigned	0.16	not detected	not detected	not detected
M684H027b + M684H031d	Not assigned	0.20	0.35	0.73	not detected
M684H031d	Not assigned	0.90	0.37	0.38	0.30
M684H031d + M684H032c	Not assigned	not detected	0.11	not detected	not detected
M684H031d + M684H033	Not assigned	not detected	not detected	not detected	0.17
M684H032b	Not assigned	0.66	1.01	1.01	0.49
M684H032c	Not assigned	not detected	0.04	not detected	not detected
M684H023	Not assigned	0.26	0.25	0.06	0.01
M684H042	Not assigned	0.14	not detected	not detected	not detected
M684H031e	Not assigned	0.46	not detected	not detected	not detected
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy} methyl)benzoic acid	not detected	0.02	not detected	not detected
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy} methyl)benzoic acid	0.21	0.09	0.01	0.02
M684H011	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy} methyl)benzoic acid	1.63	0.38	1.09	0.43
M684H025a	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.50	0.50	0.15	0.09
M684H025b	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.78	0.94	1.74	0.90
M684H025c	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.77	0.82	0.75	0.89
M684H025d	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.07	0.26	0.02	0.01
<b>M684H012a</b>	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)phenyl)methyl beta-D-glucopyranosiduronic acid	9.70	12.23	12.07	8.79
<b>M684H012b</b>	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)phenyl)methyl beta-D-glucopyranosiduronic acid	6.45	6.57	7.89	6.17
M684H025e	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.91	0.75	1.11	0.87
M684H025f	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	1.36	0.85	1.18	0.92
M684H025f + M684H030a	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	not detected	0.69	not detected	not detected
M684H025g	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.76	1.24	0.24	0.44
M684H034b + M684H025g	Not assigned	0.65	not detected	not detected	0.32
M684H034b + M684H030b	Not assigned	2.66	not detected	3.62	not detected

Compound M Code	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw)		High dose (350 mg/kg bw)	
M684H034b	Not assigned	0.28	1.63	0.10	0.56
M684H030a	Not assigned	0.37	0.42	not detected	0.35
M684H034c + M684H030a	Not assigned	not detected	0.30	not detected	not detected
M684H034c	Not assigned	1.64	1.98	1.30	1.24
M684H030b	Not assigned	0.71	3.66	0.41	1.88
M684H034d	Not assigned	2.05	2.67	2.25	2.33
M684H043c	Not assigned	not detected	not detected	0.16	not detected
M684H002	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)phenyl)methanol	0.20	0.26	not detected	not detected
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.78	2.91	1.90	1.99
M684H043b + M684H018	Not assigned	0.33	0.39	not detected	not detected
BAS 684 H	Unchanged Cinnethylin	0.70	0.72	0.82	0.60
<b>Total identified</b>		<b>49.21</b>	<b>51.76</b>	<b>52.68</b>	<b>37.25</b>
<b>Total characterised</b>		<b>6.63</b>	<b>4.42</b>	<b>4.79</b>	<b>3.32</b>
<b>Grand total</b>		<b>55.84</b>	<b>56.18</b>	<b>57.47</b>	<b>40.57<sup>1)</sup></b>

Table 6.1-19. Metabolites identified in rat bile (cyclohexane label) and their levels (expressed as % of administered dose)

Compound M Code	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw)		High dose (350 mg/kg bw)	
		male (0-18 h)	female (0-15 h)	male (0-33 h)	female (0-36 h)
M684H029a	Not assigned	0.41	not detected	1.86	0.91
M684H036	Not assigned	0.34	0.03	0.34	0.18
M684H029b	Not assigned	not detected	not detected	0.03	0.02
M684H029c	Not assigned	0.35	not detected	0.33	0.14
M684H029e	Not assigned	0.53	0.30	not detected	0.10
M684H026 + M684H029e	(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]heptan-2-ol	0.31	not detected	1.53	0.85
M684H029f	Not assigned	0.46	0.02	0.53	0.36
M684H029d	Not assigned	0.59	0.18	1.11	0.52
M684H035b	Not assigned	not detected	not detected	0.35	0.39
M684H028c	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl β-D-glucopyranosiduronic acid	0.53	not detected	1.32	0.93
M684H028a	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl β-D-glucopyranosiduronic acid	2.25	0.69	3.31	2.13
M684H028b	(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl β-D-glucopyranosiduronic acid	2.82	1.33	3.81	3.04
M684H022b	Not assigned	1.35	1.02	0.95	0.95
M684H040b	Not assigned	0.65	not	not	not

Compound M Code	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw)		High dose (350 mg/kg bw)	
		male (0- 18 h)	female (0-15 h)	male (0- 33 h)	female (0-36 h)
			detected	detected	detected
M684H040b + M684H039	Not assigned	not detected	not detected	1.52	1.41
M684H022d	Not assigned	not detected	0.11	0.16	0.03
M684H031a	Not assigned	1.09	0.70	0.62	0.49
M684H031b	Not assigned	0.40	not detected	0.09	0.18
M684H022a	Not assigned	4.78	5.39	4.48	5.29
M684H022c	Not assigned	0.01	0.13	0.51	0.99
M684H022c + M684H031c	Not assigned	0.90	not detected	0.49	not detected
M684H022c + M684H032a + M684H031c	Not assigned	0.97	not detected	not detected	not detected
M684H031c	Not assigned	0.01	not detected	not detected	0.01
M684H022c + M684H032a	Not assigned	not detected	0.86	not detected	not detected
M684H032a	Not assigned	0.02	not detected	0.15	not detected
M684H032a + M684H027a	Not assigned	0.17	not detected	not detected	not detected
M684H032c + M684H027a	Not assigned	1.29	not detected	1.10	0.41
M684H027a	Not assigned	0.05	0.47	0.04	0.32
M684H027b + M684H031d	Not assigned	1.24	0.10	0.76	0.59
M684H031d + M684H032c + M684H027b	Not assigned	not detected	0.55	not detected	not detected
M684H031d	Not assigned	0.03	0.01	not detected	not detected
M684H032b	Not assigned	0.92	0.99	0.83	0.33
M684H031d + M684H032c	Not assigned	not detected	0.04	not detected	not detected
M684H032c	Not assigned	0.11	0.07	not detected	0.04
M684H042 + M684H032b	Not assigned	not detected	not detected	not detected	0.07
M684H042	Not assigned	not detected	not detected	0.86	1.21
M684H031e + M684H032b	Not assigned	not detected	0.09	not detected	not detected
M684H031e	Not assigned	0.30	<0.01	0.16	0.11
M684H037a	Not assigned	0.08	not detected	0.09	0.10
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.18	0.03	<0.01	0.08
M684H013a + M684H032b	Not assigned	0.02	not detected	not detected	not detected

Compound M Code	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw)		High dose (350 mg/kg bw)	
		male (0- 18 h)	female (0-15 h)	male (0- 33 h)	female (0-36 h)
M684H037b	Not assigned	not detected	not detected	0.02	0.01
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy} methyl)benzoic acid	0.30	0.10	0.07	0.02
M684H011	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy} methyl)benzoic acid	1.53	0.74	0.81	1.08
M684H025a	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.46	0.40	0.33	0.47
M684H025b	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	1.40	1.31	1.57	1.51
M684H025c	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.93	0.88	0.69	1.03
M684H025d	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.19	0.58	0.40	0.44
<b>M684H012a</b>	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)phenyl]methyl beta-D-glucopyranosiduronic acid	11.61	11.53	7.02	8.53
<b>M684H012b</b>	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)phenyl]methyl beta-D-glucopyranosiduronic acid	9.26	5.84	6.15	9.09
M684H025e	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.25	not detected	0.46	0.50
M684H025f	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	1.86	1.65	1.04	1.23
M684H025g	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.49	0.81	0.34	0.52
M684H025g + M684H030a	1-O-[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy} methyl)benzoyl]-beta-D-glucopyranuronic acid	0.62	not detected	0.16	not detected
M684H034b + M684H025g	Not assigned	not detected	not detected	0.64	not detected
M684H034b	Not assigned	1.61	2.02	0.81	2.94
M684H030a	Not assigned	0.51	1.53	0.20	0.16
M684H034c +	Not assigned	not	not	0.26	6.33

Compound M Code	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw)		High dose (350 mg/kg bw)	
		male (0- 18 h)	female (0-15 h)	male (0- 33 h)	female (0-36 h)
M684H030b		detected	detected		
M684H034c	Not assigned	1.60	1.80	2.97	2.20
M684H030b	Not assigned	2.65	2.38	1.70	0.25
M684H034d + M684H030b	Not assigned	not detected	not detected	not detected	0.04
M684H034d	Not assigned	2.84	3.00	1.85	2.79
M684H041	Not assigned	0.58	not detected	0.18	1.26
M684H043c	Not assigned	not detected	not detected	0.04	0.05
M684H002	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)phenyl]methanol	0.68	0.23	0.27	0.63
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	4.07	3.28	1.61	2.92
M684H043b + M684H018	4-methyl-3-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenol	0.85	0.44	0.42	1.01
BAS 684 H	Cinmethylin	0.73	0.73	0.42	0.57
<b>Total identified</b>		<b>68.20</b>	<b>52.35</b>	<b>57.76</b>	<b>67.74</b>
<b>Total characterised</b>		<b>8.53</b>	<b>4.96</b>	<b>7.76</b>	<b>6.89</b>
<b>Grand total</b>		<b>76.73</b>	<b>57.31</b>	<b>65.52</b>	<b>74.63</b>

#### Faeces

The metabolites identified in faeces extracts are shown below (Tables 6.1-20 <sup>14</sup>C-phenyl label and 6.1-21 <sup>14</sup>C-cyclohexane label). In total, 13 biotransformation products were identified in extracts of faeces, of which 6 <sup>14</sup>C-phenyl labelled metabolites were assigned both chemical structures and chemical names and 8 <sup>14</sup>C-cyclohexyl radiolabelled metabolites were assigned both chemical structures and chemical names. No individual metabolites were present in the faeces above 6 % of the administered dose, independent of dosing regimen, position of radiolabel or sex. M684H011 (2-hydroxypropyl cinmethylin benzoate) is the most prominent metabolite; present at 1.74 to 5.75 % of the administered dose. Unchanged cinmethylin was found in the faeces at between 1.97 % and 4.5 % of the administered dose. Given that <1 % of unchanged cinmethylin was detected in the bile, there is a small amount of unabsorbed cinmethylin excreted in the faeces.

Table 6.1-20. Metabolites identified in rat faeces (phenyl label) and their levels (expressed as % of administered dose)

Compound	Chemical Name	Percent of administered dose					
		Single low dose (15 mg/kg bw)		Single high dose (350 mg/kg bw)		Repeated high dose (14 + 1 x 350 mg/kg bw)	
		male (0-96 h)	female (0-96 h)	male (0-96 h)	female (0-96 h)	male (0-96 h)	female (0-96 h)



Compound	Chemical Name	Percent of administered dose					
		Single low dose (15 mg/kg bw)		Single high dose (350 mg/kg bw)		Repeated high dose (14 + 1 x 350 mg/kg bw)	
M684H040a	Not assigned	not detected	not detected	not detected	0.15	not detected	0.19
M684H040b	Not assigned	not detected	not detected	0.40	0.24	not detected	0.34
M684H027a	Not assigned	1.07	0.54	1.40	0.55	0.95	0.66
M684H027b	Not assigned	1.11	0.65	0.83	0.32	0.66	0.52
M684H037a	Not assigned	1.19	0.72	1.35	0.95	1.67	1.04
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.53	1.47	1.50	0.77	1.33	0.89
M684H037b	Not assigned	1.27	0.97	0.90	0.85	1.12	0.76
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.92	1.07	1.36	0.92	1.29	0.72
<b>M684H011</b>	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	4.67	1.78	4.09	2.81	5.36	2.96
M684H002	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)phenyl]methanol	3.14	3.52	2.30	5.20	4.35	4.13
M684H043c	Not assigned	not detected	not detected	0.12	not detected	0.86	0.31
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.18	0.40	1.92	not detected	2.76	not detected
M684H001 + M684H043d	Not assigned	not detected	3.02	not detected	3.21	not detected	2.72
M684H043b + M684H018	4-methyl-3-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenol	2.23	3.46	2.51	4.93	1.98	4.33
BAS 684 H	Cinmethylin	2.85	4.50	2.77	5.01	3.32	3.68
<b>Total identified</b>		<b>25.16</b>	<b>22.09</b>	<b>21.44</b>	<b>25.90</b>	<b>25.66</b>	<b>23.26</b>
<b>Total characterised</b>		<b>6.56</b>	<b>6.97</b>	<b>7.03</b>	<b>9.67</b>	<b>11.12</b>	<b>8.62</b>
<b>Total identified and characterised</b>		<b>31.72</b>	<b>29.06</b>	<b>28.46</b>	<b>35.58</b>	<b>36.78</b>	<b>31.88</b>

Table 6.1-21. Metabolites identified in rat faeces (cyclohexane label) and their levels (expressed as % of administered dose)

Compound	Chemical Name	Percent of administered dose					
		Single low dose (15 mg/kg bw)		Single high dose (350 mg/kg bw)		Repeated high dose (14 + 1 x 350 mg/kg bw)	
		male (0-96 h)	female (0-96 h)	male (0-96 h)	female (0-96 h)	male (0-96 h)	female (0-96 h)
M684H026	(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]heptan-2-ol	not detected	not detected	0.46	0.50	0.37	0.46
M684H027a	Not assigned	1.36	0.57	1.06	0.80	0.86	0.49
M684H027b	Not assigned	1.05	0.64	0.77	0.41	0.60	0.43
M684H037a	Not assigned	1.28	0.71	1.18	0.82	1.34	0.89
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.82	1.45	1.55	0.79	1.60	0.84

M684H037b	Not assigned	1.38	0.74	0.93	0.64	1.38	0.64
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	3.14	1.10	1.66	0.58	1.37	0.84
<b>M684H011</b>	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	5.13	1.74	3.74	2.39	5.75	2.99
M684H002	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)phenyl]methanol	2.01	2.52	3.10	3.56	4.26	2.83
M684H043c	Not assigned	0.08	0.40	0.09	0.48	1.00	0.57
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	3.15	0.15	2.77	not detected	3.09	not detected
M684H001 + M684H043d	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	not detected	2.56	not detected	2.72	not detected	2.25
M684H043b + M684H018	4-methyl-3-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]heptan-2-yl]oxy}methyl)phenol	2.15	3.27	2.89	5.26	2.41	4.39
BAS 684 H	Cinmethylin	2.50	3.63	1.97	3.28	2.61	3.00
<b>Total identified</b>		<b>26.06</b>	<b>19.47</b>	<b>22.17</b>	<b>22.28</b>	<b>26.64</b>	<b>20.35</b>
<b>Total characterised</b>		<b>7.54</b>	<b>8.00</b>	<b>7.80</b>	<b>6.31</b>	<b>10.12</b>	<b>7.29</b>
<b>Total identified and characterised</b>		<b>33.59</b>	<b>27.46</b>	<b>29.98</b>	<b>28.59</b>	<b>36.76</b>	<b>27.63</b>

#### Plasma

Metabolites identified in plasma are shown below (Table 6.1-22 <sup>14</sup>C-phenyl label and 6.1-23 <sup>14</sup>C-cyclohexane label). Plasma samples for metabolite analysis were taken at T<sub>max</sub>, from single low and single high dose animals. No samples were analysed from the repeated dosing group. Compared to bile and urine, only a small number of plasma metabolites were identified (4 - 6) and none were present above 1 % of the administered dose. Unchanged cinmethylin was present in the plasma at <0.1 % of the administered dose.

Table 6.1-22. Metabolites identified in rat plasma analysed at T<sub>max</sub> of plasma level (phenyl label) and their levels (expressed as % of administered dose)

Compound	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw) (1 h after dosing)		High dose (350 mg/kg bw) (4 h after dosing)	
		Male	Female	Male	Female
M684H010	2-(hydroxymethyl)benzoic acid	0.05	not detected	0.04	0.02
M684H042	Not assigned	0.02	0.02	0.01	0.02
M684H011	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.12	0.03	0.12	0.01
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.22	0.33	0.29	0.18
BAS 684 H	cinmethylin	0.01	0.03	0.01	0.01
Total identified		0.41	0.41	0.47	0.24
Total characterised		0.08	0.24	--	0.03
Total identified and characterised		0.49	0.65	0.47	0.27

Table 6.1-23. Metabolites identified in rat plasma analysed at T<sub>max</sub> of plasma level (cyclohexane label) and their levels (expressed as % of administered dose)

Compound	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw) (1 h after dosing)		High dose (350 mg/kg bw) (1 h after dosing)	
		Male	Female	Male	Female
M684H026	(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]heptan-2-ol	0.04	0.02	0.02	0.01
M684H042	Not assigned	not detected	0.02	0.01	not detected
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	not detected	not detected	0.01	not detected
M684H011	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.12	0.08	0.04	not detected
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.26	0.34	0.22	0.15
BAS 684 H	cinmethylin	0.01	0.02	0.01	0.01
Total identified		0.44	0.47	0.30	0.16
Total characterised		0.03	0.07	0.07	0.04
Total identified and characterised		0.46	0.55	0.37	0.20

### Liver

The identified metabolites in the liver are shown below (Table 6.1-24 <sup>14</sup>C-phenyl label and 6.1-25 <sup>14</sup>C-cyclohexane label). Samples for metabolite analysis were taken at T<sub>max</sub>, from single low and single high dose animals. No samples were analysed from the repeated dosing group. Compared to bile and urine, only a small number of metabolites were identified in the liver (4 - 6) and none were present above 4.3 % of the administered dose. Unchanged cinmethylin was present in the liver at 0.21 - 0.9 % of the administered dose. No significant sex, dose or label specific differences were noted.

Table 6.1-24. Metabolites identified in rat liver analysed at T<sub>max</sub> of plasma level (phenyl label) and their levels (expressed as % of administered dose)

Compound	Chemical name	Percent of administered dose			
		Low dose (15 mg/kg bw) (1 h after dosing)		High dose (350 mg/kg bw) (4 h after dosing)	
		Male	Female	Male	Female
M684H027a	Not assigned	0.10	0.05	0.03	not detected
M684H027b	Not assigned	0.07	0.08	0.02	not detected
M684H042	Not assigned	0.12	0.11	0.07	0.17
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.21	0.21	0.05	not detected
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	1.35	0.52	0.16	0.11
M684H011	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.80	1.55	0.46	0.16
M684H002	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)phenyl]methanol	0.09	0.11	0.08	0.18
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.42	4.29	0.58	0.56
BAS 684 H	cinmethylin	0.29	0.21	0.27	0.90
<b>Total identified</b>		<b>7.45</b>	<b>7.13</b>	<b>1.71</b>	<b>2.07</b>
<b>Total characterised</b>		<b>0.96</b>	<b>0.45</b>	<b>0.33</b>	<b>0.27</b>
<b>Total identified and characterised</b>		<b>8.40</b>	<b>7.59</b>	<b>2.03</b>	<b>2.34</b>

Table 6.1-25. Metabolites identified in rat liver analysed at T<sub>max</sub> of plasma level (cyclohexane label) and their levels (expressed as % of administered dose)

Compound	Chemical name	Percent of administered dose			
		Low dose (15 mg/kg bw) (1 h after dosing)		High dose (350 mg/kg bw) (4 h after dosing)	
		Male	Female	Male	Female
M684H026	(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]heptan-2-ol	0.09	0.09	0.04	not detected
M684H027a	Not assigned	0.07	0.11	not detected	not detected
M684H027b	Not assigned	0.10	0.09	not detected	not detected
M684H042	Not assigned	0.11	0.21	0.12	0.07
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.21	0.31	0.04	not detected
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	1.23	0.80	0.13	0.07
M684H011	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.46	2.31	0.18	0.11
M684H002	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)phenyl]methanol	0.10	0.21	0.17	0.13
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	2.16	5.03	0.50	0.48
BAS 684 H	Cinmethylin	0.23	0.32	0.65	0.64
<b>Total identified</b>		<b>6.76</b>	<b>9.47</b>	<b>1.82</b>	<b>1.49</b>
<b>Total characterised</b>		<b>0.53</b>	<b>0.23</b>	<b>0.62</b>	<b>0.18</b>
<b>Total identified and characterised</b>		<b>7.29</b>	<b>9.70</b>	<b>2.45</b>	<b>1.67</b>

Kidney

The identified metabolites in kidney extracts are shown below (Table 6.1-26 <sup>14</sup>C-phenyl label and 6.1-27 <sup>14</sup>C-cyclohexane label). Samples for metabolite analysis were taken at T<sub>max</sub>, from single low and single high dose animals. No samples were analysed from the repeated dosing group. Apart from unchanged cinmethylin (0.01 to 0.04 % of the administered dose), 10 metabolites were identified in the kidney, representing 0.01 to 0.33 % of the administered dose. No significant sex, dose or label specific differences were noted.

Table 6.1-26. Metabolites identified in rat kidney analysed at Tmax of plasma level (phenyl label) and their levels (expressed as % of administered dose)

Compound	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw) (1 h after dosing)		High dose (350 mg/kg bw) (4 h after dosing)	
		Male	Female	Male	Female
M684H010	2-(hydroxymethyl)benzoic acid	0.04	not detected	0.03	0.01
M684H009	N-(2-methylbenzoyl)glycine (2-methyl-hippuric acid )	0.10	0.06	0.03	0.02
M684H027a	Not assigned	0.02	0.01	0.01	0.01
M684H027b	Not assigned	0.02	0.01	0.01	<0.01
M684H042	Not assigned	0.02	0.02	0.02	0.03
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.03	0.03	0.02	<0.01
M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.04	0.02	0.03	0.01
M684H011	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.33	0.30	0.14	0.05
M684H002	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)phenyl]methanol	not detected	not detected	0.01	0.02
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.04	0.06	0.04	0.03
BAS 684 H	Cinnethylin	0.01	0.01	0.01	0.04
Total identified		0.65	0.52	0.34	0.21
Total characterised		0.18	0.10	0.10	0.04
Total identified and characterised		0.83	0.62	0.44	0.25

Table 6.1-27. Metabolites identified in rat kidney analysed at Tmax of plasma level (cyclohexane label) and their levels (expressed as % of administered dose)

Compound	Chemical Name	Percent of administered dose			
		Low dose (15 mg/kg bw) (1 h after dosing)		High dose (350 mg/kg bw) (1 h after dosing)	
		Male	Female	Male	Female
M684H026	(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]heptan-2-ol	0.02	0.01	0.01	<0.01
M684H060	Not assigned	0.01	0.01	not detected	not detected
M684H027a	Not assigned	0.01	0.02	0.01	<0.01
M684H027b	Not assigned	0.01	0.01	0.01	<0.01
M684H042	Not assigned	0.01	0.03	0.02	0.01
M684H013a	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.02	0.04	0.01	0.01

M684H013b	2-({[(1SR,2RS,4RS)-4-(1-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.02	0.02	0.04	0.01
M684H011	2-({[(1SR,2RS,4RS)-4-(2-hydroxypropan-2-yl)-1-methyl-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.20	0.30	0.08	0.04
M684H002	[2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)phenyl]methanol	not detected	0.01	0.01	0.01
M684H001	2-({[(1SR,2RS,4RS)-1-methyl-4-(propan-2-yl)-7-oxabicyclo[2.2.1]hept-2-yl]oxy}methyl)benzoic acid	0.05	0.06	0.03	0.01
BAS 684 H	Cinmethylin	0.01	0.01	0.02	0.03
<b>Total identified</b>		<b>0.36</b>	<b>0.51</b>	<b>0.23</b>	<b>0.13</b>
<b>Total characterised</b>		<b>0.05</b>	<b>0.03</b>	<b>0.07</b>	<b>0.03</b>
<b>Total identified and characterised</b>		<b>0.41</b>	<b>0.55</b>	<b>0.30</b>	<b>0.16</b>

#### Results - Metabolic Pathway

The main biotransformation steps of the metabolic pathway for cinmethylin are:

- Hydroxylation at the cyclohexane and / or benzyl ring
- Hydroxylation of the alkyl groups at the benzyl and / or cyclohexane ring
- Oxidation of the hydroxylated methyl group at the benzyl ring to a carboxy group
- Cleavage of the ether bridge
- Conjugation with glucuronic acid
- Conjugation with glycine

Conjugation with sulphate and glutathione and subsequent degradation of the glutathione conjugate were noted as minor pathways. For some metabolites, a precise specification for the position of an -OH group or Phase II conjugate could not be made and a generic structure has been given.

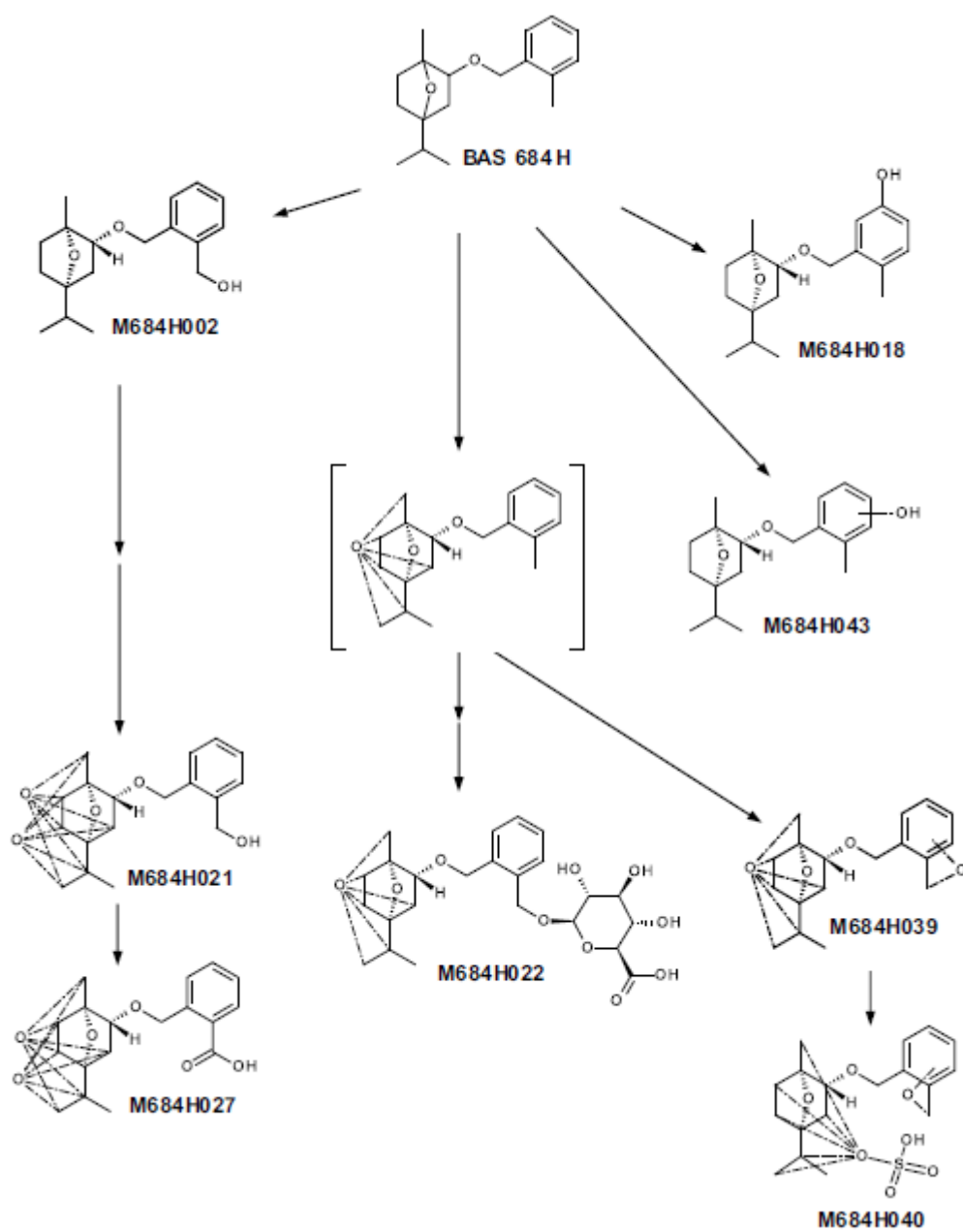
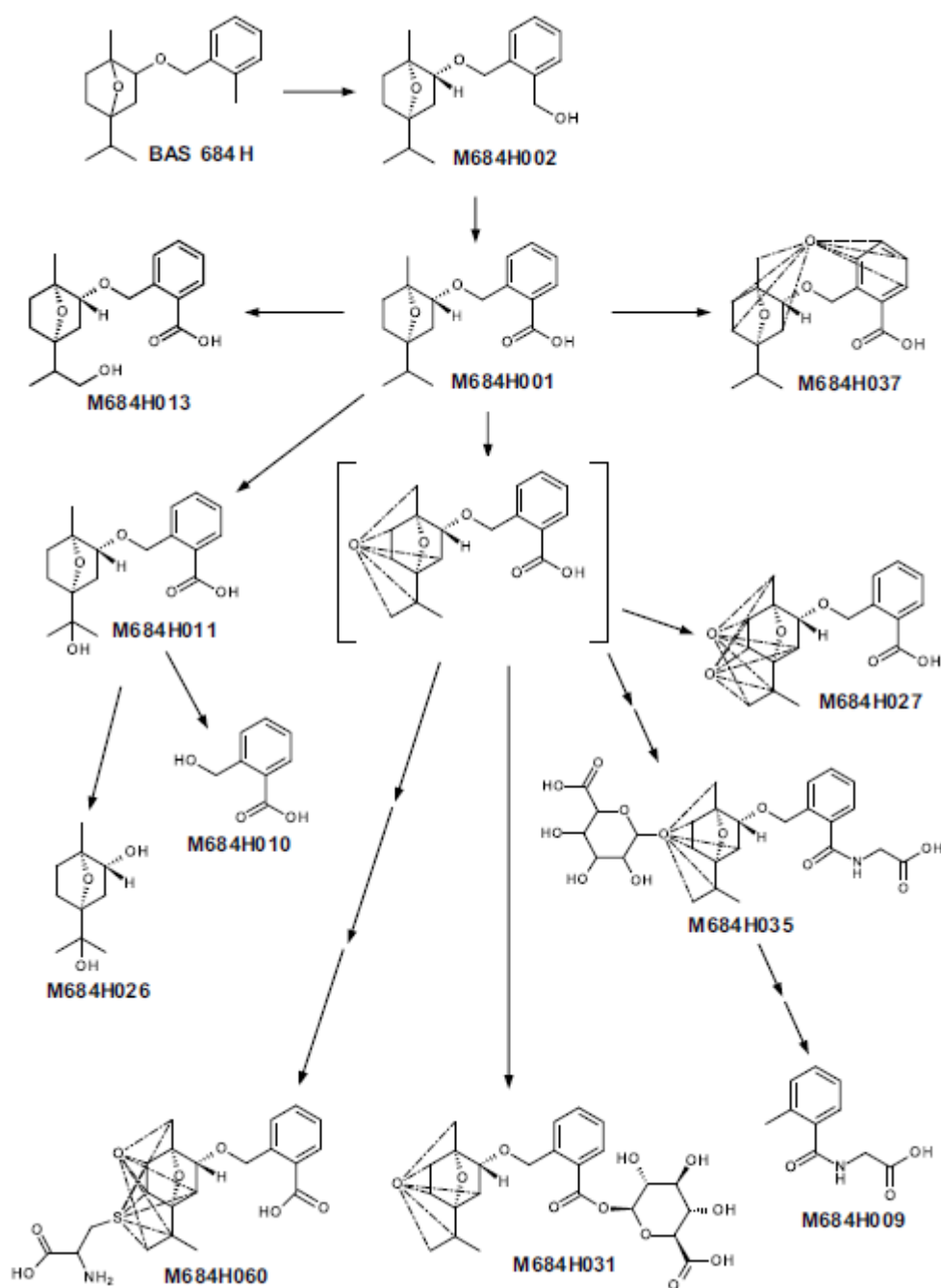
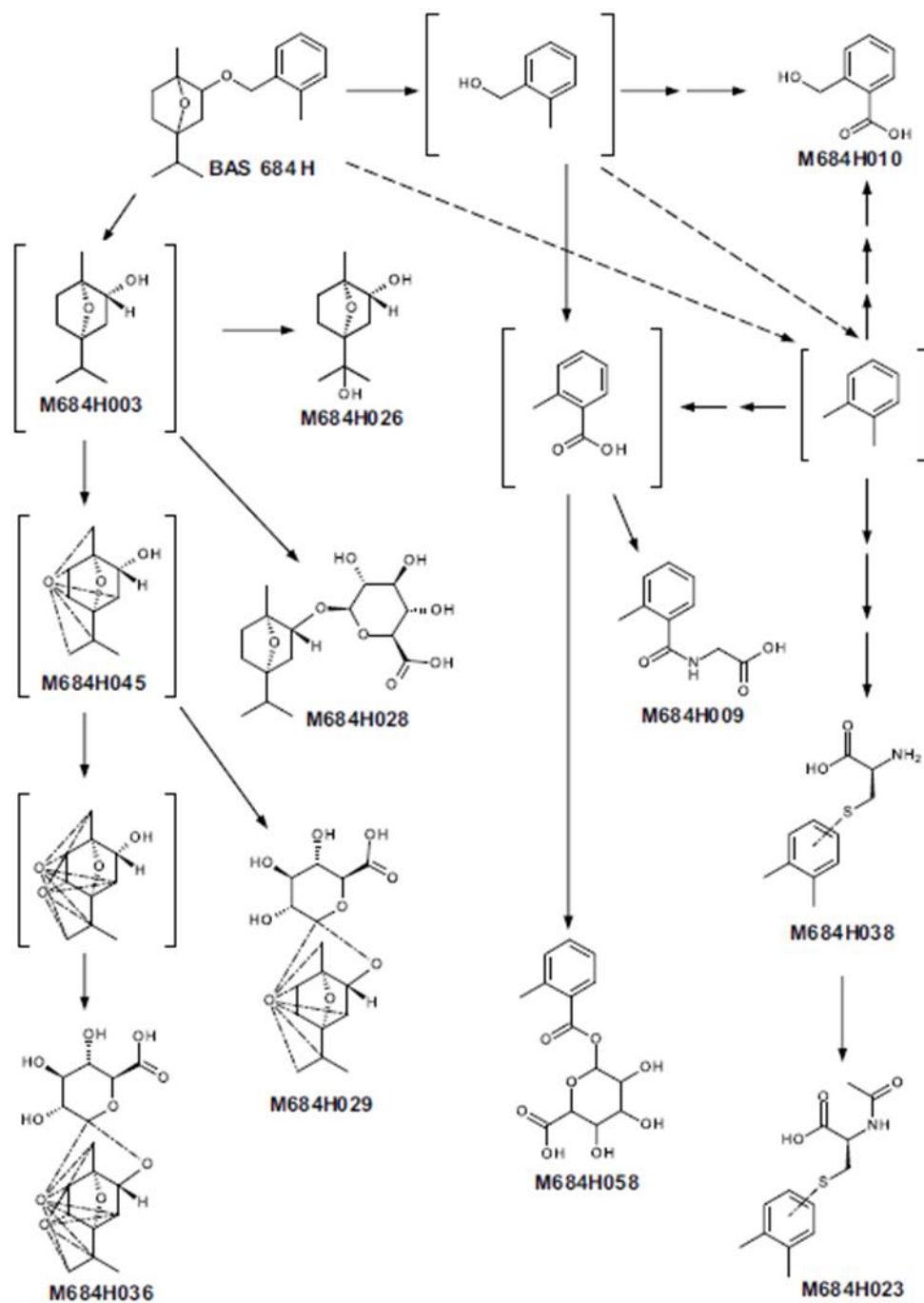
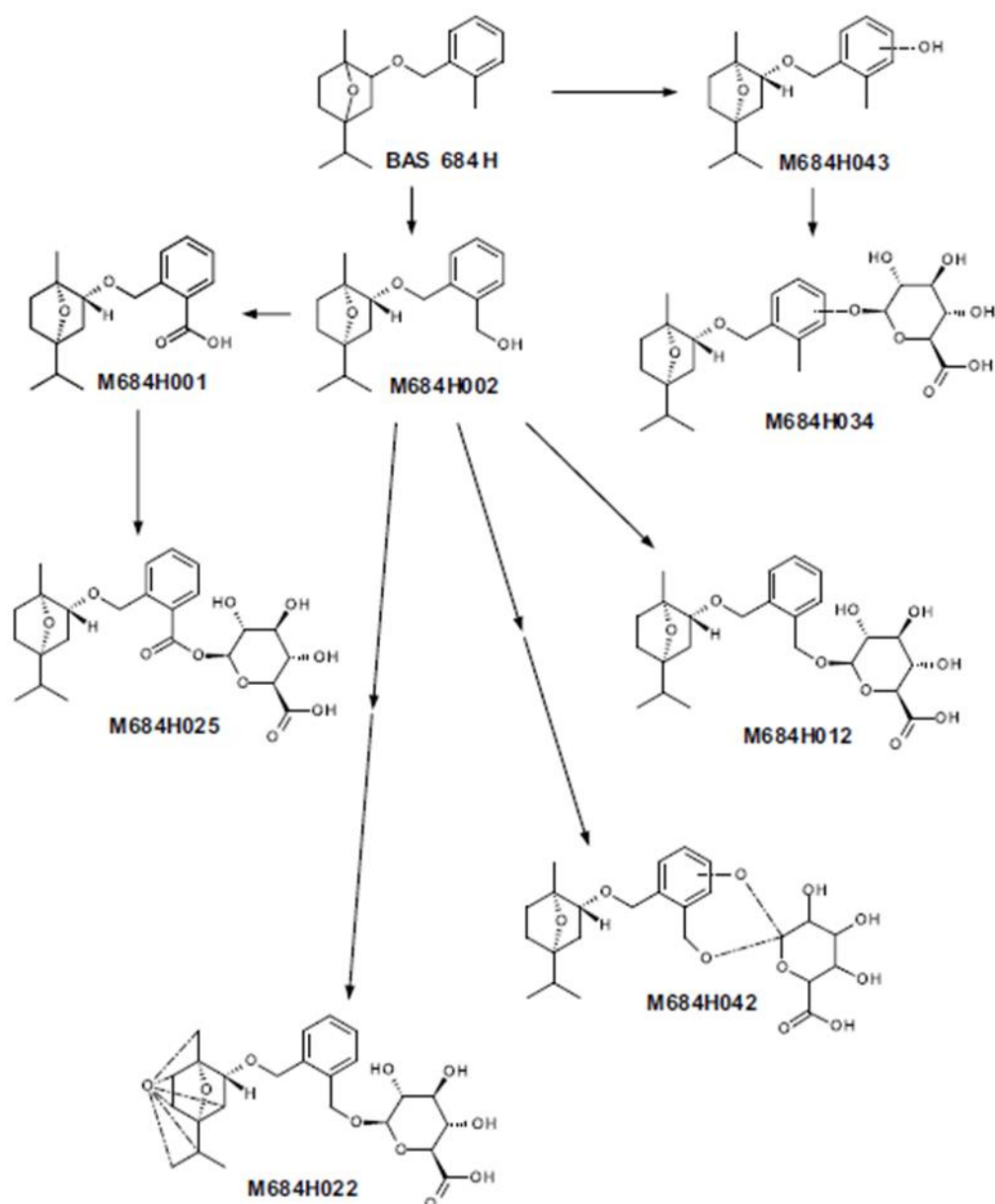


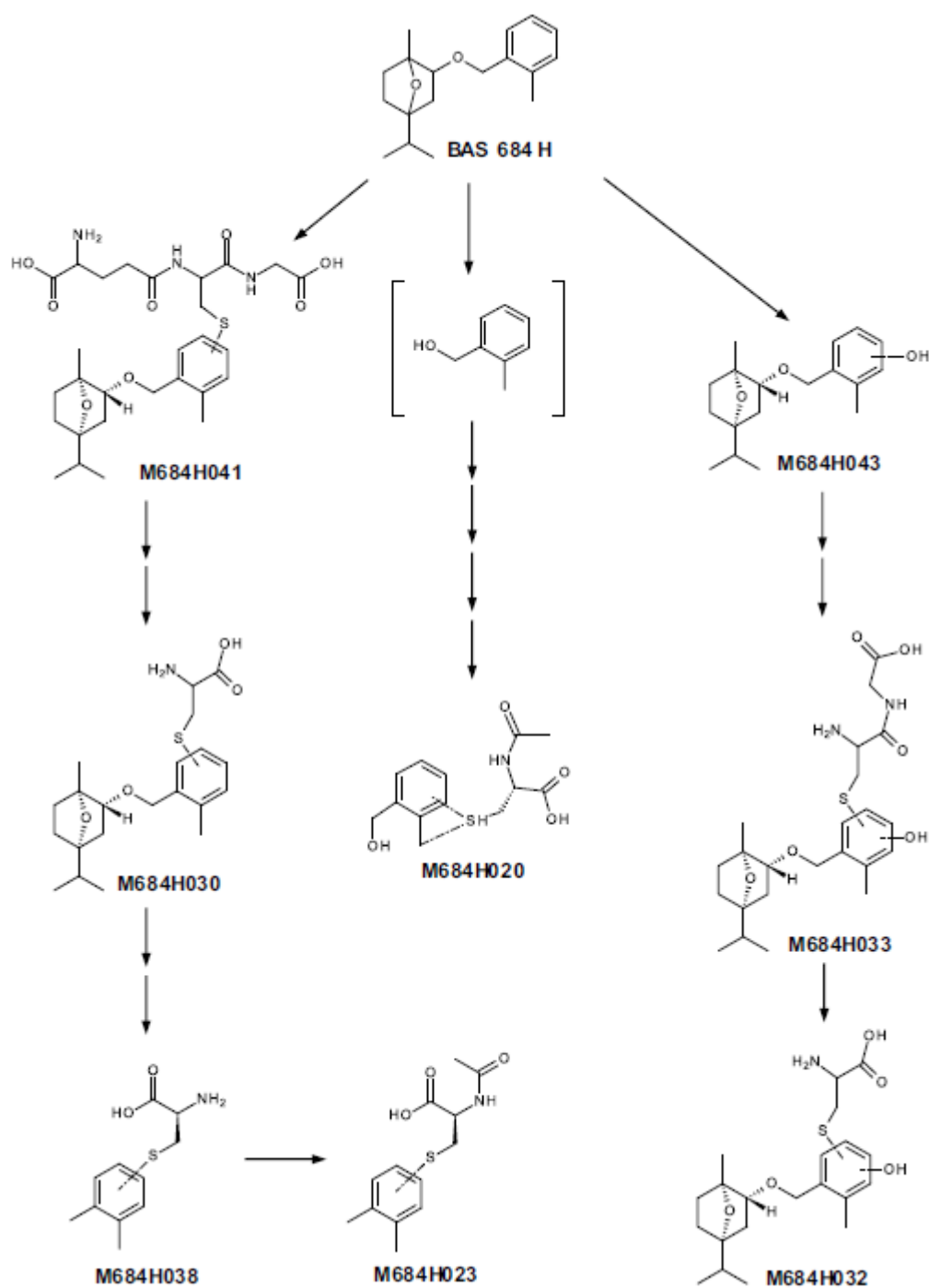
Figure 6.1-1. Proposed metabolic pathway for cinmethylin in rats



Figure 6.1-2. Proposed metabolic pathway for cinmethylin in rats (continued)

Figure 6.1-3. Proposed metabolic pathway for cinmethylin in rats (continued)

Figure 6.1-4. Proposed metabolic pathway for cinmethylin in rats (continued)

Figure 6.1-5. Proposed metabolic pathway for cinmethylin in rats (continued)

**B.6.1.2. Metabolism studies *in vitro***

Study	Comparative <i>in-vitro</i> metabolism with <sup>14</sup> C-BAS 684 H
Reference	Funk-Weyer & Ufer, 2017
Date performed	21/12/2017
Test facility	BASF SE Crop Protection, Ecology and Environmental Analytica, Speyerer Strasse 2, 67117 Limburgerhof, Germany
Report reference	BASF ID No 2017/1172468
Guideline(s)	None
Deviations from the guideline	Not Applicable
GLP	Yes
Test material	<sup>14</sup> C-cyclohexyl radiolabelled cinmethylin, radiochemical purity 97.9 % and chemical purity 95.9 % <sup>14</sup> C-phenyl radiolabelled cinmethylin, radiochemical purity 98 % and chemical purity 90.9 %
Study acceptable	Yes

An *in vitro* comparative metabolism study is available employing primary hepatocytes from humans, Wistar rats, Beagle dogs and New Zealand White rabbits exposed to <sup>14</sup>C-phenyl labelled cinmethylin and <sup>14</sup>C-cyclohexyl labelled cinmethylin.

*Method*

The human hepatocytes employed were a mixture of cells obtained from male and female donors. For the rat, rabbit and dog, hepatocytes from males and females were combined in a ratio of 1:1. The viability of the hepatocytes after incubation with 10 µM cinmethylin for 180 min was tested in parallel to the incubation for metabolic investigations, to determine whether cinmethylin affected the viability of the cells.

Human, rat, rabbit and dog hepatocytes were incubated with cinmethylin (both labels) and the positive controls 7-ethoxycoumarin and testosterone at a final concentration of 10 µM for positive controls and cinmethylin. For cinmethylin, the concentration was selected based on a viability experiment.

The dosing solutions in DMSO were diluted with the hepatocytes incubation medium. Aliquots of the application media were analysed by LSC to calculate the amounts of applied radioactivity per assay (representing 100 % AR (applied radioactivity)). The purity of the application media for the *in vitro* assays was determined by HPLC. Each sample comprised equal amounts of the application medium and of the hepatocyte cell suspension in one well of a 24-well cell culture plate (maximum concentration of DMSO: 0.5 %).

The incubations were carried out at 37°C and at 5 % CO<sub>2</sub> for 0 min (zero-incubation control), 10 min, 30 min, 60 min and 180 min. The reaction was stopped with ice-cold ethanol and hepatocytes lysed by ultra-sonication.

Subsequently, at least one sample per triplicate (for each species and each incubation time) of the supernatants was analysed by HPLC MS. The metabolite patterns formed by incubation of the animal hepatocytes were compared to the metabolite pattern formed by incubation of human hepatocytes to establish whether any unique metabolites were present.

### Results

The viability of human, rat, rabbit and dog hepatocytes with 10  $\mu$ M cinmethylin ranged from 86 – 104 % of the control values for both radiolabels. HSE considers this an acceptable level of viability. The recovery of radioactivity in the supernatants of the hepatocyte samples ranged from 82.8 - 94.8 % AR (human), 70.7 - 98.0 % AR (rat), 79.1 - 93.0 % AR (rabbit) and 83.4 - 96.1 % AR (dog). Since the recovery for some hepatocyte samples was < 90 % AR, the remaining cell pellets of the corresponding samples were further extracted with a methanol mixture and the extracts were analysed with HPLC. Samples with a total recovery of < 90 % were further extracted with an acetonitrile mixture. The total recovery in a few samples remained slightly below 90.0 % AR, but were close to 90.0 % AR and at least one corresponding replicate of these samples was  $\geq$  90.0 % AR. Overall, recoveries are considered to be acceptable.

Metabolism of the control substance testosterone was: >55 % AR, 85 % AR, 65 % AR and 75 % AR for human, rat, rabbit and dog hepatocytes respectively; and for 7-ethoxycoumarin: >65 % AR, 75 % AR, 85 % AR and 85 % AR for human, rat, rabbit and dog hepatocytes respectively. The target metabolism rates stated in the test report were >50 % AR, 85 % AR, 75 % AR and 75 % AR for human, rat, rabbit and dog hepatocytes respectively. Therefore, the primary hepatocytes used in this study are considered to have acceptable metabolic capacity.

For human hepatocytes, unchanged cinmethylin was detected for all timepoints up to 60 min for both radiolabels. In human hepatocytes, the concentration of cinmethylin decreased continuously from 84.72 % AR (phenyl label) and 88.65 % AR (cyclohexane label) after 0 min to 22.84 % AR (phenyl label) and 26.69 % AR (cyclohexane label) after 60 min. The parent compound was not detected after 180 min (both labels). Compared to human hepatocytes, metabolism of cinmethylin was faster in rat, rabbit and dog hepatocytes, where the parent compound was completely metabolised within 60 min.

Up to 6 metabolites were confirmed, based on HPLC retention-time (Tables 6.1-28 and 6.1-29). One metabolite peak was identified (M684H004) which is abbreviated as 2-hydroxypropyl cinmethylin. No unique human metabolites were identified.

#### M684H004 (2-hydroxypropyl cinmethylin)

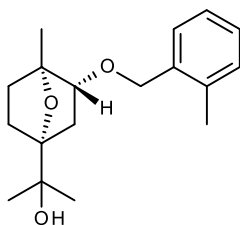


Table 6.1-28. Comparison of HPLC peaks detected after incubation of human, rat, rabbit and dog hepatocytes with cinmethylin ( $^{14}$ C-phenyl label) in % of applied radioactivity (% AR)

Analyte / Peak	Human Mean % AR	Rat Mean % AR	Rabbit Mean % AR	Dog Mean % AR
Incubation Time: 0 min (Zero Incubation Control)				
P29.1	not detected	6.04	4.35	1.78
BAS 684 H	84.72	79.95	85.76	89.19
Incubation Time: 10 min				
P21.9	not detected	7.46	8.70	3.58
P23.2	not detected	1.97	not detected	3.34
P29.1	5.69	35.27	41.82	38.99
M684H004	1.59	-- <sup>1</sup>	6.07	12.10
BAS 684 H	84.01	8.79	24.84	31.88
Incubation Time: 30 min				
P16.7	not detected	5.34	4.77	not detected
P21.9	5.20	17.35	17.13	13.95
P23.2	1.35	4.66	3.79	19.39

P29.1	14.86	not detected	15.85	20.51
M684H004	4.90	not detected	7.66	14.99
BAS 684 H	61.92	not detected	not detected	4.01
Incubation Time: 60 min				
P9.0	5.22	4.09	not detected	not detected
P16.7	not detected	7.72	14.02	4.99
P21.9	20.08	17.79	41.74	25.66
P23.2	5.52	7.00	5.74	28.21
P29.1	14.62	not detected	3.57	0.45
M684H004	9.72	not detected	5.04	9.48
BAS 684 H	22.84	not detected	not detected	not detected
Incubation Time: 180 min				
P9.0	18.64	6.93	3.89	not detected
P16.7	5.94	13.03	29.36	11.94
P21.9	37.60	14.13	34.83	25.18
P23.2	8.95	6.92	4.98	30.87
M684H004	4.98	not detected	not detected	6.75

<sup>1</sup>Detected in traces by MS, but no peak could be assigned in the respective radio-chromatogram

Table 6.1-29. Comparison of HPLC peaks detected after incubation of human, rat, rabbit and dog hepatocytes with cinnethylin (<sup>14</sup>C-cyclohexyl label) in % of applied radioactivity (%AR)

Analyte / Peak	Human Mean % AR	Rat Mean % AR	Rabbit Mean % AR	Dog Mean % AR
Incubation Time: 0 min (Zero Incubation Control)				
P14.4	1.25	3.34	3.44	2.32
P29.1	not detected	5.29	2.13	2.42
BAS 684 H	88.65	82.49	84.95	87.75
Incubation Time: 10 min				
P14.4	4.31	10.44	6.75	5.99
P21.9	not detected	2.91	5.23	3.26
P23.2	not detected	not detected	1.43	2.55
P29.1	5.10	37.05	25.61	34.92
M684H004	1.36	-- <sup>1</sup>	6.29	11.88
BAS 684 H	84.14	26.90	41.98	30.54
Incubation Time: 30 min				
P14.4	9.50	14.20	5.89	7.36
P16.7	not detected	3.68	4.54	not detected
P21.9	3.54	16.76	23.53	15.59
P23.2	0.90	6.09	10.78	20.58
P29.1	13.21	6.11	12.78	15.65
M684H004	5.03	-- <sup>1</sup>	7.03	14.06
BAS 684 H	62.08	not detected	6.69	1.24
Incubation Time: 60 min				
P14.4	19.45	11.77	0.56	5.06
P16.7	not detected	7.37	14.94	7.05
P21.9	14.84	17.85	37.04	20.99
P23.2	4.49	7.00	13.84	24.63
P29.1	14.89	0.43	not detected	not detected
M684H004	9.17	not detected	3.50	10.61
BAS 684 H	26.69	not detected	not detected	not detected
Incubation Time: 180 min				
P14.4	23.93	8.85	not detected	2.87
P16.7	6.67	9.92	25.38	11.29
P21.9	35.30	15.68	29.61	19.65

P23.2	11.32	6.04	12.44	26.38
M684H004	4.87	not detected	not detected	5.65

<sup>1</sup>Detected in traces by MS, but no peak could be assigned in the respective radio-chromatogram.

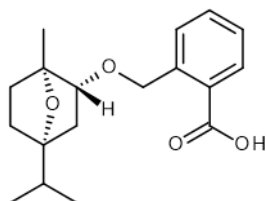
### B.6.1.3. Toxicokinetic information from Toxicodynamic Studies

Some limited toxicokinetic information is available from the rat and mouse lifetime/carcinogenicity studies. The levels of cinmethylin and selected metabolites were determined in the plasma of rats and mice from these standard lifetime studies.

#### Rats

The study details can be found in [section B.6.5.1](#) (■■■■■, 2018). Reported below (Table 6.1-30) are the plasma concentrations of cinmethylin and four metabolites (M684H001, M684H010, M684H011 and M684H026) determined throughout the study (on days 22, 43, 64, 80, 172, 262 and 353) (also Table 6.5-2). Analytical results demonstrated the clear presence of four metabolites of cinmethylin in all plasma samples of treated animals from all time-points. Levels of unchanged cinmethylin were below the LOQ; only in females at the highest dose, levels above the LOQ were seen. In general, levels of metabolites increased in a dose-dependent manner, although at the top dose considerable variation was observed.

M684H001 (Cinmethylin benzoate)



M684H026 (2-hydroxypropyl 2-hydroxycineol)

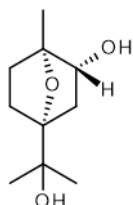


Table 6.1-30. Plasma concentration of cinmethylin and metabolites in rats (chronic toxicity group, 10/dose)

Dose [ppm]		Males				Females			
		0	200	1000	5000	0	200	1000	5000
Cinmethylin	Day 22 [ng/mL]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	105 <sup>1</sup>
	[RSD]								
	Day 43 [ng/mL]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	95 <sup>1</sup>
	[RSD]								
	Day 64 [ng/mL]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	94 <sup>1</sup>
	[RSD]								
Cinmethylin	Day 80 [ng/mL]	n. d.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	121 <sup>3</sup>
	[RSD]								10.8
Cinmethylin	Day 172 [ng/mL]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	138 <sup>1</sup>
	[RSD]								
Cinmethylin	Day 262 [ng/mL]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	112 <sup>2</sup>
	[RSD]								



Dose [ppm]		Males				Females			
		0	200	1000	5000	0	200	1000	5000
	/RSD]								22.1
	Day 353 [ng/mL]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	115 <sup>3</sup>
M684H001	/RSD]								10.7
	Day 22 [ng/mL]	n. d.	500	1476	12811	n. d.	559	2265	11487
	/RSD]		20.5	18.2	18.8		26.4	16.1	22.3
	Day 43 [ng/mL]	n. d.	300	1273	8634	n. d.	616	2729	9652
	/RSD]		19.9	26.9	26.7		24.8	32.4	46.1
	Day 64 [ng/mL]	n. d.	304	1233	5962	n. d.	534	2340	16496
	/RSD]		15.5	26.2	35.4		18.8	32.5	26.8
	Day 80 [ng/mL]	n. d.	345	1108	10020	n. d.	416	2365	19439
	/RSD]		42.8	22.8	43.3		30.2	30.1	36.4
	Day 172 [ng/mL]	n. d.	249	945	11233	n. d.	410	1760	20743
	/RSD]		32.2	22.0	30.9		30.9	35.6	32.8
	Day 262 [ng/mL]	n. d.	229	1112	5400	n. d.	384	1538	8906
	/RSD]		24.1	24.4	32.8		30.0	26.1	34.8
M684H010	Day 353 [ng/mL]	n. d.	230	819	3271	n. d.	676	2971	13305
	/RSD]		26.5	23.8	30.0		30.4	19.2	32.2
	Day 22 [ng/mL]	n. d.	<LOQ	277	1936	n. d.	<LOQ	129 <sup>8</sup>	1308.1
	/RSD]			20.3	17.8			19.0	21.7
	Day 43 [ng/mL]	n. d.	<LOQ	198	1393	n. d.	<LOQ	141 <sup>7</sup>	1204
	/RSD]			24.5	19.7			26.0	18.0
	Day 64 [ng/mL]	n. d.	<LOQ	193	1298	n. d.	<LOQ	122 <sup>5</sup>	1117
	/RSD]			13.7	16.2			9.5	22.6
	Day 80 [ng/mL]	n. d.	<LOQ	191	1191	n. d.	<LOQ	130 <sup>8</sup>	1247
	/RSD]			16.5	22.0			15.8	24.2
	Day 172 [ng/mL]	n. d.	<LOQ	192	1179	n. d.	<LOQ	121	1448
	/RSD]			12.5	27.7			18.3	13.9
	Day 262 [ng/mL]	n. d.	<LOQ	203	1058	n. d.	<LOQ	138 <sup>9</sup>	1430
	/RSD]			24.4	16.0			16.9	25.8
M684H011	Day 353 [ng/mL]	<LOQ	<LOQ	185	7816	n. d.	<LOQ	164 <sup>7</sup>	1407
	/RSD]			13.2	20.8			14.9	24.3
	Day 22 [ng/mL]	n. d.	370	1278	8683	n. d.	258	1199	4255
	/RSD]		23.3	14.4	17.1		28.1	18.1	25.2
	Day 43 [ng/mL]	n. d.	269	1279	6179	n. d.	240	1039	3269
	/RSD]		8.1	16.1	19.2		19.4	34.6	22.7
	Day 64 [ng/mL]	n. d.	219	892	3353	<LOQ	182	816	2802
	/RSD]		15.0	26.9	22.6		16.7	25.0	18.4
	Day 80 [ng/mL]	<LOQ	247	920	3079	<LOQ	206	1031	3171
	/RSD]		19.3	20.1	20.2		23.6	15.4	22.2
	Day 172 [ng/mL]	<LOQ	189	884	3135	<LOQ	208	885	3241
	/RSD]		23.2	22.9	17.8		29.9	33.8	23.4
	Day 262 [ng/mL]	<LOQ	182	1127	3039	<LOQ	198	827	2876
	/RSD]		18.8	13.2	22.0		21.3	16.1	25.4
M684H026	Day 353 [ng/mL]	n. d.	176	727	2621	n. d.	191	890	2909
	/RSD]		20.1	17.7	20.8		25.2	25.3	19.7
	Day 22 [ng/mL]	n. d.	329	1855	9282	n. d.	216	1207	12133
	/RSD]		15.7	16.2	33.5		23.5	18.6	23.2
	Day 43 [ng/mL]	n. d.	273	1601	8884	n. d.	240	1207	8570
	/RSD]		13.7	13.3	20.6		24.2	20.9	19.1
	Day 64 [ng/mL]	n. d.	303	1529	7755	n. d.	206	988	14632
	/RSD]		12.0	13.9	26.7		24.9	20.6	23.9

Dose [ppm]		Males				Females			
		0	200	1000	5000	0	200	1000	5000
	Day 80 [ng/mL] [RSD]	n. d.	295 12.8	1609 11.5	11327 16.1	n. d.	225 25.8	984 15.2	15323 24.5
	Day 172 [ng/mL] [RSD]	n. d.	297 12.5	1419 11.5	13185 19.4	n. d.	214 24.5	1021 14.8	16137 20.5
	Day 262 [ng/mL] [RSD]	n. d.	262 14.0	1431 11.9	6346 22.6	n. d.	211 30.4	1090 21.9	8849 28.7
	Day 353 [ng/mL] [RSD]	n. d.	248 20.0	1381 13.2	7816 20.8	n. d.	190 32.0	1033 25.6	10540 26.7

LOQ (limit of quantification) = 100 ng/mL

RSD = relative standard deviation in %

n. d. = not detectable

n<sup>x</sup> = superscript x is the number of animals used for plasma concentration measurement; if no x is depicted, mean is based on values of all 10 animals per dose group

#### Mice

The full study details can be found in [section B.6.5.2](#) (■■■■■, 2018d). Reported below (Table 6.1-31) are the plasma concentrations of cinmethylin and metabolites (M684H001, M684H010, M684H011 and M684H026).

Unchanged cinmethylin was observed in plasma but generally at levels below the LOQ. However, cinmethylin metabolites (M684H001, M684H010, M684H011 and M684H026) were detected and quantified in plasma samples of treated animals; the concentrations of metabolites increased (however not linearly) with dose.

Table 6.1-31. Plasma concentration of cinmethylin and metabolites in mice (satellite group, 6 animals/dose)

Dose [ppm]		Males				Females			
		0	150	1000	5000	0	150	1000	5000
Cinmethylin	Day 20 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	173.9 <sup>2</sup> 53.2	<LOQ	<LOQ	<LOQ	<LOQ
	Day 41 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	101.7 <sup>1</sup>	<LOQ	<LOQ	<LOQ	<LOQ
	Day 62 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
M684H001	Day 20 [ng/mL] [RSD]	n. d.	<LOQ	<LOQ	186.4 <sup>4</sup> 9.8	n. d.	<LOQ	<LOQ	230.6 <sup>5</sup> 26.6
	Day 41 [ng/mL] [RSD]	n. d.	<LOQ	<LOQ	131.6 <sup>4</sup> 11.8	n. d.	<LOQ	<LOQ	145.9 <sup>3</sup> 32.9
	Day 62 [ng/mL] [RSD]	n. d.	<LOQ	<LOQ	157.2 <sup>5</sup> 18.7	n. d.	<LOQ	<LOQ	171.0 <sup>4</sup> 49.3
M684H010	Day 20 [ng/mL] [RSD]	n. d.	116.3 <sup>1</sup>	532.5 <sup>6</sup> 26.2	2380.7 <sup>6</sup> 26.4	n. d.	<LOQ	382.6 <sup>6</sup> 17.9	1410.2 <sup>6</sup> 26.1
	Day 41 [ng/mL] [RSD]	n. d.	109.6 <sup>2</sup> 0.7	578.1 <sup>6</sup> 15.0	2041.3 <sup>6</sup> 21.2	n. d.	97.9 <sup>1</sup>	287.6 <sup>6</sup> 21.3	1149.9 <sup>6</sup> 31.2
	Day 62 [ng/mL] [RSD]	n. d.	99.9 <sup>2</sup> 5.4	538.3 <sup>6</sup> 19.9	2018.8 <sup>6</sup> 22.5	n. d.	<LOQ	287.7 <sup>6</sup> 16.7	1115.3 <sup>6</sup> 36.3
M684H011	Day 20 [ng/mL] [RSD]	n. d.	<LOQ	142.9 <sup>1</sup>	399.0 <sup>6</sup> 55.4	n. d.	<LOQ	170.9 <sup>4</sup> 31.2	879.9 <sup>6</sup> 35.3
	Day 41 [ng/mL] [RSD]	n. d.	<LOQ	106.3 <sup>2</sup> 2.3	266.3 <sup>6</sup> 19.8	n. d.	<LOQ	108.6 <sup>1</sup>	414.9 <sup>6</sup> 67.7
	Day 62 [ng/mL] [RSD]	n. d.	<LOQ	107.2 <sup>1</sup>	366.5 <sup>6</sup> 32.7	n. d.	<LOQ	138.0 <sup>1</sup>	548.5 <sup>6</sup> 52.4
4 H	Day 20 [ng/mL]	n. d.	102.9 <sup>6</sup>	406.0 <sup>6</sup>	964.6 <sup>6</sup>	n. d.	<LOQ	239.6 <sup>6</sup>	696.6 <sup>6</sup>

Dose [ppm]		Males				Females			
		0	150	1000	5000	0	150	1000	5000
Day 41	[RSD]		9.4	12.2	28.6			21.2	26.6
	[ng/mL]	n. d.	99.6 <sup>6</sup>	448.9 <sup>6</sup>	801.6 <sup>6</sup>	n. d.	<LOQ	130.2 <sup>6</sup>	400.8 <sup>6</sup>
	[RSD]		8.7	15.1	26.5			28.9	66.6
Day 62	[ng/mL]	n. d.	100.4 <sup>5</sup>	413.7 <sup>6</sup>	824.7 <sup>6</sup>	n. d.	<LOQ	162.6 <sup>6</sup>	435.3 <sup>6</sup>
	[RSD]		10.5	14.9	29.7			23.9	35.2

LOQ (limit of quantification) = 100 ng/mL

RSD = relative standard deviation in %

n. d. = not detectable

n<sup>x</sup> = superscript x is the number of animals used for plasma concentration measurement;

#### B.6.1.4. Absorption, distribution, metabolism and excretion by other routes

There are no toxicokinetic studies available conducted by other routes of exposure.

### B.6.1.5. Summaries of older toxicokinetic studies

A total of 10 toxicokinetic studies are available which were performed exclusively with one radiolabel ( $^{14}\text{C}$ -phenyl labelled cinmethylin). These studies are included for completeness and for comparison with the modern toxicokinetic studies. The full study reports have not been consulted. Overall, the findings from these older studies are broadly consistent with those of the new/modern dataset.

#### Study 1 - Toxicokinetics

The toxicokinetics of cinmethylin was investigated in rats following a single oral dose of 15 mg/kg bw or 450 mg/kg bw dosing. There is a reasonable fit of the experimental data by a one compartment model. The disposition of cinmethylin is linear within the two dose levels. Pharmacokinetic analysis showed rapid absorption, elimination and metabolism of the administered dose (Lee and Brown, 1984b).

#### Study 2 - Distribution, Elimination and Half-life

The elimination of  $^{14}\text{C}$ -cinmethylin and degradation products from male and female rats was rapid via both the urine and faeces following a single oral dose of 15 mg/kg bw. Approximately 95 % of the administered radioactivity was recovered from the excreta 3 and 4 days post-treatment from the male and female test animals, respectively. The majority of the eliminated radioactivity was recovered in the initial 24 hours. The major route of elimination was via urine (51 and 57 % of the administered dose in the male and female test animals, respectively). Faeces contained 42 and 36 % of the administered dose in the male and female, respectively. Radioactive volatile materials and  $^{14}\text{C}$ -carbon dioxide were not detected in the respired air of the treated animals. Tissue residue distribution data indicate the lack of bioconcentration of  $^{14}\text{C}$ -cinmethylin equivalent residues in blood, lung, heart, fat, gonad, kidney, muscle, brain, bone and spleen tissue of the test animals. Significant levels of  $^{14}\text{C}$ -cinmethylin residues were detected only in the liver tissues. A fractionation experiment indicated the majority of the  $^{14}\text{C}$ -residues in the liver tissues were recovered by organic solvent extraction. TLC autoradiograms showed similar degradation products in the liver to those identified in the animal excreta. Blood/tissue residue ratio, plasma and liver residue kinetic data clearly demonstrated the extensive and rapid clearance of  $^{14}\text{C}$ -cinmethylin and derived degradation products from the treated animals after a single oral dose administration (Lee, Stearns and Powell, 1982b).

#### Study 3 - Distribution, Elimination and Half-life

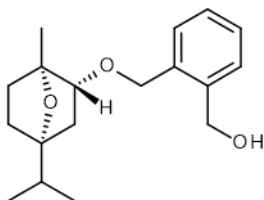
The elimination of  $^{14}\text{C}$ -cinmethylin and degradation products from male and female rats was rapid via both the urine and faeces following a single oral dose of 450 mg/kg bw. Approximately 80 and 90 % of the administered radioactivity was recovered from the excreta 7 days post-treatment from males and females, respectively. The majority of the eliminated radioactivity was recovered during the initial 24 hours. The major route of elimination was via urine (50 and 63 % of the administered dose in male and female test animals, respectively). Faeces contained 32 and 28 % of the administered dose in the males and females, respectively. Tissue residue distribution data indicate the lack of bioconcentration of  $^{14}\text{C}$ -cinmethylin equivalent residues in blood, lung, heart, fat, gonad, kidney, muscle, brain, bone and spleen tissue of the test animals. Significant levels of  $^{14}\text{C}$ -cinmethylin equivalent residues were detected only in the liver tissues as indicated by the blood/tissue residue ratio. Whole blood kinetic data clearly indicated the extensive clearance of  $^{14}\text{C}$ -cinmethylin and derived degradation products from the treated animals after a single oral dose administration of 450 mg/kg bw (Lee, 1983c).

#### Study 4 - Metabolism

The elimination of  $^{14}\text{C}$ -cinmethylin and degradation products from male and female rats was rapid via both the urine and faeces following a single oral dose of 15 mg/kg bw. A complex degradation pattern of  $^{14}\text{C}$ -cinmethylin was observed in the animal excreta. In addition to unchanged cinmethylin, seven principal metabolites were isolated and characterised by a combination of chromatographic and spectroscopic techniques. Degradation products included cinmethylin benzoate, cinmethylin benzyl alcohol, 2-hydroxypropyl cinmethylin benzoic acid, 1-hydroxypropyl cinmethylin benzoic acid, 2-benzofuran-1(3H)-one, 2-(hydroxymethyl)benzoic acid phthalic acid. Hydroxylation and oxidation reactions at the 2-methyl and in the cineole portion of the parent molecule were identified as the primary metabolic pathways. Tissue residue distribution data indicate the lack of bioconcentration of  $^{14}\text{C}$ -cinmethylin equivalent residues in the blood, lung, heart, fat, gonad, kidney, muscle, brain, bone and spleen tissue of the test animals. Significant levels of  $^{14}\text{C}$ -cinmethylin equivalent residues were

detected only in the liver tissues. A fractionation experiment indicated the majority of the  $^{14}\text{C}$ -residues in the liver tissues were recovered by organic solvent extraction. TLC autoradiograms showed similar degradation products in the liver with those identified in the animal excreta. Blood/tissue residue ratio and whole blood kinetic data clearly demonstrated the extensive and rapid clearance of  $^{14}\text{C}$ -cinmethylin and derived degradation products from the treated animals after a single oral dose administration (Lee, Stearns and Powell, 1983b).

#### Cinmethylin benzyl alcohol



#### Study 5 - Metabolism

The elimination of  $^{14}\text{C}$ -cinmethylin and degradation products from male and female rats was rapid via both the urine and faeces following a multiple oral dose regimen. Test animals were administered a single daily oral dose of 15 mg/kg bw of unlabelled cinmethylin via stomach intubation for 14 days. On the 15<sup>th</sup>-day, animals were administered a single oral dose (15 mg/kg bw) of  $^{14}\text{C}$ -cinmethylin and the metabolic fate of this radioactive dose was examined. Approximately 100 % of the administered radioactivity was recovered from the excreta 3 days post-treatment from the male and female test animals. The majority of the eliminated radioactivity was recovered in the initial 24 hours. The major route of elimination was via the urine (60 and 75 % of the administered dose in the male and female test animals, respectively). Faeces contained 40 and 24 % of the administered dose in the male and female test animals, respectively. Radioactive volatile materials and  $^{14}\text{C}$ -carbon dioxide were not monitored in the respired air of the treated animals in this study.

A complex degradation pattern of  $^{14}\text{C}$ -cinmethylin was observed in the animal excreta. In addition to unchanged cinmethylin, the following degradation products (each accounted for greater than 2 % of the administered dose) were isolated and confirmed by a combination of chromatographic and spectroscopic techniques. Degradation products included cinmethylin benzoic acid, cinmethylin benzyl alcohol, 2-hydroxypropyl cinmethylin benzoic acid, 1-hydroxypropyl cinmethylin benzoic acid, 2-benzofuran-1(3H)-one and an unnamed metabolite, 2-(hydroxymethyl)benzoic acid and 2-methyl-hippuric acid. Hydroxylation and oxidation reactions at the 2-methyl and in the cineole portion of the parent molecule were identified as the primary metabolic pathways. A majority of the urinary metabolites were recovered as organic-extractable products. Tissue residue distribution data indicated the lack of bioconcentration of  $^{14}\text{C}$ -cinmethylin equivalent residues in the blood, lung, heart, fat, gonad, kidney, muscle, brain, bone and spleen tissue of the test animals. Significant levels of  $^{14}\text{C}$ -cinmethylin equivalent residues were detected only in the liver tissues; however, accounting for less than 0.5 % of the administered dose. Blood/tissue residue ratio and whole blood kinetic data clearly demonstrated the extensive and rapid clearance of  $^{14}\text{C}$ -cinmethylin and derived degradation products from the treated animals after multiple oral dose administration (Lee, Stearns and Hernandez, 1985b).

#### Study 6 - Metabolism

The elimination of  $^{14}\text{C}$ -cinmethylin and degradation products from male and female rats was rapid via both the urine and faeces following a single oral dose of 450 mg/kg bw. A complex degradation pattern of  $^{14}\text{C}$ -cinmethylin was observed in the animal excreta. In addition to undegraded cinmethylin, ten primary degradation products (each accounted for at least 1% of the administered dose) were isolated, and characterised by a combination of various chromatographic and spectroscopic techniques. Metabolites identified included; 2-methylbenzoic acid, cinmethylin benzoate, cinmethylin benzyl alcohol, 2-hydroxypropyl cinmethylin benzoic acid, 1-hydroxypropyl cinmethylin benzoic acid, 2-benzofuran-1(3H)-one, 2-hydroxypropyl cinmethylin benzoic acid, N-(2-methylbenzoyl)glycine and an unidentified metabolite. Hydroxylation and oxidation reactions at the 2-methyl and in the cineole portion of the parent molecule were identified as the primary metabolic pathways. A majority of the urinary metabolites were recovered as water-soluble conjugates. Tissue residue distribution data indicated the lack of bioconcentration of  $^{14}\text{C}$ -cinmethylin equivalent residues in the blood, lung, heart, fat, gonad, kidney, muscle, brain, bone and spleen tissue of the test animals. Significant levels of  $^{14}\text{C}$ -cinmethylin

equivalent residues were detected only in the liver tissues. A fractionation experiment showed the majority of the  $^{14}\text{C}$ -residues in the liver tissues were bound to the tissue macromolecules and were not readily recovered by the various solvent extraction procedures. Blood/tissue residue ratio and whole blood kinetic data clearly demonstrated the extensive and rapid clearance of  $^{14}\text{C}$ -cinnethylin and derived degradation products from the treated animals after a single oral dose administration (Lee, 1984b).

#### Study 7 - Comparative metabolism in rats; single low dose, single high dose and repeated dosing

The metabolic fate of  $^{14}\text{C}$ -phenyl cinnethylin in rats (Fischer-344 strain) following a single oral low dose (15 mg/kg bw), a single oral high dose (450 mg/kg bw) and a multiple oral low dose (15 mg/kg bw) treatment was examined. The major route of elimination was via urinary excretion and greater than 85% of the administered radioactivity was eliminated in the urine and faeces during the initial 48 hours post-dosing.  $^{14}\text{CO}_2$  or other radioactive volatile material was not detected in the respired air. A complex degradation pattern of  $^{14}\text{C}$ -cinnethylin was observed in the animal excreta. In addition to the unchanged  $^{14}\text{C}$ -cinnethylin (1-7% of the administered dose, recovered only in the faeces), at least ten metabolites were isolated and identified from the urinary and fecal excreta as both organic-extractable and conjugated products.

2-hydroxypropyl cinnethylin benzoate, and 1-hydroxypropyl cinnethylin benzoate were identified as major products. The proposed metabolic pathway for cinnethylin is; hydroxylation and oxidation at the benzyl and cineole portions of the parent molecule, conjugation (with glucuronic acid and glycine) and ether cleavage. Significant levels of  $^{14}\text{C}$ -SD 95481 equivalent residues were not detected in tissues other than the liver. With the exception of the differences in the quantitative distribution of urinary metabolites (e.g., metabolites eliminated as water-soluble conjugates or as intact molecules), results showed the similarity in the disposition of cinnethylin between the male and female animals and among the various dosing regimens (Lee, 1986b).

#### Study 8 - Distribution and identification of Metabolites

After a single oral dose, the excretion of cinnethylin in male and female rats was rapid. Approximately 90% of the initial administered dose was eliminated via the urine and fecal excretion during the initial 48 hours. A complex degradation pattern of  $^{14}\text{C}$ -cinnethylin was observed in the animal excreta. In addition to unchanged cinnethylin, seven primary degradation products were isolated and characterised by a combination of various analytical techniques including TLC, radio-GLC, NMR and GC-mass spectral analysis. Hydroxylation reactions at the 2-methyl and in the cineole portion of the parent molecule were identified as the primary metabolic pathways (Lee, 1982b).

#### Study 9 - O-acetylation as a novel conjugation pathway for cinnethylin

A complex degradation pattern of [phenyl- $^{14}\text{C}$ ] cinnethylin (1) in laboratory rats following oral administration has been reported earlier. In addition to the undegraded parent, 10 metabolites were detected. In the urinary organic extractable fraction, two minor metabolites (each accounting for less than 1% of the administered radioactivity) were identified as o-(acetoxymethyl)benzoic acid and 9-(acetoxymethyl)- $\alpha$ -carboxycinnethylin. They were the corresponding O-acetyl analogues of o-(hydroxymethyl)benzoic acid and 9-hydroxy- $\alpha$ -carboxycinnethylin, the principal metabolites of cinnethylin (Lee, Woodward and Stearns, *J. of Ag. and Food Chem.*, 1988, 36, 95-97).

#### Study 10 - A Comparison of the Disposition and Metabolism of $^{14}\text{C}$ -cinnethylin in Dogs and Rats

This study was designed to compare the elimination, disposition and metabolism of cinnethylin when orally administered to dogs with those observed in a parallel experiment with rats (Lee *et al.*, 1983).

Two male and two female dogs were orally dosed with a solution of  $^{14}\text{C}$ -cinnethylin in 1,2-propanediol at a dose rate of 15 mg/kg bw. Levels of radioactivity were measured in the excreta and in the blood throughout the study and in the tissues at sacrifice, 7 days after dosing.

Cinnethylin was rapidly eliminated in the dog via the urine and faeces. As found in previous studies with the rat, urine was the major route of elimination (55% of the administered dose after 3 days) with the faeces accounting for approximately 29% of the administered dose after 3 days.

Disposition in the body at sacrifice followed a similar pattern in dog as rat with very low levels in all tissues. Levels in whole blood were consistent with this picture with mean peak concentrations in both species being achieved in the first hour after dosing, followed by a rapid depletion of radioactive residues from the blood.

Complex metabolite profiles were observed in both urine and faeces and no attempt was made to isolate and identify individual metabolites. In order to provide the appropriate samples for comparative chromatography, five male rats were dosed with <sup>14</sup>C-cinmethylin in a parallel supplementary study. Comparative chromatography of dog and rat excreta by TLC and HPLC revealed close similarities in the nature of the metabolites although quantitative differences existed. The major dog metabolite was characterised as 2-hydroxypropyl cinmethylin benzoic acid which was also the major metabolite of cinmethylin rat excreta. This metabolite is formed by oxidation of the 2-methyl group on the aromatic ring to a carboxy group and hydroxylation of the isopropyl group on the cineole portion of the molecule (Edwards, 1989b).

### B.6.1.6. Summary of ADME

The toxicokinetics of cinmethylin have been largely investigated in rats via oral dosing only. There are two data sets available, a new/modern data set (3 studies, Table 6.1-32) using both <sup>14</sup>C-phenyl labelled cinmethylin and <sup>14</sup>C-cyclohexyl labelled cinmethylin in rats only, and an older set of studies conducted with <sup>14</sup>C-phenyl labelled cinmethylin only. The earlier set of studies are available as brief summaries and the original test reports were not considered by HSE. These summaries are included for completeness and supportive information only. There is also an *in vitro* comparative metabolism study, employing primary hepatocytes from humans, rats, dogs and rabbits exposed to <sup>14</sup>C-phenyl labelled cinmethylin and <sup>14</sup>C-cyclohexyl labelled cinmethylin. Furthermore, there is some toxicokinetic information available on cinmethylin and some of its metabolites from the rat and mouse chronic dosing toxicodynamic studies.

Table 6.1-32. Summary of new/modern toxicokinetic studies

Study reference	Study type	Dose level [mg/kg bw/d]	Remarks
██████, 2018	Determination of kinetic parameters in plasma, absorption, tissue distribution, excretion balance (urine, feces, bile)	Single oral low 15. Single oral high 350. Multiple oral high 350. Single i.v. 1.	An oral absorption value of 100 % is proposed. For post-hepatic systemic bioavailability, a value of 70 % is proposed and a default inhalation absorption value of 100 % is assumed.
██████, 2018a	Sample generation for investigation of metabolism in tissues and plasma at T <sub>max</sub>	Single oral low 15. Single oral high 350.	This study was performed to investigate further the distribution of cinmethylin and collect tissue samples for analysis of metabolites.
██████, 2018	Investigation of metabolism in urine, feces, bile, tissues, plasma	Single oral low 15. Single oral high 350. Multiple oral high 350.	Cinmethylin was rapidly and extensively metabolised with no significant, post-hepatic exposure to unchanged cinmethylin. No unchanged cinmethylin was detected in the bile.  Two urinary metabolites, M684H010 (2-hydroxymethyl benzoate) and M684H011 (2-hydroxypropyl cinmethylin benzoate) were present above 10 % of the administered dose.  There is some preferential metabolism for the (+)enantiomer.
<a href="#">Funk-Weyer &amp; Ufer, 2017</a>	Comparative <i>in vitro</i> metabolism study. Primary hepatocytes from humans, Wistar rats, Beagle dogs and New Zealand White rabbits	10 µM cinmethylin	No unique metabolites were identified from human hepatocytes.



### Absorption

Cinmethylin is well absorbed from the gastrointestinal tract (75.4 % to 98.5 % of the administered dose) in the dose range 15 - 350 mg/kg bw. The maximum plasma concentrations for oral administration, were achieved 1 hour after low dose (15 mg/kg bw) administration (both radiolabels) and 4 - 8 hours after high dose (350 mg/kg bw) administration (for the  $^{14}\text{C}$ -phenyl and  $^{14}\text{C}$ -cyclohexane radiolabels, respectively). For male rats administered 350 mg/kg bw cinmethylin ( $^{14}\text{C}$ -cyclohexane label), an earlier  $C_{\text{Max}}$ -value was also observed at 1 hour post dose. It is possible this indicates some biphasic absorption at the high dose. Absorption appears to be independent of dose, sex and position of the radiolabel.

Although uptake from the GIT (gastrointestinal tract) is extensive, it appears that post-hepatic systemic exposure to unchanged cinmethylin and/or its metabolites accounts for around 50 % of the administered dose, with a significant amount excreted in bile within 6 hours of gavage dosing (33.77 % to 56.72 % of the administered dose noted at the low dose and 12.56 % to 35.36 % of the administered dose at the high dose). It is possible that cinmethylin and/or its metabolites excreted into the bile within 6 hours of gavage dosing might not be systemically available.

Therefore, HSE has proposed an **oral absorption value of 100 %**. From this toxicokinetic experiment, dose corrected post hepatic systemic bioavailability values of 66 % and 73 % for males and females respectively were obtained and an overall average value of 70% can be calculated. This value is considered to be more robust and less uncertain than that derived from the bile-duct cannulation experiment. Overall, HSE proposes a **post-hepatic systemic bioavailability value of 70 %**. There are no data to determine the absorption of cinmethylin across the respiratory tract. However, based on the extensive oral absorption, **a default inhalation absorption value of 100 % can be assumed**. Dermal absorption of cinmethylin from its representative product is addressed in the CP-B6 document.

### Distribution

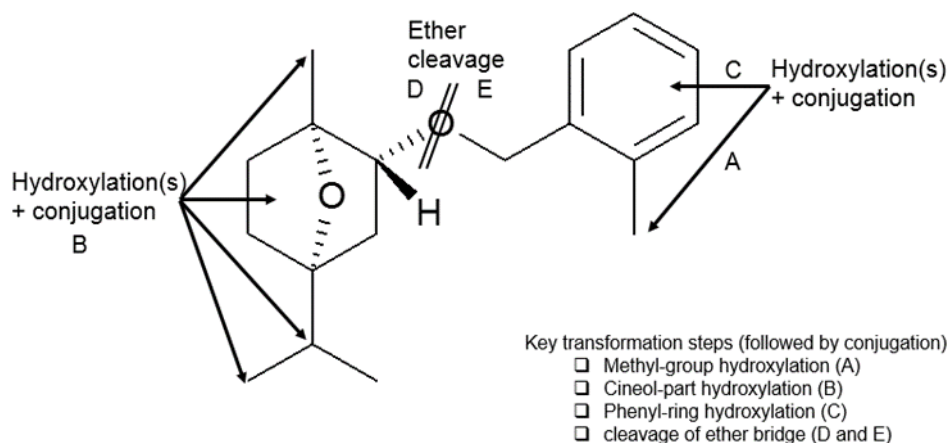
Distribution of radiolabelled cinmethylin and/or its metabolites was predominately to the organs of metabolism and excretion, the liver and kidneys and also the adrenals and adipose tissues. There was no evidence of retention in any organs/tissues at any dose level. Limited information on plasma concentrations of cinmethylin and four metabolites (M684H001, M684H010, M684H011 and M684H026) from lifetime toxicodynamic studies conducted in rats and mice confirmed that there is little or no detectable unchanged cinmethylin in blood plasma following repeated exposure. The levels of the metabolites in plasma were significantly higher than those of the parent in these studies.

### Metabolism

*In vivo* toxicokinetic investigations of metabolism found that cinmethylin was rapidly and extensively metabolised such that there was no significant, post-hepatic exposure to unchanged cinmethylin. The main biotransformation reactions identified are:

- Hydroxylation at the cyclohexane and/or benzyl ring
- Hydroxylation of the alkyl groups at the benzyl and/or cyclohexane ring
- Oxidation of the hydroxylated methyl group at the benzyl ring to a carboxy group
- Cleavage of the ether bridge
- Conjugation with glucuronic acid•
- Conjugation with glycine

Cinmethylin showing sites of key metabolic transformation reactions



Two urinary metabolites, M684H010 (2-hydroxymethyl benzoate) and M684H011 (2-hydroxypropyl cinmethylin benzoate) were present above 10 % of the administered dose. With the exception of M684H011 which was present in the urine at a higher concentration in low dose animals than in the high dose animals, there were no other sex, dose or positional differences in urinary, plasma or biliary metabolites. No unchanged cinmethylin was detected in plasma, urine or bile. The most abundant (13 – 21 % of the administered dose) metabolite in bile was M684H012 (M684H012a + M684H012b) abbreviated to cinmethylin benzyl alcohol glucuronide. No individual metabolites were present in the faeces above 6 % of the administered dose. M684H011 was the most prominent metabolite in faeces, present at 1.7 % to 5.8 % of the administered dose. Only a small number of plasma metabolites were identified (4 - 6) and none were present above 1 % of the administered dose. However, analysis of plasma levels of cinmethylin and four metabolites (M684H001 or cinmethylin benzoate, M684H010, M684H011 and M684H026 or 2-hydroxypropyl 2-hydroxycineol) from the rat and mouse chronic studies showed that the levels of these metabolites were significantly higher than those of the parent compound.

The ratio of the (-)/(+) enantiomers of unchanged cinmethylin in representative methanol extracts of liver and faeces shifted from 50:50 (-)/(+) in the starting material towards higher relative amounts of the (-) enantiomer and ranged from approximately 70:30 to 76:24 (-)/(+) in faeces extracts and from approximately 63:37 to 69:31 (-)/(+) in liver extracts. These data indicate some preferential metabolism for the (+) enantiomer.

Limited information from an *in vitro* comparative metabolism study employing primary hepatocytes from humans, rats, dogs and rabbits exposed to  $^{14}\text{C}$ -phenyl labelled cinmethylin and  $^{14}\text{C}$ -cyclohexyl labelled cinmethylin found no unique metabolites were formed by human primary hepatocytes.

#### Excretion

Excretion via both the urine and faeces is rapid, and essentially complete within 48 hours of oral dosing. There is no evidence for a preferential route, although excretion via the urine was slightly higher (52 – 60 % of the administered dose) than faecal excretion. Faecal excretion was mainly due to biliary elimination. The expired air is not a significant route of excretion for cinmethylin. There is no evidence for dose or sex-dependent differences in urinary or faecal excretion. Comparing urinary excretion in non-bile duct cannulated animals with bile duct cannulated animals suggests that there is some enterohepatic recirculation but it is of relatively minor importance. There do not appear to be any clear differences between single high dose and repeated high dose animals for both  $^{14}\text{C}$  cyclohexyl and  $^{14}\text{C}$ -phenyl labelled cinmethylin, in males or females.

There is no toxicokinetic information from other relevant routes of exposure. However, given the significant first-pass effect, quantitative differences in the degree of systemic exposure to unchanged cinmethylin and metabolites would be anticipated following inhalation or dermal exposure.

The earlier toxicokinetic studies, conducted with  $^{14}\text{C}$ -phenyl labelled cinmethylin only do not contradict the conclusions on the toxicokinetics of cinmethylin from the more modern data set.

Residue definition for body fluids and tissues in humans

The applicant has proposed a residue definition for monitoring in body fluids and tissues of cinmethylin and M684H011.

**Parent (cinmethylin):**

██████████ (2018) found that cinmethylin was rapidly and extensively metabolised with no significant, post-hepatic exposure to unchanged cinmethylin. No unchanged cinmethylin was detected in the bile. Compartments studied by ██████████ (2018) revealed the following metabolites were identified from liver, kidney and plasma of rats following oral administration at doses of 15 mg/kg and 350 mg/kg bw (██████████, 2018a) and in urine, faeces and bile samples from the bile duct cannulation experiment (██████████, 2018):

- Urine: No unchanged parent was detected.
- Bile: Unchanged cinmethylin was present at <1 % of the administered dose.
- Faeces: Unchanged cinmethylin was found at between 1.97 % and 4.5 % of the administered dose, but it is likely to represent unabsorbed material.
- Plasma: Unchanged cinmethylin was present at <0.1 % of the administered dose.
- Liver: Unchanged cinmethylin was present at 0.21 to 0.9 % of the administered dose.
- Kidney: unchanged cinmethylin (0.01 to 0.04 % of the administered dose).

In addition, information from lifetime toxicodynamic studies conducted in rats and mice (██████████, 2018 and ██████████, 2018d) confirmed that there is little or no detectable unchanged cinmethylin in blood plasma following repeated exposure.

Overall, no unchanged cinmethylin was detected in plasma, urine or bile; therefore the parent is not a suitable substance for the residue definition for body fluids in humans.

**Metabolites**

**M684H011:** ██████████ (2018) named M684H011 as one of two urinary metabolites present above 10 % of the administered dose. Compartments studied by ██████████ (2018) revealed the following:

- Urine: The principal urinary metabolite was M684H011 (>10 %)
- Bile: The prominent urinary metabolite, M684H011 was present in bile below 2 %.
- Faeces: No individual metabolites were present above 6 %. M684H011 was the most prominent metabolite, present at 1.74 to 5.75 % of the administered dose, but it could represent unabsorbed material metabolized by the intestinal flora.
- Plasma: Only a small number of plasma metabolites were identified (4 - 6) and none were present above 1 % of the administered dose.
- Liver: Only a small number of metabolites were identified (4 - 6) and none were present above 4.3 % of the administered dose.
- Kidney: Ten metabolites were identified, representing 0.01 to 0.33 % of the administered dose.

Overall, M684H011 was detected in urine at levels > 10 % and is therefore a suitable substance for the residue definition for body fluids, specifically urine, in humans. However, M684H011 was not detected at sufficient amounts in plasma to recommend it as a suitable substance for the residue definition for whole blood in humans.

**Other:** Compartments studied by ██████████ (2018) were checked for other suitable metabolites:

- Urine: Another significant metabolite, M684H010 was identified in the urine of rats.
- Bile: The most abundant component in bile from rats of all dose groups and for both labels was M684H012 (M684H012a + M684H012b), present at 13.17 to 20.87 %. None of the remaining metabolites with a chemical name were present above 2 % in the bile.
- Faeces: No individual metabolites were present above 6 %.
- Plasma: Only a small number of plasma metabolites were identified (4 - 6) and none were present above 1 % of the administered dose.
- Liver: Only a small number of metabolites were identified (4 - 6) and none were present above 4.3 % of the administered dose.
- Kidney: Apart from the unchanged cinmethylin (0.01 to 0.04 % of the administered dose), ten metabolites were identified, representing 0.01 to 0.33 % of the administered dose.

Overall, although M684H010 was identified as a significant metabolite in the urine of rats, in line with the requirement to keep the residue definition as simple as possible, HSE concludes that only M684H011 should be monitored in urine.

Overall, the following metabolite(s) may be suitable for the residue definition for body fluids and tissues in humans:

Substance (i.e. metabolite)	Compartment (in body fluid and/or tissue)	Justification (evidence from study data)
M684H011	Body fluid - urine	For both the <sup>14</sup> C-phenyl and <sup>14</sup> C-cyclohexyl radiolabelled cinmethylin, the principal urinary metabolite was M684H011 (12.87 – 29.87 %) (██████████, 2018)

No other metabolites were detected at sufficient amounts in compartments analysed to recommend as a suitable substances for the residue definition for body fluids and tissues in humans.

### B.6.2. ACUTE TOXICITY

The acute toxicity of cinmethylin was investigated in multiple studies, including existing and new/modern studies, conducted via the oral, dermal and inhalation routes. Skin and eye irritation has been investigated sequentially in *in vitro* and then *in vivo* studies. Skin sensitisation has been investigated in a Guinea Pig Maximisation Test (GPMT) and two Buehler tests. None of the submitted studies have been evaluated at EU level before. Modern studies were conducted according to relevant OECD test guidelines and are GLP compliant. Older studies were not conducted to OECD test guidelines and not all studies are GLP compliant. No phototoxicity studies have been provided as testing is not triggered according to data requirements in Reg 283/2013. Validated methods of analysis for single exposure (ie acute) studies, except inhalation studies, are not required.

The applicant submitted the following justification for acute toxicity testing:

‘The acute toxicity of BAS 684 H [cinmethylin] was already investigated between 1981-1988, partly according GLP. However, the current production process is different to that used in the 80s and especially registration in Asian countries requires acute GLP-conform toxicity studies with the material produced under the current production process. Therefore, all acute studies had to be repeated for global registration. Those new/modern studies are now used for classification, and at least for skin sensitisation resulted in the need for classification although two former studies did not indicate this property. For skin and eye irritation, the chosen approach was based on sequential *in vitro* followed by *in-vivo* testing to comply with global data requirements, including Regulation (EU) No 283/2013.’

HSE accepts the majority of the above justification for conducting *in vivo* studies. It is important that the test material is representative of the current/proposed specification for the active substance. It is also important to have reliable GLP and OECD test guideline compliant studies. New/modern studies for skin and eye effects were conducted in a sequential manner (*in vitro* followed by *in vivo*). However, conducting studies for another global regulation is not an acceptable justification for initiation of new *in vivo* tests. HSE considers that the new *in vivo* irritation study was unnecessary. The study has been evaluated for transparency but it is not relied upon.

#### B.6.2.1. Oral

The acute oral toxicity of cinmethylin was investigated in three studies, two in rats and one in mice. Two studies are older (conducted in the 1980s) and whilst they were conducted according to GLP they were not conducted according to the latest relevant OECD test guidelines. A modern (conducted in 2016) study was therefore conducted according to GLP and to the latest relevant OECD test guidelines. This study was used as they key study for the evaluation of acute oral toxicity and the other studies were regarded as supplemental.

*1) New/modern study in rats*

<b>Author(s)</b>	██████████
<b>Study title</b>	BAS 684 H (Cinmethylin) Acute oral toxicity study in rats
<b>Study reference</b>	██████████, 2016a BASF DocID 2016/1273410
<b>Study dates</b>	02/08/2016 – 24/08/2016
<b>Test facility</b>	██
<b>Test substance</b>	Cinmethylin; BAS 684 H
<b>Purity (%)</b> <b>Batch no.</b>	93.5 COD-002038 (-) / (+) ratio = 48:52
<b>Test animals</b>	Rat. Wistar / CrI:WI (Han) SPF. Female Fasted, at least 16 h before administration.
<b>Groups</b>	2 groups (administrations 1 and 2). 3/group
<b>Dose</b>	2000 mg/kg bw (administrations 1 and 2). Volume: 1.95 mL/kg bw.
<b>Route</b>	Oral gavage. Single administration.
<b>Vehicle</b>	None, undiluted.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 423 (2001) – The acute toxic class method (the current test guidelines). (EC) No 440/2008 of 30 May 2008 - Part B No. L 142, EPA 870.1100, JMAFF No 12 Nosan No 8147 (2000)
<b>Deviation</b>	None.
<b>Impact of deviation(s)</b>	N/A.
<b>Acceptable</b>	Yes. Key study used in the evaluation of acute oral toxicity.
<b>LD<sub>50</sub></b>	LD <sub>50</sub> > 2000 mg/kg bw (female only)

Methods

In a GLP and OECD test guideline compliant acute oral toxicity study, two groups of 3 female Wistar rats were sequentially administered a single oral dose (2000 mg/kg bw) via gavage of undiluted cinmethylin (i.e. a limit test). A check for any dead or moribund animals was made at least once each workday. Observations for clinical signs were performed several times on the day of administration. Thereafter, animals were observed at least once a day for a total of 2 weeks. Body weights were recorded at day 0 (prior to dosing), weekly thereafter and on the last day of observation. The animals were sacrificed by carbon dioxide inhalation and subsequently subjected to macroscopic examination on the last day of the observation period. A validated method of analysis for single exposure gavage studies is not required.

Results

*Mortality:* No mortality occurred.

*Clinical observations:* Clinical signs in the first test group revealed an impaired general state and piloerection, in all animals, from hours 3 - 5 after administration. Clinical signs in the second test group revealed impaired general state and piloerection, in all animals, from hour 0 - 5 after administration. In addition, all animals showed diarrhoea from hour 2 - 5, followed by reduced defecation on day 1.

*Body weight:* In the first test group all animals gained weight in a normal range during the first week but showed a stagnation of body weight during the second week. This effect is observed at times in the rat strain used (Wistar / CrI:WI (Han) SPF), because in the age range required in the OECD test guidelines (8 – 12 weeks old) female animals have already reached the phase of slow growth. The body weights of animals increased within the normal range throughout the study period in all animals of the second 2000 mg/kg bw test group. There were no adverse, treatment-related changes in body weight gain in treated animals.

*Necropsy:* There were no macroscopic pathological findings in the animals sacrificed at the end of the observation period.

#### Conclusion

Under the conditions of this GLP and OECD test guideline compliant study, the acute oral LD<sub>50</sub> of cinmethylin was greater than 2000 mg/kg bw in female rats. Cinmethylin is not acutely toxic by the oral route and, in accordance with Regulation (EC) 1272/2008, does not meet the criteria for classification for acute oral toxicity.

(██████, 2016a)

#### **2) Old study in rats**

<b>Author(s)</b>	██
<b>Study title</b>	Acute Oral Toxicity of SD95481 in the Rat
<b>Study reference</b>	██████████, 1982 CI-411-001
<b>Test substance</b>	SD95481 (BAS 684 H) also known as cinmethylin
<b>Study dates</b>	26/03/1981 – 09/04/1981
<b>Test facility</b>	██
<b>Purity (%)</b>	93.3
<b>Batch no.</b>	1-3-0-0
<b>Test animals</b>	Rat Fischer 344. Male and female. Fasted overnight.
<b>Groups</b>	5/sex/dose
<b>Dose</b>	0, 1.0, 1.8, 3.2 and 5.6 mL/kg Equivalent to 1016, 1829, 3251 and 5690 mg/kg bw
<b>Route</b>	Oral gavage. Single administration.
<b>Control</b>	Deionised water (5 mL/kg).
<b>GLP</b>	Compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	Albeit not stated in the study report, the study is broadly consistent with OECD TG No. 401 (1981). However, the following deviations from this test guideline occurred: <ul style="list-style-type: none"> <li>• Bodyweights of rats which died during the study were not measured at the time of death.</li> <li>• Clinical signs of toxicity were not observed on day 10.</li> </ul>
<b>Impact of deviation(s)</b>	Minor – these deviation are minimal and are not considered to affect the validity of the study.
<b>Acceptable</b>	Yes, however, regarded as supplemental information because the study was not OECD test guideline compliant.
<b>LD<sub>50</sub></b>	LD <sub>50</sub> 4550 mg/kg bw (male and female)

#### Methods

In a GLP compliant acute oral toxicity study, groups of 5 male and 5 female fasted Fischer rats were administered a single oral dose via gavage of undiluted cinmethylin at dose levels of 1.0, 1.8, 3.2 and 5.6 mL/kg bw, equivalent to 1016, 1829, 3251 and 5690 mg/kg bw (calculated using a density of 1.016 g/mL). Control rats received 5.0 mL/kg bw deionised water. A dose range-finding study was conducted to aid selection of doses used in the main study. Observations for clinical signs of toxicity were made hourly for a 6 hr period, at 24 hr and twice daily thereafter until termination of the study on day 14.

Results

**Mortality:** At 5690 mg/kg bw, four rats of each sex died on day 2, while one female dosed at 3251 mg/kg bw died on day 3. Lethalities generally occurred overnight between days 1 – 3 (Table 6.2-1). Using a computerised log-probit method of Finney, the acute oral LD<sub>50</sub> for cinmethylin in rats was calculated to be 4.49 mL/kg bw (equal to 4550 mg/kg bw) with a 95 % confidence limits 3.62 - 5.69 mL/kg bw (equal to 3672 - 5771 mg/kg bw).

Table 6.2-1. Acute oral toxicity in rats - mortality

Sex	Dose		Mortality / animals treated	Time of occurrence [day]
	[mL/kg bw]	[mg/kg bw]		
Males	5.6	5680	4/5	2
	3.2	3246	0/5	-
	1.8	1826	0/5	-
	1.0	1014	0/5	-
	0	0	0/5	-
Females	5.6	5680	4/5	2
	3.2	3246	1/5	3
	1.8	1826	0/5	-
	1.0	1014	0/5	-
	0	0	0/5	-

**Clinical observations:** The most common clinical signs (seen in 28 – 35 treated animals compared to 0 – 4 controls) were ataxia, hypoactivity, piloerection and lacrimation (eye discharge). These occurred at all doses, though more frequently with increasing dose, and generally resolved by days 2 - 4. Other signs that mainly occurred at the higher doses (seen in 2 – 13 treated animals compared to 0 – 1 controls) included hypothermia, prostration, hypotonus, pale extremities, ptosis, hypopnea, loss of righting reflex, depression of myotactic placing reflex, oral discharge, red discharge from nose and/or eye and pain response blocked. Other occasional signs (seen in 2 – 15 treated animals compared to 0 – 1 controls) were hunched posture, chewing movements, diarrhoea, hypersensitive to touch, non-retracted penis, swollen testes, dyspnea, unkempt appearance, miosis and no observed faeces.

**Body weight:** The mean body weight of the test groups increased throughout the study period within the normal range. There were no adverse, treatment-related changes in body weight gain in treated animals.

**Necroscopy:** Observations at necropsy of unscheduled deaths were limited by generalised autolysis. One female in each of the 3246 and 5680 mg/kg bw dose groups showed slight fatty change of the liver (Table 6.2-2); these findings are considered to be treatment-related. All other findings (slight enlargement of pituitary glands, yellow fluid on mucosal surface of the stomach, bilateral luminal dilation with clear fluid of the bladder, busa distended (left) with clear fluid in the ovary and reddened/dark thymus) were considered incidental and not treatment-related because they occurred at low incidences and were not seen to be dose-related and/or they also occurred in the concurrent control.

Table 6.2-2. Acute oral toxicity in rats - necropsy findings

Sex		Males					Females				
Dose	[mL/kg bw]	0	1.0	1.8	3.2	5.6	0	1.0	1.8	3.2	5.6
	[mg/kg bw]	0	1014	1826	3246	5680	0	1014	1826	3246	5680
Day of lethality		14	14	14	14	14	14	14	14	14	14
Liver											
- slight fatty change		-	-	-	-	-	-	-	-	1	1
- focal tension lipidosis		1	-	-	-	-	-	-	-	-	-
- parenchymal nodule(s)		-	-	1	-	-	-	-	-	-	-
- small nodule at site of ligamentous attachemnt		-	-	1	1	-	-	-	-	-	-



Conclusion

Under the conditions of this GLP compliant study, the acute oral LD<sub>50</sub> of cinmethylin was found to be 4.49 mL/kg bw (equal to 4550 mg/kg bw) in rats. This study supports the key acute oral toxicity study (Höger, 2016d). Cinmethylin is not acutely toxic by the oral route and, in accordance with Regulation (EC) 1272/2008, does not meet the criteria for classification for acute oral toxicity.

(██████████, 1982)

**3) Old study in mice**

<b>Author(s)</b>	██████████
<b>Study title</b>	Acute Oral Toxicity of SD95481 in the Mouse
<b>Study reference</b>	██████████, 1982 CI-411-002
<b>Study dates</b>	06/01/1982 – 20/01/1982
<b>Test facility</b>	██████████
<b>Test substance</b>	BAS 684 H (SD 95481) also known as cinmethylin
<b>Purity (%)</b>	Not specified.
<b>Batch no.</b>	513B
<b>Test animals</b>	Mouse B6C3F1 Male and female. Fasted overnight.
<b>Groups</b>	Dose range-finding study: 1/sex/dose Main study: 5/sex/dose
<b>Dose</b>	Dose range-finding study: 1.0, 2.5 and 5.0 mL/kg, equivalent to 1014, 2536 and 5072 mg/kg bw (no mice died) Main study: 5 mL/kg (limit dose), equivalent to 5072 mg/kg bw.
<b>Route</b>	Oral gavage. Single administration.
<b>Vehicle</b>	Normal saline (5.0 mL/kg).
<b>GLP</b>	Compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	Albeit not stated in the study report, the study is broadly consistent with OECD TG No. 401 (1981) with no deviations noted.
<b>Impact of deviation(s)</b>	N/A
<b>Acceptable</b>	Yes.
<b>LD<sub>50</sub></b>	LD <sub>50</sub> > 5000 mg/kg bw (male and female)

Methods

A dose range-finding study was conducted to aid selection of doses used in the main study; no mice died. In a GLP compliant acute oral toxicity study, groups of five male and female fasted B6C3F1 mice were administered a single oral dose via gavage of undiluted cinmethylin at a dose level of 5.0 mL/kg bw, equivalent to 5072 mg/kg bw (calculated using a density of 1.016 g/mL). Control mice received 5.0 mL/kg bw normal saline. Observations for clinical signs of toxicity were made hourly for a 6 hr period, at 24 hr and twice daily thereafter until termination of the study on day 14.

Results

**Mortality:** Two deaths occurred overnight on the day of dosing. One female in the control group died due to incorrect gavage and one male in the treated group died of undetermined cause (Table 6.2-3). The acute oral LD<sub>50</sub> was therefore found to be greater than 5 mL/kg bw, equivalent to 5072 mg/kg bw.

Table 6.2-3. Acute oral toxicity in mice - mortality

Sex	Dose		Mortality / animals treated	Time of occurrence [day]
	[mL/kg bw]	[mg/kg bw]		
Males	5.0	5072	1/5	1
	0	0	0/5	-
Females	5.0	5072	0/5	-
	0	0	1/5	1

*Clinical observations:* Clinical signs of toxicity were observed within 1 hr after administration; animals recovered over 1 – 4 days. The prominent treatment-related clinical signs of toxicity (seen in 3 – 9 treated animals compared to 1 control (the mouse that died overnight due to misgavage)) consisted of mucoid diarrhoea, diarrhoea, soft stool, polyuria, hypoactivity, and hunched posture. In addition the following sporadic signs of toxicity were seen (in 1 – 2 treated animals compared to 0 controls): unsteady stance and hypothermia.

*Body weight:* The mean body weight of the test groups increased throughout the study period within the normal range. There were no adverse, treatment-related changes in body weight gain in treated animals.

*Necropsy:* Observations at necropsy of unscheduled deaths revealed rupture of the oesophagus in one female in the control group; this occurred accidentally during the gavage procedure. No explanation for the death of the one male in the treated group could be obtained by necropsy. In animals that were sacrificed on schedule, no remarkable pathological changes were observed.

#### Conclusion

Under the conditions of this GLP compliant study, the acute oral LD<sub>50</sub> of cinmethylin was found to be greater than 5000 mg/kg bw in mice. This study supports the key acute oral toxicity study (■■■■■, 2016d). Cinmethylin is not acutely toxic by the oral route and, in accordance with Regulation (EC) 1272/2008, does not meet the criteria for classification for acute oral toxicity.

(■■■■■, 1982)

#### ***Summary of acute oral toxicity***

The acute oral toxicity of cinmethylin was investigated in three studies, two in rats (one old and one new/modern study) and one in mice (old study). All three studies showed low toxicity via the oral route; in both the rat and mouse. Older studies were tested to higher doses and showed the LD<sub>50</sub> to be close to 5000 mg/kg bw. The new/modern study was tested to the limit dose of 2000 mg/kg bw (according to modern OECD test guidelines) and showed the LD<sub>50</sub> to be greater than 2000 mg/kg bw.

**B.6.2.2. Dermal**

The acute dermal toxicity of cinmethylin was investigated in two studies in two different species (in rats and rabbits). One study is older (conducted in the 1980s) and was not conducted according to GLP or the latest relevant OECD test guidelines. A modern (conducted in 2016) study was therefore performed according to GLP and to relevant OECD test guidelines. This study was used as the key study for the evaluation of acute dermal toxicity and the other study was regarded as supplemental.

**1) New/modern study in rats**

<b>Author(s)</b>	[REDACTED]
<b>Study title</b>	BAS 684 H (Cinmethylin) - Acute dermal toxicity in rats
<b>Study reference</b>	[REDACTED], 2016a 2016/1225928
<b>Study dates</b>	12/07/2016 – 27/07/2016
<b>Test facility</b>	[REDACTED]
<b>Test substance</b>	BAS 684 H (cinmethylin)
<b>Purity (%)</b>	93.5
<b>Batch no.</b>	COD-002038 (-) / (+) ratio = 48:52
<b>Test animals</b>	Rat Wistar / CrI:WI (Han) SPF Male and female
<b>Groups</b>	5/sex/dose
<b>Dose</b>	5000 mg/kg bw (limit dose) Volume: 4.87 mL/kg bw Undiluted.
<b>Route</b>	Single dermal dose.
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 402 (1987) – the fixed dose procedure (the current guidelines were adopted in 2017). (EC) No 440/2008 of 30 May 2008 - Part B No. L 142, EPA 870.1200, JMAFF No 12 Nosan No 8147 (2000).
<b>Deviation</b>	Compared with the currently valid OECD TG No. 402 (2017): <ul style="list-style-type: none"> <li>A single dermal limit dose of 5,000 mg/kg bw was used in this study. The limit dose quoted in the OECD TG No. 402 1981 and 2017 is 2,000 mg/kg bw.</li> </ul>
<b>Impact of deviation(s)</b>	N/A.
<b>Acceptable</b>	Yes. Key study used in the evaluation of acute dermal toxicity.
<b>LD<sub>50</sub></b>	LD <sub>50</sub> > 5000 mg/kg bw (male and female)

**Methods**

In a GLP and OECD test guideline compliant acute dermal toxicity study, one group of 5 male and 5 female Wistar rats were exposed to a single dermal dose of 5000 mg/kg bw undiluted cinmethylin. HSE notes that this is higher than the limit dose quoted in the OECD test guidelines. Cinmethylin was applied on to the clipped skin (dorsal and dorso-lateral parts of the trunk) and covered by semi-occlusive dressing for 24 hr.

A check for dead or moribund animals was made at least once each workday. Animals were observed for clinical signs several times on the day of administration, and then at least once a day for a total of 14 days. Skin findings were scored 30 – 60 min after removal of the dressing, according to the Draize method, weekly thereafter and on the last day of observation. Individual body weights were determined shortly before administration (day 0), weekly thereafter and on the last day of administration. The animals were sacrificed and subjected to macroscopic examination on the last day of the observation period. A validated method of analysis for single exposure dermal studies is not required.

Results

*Mortality:* No mortality occurred.

*Clinical observation:* There were no signs of systemic toxicity or skin effects observed in any animal.

*Body weight:* The body weight of the male animals increased within the normal range throughout the study period. The body weight of the female animals increased within the normal range throughout the study period with two exceptions – one female showed stagnated body weight gain during the first week only and one female showed a slight increase in body weight gain during the second week. However, this was not considered adverse or treatment-related.

*Necropsy:* No macroscopic pathologic abnormalities were noted in the animals examined at the end of the study.

Conclusion

Under the conditions of this GLP and OECD test guideline compliant study the acute dermal LD<sub>50</sub> of cinmethylin was found to be greater than 5000 mg/kg bw in rats. Cinmethylin is not acutely toxic by the dermal route and, in accordance with Regulation (EC) 1272/2008, does not meet the criteria for classification for acute dermal toxicity.

(██████████ 2016a)

**2) Old study in rabbits**

<b>Author(s)</b>	██████████
<b>Study title</b>	Acute Toxicology Studies with Technical SD 95481 Acute Dermal Toxicity of Technical SD 95481
<b>Study reference</b>	██████████, 1981a CI-412-001, ROT 36
<b>Study dates</b>	27/03/1981
<b>Test facility</b>	██
<b>Test substance</b>	BAS 684 H (SD 95481) Undiluted
<b>Purity (%)</b> <b>Batch no.</b>	93.3 513A (1-3-0-0) (-) / (+) ratio = not specified.
<b>Test animals</b>	Rabbit New Zealand White Male and female
<b>Groups</b>	3/sex/treatment (treatment groups – one with normal skin and another group with intentionally abraded skin) 4/sex (control group)
<b>Dose</b>	Single dose of 2 mL/kg bw (equivalent to 2029 mg/kg bw)
<b>Route</b>	Dermal.
<b>Control</b>	Deionised water.
<b>GLP</b>	Not compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	The study is broadly consistent with OECD TG No. 402 (1981). However, the following deviations from this test guideline were noted: <ul style="list-style-type: none"> <li>Only 3 instead of 5 animals of both sexes with intact skin were exposed, since another group of 3 animals per sex had abraded skin.</li> <li>Dermal exposure was performed under occluded conditions, whereas semi-occluded exposure is used in modern studies.</li> </ul>
<b>Impact of deviation(s)</b>	Minor – these deviation are minimal and are not considered to affect the validity of the study.
<b>Acceptable</b>	Yes, however, regarded as supplemental information because the study was not GLP or

	OECD test guideline compliant.
<b>LD<sub>50</sub></b>	> 2.0 mL/kg, equivalent to 2029 mg/kg bw (male and female)

### Methods

Two groups of 3 male and 3 female New Zealand White rabbits were exposed to a single dermal dose of 2 mL/kg bw (equivalent to 2029 mg/kg bw) undiluted cinmethylin. The clipped skin of the one treatment group was intact, whereas the skin of the second treatment group was intentionally abraded. Additionally, two groups of 4 male and 4 female rabbits with intact or abraded skin were exposed to dionised water and served as controls. Cinmethylin was administered on to the clipped skin (backs of the trunks) and covered by occlusive dressing for 24 hr. Thereafter, the remaining substance was removed by damp towel. Mortality and clinical signs were recorded several times on the day of administration, and twice daily thereafter. Individual skin findings were recorded immediately after removal of the occlusive dressing (day 1) and on the last day of observation using a scoring system that is in line with Draize. Body weights were determined shortly before administration (day 0), weekly thereafter and at the end of the study. At termination, necropsy with gross pathology examinations was performed. The observation period lasted for 14 days. Only findings of the animals with the intact skin are summarised below.

### Results

*Mortality:* No mortality occurred.

*Clinical observation:* Clinical signs of toxicity were limited to diarrhoea in one control female on day 2 and in one treated male on day 1. However, due to isolated and transient occurrence, these findings were not considered treatment-related.

The majority of control rabbits showed barely perceptible erythema and oedema at 24 hours (Table 6.2-4). Relative to controls, the severity of dermal reaction was increased in most treated rabbits, the majority having erythema pale red in colour with definable edges, and a definable area of oedema, not raised more than 1 mm. No skin findings were observed on day 14 in any animal.

Table 6.2-4. Acute dermal toxicity in rabbits - skin findings

Time after application	Sex	Animal	Control group [0 mg/kg bw]		Treatment group [2000 mg/kg bw]	
			Erythema	Oedema	Erythema	Oedema
24 h	♂	1	0	1	1	1
		2	1	1	2	2
		3	1	0	1	1
		4	1	1	-	-
		<b>mean</b>	<b>0.75</b>	<b>0.75</b>	<b>1.3</b>	<b>1.3</b>
	♀	1	1	1	2	2
		2	1	0	1	0
		3	1	0	2	1
		4	1	1	-	-
		<b>mean</b>	<b>1.0</b>	<b>0.5</b>	<b>1.67</b>	<b>1.0</b>
	♂+♀	<b>overall</b>	<b>0.88</b>	<b>0.63</b>	<b>1.50</b>	<b>1.17</b>

Skin reaction scores:

Erythema: 0 = no erythema; 1 = barely perceptible (edges of area not defined); 2 = pale red in color and edges definable; 3 = definite red in color and area well-defined; 4 = beet or crimson red in color

Oedema: 0 = no oedema; 1 = barely perceptible (edges of area not defined); 2 = area definable and not raised more than 1 mm; 3 = area well-defined and raised approx. 1 mm; 4 = area raised more than 1 mm

*Body weight:* Body weight development was not affected by the treatment.

*Necropsy:* No macroscopic pathologic abnormalities were noted in the animals examined at the end of the study.

Conclusion

Under the conditions of this study the acute dermal LD<sub>50</sub> of cinmethylin was found to be greater than 2000 mg/kg bw in rabbits. Whilst not conducted according to GLP or relevant latest OECD test guidelines, this study supports the key acute dermal toxicity study ([REDACTED], 2016a). Cinmethylin is not acutely toxic by the dermal route and, in accordance with Regulation (EC) 1272/2008, does not meet the criteria for classification for acute dermal toxicity.

([REDACTED], 1981a)

**Summary of acute dermal toxicity**

The acute dermal toxicity of cinmethylin was investigated in two studies, one in rats (new/modern study) and one in rabbits (old study). Both studies showed low toxicity via the dermal route; in both the rat and rabbit. The older study was tested to a lower top dose (2029 mg/kg bw) and showed the LD<sub>50</sub> to be greater than 2000 mg/kg bw. The new/modern study was tested to the limit dose of 5000 mg/kg bw (HSE notes that this is higher than the limit dose quoted in the modern OECD test guidelines) and showed the LD<sub>50</sub> to be greater than 5000 mg/kg bw.

**B.6.2.3. Inhalation**

The acute inhalation toxicity of cinmethylin was investigated in two studies in rats, in two different strains. One study is older (conducted in the 1980s) and whilst it was conducted according to GLP it was not conducted according to the latest relevant OECD test guidelines. A modern (conducted in 2017) study was therefore conducted according to GLP and to the latest relevant OECD test guidelines. This study was used as they key study for the evaluation of acute inhalation toxicity and the other study was regarded as supplemental.

**1) New/modern study in rats**

<b>Author(s)</b>	[REDACTED]
<b>Study title</b>	BAS 684 H (Cinmethylin) Acute inhalation toxicity study in Wistar rats 4-hour liquid aerosol exposure (nose only)
<b>Study reference</b>	[REDACTED], 2017 BASF Doc ID: 2017/1068662
<b>Study dates</b>	7/02/2017 – 22/02/2017
<b>Test facility</b>	[REDACTED]
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b>	93.5
<b>Batch no.</b>	COD-002038 (-) / (+) ratio = 48:52
<b>Test animals</b>	Rat Wistar, CrI:WI(Han) Male and female
<b>Groups</b>	5/sex
<b>Dose</b>	5.268 mg/L (analytical concentration) (limit test) 4 hours
<b>Route</b>	Inhalation, nose only.
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 403 (2009) (the current test guideline) Commission Regulation (EC) No 440/2008 - Part B No. B.2, EPA 870.1300
<b>Deviation</b>	The mass median aerodynamic diameters (MMADs) were within the range recommended by the guideline (1 to 4 µm), however, the geometric standard deviations were outside of the range recommended in the guideline (1.5 to 3.0).
<b>Impact of deviation(s)</b>	Minor – this deviation is minimal and is not considered to affect the validity of the study.
<b>Acceptable</b>	Yes. Key study used in the evaluation of acute inhalation toxicity.
<b>LC<sub>50</sub></b>	> 5.3 mg/L (male and female) (4-hr, aerosol)

Methods

In a GLP and OECD test guideline compliant acute inhalation toxicity study (limit test), groups of 5 male and 5 female Wistar rats were exposed (nose only) to a liquid aerosol of cinmethylin at a concentration of 5.268 mg/L for 4 hr. Animals were observed for 14 days. The mass median aerodynamic diameters (MMADs) were within the range recommended by the guideline (1 to 4 µm), however, the geometric standard deviations were outside of the range recommended in the guideline (1.5 to 3.0) (Table 6.2-5). This was not considered to invalidate the results of the study. A method for the dose verification of cinmethylin in air (Catchpole & Hidding, 2017a; 2017/1032967) was evaluated and was considered validated (see Volume 3 CA B5, section B.5.1.2).

Table 6.2-5. Acute inhalation toxicity in rats – particle size measurements

Sample	MMAD [µm]	Geometric standard deviation
1	1.5	3.3
2	1.4	3.4

Results

*Mortality:* No mortality occurred at the tested concentration (5.268 mg/L) during the study period (14 days). Based on the absence of mortality the following LC<sub>50</sub> values were determined:

Acute 4-h inhalation LC<sub>50</sub> (both sexes combined): > 5.268 mg/L ( $p < 0.01$ )  
 Acute 4-h inhalation LC<sub>50</sub> (male rats): > 5.268 mg/L ( $p < 0.05$ )  
 Acute 4-h inhalation LC<sub>50</sub> (female rats): > 5.268 mg/L ( $p < 0.05$ )

*Clinical observations:*Table 6.2-6. Acute inhalation toxicity in rats – clinical observations

Observation	Male (n=5)		Female (n=5)	
	Animals with signs	Observed	Animals with signs	Observed
Eye, semiclosed eyelid	2	d0	-	-
Eye, closed eyelid	-	-	1	d0
Fur, substance contaminated	5	d0	5	d0 – d4
Hunched posture	2	d0	1	d0
Nose, red discharge	1	d0	2	d0
Nose, red encrusted	-	-	1	d1
Respiration, abdominal	3	d0 – d5	2	d0 – d4
Respiration, accelerated	5	h1 – d1	5	h1 – d5
Respiration, laboured	3	d2 – d8	1	d2 – d12
Respiration, sounds	-	-	1	d0 – d1; d8 – d12
Piloerection	-	-	1	d0 – d1
Plough nose-first into bedding	1	d0	4	d0

Clinical signs of toxicity were observed in some animals immediately after exposure and predominantly during the first week post-exposure. No clinical signs of toxicity were detected in male animals during the post exposure observation period from study day 9 onwards. No abnormalities were detected in female animals during the post exposure observation period from study day 13 onwards.

*Body weight:* The mean body weights of the animals decreased during the first post exposure observation day but increased thereafter. There were no adverse, treatment-related changes in body weight gain in treated animals.

*Necropsy:* No gross pathological abnormalities were detected during the necropsy in the animals at the termination of the study.

Conclusion

Under the conditions of this GLP and OECD test guideline compliant study in Wistar rats, a 4-hr LC<sub>50</sub> of > 5.3 mg/L (aerosol) was calculated for males and females, considered both separately and combined. Therefore, cinmethylin is not acutely harmful by the inhalation route and does not meet the criteria for classification for acute inhalation toxicity in accordance with Regulation (EC) 1272/2008.

(██████████, 2017)

**2) Old study in rats**

<b>Author(s)</b>	██████████
<b>Study title</b>	Acute 4-hour inhalation study in rats with CINCH technical herbicide
<b>Study reference</b>	██████████, 1986 CI-413-001
<b>Study dates</b>	22/10/1985 – 10/12/1985
<b>Test facility</b>	██████████
<b>Test substance</b>	BAS 684 H (CINCH technical)
<b>Purity (%)</b>	91.8
<b>Batch no.</b>	513 P
<b>Test animals</b>	Rats Fischer 344 Male and female
<b>Groups</b>	6/sex/dose
<b>Dose</b>	0.9, 2.2 and 3.5 mg/L (the highest concentration which could be generated) 4 hr exposure.
<b>Route</b>	Inhalation, whole body exposure.
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	Albeit not stated in the study report, the study is broadly consistent with OECD TG No. 403 (1981) with no deviations noted.
<b>Impact of deviation(s)</b>	N/A.
<b>Acceptable</b>	Yes.
<b>LC<sub>50</sub> (4-hr)</b>	> 3.5 mg/L (male and female) (maximum feasible concentration) (aerosol)

Methods

In a GLP compliant acute inhalation toxicity study, groups of 6 male and 6 female Fischer rats were exposed (whole-body) to a liquid aerosol of cinmethylin at concentrations of 0.9, 2.2 and 3.5 mg/L (maximum feasible concentration) for 4 hr. Animals were observed for 14 days.

The measurements of particle-size distribution revealed mass median aerodynamic diameters (MMAD) in the respirable range of 2.2 to 3.4 µm, with geometric standard deviations in the range of 1.7 to 2.2 µm, respectively. These values are within the ranges recommended in OECD TG No. 403 (2009).

A validated method of analysis has not been submitted (see Volume 3 CA B5, section B.5.1.2).

Results

**Mortality:** Two females of the top concentration group were found dead on days 3 and 4 respectively (Table 6.2-7). No other mortalities were observed during the study period. Based on the obtained mortality data, the LC<sub>50</sub> value was determined to be > 3.5 mg/L (the maximum technically feasible concentration).



Table 6.2-7. Acute inhalation toxicity in rats - mortality

Concentration [mg/L]	Toxicological results *	Duration of signs	Time of death	Mortality [%]	LC <sub>50</sub> [mg/L/4 h]
Male					
3.5	0/6/6	d0-d14	-	0	> 3.5
2.2	0/6/6	d0	-	0	
0.9	0/6/6	d0	-	0	
Female					
3.5	2/6/6	d0-d4	d 3, d 4	33	> 3.5
2.2	0/6/6	d0-d1	-	0	
0.9	0/1/6	d0, d7	-	0	

\* Number of animals which died/number of animals with clinical signs/number of animals used  
d = day; on day 0 observation was made direct after exposure

*Clinical observations:* Following exposure to the top concentration (3.5 mg/L), all animals were visibly wet, and exhibited chewing and rubbing the face against the cage bottom (described as "burrowing"). By day 1, the chewing and burrowing had stopped for all but one female, which had chewing recorded in the morning but not thereafter. The wet fur had disappeared in most rats by about 3 to 4 days but persisted in a few rats for the entire 14-day period. By day 3, all female rats had recovered completely except for the two which died. By day 6, all but two males had completely recovered. These two males continued to have wet fur, decreased activity, occasional chromodacryorrhea, hunched posture, and tip-toe gait during the remainder of the 14-day observation period.

Following exposure to the mid concentration (2.2 mg/L), all males and females had chewing and burrowing activity, along with nasal discharge, salivation and scratching, but no wet fur. By day 1 all rats were normal except two females, which had polyuria. One of them also showed hunched posture and tip-toe gait. Both of these rats had completely recovered by day 2.

Following exposure to the low concentration (0.9 mg/L), all males showed chewing, burrowing, nasal discharge, salivation and scratching. Only one female had signs of nasal discharge and burrowing. There was no evidence of wet fur. By day 1, all rats recovered and were free of any kind of clinical signs of toxicity, with the exception of one female that showed chromodacryorrhea in the afternoon of day 7 only.

*Body weight:* Body weight development of the low- and mid-concentration males was not affected by the treatment. Top concentration males showed transient weight loss during the first study week but gained weight during the second study week. Low- and mid-concentration females revealed a body weight stagnation during the first study week but gained weight during the second study week. Top-concentration females revealed body weight stagnation during the entire study period; this was considered treatment-related.

*Necropsy:* There were no treatment-related gross morphological lesions seen in either male or female rats in any of the exposure groups (Table 6.2-8). The two female rats which died during the observation period following exposure to the high-dose had gross lesions but these could not be directly related to any specific toxicological effect of cinmethylin.

Table 6.2-8. Acute inhalation toxicity in rats - necropsy findings

Sex	Males			Females		
Dose [mg/L]	0.9	2.2	3.5	0.9	2.2	3.5
Day of sacrifice	14	14	14	14	14	14
Number of animals	6	6	6	6	6	4
Liver						
- small nodule	0	0	0	1	1	0
Spleen						
- minimal enlargement and wet on cut surface	3	6	0	4	2	0
Thymus						
- deep red	1	1	0	0	0	0

Uterus - minimal bilateral luminal dilation with clear fluid				0	0	1	
<b>Day of lethality</b>						3	4
<b>Number of animals</b>						1	1
Body - moderate autolysis						0	1
Adipose tissue - moderate decrease in fet deposits						1	0
Nares - slight red paranasal crusts						1	0
Eyes - minimal (m) or slight (s) periocular red crusts						m	s
Small intestine - fluid / semi-fluid dark luminal contents						1	0

**Conclusion:**

Under the conditions of this GLP compliant study in Fisher rats, a 4-hr LC<sub>50</sub> of > 3.5 mg/L (aerosol; the maximum technical feasible concentration) was calculated for males and females. There were 2/12 mortalities at the top concentration of 3.5 mg/L and no mortalities in the low (0.9 mg/L) and mid (2.2 mg/L) concentration groups. Therefore, cinmethylin is not acutely harmful by the inhalation route and does not meet the criteria for classification for acute inhalation toxicity in accordance with Regulation (EC) 1272/2008.

██████████, 1986)

**Summary of acute inhalation toxicity**

The acute inhalation toxicity of cinmethylin was investigated in two studies in rats, one in the Wistar strain (new/modern study) and one in the Fischer 344 strain (old study). Both studies showed low toxicity via the inhalation route. It was noted that animals of the new/modern study were exposed to a limit concentration of 5.3 mg/L (no maximum attainable concentration was noted in the study report) whereas animals of the old study were exposed to a maximum attainable concentration of 3.5 mg/L - clearly lower. It was also noted that in the old study lethality occurred (16 %) at the top concentration of 3.5 mg/L while no deaths were observed in the new/modern study up to the higher concentration of 5.3 mg/L. It is possible that this difference might be due to experimental variation or different strain sensitivity (with Fisher rats being more susceptible than Wistar rat).

Considerations for STOT-SE classification is included in the aligned MCL dossier.

#### B.6.2.4. Skin irritation

The potential skin irritation/corrosion of cinnethylin was investigated in three studies, one *in vitro* (including skin irritation and corrosion tests) and two *in vivo* studies in rabbits. The *in vitro* skin irritation study was inconclusive and the older *in vivo* study (conducted in the 1980s) was not conducted according to GLP and/or relevant OECD test guidelines. A modern (conducted in 2016) study was therefore performed according to GLP and to the latest relevant OECD test guidelines.

##### 1) New/modern *in vitro* test

<b>Author(s)</b>	Remmele M.
<b>Study title</b>	BAS 684 H (Cinnethylin) - <i>In vitro</i> skin irritation and corrosion Turnkey testing strategy
<b>Study reference</b>	Remmele, 2017a BASF DocID 2016/1302127
<b>Test substance</b>	BAS 684 H (Cinnethylin).
<b>Study dates</b>	28/06/2016 – 27/10/2016
<b>Test facility</b>	BASF SE, Experimental Toxicology and Ecotoxicology, 67056 Ludwigshafen, Germany.
<b>Purity (%)</b> <b>Batch no.</b>	93.5 COD-002038 (-) / (+) ratio = 48:52
<b>Test system</b>	Reconstructed three-dimensional human epidermis (RhE) model (EpiDerm™)
<b>Groups</b>	2/test
<b>Dose</b>	SCT: 50 µL SIT: 30 µL Undiluted test item
<b>Route</b>	Single topical application.
<b>Controls</b>	Negative control (NC): deionised water (SCT) / PBS (SIT) Positive control (PC): 8 N KOH (for SCT) / 5% sodium dodecyl sulphate (SDS) in deionised water (for SIT). MTT reduction control (Killed Control - KC): test substance or deionised water on killed tissues
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 431 (2015) (the current test guidelines were adopted in 2016) for skin corrosion; OECD TG No. 439 (2015) (the current test guidelines) for skin irritation. (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.40 BIS. No. L 142, Commission Regulation EU No. 640/2012 of 06 July 2012 - B.46: <i>In Vitro</i> Skin Irritation: Reconstructed Human Epidermis Model Test
<b>Deviation</b>	Demonstration of the technical proficiency of the laboratory was not included in the study report.
<b>Impact of deviation(s)</b>	Minor – this deviation is minimal and is not considered to affect the validity of the study.
<b>Acceptable</b>	Yes. Key study used in the evaluation of skin corrosion and irritation.
<b>Result</b>	Cinnethylin was non-corrosive but the irritation test was inconclusive.

##### Methods

**Skin corrosion test (SCT):** In a GLP and OECD test guideline compliant *in vitro* study to determine the skin corrosion of cinnethylin, 50 µL of undiluted cinnethylin was topically applied to two EpiDerm™ skin models and incubated for 3 min and 1 hr respectively. Concurrent positive control (PC) tissues were treated with 8-N potassium hydroxide solution (8N KOH) and negative control (NC) tissues were treated with deionised water. Due to the ability of cinnethylin to directly reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), freeze-killed control tissues were applied in parallel and the resulting mean value used for the correction of the negative control values. Tissue viability (a marker of skin corrosion) relative to the concurrent negative control (KC corrected) was calculated.

**Skin irritation test (SIT):** In a GLP and OECD test guideline compliant *in vitro* study to determine the skin irritation of cinnethylin, 30 µL of undiluted cinnethylin was topically applied on groups of three EpiDerm™

skin models and incubated for 1 hr, washed out and incubated for another 42 hr. PBS (phosphate buffered saline) and 5% SDS were used as the negative and positive control, respectively. Again, due to the ability of cinmethylin to directly reduce MTT, freeze-killed control tissues were applied in parallel and the resulting mean value used for the correction of the negative control values. Tissue viability (a marker of skin irritation) relative to the concurrent negative control (KC corrected) was calculated.

### Results

#### *Skin corrosion test (SCT)*

Table 6.2-9. *In vitro* skin corrosion test results

	Negative control (NC)		Cinmethylin		Positive control (PC)
	viable tissue	KC	viable tissue	KC	viable tissue
<b>Exposure: 3 min</b>					
OD <sub>570</sub> tissue I	2.051	0.083	2.368	0.052	0.284
OD <sub>570</sub> tissue II	1.964	0.095	2.527	0.038	0.188
mean OD <sub>570</sub>	2.007	0.089	2.447	0.045	0.236
Viability (% of NC±SD)	100 ± 3	4.4 ± 0.4	122 ± 6	2.2 ± 0.5	12 ± 3
CV (%)	3.1	9.2	4.6	21.2	28.8
Viability (% of NC <sub>corr.</sub> )	-	-	<b>120</b>		-
<b>Exposure: 1 h</b>					
OD <sub>570</sub> tissue I	1.736	0.086	2.371	0.028	0.088
OD <sub>570</sub> tissue II	1.717	0.099	2.179	0.009	0.111
mean OD <sub>570</sub>	1.726	0.092	2.275	0.019	0.099
Viability (% of NC±SD)	100 ± 0.8	5.3 ± 0.6	132 ± 8	1.1 ± 0.8	5.7 ± 0.9
CV (%)	0.8	10.3	6.0	70.7	16.0
Viability (% of NC <sub>corr.</sub> )	-	-	<b>131</b>		-

Uncorrected viability is indicated as the mean ± standard deviation

CV - Coefficient of variation

Table 6.2-10. *Corrosion test Historical Control Data (HCD)*

<b>Historical control range of NC (period Jan 2014 – Jun 2016)</b>				
		Mean	SD	Mean + 2 SD range
OD <sub>570</sub>	3 min	1.942	0.171	1.600 to 2.285
	1 hr	1.971	0.169	1.633 to 2.309
<b>Historical control range of PC (period Jan 2014 – Jun 2016)</b>				
		Mean	SD	Mean + 2 SD range
OD <sub>570</sub>	3 min	0.314	0.074	0.167 to 0.462
	1 hr	0.131	0.036	0.059 to 0.204
<b>Viability (%) (period Jan 2014 – Jun 2016)</b>				
		Mean	SD	Mean + 2 SD range
3 min		16.2	3.8	8.6 to 23.8
1 hr		6.7	1.7	3.3 to 10.0

SD – standard deviation

**Acceptability:** NC results, PC results, barrier function and the range of viability between tissue replicates were all within the acceptability criteria. Concurrent NC and PC values were in the range of the historical control data, demonstrating the validity of the performed experiment as well as the ability to detect known corrosive substances.

**Viability:** Tissue viability after 3 min and 1 hr cinmethylin exposure was 120 % and 131 %, respectively (KC corrected values). As viability was > 50 % after 3 min exposure and > 15 % after 1 hr exposure, the test substance is predicted to be non-corrosive *in vitro*.

*Skin irritation test (SIT):*

Table 6.2-11. *In vitro* skin irritation test results

	Negative control (NC)		Test item		Positive control (PC)
	viable tissue	KC	viable tissue	KC	viable tissue
<b>Exposure: 1 h, 42 h recovery – 1<sup>st</sup> test run</b>					
OD <sub>570</sub> tissue I	2.268	0.055	1.354	0.0000	0.052
OD <sub>570</sub> tissue II	2.194	0.051	0.695	0.0000	0.053
OD <sub>570</sub> tissue III	2.033	0.066	0.707	0.0012	0.051
mean OD <sub>570</sub>	2.165	0.057	0.919	0.0004	0.052
Viability (% of NC)	100 ± 6	2.6 ± 0.4	42.43 ± 17	0.02 ± 0.03	2.4 ± 0.0
Viability (% of NC <sub>corr.</sub> )			<b>42.41</b>		
<b>Exposure: 1 h, 42 h recovery – 2<sup>nd</sup> test run</b>					
OD <sub>570</sub> tissue I	1.976	0.056	0.866	0.012	0.094
OD <sub>570</sub> tissue II	2.053	0.044	1.005	0.000	0.051
OD <sub>570</sub> tissue III	1.904	0.043	1.468	0.013	0.051
mean OD <sub>570</sub>	1.978	0.048	1.113	0.008	0.065
Viability (% of NC)	100 ± 4	2.4 ± 0.4	56.3 ± 15.9	0.4 ± 0.4	3.3 ± 1.3
Viability (% of NC <sub>corr.</sub> )			<b>55.9</b>		

Uncorrected viability is indicated as the mean ± standard deviation

CV - Coefficient of variation

Table 6.2-12. Irritation test Historical Control Data (HCD)

<b>Historical control range of NC (period Jan 2014 – Jun 2016)</b>			
	Mean	SD	Mean + 2 SD range
OD <sub>570</sub>	2.330	0.279	1.772 to 2.888
<b>Historical control range of PC (period Jan 2014 – Jun 2016)</b>			
	Mean	SD	Mean + 2 SD range
OD <sub>570</sub>	0.071	0.011	0.050 to 0.093
<b>Viability (%) (period Jan 2014 – Jun 2016)</b>			
	Mean	SD	Mean + 2 SD range
	3.1	0.5	2.2 to 4.0

SD – standard deviation

*Acceptability:* NC results, PC results, barrier function and the range of viability between tissue replicates were all within the acceptability criteria. Concurrent NC and PC values are in the range of the historical control data, demonstrating the validity of the performed experiment as well as the ability to detect known irritants.

*Viability:* In the 1<sup>st</sup> test run, the corrected mean viability of the treated tissues was 42 %, based on individual values of 63 %, 32 % and 33 %. However, due to non-concordant replicate measurements a 2<sup>nd</sup> test run was performed. For the 2<sup>nd</sup> test run, the corrected mean viability of the treated tissues was 56 %, based on individual values of 44 %, 51 % and 74 %. Of the six tissues, two tissues indicated non-irritancy (viability > 50 %), three tissues indicated irritancy (viability ≤ 50 %) and one tissues showed borderline results (viability 50 ± 5 %). Therefore, no conclusive prediction on the irritation potential of the test item could be made. A third test run was not completed.

#### Conclusion

Under the conditions of this GLP and OECD test guideline compliant *in vitro* skin corrosion and irritation study, it was concluded that cinmethylin does not have a corrosive potential. However, as the results of the skin irritation test were inconclusive, the irritating potential of cinmethylin cannot be determined and further testing is required.

(Remmele, 2017a)

**2) New/modern in vivo study**

<b>Author(s)</b>	
<b>Study title</b>	BAS 684 H (Cinnethylin) - Acute dermal irritation / corrosion in rabbits
<b>Study reference</b>	, 2016b BASF DocID 2016/1225929
<b>Test substance</b>	BAS 684 H (Cinnethylin)
<b>Study dates</b>	11/07/2016 – 25/07/2016
<b>Test facility</b>	
<b>Purity (%)</b> <b>Batch no.</b>	93.5 COD-002038 (-) / (+) ratio = 48:52
<b>Test animals</b>	Rabbit New Zealand white Crl:KBL(NZW) Female
<b>Groups</b>	Stepwise procedure starting with one animal, followed by two additional animals.
<b>Dose</b>	0.5 mL undiluted cinnethylin.
<b>Route</b>	Single topical application.
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 404 (2015) (the current test guidelines). (EC) No 440/2008 of 30 May 2008 - Part B No. L 142, EPA 870.2500, JMAFF No 12 Nosan No 8147 (2000)
<b>Deviation</b>	None.
<b>Impact of deviation(s)</b>	N/A.
<b>Acceptable</b>	Yes. Key study used in the evaluation of skin corrosion and irritation.
<b>Result</b>	Cinnethylin was slightly irritating to the skin but no classification is required.

**Methods**

In a GLP and OECD test guideline compliant *in vivo* study to determine the skin irritation potential of cinnethylin, 3 female NZW rabbits were treated. 0.5 mL undiluted cinnethylin, was applied to the intact shaved flank for 4 hours under a patch of 5 cm<sup>2</sup>, which was secured in position by a semi-occlusive dressing. The treated skin surface was examined at 1, 24, 48 and 72 hours after patch removal. Body weights were determined just before application and after the last reading. A check for dead or moribund animals was performed at least once each workday. A validated method of analysis for single exposure skin irritation studies is not required.

**Results**

**Bodyweight:** One animal slightly lost body weight during the study. However, as the affected animal showed a good general condition and no change in behaviour, the observed body weight loss was not considered to be treatment-related.

Table 6.2-13. Skin irritation in rabbits test results

Readings	Animal	Erythema	Edema	Additional findings
0 h	1	2	0	–
	2	2	0	–
	3	2	0	–
1 h	1	3	0	48
	2	2	0	–
	3	2	0	–
24 h	1	2	0	48
	2	1	0	–
	3	2	0	–
48 h	1	2	0	48
	2	1	0	–
	3	2	0	–
72 h	1	1	0	–
	2	0	0	SD
	3	2	0	–
7 d	1	0	0	SD
	2	–	–	–
	3	0	0	SD
Individual 24-48-72 h means	1	1.7	0.0	
	2	0.7	0.0	
	3	2.0	0.0	

48 = Erythema beyond the application area;

SD = study discontinued because the animal was free of findings

All animals revealed well-defined erythema (grade 2) immediately after removal of the patch that persisted in one of these animals until hour 72 (last reading with findings). In one animal the observation of well-defined erythema (grade 2) persisted only until 1 hr; very slight erythema (grade 1) was noted from hour 24 until hour 48 (last reading with findings). In the third animal, moderate erythema (grade 3) was seen at hour 1. Thereafter, well-defined erythema (grade 2) was noted from hour 24 until hour 48. At hour 72, very slight erythema (grade 1) was noted in this animal (last reading with findings). Erythema beyond the application area was noted in the animal from hour 1 until hour 48. The cutaneous reactions were reversible in one animal within 72 hours and in two animals within 7 days after removal of the patch. Mean scores over 24, 48 and 72 hours for each animal were 1.7, 0.7 and 2.0 for erythema and 0.0, 0.0 and 0.0 for oedema.

#### Conclusion

Under the conditions of this GLP and OECD test guideline compliant *in vivo* study, cinmethylin was slightly irritating to the skin when tested in female New Zealand White rabbits. However, cinmethylin does not meet the criteria for classification as a skin irritant in accordance with Regulation (EC) 1272/2008.

( [REDACTED] 2016b)

**3) Old in vivo study**

<b>Author(s)</b>	██████████
<b>Study title</b>	Acute Toxicity Studies with Technical SD 95481 Primary Skin Irritation of Technical SD 95481
<b>Study reference</b>	██████████, 1981b CI-412-001 ROT 35
<b>Study dates</b>	27/03/1981
<b>Test facility</b>	██
<b>Test substance</b>	BAS 684 H (SD 95481) Undiluted.
<b>Purity (%)</b>	93.3
<b>Batch no.</b>	513A (1-3-0-0)
<b>Test animals</b>	Rabbit New Zealand White Male and female
<b>Groups</b>	3/sex (treatment groups – two intact and two abraded skin test sites per animal)
<b>Dose</b>	Single dermal dose of 0.5 mL
<b>Route</b>	Dermal.
<b>Vehicle</b>	None.
<b>GLP</b>	Not compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	This study is rather a screening study with 24-hour exposure under occlusive conditions and reading time-points of 24 and 72 hours after application.
<b>Impact of deviation(s)</b>	N/A
<b>Acceptable</b>	Yes, however, regarded as supplemental information because the study was not GLP or OECD test guideline compliant.
<b>Result</b>	Cinmethylin showed a slight skin irritating potential but insufficient for classification.

**Methods**

An *in vivo* skin irritation test (not conducted to GLP or OECD test guidelines) was carried out with New Zealand White rabbits to determine the potential for cinmethylin to produce dermal irritation after a single topical exposure. 0.5 mL of undiluted cinmethylin was applied to the skin of 3 male and 3 female New Zealand White rabbits on two application sites (intact and abraded) and exposed for 24 hours under occlusive conditions. The cutaneous reactions (erythema and eschar formation, oedema formation, and any other dermal defects or irritation) were assessed 15 – 20 minutes, 72-hour and 7-day after removal of the patch. The study was terminated on day 7, since no cutaneous reaction was present in any animal. Only findings with the intact skin are summarised below (sites 1 and 4).

The study represents a worst-case scenario (24 hours skin exposure instead of 4 hours) under occlusive instead of semi-occlusive conditions.



ResultsTable 6.2-14. Skin irritation in rabbits test results

Readings	Animal	Erythema site 1 / site 4	Oedema site 1 / site 4	Additional findings
15 – 20 min (24 h)	1 ♂	2 / 2	1 / 1	–
	2 ♂	1 / 1	1 / 2	–
	3 ♂	2 / 2	2 / 2	–
	4 ♀	1 / 2	1 / 1	–
	5 ♀	2 / 2	1 / 1	–
	6 ♀	2 / 2	1 / 1	–
72 h	1 ♂	2 / 1	2 / 1	–
	2 ♂	2 / 2	2 / 2	–
	3 ♂	2 / 2	2 / 2	–
	4 ♀	1 / 1	2 / 1	–
	5 ♀	2 / 1	1 / 1	–
	6 ♀	2 / 2	2 / 1	–
7 d	1 ♂	0 / 0	0 / 0	–
	2 ♂	0 / 0	0 / 0	–
	3 ♂	0 / 0	0 / 0	–
	4 ♀	0 / 0	0 / 0	–
	5 ♀	0 / 0	0 / 0	–
	6 ♀	0 / 0	0 / 0	–
Individual 24-72 h means	1 ♂	2 / 1.5	1.5 / 1	-
	2 ♂	1.5 / 1.5	1.5 / 2	
	3 ♂	2 / 2	2 / 2	
	4 ♀	1 / 1.5	1.5 / 1	
	5 ♀	2 / 1.5	1.5 / 1.5	
	6 ♀	2 / 2	1.5 / 1	
Individual 24-72 h means – average of both application sites	1 ♂	<b>1.8</b>	<b>1.3</b>	-
	2 ♂	<b>1.5</b>	<b>1.8</b>	-
	3 ♂	<b>2.0</b>	<b>2.0</b>	-
	Mean ♂	<b>1.8</b>	<b>1.7</b>	
	4 ♀	<b>1.3</b>	<b>1.3</b>	-
	5 ♀	<b>1.8</b>	<b>1.5</b>	-
	6 ♀	<b>2.0</b>	<b>1.3</b>	-
	Mean ♀	<b>1.7</b>	<b>1.3</b>	

Dermal observation comprised very slight to well-defined erythema (grade 1 to 2) and very slight to slight oedema (grade 1 to 2) 24 and 72 hours after application start. Cutaneous reactions were reversible in all animals within 7 days. Mean scores over 24 - 72 hours for each animal were 1.8, 1.5, 2.0, 1.3, 1.8 and 2.0 for erythema and 1.3, 1.8, 2.0, 1.3, 1.5 and 1.3 for oedema.

Conclusion

Under the conditions of this study, cinmethylin showed a slight skin irritating potential when tested in New Zealand White rabbits but no classification is required. These data are regarded as supplemental as the study was not conducted according to OECD test guidelines or to GLP. However, the results support the conclusions of the modern GLP and OECD test guideline compliant *in vivo* study (██████████, 2016b).

(██████████, 1981b)

***Summary of skin irritation***

The skin irritation/corrosion potential of cinmethylin was investigated in three studies, one *in vitro* (new/modern study, including skin irritation and corrosion tests) and two *in vivo* studies in rabbits (one new/modern and one old study). The *in vitro* study concluded that cinmethylin dose not have a corrosive potential, however, due to

non-concordant replicate measurements, results of the skin irritation test were inconclusive. In both the new/modern and old *in vivo* studies in rabbits, cinnethylin was slightly irritating to the skin. However, skin reactions did not meet the criteria for classification as a skin irritant.

### B.6.2.5. Eye irritation

The eye irritation/damage potential of cinnethylin was investigated in three studies, one *in vitro* (including the Bovine Corneal Opacity and Permeability (BCOP) and EpiOcular™ Eye Irritation tests) and two *in vivo* studies in rabbits. The older *in vivo* study (conducted in the 1980s) was not conducted according to GLP and/or relevant OECD test guidelines. A new/modern (conducted in 2016) study was therefore performed according to GLP and to the latest relevant OECD test guidelines. HSE considers that the new *in vivo* study should have not been conducted. Taking into account the clear negative results of the guideline *in vitro* tests, together with the negative findings of the old *in vivo* study, there is sufficient information to reliably determine the eye irritation potential of cinnethylin. The new *in vivo* study has been evaluated by HSE for transparency but it will not be relied upon.

#### 1) New/modern *in vitro* study

<b>Author(s)</b>	Remmele M.
<b>Study title</b>	BAS 684 H (Cinnethylin) - <i>In vitro</i> eye irritation Turnkey testing strategy
<b>Study reference</b>	Remmele, 2017b BASF DocID 2016/1302128
<b>Study dates</b>	16/06/2016 – 27/10/2016
<b>Test facility</b>	BASF SE, Experimental Toxicology and Ecotoxicology, 67056 Ludwigshafen, Germany.
<b>Test substance</b>	BAS 684 H (Cinnethylin)
<b>Purity (%)</b> <b>Batch no.</b>	93.5 COD-002038 (-) / (+) ratio = 48:52
<b>Test system</b>	BCOP: isolated corneas from the eyes of freshly slaughtered cattle EpiOcular: reconstructed three-dimensional human cornea model (EpiOcular™)
<b>Groups</b>	BCOP: 3/test EpiOcular: 2/test
<b>Dose</b>	BCOP: 750 µL undiluted cinnethylin EpiOcular: 50 µL undiluted cinnethylin
<b>Controls</b>	Negative control (NC): deionised water Positive control (PC): BCOP: 100% ethanol (EtOH) and 100% dimethyl-formamide (DMF). EpiOcular: >98% methyl acetate MTT reduction control (KC): cinnethylin or deionised water on killed tissues (EpiOcular)
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 437 (2013) (the current guideline was adopted in 2017) for eye irritation/corrosion, OECD TG No. 492 (2015) (the current guideline was adopted in 2018) for eye irritation/corrosion. (EC) No 440/2008 of 30 May 2008 - B.47.
<b>Deviation</b>	Demonstration of the technical proficiency of the laboratory was not included in the study report.
<b>Impact of deviation(s)</b>	Minor – this deviation is minimal and is not considered to affect the validity of the study.
<b>Acceptable</b>	Yes. Key study used in the evaluation of eye irritation and corrosion.
<b>Result</b>	Cinnethylin does not have an eye irritating potential

#### Methods

**Bovine Corneal Opacity and Permeability Test (BCOP Test):** 750 µL of undiluted cinnethylin was applied to the surface of three isolated corneas from the eyes of freshly slaughtered cattle for 10 min followed by 2-hr recovery period. Corneal opacity (light transmission through the cornea) and permeability (sodium fluorescein dye leakage at  $\lambda = 490$  nm) were assessed quantitatively and the *In Vitro* Irritation Score (IVIS) was determined. Suitable concurrent NC (deionised water) and PCs (100% ethanol and 100 % DMF) were applied.

*EpiOcular™ Eye Irritation Test:* 50 µL of the undiluted cinnethylin was topically applied on groups of two EpiOcular™ corneal models and incubated for 30 min followed by 2-hr recovery period. Suitable concurrent NC (deionised water, sterile) and PC (>98% methyl acetate) were applied. Due to the ability of cinnethylin to directly reduce MTT, freeze-killed control tissues were applied in parallel and the resulting mean value used for the correction of the negative control values. Tissue viability relative to the concurrent negative control (KC corrected) was calculated.

#### Results

*BCOP Test:* Cinnethylin did not induce either corneal opacity or permeability, leading to the IVIS score of 0.0 (Table 6.2-15). The value was below the cut-off limit of 3 for classification as ‘no category’, therefore cinnethylin was assessed as not irritating or corrosive to the eye.

Table 6.2-15. BCOP test results

	Negative control	Positive control (PC)				Cinmethylin	
		EtOH		DMF			
		measured	corrected*	measured	corrected*	measured	corrected*
Opacity per cornea							
cornea I	8.2	25.7	20.1	96.8	91.2	3.7	0.0
cornea II	2.5	27.4	21.8	88.4	82.8	0.0	0.0
cornea III	6.1	23.7	18.2	101.6	96.1	3.0	0.0
group mean ± SD	5.6 ± 2.9	-	20 ± 1.8	-	90 ± 6.7	-	0.0 ± 0.0
Permeability per cornea							
cornea I	0.001	1.055	1.053	0.557	0.555	0.003	0.001
cornea II	0.002	1.234	1.232	0.792	0.790	0.004	0.002
cornea III	0.003	0.778	0.776	0.666	0.664	0.005	0.003
group mean ± SD	0.002 ± 0.001	-	1.020 ± 0.230	-	0.670 ± 0.118	-	0.002 ± 0.001
IVIS							
cornea I	8.2	35.9		99.5		0.0	
cornea II	2.5	40.3		94.7		0.0	
cornea III	5.1	29.8		106.0		0.0	
group mean ± SD	5.6 ± 2.9	35.3 ± 5.3		100.1 ± 5.7		0.0 ± 0.0	

\* negative values are set to zero for further calculation

*Acceptability:* The concurrent NC and PC mean values are in the range of the historical control data, demonstrating the validity of the performed experiment as well as the ability to detect known corrosive substances.

Table 6.2-16. BCOP test Historical Control Data (HCD)

<b>Historical control range of NC (period Sep 2014 – Jan 2018, no. of tests 9)</b>			
	Mean	SD	Mean + 2 SD range
Opacity	5.2	3.0	-0.8 to 11.1
Permeability	0.004	0.002	-0.001 to 0.008
<b>Historical control range of PC 100 % dimethylformamide (period Sep 2014 – Jan 2018, no. of tests 9)</b>			
	Mean	SD	Mean + 2 SD range
Opacity	89.9	5.5	78.9 to 100.8
Permeability	0.605	0.129	0.346 to 0.863
IVIS	98.9	7.0	84.9 to 113.0
<b>Historical control range of PC 100 % ethanol (period Sep 2014 – Jan 2018, no. of tests 9)</b>			
	Mean	SD	Mean + 2 SD range
Opacity	25.5	3.4	18.8 to 32.2
Permeability	0.850	0.096	0.658 to 1.043

IVIS	38.3	3.3	31.7 to 44.8
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SD – standard deviation

IVIS – *in vitro* irritation score

#### *EpiOcular*

**Acceptability:** NC results, PC results, barrier function and the range of viability between tissue replicates were all within the acceptability criteria. Concurrent NC and PC values are in the range of the historical control data (Table 6.2-18), demonstrating the validity of the performed experiment as well as the ability to detect known corrosive substances.

**Viability:** The tissue viability after 30 min cinnethylin exposure followed by 2 hr recovery was 104 % (Table 6.2-17). The value was clearly above the cut-off limit of 60 % for classification as ‘no category’, therefore cinnethylin was identified as not requiring classification and labelling for eye irritation or corrosion.

Table 6.2-17. *EpiOcular*<sup>TM</sup> test results

	Negative control (NC)		Test item		Positive control (PC)
	viable tissue	KC	viable tissue	KC	viable tissue
<b>Exposure: 1 h, recovery 2 h</b>					
OD <sub>570</sub> tissue I	1.838	0.034	1.999	0.003	0.541
OD <sub>570</sub> tissue II	1.909	0.033	1.910	0.004	0.582
mean OD <sub>570</sub>	1.873	0.033	1.954	0.004	0.562
Viability (% of NC)	100 ± 3.8	1.8 ± 0.0	104.3 ± 4.8	0.2 ± 0.0	30.0 ± 2.2
Viability (% of NC <sub>corr.</sub> )	-	-	<b>104.1</b>	-	-

Uncorrected viability is indicated as the mean ± relative standard variation in %

Table 6.2-18. *EpiOcular*<sup>TM</sup> test Historical Control Data (HCD)

<b>Historical control range of NC (period Jan 2014 – Jun 2016)</b>			
	Mean	SD	Mean + 2 SD range
OD <sub>570</sub> (for liquids)	1.926	0.270	1.386 to 2.466
<b>Historical control range of PC (period Jan 2014 – Jun 2016)</b>			
	Mean	SD	Mean + 2 SD range
OD <sub>570</sub> (for liquids)	0.580	0.145	0.289 to 0.871
<b>Viability (%)</b>			
	Mean	SD	Mean + 2 SD range
For liquids	30.1	6.8	16.4 to 43.7

SD – standard deviation

#### *Combined assessment of the eye irritation potential*

No eye irritating or corrosive potential was identified by the BCOP assay. This result was verified by the *EpiOcular* test. The overall assessment of cinnethylin in this *in vitro* eye irritation study, was negative.

#### Conclusion

Under the conditions of this GLP and OECD test guideline compliant *in vitro* eye irritation/corrosion study, it was concluded that cinnethylin does not have an eye irritating potential. Cinnethylin does not meet the criteria for classification as damaging or irritating to the eye in accordance with Regulation (EC) 1272/2008.

(Remmele, 2017b)

**2) New/modern in vivo study – not relied upon**

<b>Author(s)</b>	██████████
<b>Study title</b>	BAS 684 H (Cinmethylin) - Acute eye irritation in rabbits
<b>Study reference</b>	██████████, 2016b BASF DocID 2016/1326828
<b>Study dates</b>	08/08/2016 – 12/10/2016
<b>Test facility</b>	██
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b> <b>Batch no.</b>	93.5 COD-002038 (-) / (+) ratio = 48:52
<b>Test animal</b>	Rabbit New Zealand White HsdIff.NZW – (SPF) Female
<b>Groups</b>	Stepwise procedure starting with one animal, followed by two additional animals.
<b>Dose</b>	0.1 mL undiluted cinmethylin.
<b>Route</b>	Single application into the conjunctival sac of the right eye.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 405 (2012) (the current test guideline was adopted in 2017). (EC) No 440/2008 of 30 May 2008 - Part B No. L 142, EPA 870.2400, JMAFF No 12 Nosan No 8147 (2000)
<b>Deviation</b>	None.
<b>Impact of deviation(s)</b>	N/A
<b>Acceptable</b>	Yes. However the study is considered to be in contravention of Article 62 of Regulation (EC) 1272/2008. Reported for transparency but not relied upon.
<b>Result</b>	Cinmethylin was not an eye irritant.

**Methods**

In a GLP and OECD test guideline compliant *in vivo* eye irritation study, the eye irritation/corrosion potential of cinmethylin was determined by instillation of 0.1 mL undiluted cinmethylin into the conjunctival sac of the right eye of three New Zealand White rabbits. The application of the test substance was performed in a stepwise procedure starting with one animal and supplementing two additional animals. About 24 hours after application the eye was rinsed with tap water. The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after administration of cinmethylin. The study was terminated on day 4, due to absence of any ocular findings. A validated method of analysis for single exposure eye irritation studies is not required.

**Results**

There was no corneal opacity during the study. Moderate iritis (grade 1) was noted in one animal at 1-hour. Slight conjunctival redness was observed in all three animals during the study (at hour 1 for all three animals, at 24-hour for two animals, and at hour 48 for only one animal). All animals showed slight (grade 1 in 1/3 animals) to moderate (grade 2 in 2/3 animals) conjunctival chemosis at 1-hour; this was reversible in two animals at 24-hour whereas the recovery in one animal was still on-going and revealed a slight chemosis at 24-hour that was resolved at 48-hours. Obvious (grade 2 in 1/3 animals) to severe (grade 3 in 2/3 animals) discharge were recorded at 1-hour only. Additional findings - injected scleral vessels in a circumscribed or circular area - were noted in the animals during the first 24 hours. The ocular reactions were reversible in all animals within 24, 48 or 72 hours after application. The individual and overall mean eye irritation scores over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for corneal opacity and for iris lesions, 0.7, 0.3 and 0.0 for redness of the conjunctiva and 0.3, 0.0 and 0.0 for chemosis.

Table 6.2-19. Eye irritation in rabbits test results

Readings	Animal	Cornea		Iris	Conjunctiva			Additional findings
		Area involved	Opacity		Redness	Chemosis	Discharge	

1 h	1	0	0	0	1	2	3	#49
	2	0	0	1	1	2	2	#49
	3	0	0	0	1	1	3	#49
24 h	1	0	0	0	1	1	0	FL neg; #48
	2	0	0	0	1	0	0	FL neg; #48
	3	0	0	0	0	0	0	FL neg;
48 h	1	0	0	0	1	0	0	FL neg
	2	0	0	0	0	0	0	FL neg
	3	0	0	0	0	0	0	FL neg
72 h	1	0	0	0	0	0	0	–
	2	0	0	0	0	0	0	–
	3	0	0	0	0	0	0	–
96 h		0	0	0	0	0	0	SD
		0	0	0	0	0	0	SD
		0	0	0	0	0	0	SD
Individual 24-48-72 h means	1		0.0	0.0	0.7	0.3		
	2		0.0	0.0	0.3	0.0		
	3		0.0	0.0	0.0	0.0		

FL neg. = fluorescein-negative (= no evidence of corneal damage)

48 = scleral vessels injected, circumscribed area, central ingrown

49 = scleral vessels injected, circular, ingrown

SD = study discontinued because the animal was free of findings

### Conclusion

Under the conditions of this GLP and OECD test guideline compliant study, it was concluded that cinmethylin is slightly irritant to the eye but does not meet the criteria for classification in accordance with Regulation (EC) 1272/2008.

(██████, 2016b)

### 3) Old in vivo study

<b>Author(s)</b>	██████
<b>Study title</b>	Acute Toxicity Studies with Technical SD 95481 Rabbit Eye Irritation of Technical SD 95481
<b>Study reference</b>	██████ 1981c CI-412-001 ROT 35
<b>Study dates</b>	5/10/1981
<b>Test facility</b>	████████████████████
<b>Test substance</b>	BAS 684 H (SD 95481) Undiluted
<b>Purity (%)</b>	93.3
<b>Batch no.</b>	513A (1-3-0-0)
<b>Test animals</b>	Rabbit New Zealand White Male and female
<b>Groups</b>	Washed (3 males) and non-washed (3 male and 3 female) eyes.
<b>Dose</b>	Single dose of 0.1 mL .
<b>Route</b>	The conjunctival sac of the right eye.
<b>Vehicle</b>	None.
<b>GLP</b>	Not compliant.
<b>Guideline</b>	None but broadly consistent with OECD TG No. 405.
<b>Deviation</b>	Deviations from OECD TG No. 405 (2017) (the current test guideline): <ul style="list-style-type: none"> <li>3 males and 3 females were exposed without a wash out and additionally, 3 animals (3 males) were exposed for 30 seconds following a wash out procedure.</li> </ul>
<b>Impact of deviation(s)</b>	Minor – this deviation is minimal and is not considered to affect the validity of the study.

<b>Acceptable</b>	Yes – used in a WoE approach with the <i>in vitro</i> tests
<b>Result</b>	Cinnethylin was mildly irritant to the rabbit eye, however, these effects were fully reversible within 14 days.

### Methods

In an *in vivo* eye irritation study, the eye irritation/corrosion potential of cinnethylin was determined by instillation of 0.1 mL undiluted cinnethylin into the conjunctival sac of the right eye of nine (6 males and 3 females) New Zealand White rabbits. About 30 seconds after application, the exposed eyes of 3 males were rinsed with tap water. The eyes of the other 3 males and 3 females were exposed continuously without a wash out procedure. The ocular reactions were assessed 1, 24, 48 and 72 hours, as well as on day 7 and 14 after administration of cinnethylin. The study was terminated on day 14, due to absence of any ocular findings.

### Results

*Wash out after 30 seconds:* There was no corneal opacity or signs of iritis during the study. No discharge was noted. Slight conjunctival redness (grade 1) was noted in all three animals from hour 1 until 48 hours after application and persisted in one animal up to day 7. Slight (grade 1 in 1/3 animals) to moderate (grade 2 in 2/3 animals) conjunctival chemosis was noted 1 hour after application. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for corneal opacity, for iris lesions and for chemosis, and 1.0, 0.7 and 0.7 for redness of the conjunctiva. The ocular reactions were reversible in two animals within 72 hours and in one animal within 14 days after application.

Table 6.2-20. Eye irritation in rabbits – with wash out

Readings	Animal	Cornea		Iris	Conjunctiva			Additional findings
		Area involved	Opacity		Redness	Chemosis	Discharge	
1 h	7 ♂	0	0	0	1	2	0	–
	8 ♂	0	0	0	1	2	0	–
	9 ♂	0	0	0	1	1	0	–
24 h	7 ♂	0	0	0	1	0	0	–
	8 ♂	0	0	0	1	0	0	–
	9 ♂	0	0	0	1	0	0	–
48 h	7 ♂	0	0	0	1	0	0	–
	8 ♂	0	0	0	1	0	0	–
	9 ♂	0	0	0	1	0	0	–
72 h	7 ♂	0	0	0	1	0	0	–
	8 ♂	0	0	0	0	0	0	–
	9 ♂	0	0	0	0	0	0	–
7 d	7 ♂	0	0	0	1	0	0	–
	8 ♂	0	0	0	0	0	0	–
	9 ♂	0	0	0	0	0	0	–
14 d	7 ♂	0	0	0	0	0	0	–
	8 ♂	0	0	0	0	0	0	–
	9 ♂	0	0	0	0	0	0	–
Individual 24-48-72 h means	1		<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>0.0</b>		
	2		<b>0.0</b>	<b>0.0</b>	<b>0.7</b>	<b>0.0</b>		
	3		<b>0.0</b>	<b>0.0</b>	<b>0.7</b>	<b>0.0</b>		

*Continuous exposure without wash out:* There was no corneal opacity or signs of iritis during the study. Slight (grade 1 in 3/3 males and 2/3 females) to obvious (grade 2 in 1/3 females) conjunctival redness was noted at hour 1 and persisted as slight redness in all animals 24 hours after application. This finding was resolved by 48 hours in 1/3 males, by 72 hours in 1/3 females, by day 7 in all males and by day 14 in all females. Moderate conjunctival chemosis (grade 2) was noted in all animals 1 hour after application that was resolved in all 3 males and 1 female at 24 hours and in two remaining females at 48 hours. Slight (grade 1 in 2/3 males) to obvious (grade 2 in 2/3 females) discharge was noted 1 hour after application. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0, 0.0, 0.0, 0.0 and 0.0 for corneal opacity and for iris lesions, and 0.3, 1.0,

1.0, 0.3, 1.0 and 1.0 for redness of the conjunctiva, and 0.3, 0.3, 0.0, 0.0, 0.0 and 0.0 for chemosis. The ocular reactions were fully reversible from 48 hours to 14 days after application.

Table 6.2-21. Eye irritation in rabbits – no wash out

Readings	Animal	Cornea		Iris	Conjunctiva			Additional findings
		Area involved	Opacity		Redness	Chemosis	Discharge	
1 h	1 ♂	0	0	0	2	2	2	—
	2 ♂	0	0	0	1	2	2	—
	3 ♂	0	0	0	1	2	0	—
	4 ♀	0	0	0	1	2	0	—
	5 ♀	0	0	0	1	2	1	—
	6 ♀	0	0	0	1	2	1	—
24 h	1 ♂	0	0	0	1	1	0	—
	2 ♂	0	0	0	1	1	0	—
	3 ♂	0	0	0	1	0	0	—
	4 ♀	0	0	0	1	0	0	—
	5 ♀	0	0	0	1	0	0	—
	6 ♀	0	0	0	1	0	0	—
48 h	1 ♂	0	0	0	0	0	0	—
	2 ♂	0	0	0	1	0	0	—
	3 ♂	0	0	0	1	0	0	—
	4 ♀	0	0	0	0	0	0	—
	5 ♀	0	0	0	1	0	0	—
	6 ♀	0	0	0	1	0	0	—
72 h	1 ♂	0	0	0	0	0	0	—
	2 ♂	0	0	0	1	0	0	—
	3 ♂	0	0	0	1	0	0	—
	4 ♀	0	0	0	0	0	0	—
	5 ♀	0	0	0	1	0	0	—
	6 ♀	0	0	0	1	0	0	—
7 d	1 ♂	0	0	0	0	0	0	—
	2 ♂	0	0	0	1	0	0	—
	3 ♂	0	0	0	1	0	0	—
	4 ♀	0	0	0	0	0	0	—
	5 ♀	0	0	0	0	0	0	—
	6 ♀	0	0	0	0	0	0	—
14 d	1 ♂	0	0	0	0	0	0	—
	2 ♂	0	0	0	0	0	0	—
	3 ♂	0	0	0	0	0	0	—
	4 ♀	0	0	0	0	0	0	—
	5 ♀	0	0	0	0	0	0	—
	6 ♀	0	0	0	0	0	0	—
Individual 24-48-72 h means	1 ♂		<b>0.0</b>	<b>0.0</b>	<b>0.3</b>	<b>0.3</b>		
	2 ♂		<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>0.3</b>		
	3 ♂		<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>0.0</b>		
	4 ♀		<b>0.0</b>	<b>0.0</b>	<b>0.3</b>	<b>0.0</b>		
	5 ♀		<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>0.0</b>		
	6 ♀		<b>0.0</b>	<b>0.0</b>	<b>1.0</b>	<b>0.0</b>		

#### Conclusion

Under the conditions of this study (not conducted to OECD test guideline and not GLP compliant) cinmethylin was mildly irritant to the rabbit eye. These effects were fully reversible within 14 days. Based on the scores in this study, cinmethylin does not meet the criteria for classification for eye irritation in accordance with Regulation (EC) 1272/2008.



(██████, 1981c)

***Summary of eye irritation***

The eye irritation/damage potential of cinmethylin was investigated in three studies; one *in vitro* (a new/modern study, including the Bovine Corneal Opacity and Permeability (BCOP) and EpiOcular™ Eye Irritation tests) and two *in vivo* studies in rabbits (one new/modern and one old study). In the *in vitro* study cinmethylin was shown not to have an eye irritating and/or corrosion potential in both the BCOP and EpiOcular™ tests. HSE notes that the new/modern *in vivo* study was not required as an acceptable validated *in vitro* study was available. This study was reported for transparency but not relied upon; it concluded that cinmethylin is slightly irritant to the eye but does not meet the criteria for classification. The old *in vivo* study also showed that cinmethylin was mildly irritant to the eye, however, these effects were fully reversible within 14 days. Overall, cinmethylin is slightly irritant to the eye but does not meet the criteria for classification.

**B.6.2.6. Skin sensitisation**

The potential for skin sensitisation of cinmenthylin was investigated in three studies in guinea pigs: two Buehler tests and one Guinea Pig Maximization Test (GPMT). Two studies (one Buehler and the GPMT) are older (conducted in the 1980s) and whilst they were conducted according to GLP they were not conducted according to the latest relevant OECD test guidelines; both studies contain deficiencies which impact on the acceptability of the results. Therefore, the applicant performed a new Buehler study. HSE questioned why a Buehler assay was conducted rather than the more sensitive LLNA, as prescribed by the data requirements (Reg EU 283/2014).

The applicant provided the following justification. Both a LLNA and a Maximisation Test were initiated for a formulation under development. However, these tests were terminated within the pre-test period due to unacceptably high irritation scores after repeated application. Lowering the test concentration was not considered to be representative/suitable for hazard assessment purposes. Subsequently a Buehler study was conducted on the formulation, which allowed the use of higher test concentrations. This study was positive, thereby demonstrating the test protocol was suitable to identify the sensitising properties of the formulation containing mainly cinmethylin and was not confounded by irritation. In light of this information on a formulation, a modern (conducted in 2016) Buehler study was performed on cinmethylin alone, according to GLP and to the latest relevant OECD test guidelines, as it could not be excluded that the positive result from the formulation Buehler study was due to cinmethylin. This study was used as they key study for the evaluation of the skin sensitising potential of cinmethylin and the other studies were regarded as supplemental. A LLNA was not performed with cinmethylin as there was concern that, as for the formulation preliminary LLNA study, the result could have been confounded by irritation. HSE accepts the justification provided by the applicant.

**1) New/modern in vivo (Buehler) study**

<b>Author(s)</b>	██████████
<b>Study title</b>	BAS 684 H (Cinmethylin) - Buehler test in guinea pigs
<b>Study reference</b>	██████████, 2016c BASF DocID 2016/1330875
<b>Study dates</b>	30/08/2016 – 19/10/2016
<b>Test facility</b>	██
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b> <b>Batch no.</b>	93.5 COD-002038 (-) / (+) ratio = 48:52
<b>Test animal</b>	Guinea pigs Dunkin Hartley (CrI:HA, SPF) Female
<b>Groups</b>	Preliminary test: 3 Exposed group: 20 Control group: 10
<b>Route</b>	Epicutaneous occlusive
<b>Dose</b>	Preliminary test: 25 %, 50 %, 75 % and 100 % cinmethylin. Main test: 100% for initiation and 75% for challenge.
<b>Exposure period</b>	6 hr
<b>Vehicle</b>	Viscous paraffin
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 406 (1992) (the current test guideline) (EC) No 440/2008 of 30 May 2008 - Part B No. L 142, EPA 870.2600, JMAFF No 12 Nosan No 8147 (2000)
<b>Deviation</b>	None.
<b>Impact of deviation(s)</b>	N/A.
<b>Acceptable</b>	Yes. Key study used in the evaluation of skin sensitisation.
<b>Result</b>	Cinmethylin was shown to have sensitisation potential.

### Methods

*Preliminary test:* Cinnethylin concentrations for the main test were selected based on the results of the preliminary test. The test was performed on 3 animals; concentrations of 25 %, 50 %, 75 % and 100 % were administered. Skin reactions were determined 1, 24 and 48 hr after patch removal.

*Main test:* In a GLP and OECD test guideline compliant study to determine the skin sensitisation potential of cinnethylin, a Buehler test was conducted using groups of 20 and 10 guinea pigs for the exposed and negative control treatments respectively. All inductions were performed with the undiluted cinnethylin (100%). 4 cm<sup>2</sup> gauze patches containing 0.5 mL cinnethylin were applied to the skin of the flanks under an occlusive dressing for 6 hr. Evaluation of skin reactions was performed 1, 24 and 48 hr after patch removal. Inductions were performed on days 0, 7 and 14. A challenge was performed 14 days after the last induction. 0.5 mL of the 75 % cinnethylin preparation was applied for 6 hr under occlusive conditions to the right posterior flank; the vehicle was applied to the left posterior flank. Skin reactions were determined 24 and 48 hr after patch removal. A validated method of analysis for skin sensitisation studies is not required.

*Positive controls:* A concurrent positive control with a known sensitiser was not performed in this study. However, separate positive control studies (reliability check) were performed twice a year in the laboratory. The positive control with alpha-hexylcinnamaldehyde techn. 85 % showed that the test system was able to detect a known sensitising compounds under the laboratory conditions chosen.

### Results

*Preliminary test:* Signs of systemic toxicity were not observed. At the application site dosed with 100 % cinnethylin, two animals showed discrete erythema (grade 1) at hour 1 or from hour 1 until hour 48 after removal of the patch, while the third animal showed moderate erythema (grade 2) from hour 1 until hour 24, which regressed to discrete erythema (grade 1) at hour 48. At the lower concentrations, no skin reactions were seen in the animals. Therefore 100 % (undiluted) cinnethylin was selected for the induction phase and a 75 % cinnethylin was chosen for the challenge in the main test.

*Main test – Induction:* No local skin findings were observed in the (untreated, negative) control group after all inductions. In the exposed group 17 animals showed discrete or moderate erythema after the first induction, while 18 animals showed discrete or moderate erythema after the second induction and all 20 animals showed discrete or moderate erythema after the third induction (Table 6.2-22).

Table 6.2-22. Summary of skin reaction scores in animals treated with 100 % cinnethylin (n=20)

Description	Findings 24 hr. after beginning of application		
	1 <sup>st</sup> induction	2 <sup>nd</sup> induction	3 <sup>rd</sup> induction
Grade 0	3	2	0
Grade 1	12	11	7
Grade 2	5	7	13
Grade 3	0	0	0

*Main test – Challenge:* No local skin findings were observed in the control group after the challenge. However, cinnethylin induced discrete to moderate erythema (grade 1 to 2) in 16 out of 20 animals (80 % responded). Since borderline results were not observed, a second challenge was not performed.

Table 6.2-23. Summary of skin reactions after challenge

Treatment group	Skin reactions		Incidence	
	24 h	48 h	Overall incidence	Incidence rate [%]
Control	0/10	0/10	0/10	0
Cinnethylin (75%)	16/20	5/20	16/20	80

x/y: number of animals with findings / number of animals tested

Table 6.2-24. Challenge skin reaction scores 24 and 48 hr. after topical treatment

Findings	Control group (n=10)		Test group (n=20)	
	24 h	48 h	24 h	48 h
Grade 0	10	10	4	15
Grade 1	0	0	8	4
Grade 2	0	0	8	1
Grade 3	0	0	0	0

x/y: number of animals with findings / number of animals tested

*Observations:* No clinical signs of systemic toxicity or mortality were observed.

*Body weights:* No treatment-related effects on body weight gain were observed.

#### Conclusion

Under the conditions of this GLP and OECD test guideline compliant Buehler study, cinmethylin was shown to have sensitisation potential; > 15 % (80 %) of the test animals responded at > 20 % (75 %) topical induction dose. These findings support classification as a category 1B skin sensitiser (Skin Sens 1B ; H317) under Regulation (EC) No 1272/2008. However, it is not known if, at a topical concentration of < 20 %, whether > 60 % of the test animals would have responded, therefore, since classification in subcategory 1A cannot be fully excluded on the basis of this Buehler study, it is proposed that cinmethylin is classified with **Skin Sens 1; H317**. Further details are available in the aligned MCL dossier.

(██████, 2016c)

#### 2) Old in vivo (Buehler) study

<b>Author(s)</b>	██████████
<b>Study title</b>	Guinea pig sensitization study of SD95481
<b>Study reference</b>	██████████, 1982 CI-416-001
<b>Study dates</b>	22/07/1982 – 07/08/1982
<b>Test facility</b>	████████████████████
<b>Test substance</b>	BAS 684 H (SD 95481)
<b>Purity (%)</b> <b>Batch no.</b>	Not specified. 513D
<b>Test animal</b>	Guinea pigs Dunkin Hartley albino Male and female
<b>Groups</b>	5/sex/treatment
<b>Dose</b>	Preliminary test: 100, 50, 10 and 1 %. Main test: 1 % (induction and challenge).
<b>Controls</b>	Positive control: 2,4-dinitrochlorobenzene (DNCB), 0.1% (w/v) in diethyl ether
<b>Vehicle</b>	Test item vehicle: ethanol (EtOH) Positive control vehicle: diethyl ether
<b>GLP</b>	Compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	Albeit not stated in the study report, the study is broadly consistent with OECD TG No. 406 (1981). However, the following deviations from this test guideline occurred: <ul style="list-style-type: none"> <li>The purity of the test item is not specified.</li> <li>According to the OECD TG No. 406 (1981 and 1992), induction should be performed with a test concentration inducing mild to moderate skin irritation. However, in this study, a non-irritating dose was applied for induction as well as for the challenge.</li> </ul>
<b>Impact of deviation(s)</b>	Major - the choice of a very low induction concentration is considered to compromise the validity of the study.

<b>Acceptable</b>	No, the study was not OECD test guideline compliant and the test concentration was not maximised.
<b>Result</b>	Inconclusive - Cinmethylin was not shown to have sensitisation potential, however, the concentration of cinmethylin used for the induction phase (1 %) was not considered to be appropriate.

### Methods

To determine the skin sensitising potential of cinmethylin a Buehler test was conducted using groups of 5 male and 5 female guinea pigs for each treatment. Cinmethylin concentrations for the main test were selected based on the results of the pre-tests (in which 100 %, 50 %, 10 % and 1 % were analysed). All inductions and the challenge were performed with 1 % cinmethylin in ethanol. Ethanol (0.5 mL, absolute) and 2,4-dinitrochlorobenzene (0.5 mL of 0.1 % solution in diethyl ether) served as negative and positive controls respectively. Topical inductions were performed on days 0, 7 and 14 for 6 hr under occlusive conditions. A challenge was performed 14 days after the last induction for 6 hr under occlusive conditions. An additional treatment group was introduced to serve as irritation control.

### Results

*Preliminary test:* 10% cinmethylin induced slight irritation after 24 hours, therefore, 10 % (repeated for 24-hr only) and 1 % cinmethylin was additionally tested (Table 6.2-25). Based on skin reactions seen, a 1 % cinmethylin concentration was used for induction and challenge doses in the main test.

Table 6.2-25. Preliminary test - skin findings

Concentration [%]	Sex	Skin reaction score	
		24 h	48 h
100	♂	1	0.5
	♀	1	0.5
50	♂	3	3
	♀	2	1
10	♂	1 <sup>a</sup> / 2 <sup>b</sup>	1
	♀	1 <sup>a</sup> / 1 <sup>b</sup>	0.5
1	♂	1	-
	♀	0.5	-

Key for skin reaction scores:

0.5 – minimal erythema

1 – slight erythema

2 – moderate erythema

3 – moderate erythema with slight edema

4 – severe erythema with moderate edema and craking of skin

a – first preliminary test

b – second preliminary test

*Main test:* During induction, only 1/10 animals of the treatment group showed cutaneous findings (Table 6.2-26 – slight erythema recorded in one animal at the 1<sup>st</sup> induction at 24-hr); the dose used (1 %) did not cause mild irritation, as stipulated in the current OECD test guideline (No. 406). After the challenge 0/10 animals in the vehicle control group and 1/10 animals in the exposed group showed skin findings, leading to an affection rate of 10 % induced by cinmethylin. All animals of the positive control group revealed skin reactions after the challenge, leading to an affection rate of 100 %.

Table 6.2-26. Summary of skin reactions after topical induction

Treatment group	1 <sup>st</sup> induction		2 <sup>nd</sup> induction		3 <sup>rd</sup> induction	
	24 h	48 h	24 h	48 h	24 h	48 h
Vehicle control	0/7 0.5/3	0/10	0/6 0.5/4	0/10	0/4 0.5/6	0/8 0.5/2
Test item	0/5 0.5/4 1/1	0/9 0.5/1	0/7 0.5/3	0/9 0.5/1	0/3 0.5/7	0/10
Positive control	0.5/1 1/9	0/10	1/2 2/7 3/1	1/2 2/8	2/10	1/2 2/8

x/y: skin reading score / number of animals with findings

Key for skin reaction scores:

0.5 – minimal erythema

1 – slight erythema

2 – moderate erythema

3 – moderate erythema with slight edema

4 – severe erythema with moderate edema and craking of skin

Table 6.2-27. Summary of skin reactions after challenge

Treatment group	Skin reactions		Incidence	
	24 h	48 h	Overall affection	Incidence rate (%)
Vehicle control	0/10	0/10	0/10	0
Test item	1/10	0/10	1/10	10
Positive control	10/10	10/10	10/10	100

x/y: number of animals with findings / number of animals tested

*Observations:* No clinical signs of systemic toxicity or mortality were observed.*Body weights:* No treatment-related effects on body weight gain were observed.Conclusion

Under the conditions of this GLP study, cinnethylin was not shown to have sensitisation potential in the Buehler test. However, the concentration of cinnethylin used for the induction phase (1 %) was not considered to be appropriate; therefore, this study is not considered acceptable.

(██████████, 1982)

**3) Old in vivo (GPMT) study**

Author(s)	██████████
Study title	WL95481: skin sensitizing potential
Study reference	██████████, 1988 CI-416-002 ROT 45
Study dates	22/06/1988 – 29/07/1988
Test facility	████████████████████
Test substance	WL95481 Also known as BAS 684 H (Cinnethylin)
Purity (%)	92.0
Batch no.	513F
Test animal	Guinea pigs Dunkin Hartley Male and female

<b>Groups</b>	Preliminary test: 2/sex/treatment Exposed group: 10/sex Control group: 5/sex
<b>Dose</b>	Range finding: Intradermal injections: 0.05, 0.1, 0.5 and 1.0 % Topical: 10, 25, 50, 75 and 100 % Main test: Intradermal induction: 0.1 % Topical induction: 100 % Topical challenge: 75 %
<b>Vehicle</b>	Corn oil
<b>GLP</b>	Compliant.
<b>Guideline</b>	None, but broadly consistent with OECD TG No. 406
<b>Deviation</b>	Deviations from OECD TG No. 406 (1992) (the current test guideline): <ul style="list-style-type: none"> <li>An appropriate reliability check of the performing laboratory (or concurrent positive control) was not included in this study report.</li> </ul>
<b>Impact of deviation(s)</b>	Major – the lack of reliability data is considered to compromise the validity of the study.
<b>Acceptable</b>	No, the study was not OECD test guideline compliant, and reliability check data are not available.
<b>Result</b>	Inconclusive - Cinnethylin did not show a skin sensitising effect, but the study is compromised.

#### Methods

*Range finding:* One group of 4 animals (2/sex) were given a single intradermal injection containing 0.1 mL of several dilutions (0.05, 0.1, 0.5 and 1.0 %) of cinnethylin. Five further groups (2/sex) were given dermal patches containing 0.3 mL of several dilutions (10, 25, 50, 75 and 100%) of cinnethylin; exposure lasted 24 hr.

*Main study:* In a GLP compliant Guinea Pig Maximization Test (GPMT), to determine the skin sensitising potential of cinnethylin, 10 male and 10 female guinea pigs (exposure groups) and 5 male and 5 female (control groups) animals were used. The intradermal induction was performed with a 0.1 % cinnethylin preparation, the topical induction was conducted with 0.3 mL undiluted cinnethylin (100 %). For the challenge, a 75 % cinnethylin preparation was used.

#### Results

*Range-finding:* Mild skin reactions were observed in all tested animals.

Table 6.2-28. Range-finding study results

Intradermal injection					
	Cinnethylin concentration (%)				
	0.05	0.1	0.5	1.0	
	Response				
Male 1	0	1	1	1	
Male 2	1	1	2	2	
Female 1	0	1	1	2	
Female 2	1	1	1	1	
Topical application					
	Cinnethylin concentration (%)				
	100	75	50	25	10
	Response				
Male 1	0	0	0	0	0
Male 2	0	0	0	0	0
Female 1	1	0	0	0	0
Female 2	0	0	0	0	0

*Main study:* No results of the intradermal and topical induction are presented or mentioned in the study report. No animal, exposed or control, showed any positive response at either 24 or 48 hr after removal of the challenge patches.

Table 6.2-29. Summary of skin reactions after challenge

Treatment group	Skin reactions		Incidence	
	24 h	48 h	Overall affection	Incidence rate [%]
Vehicle control	0/10	0/10	0/10	0
Test item	0/20	0/20	0/20	0
Positive control	not applied			

x/y: number of animals with findings / number of animals tested

*Observations:* Not specified.

*Body weights:* No treatment-related effects on body weight gain were observed.

#### Conclusion

Under the conditions of this GLP compliant study, cinmethylin was not shown to have sensitisation potential in the GPMT. However, due to lack of positive control data the validity of the performed test cannot be demonstrated, and this study is not acceptable.

(████████, 1988)

#### ***Summary of skin sensitisation***

The potential for skin sensitisation of cinmethylin was investigated in three studies in guinea pigs: two Buehler tests (one new/modern and one old study) and one Guinea Pig Maximization Test (GPMT). In the new/modern Buehler study cinmethylin was shown to have sensitisation potential following topical induction with 75% – when challenged in the main study cinmethylin induced discrete to moderate erythema (grade 1 to 2) in 16 out of 20 animals (80 % responded). These findings support classification of cinmethylin as a category 1B skin sensitizer (Skin Sens 1B ; H317) under Regulation (EC) No 1272/2008. However, it is not known if, at a topical concentration of < 20 %, whether > 60 % of the test animals would have responded; therefore, since classification in subcategory 1A cannot be fully excluded on the basis of this Buehler study, it is proposed that cinmethylin is classified with Skin Sens 1; H317. Further details are available in the aligned MCL dossier. HSE notes that the old (Buehler and GPMT) studies, which did not show a sensitisation potential for cinmethylin, were not acceptable as they were not OECD test guideline compliant. In addition, in the case of the Buehler study the test concentration was not maximised and in the case of the GPMT study reliability check data were not available.

#### **B.6.2.7. Phototoxicity**

An *in vitro* 3T3 NRU phototoxicity test is not required as there is no relevant absorption in the range 290 - 700 nm and the ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L x mol<sup>-1</sup> x cm<sup>-1</sup> (see chemistry evaluation section B.2.4). The applicant provided sufficient justification for the non-provision of a phototoxicity study.

#### **B.6.2.8. Summary of acute toxicity**

The acute toxicity of cinmethylin was investigated in multiple studies conducted via the oral, dermal and inhalation routes. Studies of skin irritancy, eye irritancy and skin sensitisation were also conducted. No phototoxicity studies have been provided as testing is not triggered according to data requirements in Reg 283/2013. The available studies, some conducted in the 1980s and others more recently (2016 and 2017) have not previously been evaluated at EU level.

The following key conclusions have been made with regards to the acute toxicity of cinmethylin:

- The results of the acute toxicity studies show that classification for skin sensitisation 1; H317 (May cause an allergic skin reaction) is required. Further details are available in aligned MCL dossier



- No further classification for acute toxicity is proposed
- The data requirements of Regulation 283/2013 have been met.

Table 6.2-30. Summary of acute toxicity studies with cinmethylin

Study and Acceptability	Species/ Strain/	Sex	Acceptable	Result	Classification according to Reg. (EC) No. 1272/2008
Acute oral toxicity study  [REDACTED] 2016a <a href="#">(2016/1273410)</a>  Batch: COD-002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  <i>Acceptable Relied upon</i>	Rat (Wistar)	F* only	Y	LD <sub>50</sub> > 2000 mg/kg bw.	No classification.
Acute oral toxicity study  [REDACTED], 1982 <a href="#">(CI-411-001)</a>  Batch: 1-3-0-0 Purity (%): 93.3 (-) / (+) ratio = not specified.  <i>Acceptable Supplementary</i>	Rat (Fischer 344)	M & F *	Y	LD <sub>50</sub> 4550 mg/kg bw.	No classification.
Acute oral toxicity study  [REDACTED], 1982 <a href="#">(CI-411-002)</a>  Batch: 513B Purity (%): not specified. (-) / (+) ratio = not specified.  <i>Acceptable</i>	Mice (B6C3F1)	M & F *	Y	LD <sub>50</sub> > 5000 mg/kg bw.	No classification.
Acute dermal toxicity  [REDACTED], 2016a <a href="#">(2016/1225928)</a>  Batch: COD-002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  <i>Acceptable Relied upon</i>	Rat (Wistar)	M & F	Y	LD <sub>50</sub> > 5000 mg/kg bw.	No classification.

Study and Acceptability	Species/ Strain/	Sex	Acceptable	Result	Classification according to Reg. (EC) No. 1272/2008
<p>Acute dermal toxicity</p> <p>██████████, 1981a (CI-412-001)</p> <p>Batch: 513A (1-3-0-0) Purity (%): 93.3 (-) / (+) ratio = not specified.</p> <p><i>Acceptable Supplementary</i></p>	Rabbit (NZW)	M & F	Y	LD <sub>50</sub> > 2029 mg/kg bw.	No classification.
<p>Acute inhalation toxicity study</p> <p>██████████, 2017 (2017/1068662)</p> <p>Batch: COD-002038 Purity (%): 93.5 (-) / (+) ratio = 48:52</p> <p><i>Acceptable Relied upon</i></p>	Rat (Wistar)	M & F	Y	LC <sub>50</sub> > 5.3 mg/L	No classification.
<p>Acute inhalation toxicity study</p> <p>██████████, 1986 (CI-413-001)</p> <p>Batch: 513P Purity (%): 91.8 (-) / (+) ratio = not specified.</p> <p><i>Acceptable</i></p>	Rat (Fischer 344)	M & F	Y	LC <sub>50</sub> > 3.5 mg/L	No classification.
<p><i>In vitro</i> skin irritation and corrosion study</p> <p>Remmele, 2017a (2016/1302127)</p> <p>Batch: COD-002038 Purity (%): 93.5 (-) / (+) ratio = 48:52</p> <p><i>Acceptable Relied upon</i></p>	Human epidermis model	-	Y	Non-corrosive, inconclusive for irritation	Inconclusive.
<p><i>In vivo</i> dermal irritation study</p> <p>██████████ 2016b</p>	Rabbit (NZW)	F only	Y	Slightly irritating	No classification.

Study and Acceptability	Species/ Strain/	Sex	Acceptable	Result	Classification according to Reg. (EC) No. 1272/2008
<a href="#">(2016/1225929)</a> Batch: COD-002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  <i>Acceptable Relied upon</i>					
<i>In vivo</i> dermal irritation study  <a href="#">■■■■■. 1981b (CI-412-001)</a>  Batch: 513A (1-3-0-0) Purity (%): 93.3 (-) / (+) ratio = not specified.  <i>Acceptable Supplementary</i>	Rabbit (NZW)	M & F	Y	Slightly irritating	No classification.
<i>In vitro</i> eye irritation study  <a href="#">Remmele, 2017b (2016/1302128)</a>  Batch: COD-002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  <i>Acceptable Relied upon</i>	Bovine & human cornea models	-	Y	Not irritating	No classification.
<i>In vivo</i> eye irritation study  <a href="#">■■■■■. 2016b (2016/1326828)</a>  Batch: COD-002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  <i>Acceptable Not relied upon, reported for transparency.</i>	Rabbit (NZW)	F only	Y <sup>a</sup>	Not irritating	No classification
<i>In vivo</i> eye irritation study  <a href="#">■■■■■. 1981c (CI-412-001)</a>  Batch: 513A (1-3-0-0)	Rabbit (NZW)	M & F	Y	Mildly irritating	No classification.

Study and Acceptability	Species/ Strain/	Sex	Acceptable	Result	Classification according to Reg. (EC) No. 1272/2008
Purity (%): 93.3 (-) / (+) ratio = not specified.  <i>Acceptable Used in a WoE approach</i>					
<i>In vivo</i> skin sensitisation study (Buehler test)  [REDACTED], 2016c (2016/1330875)  Batch: COD-002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  <i>Acceptable Relied upon</i>	Guinea pigs (Dunkin Hartley)	F only	Y	Skin sensitiser	<b>Skin Sens 1; H317</b>
<i>In vivo</i> skin sensitisation study (Buehler test)  [REDACTED], 1982 (CI-416-001)  Batch: 513D Purity (%): not specified. (-) / (+) ratio = not specified.  <i>Not acceptable</i>	Guinea pigs (Dunkin Hartley)	M & F	N - concentration used was not appropriate	Inconclusive	Inconclusive
<i>In vivo</i> skin sensitisation study (GPMT)  [REDACTED], 1988 (CI-416-002)  Batch: 513F Purity (%): 92.0 (-) / (+) ratio = not specified.  <i>Not acceptable</i>	Guinea pigs (Dunkin Hartley)	M & F	N – lack of reliability data	Inconclusive	Inconclusive

M – male

F – female

\* - fasted animals

a - the study is considered to be in contravention of Article 62 of Regulation (EC) 1272/2008. This study was not relied on.

Based upon the results of these studies, cinmethylin is of low acute toxicity via the oral (LD<sub>50</sub> > 2000 mg/kg bw), dermal (LD<sub>50</sub> > 5000 mg/kg bw) and inhalation (4hr LC<sub>50</sub> > 5.3 mg/L) routes. It is not corrosive to the skin and eye and although it is slightly irritating to the skin and eye, it does not meet the CLP criteria (Regulation

(EC) No 1272/2008) and so no classification is required for skin or eye irritation. However, skin sensitisation was observed in a Buehler test. Therefore, no classification is required for acute oral, dermal and inhalation toxicity; nor is classification required for skin and eye irritation. However, classification is required for skin sensitisation.

Acute toxicity endpoints for cinmethylin		Classification (1272/2008)
Rat LD <sub>50</sub> oral	> 2000 mg/kg bw	-
Rat LD <sub>50</sub> dermal	> 5000 mg/kg bw	-
Rat 4hr-LC <sub>50</sub> inhalation	> 5.3 mg/L air/4 h, nose only	-
Skin irritation	Non-irritant	-
Eye irritation	Non-irritant	-
Skin sensitisation	Sensitising (Buehler test)	<b>Skin Sens 1 ; H317</b>
Phototoxicity	Study not required	-

Phototoxicity testing is not required as the criteria in Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances are not met.

### B.6.3. SHORT-TERM TOXICITY

The short-term toxicity of cinmethylin has been investigated in rats, mice and dogs via the oral (dietary) route of exposure in 28- and 90-day studies; two one-year studies in dogs are also available. Modern studies conducted according to GLP and OECD test guidelines are available in both rats and mice, at 28- and 90-days. In addition older studies, not all of which were conducted according to GLP and OECD test guidelines are available in rats and mice (90-day), as well as dogs (5-week, 90-day and 1-year); nevertheless, HSE considers that these studies were well-conducted and are sufficiently reliable to contribute to the overall picture of the repeated-dose toxicity of cinmethylin. The short-term toxicity via the dermal route of exposure has been investigated in a 28-day study in rats.

#### B.6.3.1. Oral 28-day study

Three 28-day oral (dietary) toxicity studies are available, one each in rats, mice and dogs. Studies in rats and mice are new/modern, GLP and OECD test guideline compliant studies. The study in dogs is an older study and was not conducted according to GLP and OECD test guidelines; it was conducted for a period of 5-weeks.

##### *Studies in rats*

##### *New/modern study*

<b>Author(s)</b>	
<b>Study title</b>	BAS 684 H (Cinmethylin) - Repeated-dose 28-day toxicity study in Wistar rats - Administration via the diet
<b>Study reference</b>	2015 BASF DocID : 2015/1076329
<b>Test facility</b>	
<b>Date</b>	20/01/2014 – 18/02/2014
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Batch no.</b>	COD-001794
<b>Purity (%)</b>	97.5 (-) / (+) ratio = 70:30
<b>Test animals</b>	Rat Wistar, CrI :WI(Han) Male and female
<b>Groups</b>	5/sex/dose
<b>Dose/concentrations</b>	0, 1500, 5000 and 15000 ppm Equivalent to 0, 137, 477 and 1522 mg/kg bw/d in males and 0, 141, 477 and 1331 mg/kg bw/d in females.
<b>Route</b>	Administered daily via the diet for 4 weeks.
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 407 (2008) (this is the current test guideline) EPA 870.3050, Commission Regulation (EC) No 440/2008 - Part B No. B.7
<b>Deviation</b>	None.
<b>Impact of deviations</b>	Not applicable.
<b>Acceptable</b>	Yes
<b>NOAEL</b>	1500 ppm Equivalent to 137 and 141 mg/kg bw/d in males and females respectively.
<b>Effects at the LOAEL</b>	Effects on liver, thyroid and water consumption at 477 mg/kg bw/day in males and females.

##### Methods

In a GLP and OECD test guideline compliant study, cinmethylin was administered via the diet to groups of 5 male and 5 female Wistar rats (the same species and strain used in acute oral toxicity, chronic and carcinogenicity studies) per test group, at concentrations of 0, 1500, 5000 and 15000 ppm, over a period of 4 weeks. Equivalent cinmethylin intakes were 0, 137, 477, 1522 mg/kg bw/d for males and 0, 141, 477, 1331 mg/kg bw/d for females, respectively. A method for the detection of cinmethylin in rat/mouse diet (Catchpole & Hidding, 2017b; 2017/1123754) was evaluated and was considered validated (see Volume 3 CA B5, section B.5.1.2).

## Results

*Mortality and general clinical observations:* There were no treatment-related deaths or clinical signs of toxicity.

*Body weight, food and water consumption:* Body weight was decreased (> 10 % change compared to control but not statistically-significant) in males of the top dose group. Body weight gain was statistically-significantly decreased in males in the top dose group over most of the study (19.8 to 34.3 %) (Table 6.3-1). There were no treatment-related effects on body weight and body change in females (all doses) and males of the low and mid dose groups. There were no treatment-related, adverse effects on food consumption. Water consumption was increased on day 14 in the top dose group (by 21.2 % in males and 30.0 % in females) and in males in the mid dose group (20.7 %). Overall, the changes in body weight and body weight gain of high dose male rats (1522 mg/kg bw/d) are considered treatment-related and adverse. In addition, there were adverse effects on water consumption from the mid-dose in males and at the top dose in females.

Table 6.3-1. Mean body weight change

Dose [ppm] [mg/kg bw/d]		Males				Females			
		0 0	1500 137	5000 477	15000 1522	0 0	1500 141	5000 477	15000 1331
Body weight change		Mean ± SD				Mean ± SD			
Day 0-7	[g]	42.9 ± 5.5	47.9 ± 3.1	44.2 ± 4.5	<b>28.2</b> ± <b>5.6**</b>	19.8 ± 7.6	18.2 ± 5.4	17.5 ± 5.1	18.9 ± 4.6
	[Δ%]	-	11.7	3.0	<b>-34.3</b>	-	-8.4	-11.7	-4.5
Day 0-14	[g]	86.5 ± 14.1	89.6 ± 5.2	92.7 ± 5.0	<b>69.4</b> ± <b>8.5*</b>	35.4 ± 6.0	33.1 ± 7.1	31.2 ± 5.7	36.3 ± 8.4
	[Δ%]	-	3.6	7.2	<b>-19.8</b>	-	-6.5	-11.8	2.6
Day 0-21	[g]	119.5 ± 18.3	120.2 ± 6.7	126.2 ± 11.2	98.9 ± 11.8	49.7 ± 3.7	47.6 ± 4.9	45.3 ± 5.4	46.5 ± 12.9
	[Δ%]	-	0.7	5.7	-17.2	-	-4.2	-8.9	-6.5
Day 0-28	[g]	141.4 ± 24.1	140.8 ± 8.2	147.9 ± 13.2	<b>111.8</b> ± <b>14.0*</b>	62.5 ± 7.8	56.4 ± 4.2	50.5 ± 6.4	55.4 ± 12.9
	[Δ%]	-	-0.4	4.6	<b>-20.9</b>	-	-9.8	-19.2	-11.4

\* = p≤0.05; \*\* = p≤0.01; DUNNETT's test (two-sided)

Δ% – percent change compared to control.

*Functional observation battery and motor activity:* No treatment-related effects were observed in functional observations and motor activity.

*Haematology:* Haematocrit (HCT) values were statistically-significantly higher compared to controls in males of the low and high dose group (Table 6.3-2), however, a dose-dependent relationship was not observed, therefore, this effect was not considered treatment-related. In females of the top dose group mean corpuscular haemoglobin concentration (MCHC) was statistically-significantly lower compared to controls. However, as only this parameter was changed whereas other red blood parameters were not altered, this effect was not considered treatment-related. Prothrombin time (PT i.e. Hepatoquick's test) was statistically-significantly reduced in rats of both sexes in the top dose group (> 10 % change compared to control) and in males of the mid dose group. The finding in the males of the mid dose group (by 6 %) was within the historical control data range. The effect on prothrombin time was considered to be the consequence of altered liver cell metabolism, resulting in a higher synthesis of coagulation factors, and was therefore considered to be treatment-related and adverse at the top dose.

Absolute and relative monocyte counts were increased (> 10 % change compared to control) in males of top dose group; absolute monocyte counts were statistically-significantly increased whereas relative counts were not (Table 6.3-3). This was due to one individual in the top dose group (No. 20), which also had high total white blood cell (WBC) counts, absolute and relative neutrophil and low relative lymphocyte cell counts. Relation to treatment of these findings cannot be excluded. Overall, there were treatment-related and adverse effects on haematology parameters (reduced prothrombin time and increased monocyte counts) at the top dose.

Table 6.3-2. Haematology: Red Blood cell and coagulation parameters

Dose	[ppm] [mg/kg bw/d]	Males				Females			
		0	1500	5000	15000	0	1500	5000	15000
		0	137	477	1522	0	141	477	1331
RBC [ $10^{12}/L$ ]	[mean]	7.77	7.98	7.83	8.27	7.51	7.48	7.78	7.65
	[SD]	0.43	0.11	0.19	0.25	0.33	0.15	0.34	0.30
	$\Delta\%$ of control		+2.7	+0.8	+6.4		-0.4	+3.6	+1.9
HGB [mmol/L]	[mean]	8.8	9.0	8.8	8.9	8.3	8.3	8.3	8.1
	[SD]	0.1	0.2	0.0	0.2	0.3	0.3	0.4	0.1
	$\Delta\%$ of control		+2.3	$\pm 0.0$	+1.1		$\pm 0.0$	$\pm 0.0$	-2.4
HCT [L/L]	[mean]	0.413	<b>0.431*</b>	0.422	<b>0.428**</b>	0.390	0.388	0.393	0.388
	[SD]	0.006	0.010	0.006	0.010	0.010	0.017	0.017	0.006
	$\Delta\%$ of control		+4.4	+2.2	+3.6		-0.5	+0.8	-0.5
MCHC [mmol/L]	[mean]	21.15	20.79	20.84	20.81	21.28	21.36	21.16	<b>20.96*</b>
	[SD]	0.45	0.24	0.26	0.16	0.16	0.18	0.14	0.20
	$\Delta\%$ of control		-1.7	-1.5	-1.6		+0.4	-0.6	-1.5
PT [sec]	[mean]	38.3	40.1	<b>35.9*</b>	<b>32.3**</b>	36.4	35.0	35.0	<b>28.6**</b>
	[SD]	1.7	1.5	0.8	1.8	2.8	1.7	2.7	1.3
	$\Delta\%$ of control		+4.7	-6.3	-15.7		-3.8	-3.8	-21.4
HCD <sup>#</sup>	[mean (range)]	36.2 (33.3 – 39.6)				-			

RBC – red blood cells (erythrocytes).

HGB – haemoglobin.

HCT – haematocrit.

MCHC – mean corpuscular haemoglobin concentration.

PT – prothrombin time.

SD – standard deviation.

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Kruskal-Wallis + Wilcoxon test (two-sided) $\Delta\%$  of control – percent change compared to control.<sup>#</sup> historical control data (HCD) based on 41 oral, 3 inhalation, 3 dermal and 1 i.v. 28-d studies with Wistar rats conducted in the testing facility under GLP conditions in the time-period between 2007 – 2010.

Table 6.3-3. Haematology: White Blood cell parameters

Dose	[ppm] [mg/kg bw/d]	Males				Females			
		0	1500	5000	15000	0	1500	5000	15000
		0	137	477	1522	0	141	477	1331
WBC absolute [ $10^9/L$ ]	[mean]	7.42	7.80	8.87	9.08	3.69	4.62	4.06	4.15
	[SD]	0.87	2.68	1.40	2.55	0.70	1.05	1.35	1.38
	$\Delta\%$ of control		+5.1	+19.5	+22.4		+25.2	+10.0	+12.5
NEUT absolute [ $10^9/L$ ]	[mean]	0.83	1.05	1.26	1.54	0.51	0.67	0.51	0.65
	[SD]	0.23	0.07	0.48	0.93	0.14	0.14	0.07	0.20
	$\Delta\%$ of control		+26.5	+51.8	+85.5		+31.4	$\pm 0.0$	+27.5
Relative [%]	[mean]	11.2	14.6	14.5	16.3	14.1	15.4	13.8	16.8
	[SD]	2.8	4.2	5.7	4.8	3.5	6.4	4.7	7.5
	$\Delta\%$ of control		+30.4	+29.5	+45.5		+9.2	-2.1	+19.1
LYMPH absolute [ $10^9/L$ ]	[mean]	6.23	6.45	7.19	6.68	3.01	3.77	3.38	3.30
	[SD]	0.71	2.62	1.49	1.11	0.64	1.09	1.33	1.28
	$\Delta\%$ of control		+3.5	+15.4	+7.2		+25.2	+12.3	+9.6
Relative [%]	[mean]	84.1	81.5	80.8	75.6	81.5	80.4	81.9	78.5
	[SD]	2.8	4.6	6.0	11.0	3.7	6.8	5.4	7.6
	$\Delta\%$ of control		-3.1	-3.9	-10.1		-1.3	+0.5	-3.7
MONO absolute [ $10^9/L$ ]	[mean]	0.14	0.12	0.17	<b>0.50*</b>	0.06	0.07	0.06	0.09
	[SD]	0.04	0.03	0.05	0.65	0.02	0.01	0.02	0.05
	$\Delta\%$ of control		-14.3	+21.4	+257.1		+16.7	$\pm 0.0$	+50.0
Relative [%]	[mean]	1.9	1.7	2.0	4.7	1.6	1.6	1.6	2.1
	[SD]	0.6	0.6	0.7	4.5	0.5	0.4	0.2	0.5



$\Delta\%$ of control	-10.5	+5.3	+147.4	$\pm 0.0$	$\pm 0.0$	+31.3
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WBC – white blood cells (leukocytes)

NEUT – polymorphonuclear neutrophils

LYMPH – lymphocytes

MONO – monocytes

SD – standard deviation.

$\Delta\%$  of control – percent change compared to control.

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Kruskal-Wallis + Wilcoxon test (two-sided)

*Clinical chemistry:*  $\gamma$ -glutamyl transferase (GGT) activities were statistically-significantly increased in rats of both sexes, from the mid dose group in males and in all dose groups in females; values of the mid and top dose groups of females were also outside the HCD range (Table 6.3-4). This effect was considered treatment-related and adverse from the mid dose group in both sexes. Statistically-significantly decreases seen in aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were not dose-dependent and they were not considered treatment-related. In addition, decreases in these enzymes are considered of no toxicological significance. Total protein levels were statistically-significantly increased in rats of both sexes of the top dose group and additionally in females of the mid dose group. This was due to a statistically-significantly increase in albumin levels in males, and globulin levels in females. Cholesterol levels were statistically-significantly increased ( $> 10\%$ ) in rats of both sexes of top dose group and additionally in males of the mid dose group. In males of the mid and high dose groups glucose levels were statistically-significantly lower ( $> 10\%$ ) and calcium levels were statistically-significantly higher ( $< 10\%$ ). Additionally, in males of the top dose group higher triglyceride levels were observed. Whilst a statistically-significantly decrease in glucose levels in females of all treatment groups was noted, values were within the HCD range, and were therefore not considered treatment-related. Inorganic phosphate levels were statistically-significantly increased ( $< 10\%$ ) in males of the mid dose group, however, changes were not dose-dependent and so not considered treatment-related. Overall, treatment-related and adverse effects on certain clinical chemistry parameters (GGT, protein, globulin, albumin, cholesterol, triglycerides, glucose, calcium), mainly indicative of liver toxicity and alterations in liver metabolism, were seen from the mid dose (477 mg/kg bw/d).

Table 6.3-4. Clinical chemistry parameters

Dose	[ppm] [mg/kg bw/d]	Males				Females			
		0	1500	5000	15000	0	1500	5000	15000
		0	137	477	1522	0	141	477	1331
AST [ $\mu$ kat/L]	[mean]	1.99	2.00	<b>1.44*</b>	1.60	1.60	1.51	1.34	1.33
	[SD]	0.44	0.40	0.19	0.26	0.34	0.31	0.11	0.22
	$\Delta\%$ of control		+0.5	-27.6	-19.6		-5.6	-16.3	-16.9
ALP [ $\mu$ kat/L]	[mean]	2.34	2.42	2.32	2.23	1.33	1.10	<b>0.86**</b>	1.24
	[SD]	0.29	0.49	0.57	0.25	0.16	0.33	0.09	0.20
	$\Delta\%$ of control		+3.4	-0.9	-4.7		-17.3	-35.3	-6.8
GGT [ $\mu$ kat/L]	[mean]	0	1	<b>36**</b>	<b>412**</b>	0	<b>6*</b>	<b>65**</b>	<b>337**</b>
	[SD]	0	1	8	153	0	5	25	75
	$\Delta\%$ of control	-	-	-	-	-	-	-	-
HCD <sup>#</sup>	[mean(range)]		-	-	-		4 (0 – 17)		
GLUC [mmol/L]	[mean]	4.98	4.87	<b>3.85**</b>	<b>3.73*</b>	5.19	<b>4.78*</b>	<b>4.53*</b>	<b>4.09**</b>
	[SD]	0.44	0.60	0.25	0.71	0.23	0.20	0.52	0.55
	$\Delta\%$ of control		-2.2	-22.7	-25.1		-7.9	-12.7	-21.2
HCD <sup>#</sup>	[mean(range)]		-	-	-		5.33 (4.07 – 6.72)		
PROT [g/L]	[mean]	62.41	64.18	64.74	<b>65.29**</b>	65.11	67.18	<b>69.17**</b>	<b>70.12*</b>
	[SD]	1.72	1.34	1.38	1.53	1.92	3.17	1.23	3.31
	$\Delta\%$ of control		+2.8	+3.7	+4.6		+3.2	+6.2	+7.7
ALB [g/L]	[mean]	38.41	39.26	39.51	<b>40.72*</b>	41.24	41.62	43.31	43.41
	[SD]	0.94	0.70	0.95	1.30	1.75	2.11	0.98	2.29
	$\Delta\%$ of control		+2.2	+2.9	+6.0		+0.9	+5.0	+5.3
GLOB [g/L]	[mean]	24.00	24.92	25.23	24.57	23.87	25.55	<b>25.86**</b>	<b>26.71**</b>
	[SD]	1.29	1.20	0.55	0.68	0.24	1.16	0.65	1.47
	$\Delta\%$ of control		+3.8	+5.1	+2.4		+7.0	+8.3	+11.9

Dose	[ppm]	Males				Females			
		0	1500	5000	15000	0	1500	5000	15000
	[mg/kg bw/d]	0	137	477	1522	0	141	477	1331
CHOL [mmol/L]	[mean]	2.05	2.13	<b>2.76**</b>	<b>3.67**</b>	1.40	1.71	1.77	<b>3.34**</b>
	[SD]	0.13	0.39	0.30	0.83	0.22	0.26	0.28	0.26
	Δ% of control		+3.9	+34.6	+79.0		+22.1	+26.4	+138.6
TRIG [mmol/L]	[mean]	0.84	1.15	1.06	<b>1.61*</b>	0.42	0.48	0.41	0.67
	[SD]	0.28	0.30	0.36	0.40	0.10	0.12	0.09	0.26
	Δ% of control		+36.9	+26.2	+91.7		+14.3	-2.4	+59.5
P (inorg.) [mmol/L]	[mean]	2.37	2.43	<b>2.60**</b>	2.32	1.80	1.75	1.80	1.85
	[SD]	0.06	0.16	0.11	0.12	0.30	0.29	0.23	0.20
	Δ% of control		+2.5	+9.7	-2.1		-2.8	±0.0	+2.8
Ca [mmol/L]	[mean]	2.65	2.64	<b>2.73*</b>	<b>2.76*</b>	2.57	2.61	2.67	2.74
	[SD]	0.03	0.07	0.05	0.05	0.06	0.07	0.03	0.14
	Δ% of control		-0.4	+3.0	+4.2		+1.6	+3.9	+6.6

AST – aspartate aminotransferase.

ALP – alkaline phosphatase.

GGT – serum-γ-glutamyltransferase.

GLUC – glucose.

PROT – protein.

ALB – albumin.

GLOB – globulins.

CHOL – cholesterol.

TRIG – triglycerides.

P – phosphate.

Ca – calcium

SD – standard deviation.

Δ% of control – percent change compared to control.

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Kruskal-Wallis + Wilcoxon test (two-sided)

# historical control data (HCD) based on 41 oral, 3 inhalation, 3 dermal and 1 i.v. 28-d studies with Wistar rats conducted in the testing facility under GLP conditions in the time-period between 2007 - 2010

**Urinalysis:** Statistically-significantly increases seen in urinalyses findings (Table 6.3-5) were not dose-dependent and therefore were not considered treatment-related.

Table 6.3-5. Urinalyses findings

Dose	[ppm]	Males				Females			
		0	1500	5000	15000	0	1500	5000	15000
	[mg/kg bw/d]	0	137	477	1522	0	141	477	1331
Transitional epithelial cells		1	2	<b>3**</b>	2	1	1	1	1
Casts		0	1	<b>3**</b>	1	0	0	0	0

\*  $p \leq 0.05$ ; \*  $p \leq 0.01$ ; Wilcoxon test (one-sided).

mean severity: 0=none; 1=few; 2=many; 3=masses;

**Organ weight:** A dose-related increase in absolute and relative liver weight was seen in both sexes. Increases were both statistically significant and > 15 % change compared to control in males from the mid dose group (both absolute and relative), and in females from the mid dose group for relative liver weight and at the top dose (1331 mg/kg bw/d) for absolute liver weight. A dose-related increase in relative kidney weight was seen in males. Increases were both statistically significant and > 10 % change compared to control in males from the mid dose group (relative). An effect was not seen in absolute kidney weight in males nor in absolute or relative kidney weight in females. A statistically-significant increase in relative weight of epididymides and testes in males of the top dose group were noted. However, these increases were related to the reduced terminal body weight (- 13 %) in this dose group and the absolute organ weights were unchanged. Overall, treatment-related and adverse effects on organ weight were seen in the liver (both sexes) and kidney (in males) from the mid dose group (477 mg/kg bw/d).

Table 6.3-6. Selected organ weight data

Sex	Males					Females				
Organ weight [mg]	Dose [ppm]/ [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Dose [ppm]/ [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Terminal weight [g]	0	266.90				0	177.44			
	1500 137	264.96	(-1)			1500 141	173.50	(-2)		
	5000 477	270.76	(+1)			5000 477	167.14	(-6)		
	15000 1522	232.62	(-13)			15000 1331	166.00	(-6)		
Liver [mg]	0	7.384		2.765		0	4.978		2.804	
	1500 137	7.674	(+4)	2.895	(+5)	1500 141	5.134	(+3)	2.955	(+5)
	5000 477	<b>9.104**</b>	<b>(+23)</b>	<b>3.361**</b>	<b>(+22)</b>	5000 477	5.536	(+11)	<b>3.305**</b>	<b>(+18)</b>
	15000 1522	<b>10.24**</b>	<b>(+39)</b>	<b>4.403**</b>	<b>(+59)</b>	15000 1331	<b>7.706**</b>	<b>(+55)</b>	<b>4.642**</b>	<b>(+66)</b>
Kidney [mg]	0	1.964		0.734		0	1.332		0.751	
	1500 137	2.096	(+7)	0.791	(+8)	1500 141	1.356	(+2)	0.780	(+4)
	5000 477	2.294	(+17)	<b>0.848*</b>	<b>(+15)</b>	5000 477	1.292	(-3)	0.773	(+3)
	15000 1522	2.042	(+4)	<b>0.876**</b>	<b>(+19)</b>	15000 1331	1.354	(+2)	0.816	(+9)
Epididymides [mg]	0	0.648		0.244		0				
	1500 137	0.660	(+2)	0.249	(+2)	1500 141				
	5000 477	0.658	(+2)	0.243	(±0)	5000 477				
	15000 1522	0.658	(+2)	<b>0.283*</b>	<b>(+16)</b>	15000 1331				
Testes [mg]	0	3.212		1.204		0				
	1500 137	3.314	(+3)	1.249	(+4)	1500 141				
	5000 477	3.386	(+5)	1.250	(+4)	5000 477				
	15000 1522	3.310	(+3)	<b>1.423**</b>	<b>(+18)</b>	15000 1331				

Bold: statistical significant values; \* p ≤ 0.05; \*\* p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided).

# Values may not calculate exactly due to rounding of figures.

Δ% - percent change compared to control.

*Gross pathology:* The livers of all males and females in the top dose group were enlarged and showed a dark brown discoloration. These effects on the liver were considered treatment-related and adverse and correlated also with the increases in liver weight seen. All other findings were not considered treatment-related.

*Histopathology:* Histopathological findings were noted in the liver, thyroid and kidney. The finding of enlarged livers correlated with hepatocellular hypertrophy that was observed in all males and females of the top dose group. Hypertrophy was diffuse (minimal or slight) in males and centrilobular (slight or moderate) in females. Hypertrophy observed in all animals of the top dose group correlates with the increased absolute and relative liver weights of this dose group. The increased liver weights in males (absolute and relative) and females (relative) of the mid dose group was not accompanied with adverse histopathological effects; only one male in the mid dose group showed a minimal centrilobular hepatocellular hypertrophy. However, at this dose they were accompanied by changes in clinical chemistry. The macroscopically observed dark brown discoloration of the

liver in the top dose group was not supported by histopathological findings. Overall, liver histopathology was seen from the mid dose.

Table 6.3-7. Incidence and severity of hepatocellular hypertrophy

Sex		Males				Females				
Dose	[ppm]	Gradings	0	1500	5000	15000	0	1500	5000	15000
	[mg/kg bw/d]		0	137	477	1522	0	141	477	1331
Animals examined		N	5	5	5	5	5	5	5	5
Liver										
- Hypertrophy, centrilobular	N	0	0	1	0	0	0	0	5	
	1	-	-	1	-	-	-	-	-	
	2	-	-	-	-	-	-	-	2	
	3	-	-	-	-	-	-	-	3	
	[mean] <sup>#</sup>	[0.0]	[0.0]	[1.0]	[0.0]	[0.0]	[0.0]	[0.0]	[2.6]	
- Hypertrophy, diffuse		0	0	0	5	0	0	0	0	
	1	-	-	-	4	-	-	-	-	
	2	-	-	-	1	-	-	-	-	
	[mean] <sup>#</sup>	[0.0]	[0.0]	[1.0]	[1.2]	[0.0]	[0.0]	[0.0]	[0.0]	

<sup>#</sup> = mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding.

An increase in the incidence of thyroid follicular hypertrophy/hyperplasia was observed in males and females of the mid and top dose groups (Table 6.3-8). The incidence and severity of this finding were dose-dependently increased. In affected animals, the number of small follicles was increased or the follicular epithelium was higher, varying in size from cuboidal cells to columnar cells. The incidence of altered colloid was increased in males and females of the top dose group. The colloid in affected animals showed a flaky appearance. Overall, thyroid histopathology was seen in both sexes from the mid dose.

Table 6.3-8. Thyroid gland findings

Sex		Males				Females				
Dose	[ppm]	Gradings	0	1500	5000	15000	0	1500	5000	15000
	[mg/kg bw/d]		0	137	477	1522	0	141	477	1331
Animals examined	N		5	5	5	5	5	5	5	
Thyroid										
- Hypertrophy/ hyperplasia, foll	N		0	0	4	5	0	0	2	5
	1		-	-	3	2	-	-	2	2
	2		-	-	1	3	-	-	-	2
	3		-	-	-	-	-	-	-	1
	[mean] <sup>#</sup>		[0.0]	[0.0]	[1.3]	[1.6]	[0.0]	[0.0]	[1.0]	[1.8]
- Altered colloid	N		1	2	2	3	-	-	-	2
	1		1	2	2	2	-	-	-	2
	2		-	-	-	1	-	-	-	-
	[1.0]		[1.0]	[1.0]	[1.0]	[1.3]	[0.0]	[0.0]	[0.0]	[1.0]

# = mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding.

Eosinophilic droplets (very few to moderate) were observed in the cytoplasm of proximal tubules in all males (both treated and control), however, the severity of this finding was increased in a dose-dependent manner especially from the mid dose (Table 6.3-9). This finding may have been the cause of the increased relative kidney weights observed in males from the mid dose group (Table 6.3-6). These droplets were shown in the subsequent 90-day study (■■■■■, 2018a) to be due to accumulation of  $\alpha_2$ -globulin, a male rat specific phenomenon of no relevance to humans.

Table 6.3-9. Kidney findings

		Males			
Dose	[ppm]	0	1500	5000	15000
	[mg/kg bw/d]	0	137	477	1522
Organs examined [N]		5	5	5	5
Eosinophilic droplets		5	5	5	5
Grade 1		4	3	1	-
Grade 2		1	2	4	-
Grade 3		-	-	-	5

No histopathological findings were detected upon examination of the immuno-relevant organs and tissues (thymus, spleen, lymph nodes, peyer's patches and bone marrow) besides a singular myeloid hyperplasia in the one male (No. 20) of the top dose group, which also showed increased white blood cell, monocyte and neutrophil and reduced lymphoid counts indicating a systemic inflammation. A relation to treatment could not be excluded.

*Oestrous cycle:* No abnormality was detected with regard to synchrony of the morphology of the estrous cycle in ovaries, uterus, cervix, and vagina.

Overall, treatment-related and adverse histopathology was observed in the liver (both sexes), thyroid (both sexes) and kidney (in males) from the mid dose group (477 mg/kg bw/d). The kidney findings are not relevant to humans.

In conclusion, under the conditions of this GLP and OECD test guideline compliant rat study, dietary administration of cinmethylin for 28-days resulted in lower body weight and body weight gains in males at the top dose (1522 mg/kg bw/d, 15000 ppm) throughout the study, hematological changes at the top dose and increases in water consumption from the mid dose of 5000 ppm. The target organs of toxicity were the liver, thyroid and kidney, with changes seen from the mid dose. Increased liver weights with associated hypertrophy and changes in some clinical-chemistry parameters were seen in both sexes. Thyroid follicular cell hypertrophy was also seen in both sexes. Increased kidney weights with associated droplets were seen in males only. These droplets were shown in the subsequent 90-day study (██████████, 2018a) to be due to accumulation of  $\alpha$ 2u-globulin, a male rat specific phenomenon of no relevance to humans.

A NOAEL of 1500 ppm in males and females (equivalent to 137 and 141 mg/kg bw/d respectively) is proposed by HSE since no adverse effects were seen at this dose. A LOAEL of 5000 ppm (477 mg/kg bw/d in both males and females) is proposed based on effects on liver (increased weight, hypertrophy and clinical chemistry), thyroid (follicular cell hypertrophy) and water consumption at 477 mg/kg bw/day in both sexes.

([REDACTED], 2015)

### *New/Modern Study*

<b>Author(s)</b>	[REDACTED]
<b>Study title</b>	BAS 684 H (Cinmethylin) - Repeated-dose 28-day toxicity study in C57BL/6J Rj mice - Administration via the diet
<b>Study reference</b>	[REDACTED], 2016 BASF DocID : 2014/1162710
<b>Test facility</b>	[REDACTED]
<b>Date</b>	07/05/2014 - 05/06/2014
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b>	96.2
<b>Batch no.</b>	COD-001919 (-) / (+) ratio = 51:49
<b>Test animals</b>	Mice C57BL/6J Rj Male and female
<b>Groups</b>	5/sex/dose
<b>Dose (mg/kg bw/day)</b>	0, 400, 1200 and 4000 ppm Equivalent to 0, 95.1, 295.9 and 791.4 mg/kg bw/d for males and 0, 92.4, 254, 1015.6 mg/kg bw/d for females.
<b>Route</b>	Administered daily via the diet for 4 weeks.
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 407 (2008) (this is the current test guideline) Commission Regulation (EC) No 440/2008 - Part B No. B.7, EPA 870.3050
<b>Deviation</b>	None.
<b>Impact of deviations</b>	Not applicable.
<b>Acceptable</b>	Yes
<b>NOAEL</b>	1200 ppm Equivalent to 296 and 254 mg/kg bw/d in males and females respectively.
<b>Effects at the LOAEL</b>	Decreased body weight gain, changes in clinical chemistry parameters indicative of liver toxicity and increased liver weights (> 15 %) at 791 and 1016 mg/kg bw/day in males and females respectively.

In a GLP and OECD test guideline compliant study, cinmethylin was administered via the diet to groups of 5 male and 5 female C57BL/6J Rj mice per test group, at concentrations of 0, 400, 1200 and 4000 ppm over a period of 4 weeks. Corresponding test substance intake was 95.1, 295.9, 791.4 mg/kg bw/d for males and 92.4,

254, 1015.6 mg/kg bw/d for females, respectively. A method for the detection of cinmethylin in rat/mouse diet (Catchpole & Hidding, 2017b; 2017/1123754) was evaluated and was considered validated (see Volume 3 CA B5, section B.5.1.2).

### Results

*Mortality and general clinical observations:* There were no treatment-related deaths or clinical signs of toxicity.

*Body weight and food consumption:* Cinmethylin did not affect the body weight development of males at the mid or low doses, or females at any dose (Table 6.3-10). A decrease in mean body weight gain was seen in males at the top dose group between study day 0 – 7; this decrease was statistically significant, > 10 % compared to control and showed a dose-response relationship. Over the total study period (day 0 – 28) the mean body weight gain in males at the top dose was non statistically-significantly decreased and no dose-response relationship was evident, however, there was a -44 % change compared to control. In females an increase in body weight gain was seen, and whilst not statistically-significant, a dose-response was evident and a relative change of 56 % compared to controls was observed. As an increase in body weight gain is, in general, considered beneficial, the findings in females are not considered of toxicological significance. Overall, HSE considers decrease in body weight gain observed in the top dose males to be treatment-related and adverse.

There were no treatment-related and adverse effects on food consumption. Non statistically-significant decreases in food consumption were seen in both males (in the top dose group only) and females (in all treatment groups), however, changes were inconsistent (times observed) and a dose-response relationship was not evident, therefore this finding was not considered treatment-related.

Table 6.3-10. Body weight change

Dose level	[ppm]	Males				Females			
		0	400	1200	4000	0	400	1200	4000
	[mg/kg bw/d]	0	95.1	496.0	791.4	0	92.4	254.0	1015.6
Body weight change		Mean ± SD				Mean ± SD			
Day 0-7	[g]	0.8 ± 0.5	0.7 ± 0.4	0.4 ± 0.5	-0.5 ± 0.5**	0.0 ± 0.7	0.0 ± 0.5	0.1 ± 0.4	0.2 ± 1.2
	[Δ%]	-	-21.4	-50.0	-161.9	-	-	-	-
Day 0-28	[g]	2.3 ± 1.8	2.0 ± 1.3	2.3 ± 1.1	1.3 ± 1.0	1.1 ± 0.9	1.0 ± 0.3	1.3 ± 0.7	1.7 ± 1.7
	[Δ%]	-	-12.1	-2.6	-44.0	-	-10.9	20.0	56.4

\* = p≤0.05; \*\* = p≤0.01; DUNNETT's test (two-sided)

SD – standard deviation.

Δ% - percent change compared to control.

*Haematology:* Small but statistically-significant changes in haematology parameters were observed in top dose males and females (Table 6.3-11). In the top dose group red blood cell (RBC) counts and haematocrit (HCT) values were decreased in males and mean corpuscular hemoglobin (MCH) content and platelet counts (PLT) were increased in females. Platelet counts were already statistically-significant higher compared to controls in females of the mid dose group. However, the values were all within historical control data ranges and were therefore not considered treatment-related or adverse. Overall, there were no treatment-related effects on haematological parameters.

Table 6.3-11. Haematology results (group means)

Dose		RBC	HGB	HCT	MCH	PLT
[ppm]	[mg/kg bw/d]	[tera/L]	[mmol/L]	[L/L]	[fmol]	[giga/L]
<b>Males</b>						
0	0	10.39 ± 0.54	9.0 ± 0.5	0.475 ± 0.029	0.86 ± 0.01	1342 ± 117
400	95.1	10.34 ± 0.37	8.9 ± 0.3	0.465 ± 0.016	0.86 ± 0.01	1407 ± 136
1200	296.0	10.10 ± 0.36	8.8 ± 0.3	0.465 ± 0.013	0.87 ± 0.01	1402 ± 54
4000	791.4	<b>9.56 ± 0.25*</b>	8.4 ± 0.3	<b>0.436 ± 0.010*</b>	0.88 ± 0.02	1483 ± 100
HCD		Mean 10.37 Min 9.47 Max 11.33	-	Mean 0.467 Min 0.434 Max 0.514	-	-
<b>Females</b>						
0	0	10.05 ± 0.62	8.7 ± 0.5	0.447 ± 0.028	0.87 ± 0.01	1202 ± 32
400	92.4	9.63 ± 0.74	8.3 ± 0.7	0.429 ± 0.033	0.87 ± 0.01	1184 ± 84
1200	254.0	10.26 ± 0.23	8.9 ± 0.1	0.458 ± 0.009	0.86 ± 0.01	<b>1318 ± 35**</b>
4000	1015.6	9.86 ± 0.54	8.7 ± 0.4	0.440 ± 0.023	<b>0.88 ± 0.01*</b>	<b>1286 ± 30*</b>
HCD		-	-	-	Mean 0.92 Min 0.86 Max 0.97	Mean 1305 Min 1149 Max 1465

\* = p≤0.05; \*\* = p≤0.01; Kruskal-Wallis and Wilcoxon-test, two sided

HCD: Historical control data. Species: mouse, strain: C57BL/6J Rj, administration via the diet, same laboratory, 9 studies conducted between 11/09 to 01/13 (contemporus to the study dated 05/14 to 06/14), 9 animals/study.

bold: statistical significant values.

RBC – red blood cells (erythrocytes).

HGB – haemoglobin.

HCT – haematocrit.

MCH – mean corpuscular haemoglobin.

PLT – platelet counts.

**Clinical chemistry:** Statistically-significant changes in some clinical chemistry measurements were noted in males. Bilirubin (BIL) was statistically-significantly decreased in males at the top dose; a clear dose-response relationship was evident and percent change compared to controls increased from --10.9 % in the low dose group to -30 % in the top dose group. Due to its magnitude, the decrease in BIL observed at the top dose is considered to be treatment-related and adverse. Statistically-significantly decreases in urea were observed in males in the low and mid dose groups; however, values recovered and were increased in males of the top dose group. Therefore, due to the lack of a dose-response, the reduction in urea was considered a chance finding. Protein (PROT) levels showed statistically-significantly decreases in males from the mid dose. In the top dose a percent change of > 10 % was observed which was outside the historical control data range. In addition a dose-response relationship was evident. Therefore, the decreases in protein levels observed in males at the top dose are considered treatment-related and adverse. A statistically-significantly decrease in albumin (ALB) was seen in males in all treatment groups. These decreases in ALB were all within the HCD ranges; however, given the presence of a clear dose-response and the fact that the decrease at the top dose was very close to the limit of the HCD, the effect at the top dose was considered treatment-related and adverse. In males of the top dose group statistically-significantly decreases were seen in globulins (GLOB), cholesterol (CHOL) and triglycerides (TRIG). A dose-response relationship was evident in both globulins and cholesterol. Statistically-significant changes in certain clinical chemistry parameters were noted in females, however, no dose-responses were evident. Overall, treatment-related and adverse effects in some clinical chemistry parameters (BIL, PROT, ALB, GLOB, CHOL, TRIG), indicative of liver toxicity were seen in males at the top dose (791 mg/kg/bw/d).

Table 6.3-12. Clinical chemistry parameters

		Males				Females			
Dose	[ppm]	0	400	1200	4000	0	400	1200	4000
	[mg/kg bw/d]	0	95.1	296.0	791.4	0	92.4	254.0	1015.6



		Males				Females			
Dose	[ppm]	0	400	1200	4000	0	400	1200	4000
	[mg/kg bw/d]	0	95.1	296.0	791.4	0	92.4	254.0	1015.6
ALT [ $\mu$ kat/L]	[mean]	0.76	0.70	0.68	0.78	0.85	<b>1.18*</b>	<b>1.19*</b>	0.95
	[SD]	0.17	0.09	0.07	0.06	0.16	0.14	0.25	0.05
	$\Delta\%$ of control		-7.9	-10.5	+2.6		+38.8	+40.0	+11.8
UREA [mmol/L]	[mean]	11.71	<b>9.44*</b>	<b>10.02*</b>	12.21	11.35	11.56	11.08	9.99
	[SD]	1.20	0.97	0.35	1.72	3.21	2.27	1.07	0.56
	$\Delta\%$ of control		-19.4	-14.4	+4.3		+1.9	-2.4	-12.0
BIL [ $\mu$ mol/L]	[mean]	1.10	0.98	0.88	<b>0.77**</b>	1.44	1.15	1.02	1.21
	[SD]	0.15	0.14	0.12	0.14	0.44	0.26	0.20	0.29
	$\Delta\%$ of control		-10.9	-20.0	-30.0		-20.1	-29.2	-16.0
PROT [g/L]	[mean]	51.96	50.15	<b>49.16**</b>	<b>46.32**</b>	48.08	47.06	48.56	45.65
	[SD]	1.41	1.18	0.69	1.44	2.55	2.29	1.53	2.69
	$\Delta\%$ of control		-3.5	-5.4	-10.9		-2.1	+1.0	-5.1
HCD <sup>#</sup>	[mean (range)]	51.99 (48.66 – 55.13)				-			
ALB [g/L]	[mean]	34.82	<b>33.25*</b>	<b>32.44**</b>	<b>31.30**</b>	33.41	32.62	34.11	<b>31.51*</b>
	[SD]	0.43	1.15	0.87	0.70	1.26	1.36	0.83	1.54
	$\Delta\%$ of control		-4.5	-6.8	-10.1		-2.4	+2.1	-5.7
HCD <sup>#</sup>	[mean (range)]	35.26 (31.22 – 37.14)				-			
GLOB [g/L]	[mean]	17.14	16.91	16.72	<b>15.02**</b>	14.67	14.43	14.45	14.14
	[SD]	1.22	0.61	0.62	0.80	1.36	1.34	0.90	1.26
	$\Delta\%$ of control		-1.3	-2.5	-12.4		-1.6	-1.5	-3.6
CHOL [mmol/L]	[mean]	2.38	2.28	2.20	<b>1.46**</b>	1.88	<b>1.54*</b>	1.61	<b>1.51*</b>
	[SD]	0.24	0.18	0.12	0.20	0.20	0.14	0.08	0.18
	$\Delta\%$ of control		-4.2	-7.6	-38.7		-18.1	-14.4	-19.7
HCD <sup>#</sup>	[mean (range)]	-				1.83 (1.46 – 2.85)			
TRIG [mmol/L]	[mean]	0.75	0.81	0.79	<b>0.40**</b>	0.66	0.72	0.44	0.43
	[SD]	0.16	0.16	0.11	0.09	0.18	0.22	0.16	0.17
	$\Delta\%$ of control		+8.0	+5.3	-46.7		+9.1	-33.3	-34.8

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Kruskal-Wallis and Wilcoxon test (two-sided)

<sup>#</sup> historical control data (HCD) based on 9 dietary 28-d studies with C57BL/6J Rj mice conducted in the testing facility under GLP conditions in the time-period between 2009 – 2013.

ALT – alanine aminotransferase.

UREA – urea.

BIL – bilirubin.

PROT – protein.

ALB – albumin.

GLOB – globulins.

CHOL – cholesterol.

TRIG – triglycerides.

**Organ weight:** Liver weights (absolute and relative) were increased in both males and females in all treatment groups; a dose-response relationship was seen. In males whilst absolute liver weights showed a > 15 % change compared to control in the mid (15 %) and top dose (16 %) groups and values were outside the historical control data range, changes were not statistically-significant. In males relative liver weights were statistically-significantly increased and outside the historical control data range from the mid dose group, with a change compared to control of > 15 % in the top dose group only. In females absolute and relative liver weights were statistically-significantly increased (also > 15 % change compared to control) at the top dose; a statistically-significant increase in relative liver weight was also seen in the mid dose group (11 %). Values were outside the historical control data range in the top dose group. A statistically-significant increase in absolute ovary weight was seen in the top dose group, however, no dose-response relationship in ovary weights (absolute and relative) was seen and relative ovary weight was statistically-significant decreased in the mid dose group alone. Therefore, the ovary weight changes were considered unrelated to treatment. The lack of associated histopathology further supports this conclusion. The target organ was the liver. In combination with clinical chemistry findings, the increases in liver weights in the top dose group were considered adverse while the increases in the mid dose group were considered adaptive. Overall, treatment-related and adverse increased in

liver weights (> 15 %) were seen in both sexes at the top dose (791 and 1016 mg/kg bw/d in males and females respectively).

Table 6.3-13. Selected organ weights

Sex	Males					Females				
Organ weight [mg]	Dose [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Dose [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
<b>Terminal weight [g]</b>	<b>0</b>	23.34				<b>0</b>	17.58			
	<b>95.1</b>	24.2	(+4)			<b>92.4</b>	17.62	(±0)		
	<b>296.0</b>	24.26	(+4)			<b>254.0</b>	17.94	(+2)		
	<b>791.4</b>	22.7	(-3)			<b>1015.6</b>	18.16	(+3)		
<b>Liver (mg)</b>	<b>0</b>	1039.8		4.457		<b>0</b>	794.8		4.526	
	<b>95.1</b>	1111.4	(+7)	4.584	(+3)	<b>92.4</b>	872.4	(+10)	4.956	(+9)
	<b>296.0</b>	1191.0	(+15)	<b>4.911**</b>	<b>(+10)</b>	<b>254.0</b>	901.2	(+13)	<b>5.02*</b>	<b>(+11)</b>
	<b>791.4</b>	1208.8	(+16)	<b>5.317**</b>	<b>(+19)</b>	<b>1015.6</b>	<b>1003.0**</b>	<b>(+26)</b>	<b>5.523**</b>	<b>(+22)</b>
<b>HCD (n=7, 2010-2013)</b>		Abs.: mean: 900.2 mg [854.0-1006 mg] Rel.: mean: 4.9% [3.716 - 4.603%]					Abs.: mean: 771.4 mg [685.0-856.2 mg] Rel.: mean: 4.6% [4.172 – 5.447%]			
<b>Ovaries (mg)</b>	<b>0</b>					<b>0</b>	15.2		0.086	
	<b>95.1</b>					<b>92.4</b>	14.4	(-5)	0.081	(-6)
	<b>296.0</b>					<b>254.0</b>	14.0	(-8)	<b>0.078**</b>	<b>(-10)</b>
	<b>791.4</b>					<b>1015.6</b>	<b>16.8*</b>	<b>(+11)</b>	0.093	(+7)

\* p ≤ 0.05; \*\* p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

# Values may not calculate exactly due to rounding of figures

**Gross pathology:** The only gross lesion identified was a black focus in the left lateral lobe in the liver of one male of the top dose group; this finding correlated with a peliosis, histopathologically. This isolated finding was not considered treatment-related.

**Histopathology:** There were no treatment-related histopathological findings. Changes in liver weight were not supported by histopathological findings.

### Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant study, dietary administration of cinmethylin for 28 days in mice resulted in treatment-related and adverse decreases in body weight development in males, changes in some clinical chemistry parameters in males and increases (> 15%) in liver weights in both sexes at the top dose of 4000 ppm. However, HSE notes that concomitant histopathology was not observed in the liver. The target organ was the liver.

**A NOAEL of 1,200 ppm in males and females (equivalent to 296 and 254 mg/kg bw/d in M/F respectively)** is proposed by HSE since no adverse effects were seen at this dose. A LOAEL of 4,000 ppm (791 and 1016 mg/kg bw/d in males and females respectively) is proposed based on decreases in body weight, changes in clinical chemistry parameters, indicative of liver toxicity and increased liver weights at the top dose.

(██████████, 2016)

**Studies in dogs****Old study**

<b>Author(s)</b>	██████████
<b>Study title</b>	Five week dietary feeding study in dogs - SD95481 technical
<b>Study reference</b>	██████████, 1984 BASF DocID : CI-420-004
<b>Test facility</b>	██
<b>Date</b>	12/06/1983 – 01/11/1984
<b>Test substance</b>	SD 95481 (Cinmethylin)
<b>Purity (%)</b>	92.4
<b>Batch no.</b>	513 J
<b>Test animals</b>	Beagle dogs Male and female
<b>Groups</b>	2/sex/dose
<b>Dose</b>	0, 300, 3000, 10000 and 30000 ppm Equivalent to 0, 8.8, 131.1, 338.7 and 330.0 mg/kg bw/d in males and 0, 10.5, 103.6, 334.2 and 433.6 mg/kg bw/d in females.
<b>Route</b>	Administered daily via the diet for 5 weeks.
<b>Vehicle</b>	Acetone <sup>1</sup>
<b>GLP</b>	Not compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	Not applicable.
<b>Impact of deviations</b>	Not applicable.
<b>Acceptable</b>	Yes in a WoE approach with the other available dog studies
<b>NOAEL</b>	3000 ppm in both sexes equivalent to 131 and 104 mg/kg bw/d in males and females respectively.
<b>Effects at the LOAEL</b>	Increased liver weight and corresponding histopathological effects at 10000 ppm equivalent to 339 and 334 mg/kg bw/d in males and females respectively.

**Methods**

In a relatively old, non-GLP and non-guideline study, cinmethylin was administered via the diet to groups of 2 male and 2 female Beagle dogs per test group, at concentrations of 0, 300, 3000, 10000 and 30000 ppm, over a period of 5 weeks. Equivalent cinmethylin intakes were 0, 8.8, 131.1, 338.7 and 330.0 mg/kg bw/d in males and 0, 10.5, 103.6, 334.2 and 433.6 mg/kg bw/d in females, respectively. Cinmethylin intake was not proportionally increased for the high dose animals; males had a higher intake in the 10,000 ppm (338.7 mg/kg bw/d) dose group compared to the top dose group of 30,000 ppm (330.0 mg/kg bw/d).

Food consumption and body weight was determined weekly. Animals were examined for signs of toxicity or mortality at twice daily. Haematology, clinical chemistry and urinalysis parameters were examined at 3 and 5 weeks. Liver and kidney function tests were performed on all dogs at 5 weeks. Complete gross necroscopy examinations were performed on all dogs at study termination. Organ and organ/body weight ratios of brain, heart, liver, kidney, spleen, pituitary, thyroid, gonads and adrenals were recorded. Sections of the liver and kidneys were processed for histopathologic examination.

A method for the dose verification of cinmethylin in feed was evaluated and was regarded as fit for purpose (see Volume 3 CA B5, section B.5.1.2).

**Results**

**Mortality and general clinical observations:** There were no treatment-related deaths or clinical signs of toxicity. Emaciation and dehydration was observed in dogs of the top dose group (30,000 ppm) at weeks 3 – 5.

**Body weight and food consumption:** Most dose groups gained weight except for the top dose group, which lost weight in each week of the study; final body weights were -38 % (male) and -33 % (female) lower compared to controls (Table 6.3-14). Statistically-significant differences in body weights were reached in high dose females

<sup>1</sup> Studies to determine the solubility of cinmethylin in DMSO and acetone have been submitted and are evaluated in detail in Volume 3 – B.2, section B.2.6. Cinmethylin was found to be readily soluble in acetone, as confirmed by a GLP study.

in week 3 and 4. Body weight gain was statistically-significantly decreased in both males and females at the top dose, in weeks 1 and 5; this corresponds with the reduced food consumption observed in this dose group (see below). The effects on body weight was considered treatment-related and adverse at the top dose (330 and 434 mg/kg bw/d in males and females respectively).

Food consumption was severely decreased in the high dose group throughout the study (Table 6.3-15). Statistical-significance was reached in females in week 1 and 2, and in males in week 2, 3 and 5. Food consumption of the high dose animals across the 5 weeks ranged between 22 – 40 % of controls in males, and 3 – 61 % of controls in females. No dose-dependent change on food consumption was observed in other dose groups. The effects on food consumption was considered treatment-related and adverse at the top dose (330 and 434 mg/kg bw/d in males and females respectively). Overall, treatment-related and adverse effects on body weights and food consumption were seen at the top dose (30,000 ppm) in both sexes. It is unclear why at 10,000 ppm which resulted in similar dose levels as the top concentration of 30,000 ppm there were no effects on body weight gain and food consumption.

Table 6.3-14. Body weight and body weight change

		Males					Females				
Dose level	[ppm]	0	300	3000	10000	30000	0	300	3000	10000	30000
	[g/kw bw/d]	0	8.8	131.1	338.7	330.0	0.0	10.5	103.6	334.2	433.6
Mean body weight[g] (Δ%)											
Day -1		7780	6925	7975	8470	8045	7330	6715	7020	6890	7285
Day 0		7615	6865	7765	8240	7780	7055	6595	6895	6820	7110
Week 1		7935	6805	8200	7920	6765	7355	6595	7155	6870	5845
Week 2		8450	7235	8520	8545	6360	7705	7065	7435	7235	5645
Week 3		8510	7335	8790	8685	5865	7755	7090	7660	7380	5655*
Week 4		8660	7315	9035	9035	5610	7850	7260	8140	7385	5440*
Week 5		8750	7360	9225	9010	5400 (-38)	7845	7310	7885	7470	5265 (-33)
Mean body weight change [g]											
Week 1		155	-120	225	-550	-1280**	25	-120	135	-20	-1440**
Week 5		970	435	1250	540	-2645**	515	595	865	580	-2020*

\* = p≤0.05; \*\* = p≤0.01

Body weight change was calculated from Day -1 after the test material was made available in the diet.

Δ% - percent of change compared to control.

Table 6.3-15. Food consumption

		Males					Females				
Dose level	[ppm]	0	300	3000	10000	30000	0	300	3000	10000	30000
	[mg/kg bw/d]	0	8.8	131.1	338.7	330.0	0.0	10.5	103.6	334.2	433.6
Mean food consumption [g/kg bw/day]											
- Day 0		37.2	34.2	45.7*	40.9	39.8	41.9	36.4	37.9	38.0	38.3
- Week 1	[g/kg bw/day]	33.3	26.6	43.7	24.2	7.2	35.8	34.9	33.8	30.9	1.0**
	[Δ%]					21.6					2.8
- Week 2	[g/kg bw/day]	38.6	34.0	50.3	44.4	10.9*	38.8	40.3	40.3	38.4	11.1*
	[Δ%]					28.2					28.6
- Week 3	[g/kg bw/day]	33.4	28.5	41.8	35.6	10.4*	32.0	33.6	33.7	33.3	19.4
	[Δ%]					31.1					60.6
- Week 4	[g/kg bw/day]	33.7	29.8	43.8	36.0	10.7	35.2	36.6	33.3	35.4	19.2
	[Δ%]					31.8					54.5
- Week 5	[g/kg bw/day]	31.4	27.8	36.8	30.2	12.5*	33.0	35.1	32.1	28.9	17.1
	[Δ%]					39.8					51.8

Dose level	[ppm]	Males					Females				
	[mg/kg bw/d]	0	300	3000	10000	30000	0	300	3000	10000	30000
		0	8.8	131.1	338.7	330.0	0.0	10.5	103.6	334.2	433.6

\* =  $p \leq 0.05$ ; \*\* =  $p \leq 0.01$ ; Dunnett's test (two-sided)

Δ% - percent of control.

**Haematology:** In week 3, mean red blood cells (RBC) and hemoglobin (HBG) levels were statistically-significantly increased in males of the high dose group. However, these were not reproduced at week 5. Hemoglobin was statistically-significantly increased in females of the 3000 ppm dose group; however, an increase was not seen in the higher dose groups. Increases in eosinophils (in top dose males), increase of neutrophils and reduction of lymphocytes (in top dose females) were observed but were considered to be a secondary effect of weight loss in animals of the top dose. Overall, there were no consistent treatment-related and adverse effects on haematology.

Table 6.3-16. Haematology data

Dose [ppm] [mg/kg bw/d]	RBC [10 <sup>6</sup> /L]	HBG [g/dL]	WBC [10 <sup>3</sup> /L]	Eosinophils (%)	Neutrophils (%)	Lymphocytes (%)
Week	3	3	5	5	5	5
<b>Males</b>						
<b>0</b>	5.76	12.9	8.30	0.5	65.5	31.5
<b>300</b> <b>8.8</b>	6.99	15.7	7.75	1.0	55.0	43.5
<b>3000</b> <b>131.1</b>	6.21	13.9	9.05	2.0	60.5	34.0
<b>10000</b> <b>338.7</b>	6.18	14.1	8.85	1.0	64.0	31.0
<b>30000</b> <b>330.0</b>	<b>7.59*</b>	<b>17.1*</b>	6.75	<b>4.0*</b>	69.0	24.5
<b>Females</b>						
<b>0</b>	6.46	15.2	5.80	3.5	54.5	39.5
<b>300</b> <b>10.5</b>	6.93	16.0	5.20	2.5	49.0	46.5
<b>3000</b> <b>103.6</b>	7.68	<b>18.2*</b>	7.15	4.5	54.0	39.0
<b>10000</b> <b>334.2</b>	6.24	14.6	7.70	3.5	53.5	41.5
<b>30000</b> <b>433.6</b>	6.59	14.7	6.70	4.0	<b>71.0*</b>	<b>20.0*</b>

\* =  $p \leq 0.05$ ; \*\* =  $p \leq 0.01$  by Dunnett's test

RBC – red blood cells (erythrocytes).

HGB – haemoglobin.

WBC – white blood cells (leukocytes)

**Clinical chemistry:** There were no treatment-related effects on clinical chemistry parameters. A statistically-significant reduction in cholesterol levels was observed in males of the 300 and 10,000 ppm dose groups (Table 6.3-17). However, as values seen in the control group were unusually high and there was no dose-response relationship, these observations were not considered treatment-related or adverse.

Table 6.3-17. Selected clinical chemistry findings

Dose	[ppm]	Males					Females				
		0	300	3000	10000	30000	0	300	3000	10000	30000
	[mg/kg bw/d]	0	8.8	131.1	338.7	330.0	0	10.5	103.6	334.2	433.6
CHOL [mg/100 mL]		269.5	150.0*	187.0	181.0*	199.5	165.5	177.5	213.5	248.5	191.5

\* = p≤0.05; \*\* = p≤0.01 by Dunnett's test

*Urinalysis:* Treatment-related and adverse effects were observed in urine parameters at the top dose. Small urine volumes were observed in animals of the high dose group. Urinary excretion values for Phenolsulfonphthalein (PSP) recovery (as a kidney function test) were lower in the top dose group (Table 6.3-18). These results reflected the reduced urine output associated with lowered food consumption and the clinical observation of dehydration in the top dose group.

Table 6.3-18. Kidney function test (% PSP excreted\*)

Dose	[ppm]	Males					Females				
		0	300	3000	10000	30000	0	300	3000	10000	30000
	[mg/kg bw/d]	0	8.8	131.1	338.7	330.0	0	10.5	103.6	334.2	433.6
Dog 1		46.0	38.6	37.5	54.9	24.0	47.3	40.5	41.0	45.4	30.7
Dog 2		48.8	54.9	45.3	47.3	33.8	46.7	52.5	29.5	42.2	3.8

\*Normal range of Phenolsulfonphthalein (PSP) excretion is 40-60%

PSP - phenolsulfonphthalein

*Organ weight:* Changes in liver weight were noted. In females, absolute and relative liver weight was increased (> 15%) at 10,000 and 30,000 ppm, with relative weight being statistically significant at the top dose. In males, absolute and relative liver weight was increased (> 15%) from 3,000 ppm; however, as histopathology occurred from 10,000 ppm only and taking into account the very small group size (2 dogs), treatment-related and adverse liver weights were seen from 10,000 ppm in both sexes.

Table 6.3-19. Selected organ weight

Sex		Males					Females			
Organ weight [mg]	Dose [ppm] [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Dose [ppm] [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Terminal weight [g]	0	8341.5				0	7471.0			
	300 8.8	6955.0	(-17)			300 10.5	7008.5	(-6.2)		
	3000 131.1	8752.0	(+5)			3000 103.6	7411.5	(-0.8)		
	10000 338.7	8601.0	(+3)			10000 334.2	7033.5	(-5.9)		
	30000 330.0	5218.5	(-37)			30000 433.6	5036.5*	(-32)		
Liver [mg]	0	250.2		3.007		0	230.4		3.088	
	300 8.8	183.8	(-27)	2.638	(-12)	300 10.5	225.7	(-2)	3.221	(+4)
	3000 131.1	304.9	(+22)	3.490	(+16)	3000 103.6	261.4	(+13)	3.517	(+14)
	10000 338.7	346.3	(+38)	4.064	(+35)	10000 334.2	275.9	(+20)	3.929	(+27)
	30000 330.0	204.7	(-18)	3.900	(+30)	30000 433.6	235.7	(+2)	4.633**	(+50)

Sex		Males					Females			
Organ weight [mg]	Dose [ppm] [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Dose [ppm] [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #

\* p ≤ 0.05; \*\* p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

# Values may not calculate exactly due to rounding of figures

Δ% -

*Gross pathology:* There were no treatment-related macroscopic findings.

*Histopathology:* Findings were noted in the kidney and liver. In the kidney, tubular nephropathy (vacuolated epithelium) was observed in animals (both sexes) of the 30,000 ppm dose group; this was considered treatment-related and adverse. In the liver, hepatopathy (hypertrophy and congestion) was observed most commonly in the 10,000 ppm dose group but also in the top dose group. Both kidney and liver effects were not considered severe as no cellular necrosis or fibrosis was observed. Overall, treatment-related and adverse effects on histopathology were seen from 10,000 ppm in the liver and at the top dose in the kidney in both sexes. The liver findings were associated with the increased liver weight and the kidney findings with the urinalysis observations and the dehydration observed at the top dose.

Table 6.3-20. Selected histopathology

Dose [ppm] [mg/kg bw/d]	Males					Females				
	0	300	3000	10000	30000	0	300	3000	10000	30000
No. of animals	2	2	2	2	2	2	2	2	2	2
<b>Liver</b>										
Hepatocytes with clear cytoplasm (central)	0	0	0	0	1	0	0	0	0	0
Hepatocytes with clear cytoplasm and enlargement (midzonal)	0	0	0	2	0	0	0	0	0	0
Centrilobular congestion	0	0	0	0	0	1	0	0	0	2
<b>Kidney</b>										
Pars recta: vacuolated and enlarged epithelium	0	0	0	0	1	0	0	0	0	2
Pars recta: vacuolated epithelium	0	0	0	0	1	0	0	0	0	0

#### Conclusion

In conclusion, under the conditions of this non-GLP and non-guideline study in dogs, dietary administration of cinmethylin for 5 weeks resulted in clinical signs of toxicity, effects on body weight and food consumption, small urine volumes and kidney histopathology at the top dose of 30,000 ppm. In addition, increased liver weights with associated histopathology were seen from 10,000 ppm.

A NOAEL of 3,000 ppm in males and females (equivalent to 131 and 104 mg/kg bw/d in M/F respectively) is proposed by HSE since no adverse effects were seen at this dose. A LOAEL of 10,000 ppm (330 mg/kg bw/d in males and 334 mg/kg bw/d in females) is proposed based on increased liver weights and corresponding histopathological effects.

(██████████, 1984)

#### B.6.3.2. Oral 90- day study

Five 90-day oral toxicity studies are available, two in rats, two in mice and one in dogs. A new/modern, GLP and OECD test guideline compliant study is available for both rats and mice. In addition an older study, which was not conducted according to GLP and OECD test guidelines, is available for both the rat and mouse; these were evaluated as supplementary studies. The study in the dog is an older study and was conducted according to GLP but not according to OECD test guidelines.

**Studies in rats**

The repeated dose toxicity of cinmethylin have been investigated in rats via the oral (dietary) route in one standard guideline 90-day study (new/modern) in Wistar rats and one previously performed study with Fischer 344 rats.

**1) New/modern study**

<b>Author(s)</b>	
<b>Study title</b>	BAS 684 H - Repeated-dose 90-day oral toxicity study in Wistar rats - Administration via the diet
<b>Study reference</b>	2018a BASF DocID : 2014/1228370
<b>Test facility</b>	
<b>Date</b>	09/06/2014 - 10/09/2014
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b>	96.2
<b>Batch no.</b>	COD-001919 (-) / (+) ratio = 51:49
<b>Test animals</b>	Rat Wistar, CrI : WI(Han) Male and female
<b>Groups</b>	10/sex/dose
<b>Dose/concentrations</b>	0, 1000, 3000 and 10000 ppm Equivalent to 0, 67, 211 and 792 mg/kg bw/d in males and 0, 79, 240 and 814 mg/kg bw/d in females.
<b>Route</b>	Administered daily via the diet for 3 months
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 408 (1998) (note the current test guideline was adopted in 2018) OPPTS 870.3100, Commission Regulation (EC) No 440/2008, JMAFF No. 12-Nousan-8147
<b>Deviation</b>	Clinical examination was not performed for all animals on study day 74 (daily examination was planned).
<b>Impact of deviations</b>	The deviation identified is not considered to compromise the validity of the study.
<b>Acceptable</b>	Yes
<b>NOAEL</b>	1000 ppm Equivalent to 67 and 79 mg/kg bw/d in males and females respectively.
<b>Effects at the LOAEL</b>	Increased liver weight with corresponding clinical chemistry and histopathology, histopathology findings of thyroid and nasal cavity and increased prothrombin time from 3,000 ppm (211 and 240 mg/kg bw/d in males and females respectively).

**Methods**

In a GLP and OECD test guideline compliant study, cinmethylin was administered via the diet to groups of 10 male and 10 female Wistar rats per test group, at concentrations of 0, 1000, 3000 and 10000 ppm, over a period of 3 months. Equivalent cinmethylin intakes were 0, 67, 211 and 792 mg/kg bw/d for males and 0, 79, 240 and 814 mg/kg bw/d for females, respectively. No satellite groups were included in this study. A method for the detection of cinmethylin in rat/mouse diet (Catchpole & Hidding, 2017b; 2017/1123754) was evaluated and was considered validated (see Volume 3 CA B5, section B.5.1.2).

**Results**

*Mortality and general clinical observations:* There were no treatment-related deaths or clinical signs of toxicity.

*Functional observation battery and motor activity:* There were no treatment-related findings seen across all the FOB examinations. A statistically-significant reduction in grip strength of hindlimbs in males in the top dose group was observed. However, as the value was within the range of the respective HCD, grip strength was not affected in females, and no other related parameters were affected, it was not considered treatment-related. There were no treatment-related findings in any motor activity measurements.



Table 6.3-21. Grip strength

Dose	[ppm]	Males				Females			
		0	1000	3000	10000	0	1000	3000	10000
	[mg/kg bw/d]	0	67	211	792	0	79	240	814
GSH [Newton]									
Week 12	[mean]	7.1	6.6	6.3	5.7**	6.4	6.2	6.6	6.5
	[SD]	1.0	1.0	1.0	0.9	1.4	1.4	1.0	1.2
	Δ% of control	-	-7.1	-12.0	-20.2	-	-2.6	+3.6	+1.3
HCD#	[mean (range)]	7.7 (4.6 – 10.9)				6.4 (3.6 – 10.2)			

Statistical evaluation: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Kruskal-Wallis + Wilcoxon test (two-sided)

# historical control data (HCD) based on 26 dietary 90-d studies with Wistar rats (supplier: [REDACTED]) conducted in the testing facility under GLP conditions in the time period 2013 - 2015

*Ophthalmoscopy:* There were no treatment-related ophthalmological findings.

*Body weight and body weight gain:* Statistically-significant reductions in mean body weight were seen in the top dose group of males and females on study day 28 and 91. Mean body weights of the high dose animals were > 10 % lower than controls for males at both time points and for females on day 91. This corresponded with statistically-significant reductions in body weight gain in the top dose group of males and females at all time points. The overall (day 0 – 91) body weight gain in the high dose group was 27 % lower for males and 33 % lower for females compared to their respective controls. Neither body weights nor body weight gains were reduced by > 10 % at any other dose. Mean food consumption was not affected by treatment with cinmethylin in males or females at any dose. Overall, HSE considered the changes in body weight and body weight gain to be treatment-related and adverse in the top dose for both males and females (792 and 814 mg/kg bw/d for males and females respectively).

Table 6.3-22. Body weight development

Dose level	[ppm]	Males				Females			
		0	1000	3000	10000	0	1000	3000	10000
	[mg/kg bw/d]	0	67	211	792	0	79	240	814
Body weight [g]									
Day 0	[mean]	153.5	155.9	154.0	155.0	124.6	127.5	127.1	125.4
	[SD]	8.0	6.3	9.1	7.5	6.7	6.0	3.7	4.6
	Δ% of control	-	+1.6	+0.3	+1.0	-	+2.3	+2.0	+0.6
Day 28	[mean]	292.9	286.1	282.1	254.7**	185.0	185.1	186.6	170.9*
	[SD]	11.1	15.2	30.8	21.3	17.3	12.9	10.8	7.1
	Δ% of control	-	-2.3	-3.7	-13.1	-	±0.0	0.9	-7.6
Day 91	[mean]	399.8	386.4	380.3	334.9**	230.4	220.9	226.9	196.0**
	[SD]	15.7	28.5	39.6	30.7	21.9	17.4	11.3	8.1
	Δ% of control	0	-3.4	-4.9	-16.2	0	-4.2	-1.5	-15.0
Body weight gain [g]									
Day 0 – 7	[mean]	39.5	40.3	39.2	21.7**	19.5	14.5	19.7	13.0*
	[SD]	2.1	2.5	6.6	5.7	6.7	4.8	3.2	4.1
	Δ% of control	-	+2.0	-0.6	-45.1	-	-25.4	+1.3	-33.2
Day 0 – 28	[mean]	139.4	130.2	128.1	99.7**	60.4	57.6	59.5	45.5**
	[SD]	7.8	14.1	23.0	15.5	13.5	7.9	10.5	6.1
	Δ% of control	-	-6.6	-8.1	-28.5	-	-4.7	-1.5	-24.7
Day 0 – 42	[mean]	179.0	167.6	165.5	125.2**	81.1	72.7	75.9	51.7**
	[SD]	9.9	18.5	31.8	18.9	14.4	9.6	7.3	7.2
	Δ% of control	-	-6.3	-7.5	-30.0	-	-10.4	-6.4	-36.2
Day 0 – 91	[mean]	246.3	230.5	226.4	179.9**	105.8	93.4	99.8	70.6**
	[SD]	15.0	27.3	33.0	25.1	18.5	14.2	10.5	8.6
	Δ% of control	-	-6.4	-8.1	-27.0	-	-11.8	-5.8	-33.3

		Males				Females			
Dose level	[ppm]	0	1000	3000	10000	0	1000	3000	10000
	[mg/kg bw/d]	0	67	211	792	0	79	240	814

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Dunnett test (two-sided)

SD – standard deviation.

Δ% of control – percent change compared to control.

**Haematology:** Statistically-significant changes to certain haematology parameters were observed (Table 6.3-23). Statistically-significant decreases in haemoglobin (HGB) and haematocrit (HCT) levels were observed in top dose females. However, a change of < 10 % compared to controls was seen; in addition HCT values were within the HCD range. A statistically-significant increase in relative reticulocyte count (RET) was observed in top dose males, with a relative change of > 10 % compared to controls. However, the value was equal to the HCD mean, whereas that of the concurrent control males was below HCD range. It is noted that the increase in RET occurred in males, whilst the decreases in HGB and HCT occurred in females. Overall, there was no consistent pattern of effects on red blood parameters. The platelet count was statistically-significantly increased in low dose females, but this is not considered to be treatment-related due to absence of a dose-response relationship. The measured coagulation parameter, prothrombin time (PT), was statistically-significantly shortened in top dose animals of both sexes and in mid dose females. This finding was considered to be treatment-related and adverse, but very likely secondary to induced enzyme activity in the liver triggering increased biosynthesis of coagulation factors. The same effect was seen in the 28-day study at the top dose. Statistically-significantly decreased relative eosinophil counts (EOS) in top dose animals of both sexes and in mid dose males, as well as a reduced absolute eosinophil count in top dose females were observed. Relative change compared to controls was > 10 % for all mid and top dose animals. However, in isolation, without related effects on other WBC parameters, these changes are considered chance findings. Statistically-significant increases in absolute and relative counts of large unstained cells (LUC) were observed in top dose males and females, respectively. Relative change compared to controls was > 10 % for top dose animals. Whilst values obtained for top dose males (absolute) and females (relative) were within the HCD range they were greater than their respective HCD means. However, a dose-response relationship was lacking. Overall, HSE does not consider these LUC changes to be related to treatment. Overall, treatment-related and adverse effects on prothrombin time were seen from the mid dose.

Table 6.3-23. Haematology

		Males				Females			
Dose	[ppm]	0	1000	3000	10000	0	1000	3000	10000
	[mg/kg bw/d]	0	67	211	792	0	79	240	814
<b>Red Blood cell and coagulation parameters on day 92/93</b>									
RBC [ $10^{12}/L$ ]	[mean]	8.50	8.57	8.48	8.35	7.97	7.79	7.79	7.51
	[SD]	0.17	0.49	0.23	0.36	0.45	0.53	0.34	0.25
	Δ% of control		+0.8	-0.2	-1.8		-2.3	-2.3	-5.8
HGB [mmol/L]	[mean]	8.9	9.1	9.0	8.8	8.8	8.7	8.7	<b>8.3**</b>
	[SD]	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2
	Δ% of control		+2.2	+1.1	-1.1		-1.1	-1.1	-5.7
HCD <sup>#</sup>	[mean (range)]	-				9.0 (8.6 – 9.5)			
HCT [L/L]	[mean]	0.417	0.425	0.418	0.411	0.409	0.400	0.399	<b>0.384**</b>
	[SD]	0.008	0.014	0.011	0.009	0.015	0.015	0.011	0.010
	Δ% of control		+1.9	+0.2	-1.4		-2.2	-2.4	-6.1
HCD <sup>#</sup>	[mean (range)]	-				0.399 (0.378 – 0.424)			
RET [%]	[mean]	1.3	1.4	1.4	<b>1.8**</b>	1.5	1.5	1.7	1.8
	[SD]	0.2	0.3	0.2	0.3	0.6	0.4	0.3	0.2
	Δ% of control		+7.7	+7.7	+38.5		±0.0	+13.3	+20.0
HCD <sup>#</sup>	[mean (range)]	1.8 (1.4 – 2.5)							
PLT [ $10^9/L$ ]	[mean]	695	662	672	699	679	<b>751*</b>	702	696
	[SD]	74	62	51	85	100	38	66	57
	Δ% of control		-4.7	-3.3	+0.6		+10.6	+3.4	+2.5
PT [sec]	[mean]	36.2	36.7	35.7	<b>33.7**</b>	34.1	33.6	<b>32.1**</b>	<b>29.4**</b>
	[SD]	1.4	2.3	1.7	3.2	1.4	1.9	1.1	1.6
	Δ% of control		+1.4	-1.4	-6.9		-1.5	-5.9	-13.8

		Males				Females			
Dose	[ppm]	0	1000	3000	10000	0	1000	3000	10000
	[mg/kg bw/d]	0	67	211	792	0	79	240	814
White Blood cell parameters on day 92/93									
EOS [ $10^9/L$ ]	[mean]	0.10	0.10	0.07	0.09	0.09	0.06	0.08	<b>0.05**</b>
	[SD]	0.03	0.04	0.03	0.03	0.03	0.02	0.03	0.02
	Δ% of control		±0.0	-30.0	-10.0		-33.3	-11.1	-44.4
EOS [%]	[mean]	1.90	1.70	<b>1.40*</b>	<b>1.40*</b>	2.00	1.70	1.80	<b>1.30**</b>
	[SD]	0.4	0.4	0.4	0.8	0.6	0.5	0.7	0.4
	Δ% of control		-10.5	-26.3	-26.3		-15.0	-10.0	-35.0
LUC [ $10^9/L$ ]	[mean]	0.01	0.03	0.02	<b>0.03**</b>	0.01	0.01	0.01	0.02
	[SD]	0.00	0.03	0.01	0.01	0.01	0.01	0.01	0.01
	Δ% of control		+200	+100	+200		±0.0	±0.0	+100
HCD <sup>#</sup>	[mean (range)]	0.02 (0.01 – 0.03)				-			
LUC [%]	[mean]	0.3	0.4	0.3	0.4	0.3	0.4	0.3	<b>0.5*</b>
	[SD]	0.1	0.3	0.1	0.1	0.1	0.2	0.1	0.2
	Δ% of control		+33.3	±0.0	+33.3		+33.3	±0.0	+66.7
HCD <sup>#</sup>	[mean (range)]	-				0.4 (0.2 – 0.6)			

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Kruskal-Wallis + Wilcoxon test (two-sided)

<sup>#</sup> historical control data (HCD) based on 24 dietary and 3 inhalation 90-d studies with Wistar rats conducted in the testing facility under GLP conditions in the time-period between 2007 – 2011

SD – standard deviation.

Δ% of control – percent change compared to control.

RBC – red blood cells (erythrocytes).

HGB – haemoglobin.

HCT – haematocrit.

RET – reticulocytes.

PLT – platelets.

PT – prothrombin time.

EOS – eosinophils.

LUC – large unstained cells.

*Clinical chemistry:* Statistically-significant changes in clinical chemistry measurements were noted in males and females, across different parameters, mainly in the top dose but occasionally in mid dose groups (Table 6.3-24). GGT levels were statistically-significantly increased for both sexes from the mid dose group; in addition a dose-response relationship was evident. Cholesterol levels were statistically-significantly increased for both sexes at the top dose and additionally for females at the mid dose; changes were > 10 % compared to controls across these groups and a dose-response relationship was evident. These effects are considered treatment-related and adverse. Bilirubin levels were decreased in a dose-dependent manner for both males and females. Values were statistically-significantly decreased for females of the mid and top dose group, with a relative change of > 10 % compared to controls. HSE dose not consider this effect to be adverse as a reduction in bilirubin is not toxicologically significant.

Treatment-related and adverse effects were also seen at the top dose on glucose levels (decrease) in both sexes, creatinine levels (decrease) in females and triglycerides (increase) in females. In addition, protein, albumin, globulin and some ion levels were adversely affected from the mid dose. ALP was increased in males at all doses, but as no clear dose-response was seen and values were within the HCD ranges, these were considered unrelated to treatment. Urea was also increased at all dose levels in males but decreased in females from the mid-dose. Given this inconsistency and the fact that values were within the HCD ranges, they were considered unrelated to treatment.

Overall, treatment-related and adverse effects in some clinical chemistry parameters (GGT, CHOL, PROT, ALB, GLOB, ions from the mid dose and GLU, CREAT and TRI at the top dose), indicative of liver toxicity were seen in both sexes from the mid dose (3000 ppm equivalent to 211 and 240 mg/kg bw/d in males and females respectively).

Table 6.3-24. Clinical chemistry parameters

		Males				Females			
Dose level	[ppm]	0	1000	3000	10000	0	1000	3000	10000
	[mg/kg bw/d]	0	67	211	792	0	79	240	814
ALP [ $\mu$ kat/L]	[mean]	1.08	<b>1.35**</b>	<b>1.38*</b>	<b>1.37*</b>	0.57	0.58	0.53	0.59
	[SD]	0.20	0.20	0.26	0.34	0.10	0.14	0.09	0.14
	$\Delta\%$ of control		+25.0	+27.8	+26.9		+1.8	-7.0	+3.5
HCD <sup>#</sup>	[mean (range)]	1.18 (0.91 – 1.45)				-			
GGT [ $\mu$ kat/L]	[mean]	0	1	<b>27*</b>	<b>292**</b>	0	2	<b>46**</b>	<b>268**</b>
	[SD]	0	0	17	701	0	4	17	50
	$\Delta\%$ of control		-	-	-		-	-	-
UREA [mmol/L]	[mean]	4.48	<b>5.00*</b>	<b>5.18**</b>	<b>5.98**</b>	6.49	6.01	<b>5.61**</b>	<b>5.85*</b>
	[SD]	0.47	0.61	0.42	0.69	0.66	0.63	0.60	0.68
	$\Delta\%$ of control		+11.6	+15.6	+33.5		-7.4	-13.6	-9.9
HCD <sup>#</sup>	[mean (range)]	5.90 (4.91 – 7.42)				-			
CREA [ $\mu$ mol/L]	[mean]	25.5	23.9	23.8	22.6	31.1	29.8	29.1	<b>25.6**</b>
	[SD]	2.2	2.8	3.6	1.2	3.9	3.1	2.5	2.2
	$\Delta\%$ of control		-6.3	-6.7	-11.4		-4.2	-6.4	-17.7
GLUC [mmol/L]	[mean]	5.96	5.77	5.48	<b>4.41**</b>	5.44	<b>4.75*</b>	4.92	<b>4.16**</b>
	[SD]	0.37	0.53	0.86	0.37	0.60	0.71	0.51	0.36
	$\Delta\%$ of control		-3.2	-8.1	-26.0		-12.7	-9.6	-23.5
BIL [ $\mu$ mol/L]	[mean]	1.74	1.81	1.56	1.55	2.57	2.23	<b>2.08*</b>	<b>1.87**</b>
	[SD]	0.26	0.34	0.12	0.24	0.42	0.36	0.41	0.33
	$\Delta\%$ of control		+4.0	-10.3	-10.9		-13.2	-19.1	-27.2
PROT [g/L]	[mean]	63.99	62.89	63.64	<b>66.72**</b>	64.68	66.45	<b>68.70**</b>	<b>69.42**</b>
	[SD]	1.40	1.75	1.96	1.92	2.33	2.55	2.71	3.40
	$\Delta\%$ of control		-1.7	-0.5	+4.3		+2.7	+6.2	+7.3
ALB [g/L]	[mean]	37.39	37.72	38.19	<b>39.76**</b>	39.72	40.69	<b>41.48*</b>	<b>41.93**</b>
	[SD]	0.77	1.01	1.06	1.30	1.05	1.56	1.69	1.81
	$\Delta\%$ of control		+0.9	+2.1	+6.3		+2.4	+4.4	+5.6
GLOB [g/L]	[mean]	26.6	<b>25.17*</b>	<b>25.45*</b>	26.96	24.96	25.76	<b>27.21**</b>	<b>27.49**</b>
	[SD]	1.19	1.65	1.64	1.17	1.53	1.37	1.24	1.83
	$\Delta\%$ of control		-5.4	-4.3	+1.4		+3.2	+9.0	+10.1
CHOL [mmol/L]	[mean]	2.15	2.20	2.33	<b>2.71**</b>	1.46	1.56	<b>2.11**</b>	<b>2.84**</b>
	[SD]	0.32	0.35	0.38	0.37	0.42	0.21	0.19	0.57
	$\Delta\%$ of control		+2.3	+8.4	+26.0		+6.8	+44.5	+94.5
TRIG [mmol/L]	[mean]	1.12	1.17	1.11	1.10	0.58	0.54	0.50	<b>0.84*</b>
	[SD]	0.36	0.34	0.29	0.29	0.23	0.18	0.12	0.38
	$\Delta\%$ of control		+4.5	-0.9	-1.8		-6.9	-13.8	+44.8
NA [mmol/L]	[mean]	142.7	142.3	142.8	<b>141.5**</b>	141.0	141.0	141.2	140.9
	[SD]	0.8	0.8	0.8	0.8	0.7	0.7	0.8	1.4
	$\Delta\%$ of control		-0.3	+0.1	-0.8		$\pm 0.0$	+0.1	-0.1
K [mmol/L]	[mean]	4.71	4.83	4.74	<b>5.03**</b>	3.86	4.03	<b>4.13*</b>	<b>4.37**</b>
	[SD]	0.19	0.22	0.20	0.28	0.30	0.28	0.22	0.24
	$\Delta\%$ of control		+2.5	+0.6	+6.8		+4.4	+7.0	+13.2
Cl [mmol/L]	[mean]	100.3	100.0	99.5	<b>98.1**</b>	99.5	99.6	99.0	99.5
	[SD]	0.8	0.9	1.3	1.1	1.0	1.6	0.9	1.3
	$\Delta\%$ of control		-0.3	-0.8	-2.2		+0.1	-0.5	$\pm 0.0$
P (inorg.) [mmol/L]	[mean]	1.63	1.75	1.80	<b>1.95**</b>	1.40	1.43	1.47	1.48
	[SD]	0.17	0.15	0.20	0.18	0.20	0.29	0.23	0.17
	$\Delta\%$ of control		+7.4	+10.4	+19.6		+2.1	+5.0	+5.7
Ca [mmol/L]	[mean]	2.53	2.55	<b>2.59**</b>	<b>2.62**</b>	2.53	2.55	<b>2.63**</b>	<b>2.64**</b>
	[SD]	0.03	0.07	0.04	0.07	0.07	0.07	0.04	0.07
	$\Delta\%$ of control		+0.8	+2.4	+3.6		+0.8	+4.0	+4.3

Dose level	[ppm]	Males				Females			
		0	1000	3000	10000	0	1000	3000	10000
	[mg/kg bw/d]	0	67	211	792	0	79	240	814

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Kruskal-Wallis + Wilcoxon test (two-sided)

# historical control data (HCD) based on 24 dietary and 3 inhalation 90-d studies with Wistar rats conducted in the testing facility under GLP conditions in the time-period between 2007 – 2011.

SD – standard deviation.

$\Delta\%$  of control – percent change compared to control.

ALP – alkaline phosphatase.

GGT – serum- $\gamma$ -glutamyltransferase.

CREA – creatinine

GLUC – glucose.

BIL – bilirubin.

PROT – protein.

ALB – albumin.

GLOB – globulins.

CHOL – cholesterol.

TRIG – triglycerides.

NA – sodium.

K – potassium.

Cl – chlorine.

P – phosphate.

Ca – calcium.

**Urinalyses:** In males at the mid and top dose, the incidences of transitional cells and granulated and epithelial casts in the urine sediment were statistically-significantly increased (Table 6.3-25). These changes were not observed in females and were not accompanied by any other change in the renal system. The findings in males correlated with the occurrence of  $\alpha$ 2u-globulins in the histopathologic investigations and are regarded as species-specific and not relevant to humans.

Table 6.3-25. Urinalyses findings

Dose level	[ppm]	Males				Females			
		0	1000	3000	10000	0	1000	3000	10000
	[mg/kg bw/d]	0	67	211	792	0	79	240	814
Transitional epithelial cells		1	1	2**	2**	1	1	1	1
Casts		0	0	2**	1**	0	0	0	0

\*  $p \leq 0.05$ ; \*  $p \leq 0.01$ ; Wilcoxon test (one-sided).

mean severity: 0=none; 1=few; 2=many; 3=masses;

**Organ weight:** Statistically-significant changes to organ weights were seen in liver, kidney, thyroid, adrenal glands, brain, epididymides, heart, ovaries, spleen, testes and thymus (Table 6.3-26).

Liver weights were statistically-significantly and dose-dependently increased in both males and females. Relative changes were  $> 15\%$  for the top dose and between  $11 - 18\%$  for the mid dose. In both sexes of the high dose, and in mid dose females, liver weight increase was corroborated by several microscopic liver findings as well as clinical chemistry effects and therefore considered treatment-related and adverse from the mid dose.

Absolute kidney weights in all treated male groups were not significantly changed and were within the range of HCD, however, they did show a dose-dependent increase. Relative kidney weights were statistically-significantly increased in all treatment groups, relative changes were  $> 10\%$  compared to controls and were outside the HCD range from the mid dose. Therefore, this effect was considered treatment-related. Considering the corroborative histopathological findings ( $\alpha$ 2u-globulin accumulation), the effect was also considered to be adverse but not human relevant.

Relative thyroid weight was statistically-significant increased in females of the top dose (relative change  $25\%$  compared to controls). Both the absolute and relative values were outside the HCD range, therefore, this effect was considered to be treatment-related and adverse but most likely secondary to liver hypertrophy by hepatic

enzyme induction. An increase (> 10 %) in relative thyroid weight was also seen in top dose males, although no statistical significance was reached.

Despite statistically-significant changes in the weights of other organs, mostly seen in the top dose group, changes were not considered to be treatment-related. This was due to a lack of a dose-response relationship (for adrenal gland weights, kidney weights in females and ovary weights) and/or values falling within the range of the HCD (for brain weight, thymus weight, ovary weights and epididymides weights), and/or changes less than 10 % of controls (for brain, heart and spleen weights) and a lack of correlated histopathological changes (for kidney weights in females, thymus, brain, heart, adrenals, ovaries, epididymids, spleen and testes weights).

Overall, treatment-related, adverse and human relevant changes in organ weights were seen in the liver from the mid dose in both sexes and in the thyroid at the top dose in both sexes.

Table 6.3-26. Terminal body and organ weights

Sex		Males				Females			
	Dose [ppm]	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
Terminal weight [g]	0	373.77				212.97			
	1000	363.42	(-3)			208.97	(-2)		
	3000	355.52	(-5)			213.14	(±0)		
	10000	<b>310.85**</b>	<b>(-17)</b>			<b>182.31**</b>	<b>(-14)</b>		
Adrenal glands [mg]	0	59.2		0.016		68.5		0.032	
	1000	58.3	(-3)	0.016	(+1)	<b>59.2*</b>	<b>(-14)</b>	<b>0.028*</b>	<b>(-12)</b>
	3000	56.1	(-5)	0.016	(+1)	68.3	(±0)	0.032	(-1)
	10000	55.2	(-17)	0.018	(+12)	<b>56.3*</b>	<b>(-18)</b>	0.031	(-4)
Brain [g]	0	2.188		0.586		1.944		0.918	
	1000	2.124	(-3)	0.587	(±0)	1.958	(+1)	0.940	(+2)
	3000	<b>2.098*</b>	<b>(-4)</b>	0.596	(+2)	1.919	(-1)	0.903	(-2)
	10000	<b>2.051**</b>	<b>(-6)</b>	<b>0.664**</b>	<b>(+13)</b>	1.866	(-4)	<b>1.025**</b>	<b>(+12)</b>
	HCD#	Mean (g)	2.095	Mean (%)	0.556				
		Min (g)	1.990	Min (%)	0.516				
		Max (g)	2.175	Max(%)	0.642				
Heart [g]	0	1.085		0.291		0.698		0.328	
	1000	1.047	(-4)	0.289	(-1)	0.687	(-2)	0.329	(±0)
	3000	1.054	(-3)	0.298	(+3)	0.726	(+4)	0.340	(+4)
	10000	1.007	(-7)	<b>0.325*</b>	<b>(+12)</b>	<b>0.638*</b>	<b>(-9)</b>	<b>0.350**</b>	<b>(+7)</b>
Kidneys [g]	0	2.211		0.592		1.511		0.709	
	1000	2.341	(+6)	<b>0.646*</b>	<b>(+9)</b>	<b>1.417*</b>	<b>(-6)</b>	0.680	(-4)
	3000	2.378	(+8)	<b>0.671**</b>	<b>(+13)</b>	1.459	(-3)	0.684	(-4)
	10000	2.444	(+11)	<b>0.790**</b>	<b>(+33)</b>	<b>1.331*</b>	<b>(-12)</b>	0.730	(+3)
	HCD#	Mean (g)	2.281	Mean (%)	0.603				
		Min (g)	2.010	Min (%)	0.551				
		Max (g)	2.461	Max(%)	0.649				
Liver [g]	0	8.676		2.318		5.312		2.498	
	1000	8.828	(+2)	2.426	(+5)	4.955	(-7)	2.375	(-5)
	3000	9.709	(+12)	<b>2.725**</b>	<b>(+18)</b>	<b>5.943*</b>	<b>(+12)</b>	<b>2.784**</b>	<b>(+11)</b>
	10000	<b>11.697**</b>	<b>(+35)</b>	<b>3.752**</b>	<b>(+62)</b>	<b>6.477**</b>	<b>(+22)</b>	<b>3.553**</b>	<b>(+42)</b>
Spleen [g]	0	0.624		0.166		0.424		0.200	
	1000	0.622	(±0)	0.171	(+3)	0.383	(-10)	0.183	(-8)
	3000	0.574	(-8)	0.161	(-3)	0.420	(-1)	0.197	(-1)
	10000	0.610	(-2)	<b>0.195*</b>	<b>(+17)</b>	0.382	(-10)	0.210	(+5)
Thymus [mg]	0	362.2		0.097		298.8		0.140	
	1000	<b>294.4*</b>	<b>(-19)</b>	0.081	(-17)	265.4	(-11)	0.126	(-10)
	3000	<b>275.0**</b>	<b>(-24)</b>	0.078	(-20)	281.3	(-6)	0.132	(-6)
	10000	<b>266.4**</b>	<b>(-26)</b>	0.085	(-12)	<b>234.3**</b>	<b>(-22)</b>	0.129	(-8)

Sex		Males				Females			
	Dose [ppm]	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
	HCD#	Mean (mg)	310.489	Mean (%)	0.082	Mean (mg)	288.35	Mean (%)	0.132
		Min (mg)	241.500	Min (%)	0.064	Min (mg)	240.90	Min (%)	0.115
		Max (mg)	360.300	Max (%)	0.095	Max (mg)	345.00	Max (%)	0.156
Thyroids [mg]	0	24.2		0.006		19.3		0.009	
	1000	25.8	(+7)	0.007	(+10)	19.3	(±0)	0.009	(+1)
	3000	25.3	(+5)	0.007	(+10)	19.9	(+3)	0.009	(+3)
	10000	23.6	(-2)	0.008	(+18)	20.9	(+8)	<b>0.011*</b>	<b>(+25)</b>
	HCD#					Mean (mg)	17.284	Mean (%)	0.008
						Min (mg)	13.100	Min (%)	0.006
						Max (mg)	20.800	Max (%)	0.009
Epididymides (♂) / Ovaries (♀) [g/mg]	0	1.035		0.277		109.6		0.052	
	1000	1.118	(+8)	<b>0.309*</b>	<b>(+12)</b>	<b>88.3*</b>	<b>(-19)</b>	0.043	(-17)
	3000	1.084	(+5)	0.307	(+11)	98.8	(-10)	0.046	(-10)
	10000	1.109	(+7)	<b>0.359**</b>	<b>(+29)</b>	<b>81.8**</b>	<b>(-25)</b>	0.045	(-13)
	HCD#	Mean (g)	1.116	Mean (%)	0.296	Mean (g)	98.133	Mean (%)	0.045
		Min (g)	1.027	Min (%)	0.270	Min (g)	80.700	Min (%)	0.036
		Max (g)	1.204	Max (%)	0.341	Max (g)	113.300	Max (%)	0.051
Testes (♂) [g]	0	3.412		0.914					
	1000	3.577	(+5)	0.986	(+8)				
	3000	3.598	(+5)	<b>1.019*</b>	<b>(+12)</b>				
	10000	3.655	(+7)	<b>1.178**</b>	<b>(+29)</b>				

\* p ≤ 0.05; \*\* p ≤ 0.01; Kruskal-Wallis and Wilcoxon-test (two-sided)

& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

# historical control data (HCD) based on 35/36 dietary 90-d studies with Wistar rats (supplied by [REDACTED]) conducted in the testing facility under GLP conditions in the time-period between 2007 - 2011

**Gross pathology:** At top dose, the livers of 9/10 males and all females showed a dark brown discoloration (Table 6.3-27); this was considered to be treatment-related due to correspondent histopathological findings (minimal peripheral pigment storage). All other findings were of isolated occurrence and therefore were not considered treatment-related.

Table 6.3-27. Selected gross necropsy findings

Dose level [ppm] [mg/kg bw/d]	Males				Females			
	0	1000	3000	10000	0	1000	3000	10000
	0	67	211	792	0	79	240	814
Animals examined	10	10	10	10	10	10	10	10
Liver								
Discoloration, dark brown	-	-	-	9	-	-	-	10

**Histopathology:** Treatment-related findings were observed in liver, kidneys, nasal cavity, ovaries and thyroid glands (Table 6.3-28).

**Liver:** At top dose, diffuse hypertrophy was observed in the most male rats (9 of 10) while all female rats (10/10) showed centrilobular hypertrophy. Additionally, minimal centrilobular hypertrophy was observed in 4/10 males and 3/10 females of the mid dose group. Minimal to slight fatty change of hepatocytes in the peripheral area (zone 1) was observed in top dose males (7/10) and minimal pigment storage was noted in top dose males (9/10) and females (10/10). Corresponding to the gross necropsy observation of dark brown discoloration in top dose animals (Table 6.3-28), light brown pigment was detected in hepatocytes of the peripheral area (zone 1). This pigment did not stain positive with Halls stain (indicative of bile), Perl's or Turnbull stain (indicative of iron), and showed no fluorescence on unstained slides (indicative of lipofuscin); the ORO stain was positive, indicative for an increase of fat storage within the hepatocytes. Hypertrophy in the mid and high dose animals correlates with the increased liver weights (Table 6.3-26); this was considered likely to be

the consequence of hepatic enzyme-induction. Overall, adverse histopathological findings in the liver were seen from the mid dose.

**Kidneys:** Males of all treatment groups revealed minimal to slight chronic nephropathy; both incidence and severity increased in a dose-related manner. Granular casts were noted in some males of all treated dose groups. Incidence lacked a clear dose-dependency, however, severity was increased in a dose-related fashion. Eosinophilic droplets in the cytoplasm of the proximal convoluted tubules occurred in all males of all treatment groups, including the control, however, with a dose-related increase in observations (severity). Eosinophilic droplets were shown to be  $\alpha$ 2u-globulin (by immunohistochemical staining), a poorly hydrolysable, low molecular weight protein, characteristic for male rats. The increase of eosinophilic droplets was therefore regarded to be treatment-related, but not of human relevance. The increase of eosinophilic droplets could also be responsible for the higher incidence of chronic nephropathy and granular casts. Histopathological findings in the kidneys are therefore regarded as treatment-related and adverse, but not relevant for humans. The increased relative kidney weights observed in males of all treated groups may have been caused by the increase of eosinophilic droplets. No relevant kidney findings were observed in females of any dose group.

**Nasal cavity:** In mid and top dose animals of both sexes, minimal to moderate proteinaceous exudation and minimal to slight degeneration of the olfactory epithelium was observed. The dorsal meatus was the area mostly affected in the nasal cavity. In addition to the proteinaceous exudate, granulocytic infiltrates within the olfactory epithelium and within the exudate were observed in each one top dose animal of both sexes. These findings were considered to be treatment-related and adverse in both sexes from the mid dose. Similar findings were not noted in the rat 28-day study (■■■■■, 2015), however, treatment-related and adverse nasal cavity findings were recorded in the long-term toxicity studies in the rat (■■■■■, 2018) and mouse (■■■■■, 2018d).

**Thyroid glands:** In mid and top dose males, hypertrophy/hyperplasia of the follicular cells was observed with a dose-related increase of incidence and severity. The same was seen in females but to a lower extent. Most top dose males (8/10) and some top dose females (2/10) revealed altered (flaky) colloid of increased incidence and grading. Overall, treatment-related and adverse thyroid histopathology was seen in both sexes from the mid dose.

**Ovaries:** In mid and high dose females increased incidence and severity of interstitial glands vacuolation was observed. This finding was not seen in the 28-day study and was not reproduced in the chronic study (■■■■■, 2018). Therefore, it is considered to be either a chance finding or of limited toxicological significance.

All other findings were not considered treatment-related.

Overall, treatment-related and adverse histopathological changes were observed in the liver, thyroid and nasal cavity in both sexes from the mid dose (3,000 ppm equivalent to 211 and 240 mg/kg bw/d in males and females respectively).

Table 6.3-28. Selected histopathological findings

Dose level [ppm] [mg/kg bw/d]	Gradings	Males				Females			
		0	1000	3000	10000	0	1000	3000	10000
		0	67	211	792	0	79	240	814
Number of animals		10	10	10	10	10	10	10	10
<b>Liver</b>	N	10	10	10	10	10	10	10	10
- Hypertrophy, centrilobular	N	0	0	4	0	0	0	3	10
	1	-	-	4	-	-	-	3	2
	2	-	-	-	-	-	-	-	7
	3	-	-	-	-	-	-	-	1
	[mean] <sup>#</sup>	[0.0]	[0.0]	[1.0]	[0.0]	[0.0]	[0.0]	[1.0]	[1.9]
- Hypertrophy, diffuse	N	0	0	0	9	0	0	0	0
	1	-	-	-	6	-	-	-	-
	2	-	-	-	3	-	-	-	-
	[mean] <sup>#</sup>	[0.0]	[0.0]	[0.0]	[1.3]	[0.0]	[0.0]	[0.0]	[0.0]
- Fatty change, peripheral	N	0	0	0	7	0	0	0	0
	1	-	-	-	4	-	-	-	-
	2	-	-	-	3	-	-	-	-



Dose level [ppm] [mg/kg bw/d]	Gradings	Males				Females			
		0	1000	3000	10000	0	1000	3000	10000
		0	67	211	792	0	79	240	814
	[mean] <sup>#</sup>	[0.0]	[0.0]	[0.0]	[1.4]	[0.0]	[0.0]	[0.0]	[0.0]
- Pigment storage, peripheral.	N	0	0	0	9	0	0	0	10
	1	-	-	-	9	-	-	-	10
	[mean] <sup>#</sup>	[0.0]	[0.0]	[0.0]	[1.0]	[0.0]	[0.0]	[0.0]	[1.0]
<b>Kidney</b>	N	10	10	10	10	10	0	0	10
- Eosinophilic droplets	N	10	10	10	10	0	-	-	0
	1	10	5	-	-	-	-	-	-
	2	-	5	10	9	-	-	-	-
	3	-	-	-	1	-	-	-	-
	[mean] <sup>#</sup>	[1.0]	[1.5]	[2.0]	[2.1]	[0.0]	-	-	[0.0]
- Nephropathy, chronic	N	2	7	8	9	0	-	-	0
	1	2	6	5	4	-	-	-	-
	2	-	1	3	5	-	-	-	-
	[mean] <sup>#</sup>	[1.0]	[1.1]	[1.4]	[1.6]	[0.0]	-	-	[0.0]
- Cast, granular, tubular	N	0	1	5	3	0	-	-	0
	1	-	1	3	-	-	-	-	-
	2	-	-	1	2	-	-	-	-
	3	-	-	1	1	-	-	-	-
	[mean] <sup>#</sup>	[0.0]	[1.0]	[1.6]	[2.3]	[0.0]	-	-	[0.0]
<b>Nasal cavity III</b>	N	10	10	10	10	10	10	10	10
- Infiltration, granulocytic	N	-	-	-	1	-	-	-	1
	1	-	-	-	1	-	-	-	-
	2	-	-	-	-	-	-	-	1
	[mean] <sup>#</sup>	[0.0]	[0.0]	[0.0]	[1.0]	[0.0]	[0.0]	[0.0]	[2.0]
- Exudate, proteinaceous	N	-	-	9	10	-	-	5	9
	1	-	-	7	5	-	-	4	2
	2	-	-	2	5	-	-	1	6
	3	-	-	-	-	-	-	-	1
	[mean] <sup>#</sup>	[0.0]	[0.0]	[1.2]	[1.5]	[0.0]	[0.0]	[1.2]	[1.9]
- Degeneration olfactory ep.	N	-	-	10	10	-	-	4	7
	1	-	-	8	6	-	-	4	3
	2	-	-	2	4	-	-	-	4
	[mean] <sup>#</sup>	[0.0]	[0.0]	[1.2]	[1.4]	[0.0]	[0.0]	[1.0]	[1.6]
<b>Thyroid</b>	N	10	10	10	10	10	10	10	10
-	N	0	0	4	9	0	0	1	1
	1	-	-	2	1	-	-	1	-
	2	-	-	2	7	-	-	-	1
	3	-	-	-	1	-	-	-	-
	[mean] <sup>#</sup>	[0.0]	[0.0]	[1.5]	[2.0]	[0.0]	[0.0]	[1.0]	[2.0]
- Altered colloid.	N	1	1	0	8	0	1	0	2
	1	1	1	-	5	-	1	-	2
	2	-	-	-	3	-	-	-	-
	[mean] <sup>#</sup>	[1.0]	[1.0]	[0.0]	[1.4]	[0.0]	[1.0]	[0.0]	[1.0]
<b>Ovaries</b>	N	-	-	-	-	10	10	10	10
- Vacuolation, interst. glands	N	-	-	-	-	0	0	7	9
	1	-	-	-	-	-	-	1	-
	2	-	-	-	-	-	-	6	2
	3	-	-	-	-	-	-	-	6
	4	-	-	-	-	-	-	-	1
	[mean] <sup>#</sup>	-	-	-	-	[0.0]	[0.0]	[1.9]	[2.9]

# = mean severity grading; histopathological findings were graded minimal/very few (Grade 1); slight/few (Grade 2); moderate (Grade 3); marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding

In conclusion, under the conditions of this GLP and OECD test guideline compliant study in rats, dietary administration of cinmethylin for 90 days resulted in the following effects from the mid dose (3,000 ppm = 211/240 mg/kg bw/d in M/F respectively): changes in some clinical chemistry parameter in both sexes, increased prothrombin time, increased liver weight with associated histopathology, and histopathological findings of thyroid and nasal cavity.

A NOAEL of 1,000 ppm in males and females (equivalent to 67 and 79 mg/kg bw/d respectively) is proposed by HSE since no adverse effects were seen at this dose. A LOAEL of 3,000 ppm (211 and 240 mg/kg bw/d in males and females respectively) is proposed based on increased liver weight with corresponding clinical chemistry and histopathology, histopathological findings of thyroid and nasal cavity and increased prothrombin time.

[REDACTED], 2018a)

<b>Author(s)</b>	[REDACTED]
<b>Study title</b>	Subchronic feeding study of SD95481 in the rat. Volume I
<b>Study reference</b>	[REDACTED], 1983 CI-425-001
<b>Test facility</b>	[REDACTED]
<b>Date</b>	November 1983
<b>Test substance</b>	SD95481 (Cinmethylin)
<b>Purity (%)</b>	Not specified in the study report.
<b>Batch no.</b>	WRC-Tox Sample No. 513D
<b>Test animals</b>	Rat Fischer 344 Male and female
<b>Groups</b>	30/sex/dose in total. 10/sex/dose sacrificed at week 7, 20/sex/dose sacrificed at week 13.
<b>Dose/concentrations</b>	0, 30, 100, 300 and 1000 ppm Equivalent to 0, 2.18, 7.51, 22.51 and 75.78 for males and 0, 2.61, 8.73, 26.08 and 88.56 for females respectively.
<b>Route</b>	Administered daily via the diet for 13 weeks.
<b>Vehicle</b>	None.
<b>GLP</b>	Not compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	Relevance and reliability of the study is limited due to: <ul style="list-style-type: none"> <li>• Purity was not reported.</li> <li>• Lack of toxicity up to the top dose.</li> <li>• Further details of test substance stability and homogeneity of the preparation were not reported.</li> <li>• Ophthalmological examination prior to administration of the test substance was not performed.</li> <li>• In haematology and clinical biochemistry, blood clotting time/potential, creatinine and gamma glutamyl transpeptidase were not determined.</li> </ul>

	<ul style="list-style-type: none"> <li>Protein measurement was not conducted in the urine.</li> <li>At gross necropsy, weight of adrenals, ovaries, thymus and spleen were not recorded. Histopathology was not performed on parathyroid.</li> </ul>
<b>Impact of deviations</b>	The above listed deficiencies impact the relevance and reliability of the study.
<b>Acceptable</b>	This study is considered supplemental information only, used as part of the weight of evidence approach.
<b>NOAEL</b>	1000 ppm Equivalent to 76 and 89 mg/kg bw/d in males and females respectively.
<b>Effects at the LOAEL</b>	Not applicable, no adverse effects were seen at the top dose.

### Methods

In a relatively old, non-GLP and non-guideline study, cinmethylin was administered via the diet to groups of 30 male and 30 female Fischer rats per test group, at concentrations of 0, 30, 100, 300 and 1000 ppm, over a period of 13 weeks. Equivalent cinmethylin intakes were 0, 2.18, 7.51, 22.51 and 75.78 mg/kg bw/d for males and 0, 2.61, 8.73, 26.08 and 88.56 mg/kg bw/d for females, respectively. An interim treatment group, consisting of 10 males and 10 females, necropsied at 7 weeks, was included in this study. The remaining 20 males and 20 females were necropsied at week 13 (terminal sacrifice).

Food consumption and body weight was determined weekly. Water consumption was determined on day 14 and weekly thereafter. Animals were examined for signs of toxicity or mortality twice daily. Detailed clinical observations were conducted prior to administration and weekly thereafter. Analysis of urine was performed in 14/20 rats in each group. Clinical biochemistry and haematological examinations were performed after 7 and 13 weeks, in 14/20 rats in each group. After the administration period (7 or 13 weeks) rats were euthanised and necropsied, followed by histopathological examinations.

A method for the dose verification of cinmethylin in feed was evaluated and was regarded as fit for purpose (see Volume 3 CA B5, section B.5.1.2).

### Results

*Mortality and general clinical observations:* There were no treatment-related deaths or clinical signs of toxicity.

*Body weight and food consumption:* Mean body weight of the high dose males was increased on five occasions (days 7, 56, 70, 84 and 91). However, this was not considered to be treatment-related; cinmethylin did not affect the body weight of treated animals. Food consumption was not affected by treatment with cinmethylin in both sexes at any dose.

*Haematology:* Mean platelet count was significantly increased in males of the top dose at the interim and terminal kill, and in females at the interim kill. These changes were not considered biologically-significant as no abnormalities in platelet function or morphologic changes were observed in the platelets themselves or in the bone marrow. Single hemocellular or hemochemical parameters were significantly different from controls. These changes were not dose-related and were not supported by either morphological changes, data from the opposite sex or sacrifice interval.

*Clinical chemistry:* Various serum enzymes (lactate dehydrogenase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and alkaline phosphatase) normally associated with hepatic integrity showed variable and statistically-significant decreases compared to controls at different kill intervals and dose levels. These lower values were not considered to be physiologically-relevant as no toxicological significance is attached to decreases in these enzymes. Statistically-significant differences between treatment and control rats occurred in serum total protein, globulin, cholesterol, total bilirubin, blood urea nitrogen and electrolyte (potassium, calcium and phosphorus) values. Due to the absence of consistent differences among sexes and at the kill intervals and the lack of supporting clinical and morphologic parameters, these alterations were not considered treatment-related.

*Urinalysis:* No treatment-related changes among urinalysis parameters were observed.

*Organ weight:* Absolute and relative liver weight of males in the top dose group, at the interim and terminal sacrifices were statistically-significantly increased (Table 6.3-29). However, increases were < 15 % compared to controls, only reaching > 10 % compared to controls in absolute liver weight at 13-weeks. In addition no dose-

response was evident. In females, statistically-significant increases were seen in the top dose for relative liver weight at 7-weeks and absolute liver weight at 13-weeks. Similar to males, increases were < 10 % change compared to controls and no dose-response was seen. Females of the top dose group showed a statistically-significant increase in absolute (right only) and relative kidney weight at the interim kill. Males of the top dose group showed a statistically-significant increase in absolute and relative kidney weight at the terminal sacrifice. However, a relative change of < 10 % change compared to control was noted for these increases in males and females of the top dose. Without consistency across sexes and sacrifice intervals, and without correlating findings in urinalysis and histopathology, these organ weight findings were not considered treatment-related. There were no treatment-related effects on the weights of any other organs.

Table 6.3-29. Selected organ weight data - liver and kidney weight (group mean)

Concentration [ppm] [mg/kg bw/d]	Absolute [g] (Δ%)		Relative [%] (Δ%)		Absolute [g] (Δ%)		Relative [%] (Δ%)	
	7-week necropsy			13-week necropsy				
Liver weight (% of body weight)								
Males								
0	7.468		3.009		8.113		2.708	
30 2.18	7.242		2.963		8.274		2.729	
100 7.51	7.718		3.120		8.156		2.744	
300 22.51	7.488		3.058		8.293		2.786	
1000 75.78	8.062* (8)		3.195* (6.1)		8.997* (10.9)		2.935* (8.4)	
Females								
0	4.698		3.051		4.757		2.778	
30 2.61	4.745		3.101		4.838		2.824	
100 8.73	4.618		3.035		4.855		2.791	
300 26.08	4.665		3.110		4.814		2.796	
1000 88.56	4.910 (4.5)		3.309* (8.5)		5.036* (5.9)		2.935 (5.7)	
Kidney weight (% of body weight)								
Males	Left	Right			Left	Right		
0	0.936	0.927	0.751		1.010	1.012	0.675	
30 2.18	0.889	0.900	0.732		1.031	1.027	0.678	
100 7.51	0.944	0.926	0.756		1.044	1.036	0.700*	
300 22.51	0.925	0.909	0.750		1.037	1.039	0.698	
1000	0.953	0.942	0.752		1.083* (7.2)	1.071* (5.8)	0.703* (4.2)	
Females	Left	Right			Left	Right		
0	0.594	0.576	0.760		0.613	0.603	0.709	
30 2.61	0.597	0.582	0.771		0.625	0.609	0.720	
100 8.73	0.585	0.571	0.759		0.628	0.632*	0.724	
300 26.08	0.615	0.589	0.804		0.629	0.621	0.725	
1000 88.56	0.611 (2.8)	0.610* (3.5)	0.825* (8.6)		0.632 (3.1)	0.623 (3.3)	0.731 (3.1)	

\* =  $p \leq 0.05$ ; \*\* =  $p \leq 0.01$

$\Delta\%$  - percent change compared to control.

**Gross pathology:** There were no treatment-related macroscopic finding at any dose. Uniform enlargement of the liver of one high dose male, at both the interim and terminal kill was noted. Occasional control and treated animals showed focal parenchymal protrusions on the diaphragmatic surface of the median lobe of the liver. These findings were not considered treatment-related due to their sporadic nature.

**Histopathology:** No treatment-related microscopic changes were observed in the any dose group, at either the interim or terminal kill. Pale centrilobular hepatocytes were observed in 4 of 10 males of the top dose group at 7 weeks. Affected cells were characterised by slightly pale eosinophilic cytoplasm that contained relatively more prominent oblong basophilic bodies presumed to be mitochondria. However, this histopathological finding was not accompanied by a biologically-significant change in liver weight (a change of < 10 % was seen for absolute and relative liver weight in this group), or changes in clinical chemistry and was not seen in the terminal sacrifice. Therefore this finding was not considered treatment-related and adverse. Other observations were considered to be incidental or commonly observed in Fischer 344 rats. The incidence, severity and distribution of these lesions were randomly observed among rats in all dose groups including controls.

## Conclusion

In conclusion, under the conditions of this limited study, dietary administration of cinmethylin for 90 days in rats resulted in no treatment-related and adverse effects up to the top dose of 1,000 ppm. It is noted that the top dose used in this study (76/89 mg/kg bw/d for M/F respectively) is lower than the LOAEL identified in the previous (new/modern) 90-day study in the rat (3,000 ppm = 211/240 mg/kg bw/d for M/F respectively; [REDACTED], 2018a). Therefore, the results of this study are consistent with those of the new/modern study.

A NOAEL of 1,000 ppm (equivalent to 76 and 89 mg/kg bw/d in males and females respectively) is proposed by HSE since no adverse effects were seen at this dose.

(██████████, 1983)

### *Studies in mice*

The repeated dose toxicity of cinmethylin have been investigated in mice via the oral (dietary) route in one standard guideline 90-day study (new/modern) in C57BL/6J Rj mice and one previously performed study with B6C3F1 mice.

### 1) *New/modern study*

<b>Author(s)</b>	[REDACTED]
<b>Study title</b>	BAS 684 H - Repeated dose 90-day oral toxicity in C5BL/6JRj mice - Administration via the diet
<b>Study reference</b>	[REDACTED], 2018b BASF DocID : 2015/1005983
<b>Test facility</b>	[REDACTED]
<b>Date</b>	30/07/2014 – 31/10/2014
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b>	96.2
<b>Batch no.</b>	COD-001919 (-) / (+) ratio = 51:49
<b>Test animals</b>	Mice C57BL/6J Rj Male and female
<b>Groups</b>	10/sex/dose
<b>Dose/concentrations</b>	0, 200, 1000 and 5000 ppm Equivalent to 0, 43, 201 and 1200 mg/kg bw/d in males and 0, 58, 285 and 1304 mg/kg bw/d in females.
<b>Route</b>	Administered daily via the diet for 90 days.
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.

<b>Guideline</b>	OECD TG No. 408 (1998) (note the current test guideline was adopted in 2018) (EC) No 440/2008 of 30 May 2008 - Part B No. L 142, EPA 870.3100, JMAFF No 12 Nosan No 8147 (2000)
<b>Deviation</b>	None.
<b>Impact of deviations</b>	Not applicable.
<b>Acceptable</b>	Yes.
<b>NOAEL</b>	200 ppm (43 and 58 mg/kg bw/d in males and females respectively)
<b>Effects at the LOAEL</b>	A LOAEL of 1000 ppm (201 and 285 mg/kg bw/d in males and females respectively) is proposed based on decreases in body weight gain and food consumption and changes in some clinical chemistry parameters (TRIG, TPROT, ALB, CHOL), indicative of liver toxicity, with associated increases in liver weights.

### Methods

In a GLP and OECD test guideline compliant study, cinmethylin was administered via the diet to groups of 10 male and 10 female C57BL/6J Rj mice per test group, at concentrations of 0, 200, 1000 and 3000 ppm, over a period of 90 days. Equivalent cinmethylin intakes were 0, 43, 201 and 1200 mg/kg bw/d for males and 0, 58, 285 and 1304 mg/kg bw/d for females, respectively. No satellite groups were included in this study. A method for the detection of cinmethylin in rat/mouse diet (Catchpole & Hidding, 2017b; 2017/1123754) was evaluated and was considered validated (see Volume 3 CA B5, section B.5.1.2).

### Results

*Mortality and general clinical observations:* There were no treatment-related deaths or clinical signs of toxicity. One female of the mid dose showed alopecia at the tail and right hindlimb, the later corroborated by a skin lesion. However, due to its isolated occurrence these findings were not considered to be treatment-related.

*Body weight and body weight gain:* A statistically-significant decrease in mean body weight was observed in males at the top dose in the last two weeks of the study. A dose-response was evident, and although relative changes compared to control were relatively low (7 %) they were considered treatment-related and adverse. Females in all treatment groups and males of the low and mid dose groups did not reveal any effects on mean body weight. In top dose males, statistically-significantly decreased mean body weight gain was observed for the treatment periods 0 – 77, 0 – 84 and 0 – 91 days. Over the complete study period (0 – 91 days) a 27 % reduction in body weight gain compared to controls was noted. In females, statistically-significantly decreases in body weight gain were observed in mid and high dose groups; in the top dose during the periods 0 – 14, 0 – 21, 0 – 28 and 0 – 84, and in the mid dose during the periods 0 – 21, 0 – 28 and 0 – 35. Relative changes compared to controls of > 10 % were consistently seen; in the top dose decreases between 19 % (0 – 84 days) up to 58 % (0 – 14 days) were noted, and in the mid dose a maximum change of 44 % was seen (0 – 21 days). Overall, treatment-related and adverse effects on body weight and body weight gain were seen in males at the top dose (1200 mg/kg bw/d). In addition, adverse effects on body weight gain (especially during the first five weeks of treatment) were seen in females from the mid dose (285 mg/kg bw/d).

Table 6.3-30. Body weight and body weight gain

Dose level [ppm] [mg/kg bw/d]		Males				Females			
		0	200	1000	5000	0	200	1000	5000
		0	43	201	1200	0	58	285	1304
Body weight [g]									
Day 0	[mean]	21.9	21.9	21.9	21.8	19.7	17.9	17.9	17.8
	[SD]	0.8	0.9	1.0	0.9	1.0	1.1	1.1	0.8
	Δ% of control	-	+0.1	-0.1	-0.4	-	-0.1	-0.4	-0.5
Day 28	[mean]	24.2	24.6	24.5	23.7	20.4	20.0	19.5	19.5
	[SD]	0.9	1.2	1.2	1.0	0.9	1.1	1.2	0.9
	Δ% of control	-	+1.7	+1.2	-2.1	-	-1.5	-4.2	-4.1
Day 84	[mean]	28.7	28.7	28.3	26.7*	22.6	22.1	21.8	21.7
	[SD]	0.8	1.9	1.9	1.1	1.4	1.1	1.4	0.9
	Δ% of control	-	-0.2	-1.6	-6.9	-	-2.3	-3.8	-4.3
Day 91	[mean]	29.0	29.1	28.5	27.0*	22.6	22.7	21.7	21.9
	[SD]	0.9	2.3	2.1	1.2	1.3	1.3	1.4	1.1
	Δ% of control	-	0.5	-1.6	-6.9	-	+0.4	-3.9	-2.8

		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	43	201	1200	0	58	285	1304
Body weight gain [g]									
Day 0 - 14	[mean]	0.9	1.4	1.2	0.5	1.4	1.1	0.9	<b>0.6**</b>
	[SD]	0.7	0.6	0.4	0.7	0.5	0.4	0.4	0.6
	Δ% of control	-	+53.7	+30.9	-47.9	-	-21.5	-35.6	-57.8
Day 0 - 21	[mean]	1.5	1.9	2.1	1.1	1.9	1.5	<b>1.1**</b>	<b>1.1**</b>
	[SD]	0.9	0.7	0.5	0.6	0.6	0.5	0.7	0.5
	Δ% of control	-	+26.2	+40.3	-23.5	-	-19.4	-43.5	-42.9
Day 0 - 28	[mean]	2.3	2.7	2.6	1.9	2.4	2.1	<b>1.6*</b>	<b>1.7*</b>
	[SD]	1.0	0.6	0.6	0.8	0.6	0.4	0.7	0.6
	Δ% of control	-	+16.1	+14.3	-18.3	-	-12.4	-32.6	-30.6
Day 0 - 35	[mean]	3.1	3.4	3.0	2.6	2.9	2.8	<b>2.1*</b>	2.3
	[SD]	0.9	0.7	0.7	0.6	0.6	0.4	0.7	0.5
	Δ% of control	-	+10.1	-1.9	-15.9	-	-2.4	-25.2	-20.6
Day 0 - 77	[mean]	6.0	6.4	5.8	<b>4.4*</b>	4.0	4.0	3.8	3.6
	[SD]	1.0	1.9	1.0	0.8	0.8	0.5	0.9	0.6
	Δ% of control	-	+7.7	-2.9	-26.5	-	-2.0	-4.7	-10.2
Day 0 - 84	[mean]	6.8	6.7	6.4	<b>4.9**</b>	4.7	4.2	3.9	<b>3.8*</b>
	[SD]	1.1	1.3	1.4	1.1	0.9	0.5	0.8	0.6
	Δ% of control	-	-1.2	-6.2	-27.9	-	-11.0	-16.5	-18.9
Day 0 - 91	[mean]	7.1	7.2	6.6	<b>5.2**</b>	4.6	4.7	3.8	4.1
	[SD]	1.2	1.6	1.5	0.9	1.2	0.8	0.9	0.8
	Δ% of control	-	+1.6	-6.1	-27.0	-	+2.4	-17.3	-11.9

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Dunnett test (two-sided)

SD – standard deviation.

Δ% of control – percent change compared to control.

*Food and water consumption:* There were no treatment-related changes in water consumption. Decreases in food consumption were noted for females of the top dose at different time points (Table 6.5-31); these were consistent with the decreased body weight gain observed in these animals (Table 6.3-30).

Table 6.3-31. Food consumption

		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	43	201	1200	0	58	285	1304
Food consumption [g/animal/day]									
Day 0 – 7	[mean]	5.2	5.2	4.7	5.5	5.3	4.1*	4.2	3.5**
	[SD]	0.8	1.1	0.7	2.1	1.1	0.3	0.2	0.7
	Δ% of control	-	+1.2	-9.7	+6.8	-	-21.9	-20.4	-33.2
Day 7 – 14	[mean]	5.1	5.1	5.2	6.6*	6.1	5.3	5.0	5.4
	[SD]	1.0	0.6	0.8	1.7	0.8	0.4	0.7	1.0
	Δ% of control	-	-1.3	+2.0	+29.1	-	-13.4	-19.4	-11.7
Day 14 – 21	[mean]	5.2	5.4	4.9	5.6	6.3	5.2	5.5	4.8*
	[SD]	1.1	1.3	0.6	1.9	0.6	0.4	0.4	1.1
	Δ% of control	-	+3.8	-6.7	+6.9	-	-17.1	-13.3	-24.4
Day 42 – 49	[mean]	5.6	5.9	5.2	5.4	6.3	5.7	5.6	4.4**
	[SD]	0.8	1.0	0.8	1.5	0.4	1.0	0.6	0.9
	Δ% of control	-	+5.2	-7.5	-3.6	-	-10.1	-12.0	-30.7
Day 0 - 91	[mean]	5.4	5.3	5.1	5.3	6.2	6.1	5.8	5.3*
	[SD]	0.7	0.7	0.7	0.8	0.2	0.5	0.6	0.8
	Δ% of control	-	-1.2	-5.3	-1.1	-	-3.0	-7.8	-15.5

\* p ≤ 0.05; \*\* p ≤ 0.01; Dunnett test (two-sided)

Note that some weeks have been left out of this table as they did not show any statistically-significant changes in food consumption.

**Haematology:** Statistically-significantly increased haemoglobin (HGB) was observed in top dose females (10 % change compared to controls) and increased corpuscular volume (MCV) and corpuscular hemoglobin content (MCH) were observed in top dose animals of both sexes (< 10 % change compared to control). These findings were considered to be treatment-related due to the magnitude of change compared to control (for HGB) or exceedance of the respective HCD range (for MCV and MCH). However, due to the size of the effect, they were not considered adverse. It should be noted that in the previous 28-day study (██████████, 2016), a slight opposite effect (decrease in HGB and MCV) was observed. MCV and MCH were also statistically-significantly increased in the low and/or mid dose males and mean corpuscular hemoglobin concentration (MCHC) was statistically-significantly increased in the top dose females. However, the observed changes were marginal and/or within the respective HCD range. An isolated but statistically-significant decrease in relative reticulocyte count (RET) was observed in low dose males; however, there was a notable absence of a dose-response relationship. Whilst not statistically-significant, there was a prominent increase in RET in females at the mid dose group (71 % change compared to control) but not in the top dose group. Platelet counts (PLT) were statistically-significantly increased in top dose animals of both sexes and in mid dose females, however, this lacked a clear dose-response relationship and values were within the range of HCD. Overall, therefore, there were no adverse effects on red blood parameters and platelets at any dose.

Statistically-significantly decreased total white blood cell (WBC), absolute neutrophil (NEUT), absolute lymphocyte (LYMPH) and eosinophil (EOS) counts were observed in top dose males. Relative changes compared to control were > 10 %. In females of the top dose, statistically-significantly decreased absolute and relative EOS counts were observed (well > 10 % change compared to control). Absolute and relative monocyte (MONO) count was statistically-significantly increased in the mid and/or top dose in males, however, no dose-response relationship was evident.

Overall, treatment-related and adverse changes in some white blood cell (WBC, NEUT, LYMPH, EOS) parameters were seen in males and females at the top dose (1200 and 1304 mg/kg bw/d in males and females respectively).



Table 6.3-32. Haematology

		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	43	201	1200	0	58	285	1304
Red Blood cell and coagulation parameters on day 92/93									
HGB [mmol/L]	[mean]	8.9	8.9	8.9	9.0	8.9	9.3	8.9	9.8**
	[SD]	0.2	0.4	0.5	0.5	0.6	0.6	1.1	0.2
	Δ% of control		±0.0	±0.0	+1.1		+4.5	±0.0	+10.1
MCV [fL]	[mean]	45.3	45.5	45.9**	47.0**	44.7	45.4	45.8	46.0**
	[SD]	0.3	0.4	0.4	0.4	0.6	1.1	2.0	0.4
	Δ% of control		+0.4	+1.3	+3.8		+1.6	+2.5	+2.9
HCD#	[mean (range)]	44.5 (43.0 – 45.6)				-			
MCH [fmol]	[mean]	0.88	0.89*	0.90**	0.93**	0.90	0.90	0.91	0.94**
	[SD]	0.01	0.02	0.01	0.04	0.03	0.02	0.03	0.02
	Δ% of control		+1.1	+2.3	+5.7		±0.0	+1.1	+4.4
HCD#	[mean (range)]	0.88 (0.84 – 0.92)				-			
MCHC [mmol/L]	[mean]	19.41	19.69	19.63	19.88	20.08	19.88	19.80	20.45*
	[SD]	0.24	0.39	0.28	0.89	0.72	0.41	1.07	0.43
	Δ% of control		+1.4	+1.1	+2.4		-1.0	-1.4	+1.8
HCD#	[mean (range)]	-				19.42 (18.77 – 20.55)			
RET [%]	[mean]	2.6	2.4**	2.5	2.6	2.4	2.6	4.1	2.6
	[SD]	0.2	0.2	0.2	0.2	0.4	0.7	6.1	0.3
	Δ% of control		-7.7	-3.8	±0.0		+8.3	+70.8	+8.3
PLT [10 <sup>9</sup> /L]	[mean]	1333	1365	1342	1538**	1265	1272	1441*	1375*
	[SD]	131	81	82	87	99	145	309	67
	Δ% of control		+2.4	+0.7	+15.4		+0.6	+13.9	+8.7
HCD#	[mean (range)]	1425 (1240 – 1571)				1298 (1225 – 1451)			
White Blood cell parameters on day 92/93									
WBC [10 <sup>9</sup> /L]	[mean]	6.64	7.10	7.04	4.60**	4.64	4.62	4.12	4.35
	[SD]	1.51	2.00	1.51	1.20	1.79	2.23	1.48	1.32
	Δ% of control		+6.9	+6.0	-30.7		-0.4	-11.2	-6.3
NEUT [10 <sup>9</sup> /L]	[mean]	0.58	0.65	0.73	0.45*	0.47	0.45	0.44	0.43
	[SD]	0.13	0.10	0.19	0.16	0.09	0.21	0.19	0.14
	Δ% of control		+12.1	+25.9	-22.4		-4.3	-6.4	-8.5
NEUT [%]	[mean]	8.8	9.9	10.8	9.9	12.3	10.3	12.1	10.2
	[SD]	1.6	3.8	3.2	2.8	7.6	2.6	6.7	2.8
	Δ% of control		+12.5	+22.7	+12.5		-16.3	-1.6	-17.1
LYMPH [10 <sup>9</sup> /L]	[mean]	5.89	6.22	6.08	4.04**	4.06	4.05	3.59	3.84
	[SD]	1.37	1.93	1.45	1.10	1.81	1.98	1.33	1.23
	Δ% of control		+5.6	+3.2	-31.4		-0.2	-11.6	-5.4
LYMPH [%]	[mean]	88.7	86.9	85.9	87.5	84.6	87.2	85.7	87.8
	[SD]	1.8	4.0	3.3	3.2	9.0	2.7	7.1	3.2
	Δ% of control		-2.0	-3.2	-1.4		+3.1	+1.3	+3.8
MONO [10 <sup>9</sup> /L]	[mean]	0.05	0.12	0.11**	0.06	0.05	0.05	0.04	0.04
	[SD]	0.03	0.13	0.04	0.03	0.02	0.04	0.03	0.02
	Δ% of control		+140	+120	+20		±0.0	-20.0	-20.0
MONO [%]	[mean]	0.8	1.5	1.6**	1.3**	1.4	1.1	1.0	1.0
	[SD]	0.5	1.4	0.9	0.5	1.0	0.4	0.5	0.5
	Δ% of control		+87.5	+100	+62.5		-21.4	-28.6	-28.6
EOS [10 <sup>9</sup> /L]	[mean]	0.10	0.10	0.09	0.04*	0.06	0.05	0.04	0.03**
	[SD]	0.05	0.07	0.04	0.01	0.2	0.4	0.2	0.1
	Δ% of control		±0.0	-10	-60		-16.7	-33.3	-50.0
EOS [%]	[mean]	1.4	1.3	1.3	1.0	1.4	1.1	1.0	0.8**
	[SD]	0.6	0.6	0.4	0.2	0.6	0.7	0.4	0.3
	Δ% of control		-7.1	-7.1	-28.6		-21.4	-28.6	-42.9

Dose level	[ppm]	Males				Females			
		0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	43	201	1200	0	58	285	1304

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Kruskal-Wallis + Wilcoxon test (two-sided);

# historical control data (HCD) based on 6 dietary 90-d studies in C57BL/6J Rj mice conducted in the testing facility under GLP conditions in the time period 2010 – 2013

SD – standard deviation.

$\Delta\%$  of control – percent change compared to control.

HGB – haemoglobin.

MCV – mean corpuscular volume.

MCH – mean corpuscular haemoglobin.

MCHC – mean corpuscular haemoglobin concentration.

RET – reticulocytes.

WBC – white blood cells (leukocytes).

NEUT – polymorphonuclear neutrophils.

LYMPH – lymphocytes (absolute).

MONO – monocytes.

PLT – platelets.

EOS – eosinophils.

**Clinical chemistry:** Statistically-significantly decreased triglycerides level (TRIG, > 10 % change compared to control) were observed in top dose animals of both sexes. Additionally, statistically-significant decrease of the total protein (TPROT), albumin (ALB) and cholesterol (CHOL) concentrations was observed in mid and high dose males. Statistically-significantly decreased total bilirubin (TBIL) levels were observed in mid and high dose males. However, in absence of anaemia (i.e. absence of any significant alteration of RBC parameter), the lower plasma bilirubin levels were most probably due to an increased conjugation rate of bilirubin in consequence of liver enzyme induction followed by an accelerated excretion of bilirubin via the bile. This mechanism is regarded as adaptive and not adverse. Statistically-significantly increased alkaline phosphatase (ALP) activity and chloride (Cl) level was observed in top dose males but both parameters were within the respective HCD ranges. In low dose females, statistically-significant increases in alanine aminotransferase aspartate (ALT) and aminotransferase (AST) activity was observed (> 10 % change compared to control); however, a dose-response relationship was not evident. In all treated females, sodium levels were statistically-significantly increased, but did not exceed the HCD range. Overall, treatment-related and adverse changes in some clinical chemistry parameters (TRIG, TPROT, ALB and CHOL), indicative of liver toxicity were observed in males from the mid dose (201 mg/kg bw/d) and in females at the top dose (1304 mg/kg bw/d).

Table 6.3-33. Clinical chemistry

Dose level	[ppm]	Males				Females			
		0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	43	201	1200	0	58	285	1304
ALT [ $\mu$ kat/L]	[mean]	0.78	0.81	0.77	1.09	0.71	<b>0.57*</b>	0.80	0.77
	/SD	0.17	0.24	0.18	0.36	0.15	0.08	0.15	0.12
	$\Delta\%$ of control		+3.8	-1.3	+39.7		-19.72	+12.68	+8.45
AST [ $\mu$ kat/L]	[mean]	5.53	5.32	4.90	6.13	4.29	<b>3.19**</b>	3.65	3.78
	/SD	1.61	1.32	1.81	2.84	1.24	0.43	0.72	0.61
	$\Delta\%$ of control		-3.8	-11.4	10.8		-25.6	-14.9	-11.9
ALP [ $\mu$ kat/L]	[mean]	1.13	1.06	1.08	<b>1.23*</b>	1.63	1.67	1.66	1.74
	/SD	0.08	0.04	0.06	0.09	0.16	0.14	0.41	0.22
	$\Delta\%$ of control		-6.2	-4.4	+8.8		+2.5	+1.8	+6.7
HCD#	[mean (range)]	1.12 (1.05 – 1.28)				-			
TBIL [ $\mu$ mol/L]	[mean]	1.61	1.54	<b>1.47**</b>	<b>1.30**</b>	1.69	1.78	1.59	1.51
	/SD	0.13	0.21	0.10	0.11	0.29	0.22	0.20	0.22
	$\Delta\%$ of control		-4.3	-8.7	-19.3		+5.3	-5.9	-10.7
TPROT [g/L]	[mean]	52.08	51.89	<b>50.30**</b>	<b>49.14**</b>	48.89	50.26	49.74	49.21
	/SD	1.24	1.43	0.86	1.05	2.30	2.14	1.90	0.78
	$\Delta\%$ of control		-0.4	-3.4	-5.6		+2.8	+1.7	+0.7
ALB [g/L]	[mean]	32.97	32.96	<b>31.90**</b>	<b>30.80**</b>	31.43	32.11	31.80	31.57
	/SD	0.71	0.85	0.65	0.70	0.91	1.53	1.71	0.59

Dose level		Males				Females			
	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	43	201	1200	0	58	285	1304
	Δ% of control		±0.0	-3.2	-6.6		+2.2	+1.2	+0.4
CHOL [mmol/L]	[mean]	2.55	2.52	<b>2.25**</b>	<b>1.76**</b>	2.03	2.04	1.90	1.97
	[SD]	0.17	0.22	0.20	0.23	0.31	0.26	0.37	0.25
	Δ% of control		-1.2	-11.8	-31.0		-16.1	+19.4	-19.4
TRIG [mmol/L]	[mean]	0.93	0.92	0.91	<b>0.53**</b>	0.78	0.75	0.64	<b>0.52**</b>
	[SD]	0.16	0.12	0.18	0.11	0.20	0.25	0.22	0.12
	Δ% of control		-1.1	-2.2	-43.0		-3.8	-17.9	-33.3
Na [mmol/L]	[mean]	150.07	150.4	150.7	151.7	148.3	<b>149.3*</b>	<b>150.2**</b>	<b>151.3**</b>
	[SD]	1.4	1.0	1.1	1.4	1.9	0.6	1.0	0.8
	Δ% of control		+0.2	+0.4	+1.1		+0.7	+1.3	+2.0
HCD <sup>#</sup>	[mean (range)]	-				149.9 (148.8 – 151.8)			
Cl [mmol/L]	[mean]	114.4	115.1	115.3	<b>117.2**</b>	113.8	113.9	115.7	115.5
	[SD]	1.7	1.7	0.8	1.9	2.2	1.8	1.7	1.8
	Δ% of control		+0.6	+0.8	+2.4		+0.1	+1.7	+1.5
HCD <sup>#</sup>	[mean (range)]	115.2 (112.2 – 117.2)				-			

\* p ≤ 0.05; \*\* p ≤ 0.01; Kruskal-Wallis + Wilcoxon test (two-sided)

<sup>#</sup> historical control data (HCD) based on 6 dietary 90-d studies in C57BL/6J Rj mice conducted in the testing facility under GLP conditions in the time period 2010 – 2013.

ALT – alanine aminotransferase.

AST – aspartate aminotransferase.

ALP – alkaline phosphatase.

TBIL – total bilirubin.

TPROT – total protein.

ALB – albumin.

CHOL – cholesterol.

TRIG – triglycerides.

Na – sodium

Cl – chloride.

**Organ weight:** Absolute and relative liver weights were statistically-significantly increased in mid dose males (< 15 % change compared to control) and in top dose animals of both sexes (> 15 % change compared to control). A clear dose-response relationship was evident. No corresponding macro- and microscopic effects were observed; however, observed clinical chemistry findings support these findings. Overall, adverse effects on liver weight were seen from the mid dose (201 mg/kg bw/d). All other organ weight changes were considered to be incidental in nature due to lack of a dose-response relationship, lack of any correlated macro- or microscopic findings and/or magnitude of effect.

Table 6.3-34. Terminal body and organ weights

	Males					Females				
	Dose [ppm] [mg/kg bw/d]	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&	Dose [ppm] [mg/kg bw/d]	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
Terminal weight [g]	0	25.09				0	19.75			
	200 43	25.27	+0.7			200 58	19.71	-0.2		
	1000 201	24.90	-0.8			1000 285	18.90	-4.3		
	5000 1200	<b>23.25**</b>	-7.3			5000 1304	19.11	-3.2		
Heart [mg]	0	139.2		0.556		0	118.3		0.600	
	200 43	141.8	+1.9	0.563	+1.3	200 58	115.5	-2.4	0.588	-2.0

	Males					Females				
	Dose [ppm] [mg/kg bw/d]	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&	Dose [ppm] [mg/kg bw/d]	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
	1000 201	135.9	-2.4	0.549	-1.3	1000 285	114.5	-3.2	0.608	+1.3
	5000 1200	138.9	-0.2	0.599	+7.7	5000 1304	<b>109.7*</b>	-7.3	0.575	-4.2
Liver [mg]	0	1087.4		4.342		0	869.6		4.399	
	200 43	1109.4	+2.0	4.392	+1.2	200 58	833.3	-4.2	4.222	-4.0
	1000 201	<b>1182.9**</b>	+8.8	<b>4.759**</b>	+9.6	1000 285	901.2	+3.6	<b>4.776**</b>	+8.6
	5000 1200	<b>1316.4**</b>	21.1	<b>5.665**</b>	+30.5	5000 1304	<b>1043.5**</b>	+20.0	<b>5.465**</b>	+24.2
Thymus [mg]	0	29.9		0.119		0	36.70		0.186	
	200 43	31.2	+4.3	0.124	+4.2	200 58	38.50	+4.9	0.195	+4.8
	1000 201	31.0	+3.7	0.124	+4.2	1000 285	34.40	-6.3	0.183	-1.6
	5000 1200	26.9	-10.0	0.116	-2.5	5000 1304	<b>41.60*</b>	+13.4	0.219	+17.7
Ovaries [mg]	0					0	13.70		0.070	
	200 43					200 58	13.50	-1.5	0.069	-1.4
	1000 201					1000 285	<b>11.90*</b>	-13.1	0.063	-10.0
	5000 1200					5000 1304	14.00	+2.2	0.073	+4.3

Statistical analysis: \* p ≤ 0.05, \*\* p ≤ 0.01 [Kruskal-Wallis and Wilcoxon-test (two-sided)]

& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

Δ% - percent change compared to control.

*Gross pathology:* There were no treatment-related and adverse findings in gross pathology.

*Histopathology:* In the liver, diffuse fatty change was observed in all animals, including the control. However, the lowest severity was observed in the top dose animals (Table 6.3-35). No relevant liver finding was observed that would explain the liver weight increase in the top dose animals. In the kidney, a cytoplasmatic vacuolation of tubule cells was observed in most males; however, a lower incidence and severity was observed in the top dose group. Findings in the liver and kidney were considered to be chance findings. All other histopathological findings were not treatment-related.

Table 6.3-35. Selected histopathological findings

Dose level [ppm] [mg/kg bw/d]	Gradings	Males				Females			
		0	200	1000	5000	0	200	1000	5000
		0	43	201	1200	0	58	285	1304
Number of animals		10	10	10	10	10	10	10	10
Liver - Fatty change, diffuse	N	10	10	10	10	10	10	10	10
	N	10	10	10	10	10	10	10	10
	1	0	0	0	3	1	0	0	1
	2	4	2	1	7	7	6	4	9
	3	6	8	9	0	2	4	6	0
	[mean] <sup>#</sup>	[2.6]	[2.8]	[2.9]	[1.7]	[2.1]	[2.4]	[2.6]	[1.9]
Kidney - Vacuoles, tubules	N	10	10	10	10	10	0	0	10
	N	10	10	10	5	0	-	-	0

Dose level [ppm] [mg/kg bw/d]	Gradings	Males				Females			
		0	200	1000	5000	0	200	1000	5000
		0	43	201	1200	0	58	285	1304
	1	5	1	5	5	0	-	-	0
	2	5	8	4	0	0	-	-	0
	3		1	1	0	0	-	-	0
	[mean] <sup>#</sup>	[1.5]	[2.0]	[1.6]	[1.0]	[0.0]	-	-	[0.0]

N = number of animals examined

<sup>#</sup> = mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding

### Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant study in mice, dietary administration of cinmethylin for 90 days resulted in decreases in body weight gain and changes in some clinical chemistry parameters (TRIG, TPROT, ALB and CHOL), indicative of liver toxicity, with associated increases in liver weights from the mid dose (1,000 ppm = 201 and 285 mg/kg bw/d in males and females).

In addition, at the top dose (5,000 ppm = 1200/1304 mg/kg bw/d in M/F respectively) there were effects on body weight and food consumption and changes in some white blood cell parameters.

A NOAEL of 200 ppm (43 and 58 mg/kg bw/d in M/F respectively) is proposed by HSE since no adverse effects were seen at this dose. A LOAEL of 1,000 ppm is proposed based on decreases in body weight gain and food consumption and changes in some clinical chemistry parameters (TRIG, TPROT, ALB and CHOL), indicative of liver toxicity, with associated increases in liver weights.

(██████████, 2018b)

### 2) Old study

<b>Author(s)</b>	██████████
<b>Study title</b>	Subchronic Feeding Study of SD95481 in the Mouse
<b>Study reference</b>	██████████, 1983 CI-425-002
<b>Test facility</b>	██
<b>TDate</b>	1983
<b>Test substance</b>	SD95481 (Cinmethylin)
<b>Purity (%)</b>	Not specified in the study report.
<b>Batch no.</b>	WRC-Tox Sample No. 513D
<b>Test animals</b>	Mice B6C3F1 Male and female
<b>Groups</b>	30/sex/dose in total. 10/sex/dose sacrificed at week 7, 20/sex/dose sacrificed at week 13.
<b>Dose/concentrations</b>	0, 30, 100, 300 and 1000 ppm Equivalent to 0, 3.81, 11.50, 39.57 and 123.11 for males and 0, 4.36, 13.85, 42.57 and 129.66 for females respectively.
<b>Route</b>	Administered daily via the diet for 13 weeks.
<b>Vehicle</b>	None.
<b>GLP</b>	Not compliant (GLP was not established when this study was conducted).
<b>Guideline</b>	None.
<b>Deviation</b>	Compared to the current test guideline OECD TG No. 408 (2018) : <ul style="list-style-type: none"> <li>Detailed clinical observations (weekly) were not conducted.</li> <li>Lack of toxicity up to the top dose.</li> <li>Sensory reactivity and functional observation were not performed.</li> <li>Purity and further details of test substance stability and homogeneity of the preparation were not reported.</li> <li>Additional satellite groups were not included.</li> </ul>

	<ul style="list-style-type: none"> <li>• Ophthalmological examination prior to administration of the test substance was not performed.</li> <li>• In haematology and clinical biochemistry, blood clotting time/potential, creatinine and gamma glutamyl transpeptidase were not determined.</li> <li>• Urinalysis was not conducted.</li> <li>• At gross necropsy, weight of adrenals, ovaries, thymus and spleen were not recorded.</li> <li>• Histopathology on parathyroid was not performed.</li> </ul>
<b>Impact of deviations</b>	The above listed deficiencies impact the relevance and reliability of the study.
<b>Acceptable</b>	This study is considered supplemental information only, used as part of the weight of evidence approach.
<b>NOAEL</b>	1000 ppm Equivalent to 123 and 130 mg/kg bw/d in males and females respectively.
<b>Effects at the LOAEL</b>	Not applicable, no adverse effects were seen at the top dose.

### Methods

In a relatively old, non-GLP and non-guideline study, cinmethylin was administered via the diet to groups of 30 male and 30 female B6C3F1 mice per test group, at concentrations of 0, 30, 100, 300 and 1000 ppm, over a period of 13 weeks. Equivalent cinmethylin intakes were 0, 3.81, 11.50, 39.57 and 123.11 mg/kg bw/d for males and 0, 4.36, 13.85, 42.57, 129.66 mg/kg bw/d for females, respectively. An interim treatment group, consisting of 10 males and 10 females, necropsied at 7 weeks, was included in this study. The remaining 20 males and 20 females were necropsied at week 13 (terminal sacrifice).

Food consumption and body weight was determined weekly. Animals were examined for signs of toxicity or mortality twice daily. Detailed clinical observations and urinalysis were not conducted. Clinical biochemistry and haematological examinations were performed after 7 and 13 weeks. After the administration period (7 or 13 weeks) mice were euthanised and necropsied, followed by histopathological examinations.

A method for the dose verification of cinmethylin in feed was evaluated and was regarded as fit for purpose (see Volume 3 CA B5, section B.5.1.2).

### Results

*Mortality and general clinical observations:* There were no treatment-related deaths or clinical signs of toxicity.

*Body weight and food consumption:* No consistent dose-related effects on body weight were observed. Statistically-significant differences between treated animals and controls were occasionally noted; in particular at 21 days in females and 28 days in males. At these time points all treatment groups were statistically-significantly different from controls. In some instances, exposed groups had increased means compared to controls, whilst at other times there was a body weight depression among treatment groups. Based on a lack of dose-response, these findings could not be attributed directly to exposure to cinmethylin.

No consistent treatment-related effects were noted in food consumption. Statistically-significant differences (both increases and decreases) between treatment and control means were observed during some weeks, especially in male mice (week 4). However, a low food consumption for control males was noted. Based on a lack of dose-response, these findings were not considered treatment-related.

*Haematology:* Single haemocellular or haemochemical parameters were significantly different from control values. These changes were not dose-related and were not supported by morphological changes or by data from the opposite sex or sacrifice interval and were therefore not considered treatment-related.

*Clinical chemistry:* Serum globulin levels were significantly higher in all treated females at the 7-week interim necropsy, showing dose-response characteristics. This effect was not observed at the terminal sacrifice and was not supported by organ specific pathology or other related clinical chemistry parameters and were therefore not considered treatment-related.

*Organ weight:* Statistically-significant increases in mean absolute and relative liver weights occurred in females in the top dose at both 7 and 13-week necropsy (Table 6.3-36). In males statistically-significant increases were

noted for mean absolute and relative liver weights in the top dose at 13-weeks but also in relative liver weight in the 300 ppm dose at both 7 and 13-weeks. However, a clear dose-response relationship was not evident and relative change compared to control was < 15 %. Concomitant clinical chemistry and/or histopathology were not observed. Exposure of mice to 30 or 100 ppm cinmethylin had no effect on liver weight. Other organ weight effects did not show dose-response relationships. Overall, liver weight changes seen were not considered by HSE to be adverse.

Table 6.3-36. Liver weight data

Concentration		Liver weight			
		Absolute [g] (Δ %)	Relative [%] (Δ%)	Absolute [g] (Δ%)	Relative [%] (Δ %)
[ppm]	[mg/kg bw/d]	7-week necropsy		13-week necropsy	
Males					
0	0	1.133	4.546	1.195	4.110
30	3.81	1.154	4.735	1.225	4.121
100	11.50	1.139	4.658	1.182	4.082
300	39.57	1.156 (2)	4.886** (7.5)	1.251 (4.7)	4.415** (7.4)
1000	123.11	1.179 (4)	4.809 (5.8)	1.304** (9.1)	4.493** (9.3)
Females					
0	0	0.979	4.816	1.119	4.722
30	4.36	1.020	4.934	1.147	4.820
100	13.85	1.030	4.892	1.114	4.635
300	42.57	1.013	4.993	1.105	4.696
1000	129.66	1.090* (11.3)	5.272** (9.5)	1.190* (6.3)	5.104** (8.1)

\* = p<0.05; \*\* = p<0.01; Dunnett's test (two-sided).

Δ % - percent change compared to control.

*Gross pathology and histopathology:* Macroscopic and microscopic pathology of mice revealed no lesions that could be attributed to exposure to cinmethylin. Identifiable lesions were generally uncommon or were consistently seen throughout all experimental groups. A dose-response was not evident in the incidence, severity and distribution of these lesions. Histopathological findings to support the liver enlargement (Table 6.3-35) were not identified.

#### Conclusion

In conclusion, under the conditions of this limited study, dietary administration of cinmethylin for 90 days resulted in no treatment-related and adverse effects in mice. At 1000 ppm only the liver weights were slightly increased (below 10 % increase for absolute and relative liver weight in both sexes), and without any supporting clinical chemistry and/or adverse histopathology, this effect is not considered adverse.

A NOAEL of 1000 ppm (equivalent to 123 and 130 mg/kg bw/d in males and females respectively) is proposed by HSE since no adverse effects were seen at this dose. It is noted that a lower NOAEL was identified in the mouse in a modern study (██████████, 2018b); therefore the reliability of this NOAEL is questionable.

(██████████, 1983)

#### Studies in dogs

##### Old Study

Author(s)	██████████
Study title	Thirteen week dietary feeding study in beagle dogs : cinch herbicide (technical)
Study reference	██████████, 1987 CI-425-003
Test facility	██
Date	19/08/1986 – 21/11/1986
Test substance	SD 95481 (Cinmethylin)

<b>Purity (%)</b>	Not specified in the study report.
<b>Batch no.</b>	WRC TOX #925
<b>Test animals</b>	Dogs Beagle Male and female
<b>Groups</b>	6/sex/dose
<b>Dose/concentrations</b>	0, 2, 100, 200, 3000 and 6000 ppm Equivalent to 0, 0.06, 2.9, 5.6, 96.5 and 180.5 for males and 0, 0.06, 3.0, 5.8, 91.9 and 192.3 for females respectively.
<b>Route</b>	Administered daily via the diet for 13 weeks.
<b>Vehicle</b>	Acetone
<b>GLP</b>	Compliant.
<b>Guideline</b>	US EPA Pesticide Assessment Guidelines.
<b>Deviation</b>	<p>Compared to the previous test guideline OECD TG No. 409 (1981) :</p> <ul style="list-style-type: none"> <li>• Batch and purity were not reported.</li> <li>• Ophthalmological examination were not conducted.</li> <li>• Ornithine decarboxylase and gamma glutamyl transpeptidase were not determined.</li> </ul> <p>Compared to the current OECD TG No. 409 (1998) :</p> <ul style="list-style-type: none"> <li>• Weekly detailed clinical observations were not performed.</li> <li>• Cinmethylin purity was not reported.</li> <li>• Ophthalmological examination were not conducted.</li> <li>• Additional satellite groups were not included for recovery.</li> <li>• In haematology and clinical biochemistry, blood clotting potential, ornithine decarboxylase and gamma glutamyl transpeptidase were not determined.</li> <li>• At gross necropsy, weight of gall bladder, thymus, epididymides and uterus were not recorded.</li> <li>• Histopathology was not performed on larynx, pharynx, seminal vesicles, coagulating gland, harderian gland and vagina.</li> <li>• A discussion of the observed significantly changed parameters in relation to historical control data is missing.</li> </ul>
<b>Impact of deviations</b>	<ul style="list-style-type: none"> <li>• Due to reduced haematology data collected, determination of liver findings with regard to adversity is hampered.</li> <li>• Due to the lack of historical control data, it is difficult to evaluate reported findings in test and concurrent control groups in relation to the normal range of fluctuations.</li> </ul>
<b>Acceptable</b>	Despite the above listed deviations, this study, together with 2 further dog studies (■■■■■, 1985a and ■■■■■, 1988a) is considered to satisfy regulatory requirements and is therefore used for hazard and risk assessment.
<b>NOAEL</b>	200 ppm (5.6 and 5.8 mg/kg bw/d in males and females respectively).
<b>Effects at the LOAEL</b>	Increased liver weight and delayed glandular development of prostate from 3,000 ppm (96.5 and 92 mg/kg bw/d in males and females respectively)

#### Methods

In a relatively old study, cinmethylin was administered via the diet to groups of 6 male and 6 female Beagle dogs per test group, at concentrations of 0, 2, 100, 200, 3000 and 6000 ppm, over a period of 13 weeks. Equivalent cinmethylin intakes were 0, 0.06, 2.9, 5.6, 96.5 and 180.5 mg/kg bw/d for males and 0, 0.06, 3.0, 5.8, 91.9 and 192.3 mg/kg bw/d for females, respectively. An interim treatment group was not included in this study.

Food consumption and body weight was determined weekly. Animals were examined for signs of toxicity or mortality twice daily. Urinalysis, clinical biochemistry and haematological examinations were performed after 7 and 13 weeks. After the administration period dogs were euthanised and necropsied, followed by histopathological examinations.

A method for the dose verification of cinmethylin in feed was evaluated and was regarded as fit for purpose (see Volume 3 CA B5, section B.5.1.2).



### Results

**Mortality and general clinical observations:** One dog of the 200 ppm dose group was found dead during week 7 with an acute haemorrhagic lung problem, however, the death was not considered treatment-related. The only treatment-related finding observed was an increased frequency of stool abnormalities; loose stool or blood in stool was noted in 5 of 12 high dose dog and in only one other dog (control). There was no consistent pattern of further abnormal clinical signs associated with exposure to cinmethylin.

**Body weight and food consumption:** Body weights, body weight changes and food consumption parameters were considered representative of healthy young dogs for all treatment groups. No treatment-related effects were observed in these parameters.

**Haematology:** The week 7 haematology data revealed no treatment related differences (Table 6.3-37). At study termination, statistically-significant increase in red blood cell (RBC) count, haematocrit (HCT) and haemoglobin (HGB) were observed in males of the 200 ppm dose group. Considering the lack of a dose-response, these findings were not considered treatment-related but reflected abnormal findings in 2 dogs of this group. There were no treatment-related effects at 13 weeks.

Table 6.3-37. Selected haematology findings

Concentration		Week 13					
[ppm]	[mg/kg bw/d]	RBC [ $10^6/L$ ]		HCT [%]		HGB [g/dL]	
Males		mean	SD	mean	SD	mean	SD
0	0	6.40	0.33	44.4	2.8	14.9	1.6
2	0.06	7.20	0.67	48.7	3.6	15.9	1.4
100	2.9	6.73	0.24	46.1	1.3	14.9	0.5
200	5.6	7.76**	1.16	53.9**	9.8	17.4*	2.9
3000	96.5	6.63	0.32	46.1	1.1	15.0	0.8
6000	180.5	6.53	0.50	45.2	2.1	14.9	1.2
Females		mean	SD	mean	SD	mean	SD
0	0	6.70	0.39	46.9	2.4	15.3	0.8
2	0.06	6.57	0.28	47.0	1.9	15.4	0.6
100	3.0	6.89	0.56	49.4	3.7	16.5	1.8
200	5.8	6.97	0.48	49.5	3.3	16.5	1.4
3000	91.9	6.74	0.64	47.6	2.8	15.6	1.1
6000	192.3	6.64	0.16	47.7	1.3	15.5	0.7

\*  $p < 0.05$ ; \*\*  $p < 0.01$  by Dunnett's test

RBC – red blood cells (erythrocytes).

HCT – haematocrit.

HGB – haemoglobin.

SD – standard deviation.

**Clinical chemistry:** At week 7 and 13, statistically-significant differences in clinical chemistry parameters were observed in treated dogs compared to controls but none appeared to show a consistent, time-related and/or dose-related effect of treatment (Table 6.3-38). The only parameter where consistency between sexes and time-points was seen was an increase in ALP at the top dose. This finding is considered treatment-related and adverse.

Table 6.3-38. Selected clinical chemistry findings

		Males						Females					
Dose level	[ppm]	0	2	100	200	3000	6000	0	2	100	200	3000	6000
	[mg/kg bw/d]	0	0.06	2.9	5.6	96.5	180.5	0	0.06	3.0	5.8	91.9	192.3
ALP [U/L]													
Week 7	[mean]	50.3	46.0	42.7	49.9	56.3	65.0	56.4	49.5	48.8	50.3	74.2	82.4*
	[SD]	6.5	13.3	7.1	15.8	9.2	23.5	7.8	10.3	12.1	13.3	20.9	21.2
	$\Delta\%$		-8.5	-15.1	-0.8	+11.9	+29.2		-12.2	-13.5	-10.8	+31.6	+46.1
Week 13	[mean]	48.3	43.6	41.8	41.5	55.0	66.1	53.4	47.4	43.9	44.2	72.8	87.3*

		Males						Females					
Dose level	[ppm]	0	2	100	200	3000	6000	0	2	100	200	3000	6000
	[mg/kg bw/d]	0	0.06	2.9	5.6	96.5	180.5	0	0.06	3.0	5.8	91.9	192.3
	[SD]	12.2	13.5	6.8	12.2	12.9	21.5	12.9	14.7	10.5	12.5	17.2	20.9
	Δ%		-9.7	-13.5	-14.1	+13.9	+36.9		-11.2	-17.8	-17.2	+36.3	+63.5
<b>ALT [U/L]</b>													
Week 7	[mean]	23.8	22.5	21.9	22.1	18.9	<b>16.6*</b>	23.2	23.7	23.5	23.4	23.6	19.5
	[SD]	3.4	3.7	3.0	3.2	2.8	3.2	4.2	5.2	0.7	3.7	7.9	3.7
	Δ%		-5.5	-8.0	-7.1	-20.6	-30.3		+2.2	+1.3	+0.9	+1.7	-15.9
Week 13	[mean]	26.0	20.1	21.4	21.0	20.4	19.2	23.0	22.3	23.3	22.6	19.6	19.0
	[SD]	6.5	4.5	2.7	6.3	4.3	5.2	4.1	5.5	5.7	3.4	5.0	3.6
	Δ%		-22.7	-17.7	-19.2	-21.5	-26.2		-3.0	+1.3	-1.7	-14.8	-17.4
<b>CPK [U/L]</b>													
Week 7	[mean]	61.5	61.2	50.5	71.3	69.5	55.0	70.8	82.3	80.4	75.6	61.7	<b>114.8*</b>
	[SD]	32.7	21.8	17.8	27.5	31.1	22.0	17.4	25.0	25.6	11.2	13.0	44.5
	Δ%		-0.5	-17.9	+15.9	+13.0	-10.6		+16.2	+13.6	+6.8	-12.9	+62.1
Week 13	[mean]	48.3	62.1	58.5	67.6	<b>80.1*</b>	66.7	54.7	62.5	97.3	63.8	49.4	80.0
	[SD]	14.1	10.5	23.6	7.5	30.9	11.2	12.3	14.2	77.2	22.6	7.6	42.2
	Δ%		+28.6	+21.1	+40.0	+65.8	+38.1		+14.3	+77.9	+16.6	-9.7	+46.3
<b>PROT [g/dL]</b>													
Week 7	[mean]	7.1	7.7	7.5	7.6	7.3	7.0	7.4	7.2	7.3	7.2	7.0	6.9
	[SD]	0.3	0.5	0.7	0.5	0.3	0.8	0.4	0.5	0.1	0.3	0.2	0.5
	Δ%		+8.5	+5.6	+7.0	+2.8	-1.4		-2.7	-1.4	-2.7	-5.4	-6.8
Week 13	[mean]	6.4	6.6	6.6	<b>7.0*</b>	6.4	6.3	6.4	6.4	6.5	6.3	6.2	6.3
	[SD]	0.4	0.4	0.4	0.3	0.2	0.3	0.5	0.2	0.2	0.3	0.3	0.3
	Δ%		+3.1	+3.1	+9.4	±0.0	-1.6		±0.0	+1.6	-1.6	-3.1	-1.6
<b>ALB [g/dL]</b>													
Week 7	[mean]	3.4	<b>3.8*</b>	3.5	3.7	3.5	3.3	3.6	3.6	3.7	3.6	3.4	3.4
	[SD]	0.2	0.2	0.3	0.2	0.1	0.3	0.2	0.2	0.1	0.2	0.1	0.3
	Δ%		+11.8	+2.9	+8.8	+2.9	-2.9		±0.0	+2.8	±0.0	-5.6	-5.6
Week 13	[mean]	3.1	<b>3.4*</b>	3.1	<b>3.5*</b>	3.2	3.1	3.3	3.3	3.4	3.2	3.2	3.1
	[SD]	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.2
	Δ%		+9.7	±0.0	+12.9	+3.2	±0.0		±0.0	+3.0	-3.0	-3.0	-6.1
<b>PHOS (inorg.) [mg/dL]</b>													
Week 7	[mean]	7.0	7.3	7.7	<b>8.0*</b>	<b>7.9*</b>	7.9	6.8	6.8	6.7	7.0	6.6	6.3
	[SD]	0.3	0.7	0.9	0.5	0.3	0.5	0.5	0.2	0.5	0.6	0.4	0.6
	Δ%		+4.3	+10.0	+14.3	+12.9	+12.9		±0.0	-1.5	+2.9	-2.9	-7.4
Week 13	[mean]	5.0	4.9	5.5	5.1	5.4	5.4	5.1	5.1	5.1	5.1	5.3	4.8
	[SD]	0.6	0.6	0.9	0.6	0.6	0.6	0.6	0.3	0.3	0.4	0.6	0.5
	Δ%		-2.0	+10.0	+2.0	+8.0	+8.0		±0.0	±0.0	±0.0	+3.9	-5.9
<b>Ca [mg/dL]</b>													
Week 7	[mean]	13.1	<b>14.0*</b>	13.7	<b>13.9*</b>	13.7	13.4	13.4	13.0	13.2	13.1	13.3	12.6
	[SD]	0.3	0.3	0.7	0.5	0.5	0.7	0.5	0.8	0.5	0.6	0.5	1.1
	Δ%		+6.9	+4.6	+6.1	+4.6	+2.3		-3.0	-1.5	-2.2	-0.7	-6.0
Week 13	[mean]	11.3	11.8	11.5	<b>12.2*</b>	11.7	11.6	11.7	11.3	11.5	11.3	11.5	<b>10.8*</b>
	[SD]	0.4	0.5	0.5	0.5	0.2	0.4	0.6	0.5	0.4	0.3	0.5	0.5
	Δ%		+4.4	+1.8	+8.0	+3.5	+2.7		-3.4	-1.7	-3.4	-1.7	-7.7

\* p ≤ 0.05; \*\* p ≤ 0.01 by Dunnett's test.

Δ% - percent change compared to control.

SD - standard deviation.

ALP - alkaline phosphatase.

ALT - alanine aminotransferase.

CPK - creatinine phosphokinase

PROT - protein.

ALB - albumin.

PHOS - phosphorus.

Ca - calcium.

*Urinalyses:* No treatment-related changes among urinalysis parameters were observed.

*Organ weight:* Absolute and relative liver weights were statistically-significantly increased for both sexes in the high dose; the relative change compared to control was > 15 % (Table 6.3-39). In the 3000 ppm dose group, absolute and relative liver weights were also increased in males (> 15 % change compared to control but not statistically-significant) and females (> 15 % change compared to control as well as statistically-significant). No other organ weights were statistically significantly-affected by treatment. Overall, liver weight effects in the two highest dose groups (3,000 and 6,000 ppm) were considered adverse.

Table 6.3-39. Liver weight data

Concentration		Liver weight	
[ppm]	[mg/kg bw/d]	Absolute [g] (Δ %)	Relative [%] (Δ %)
<b>Males</b>			
0	0	276.7 ± 26.2	2.73 ± 0.22
2	0.06	279.1 ± 45.4	2.75 ± 0.30
100	2.9	309.3 ± 60.4	2.82 ± 0.32
200	5.6	298.3 ± 46.7	2.71 ± 0.40
3000	96.5	333.6 ± 59.2 (+20.6%)	3.17 ± 0.20 (+16.1%)
6000	180.5	<b>358.3 ± 32.8* (+29.5%)</b>	<b>3.36 ± 0.32** (+23.1%)</b>
<b>Females</b>			
0	0	251.9 ± 39.9	2.61 ± 0.28
2	0.06	256.0 ± 44.2	2.80 ± 0.33
100	3.0	240.5 ± 37.6	2.72 ± 0.35
200	5.8	264.1 ± 42.4	2.87 ± 0.27
3000	91.9	293.3 ± 49.6 (+16.4%)	<b>3.19 ± 0.29* (+22.2%)</b>
6000	192.3	<b>329.3 ± 54.7** (+30.7%)</b>	<b>3.76 ± 0.30** (+44.1%)</b>

\* = p≤0.05; \*\* = p≤0.01; Dunnett's test (two-sided)

Δ% - percent change compared to control.

*Gross pathology:* There were no treatment-related gross necropsy findings noted during the post-mortem examinations. One dog of the top dose group (6000 ppm: male #3) revealed a small prostate. This is consistent with the findings from the histopathology investigations (see below).

*Histopathology:* The following findings were considered to be potentially treatment-related by the pathologist of the study:

**Prostate and testes:** Prostatic glandular development (severity) was slightly reduced in males of the 3,000 and 6,000 ppm dose groups (Table 6.3-40). The decrease of the prostatic glandular development in the 200 ppm dog that died intercurrently in week 7, at an age of 6-7 months, is clearly age-related rather than treatment-related as the animal was sacrificed at a younger age. An effect related to body weight development is likely for the male of the 3000 ppm dose group and one of the top dose males; both showed low body weight and high food consumption, typical of young animals. The other top dose male revealed a starting body weight that was comparable with the group means. In this dog, however, reduced prostatic glandular development was supported by the reduced organ size (see above gross pathology). Therefore, taking into account variations in the age of reaching maturity and the individual body weights of the animals showing the finding, the delay in glandular development of the prostate at 3,000 and 6,000 ppm appears to be the consequence of impaired body weight development and not a direct effect of treatment. There were no clear effects on the testes and spermatogenesis.

Stomach: The incidence of reactive lymphoid hyperplasia was slightly increased for the 3000 and 6000 ppm dose groups in males. However, lymphoid hyperplasia in the intestinal tract is not an unusual finding in dogs<sup>2</sup> and this finding was seen in control animals in this study (1/6 in females) as well as control animals in the 1-year dog study (■■■■■, 1985) (1/6 in males and 2/6 in females). The effect was not dose-dependent; severity of the effect did not increase by dose or duration, as demonstrated in the 1-year dog study (■■■■■, 1985).

There were no other distinct or consistent, treatment or group-related changes noted in tissues from the treated groups. In the liver, no supporting histopathological finding for the observed weight increase was detected. Overall, treatment-related and adverse histopathological findings were observed in the prostate from 3,000 ppm.

Table 6.3-40. Selected histopathological findings

Sex			Males						Females					
Dose level	[ppm]	Grading	0	2	100	200	3000	6000	0	2	100	200	3000	6000
	[mg/kg bw/d]		0	0.06	2.9	5.6	96.5	180.5	0	0.06	3.0	5.8	91.9	192.3
Animals examined		N	6	6	6	6	6	6	6	6	6	6	6	6
Stomach														
- reactive lymphoid hyperplasia	N		0	0	1	1	3	3	1	2	1	1	3	2
	1		-	-	-	-	-	-	-	-	-	-	-	-
	2		-	-	1	1	3	3	1	2	1	1	3	2
	[mean] <sup>#</sup>		[0.0]	[0.0]	[2.0]	[2.0]	[2.0]	[2.0]	[2.0]	[2.0]	[2.0]	[2.0]	[2.0]	[2.0]
Prostate														
- glandular development	N		6	6	6	6	6	6						
	1		-	-	-	1 <sup>§</sup>	1	2						
	2		4	3	2	1	4	3						
	3		2	3	3	3	1	1						
	4		-	-	1	1	-	-						
	[mean] <sup>#</sup>		[2.3]	[2.5]	[2.8]	[3.0]	[2.0]	[1.8]						
Testes														
- atrophy	N		0	1	0	0	3	1						
	1		-	-	-	-	3	1						
	[mean] <sup>#</sup>		[0.0]	[1.0]	[0.0]	[0.0]	[1.0]	[1.0]						
- spermatogenesis	N		6	6	6	6	6	6						
	1		-	-	-	1 <sup>§</sup>	-	-						
	2		3	1	1	3	3	2						
	3		3	5	5	2	3	4						
	[mean] <sup>#</sup>		[2.5]	[2.8]	[2.8]	[2.4]	[2.5]	[2.7]						
Liver														
- congestion	N		0	0	0	1	0	0	0	0	0	0	0	0
- granuloma	N		0	0	0	1	0	0	0	0	0	0	0	0
- focal fibrosis	N		0	0	1	0	0	0	0	0	0	0	0	0
- inflammatory foci	N		1	3	1	1	1	1	1	0	0	0	0	2
- lymphoid infiltrate	N		6	6	6	6	6	6	6	6	6	6	6	6
	1		6	6	6	6	5	6	6	6	6	6	5	6
	2		-	-	-	-	1	-	-	-	-	-	1	-
	[mean] <sup>#</sup>		[1.0]	[1.0]	[1.0]	[1.0]	[1.2]	[1.0]	[1.0]	[1.0]	[1.0]	[1.0]	[1.2]	[1.0]
- irregular staining cytoplasm, focal			0	0	0	0	0	0	1	0	0	0	0	0

<sup>2</sup> Handbook of toxicology, second edition, editor Derelanko & Hollinger., 2002 CRC Press LLC; online available and Sato *et al.* (2012), Figure 55.

Sex			Males						Females					
Dose level	[ppm]	Grading	0	2	100	200	3000	6000	0	2	100	200	3000	6000
	[mg/kg bw/d]		0	0.06	2.9	5.6	96.5	180.5	0	0.06	3.0	5.8	91.9	192.3

N = number of incidences

# = mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding

§ = finding occurred in a dog that died prematurely on week 7 and was considered to be a consequence of the age difference, and thus, not included in the mean calculation

### Conclusion

In conclusion, under the conditions of this relatively old study in dogs, dietary administration of cinmethylin for 90 days resulted in an increased frequency of stool abnormalities and increases in ALP at the top dose of 6,000 ppm. An increase in liver weights and a delay in glandular development of the prostate occurred from 3,000 ppm.

A NOAEL of 200 ppm (5.6 and 5.8 mg/kg bw/d in M/F respectively) is proposed by HSE as no adverse effects were seen at this dose. A LOAEL of 3,000 ppm (96.5 and 92 mg/kg bw/d in M/F respectively) is identified based on increased liver weights and a delay in glandular development of the prostate.

(██████, 1987)

### B.6.3.3. Oral 1-year study

Two 1-year oral toxicity studies, and one 1-year oral study with 6 months recovery, are available in dogs; these are relatively old and were conducted according to GLP but not according to OECD test guidelines.

#### Studies in dogs

##### 1) Old study

Author(s)	██████
Study title	A One Year Dietary Feeding Study in Dogs - SD95481 Technical
Study reference	██████, 1985 CI-427-002
Test facility	██
Date	13/03/1984 – 15/03/1985
Test substance	SD95481 (Cinmethylin)
Batch no. and purity (%)	Batches: 513L and 513N; Purity: 91 and 93 respectively.
Test animals	Dogs Beagle Male and female
Groups	6/sex/dose
Dose/concentrations	0, 300, 3000 and 10000 ppm Equivalent to 0, 7.9, 83.4, and 253.9 for males and 0, 7.9, 81.4 and 284.8 for females respectively.
Route	Administered daily via the diet for 52 weeks.
Vehicle	Acetone
GLP	Compliant.
Guideline	US EPA Pesticide Assessment Guidelines.
Deviation	Deviations from OECD test guideline No. 452 (1981) : <ul style="list-style-type: none"> <li>Clinical biochemistry: gamma glutamyl transpeptidase was not determined.</li> <li>The body weight data were recorded with the precision of 0.1 kg only.</li> </ul> Deviations from OECD test guideline No. 452 (2009) : <ul style="list-style-type: none"> <li>Humidity was 25-75% instead of 30–70%.</li> <li>Detailed clinical observations were not performed.</li> </ul>

	<ul style="list-style-type: none"> <li>In haematology and clinical biochemistry : mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, prothrombin time, activated partial thromboplastin time and gamma glutamyl transpeptidase were not determined.</li> <li>Osmolality and specific gravity were not recorded.</li> <li>At gross necropsy, weight of epididymides and uterus were not recorded.</li> <li>Histopathology was not performed on coagulating gland, Harderian gland, lacrimal gland, deep lymph nodes, seminal vesicle, uterus and vagina.</li> <li>A discussion of the observed significantly changed parameters in relation to historical control data is missing.</li> </ul>
<b>Impact of deviations</b>	Due to the lack of historical control data, it is difficult to evaluate reported findings in test and concurrent control groups in relation to the normal range of fluctuations.
<b>Acceptable</b>	Despite the above listed deviations, this study, together with the other two 1-year dog studies (■■■■■, 1988a and 1988b), is considered to satisfy regulatory requirements and is therefore used for hazard and risk assessment.
<b>NOAEL</b>	300 ppm in males and females (equivalent to 7.9 mg/kg bw/d).
<b>Effects at the LOAEL</b>	3000 ppm (equivalent to 83 and 81 mg/kg bw/d in males and females respectively) is proposed based on reductions in body weight and body weight gain, effects on white blood cell parameters, alterations in clinical chemistry parameters (ALP and ALB) indicative of liver toxicity, increased liver weights (> 15 %) and a delay in glandular development of the prostate

#### Methods

Cinmethylin was administered via the diet to groups of 6 male and 6 female Beagle dogs per test group, at concentrations of 0, 300, 3000 and 10000 ppm, over a period of 1-year. Equivalent cinmethylin intakes in mg/kg bw/d are shown in Table 6.3-41.

Table 6.3-41. Equivalent cinmethylin intakes

Concentration in the vehicle (ppm)	Mean theoretical daily test-substance intake (mg/kg bw/d)	
	Males	Females
0	0	0
300	7.9 ± 1.24	7.9 ± 1.09
3000	83.4 ± 10.31	81.4 ± 9.83
10000	253.9 ± 36.60	284.8 ± 33.63

A method for the dose verification of cinmethylin in feed was evaluated and was regarded as fit for purpose (see Volume 3 CA B5, section B.5.1.2).

#### Results

*Mortality and general clinical observations:* Remarkably low food consumption followed by weakness and various clinical signs (hypoactivity, ataxia and no/bloody mucoid stool) were observed in one male and one female of the top dose group; they were both sacrificed after 12 weeks. One additional female of the top dose group showed a reduced food consumption later in the study (week 21) followed by similar clinical signs (hypoactivity, ataxia, dehydration and pale mucus membranes). Several times the animal was switched to the control group and low dose groups, but when switched back, the animal refused the diet and was subsequently sacrificed at week 48. Overall, clinical signs of toxicity leading to early sacrifice were seen in 2 dogs at the top dose.

*Body weight and food consumption:* Throughout the study, mean body weights of males and females in the top dose groups were statistically-significantly lower compared to the controls (Table 6.3-42). In addition, body weights of females of the mid dose group were statistically-significantly reduced at some timepoints.

A significantly increased body weight gain was observed from the mid dose in both sexes, reaching statistical-significance in the top dose females. Overall, treatment-related and adverse effects on body weight and body weight gain were seen from the mid dose.

Food consumption in males was statistically-significantly and consistently reduced in the top dose group. No statistically-significant effect on food consumption is seen in the low and mid dose group males. In the top dose group females, food consumption was statistically-significantly reduced in a sporadic manner (isolated weeks without statistical significance). Mean cumulative food consumption over the 52 weeks in the top dose was 39 % and 44 % compared to controls for males and females respectively. Especially at the beginning of treatment a food intake reduction was seen; this finding was considered treatment-related, while at later timepoints the difference disappears. Whilst statistically-significant difference were noted in the low and mid dose groups, they did not display a dose-dependent effect. The statistical-significance in all dose groups mainly depended on fluctuations in the control. Therefore, to aid evaluation of these results food consumption data from the 90-day and 1-year study (■■■■■, 1987 and ■■■■■ 1988a) were compared. Control data from those studies indicate that food consumption of the treated groups up to the mid dose was within the normal range. Overall, treatment-related and adverse decreases in food consumption were seen at the top dose in males and females.

Table 6.3-42. Body weight and body weight gain data

Dose level	Males								Females							
[ppm]	0	300		3000		10000		0	300		3000		10000			
[mg/kg bw/d]	0	7.9		83.4		253.9		0	7.9		81.4		284.8			
Week	[kg]	[kg]	[%]	[kg]	[%]	[kg]	[%]	[kg]	[kg]	[%]	[kg]	[%]	[kg]	[%]		
Body weight																
0	7.7	7.7	100.0	7.8	101.7	7.8	101.5	7.2	7.1	98.6	7.3	101.4	7.2	100.0		
1	7.9	8.0	101.9	8.1	102.5	7.1*	90.2	7.5	7.3	97.3	7.4	98.7	6.6*	88.0		
13	9.8	10.3	104.9	10.0	102.0	8.5*	87.0	9.3	8.9	95.7	9.2	98.9	7.6*	81.7		
26	10.6	11.2	105.7	10.6	100.0	9.2	86.8	10.7	9.5	88.8	9.9	92.5	7.5*	70.1		
43	11.4	11.7	102.6	11.1	97.4	9.3*	81.6	11.6	10.4	89.7	10.1*	87.1	8.7*	75.0		
44	11.5	12.0	104.3	11.2	97.4	9.4*	81.7	11.7	10.6	90.6	10.2*	87.2	8.8*	75.2		
45	11.3	11.8	104.4	11.1	98.2	9.3*	82.3	11.6	10.6	91.4	10.4*	89.7	8.7*	75.0		
51	11.5	11.7	101.7	11.0	95.7	9.0*	78.3	11.9	10.5	88.2	10.6	89.1	8.2*	68.9		
52	11.6	12.0	103.4	11.1	95.7	9.2*	79.3	12.1 <sup>s</sup>	10.8	89.3	10.6*	87.6	8.6*	71.1		
Body weight gain																
Week 0-52	[kg]	[kg]	[%]	[kg]	[%]	[kg]	[%]	[kg]	[kg]	[%]	[kg]	[%]	[kg]	[%]		
	3.88	4.37	112.6	3.27	84.3	1.66	42.8	4.92	3.72	75.6	3.30	67.1	1.33**	27.0		

\* p ≤ 0.05; \*\* p ≤ 0.01; Dunnett test (two-sided); # p ≤ 0.05; Tukey-Kramer and Kruskal-Wallis tests (two sided);

<sup>s</sup> Week 52 terminal body weight of the control without the control animal #260;

[%] – percent compared to control (set to 100 %)

*Ophthalmoscopy:* There were no treatment-related ophthalmological findings.

*Haematology:* Red blood cell (RBC), haemoglobin (HGB), and haematocrit (HCT) levels in both male and female animals of the top dose group were statistically-significantly decreased (> 10 %) in a dose-dependent and time-dependent manner (Table 6.3-43). These findings were therefore considered treatment-related and adverse. Compared to the normal range of young adult Marshall beagle dogs with 14.2 – 19.0 mmol/L hemoglobin in males and 14.3 - 19.0 mmol/L in females, these values indicate a slight reduction of red blood cell parameters below the normal range. Platelet counts were statistically-significantly increased (> 10 %) in the top dose in both sexes. Statistically-significant increases in RBC, HGB and HCT levels at the mid dose in females were not considered treatment-related based on the lack of dose-response. Overall, treatment-related and adverse effects on red blood cell parameters and platelets were seen at the top dose.

After 13 weeks there were no statistically-significant changes in white blood cell parameters in treated animals. After 26 weeks statistically-significant increases in total WBC and absolute neutrophil counts were observed in

males from the mid dose group. However, neutrophil cell counts were not changed dose-dependently and the WBC count means were in the same range as seen in pre-tests, whereas the control mean was quite low. In females of the mid dose group WBC and absolute neutrophil counts were significantly increased, however, a dose-response relationship was not evident. After 52 weeks, in males of the top dose group WBC and neutrophil counts were increased, although not statistically-significantly, in addition lymphocyte and monocyte counts were statistically-significantly increased in this group. Both a dose-response and relative changes of > 10 % compared to control were evident in these parameters in top dose males. In females WBC and neutrophil counts were statistically-significantly increased from the mid dose group and absolute lymphocyte counts were statistically-significantly increased at the top dose. Again a dose-response and relative changes of > 10 % compared to control were evident for statistically-significant values. These findings were considered treatment-related and adverse. They reflect most probably an inflammatory reaction. Overall, treatment-related and adverse effects on white blood cell parameters were seen from the mid dose.

Table 6.3-43. Haematology: Red blood cell parameters

Dose level		Males				Females			
		0	300	3000	10000	0	300	3000	10000
	[mg/kg bw/d]	0	7.9	83.4	253.9	0	7.9	81.4	284.8
Red blood cell parameters									
RBC [ $10^{12}/L$ ]									
Pre-test	[mean]	6.28	6.11	6.04	6.10	6.2	6.5	6.2	6.2
	[SD]	0.386	0.432	0.385	0.775	0.52	0.45	0.43	0.65
	$\Delta\%$ of control		-2.7	-3.8	-2.9		4.5	-0.2	-0.8
Week 13	[mean]	7.1	7.4	6.8	<b>5.8*</b>	7.3	7.9	7.0	<b>6.2*</b>
	[SD]	0.34	0.77	0.63	0.55	0.36	0.66	0.48	0.25
	$\Delta\%$ of control		3.8	-4.5	-18.1		8.3	-3.7	-15.6
Week 26	[mean]	7.8	7.9	7.2	<b>6.4*</b>	7.3	8.2*	7.2	6.5
	[SD]	0.29	0.58	0.74	0.82	0.70	0.67	0.45	0.39
	$\Delta\%$ of control		1.0	-7.1	-17.9		12.5	-0.7	-10.8
Week 52	[mean]	7.2	7.2	6.7	<b>5.9*</b>	6.6	7.5	6.3	<b>5.3*</b>
	[SD]	0.43	0.49	0.85	0.66	0.67	0.42	0.55	0.58
	$\Delta\%$ of control		0.1	-6.8	-17.7		12.3	-4.5	-20.3
HGB [mmol/L]									
Pre-test	[mean]	14.6	14.2	14.1	14.5	14.2	15.4	14.7	14.5
	[SD]	0.86	0.98	0.59	1.76	0.91	1.24	0.90	1.42
	$\Delta\%$ of control		-2.7	-3.4	-0.7		8.5	3.5	2.1
Week 13	[mean]	16.1	16.7	15.7	<b>13.7*</b>	16.3	18.3*	16.2	<b>14.4*</b>
	[SD]	0.99	1.24	1.01	0.99	0.89	1.71	0.84	0.72
	$\Delta\%$ of control		3.7	-2.5	-14.9		12.3	-0.6	-11.7
Week 26	[mean]	17.3	17.6	16.1	<b>14.4*</b>	16.0	18.6*	16.3	14.8
	[SD]	0.69	1.19	1.41	1.63	1.36	1.64	1.01	0.79
	$\Delta\%$ of control		1.7	-6.9	-16.8		16.3	1.9	-7.5
Week 52	[mean]	16.5	16.7	15.6	<b>13.8*</b>	15.3	17.5*	14.9	<b>12.3*</b>
	[SD]	1.03	0.87	1.58	1.30	1.45	0.96	1.06	2.12
	$\Delta\%$ of control		1.2	-5.5	-16.4		14.4	-2.6	-19.6
HCT [L/L]									
Pre-test	[mean]	43.9	43.0	42.2	42.9	43.5	46.5	44.0	44.1
	[SD]	2.37	2.72	2.41	5.14	3.51	3.34	2.37	4.32
	$\Delta\%$ of control		-2.1	-3.9	-2.3		6.9	1.1	1.4
Week 13	[mean]	52.3	59.6	50.1	<b>43.5*</b>	53.1	58.6*	52.3	<b>46.3*</b>
	[SD]	2.56	4.68	3.93	3.40	2.91	4.34	2.86	2.17
	$\Delta\%$ of control		14.0	-4.2	-16.8		10.4	-1.5	-12.8
Week 26	[mean]	57.2	58.6	53.6	<b>47.8*</b>	53.6	61.2*	54.5	49.0
	[SD]	2.12	3.86	5.11	5.64	4.56	4.47	3.48	2.14
	$\Delta\%$ of control		2.4	-6.3	-16.4		14.2	1.7	-8.6
Week 52	[mean]	51.5	52.1	48.3	<b>42.8*</b>	47.7	54.8*	46.9	<b>39.2*</b>
	[SD]	2.95	3.08	5.38	3.91	4.64	2.82	3.99	5.69



Dose level	Males					Females			
	[ppm]	0	300	3000	10000	0	300	3000	10000
	[mg/kg bw/d]	0	7.9	83.4	253.9	0	7.9	81.4	284.8
	Δ% of control		1.2	-6.2	-16.9		14.9	-1.7	-17.8
<b>PLT [<math>10^9/L</math>]</b>									
Pre-test	[mean]	529.0	481.0	465.0	494.0	502.0	424.0	425.0	545.0
	[SD]	121.30	76.30	56.00	101.00	50.70	24.80	103.30	105.50
	Δ% of control		-9.1	-12.1	-6.6		-15.5	-15.3	8.6
Week 13	[mean]	561	557	647	<b>855*</b>	534	472	593	<b>835*</b>
	[SD]	60.10	87.20	63.90	133.30	97.10	53.10	62.00	245.60
	Δ% of control		-0.7	15.3	52.4		-11.6	11.0	56.4
Week 26	[mean]	408	417	487	<b>698*</b>	360	403	508	<b>611*</b>
	[SD]	36.60	51.30	72.30	146.90	153.40	57.80	102.10	172.40
	Δ% of control		2.2	19.4	71.1		11.9	41.1	69.7
Week 52	[mean]	333	307	375	<b>655*</b>	330	301	428	<b>791*</b>
	[SD]	30.60	81.90	83.20	137.60	74.70	37.20	116.40	270.40
	Δ% of control		-7.8	12.6	96.7		-8.8	29.7	139.7

\*  $p \leq 0.05$ ; Dunnett's Test

RBC – red blood cells (erythrocytes).

HGB – haemoglobin.

HCT – haematocrit.

PLT – platelet counts.

SD – standard deviation.

Δ% of control - percent change compared to control.

Table 6.3-44. Haematology: White blood cell parameters

Dose level	Males					Females			
	[ppm]	0	300	3000	10000	0	300	3000	10000
	[mg/kg bw/d]	0	7.9	83.4	253.9	0	7.9	81.4	284.8
<b>White blood cell parameters</b>									
<b>WBC [<math>10^9/L</math>]</b>									
Pre-test	[mean]	8.0	9.0	7.7	8.2	9.7	9.0	9.1	7.5
	[SD]	1.0	1.1	1.7	1.5	2.3	3.1	2.4	1.7
	Δ% of control		12.5	-3.8	2.5		-7.2	-6.2	-22.7
Week 26	[mean]	5.0	6.7	<b>7.4*</b>	<b>7.6*</b>	6.0	5.9	<b>9.1*</b>	8.2
	[SD]	1.2	1.8	1.5	1.3 #	1.1	1.4	1.9	2.1
	Δ% of control		34.0	48.0	52.0		-1.7	51.7	36.7
Week 52	[mean]	10.3	10.2	12.1	<b>15.6</b>	9.5	10.4	<b>13.8*</b>	<b>17.8*</b>
	[SD]	2.8	2.0	3.1	5.4 #	2.0	3.6	2.7	2.1 ##
	Δ% of control		-1.0	17.5	51.5		9.5	45.3	87.4
<b>Neutrophils N-Seg [<math>10^9/L</math>]</b>									
Pre-test	[mean]	5.5	5.87	4.95	4.7	6.1	5.8	5.8	4.3
	[SD]	1.5	1.2	1.2	1.2	1.8	2.9	2.9	1.5
	Δ% of control		6.7	-10.0	-14.5		-4.9	-4.9	-29.5
Week 26	[mean]	3.2	4.39	<b>5.26*</b>	<b>5.2*</b>	3.9	4.0	<b>6.5*</b>	5.7
	[SD]	0.5	1.3	1.1	1.3 #	1.0	1.2	1.5	1.7
	Δ% of control		37.2	64.4	62.5		2.6	66.7	46.2
Week 52	[mean]	6.9	6.5	7.6	<b>9.9</b>	8.6	7.5	<b>9.8*</b>	<b>11.3*</b>
	[SD]	2.1	1.7	2.1	4.9 #	1.7	3.3	2.6	2.0 ##
	Δ% of control		-5.8	10.1	43.5		-12.8	14.0	31.4
<b>Lymphocytes [<math>10^9/L</math>]</b>									
Pre-test	[mean]	2.2	3.0	2.4	2.8	3.1	2.8	2.9	2.8

		Males				Females			
Dose level	[ppm]	0	300	3000	10000	0	300	3000	10000
	[mg/kg bw/d]	0	7.9	83.4	253.9	0	7.9	81.4	284.8
	[SD]	0.6	0.7	0.7	0.7	0.6	0.4	0.8	1.0
	Δ% of control		36.4	9.1	27.3		-9.7	-6.5	-9.7
Week 26	[mean]	1.5	1.8	1.8	2.0	2.0	1.7	2.2	2.3
	[SD]	0.6	0.7	0.4	0.6 <sup>#</sup>	0.2	0.4	0.5	0.4
	Δ% of control		20.0	20.0	33.3		-15.0	10.0	15.0
Week 52	[mean]	2.7	3.0	3.2	4.3*	2.9	2.2	3.1	5.4*
	[SD]	0.9	0.6	0.6	0.8 <sup>#</sup>	0.8	0.3	0.9	1.7 <sup>##</sup>
	Δ% of control		11.1	18.5	59.3		-24.1	6.9	86.2
Monocytes [10 <sup>9</sup> /L]									
Pre-test	[mean]	0.3	0.3	0.2	0.4*	0.2	0.2	0.2	0.2
	[SD]	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.1
	Δ% of control		0.0	-33.3	33.3		0.0	0.0	
Week 26	[mean]	0.1	0.2	0.1	0.1	0.0	0.0	0.0	0.1
	[SD]	0.0	0.1	0.1	0.1 <sup>#</sup>	0.1	0.0	0.1	0.1
	Δ% of control		100.0	0.0	0.0		-	-	-
Week 52	[mean]	0.2	0.4	0.50	0.8*	0.4	0.3	0.5	0.2
	[SD]	0.2	0.3	0.2	0.4 <sup>#</sup>	0.2	0.2	0.1	0.3 <sup>##</sup>
	Δ% of control		100.0	150.0	300.0		-25.0	25.0	-50.0

\* p ≤ 0.05; Dunnett's Test

SD – standard deviation.

Δ% of control – percent change compared to control.

# 5 animals instead of 6; ## 4 animals instead of 6.

*Clinical chemistry:* Alkaline phosphatase (ALP) levels increased in a dose-related manner across all time points, in both sexes. Statistically-significant increases were observed in both sexes in the top dose group at all time points and additionally in the mid dose group in females at weeks 26 and 52 and in males at week 52. Values which gained statistical-significance also showed a relative change compared to controls of > 10 %. Albumin (ALB) values were statistically-significantly decreased in the mid (weeks 26 and 52) and top dose (all time points) groups in males and in the top dose group in females (week 52 only). Alanine aminotransferase (ALT) was statistically-significantly reduced in mid (week 26) and top dose (all time points) group in males, with females showing the similar pattern but without significance. However, lowered aminotransferase is not considered of toxicological relevance. Total protein, calcium and inorganic phosphorous values were generally lower in treated dogs, however, these changes were considered sporadic. The statistically-significant increases in glucose levels observed in males and females did not follow a dose-response relationship and were therefore not considered treatment-related.

Overall, treatment-related and adverse changes in some clinical chemistry parameters (ALB and ALP) indicative of liver toxicity were observed in males and females from the mid dose (3,000 ppm, equivalent to 83.4 and 81.4 mg/kg bw/d in males and females respectively).

Table 6.3-45. Statistical significant clinical chemistry findings

		Males				Females			
Dose level	[ppm]	0	300	3000	10000	0	300	3000	10000
	[mg/kg bw/d]	0	7.9	83.4	253.9	0	7.9	81.4	284.8
ALB [g/dL]									
Week 13	[mean]	3.3	3.3	3.2	<b>3.0*</b>	3.3	3.6	3.8	3.4
	[SD]	0.1	0.1	0.3	0.2	0.2	0.2	0.3	0.3
	Δ% of control		0	-3	-9		9	15	3
Week 26	[mean]	3.7	3.6	<b>3.3*</b>	<b>3.3*</b>	3.6	3.7	3.6	3.3
	[SD]	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.4
	Δ% of control		-3	-11	-11		3	0	-8
Week 52	[mean]	3.8	3.7	<b>3.4*</b>	<b>3.3*</b>	3.8	4.2	3.8	<b>3.2*</b>
	[SD]	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.4
	Δ% of control		-3	-11	-13		11	0	-16
ALT [IU/L]									
Week 13	[mean]	24	24	20	<b>17*</b>	22	21	18	17
	[SD]	3.6	3.3	1.3	3.7	6.6	2.8	2.1	1.1
	Δ% of control		0	-17	-29		-5	-18	-23
Week 26	[mean]	27	25	<b>19*</b>	<b>16*</b>	21	21	18	17
	[SD]	3.9	3.7	2.6	3.7	4.7	4.1	3.6	3.2
	Δ% of control		-7	-30	-41		0	-14	-19
Week 52	[mean]	26	26	24	<b>18*</b>	22	22	19	20
	[SD]	4.3	2.9	3.2	2.0	4.3	2.8	1.8	2.4
	Δ% of control		0	-8	-31		0	-14	-9
ALP [IU/L]									
Week 13	[mean]	70	71	82	<b>154*</b>	65	58	82	<b>108*</b>
	[SD]	26.3	18.8	24.4	60.2	13.0	7.7	29.3	21.7
	Δ% of control		1	17	120		-11	26	66
Week 26	[mean]	47	54	66	<b>137*</b>	46	46	<b>76*</b>	<b>125*</b>
	[SD]	17.2	14.2	20.0	42.4	14.5	9.4	17.5	41.4
	Δ% of control		15	40	191		0	65	172
Week 52	[mean]	38	49	<b>70*</b>	<b>171*</b>	34	42	<b>64*</b>	<b>148*</b>
	[SD]	15.9	19.1	24.3	73.1	8.4	6.8	21.4	71.7
	Δ% of control		29	84	350		24	88	335
Ca [mg/dL]									
Week 13	[mean]	11.1	11.5	11.2	10.7	11.0	11.3	11.2	<b>10.4*</b>
	[SD]	0.29	0.25	0.56	0.22	0.2	0.3	0.4	0.2
	Δ% of control		4	1	-4		3	2	-5
Week 26	[mean]	11.0	11.1	10.7	10.6	11.1	10.7	10.8	<b>9.9*</b>
	[SD]	0.28	0.41	0.44	0.34	0.25	0.5	0.4	0.5
	Δ% of control		1	-3	-4		-4	-3	-11
Week 52	[mean]	10.7	10.6	10.4	10.2	11.3	11.0	<b>10.7*</b>	<b>9.7*</b>
	[SD]	0.26	0.24	0.47	0.15	0.2	0.2	0.4	0.5
	Δ% of control		-1	-3	-5		-3	-5	-14
I Phos [mg/dL]									
Week 13	[mean]	5.2	5.2	4.8	<b>4.4*</b>	5.3	5.2	5.0	5.0
	[SD]	0.6	0.3	0.7	0.3	0.4	0.2	0.3	0.4
	Δ% of control		0	-8	-15		-2	-6	-6
Week 26	[mean]	3.9	3.9	3.1	3.7	4.6	4.0	4.4	<b>3.5*</b>
	[SD]	0.9	0.8	0.6	0.4	0.4	0.4	0.4	0.5
	Δ% of control		0	-21	-5		-13	-4	-24
Week 52	[mean]	3.6	3.1	<b>2.6*</b>	<b>2.8*</b>	3.7	3.2	3.2	2.8
	[SD]	0.5	0.4	0.3	0.5	0.5	0.6	0.5	0.6
	Δ% of control		-14	-28	-22		-14	-14	-24

		Males				Females			
Dose level	[ppm]	0	300	3000	10000	0	300	3000	10000
	[mg/kg bw/d]	0	7.9	83.4	253.9	0	7.9	81.4	284.8
Glu [mg/dL]									
Week 13	[mean]	87.0	93.3	99.8	93.6	90.4	101.4	101.8*	100.3*
	[SD]	6.0	4.2	10.3	9.4	5.6	12.1	4.3	2.9
	Δ% of control		7	15	8		12	13	11
Week 26	[mean]	74.6	88.8*	76.8	79.4	96.2	98.8	97.0	101.0
	[SD]	6.1	9.7	5.3	9.6	7.12	11.3	8.24	9.17
	Δ% of control		19	3	6		3	1	5
PROT [g/dL]									
Week 52	[mean]	7.2	7.0	6.7	6.9	7.0	6.7	6.4*	6.2*
	[SD]	0.5	0.4	0.3	0.3	0.4	0.3	0.4	0.4
	Δ% of control		-3	-7	-4		-4	-9	-11
CHOL [g/d]									
Week 52	[mean]	132	137	152	169*	213	170	186	154
	[SD]	14.7	18.9	16.6	19.3	57.1	45.2	69.8	21.9
	Δ% of control		4	15	28		-20	-13	-28

\* p ≤ 0.05; \*\* p ≤ 0.01 by Dunnett's test

Δ% = deviation as compared with the control in percent;

ALB – albumin.

ALT – alanine aminotransferase.

ALP – alkaline phosphatase.

Ca – calcium.

I Phos – inorganic phosphorus.

Glu – glucose.

PROT – protein.

CHOL – cholesterol.

*Urinalysis:* There were no treatment-related urinalysis findings.

*Organ weight:* Absolute liver weights were statistically-significantly increased at the top dose in females and from the mid dose in males. Relative liver weights were statistically-significantly increased from the mid dose in both sexes. A dose-response relationship was evident and statistically-significant values were increased by > 15 % compared to controls. These findings were considered treatment-related and adverse. Statistically-significant higher relative heart, adrenal, kidney and brain weights in the top dose females mirrors the statistically-significant lower terminal body weight in that group. Organ weights in males were unremarkable. The relative thyroid weight in the 10,000 ppm males and females and in the 3,000 ppm females was increased, however, without dose-dependency or microscopic correlate. The main cause is most likely the reduced body weight. Further evidence to support a lack of relation to treatment comes from the other dog studies, which did not identify the thyroid as being a target organ in beagle dogs. Historical control data to evaluate the extremely low control data and the treatment groups values more comprehensively were not available. Overall, there were treatment-related and adverse effects on liver weight from the mid dose. No other specific effects on organ weights were seen.

Table 6.3-46. Terminal body and organ weights<sup>a</sup>

Sex	Dose [ppm] [mg/kg bw/d]	Males				Dose [ppm] [mg/kg bw/d]	Females			
		Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&		Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
Terminal weight [g]	0	11300				0	11800			
	300	11600	2.7			300	10567	-10.5		
	7.9					7.9				
	3000	10800	-4.4			3000	10250	-13.1		
	83.4					81.4				

Sex	Dose [ppm] [mg/kg bw/d]	Males				Dose [ppm] [mg/kg bw/d]	Females			
		Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&		Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
	10000 253.9	8860	-21.6			10000 284.8	8275**	-29.9		
Liver [g]	0	254		2.32		0	287		2.47	
	300 7.9	301		2.60		300 7.9	256		2.45	
	3000 83.4	315*	+24	2.94*	+27	3000 81.4	319	+11	3.12*	+26
	10000 253.9	361**	+42	4.08**	+76	10000 284.8	374**	+30	4.54**	+84
Thyroids <sup>#</sup> [mg]	0	0.406		0.0039		0	0.443		0.0038	
	300 7.9	0.538	+32.4	0.0046	+19.5	300 7.9	0.447	+0.9	0.0043	11.8
	3000 83.4	0.477	+17.5	0.0044	+13.0	3000 81.4	0.616	+38.9	0.0059**	+55.3
	10000 253.9	0.587	+44.5	0.0066*	+71.4	10000 284.8	0.480	+8.2	0.0059**	+53.9
Heart [g]	0	90.195		0.8240		0	75.704		0.6434	
	300 7.9	90.242	0.1	0.7851	-4.7	300 7.9	76.263	0.7	0.7300	+13.5
	3000 83.4	94.341	4.6	0.8798	+6.8	3000 81.4	73.719	-2.6	0.7319	+13.8
	10000 253.9	81.999	-9	0.9270	+12.5	10000 284.8	79.201	4.6	0.9627**	+49.6
Adrenal <sup>#</sup> [g]	0	0.592		0.0055		0	0.707		0.006	
	300 7.9	0.652	+10	0.0058	+6	300 7.9	0.689		0.007	+9
	3000 83.4	0.640	+8	0.0060	+9	3000 81.4	0.7		0.007	+17
	10000 253.9	0.599	+1	0.0067	+22	10000 284.8	0.7035	-0.5	0.008*	+41
Brain [g]	0	75.704		0.7032		0	75.008		0.6433	
	300 7.9	76.087	+0.5	0.6707	-4.6	300 7.9	76.398	+1.9	0.7270	+13
	3000 83.4	79.388	+4.9	0.7469	+6	3000 81.4	73.914	-1.5	0.7333	+14
	10000 253.9	73.775	-2.6	0.8318	+18	10000 284.8	70.724	-5.7	0.8530**	+32
Kidney <sup>#</sup> [g]	0	26.783		0.2443		0	19.553		0.167	
	300 7.9	28.738	+7.3	0.2476	+1	300 7.9	21.523		0.205	+22.5
	3000 83.4	26.813	+0.1	0.2506	+2.6	3000 81.4	19.398		0.191	+14.4
	10000 253.9	24.031	-10.3	0.2727	+11.6	10000 284.8	21.712	+11.0	0.264**	+57.8

\* p ≤ 0.05, \*\* p ≤ 0.01; Dunnett's test

& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

<sup>a</sup> Dogs which died prior to scheduled death at 1 year were excluded as well as outliers

<sup>#</sup> Paired organs were taken as mean organ weights

*Gross pathology and histopathology:* There were no treatment-related effects on gross pathology. There were no histopathological findings in the liver or thyroid. Effects were only seen in the prostate and testes. A retardation in glandular development of the prostate was observed from the mid dose (Table 6.3-47). As in the

90-day study, this finding was considered treatment-related and adverse but secondary to the impaired body weight development seen at these doses. An increase in the incidence of testicular atrophy was seen mainly at the top dose. It is most likely this was the consequence of the impaired body weight development observed at this dose, especially considering that there were no effects on spermatogenesis. Overall, treatment-related and adverse histopathology was seen in the prostate (delayed glandular development) from the mid dose and in the testes (atrophy) at the top dose.

Table 6.3-47. Selected histopathological findings

Sex		Grading	Males				Females			
Dose level	[ppm]		0	300	3000	10000	0	300	3000	10000
	[mg/kg bw/d]		0	7.9	83.4	253.9	0	7.9	81.4	284.8
Animals examined			6	6	6	6	6	6	6	6
Prostate							-	-	-	-
		N	6	6	6	6	-	-	-	-
		1	-	-	-	1 <sup>§</sup>	-	-	-	-
- glandular		2	-	-	-	2	-	-	-	-
development		3	-	1	4	3	-	-	-	-
		4	6	5	2	-	-	-	-	-
		<i>[mean]</i> <sup>#</sup>	<i>[4.0]</i>	<i>[3.8]</i>	<i>[3.3]</i>	<i>[2.6]</i>	-	-	-	-
Testes							-	-	-	-
		N	6	6	6	6	-	-	-	-
		1	-	-	-	-	-	-	-	-
- spermatogenesis		2	1	3	1	1	-	-	-	-
		3	5	3	5	5 <sup>§</sup>	-	-	-	-
		<i>[mean]</i> <sup>#</sup>	<i>[2.8]</i>	<i>[2.5]</i>	<i>[2.8]</i>	<i>[2.8]</i>	-	-	-	-
		N	1	2	2	3	-	-	-	-
		1	1		2	2 <sup>§</sup>	-	-	-	-
- atrophy		2	-	2	-	-	-	-	-	-
		3	-	-	-	1	-	-	-	-
		<i>[mean]</i> <sup>#</sup>	<i>[1.0]</i>	<i>[2.0]</i>	<i>[1.0]</i>	<i>[2.0]</i>	-	-	-	-

<sup>#</sup> = mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding

<sup>§</sup> = finding occurred in a dog died prematurely on week 13 (day 86 -moribund sacrifice) and was considered to be a consequence of the age difference, and thus, not included in the mean calculation

N – total number of animals with findings.

Table 6.3-48. Correlation of testes and prostate findings in Control and dogs administered 3000 ppm

<b>Control</b>	<b># 219</b>	<b># 221</b>	<b># 223</b>	<b># 226</b>	<b># 229</b>	<b># 242</b>
<b>Terminal body weight [g]</b>	<b>9300</b>	<b>13400</b>	<b>8800</b>	<b>11800</b>	<b>15300</b>	<b>9200</b>
<b>Testes</b>						
-Spermatogenesis	3	3	3	3	2	3
-Testicular Atrophy					1 <sup>u</sup>	
<b>Prostate</b>						
-Glandular development	4	4	4	4	4	4
<b>3000ppm</b>	<b># 231</b>	<b># 232</b>	<b># 234</b>	<b># 235</b>	<b># 236</b>	<b># 241</b>
<b>Terminal body weight [g]</b>	<b>10100</b>	<b>10200</b>	<b>13500</b>	<b>13700</b>	<b>10200</b>	<b>10100</b>
<b>Testes</b>						
-Spermatogenesis	3	3	3	3	2	3
-Testicular Atrophy				1 <sup>u</sup>		1 <sup>u</sup>
<b>Prostate</b>						
-Glandular development	3	4	3	4	3	3

U: unilateral

### Conclusion

In conclusion, under the conditions of this relatively old GLP compliant study in dogs, dietary administration of cinmethylin for 52 weeks resulted in clear signs of systemic toxicity at the top dose (10,000 ppm, equivalent to 254 and 285 mg/kg bw/d in M/F respectively) based on deaths, clinical signs of toxicity and reductions in body weight (-21 % / -29 %), body weight gain (-57 % / -73 %) and food consumption. Several red blood cell (RBC, HGB, HCT and platelet counts) parameters were also altered at the top dose and an increased incidence of testis atrophy was observed. In addition, reductions in body weight and body weight gain, effects on white blood cell parameters, alterations in clinical chemistry parameters (ALP and ALB) indicative of liver toxicity, increased liver weights (> 15%) and a delay in glandular development of the prostate were seen from the mid dose of 3,000 ppm.

A NOAEL of 300 ppm (equivalent to 7.9 mg/kg bw/d in M/F respectively) is proposed by HSE since no adverse effects were seen at this dose. A LOAEL of 3,000 ppm (83 and 81 mg/kg bw/d in M/F respectively) is proposed based on reductions in body weight and body weight gain, effects on white blood cell parameters, alterations in clinical chemistry parameters (ALP and ALB) indicative of liver toxicity, increased liver weights (> 15%) and a delay in glandular development of the prostate.

(██████, 1985)

### 2) Old study

<b>Author(s)</b>	██████
<b>Study title</b>	One year dietary feeding study in beagle dogs of cinch herbicide
<b>Study reference</b>	██████, 1988a BASF Doc ID : CI-427-003
<b>Test facility</b>	██
<b>Date</b>	18/09/1986 – 23/09/1987
<b>Test substance</b>	SD 95481 (Cinmethylin)
<b>Batch no. and purity (%)</b>	925, code 6-4-0-0 92.4
<b>Test animals</b>	Dogs Beagle Male and female
<b>Groups</b>	6/sex/dose
<b>Dose/concentrations</b>	0, 2, 30, 100, 200 and 3000 ppm Equivalent to 0, 0.044, 0.68, 2.3, 4.7 and 80.8 mg/kg bw/d for males and 0, 0.048, 0.74, 2.4, 4.3 and 70.7 mg/kg bw/d for females respectively.
<b>Route</b>	Administered daily via the diet for 52 weeks.
<b>Vehicle</b>	Acetone.
<b>GLP</b>	Compliant.
<b>Guideline</b>	US EPA Pesticide Assessment Guidelines.
<b>Deviation</b>	Deviations from OECD test guideline No. 452 (1981) : <ul style="list-style-type: none"> <li>Clinical biochemistry: gamma glutamyl transpeptidase was not determined.</li> </ul> Deviations from OECD test guideline No. 452 (2009) : <ul style="list-style-type: none"> <li>Detailed clinical observations were not performed.</li> <li>Dose level spacing factors were greater than 10.</li> <li>In haematology no prothrombin time or activated partial thromboplastin time were recorded.</li> <li>At gross necropsy, weight of epididymides and uterus were not recorded.</li> <li>Histopathology was not performed on coagulating gland, lacrimal gland, seminal vesicle and uterus.</li> <li>A discussion of the observed significantly changed parameters in relation to historical control data is missing.</li> </ul>
<b>Impact of deviations</b>	Due to the lack of historical control data, it is difficult to evaluate reported findings in test and concurrent control groups in relation to the normal range of fluctuations.
<b>Acceptable</b>	Despite the above listed deviations, this study, together with the other two 1-year dog study (██████, 1985 and ██████, 1988b), is considered to satisfy regulatory requirements and is therefore used for hazard and risk assessment.

<b>NOAEL</b>	200 ppm (equivalent to 4.7 and 4.3 mg/kg bw/d in males and females respectively).
<b>Effects at the LOAEL</b>	Effects on some white blood cell parameters (WBC and neutrophil counts) in males and females, increases in alkaline phosphatase levels in females, increases (> 15%) in liver weights in males (without concomitant histopathology) and a higher incidence of small prostate at 3,000 ppm (equivalent to 80.8 and 70.7 mg/kg bw/d in males and females respectively).

#### Methods

Cinmethylin was administered via the diet to groups of 6 male and 6 female Beagle dogs per test group, at concentrations of 0, 2, 30, 100, 200 and 3000 ppm, over a period of 1-year. Equivalent cinmethylin intakes in mg/kg bw/d are shown in Table 6.3-49. An interim treatment group was not included in this study.

Table 6.3-49. Equivalent cinmethylin intakes

Concentration in the vehicle (ppm)	Mean daily cinmethylin intake (mg/kg bw/d)	
	Males	Females
0	0	0
2	0.044	0.048
30	0.68	0.74
100	2.3	2.4
200	4.7	4.3
3000	80.8	70.7

Food consumption and body weight was determined weekly. Animals were examined for signs of toxicity or mortality twice daily. Clinical biochemistry, urinalysis and haematological examinations were performed at weeks 13, 26, and 52. Ophthalmological examinations were conducted prior to administration and at termination of the test period. After the administration period dogs were euthanised and necropsied and specific organs weighed, selected organs and tissues were histopathologically examined. Evaluation of the prostate was of particular interest since potential treatment-related effects occurred in the previous 1-year study (■■■■■, 1985). A method for the dose verification of cinmethylin in feed was evaluated and was regarded as fit for purpose (see Volume 3 CA B5, section B.5.1.2).

#### Results

*Mortality and general clinical observations:* There were no treatment-related deaths or clinical signs of toxicity.

*Body weight, food and water consumption:* There were no treatment-related effects observed on food consumption, body weight or body weight gain. All dogs either gained or maintained body weight over the 52-week study period. Isolated statistically-significant body weight changes were observed but did not show a dose-response relationship.

Table 6.3-50. Body weight change

		Males						Females					
Dose level	[ppm]	0	2	30	100	200	3000	0	2	30	100	200	3000
	[mg/kg bw/d]	0	0.044	0.68	2.3	4.7	80.8	0	0.048	0.74	2.4	4.3	70.7
Body weight		Mean ± SD						Mean ± SD					
Week 1-2	[g]	282	306	286	173	91	88	294	98	81	149	97	-13*
	[SD]	± 176	± 318	± 158	± 201	± 201	± 164	± 157	± 110	± 172	± 176	± 159	± 246
	[Δ%]		9	1	-39	-68	-69		-67	-72	-49	-67	-104
Week 9-10	[g]	398	318	433	211	16*	67	66	79	-88	100	82	133
	[SD]	± 233	± 119	± 146	± 318	± 197	± 237	± 122	± 261	± 365	± 238	± 307	± 90
	[Δ%]		-20	9	-47	-96	-83		20	-233	52	24	102
Week 13-14	[g]	25	146	197	349	361*	133	92	-41	238	237	125	176
	[SD]	± 182	± 247	± 243	± 191	± 132	± 263	± 176	± 192	± 238	± 303	± 158	± 113



		Males						Females					
Dose level	[ppm]	0	2	30	100	200	3000	0	2	30	100	200	3000
[ppm]	[mg/kg bw/d]	0	0.044	0.68	2.3	4.7	80.8	0	0.048	0.74	2.4	4.3	70.7
	[Δ%]		484	688	1296	1344	432		-145	159	158	36	91
Week 17-18	[g]	295	104	46	285	85	36	183	193	227	-107**	106	140
	[SD]	± 174	± 139	± 150	± 274	± 283	± 58	± 166	± 84	± 209	± 180	± 104	± 111
	[Δ%]		-65	-84	-3	-71	-88		5	24	-158	-42	-23
Week 19-20	[g]	225	92	-83*	-86*	26	-6	-8	38	-92	-87	26	-137
	[SD]	± 175	± 97	± 179	± 77	± 195	± 246	± 223	± 204	± 123	± 466	± 91	± 162
	[Δ%]		-59	-137	-138	-88	-103		-575	1050	988	-425	1613
Week 23-24	[g]	289	202	21	234	-68*	17	212	16	320	77	21	130
	[SD]	± 216	± 144	± 274	± 298	± 202	± 147	± 232	± 303	± 191	± 361	± 214	± 211
	[Δ%]		-30	-93	-19	-124	-94		-92	51	-64	-90	-39
Week 37-38	[g]	666	232*	378	251*	359	325	284	317	242	87	216	255
	[SD]	± 310	± 165	± 65	± 357	± 255	± 322	± 159	± 344	± 240	± 312	± 260	± 336
	[Δ%]		-65	-43	-62	-46	-51		12	-15	-69	-24	-10
Week 38-39	[g]	-316	31	-46	0	-83	114*	-171	-166	-61	-80	28	-34
	[SD]	± 337	± 186	± 241	± 178	± 330	± 106	± 251	± 173	± 136	± 267	± 190	± 128
	[Δ%]		-110	-85	-100	-74	-136		-3	-64	-53	-116	-80
Week 41-42	[g]	148	176	32	-65	244	307	-232	29	59	246*	277*	177
	[SD]	± 278	± 344	± 229	± 353	± 352	± 272	± 506	± 359	± 244	± 198	± 81	± 212
	[Δ%]		19	-78	-144	65	107		-113	-125	-206	-219	-176
Week 46-47	[g]	12	-82	-46	-266*	-88	72	-10	15	180	-10	-17	-24
	[SD]	± 167	± 139	± 256	± 109	± 134	± 195	± 100	± 300	± 162	± 152	± 218	± 122
	[Δ%]		-783	-483	-2317	-833	500		-250	-1900	0	70	140
Week 48-49	[g]	-314	-233	-193	-149	78	-286	-324	-115	-170	37**	-130	-165
	[SD]	± 253	± 235	± 185	± 329	± 401	± 115	± 220	± 216	± 258	± 93	± 183	± 83
	[Δ%]		-26	-39	-53	-125	-9		-65	-48	-111	-60	-49
Week 49-50	[g]	234	41	207	181	-118*	374	219	9	156	77	269	10
	[SD]	± 220	± 197	± 213	± 223	± 216	± 182	± 222	± 180	± 196	± 229	± 322	± 205
	[Δ%]		-82	-12	-23	-150	60		-96	-29	-65	23	-95
Week 0-52	[g]	3643	4339	2199	2292	2391	2969	1352	1440	1880	1891	2336	1166
	[SD]	± 1458	± 1585	± 981	± 729	± 928	± 1358	± 528	± 623	± 1682	± 744	± 2261	± 480
	[Δ%]		19	-40	-37	-34	-19		7	39	40	73	-14

\* = p≤0.05; \*\* = p≤0.01 Dunnett's test

SD – standard deviation.

Δ% - percent change compared to control.

*Ophthalmoscopy:* There were no treatment-related ophthalmological findings.

*Haematology:* Slight but statistically-significant differences in mean corpuscular volume (MCV) was observed in males of the 100 ppm dose group (Table 6.3-51). However, due to the lack of dose-response, this was not considered treatment-related. After 13 weeks, total white blood cell (WBC) counts and absolute neutrophil counts were significantly increased in both males and females of the top dose group (Table 6.3-52). After 26 and 52 weeks, WBC and absolute neutrophil counts were significantly higher only in males of the top dose group. This alteration results in increased relative neutrophil counts but decreased relative lymphocyte counts. Overall, changes in white blood cell parameters were considered treatment-related and adverse in males and females in the top dose group.

Table 6.3-51. Haematology: Red Blood cell parameters

		Males						Females					
Dose level	[ppm]	0	2	30	100	200	3000	0	2	30	100	200	3000
	[mg/kg bw/d]	0	0.044	0.68	2.3	4.68	80.78	0	0.048	0.74	2.39	4.34	70.69
MCV													
Week 52	[mean]	66.7	66.2	65.3	64.3*	64.8	65.8	68.7	67.5	67.3	66.3	68.5	67.2
	[SD]	1.6	1.7	1.2	1.0	1.6	1.5	3.2	2.3	1.9	2.3	3.1	3.1
	[Δ%]		-1	-2	-4	-3	-1		-2	-2	-3	0	-2

\* = p ≤ 0.05; \*\* = p ≤ 0.01 Dunnett's test

SD – standard deviation.

Δ% - percent change compared to control.

Table 6.3-52. Haematology: White Blood cell parameters

Dose [ppm]	0	2	30	100	200	3000
Sex	Males					
Animals per dose	6	6	6	6	6	6
WBC [10 <sup>9</sup> /L]						
13 weeks	11.3 ± 2.2	11.2 ± 2.5	10.3 ± 1.4	10.7 ± 0.8	11.8 ± 2.1	<b>15.4* ± 4.0</b>
26 weeks	9.5 ± 2.3	10.3 ± 1.6	10.0 ± 1.6	10.6 ± 1.2	11.4 ± 1.6	<b>12.5* ± 2.8</b>
52 weeks	8.9 ± 0.7	8.8 ± 1.6	8.9 ± 2.1	9.0 ± 0.8	8.6 ± 1.2	<b>11.1* ± 1.4</b>
Neutrophils 10 <sup>9</sup> /L]						
13 weeks	7.40 ± 1.5	7.75 ± 1.8	6.92 ± 1.3	6.71 ± 1.1	7.67 ± 2.2	<b>10.98* ± 3.1</b>
26 weeks	5.40 ± 1.6	6.66 ± 1.8	5.83 ± 1.3	6.58 ± 1.2	7.53 ± 2.2	<b>9.09** ± 2.4</b>
52 weeks	5.27 ± 1.1	5.66 ± 1.1	5.90 ± 1.5	5.67 ± 0.5	5.46 ± 0.7	<b>7.78** ± 1.3</b>
Neutrophils [%]						
13 weeks	65.5 ± 4.3	69.3 ± 5.1	67.3 ± 8.9	62.3 ± 6.7	64.3 ± 7.8	71.0 ± 6.9
26 weeks	56.3 ± 4.7	64.2 ± 11.7	57.8 ± 9.1	61.8 ± 6.0	65.0 ± 10.2	<b>72.7* ± 7.2</b>
52 weeks	59.0 ± 9.1	64.7 ± 5.5	66.5 ± 7.3	63.3 ± 2.8	64.0 ± 7.9	<b>69.8* ± 5.5</b>
Lymphocytes [%]						
13 weeks	33.3 ± 4.6	27.0 ± 3.8	29.3 ± 6.4	35.3 ± 5.6	32.5 ± 8.1	27.2 ± 6.2
26 weeks	42.0 ± 4.6	33.8 ± 10.8	40.5 ± 8.8	37.2 ± 6.1	32.2 ± 7.9	<b>25.2** ± 5.8</b>
52 weeks	40.2 ± 9.1	35.0 ± 5.7	33.2 ± 7.1	36.3 ± 2.9	35.7 ± 8.2	<b>29.7* ± 5.8</b>
Sex	Females					
Animals per dose	6	6	6	6	6	6
WBC [10 <sup>9</sup> /L]						
13 weeks	10.2 ± 1.0	10.1 ± 0.3	8.7 ± 1.3	11.1 ± 1.8	11.7 ± 2.5	<b>12.8* ± 1.6</b>
26 weeks	9.0 ± 1.4	11.2 ± 2.2	8.4 ± 1.3	9.7 ± 1.4	9.4 ± 1.2	10.5 ± 1.1
52 weeks	9.6 ± 2.7	8.8 ± 0.9	7.9 ± 1.1	8.8 ± 1.3	8.7 ± 1.4	11.6 ± 2.9
Neutrophils [10 <sup>9</sup> /L]						
13 weeks	5.87 ± 0.9	6.44 ± 0.7	4.7 ± 0.7	6.62 ± 1.2	6.82 ± 1.0	<b>7.56* ± 1.0</b>
26 weeks	5.85 ± 1.0	<b>8.55* ± 1.5</b>	5.48 ± 1.5	6.50 ± 1.9	6.06 ± 1.1	6.56 ± 1.3
52 weeks	6.43 ± 2.1	6.32 ± 0.7	4.79 ± 1.0	5.66 ± 1.0	5.46 ± 0.9	8.03 ± 2.9
Neutrophils [%]						
13 weeks	57.3 ± 6.4	64.2 ± 9.5	54.2 ± 4.2	59.7 ± 8.3	59.2 ± 7.1	59.0 ± 4.5
26 weeks	65.2 ± 7.3	76.5 ± 5.0	64.7 ± 9.3	66.2 ± 11.1	64.8 ± 10.1	61.8 ± 6.1
52 weeks	66.7 ± 2.9	72.0 ± 6.8	60.8 ± 11.3	64.8 ± 10.5	62.7 ± 7.7	68.3 ± 8.4
Lymphocytes [%]						
13 weeks	40.7 ± 7.1	33.7 ± 8.0	44.0 ± 3.9	37.5 ± 10.6	38.5 ± 6.9	39.0 ± 3.1
26 weeks	33.0 ± 6.5	22.5 ± 5.9	33.8 ± 10.7	32.7 ± 12.2	32.7 ± 8.9	35.3 ± 7.2
52 weeks	32.5 ± 2.3	27.5 ± 6.3	38.7 ± 11.3	35.2 ± 10.5	36.7 ± 7.5	30.7 ± 7.9

\* p ≤ 0.05; \*\* p ≤ 0.01; Dunnett's Test;

*Clinical chemistry:* Alkaline phosphatase levels were consistently statistically-significantly elevated for high dose females at weeks 13, 26 and 52 (Table 6.3-53). This change is considered treatment-related and adverse. All other findings were not treatment-related.

Table 6.3-53. Selected clinical chemistry findings

		Males						Females					
Dose level	[ppm]	0	2	30	100	200	3000	0	2	30	100	200	3000
	[mg/kg bw/d]	0	0.044	0.68	2.3	4.68	80.78	0	0.048	0.74	2.39	4.34	70.69
APAS / Alkaline Phosphatase [IU/L]													
Pre-test	[mean]	81.6	75.8	67.5	57.8	73.2	71.2	62.8	60.3	58.4	73.2	78.3	77.9
	[SD]	± 14.8	± 17.2	± 20.0	± 11.1	± 18.0	± 14.1	± 11.9	± 15.2	± 16.1	± 30.2	± 27.7	± 7.2
	[Δ%]		-7.1	-17.3	-29.2	-10.3	-12.7		-4.0	-7.0	16.6	24.7	24.0
Week 13	[mean]	48.1	52.0	37.3	33.9	42.3	51.8	40.2	35.8	29.5	42.0	43.7	63.7*
	[SD]	± 10.5	± 10.1	± 8.1	± 14.4	± 11.1	± 11.3	± 15.9	± 11.3	± 13.4	± 15.8	± 16.3	± 13.5
	[Δ%]		8.1	-22.5	-29.5	-12.1	7.7		-10.9	-26.6	4.5	8.7	58.5
Week 26	[mean]	32.1	31.4	22.3	23.4	28.1	36.1	30.2	30.1	23.6	28.8	31.2	53.5*
	[SD]	± 12.4	± 6.4	± 7.3	± 13.9	± 9.3	± 9.8	± 10.7	± 11.6	± 9.7	± 10.7	± 11.8	± 16.0
	[Δ%]		-2.2	-30.5	-27.1	-12.5	12.5		-0.3	-21.9	-4.6	3.3	77.2
Week 52	[mean]	26.0	27.4	21.3	20.0	28.3	35.2	26.0	26.8	18.0	24.6	30.9	50.8*
	[SD]	± 9.3	± 8.7	± 3.9	± 10.0	± 15.0	± 10.6	± 15.4	± 12.7	± 9.7	± 9.5	± 11.7	± 15.3
	[Δ%]		5.4	-18.1	-23.1	8.8	35.4		3.1	-30.8	-5.4	18.8	95.4

\* p ≤ 0.05; \*\* p ≤ 0.01 by Dunnett's test

Δ% = percent change compared to control.

*Urinalysis:* No treatment-related changes among urinalysis parameters were observed.

*Organ weight:* Absolute and relative liver weights were increased for both males and females at the top dose. Increases for females were < 10 % compared to controls, whereas increases for males were > 15 % compared to controls, though only the change in relative weight of males at the top dose was statistically-significant (Table 6.3-54). There were no histopathological findings associated with liver weight increase. No other organ weights were affected. Overall, treatment-related and adverse increases in relative liver weight (> 15 %) were seen in males at the top dose.

Table 6.3-54. Terminal body and organ weights

		Males				Dose		Females			
	Dose [ppm] [mg/kg bw/d]	Absolute weight		Relative weight		[ppm] [mg/kg bw/d]		Absolute weight		Relative weight	
		[g or mg]	Δ%&	[% of bw]	Δ%&			[g or mg]	Δ%&	[% of bw]	Δ%&
Terminal weight [g]	0					0		9.47			
	2 0.044	14.18	6.8			2 0.048		8.9	-6.0		
	30 0.68	12.45	-6.3			30 0.74		9.86	4.1		
	100 2.3	12.23	-7.9			100 2.4		9.45	-0.2		
	200 4.7	12.42	-6.5			200 4.3		10.45	10.3		
	3000 80.8	12.99	-2.2			3000 70.7		9.39	-0.8		
Liver [g]	0	347.6		2.6113		0		264.6		2.7818	
	2 0.044	350.8	0.9	2.4807	-5.0	2 0.048		239.7	-9.4	2.7293	-1.9
	30 0.68	300.5	-13.6	2.3815	-8.8	30 0.74		247.2	-6.6	2.5403	-8.7

	Dose [ppm] [mg/kg bw/d]	Males				Dose [ppm] [mg/kg bw/d]	Females			
		Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&		Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
	100 2.3	342.9	-1.4	2.8225	8.1	100 2.4	224.4	-15.2	2.4005	-13.7
	200 4.7	314.1	-9.6	2.5368	-2.9	200 4.3	240.4	-9.1	2.3895	-14.1
	3000 80.8	428	23.1	3.2981*	26.3	3000 70.7	279.4	5.6	2.9584	6.3

\* p ≤ 0.05, \*\* p ≤ 0.01; Dunnett's test

& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

*Gross pathology:* Prostates were small in one control, one 200 ppm (4.68 mg/kg bw/d) and in two top dose group (3000 ppm or 80.78 mg/kg bw/d) males (Table 6.3-55). There were no other treatment-related gross necropsy findings noted.

Table 6.3-55. Gross necropsy observations

Dose level	[ppm] [mg/kg bw/d]	Males						Females					
		0	2	30	100	200	3000	0	2	30	100	200	3000
		0	0.044	0.68	2.3	4.68	80.78	0	0.048	0.74	2.39	4.34	70.69
Animals examined		6	6	6	6	6	6	6	6	6	6	6	6
Prostate, small		1				1	2						

*Histopathology:* At gross necropsy, two top dose dogs showed small prostate compared to 1 control dog. Histological evaluation revealed no treatment-related findings. Of special interest were the histopathological evaluation of the prostate glands in view of the findings in the first 1 year dog study (■■■■■, 1985) and in view of the findings in the gross necropsy. The prostate glands which were recorded small (Table 6.3-55) had no correlating lesions at microscopic examination. Prostates from all males were examined by six veterinary pathologists, and prostates from treated and control dogs were considered to be histologically similar and normal for Beagle dogs of this age.

#### Conclusion

In conclusion, under the conditions of this relatively old, GLP compliant study in dogs, dietary administration of cinmethylin for 52 weeks resulted in the following effects at the top dose (3,000 ppm, equivalent to 80.0 and 70.7 mg/kg bw/d in M/F respectively): effects on some white blood cell parameters (WBC and neutrophil counts) in males and females, increases in alkaline phosphatase levels in females, increases (> 15 %) in liver weights in males (without concomitant histopathology) and a higher incidence of small prostate. It is noted that the prostate histopathological findings observed at 3,000 ppm in the previous 1-year study were not reproduced here (although a higher incidence of small prostate was seen). It is also noted that body weights were unaffected at 3,000 ppm in this study, but not in the previous 1-year study. Therefore, it is not surprising that, due to experimental variation and consistent with the lack of body weight effects, no prostate histopathology was seen in this second study. The lack of these findings in this study does not override the toxicological-relevance of the prostate findings from the first 1-year study and 90-day study. However, this study confirms that the histopathology findings in the prostate are secondary to impaired body weight development.

A NOAEL of 200 ppm (equivalent to 4.7 and 4.3 mg/kg bw/d in M/F respectively) is proposed by HSE since no adverse effects were seen at this dose. A LOAEL of 3000 ppm (equivalent to 80.8 and 70.7 mg/kg bw/d in M/F respectively) is proposed based on effects on some white blood cell parameters (WBC and neutrophil counts) in males and females, increases in alkaline phosphatase levels in females, increases (> 15 %) in liver weights in males (without concomitant histopathology) and a higher incidence of small prostate.

(■■■■■, 1988a)

### 3) Old study

<b>Author(s)</b>	██████████
<b>Study title</b>	Cinch herbicide: reversibility of toxicity in beagle dogs (a 12 month feeding with 6 months reversibility)
<b>Study reference</b>	██████████, 1988b CI-427-004
<b>Test facility</b>	██
<b>Date</b>	14/11/1986 – 18/05/1988
<b>Test substance</b>	BAS 684 H (SD 95481) (Cinmethylin)
<b>Batch no. and purity (%)</b>	Batche: WRC TOX #925 Purity: no data.
<b>Test animals</b>	Dogs Beagle Male and female
<b>Groups</b>	6/sex/dose
<b>Dose/concentrations</b>	0, 2, 30, 100, 200 and 3000 ppm Equivalent to 0, 0.04, 0.63, 2.3, 4.1 and 64.3 mg/kg bw/d for males and 0, 0.04, 0.62, 2.1, 4.1 and 71.2 mg/kg bw/d for females respectively. Note: The top dose exceeded the limit dose stated in the guideline (1000 mg/kg bw/d).
<b>Route</b>	Administered daily via the diet for 52 weeks, followed by 26 weeks of recovery (no treatment).
<b>Vehicle</b>	Acetone
<b>GLP</b>	Compliant.
<b>Guideline</b>	US EPA Pesticide Assessment Guidelines.
<b>Deviation</b>	<p>Deviations from OECD test guideline No. 452 (2018) :</p> <ul style="list-style-type: none"> <li>• Test conducted in non-rodents and not justified.</li> <li>• Humidity was 25-75% instead of 30–70%.</li> <li>• Satellite groups such as these (to monitor reversibility) is normally restricted to the highest dose plus control.</li> <li>• The top dose exceeded the limit dose stated in the guideline (1000 mg/kg bw/d).</li> <li>• Purity of the test item was not given.</li> <li>• Data on actual doses (mg/kg body weight/day) were not given.</li> <li>• Dose level spacing factors were greater than 10.</li> <li>• Detailed clinical observations were not performed.</li> <li>• In haematology and clinical biochemistry : mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, prothrombin time, activated partial thromboplastin time and gamma glutamyl transpeptidase were not determined.</li> <li>• Osmolality and specific gravity were not recorded.</li> <li>• At gross necropsy, weight of epididymides and uterus were not recorded.</li> <li>• Histopathology was not performed on coagulating gland, lacrimal gland and seminal vesicle.</li> </ul>
<b>Impact of deviations</b>	The deviations identified are not considered to compromise the validity of the study.
<b>Acceptable</b>	Despite the above listed deviations, this study, together with the other 1-year dog studies, is considered to satisfy regulatory requirements and is therefore used for hazard and risk assessment in a WoE approach.
<b>NOAEL</b>	200 ppm in males (equivalent to 4.1 mg/kg bw/d).
<b>Effects at the LOAEL</b>	3,00 ppm (equivalent to 64.3 mg/kg bw/d in males) is proposed based on effects on haematology (WBC counts) in males.

## Methods

This study was conducted in parallel with the study previously described (■■■■■, 1988a, CI-427-003). The same study plan and procedures were employed. Cinmethylin was administered via the diet to groups of 6 male and 6 female Beagle dogs per test group, at concentrations of 0, 2, 30, 100, 200 and 3000 ppm, over a period of 1-year. Equivalent cinmethylin intakes in mg/kg bw/d are shown in Table 6.3-56. However, instead of the dogs being sacrificed after 12-months of treatment, they were subsequently maintained for a recovery period of 6 months, during which all groups were fed the basal diet containing no cinmethylin.

Table 6.3-56. Equivalent cinmethylin intakes

Concentration in the vehicle (ppm)	Mean daily cinmethylin intake (mg/kg bw/d)	
	Males	Females
0	0	0
2	0.04	0.04
30	0.63	0.62
100	2.28	2.05
200	4.11	4.11
3000	64.26	71.22

Food consumption and body weight was determined weekly. Animals were examined for signs of toxicity or mortality twice daily. Clinical biochemistry, urinalysis and haematological examinations were performed at weeks 13, 26, and 52. Macroscopic examinations of all surviving animals were conducted at necropsy and specific organs were weighed. Selected organs and tissues from all animals in all groups were preserved and histopathologically examined. A method for the dose verification of cinmethylin in feed was evaluated and was regarded as fit for purpose (see Volume 3 CA B5, section B.5.1.2).

#### Results

*Mortality and general clinical observations:* Two dogs died during the recovery period. One female of the low dose group (2 ppm) died in week 69; lymphosarcoma in several tissues was identified as the cause of death. One female of the 100 ppm group was found dead in week 53; this death was considered to have resulted from chronic inflammatory lesions of the urinary tract. Neither of these deaths were considered treatment-related.

Clinical signs observed (including emesis, various sores, scabs, irritated skin, lumps, swellings, hair loss, blood in stool/under cage and an enlarged Harderian gland), occurred across all groups without dose-response relationship. They were of short duration and/or did not affect the wellbeing of the animals. Overall, there were no treatment-related deaths or clinical signs of toxicity.

*Body weight, food and water consumption:* Body weight (Table 6.3-57) and food consumption were not affected by treatment.

Table 6.3-57. Body weights

Concentration [ppm]	Mean absolute bodyweight [g]			
	Day 0 – Study initiation	Week 13	Week 52	Week 79 – Terminal necropsy
<b>Males</b>				
0	9689 ± 1630	12119 ± 2005	13045 ± 2602	13969 ± 2788
2	10306 ± 1003	12633 ± 1057	13508 ± 1585	13922 ± 2468
30	10393 ± 1392	12537 ± 1472	13834 ± 2138	14134 ± 2791
100	10256 ± 1248	12857 ± 1663	13750 ± 1638	14226 ± 1632
200	9729 ± 1551	11932 ± 1918	12853 ± 1745	13114 ± 1907
3000	10038 ± 1577	11936 ± 1697	12777 ± 2365	13536 ± 2879
<b>Females</b>				
0	8302 ± 1662	9733 ± 2258	10417 ± 2506	11048 ± 2940
2	8392 ± 1146	10436 ± 1725	10838 ± 2786	11239 ± 3440
30	8385 ± 1016	9863 ± 1448	10582 ± 2295	11330 ± 2952
100	8109 ± 1270	9585 ± 1525	9613 ± 2676	10778 ± 3207

Concentration [ppm]	Mean absolute bodyweight [g]			
	Day 0 – Study initiation	Week 13	Week 52	Week 79 – Terminal necropsy
200	7999 ± 1045	9744 ± 1529	9764 ± 1535	10167 ± 2201
3000	8358 ± 942	9528 ± 1140	10382 ± 1193	10722 ± 1700

*Ophthalmoscopy:* There were no treatment-related ophthalmological findings.

*Haematology:* In the top (3000 ppm) dose group an increase in white blood cell counts (WBC) and total neutrophils was observed in males (statistically-significant increase from 200 ppm) and females (similar trends without statistical significance) at week 52 (Table 6.3-58). However, the percentage of neutrophils was not changed. Following 6 months recovery, these differences were no longer apparent. Total neutrophils were not increased at 200 ppm in the previous study. Overall, therefore, treatment-related and adverse changes in certain white blood cell parameters were seen in males in the top dose group, however, these changes were reversed following a recovery period.

Table 6.3-58. *Haematology:* at 52 and 78 weeks

Concentration [ppm]	WBC [1000x]		Total neutrophils [1000x]		Neutrophils [%]		Lymphocytes [%]	
	Week 52	Week 78	Week 52	Week 78	Week 52	Week 78	Week 52	Week 78
<b>Males</b>								
0	9.5 ± 0.5	10.5 ± 1.2	6.8 ± 0.6	7.8 ± 0.7	71.3 ± 6.1	74.3 ± 6.3	28.2 ± 5.8	24.5 ± 6.8
2	10.5 ± 1.9	12.8 ± 2.5	7.8 ± 0.9	9.9 ± 0.5	74.2 ± 8.9	77.5 ± 3.9	24.8 ± 9.5	21.7 ± 4.0
30	8.1 ± 0.7	9.2 ± 1.8	6.1 ± 0.3	7.0 ± 0.5	75.8 ± 4.0	76.0 ± 5.0	22.7 ± 3.8	23.3 ± 4.8
100	9.9 ± 1.3	12.3 ± 4.4	7.4 ± 0.8	9.4 ± 0.8	74.8 ± 7.7	76.3 ± 6.7	24.8 ± 7.3	22.8 ± 7.3
200	10.8 ± 1.4	11.9 ± 2.0	<b>8.7* ± 0.9*</b>	9.3 ± 0.9	80.5 ± 8.3	77.8 ± 7.6	18.7 ± 7.8	21.8 ± 7.8
3000	<b>12.4 ± 1.2**</b>	11.2 ± 1.4	<b>9.6** ± 1.0</b>	8.5 ± 0.9	77.0 ± 8.3	75.5 ± 8.4	21.0 ± 7.6	23.8 ± 8.6
<b>Females</b>								
0	9.3 ± 1.1	12.0 ± 2.0	6.4 ± 1.1	8.7 ± 1.6	68.5 ± 12.3	72.3 ± 13.4	30.5 ± 12.6	27.2 ± 12.9
2	9.3 ± 2.6	11.3 ± 2.8	7.0 ± 0.5	7.8 ± 0.9	75.0 ± 5.1	69.2 ± 8.1	24.3 ± 5.0	30.2 ± 7.8
30	9.6 ± 2.5	11.9 ± 3.0	7.1 ± 0.6	8.5 ± 0.8	73.5 ± 5.8	71.7 ± 6.4	25.5 ± 6.2	27.5 ± 7.4
100	15.6 ± 18.9	10.6 ± 4.3	12.4 ± 1.6	8.0 ± 0.7	79.7 ± 10.2	75.6 ± 6.3	21.3 ± 12.4	23.2 ± 5.5
200	8.2 ± 1.7	10.5 ± 3.2	5.7 ± 0.8	6.8 ± 0.8	70.0 ± 9.5	64.7 ± 7.5	29.8 ± 9.2	34.2 ± 6.9
3000	11.1 ± 3.0	10.2 ± 1.5	8.3 ± 1.4	7.0 ± 0.5	74.8 ± 12.7	69.0 ± 4.5	24.3 ± 13.4	30.0 ± 3.8

\* = p ≤ 0.05; \*\* = p ≤ 0.01, Dunnett's test

WBC – white blood cell count

*Clinical chemistry:* Potassium levels were statistically-significantly decreased (-13.5 % change compared to controls) in males from 200 ppm (4.11 mg/kg bw/d) at study termination (Table 6.3-59). In isolation, without effects on other ion levels or other related clinical chemistry parameters, and considering that potassium levels were not affected in the previous study (employing the same dose levels), this finding is not considered treatment-related. Other statistically-significant findings in clinical chemistry (increased aspartate aminotransferase levels (30 ppm, males) at week 13, decrease in phosphorus levels (3000 ppm, males) at week 26, decrease in total bilirubin levels (100 ppm, females) at week 52) represent single incidental events without consistency between males and females and without dose-dependency and were therefore not considered to be treatment-related. Overall, there were no treatment-related changes in clinical chemistry parameters.

Table 6.3-59. Selected clinical chemistry - potassium levels (mEq/L)

Dose level	[ppm]	Males						Females					
		0	2	30	100	200	3000	0	2	30	100	200	3000
	[mg/kg bw/d]	0	0.04	0.63	2.28	4.11	64.26	0	0.04	0.62	2.05	4.11	71.22
Week 52	[mean]	4.5	4.5	4.4	4.4	4.4	4.3	4.9	4.7	4.4	4.8	4.5	4.7
Study	[mean]	5.2	4.7	4.6	4.6	4.5*	4.4*	4.6	4.7	4.5	4.7	4.6	4.7
termination	$\Delta\%$	-	-9.6	-11.5	-11.5	-13.5	-15.4						

$\Delta\%$  = percent change compared to control.

*Urinalysis:* The urinalysis of the one female of the 100 ppm group found dead in week 53 was positive for blood and protein reactions before initiation of the study and throughout the study. This was not considered treatment-related. Urinalysis parameters of all other animals revealed no treatment-related changes. Due to a lack of dose-response, statistically-significant changes in the pH of urine (Table 6.3-60) (in females at 4.11 mg/kg bw/d) were not considered to be treatment-related. Overall, there were no treatment-related effects in urine parameters.

Table 6.3-60. Selected urinalysis - pH

Dose level	[ppm]	Males						Females					
		0	2	30	100	200	3000	0	2	30	100	200	3000
	[mg/kg bw/d]	0	0.04	0.63	2.28	4.11	64.26	0	0.04	0.62	2.05	4.11	71.22
Week 52	[mean]	8.3	7.8	8.2	7.9	8.2	8.2	8.1	8.1	8.2	7.8	7.6	7.8
	[SD]	0.4	0.8	0.3	0.5	0.6	0.4	0.6	0.2	0.4	1.2	0.9	0.7
Study	[mean]	7.8	7.5	8.3	8.2	7.8	8.2	8.5	8.0	8.5	7.4	6.8*	7.7
termination	[SD]	0.8	0.6	0.8	0.8	1.0	0.8	0.6	1.0	0.3	1.8	0.8	1.1

*Organ weight:* Statistically-significant increases in absolute (45 % change compared to control) and relative (48 % change compared to control) thyroid weights were seen in male dogs of the top (3000 ppm) dose group. However, a clear dose-response was not evident and no associated histopathology was observed. Increases in the thyroid weight of females of the top dose were not statistically-significant (19 and 25 % change compared to control for absolute and relative weight, respectively) (Table 6.3-61). However, a dose-response is not clear and there was no concomitant changes in the histopathology of the thyroid. HSE notes that the thyroid was not affected in the previous 1-year dog studies up to the same dose of 3000 ppm. Overall, the increases in thyroid weight reported in this study at the top dose are considered unrelated to treatment. Due to the lack of dose-response, other statistically-significant finding (increased lung and spleen weight of 100 ppm males) were also not considered to be treatment-related. No other organ weights were affected. Overall, there were no treatment-related changes in organ weights.

Table 6.3-61. Selected organ weight - thyroid weights (after 1 year treatment and 6 months recovery period)

Concentration [ppm]	Mean absolute and relative thyroid weight			
	Absolute [g]	$\Delta\%$	Relative [% of bw]	$\Delta\%$
Males				
0	0.853 $\pm$ 0.157		0.0062 $\pm$ 0.0013	
2	1.047 $\pm$ 0.168	22.7	0.0076 $\pm$ 0.0013	22.6
30	0.905 $\pm$ 0.258	6.1	0.0064 $\pm$ 0.0008	3.2
100	1.155 $\pm$ 0.235	35.4	0.0084 $\pm$ 0.0027	35.5
200	0.960 $\pm$ 0.213	12.5	0.0073 $\pm$ 0.0015	17.7
3000	1.235 $\pm$ 0.330*	44.8	0.0092 $\pm$ 0.0021*	48.4
Females				
0	0.730 $\pm$ 0.269		0.0065 $\pm$ 0.0007	
2	0.800 $\pm$ 0.136	9.6	0.0074 $\pm$ 0.0011	13.8



Concentration [ppm]	Mean absolute and relative thyroid weight			
	Absolute [g]	Δ%	Relative [% of bw]	Δ%
30	0.844 ± 0.168	15.6	0.0079 ± 0.0025	21.5
100	0.801 ± 0.242	9.7	0.0076 ± 0.0022	16.9
200	0.732 ± 0.235	0.3	0.0073 ± 0.0020	12.3
3000	0.869 ± 0.225	19.0	0.0081 ± 0.0017	24.6

\* =  $p \leq 0.05$ , Dunnett's test

Δ% = percent change compared to control.

*Gross pathology:* There were no treatment-related gross necropsy findings noted. Prostate gland size was within the normal range.

*Histopathology:* There were no treatment-related changes in tissues examined (Table 6.3-62).

Table 6.3-62. Histopathology – after 12-month treatment and 6-month recovery period

Dose level [ppm]	Males						Females					
	0	2	30	100	200	3000	0	2	30	100	200	3000
No. of animals	6	6	6	6	6	6	6	6	6	6	6	6
<b>TESTES</b>												
Hypospermatogenesis	0	0	0	1	0	1	-	-	-	-	-	-
<b>PITUITARY</b>												
Pituitary cysts	1	1	1	3	1	2	1	1	1	2	0	2
<b>LUNG</b>												
Focal inflammation	0	1	0	0	0	2	3	0	0	1	2	0
<b>STOMACH</b>												
Focal inflammation	0	0	0	0	0	1	0	0	0	0	0	0
<b>OVARY</b>												
Cyst	-	-	-	-	-	-	0	0	1	1	0	2

#### Conclusion

In conclusion, under the conditions of this relatively old, GLP compliant study in dogs, dietary administration of cinmethylin for 52 weeks, with a 6 month recovery period, resulted in effects on some white blood cell parameters (WBC and total neutrophil counts) in males at the top dose, which were reversed following the recovery period

A NOAEL of 200 ppm (equivalent to 4.1 mg/kg bw/d) is proposed by HSE since no adverse effects were seen at this dose. A LOAEL of 3,000 ppm (equivalent to 64.3 mg/kg bw/d in males) is proposed based on effects on haematology (WBC counts) in males.

(██████, 1988b)

#### B.6.3.4. Other routes

A study investigating repeated-dose toxicity via the dermal route for 28-days is available. This is a modern study, conducted in rats, according to GLP and OECD test guidelines. An amendment to the study is included.

***Dermal route***

<b>Author(s)</b>	
<b>Study title</b>	BAS 684 H - Repeated-dose 28-day dermal toxicity study in Wistar rats and Amendment No. 1 to the Report.
<b>Study reference</b>	██████████, 2018c BASF DocID : 2017/1094162 and ██████████, 2018. BASF DocID : 2018/1091459
<b>Reason for amendment</b>	The detailed descriptions of Functional Observation Battery (FOB) examinations, ranking and documentation procedures was omitted from the original study report.
<b>Test facility</b>	BASF SE, Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany
<b>Date</b>	03/05/2016 – 02/06/2016
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Batch no.</b>	COD-002038
<b>Purity (%)</b>	93.5 (-) / (+) ratio = 48:52
<b>Test animals</b>	Rat Wistar, CrI :WI(Han) Male and female
<b>Groups</b>	10/sex/dose
<b>Dose/concentrations</b>	0, 100, 300 and 1000 mg/kg bw/d Volume : 4 mL/kg bw
<b>Route</b>	Administered daily via the dermal route for 4 weeks. Semioclusive dressing.
<b>Vehicle</b>	Drinking water containing 0.5 % carboxymethylcellulose (CMC) with Tween 80.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 410 (1981) (this is the current test guideline) US EPA OPPTS 870.3200
<b>Deviation</b>	None.
<b>Impact of deviations</b>	Not applicable.
<b>Acceptable</b>	Yes
<b>NOAEL</b>	Systemic : 1000 mg/kg bw/d (both sexes). Local : 100 mg/kg bw/d
<b>Effects at the LOAEL</b>	Systemic : N/A – no adverse treatment-related findings were observed at the top dose. Local : erythema at treated site from 300 mg/kg bw/d

**Methods**

In a GLP and OECD test guideline compliant study, cinmethylin was administered via dermal application to groups of 10 male and 10 female Wistar rats per test group, at dose levels of 0, 100, 300 and 1000 mg/kg bw/d. Application was for 6 hr/day, 5 day/week, over 4 weeks (males received 21 applications, females received 22 applications). The stability and homogeneity of cinmethylin in the carrier was determined prior to the start of the study. A method for the analysis of cinmethylin in aqueous cellulose solution (██████████, 2017a; 2017/1166508) was evaluated and was considered validated (see Volume 3 CA B5, section B.5.1.2).

**Results**

*Mortality and general clinical observations:* There were no treatment-related deaths or clinical signs of toxicity.

*Dermal observations:* Slight erythema was observed at the treated skin site, in one male and female from the mid dose group, as well as in two high dose males and females. Additionally, 4 males and 2 females of the top dose revealed crust formation. These findings, which occurred from the mid dose, were considered to be treatment-related local dermal effects.

*Ophthalmoscopy:* There were no treatment-related ophthalmological findings.

*Functional observation battery and motor activity:* There were no treatment-related findings seen across most FOB examinations, with the exception of treatment-related local dermal findings noted in the open field observations. There were no treatment-related findings in any motor activity measurements.

*Body weight, food and water consumption:* There were no treatment-related body weight or body weight gain findings. There were no accompanying treatment-related food or water observations.

*Haematology:* There was a statistically-significantly decreased mean corpuscular haemoglobin (MCH) and relative lymphocyte count in high dose females as well as statistically-significantly increased relative neutrophil counts in mid and high dose females (Table 6.3-63). These parameters were within HCD range; only neutrophil counts showed a relative change > 10 % compared to controls. However, they were not affected in males and were not corroborated by changes in other parameters (total red blood and white blood counts). Therefore they were considered unrelated to treatment. Overall, no treatment-related effects on haematology were seen up to the top dose.

Table 6.3-63. Selected haematology findings

Dose level [mg/kg bw/d]		Males				Females			
		0	100	300	1000	0	100	300	1000
MCH [fmol]									
[mean]		1.1	1.08	1.11	1.12	1.13	1.12	1.10	1.09**
[SD]		0.04	0.03	0.04	0.04	0.03	0.03	0.03	0.03
Δ% of control		-	-3.3	-0.1	+0.3	-	-0.6	-2.1	-3.6
HCD <sup>#</sup>	[mean (range)]	-				1.11 (1.05 – 1.19)			
Neutrophils									
[mean]		1.06	1.23	0.98	1.13	0.55	0.53	0.62	0.73
[giga/L]		0.35	0.27	0.19	0.36	0.12	0.10	0.18	0.20
Δ% of control		-	+16.0	-7.2	+6.7	-	-3.7	+13.4	+34.6
[mean]		17.9	19.7	17.9	19.1	12.1	12.3	14.0*	16.0**
[%]		3.9	5.5	2.7	6.6	1.4	2.9	2.0	3.5
Δ% of control		-	+10.4	+0.3	+7.1	-	+1.7	+16.2	+32.4
HCD <sup>#</sup>	[mean (range)]	-				13.8 (9.4 – 18.3)			
Lymphocytes									
[mean]		4.58	4.89	4.26	4.62	3.76	3.68	3.57	3.64
[giga/L]		1.24	1.13	0.74	1.16	0.82	1.02	0.94	0.65
Δ% of control		-	+6.7	-7.2	+0.8	-	-2.0	-4.9	-3.2
[mean]		76.4	75.7	77.1	76.0	82.8	82.9	81.1	78.7*
[%]		2.9	6.5	3.0	6.9	2.6	3.2	2.4	3.6
Δ% of control		-	-0.9	+0.9	-0.5	-	+0.1	-2.0	-4.9
HCD <sup>#</sup>	[mean (range)]	-				81.7 (74.7 – 87.0)			

Statistical evaluation: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Dunnett test (two-sided)

# Historical control data based on 43-45 GLP studies using Wistar rats subjected to a 4-week exposure (oral and inhalation) that were conducted between 2011 and 2016 in the same testing facility (■■■■■).

MCH – mean corpuscular haemoglobin.

*Clinical chemistry:* Statistically-significant increases in the total protein were observed - in all treated females, albumin - in mid and high dose females and globulin - in low and high dose females (Table 6.3-64). Total protein and albumin values were within HCD range, however, HSE notes that the route of exposure for HCD differs. Globulin values exceeding the HCD range, however, this was true for the concurrent control group. The deviations were of low magnitude (< 10 %) as compared to controls. A dose-response was evident for albumin levels only.

Overall, there were no treatment-related clinical chemistry findings.

Table 6.3-64. Selected clinical chemistry findings

Dose level [mg/kg bw/d]	Males				Females			
	0	100	300	1000	0	100	300	1000
<b>Total protein [g/L]</b>								
[mean]	63.31	63.23	62.90	63.46	63.10	<b>65.86*</b>	<b>65.76*</b>	<b>67.51**</b>
[SD]	1.67	1.87	1.33	2.98	2.04	3.61	2.27	2.12
Δ% of control	-	-0.1	-0.7	+0.2	-	+4.4	+4.2	+7.0

Dose level [mg/kg bw/d]		Males				Females			
		0	100	300	1000	0	100	300	1000
HCD <sup>#</sup>	[mean (range)]	-				61.86 (58.57 – 67.91)			
Albumin [g/L]									
[mean]		35.49	35.68	35.34	35.34	37.24	38.47	38.79*	39.28*
[SD]		0.81	0.76	0.78	1.15	1.32	2.26	1.45	1.76
Δ% of control		-	+0.5	-0.4	-0.4	-	+3.3	+4.2	+5.5
HCD <sup>#</sup>	[mean (range)]	-				39.58 (35.95 – 42.64)			
Globulin [g/L]									
[mean]		27.82	27.54	27.55	28.12	25.86	27.39*	26.97	28.23**
[SD]		1.23	1.22	1.50	2.09	1.41	1.71	1.00	1.05
Δ% of control		-	-1.0	-0.9	+1.1	-	+5.9	+4.3	+9.2
HCD <sup>#</sup>	[mean (range)]	-				21.73 (19.62 – 25.27)			

Statistical evaluation: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Dunnett test (two-sided)

<sup>#</sup> Historical control data based on 38-43 GLP studies using Wistar rats subjected to a 4-week exposure (oral and inhalation) that were conducted between 2011 and 2016 in the same testing facility (■■■■■).

**Organ weight:** Changes were observed in the liver and uterus. In top dose females, absolute and relative liver weights were increased by 8 % and 7 %, respectively; relative liver weight gained statistical-significance and slightly exceeded the respective HCD range (Table 6.3-65). A dose-response was evident for both absolute and relative liver weight. However, the magnitude of change was < 15 % compared to control; in addition accompanying histopathological observations were lacking and no associated clinical-chemistry was noted. Therefore, the increased liver weight in top dose females is considered to be treatment-related but adaptive rather than adverse.

The increased (not statistically-significant) absolute and relative uterus weights in females of the mid and top dose groups were within the HCD range (studies were conducted via the same route) and therefore not regarded as treatment-related. However, a clear dose-response was evident and the relative change compared to controls was markedly greater than 10 %. Concomitant histopathological findings were not present. The values reflect the large variance of uterus weights within the oestrous cycle. Overall, there were no treatment-related and adverse organ weight findings.

Table 6.3-65. Selected organ weight findings

Sex		Males				Females			
Organ weight	Dose [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% <sup>§</sup>	Absolute weight	Δ%	Relative weight [% of bw]	Δ% <sup>§</sup>
Terminal weight [g]	0	315.27				199.02			
	100	309.53	-1.8			200.08	+0.5		
	300	312.89	-0.8			200.12	+0.6		
	1000	321.51	+2.0			201.09	+1.0		
Liver [g]	0	7.991		2.536		5.100		2.563	
	100	8.055	+0.8	2.602	+2.6	5.171	+1.4	2.586	+0.9
	300	7.836	-1.9	2.504	-1.3	5.362	+5.1	2.672	+4.3
	1000	8.505	+6.4	2.644	+4.3	5.530	+8.4	2.748**	+7.2
	HCD <sup>#</sup>					Mean: 5.000 g Min: 4.600 g Max: 5.626 g		Mean: 2.574% Min: 2.469% Max: 2.738%	
Uterus [g]	0					0.499		0.251	
	100					0.497	-0.4	0.250	-0.4
	300					0.625	+25.3	0.311	+23.9
	1000					0.729	+46.1	0.363	+44.6
	HCD <sup>#</sup>					Mean: 0.588 g Min: 0.482 g Max: 0.817 g		Mean: 0.305 % Min: 0.245% Max: 0.435%	

Sex		Males				Females			
Organ weight	Dose [mg/kg bw/d]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% <sup>§</sup>	Absolute weight	Δ%	Relative weight [% of bw]	Δ% <sup>§</sup>

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$  (Kruskal-Wallis and Wilcoxon-test, two sided)

<sup>§</sup> Values may not calculate exactly due to rounding of figures

<sup>#</sup> Historical control data based on 11/12 GLP studies using Wistar rats subjected to a 4-week exposure (dermal) that were conducted between 2010 and 2016 in the same testing facility (■■■■■).

*Gross pathology:* There were no treatment-related gross pathology findings.

*Histopathology:* There were no treatment-related histopathology findings. The occurrence of skin erosion/ulcers, observed in the mid and top doses, was similar in treated and untreated skin (Table 6.5-66). Therefore they were not considered treatment-related.

Table 6.3-66. Selected histopathological findings

Dose level [mg/kg bw/d]		Males				Females			
		0	100	300	1000	0	100	300	1000
<b>Skin, untreated</b> - Erosion / ulcer	No. examined	10	0	1	10	10	0	0	10
	N (grade)	0	-	0	1(3)	0	-	-	3(3)
	[mean] <sup>#</sup>	[0.0]	-	[0.0]	[3.0]	[0.0]	-	-	[3.0]
<b>Skin, treated</b> - Erosion / ulcer	No. examined	10	0	0	10	10	0	1	10
	N (grade)	0	-	-	1(3)	0	-	1(2)	1(3)
	[mean] <sup>#</sup>	[0.0]	-	-	[3.0]	[0.0]	-	[2.0]	[3.0]

<sup>#</sup> = mean severity grading;

Histopathological findings were graded minimal (Grade 1). slight (Grade 2). moderate (Grade 3). marked (Grade 4) and massive/severe (Grade 5).

The mean severity is the sum of the gradings divided by the incidence of the respective finding,

N – total number of animals with findings

### Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant study in rats, dermal administration of cinmethylin for 28 days resulted in only localised dermal effect, erythema from the mid dose (300 mg/kg bw/d) and crust formation at the top dose (1000 mg/kg bw/d). A NOAEL of 100 mg/kg bw/d for local dermal toxicity is proposed by HSE.

A NOAEL of 1000 mg/kg bw/d for systemic toxicity in both sexes is proposed by HSE since no adverse effects were seen at this dose.

(■■■■■, 2018c)

### **B.6.3.5. Summay of short-term toxicity**

The short-term toxicity of cinmethylin has been investigated in rats, mice and dogs via the oral (dietary) route of exposure in 28- and 90-day studies; two 1-year studies and one 18-month study in dogs are also available. Modern studies conducted according to GLP and OECD test guidelines are available in both rats and mice, at 28- and 90-days. In addition older studies, not all of which were conducted according to GLP and OECD test guidelines are available in rats and mice (90-day), as well as dogs (5-week, 90-day, 1-year and 18-months); nevertheless, HSE considers that these studies were well-conducted and are sufficiently reliable to contribute to the overall picture of the repeated-dose toxicity of cinmethylin. The short-term toxicity via the dermal route of exposure has been investigated in a 28-day study in rats. The main findings are summarised (Table 6.3-67) below. Further information on the repeated dose toxicity of cinmethylin is also available from the rat 2-generation study (see section B.6.6.1.) and in the chronic studies in rats and mice (see section B.6.5.).

The following key conclusions were obtained from the evaluation of the short-term toxicity information:

- In studies in rats, mice and dogs up to 12 months' duration, the target organs were the liver, thyroid,

- nasal cavities
- Classification for repeated dose toxicity is not required. Further details are available in the aligned MCL dossier
- The data requirements of Regulation 283/2013 have been met.

Table 6.3-67. Summary of short-term toxicity studies with cinmethylin

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
28-day oral (dietary)  GLP compliant Guideline compliant  Cinmethylin Batch: COD- 001794 Purity (%): 97.5 (-) / (+) ratio = 70:30  <b>[REDACTED]</b> , 2015 <a href="#">(2015/1076329)</a>  <i>Acceptable</i>	Rat (Wistar)  5/sex/dose	0, 1500, 5000 and 15000 ppm  Equivalent to: M: 0, 137, 477 and 1522 mg/kg bw/d  F: 0, 141, 477 and 1331 mg/kg bw/d	M: 137 F: 141 [1,500]	<u>5,000 ppm (477 mg/kg bw/d)</u> ↑water consumption (21 % in ♂). Changes in clinical chemistry parameters (GGT, protein, globulin, albumin, cholesterol, triglycerides, glucose, calcium) (♂+♀). ↑liver weight, absolute (23 % in ♂, 11 % in ♀) and relative (22 % in ♂, 18 % in ♀). ↑kidney weight, relative (15 % in ♂). Histopathology of the liver – enlarged with hepatocellular hypertrophy (♂+♀). Histopathology of the thyroid - follicular hypertrophy/hyperplasia (♂+♀). Histopathology of the kidney - eosinophilic droplets (♂) (not relevant to humans).
28-day oral (dietary)  GLP compliant Guideline compliant  Cinmethylin Batch: COD- 001919 Purity (%): 96.2 (-) / (+) ratio = 51:49  <b>[REDACTED]</b> , 2016 <a href="#">(2014/1162710)</a>  <i>Acceptable</i>	Mice (C57BL/6J Rj)  5/sex/dose	0, 400, 1200 and 4000 ppm  Equivalent to : M: 0, 95.1, 295.9 and 791.4 mg/kg bw/d  F: 0, 92.4, 254, 1015.6 mg/kg bw/d	M: 296 F: 254 [1,200]	<u>4,000 ppm (791/1016 mg/kg bw/d)</u> ↓body weight gain (♂). Changes in clinical chemistry parameters (BIL, PROT, ALB, GLOB, CHOL, TRIG) (♂). ↑liver weight, absolute (16 % in ♂, 26 % in ♀) and relative (19 % in ♂, 22 % in ♀).

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
5-week oral (dietary)  Non-GLP Non-guideline  Cinmethylin Batch: 513J Purity (%): 92.4 (-) / (+) ratio = not specified. [REDACTED], 1984 <a href="#">(CI-420-004)</a>  <i>Acceptable in a WoE approach</i>	Beagle dogs  2/sex/dose	0, 300, 3000, 10000 and 30000 ppm  Equivalent to: M: 0, 8.8, 131.1, 338.7 and 330.0 mg/kg bw/d  F: 0, 10.5, 103.6, 334.2 and 433.6 mg/kg bw/d	M: 131 F: 104 [3,000]	<u>≥ 10,000 ppm (330/334 mg/kg bw/d):</u> ↑liver weight, absolute (38 % in ♂, 20 % in ♀) and relative (35 % in ♂, 27 % in ♀). Histopathology of the liver – hepatopathology.
90-day oral (dietary)  GLP compliant Guideline compliant  Cinmethylin Batch: COD- 001919 Purity (%): 96.2 (-) / (+) ratio = 51:49  [REDACTED], 2018a <a href="#">(2014/1228370)</a>  <i>Acceptable</i>	Rat (Wistar)  10/sex/dose	0, 1000, 3000 and 10000 ppm  Equivalent to: M: 0, 67, 211 and 792 mg/kg bw/d  F: 0, 79, 240 and 814 mg/kg bw/d	M: 67 F: 79 [1,000]	<u>3,000 ppm (211/240 mg/kg bw/d):</u> Changes in haematology parameters (↓prothrombin time) (♀). Changes in clinical chemistry parameters (♂+♀). ↑liver weight, absolute (12 % in ♂, 12 % in ♀) and relative (18 % in ♂, 11 % in ♀). Histopathology of the liver – hypertrophy (♂+♀). Histopathology of the thyroid - follicular hypertrophy/hyperplasia (♂+♀). Histopathology of the nasal cavity – proteinaceous exudation and degeneration of the olfactory epithelium (♂+♀).
90-day oral (dietary)  Non-GLP Non-guideline  Cinmethylin Batch: 513D Purity (%): not specified. (-) / (+) ratio = not specified.	Rat (Fischer 344)  30/sex/dose (total) 10/sex/dose (sacrificed at week 7) 20/sex/dose (sacrificed at week 13)	0, 30, 100, 300 and 1000 ppm  Equivalent to: M: 0, 2.18, 7.51, 22.51 and 75.78 mg/kg bw/d  F: 0, 2.61, 8.73, 26.08 and 88.56 mg/kg bw/d	M: 76 F: 89 [1,000]	Not applicable, no adverse effects were seen at the top dose.

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
<p>██████████, 1983 (CI-425-001)</p> <p><i>Supplementary</i></p>				
<p>90-day oral (dietary)</p> <p>GLP compliant Guideline compliant</p> <p>Cinmethylin Batch: COD- 001919 Purity (%): 96.2 (-) / (+) ratio = 51:49</p> <p>██████████, 2018b (2015/1005983)</p> <p><i>Acceptable</i></p>	<p>Mice (C57BL/6J Rj)</p> <p>10/sex/dose</p>	<p>0, 200, 1000 and 5000 ppm</p> <p>Equivalent to : M: 0, 43, 201 and 1200 mg/kg bw/d</p> <p>F: 0, 58, 285 and 1304 mg/kg bw/d</p>	<p>M: 43 F: 58 [200]</p>	<p><u>1,000 ppm (201/285 mg/kg bw/d):</u> ↓body weight gain (17 % in ♀). Changes in clinical chemistry parameters (TRIG, TPROT, ALB, CHOL) (♂). ↑liver weight, absolute (9 % in ♂) and relative (10 % in ♂).</p>
<p>90-day oral (dietary)</p> <p>Non-GLP Non-guideline</p> <p>Cinmethylin Batch: 513D Purity (%): not specified. (-) / (+) ratio = not specified.</p> <p>██████████, 1983 (CI-425-002)</p> <p><i>Supplementary</i></p>	<p>Mice (B6C3F1)</p> <p>30/sex/dose (total) 10/sex/dose (sacrificed at week 7) 20/sex/dose (sacrificed at week 13)</p>	<p>0, 30, 100, 300 and 1000 ppm</p> <p>Equivalent to : M: 0, 3.81, 11.50, 39.57 and 123.11 mg/kg bw/d</p> <p>F: 0, 4.36, 13.85, 42.57 and 129.66 mg/kg bw/d</p>	<p>M: 123 F: 130 [1,000]</p>	<p>Not applicable, no adverse effects were seen at the top dose.</p>
<p>90-day oral (dietary)</p> <p>GLP compliant Non-guideline (OECD)</p> <p>Cinmethylin Batch: #925 Purity (%): not specified. (-) / (+) ratio = not specified.</p> <p>██████████, 1987 (CI-425-003)</p>	<p>Beagle dogs</p> <p>6/sex/dose</p>	<p>0, 2, 100, 200, 3000 and 6000 ppm</p> <p>Equivalent to : M: 0, 0.06, 2.9, 5.6, 96.5 and 180.5 mg/kg bw/d</p> <p>F: 0, 0.06, 3.0, 5.8, 91.9 and 192.3 mg/kg bw/d</p>	<p>M: 5.6 F: 5.8 [200]</p>	<p><u>3,000 ppm (96.5/91.9 mg/kg bw/d):</u> ↑liver weight, absolute (21 % in ♂, 16 % in ♀) and relative (16 % in ♂, 22 % in ♀). Histopathology of the prostate - delay in glandular development.</p>



Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
<i>Acceptable in a WoE approach</i>				
1-year oral (dietary)  GLP compliant Non-guideline (OECD)  Cinmethylin Batch: 513L Purity (%): 91 (-) / (+) ratio = not specified. and Batch: 513N Purity (%): 93 (-) / (+) ratio = not specified.  [REDACTED] 1985 (CI-427-002)  <i>Acceptable in a WoE approach</i>	Beagle dogs  6/sex/dose	0, 300, 3000 and 10000 ppm  Equivalent to : M: 0, 7.9, 83.4, and 253.9 mg/kg bw/d  F: 0, 7.9, 81.4 and 284.8 mg/kg bw/d	M: 7.9 F: 7.9 [300]	<u>3,000 ppm (83.4/81.4 mg/kg bw/d):</u> ↓body weight (4 % in ♂, 12 % in ♀). ↓body weight gain (16 % in ♂, 33 % in ♀). Changes in white blood cell parameters (♀). Alterations in clinical chemistry parameters (ALP+ALB) (♂+♀). ↑liver weight, absolute (24 % in ♂, 11 % in ♀) and relative (27 % in ♂, 26 % in ♀). Histopathology of the prostate - delay in glandular development.
1-year oral (dietary)  GLP compliant Non-guideline (OECD)  Cinmethylin Batch: 925 (6-4-0- 0) Purity (%): 92.4 (-) / (+) ratio = not specified.  [REDACTED] 1988a (CI-427-003)  <i>Acceptable in a WoE approach</i>	Beagle dogs  6/sex/dose	0, 2, 30, 100, 200 and 3000 ppm  Equivalent to : M: 0, 0.044, 0.68, 2.3, 4.7 and 80.8 mg/kg bw/d  F: 0, 0.048, 0.74, 2.4, 4.3 and 70.7 mg/kg bw/d	M: 4.7 F: 4.3 [200]	<u>3,000 ppm (80.8/70.7 mg/kg bw/d):</u> White blood cell parameters (♂+♀). ↑ALP (♀). ↑liver weight, absolute (23 % in ♂) and relative (26 % in ♂). ↑incidence small prostates.
1-year oral with 6 months recovery (dietary)  GLP compliant Non-guideline (OECD)  Cinmethylin Batch: #925 Purity (%): not specified.	Beagle dogs  6/sex/dose	0, 2, 30, 100, 200 and 3000 ppm  Equivalent to : M: 0, 0.04, 0.63, 2.3, 4.1 and 64.3 mg/kg bw/d  F: 0, 0.04, 0.62, 2.1, 4.1 and 71.2 mg/kg bw/d	M: 4.1 [200]  F: 71.2 [3000]	<u>3,000 ppm (64.3 mg/kg bw/d):</u> ↑ White blood cell parameters at week 52, recovering after 6 months (♂).

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
(-) / (+) ratio = not specified.  [REDACTED] 1988b (CI-427-004)  <i>Acceptable in a WoE approach</i>				
28-day dermal  GLP compliant Guideline compliant  Cinmethylin Batch: COD-002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  [REDACTED] 2018c (2017/1094162 and 2018/1091459)  <i>Acceptable</i>	Rat Wistar, Crl :WI(Han)  10/sex/dose	M & F: 0, 100, 300 and 1000 mg/kg bw/d	Local dermal toxicity: 100 (M & F).  Systemic toxicity: 1000 (M & F).	Local dermal toxicity: <u>300 mg/kg bw/d</u> : Slight erythema at treated skin site.  Systemic toxicity: Not applicable, no adverse effects were seen at the top dose.

OralRat

In rats the main target organs of toxicity were the liver, thyroid, kidney and nasal cavity.

Adverse increases ( $\geq 15\%$ ) in liver weight were seen from 477 mg/kg bw/d in the 28-day study and from 211 mg/kg bw/d in the new/modern 90-day study. In addition, similar effects on the liver were seen from 115 mg/kg bw/d in the 2-generational study and at 144 mg/kg bw/d in the chronic toxicity study. These effects were associated with histopathological findings (hepatocellular hypertrophy) and alterations in some clinical chemistry parameters indicative of liver toxicity (e.g. GGT, protein, globulin, albumin, cholesterol, triglycerides and glucose) from 477 mg/kg bw/d in the 28-day study and from 211 mg/kg bw/d in the new/modern 90-day study.

Thyroid weight was increased at 792 mg/kg bw/d in the new/modern 90-day study. In addition, adverse thyroid histopathology (follicular hypertrophy/hyperplasia) was seen from 477 mg/kg bw/d in the 28-day study and from 211 mg/kg bw/d in the new/modern 90-day study. Similar effects on the thyroid were seen at 394 mg/kg bw/d in the 2-generational study and at 265 mg/kg bw/d in the chronic toxicity study.

Adverse nasal cavity histopathology (proteinaceous exudation and olfactory epithelium degeneration) was seen from 211 mg/kg bw/d in the new/modern 90-day study. Similar findings were not noted in the 28-day study but were recorded at 394 mg/kg bw/d in the 2-generational study and from 45 mg/kg bw/d in the chronic toxicity study.

Adverse effects on the kidney (increased weight with associated histopathology (eosinophilic droplets)) were seen in males only, from 477 mg/kg bw/d the 28-day study and from 211 mg/kg bw/d the 90-day study. These droplets were shown (in the 90-day study) to be due to accumulation of  $\alpha_2$ -globulin, a male rat specific phenomenon of no relevance to humans.

In addition to toxic effects in these organs, decreases in body weight and/or body weight gain were observed at 1522 mg/kg bw/d in the 28-day study and from 792 mg/kg bw/d in the new/modern 90-day study. Changes in haematology parameters (e.g. prothrombin time and monocyte counts) were observed at 1331 mg/kg bw/d in the 28-day study and from 240 mg/kg bw/d in the new/modern 90-day study. Increases in water consumption were observed from 477 mg/kg bw/d in the 28-day study.

Overall, taking into account the full range of observation, **the lowest relevant subchronic NOAEL in the rat was 67 mg/kg bw/d.** The LOAEL was 211 mg/kg bw/d based on changes in clinical chemistry parameters, increases in liver weight, adverse liver, thyroid and nasal cavity histopathology in the new/modern 90-day study.

#### *Mouse*

In mice the main target organ of toxicity was the liver; however, on chronic exposure, effects on the nasal cavity were also seen.

Adverse increases ( $\geq 15\%$ ) in liver weight were seen at 791 mg/kg bw/d in the 28-day study and from 201 mg/kg bw/d in the new/modern 90-day study. These effects were associated with alterations in some clinical chemistry parameters indicative of liver toxicity (e.g. protein, globulin, albumin, cholesterol and triglycerides) at 791 mg/kg bw/d in the 28-day study and from 201 mg/kg bw/d in the new/modern 90-day study.

Adverse histopathological findings were seen in the nasal cavity after 18-month exposure (in the long-term toxicity study) from the mid dose in males (162 mg/kg bw/d) and at the top dose in females (939 mg/kg bw/d). Nasal cavity findings included an increase in metaplasia and degeneration/regeneration of olfactory epithelium in males and females.

In addition to toxic effects in the liver and the nasal cavity, decreases in body weight were observed at 1200 mg/kg bw/d in the new/modern 90-day study. Decreases in body weight gain were observed at 791 mg/kg bw/d in the 28-day study and from 285 mg/kg bw/d in the new/modern 90-day study. Decreases in food consumption were observed at 1304 mg/kg bw/d in the new/modern 90-day study. Changes in some white blood cell parameters were also observed at 1200 mg/kg bw/d in the new/modern 90-day study.

Overall, taking into account the full range of observation, **the lowest relevant subchronic NOAEL in the mouse was 48 mg/kg bw/d.** The LOAEL was 201 mg/kg bw/d based on decreases in body weight gain, changes in clinical chemistry parameters and increases in liver weight in the new/modern 90-day study.

#### *Dog*

In dogs the main target organs of toxicity were the liver, haematological system, kidney, prostate and testes.

Adverse increases ( $\geq 15\%$ ) in liver weight were seen from 334 mg/kg bw/d in the 5-week study, from 92 mg/kg bw/d in the 90-day study, from 81 mg/kg bw/d in the first 1-year study (■■■■■, 1985) and at the top dose of 81 mg/kg bw/d in the second 1-year study (■■■■■, 1988a). These effects were associated with histopathological findings (hepatocellular hypertrophy) from 334 mg/kg bw/d in the 5-week study and alterations in some clinical chemistry parameters indicative of liver toxicity (e.g. ALP and ALB) at 180 mg/kg bw/d in the 90-day study, from 81 mg/kg bw/d in the first 1-year study and at the top dose of 71 mg/kg bw/d in the second 1-year study.

Haematological effects were seen from 81 mg/kg bw/d in the first 1-year study, from 71 mg/kg bw/d in the second 1-year study and from 64.3 mg/kg bw/d in the third 1-year study (however, these changes were reversed following a 6-month recovery period).

Adverse kidney histopathology (tubular nephropathy) was seen from 330 mg/kg bw/d in the 5-week study.

Prostate histopathology revealed a delay in glandular development from 96.5 mg/kg bw/d in the 90-day study and from 83.4 mg/kg bw/d in the first 1-year study. In addition, an increase in the incidence of small prostates was seen at 81 mg/kg bw/d in the second 1-year study. Adverse testes histopathology (atrophy) was seen at 254 mg/kg bw/d in the first 1-year study. The effects on prostate and testes were considered the secondary consequence of the delayed body weight development caused by the treatment.

In addition, decreases in body weight were observed at 330 mg/kg bw/d in the 5-week study and from 81 mg/kg bw/d in the first 1-year study. Decreases in body weight gain were observed from 81 mg/kg bw/d in the first 1-year study; these were observed were associated with a delayed glandular development of the prostate and

testes. Decreased in food consumption were observed at 330 mg/kg bw/d in the 5-week study and at 254 mg/kg bw/d in the first 1-year study. Clinical signs of toxicity were observed at 330 mg/kg bw/d in the 5-week study (emaciation and dehydration), at 180 mg/kg bw/d in the 90-day study (stool abnormalities) and at 254 mg/kg bw/d in the first 1-year study.

Overall, taking into account the full range of observations, the lowest subchronic NOAEL in the dog was 4.3 mg/kg bw/d from the second 1-year study in the dog; the LOAEL in this study was 71 mg/kg bw/d. However, in the first 1-year study in the dog, the highest NOAEL was 7.9 mg/kg bw/d, with a LOAEL of 81 mg/kg bw/d. Since the first study provides the highest NOAEL which lies below the lowest LOAEL in this relevant species and study type, **the most reliable subchronic NOAEL in the dog was 7.9 mg/kg bw/d.**

#### Dermal

In a 28-day dermal study in rats no systemic effects were recorded at the top dose of 1000 mg/kg bw/d. However, localised dermal effects - slight erythema were observed from 300 mg/kg bw/d. **The NOAEL was 1000 mg/kg bw/d for systemic toxicity and 100 mg/kg bw/d for local dermal toxicity.**

#### Conclusion

Several effects were observed consistently between species; the main target organ of toxicity was the liver, with increases in liver weights, effects on some clinical chemistry parameters (indicative of liver damage) and/or changes to liver histopathology consistently seen in all three species. Adverse effects on the thyroid, including weight increases and changes to its histopathology (e.g. follicular hypertrophy/hyperplasia), were also seen in rat studies but not in mice and dogs. Histopathology of the nasal cavity was noted in rats and mice and tubular nephropathy was observed in the dog after exposure for 5 weeks. Adverse effects on the prostate, including increases in the incidence of small prostates and changes to its histopathology, and testes including changes to its histopathology (e.g. atrophy), were also seen in dog studies but not in rats and mice. However, these were considered the secondary consequence of the delayed body weight development caused by the treatment. Adverse effects on body weight development and on some haematological parameters were observed in the rat, mouse and dog. Adverse effects on the kidney were observed in the dog. Localised dermal effects (slight erythema) were observed in the rat.

The dog was the most sensitive species, with adverse effects being observed at lower dose levels than in other species (e.g. observed LOAELs of 71 mg/kg bw/d in the dog compared to 211 mg/kg bw/d and 201 mg/kg bw/d in the rat and mouse, respectively).

**The lowest relevant NOAEL from all the available short-term toxicity studies therefore was 7.9 mg/kg bw/d** from the first 1-year study in the dog (■■■■■, 1985). The LOAEL in this study was 81 mg/kg bw/d, based on decreases in body weight and body weight gain, changes in some haematology and clinical chemistry parameters, increases in liver weight and histopathology of the prostate.

### B.6.4. GENOTOXICITY

The genotoxicity of cinmethylin was tested in a range of *in vitro* and *in vivo* studies. *In vitro* studies consisted of: two bacterial reverse mutation assays (Ames test), an *in vitro* mammalian cell gene mutation assay in mouse lymphoma cells and an *in vitro* micronucleus test in human lymphocytes. *In vivo* studies include: a modern *in vivo* micronucleus test in mouse bone marrow (for the detection of structural and numerical chromosome aberrations) and an old *in vivo* chromosome aberration assay in rat bone marrow. All but one study (the *in vivo* chromosomal aberration assay in the rat) were modern studies conducted according to the current OECD test guidelines and were GLP compliant.

#### B.6.4.1. *In vitro* studies

##### *Ames tests*

Two new/modern Ames tests are available, the first was conducted with a low purity batch of cinmethylin, with increased impurity levels (artificially produced; purity: 89.6 %); the second was conducted with a very pure batch of cinmethylin (97.5 %) but which contains a new impurity (see confidential section Volume 4 for more information).

##### 1) *New/modern study*

<b>Author(s)</b>	Woitkowiak C.
<b>Study title</b>	BAS 684 H + impurities (artificial batch) - <i>Salmonella typhimurium</i> / <i>Escherichia coli</i> reverse mutation assay
<b>Study reference</b>	Woitkowiak, 2018a BASF Doc ID: 2018/1029052 Study ID: 858509
<b>Laboratory</b>	BASF SE, experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany
<b>Dates of work</b>	20/02/18 – 02/03/18
<b>Test substance</b>	Cinmethylin (BAS 684 H) + impurities
<b>Purity (%)</b> <b>Batch no.</b>	89.6 COD-002345 - artificial batch with increased impurity levels (-) / (+) ratio = not specified.
<b>Test organisms</b>	<i>S. typhimurium</i> strains TA 98, TA 100, TA 1535 and TA 1537, and a strain of <i>E. coli</i> WP2 uvrA
<b>Test concentrations</b>	0, 33, 100, 333, 1000, 2800, 5600 µg/plate SPT and PIT (± S9)
<b>Vehicle</b>	Acetone <sup>3</sup> .
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 471 (1997 – the current test guideline), Commission Regulation (EC) No 440/2008 - Part B No. B.13/B.14, EPA 870.5100.
<b>Deviation</b>	None.
<b>Impact of deviations</b>	N/A.
<b>Acceptable</b>	Yes.
<b>Conclusion</b>	Cinmethylin was not mutagenic.

##### Methods

The potential for cinmethylin to induce gene mutations in bacteria was investigated in two experiments, one each using the standard plate test (SPT) and the pre-incubation test (PIT), with *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537, and a strain of *E. coli* WP2 uvrA. The assay was performed, both in the presence and absence of metabolic activation (S9 mix - phenobarbital/β-naphthoflavone-induced rat liver S9 fraction) for 48 - 72 hours. Vehicle (acetone) and positive controls (Table 6.4-1) were included in each experiment. Each concentration, including the controls, was tested in triplicate. The test item was tested at concentrations of 0, 33,

<sup>3</sup> Studies to determine the solubility of cinmethylin in DMSO and acetone have been submitted and are evaluated in detail in Volume 3 – B.2, section B.2.6. Cinmethylin was found to be readily soluble in acetone, as confirmed by a GLP study.

100, 333, 1000, 2800 and 5600 µg/plate. A validated method of analysis for *in vitro* genotoxicity studies is not required.

Table 6.4-1. Positive control compounds tested

Strain	Mutagen	Solvent	Conc. [µg/plate]
<b>Without addition of metabolic activation system</b>			
TA 100	N-methyl-N'-nitrosoguanidine (MNNG)	DMSO	5
TA 1535	N-methyl-N'-nitrosoguanidine (MNNG)	DMSO	5
TA 1537	9-Aminoacridine (AAC)	DMSO	100
TA 98	4-nitro-o-phenylenediamine (NOPD)	DMSO	10
WP2 uvrA	4-Nitroquinoline-N-oxide (4-NQO)	DMSO	5
<b>With addition of metabolic activation system</b>			
TA 100	2-Aminoanthracene	DMSO	2.5
TA 1535	2-Aminoanthracene	DMSO	2.5
TA 1537	2-Aminoanthracene	DMSO	2.5
TA 98	2-Aminoanthracene	DMSO	2.5
WP2 uvrA	2-Aminoanthracene	DMSO	60

### Results

#### Mutagenicity:

No treatment related increase in the number of revertants was observed in the SPT and PIT either with or without metabolism by S9 mix (Table 6.4-2). Consequently, there was no evidence of a mutagenic effect of the test item in this study, under any conditions.

#### Solubility:

Precipitation of the test substance was observed at a concentration of 5600 µg/plate, with and without S9 mix, however, this had no influence on scoring of the plates. Therefore it can be concluded that the test was performed up to the limit of solubility.

#### Toxicity:

A bacteriotoxic effect (defined as reduced his<sup>+</sup> and trp<sup>+</sup> background growth and/or a decrease in the number of his<sup>+</sup> revertants) was observed depending on the strain and test conditions from about 1000 µg/plate and upward in the SPT assay and from 333 µg/plate and upward in the PIT assay. Based on this observation, it can be concluded that the test was performed up to the limit of cytotoxicity.

#### Validity:

The positive controls induced an appropriate response in the corresponding strains - a clear, biologically-relevant increase in the number of revertants, within the range of the historical positive control data (Table 6.4-3), thus demonstrating the sensitivity of the test system. The vehicle control induced number of revertants was within the range of the historical negative control data for each strain (Table 6.4-3). On this basis the performance of the test was shown to be acceptable.

Table 6.4-2. Standard plate Ames tests with cinmethylin (BAS 684 H) - Mean number of revertants.

<b>Experiment 1: Standard plate test (SPT)</b>										
Strain	TA 100		TA 1535		TA 1537		TA 98		WP2 uvrA	
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Vehicle control										
Acetone	106.0	113.0	12.3	10.7	9.3	7.3	13.7	29.3	24.0	24.0
SD	12.0	4.0	5.7	2.5	5.0	0.6	1.5	9.8	13.7	3.0
Test item [µg/plate]										
33	118.7	113.3	10.0	11.3	10.3	11.0	19.0	22.7	26.3	31.3
SD	8.1	4.9	5.0	0.6	2.3	2.6	1.7	5.9	3.5	2.1
100	98.0	116.0	8.3	10.0	9.7	6.3	19.0	20.3	28.3	30.0
SD	2.6	14.7	2.5	3.0	1.5	4.9	3.0	3.8	0.6	7.2

Experiment 1: Standard plate test (SPT)										
Strain	TA 100		TA 1535		TA 1537		TA 98		WP2 uvrA	
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
333	102.0	102.7	6.7	10.3	10.7	6.7	20.7	22.0	27.7	32.0
SD	13.5	4.5	1.5	5.0	3.2	2.1	0.6	1.7	5.0	5.6
1000	81.0	91.0	10.0	9.3	6.0	7.3	13.3	19.0	21.3	27.3
SD	11.3	12.3	6.6	2.1	1.0	2.1	1.2	3.6	3.2	10.3
2800	81.7	77.3	8.3	11.3	6.3	5.7	10.0	14.3	30.0	20.0
SD	3.8	10.6	2.5	3.2	3.2	2.5	2.0	1.5	5.2	2.6
5600	86.3 <sup>P</sup>	81.0 <sup>P</sup>	9.3 <sup>P</sup>	11.0 <sup>P</sup>	3.3 <sup>P</sup>	7.7 <sup>P</sup>	12.7 <sup>P</sup>	23.0 <sup>P</sup>	21.0 <sup>P</sup>	19.7 <sup>P</sup>
SD	11.0	4.4	2.9	3.0	0.6	2.5	3.1	2.0	3.5	6.1
Positive Control										
§	3013.3	1671.3	4190.0	216.3	650.7	102.7	967.7	1346.3	689.3	105.3
SD	291.0	41.2	81.0	24.5	98.8	15.1	21.1	56.1	31.2	5.1
Experiment 2: Pre-incubation test (PIT)										
Strain	TA 100		TA 1535		TA 1537		TA 98		WP2 uvrA	
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Vehicle control										
Acetone	106.0	96.7	11.3	12.0	9.0	8.0	21.0	23.0	21.7	26.3
SD	6.2	3.2	4.2	2.6	2.6	3.0	6.6	4.4	1.2	4.5
Test item [µg/plate]										
33	106.7	98.0	11.0	10.0	9.7	7.3	16.7	25.3	21.7	30.0
SD	3.8	12.5	1.7	3.6	0.6	3.2	9.0	5.5	4.2	1.0
100	91.0	98.3	9.0	12.0	6.3	6.3	15.3	21.7	18.7	30.7
SD	13.5	16.2	1.0	3.5	1.5	3.2	1.5	2.9	1.2	3.2
333	82.7	96.0	11.0	10.7	4.3 <sup>B</sup>	8.3	14.3 <sup>B</sup>	19.7	22.0	28.3
SD	8.1	11.3	1.7	5.5	0.6	5.0	0.6	3.1	2.0	2.1
1000	69.7 <sup>B</sup>	85.3	7.7 <sup>B</sup>	7.3	1.3 <sup>B</sup>	10.3	13.7 <sup>B</sup>	23.0	19.7 <sup>B</sup>	29.3
SD	32.8	2.1	3.1	0.6	0.6	1.5	4.0	6.2	7.0	3.1
2800	72.0 <sup>B</sup>	78.7	6.7 <sup>B</sup>	11.3	2.7 <sup>B</sup>	8.7	11.0 <sup>B</sup>	18.0	22.7 <sup>B</sup>	27.3
SD	23.4	2.9	3.1	1.2	0.6	4.2	2.0	1.7	5.7	1.5
5600	0.0 <sup>PB</sup>	58.7 <sup>P</sup>	3.7 <sup>PB</sup>	7.3 <sup>P</sup>	0.0 <sup>PB</sup>	3.0 <sup>P</sup>	0.0 <sup>PB</sup>	15.0 <sup>P</sup>	21.0 <sup>P</sup>	21.0 <sup>P</sup>
SD	0.0	13.4	1.5	3.5	0.0	1.0	0.0	1.7	3.6	4.4
Positive Control										
§	2880.7	1692.3	4089.7	191.7	471.0	97.3	1054.3	910.0	176.3	105.7
SD	94.3	210.1	217.1	18.5	1.0	4.7	42.4	107.4	14.0	28.4
§ = Compound and concentrations see Material and Methods (I.A.2.) above										
<sup>B</sup> = reduced background growth										
<sup>P</sup> = precipitation										

Table 6.4-3. Historical control data from the study report

Experiment 1: Standard plate test (SPT)										
Strain	TA 100		TA 1535		TA 1537		TA 98		WP2 uvrA	
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
<b>Historical negative control data</b>										
Based on Sorcerer Counter / Ames Study Manager System (Feb 2016 – Feb 2017)										
HCD										
Min	71	70	7	6	5	5	14	12	15	17
Max	132	147	16	18	13	16	34	38	34	36
Mean	100	107	10	10	8	9	21	28	24	25
SD	11.4	13.7	2.0	2.0	1.7	2.1	3.3	4.5	3.9	4.4
<b>Historical positive control data</b>										

Experiment 1: Standard plate test (SPT)										
Strain	TA 100		TA 1535		TA 1537		TA 98		WP2 uvrA	
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Based on Sorcerer Counter / Ames Study Manager System (Feb 2016 – Feb 2017)										
HCD										
Min	1126	272	1541	105	253	50	324	493	164	61
Max	5557	3021	6171	520	2190	399	1746	3096	1721	537
Mean	3298	1748	3967	197	1044	141	863	1524	860	133
SD	1109.6	587.2	1253.9	56.3	404.3	50.3	206.8	529.1	431.9	61.8

#### Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant study, cinmethylin (batch COD-002345, low purity batch with relatively high impurity levels) was not mutagenic in the bacterial reverse mutation (Ames) test, either in the presence or absence of metabolic activation, up to the limit concentration for this test.

(Woitkowiak, 2018a)

#### 2) New/modern study

Author(s)	Woitkowiak C.
Study title	BAS 684 H with new impurity - <i>Salmonella typhimurium</i> / <i>Escherichia coli</i> reverse mutation assay
Study reference	Woitkowiak, 2018b BASF Doc ID: 2018/1029051 Study ID: 858653
Laboratory	BASF SE, experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany
Dates of work	20/02/18 – 02/03/18
Test substance	Cinmethylin (BAS 684 H)
Purity (%)	97.5
Batch no.	COD-002314 - batch with new impurity (-) / (+) ratio = 50:50
Test organisms	<i>S. typhimurium</i> strains TA 98, TA 100, TA 1535 and TA 1537, and a strain of <i>E. coli</i> WP2 uvrA
Test concentrations SPT/PIT (± S9)	0, 33, 100, 333, 1000, 2600, 5200 µg/plate
Vehicle	Acetone
GLP	Compliant.
Guideline	OECD TG No. 471 (1997 – the current test guideline), Commission Regulation (EC) No 440/2008 - Part B No. B.13/B.14, EPA 870.5100.
Deviation	None.
Impact of deviations	N/A
Acceptable	Yes
Conclusion	Cinmethylin was not mutagenic.

#### Methods

The potential of cinmethylin to induce gene mutations in bacteria was investigated in two experiments using the standard plate test (SPT) and the pre-incubation test (PIT), with *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537, and a strain of *E. coli* (WP2 uvrA). The assay was performed, both in the presence and absence of metabolic activation (S9 mix - phenobarbital/β-naphthoflavone-induced rat liver S9 fraction) for 48 - 72 hours. Vehicle (acetone) and positive controls (Table 6.4-4) were included in each experiment. Each concentration, including the controls, was tested in triplicate. Cinmethylin was tested at concentrations of 0, 33, 100, 333, 1000, 2600 and 5200 µg/plate. A validated method of analysis for *in vitro* genotoxicity studies is not required.



Table 6.4-4. Positive control compounds tested

Strain	Mutagen	Solvent	Conc. [µg/plate]
<b>Without addition of metabolic activation system</b>			
TA 100	N-methyl-N'-nitrosoguanidine (MNNG)	DMSO	5
TA 1535	N-methyl-N'-nitrosoguanidine (MNNG)	DMSO	5
TA 1537	9-Aminoacridine (AAC)	DMSO	100
TA 98	4-nitro-o-phenylenediamine (NOPD)	DMSO	10
WP2 uvrA	4-Nitroguinoline-N-oxide (4-NQO)	DMSO	5
<b>With addition of metabolic activation system</b>			
TA 100	2-Aminoanthracene	DMSO	2.5
TA 1535	2-Aminoanthracene	DMSO	2.5
TA 1537	2-Aminoanthracene	DMSO	2.5
TA 98	2-Aminoanthracene	DMSO	2.5
WP2 uvrA	2-Aminoanthracene	DMSO	60

Results*Mutagenicity:*

Treatment with cinmethylin did not cause an increase in the number of revertants in either the SPT or PIT, under any tested conditions (Table 6.4-5). Consequently, there was no evidence of a mutagenic effect of cinmethylin in this study.

*Solubility:*

Precipitation of the test substance was observed, at a concentration of 5200 µg/plate, with and without S9 mix, however, this had no influence on scoring of the plates. Therefore, it can be concluded that the test was performed up to the limit of solubility.

*Toxicity:*

A bacteriotoxic effect (defined as reduced his<sup>+</sup> and trp<sup>+</sup> background growth and/or a decrease in the number of his<sup>+</sup> revertants) was observed depending on the strain and test conditions from about 333 µg/plate onward in both the SPT and PIT assays. On this basis, it can be concluded that the test was performed up to the limit of cytotoxicity.

*Validity:*

The positive controls induced the appropriate response in the corresponding strains - a clear, treatment-related increase in the number of revertants, within the range of the historical positive control data (Table 6.4-6), thus demonstrating the sensitivity of the test system. The vehicle control induced number of revertants was within the range of the historical control data for each strain (Table 6.4-6). On this basis the performance of the test was shown to be acceptable.

Table 6.4-5. Standard plate Ames tests with cinmethylin (BAS 684 H) - Mean number of revertants.

<b>Experiment 1: Standard plate test (SPT)</b>										
Strain	TA 100		TA 1535		TA 1537		TA 98		WP2 uvrA	
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Vehicle control										
Acetone	106.0	113.0	12.3	10.7	9.3	7.3	13.7	29.3	24.0	24.0
SD	12.0	4.0	5.7	2.5	5.0	0.6	1.5	9.8	13.7	3.0
Test item [µg/plate]										
33	106.0	110.0	11.7	13.7	8.0	6.7	12.0	26.7	27.3	23.0
SD	8.5	9.5	2.3	2.5	3.6	1.2	4.6	4.5	3.5	6.6
100	104.7	101.0	13.0	9.0	10.0	8.7	14.3	26.3	21.7	15.3
SD	3.1	26.9	6.1	3.6	1.7	1.5	5.8	2.1	4.7	4.7
333	89.0	101.3	9.3	10.3	6.3	7.0	10.7	16.3	28.0	20.7
SD	3.0	5.9	4.9	2.9	2.1	4.4	2.5	1.5	5.2	8.1
1000	88.0	95.7	11.3	10.0	5.7	8.0	7.7	18.3	23.0	27.0

Experiment 1: Standard plate test (SPT)										
Strain	TA 100		TA 1535		TA 1537		TA 98		WP2 uvrA	
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
<i>SD</i>	2.0	3.5	0.6	0.0	1.5	4.4	3.2	0.6	4.6	2.6
2600	88.0	80.0	8.7	6.0	6.7	5.7	10.7	13.0	23.7	19.7
<i>SD</i>	6.0	9.0	1.5	2.0	2.1	2.5	2.1	4.6	4.2	3.1
5200	81.0 <sup>P</sup>	77.3 <sup>P</sup>	5.7 <sup>P</sup>	9.0 <sup>P</sup>	3.7 <sup>P</sup>	5.3 <sup>P</sup>	11.7 <sup>P</sup>	17.0 <sup>P</sup>	25.3 <sup>P</sup>	20.7 <sup>P</sup>
<i>SD</i>	3.6	3.1	1.2	3.6	2.3	2.5	2.1	4.4	5.5	2.3
Pos. Control										
§	3013.3	1671.3	4190.0	216.3	650.7	102.7	967.7	1346.3	689.3	105.3
<i>SD</i>	291.0	41.2	81.0	24.5	98.8	15.1	21.1	56.1	31.2	5.1
Experiment 2: Pre-incubation test (PIT)										
Strain	TA 100		TA 1535		TA 1537		TA 98		WP2 uvrA	
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Vehicle control										
Acetone	106.0	96.7	11.3	12.0	9.0	8.0	21.0	23.0	21.7	26.3
<i>SD</i>	6.2	3.2	4.2	2.6	2.6	3.0	6.6	4.4	1.2	4.5
Test item [µg/plate]										
33	111.0	115.3	11.7	12.0	9.3	7.0	14.3	25.0	32.0	28.7
<i>SD</i>	4.4	12.4	3.8	2.6	1.5	3.6	1.5	4.4	4.6	1.5
100	111.3	118.3	8.7	9.0	6.0	7.0	17.3	23.0	27.3	29.3
<i>SD</i>	5.9	8.0	4.5	3.6	1.7	1.7	4.7	1.7	3.8	5.1
333	95.0	100.3	13.7	9.0	6.7 <sup>B</sup>	7.0	15.7	21.0	17.7	28.7
<i>SD</i>	7.5	10.1	3.5	3.6	1.2	3.0	3.1	4.0	2.5	2.9
1000	97.0 <sup>B</sup>	98.0	10.3 <sup>B</sup>	11.0	6.0 <sup>B</sup>	6.0	17.7 <sup>B</sup>	24.7	26.3	34.7
<i>SD</i>	6.9	14.8	2.1	0.0	1.0	1.0	8.1	5.5	4.9	7.6
2600	88.3 <sup>B</sup>	80.3	12.3 <sup>B</sup>	11.0	6.3 <sup>B</sup>	6.3	13.7 <sup>B</sup>	24.3	25.0	29.7
<i>SD</i>	3.1	8.1	1.5	1.0	3.2	1.5	2.9	9.9	2.6	5.7
5200	102.0 <sup>PB</sup>	63.7 <sup>P</sup>	8.7 <sup>PB</sup>	6.0 <sup>P</sup>	1.7 <sup>PB</sup>	3.0 <sup>P</sup>	9.7 <sup>PB</sup>	14.0 <sup>P</sup>	19.3 <sup>P</sup>	19.0 <sup>P</sup>
<i>SD</i>	7.9	2.9	4.0	1.0	0.6	1.7	1.5	5.3	6.7	2.6
Pos. Control										
§	2880.7	1692.3	4089.7	191.7	471.0	97.3	1054.3	910.0	176.3	105.7
<i>SD</i>	94.3	210.1	217.1	18.5	1.0	4.7	42.4	107.4	14.0	28.4

§ = Compound and concentrations see Material and Methods (I.A.2.) above

<sup>B</sup> = reduced background growth

<sup>P</sup> = precipitation

Table 6.4-6. Historical control data from the study report

<b>Experiment 1: Standard plate test (SPT)</b>										
<b>Strain</b>	<b>TA 100</b>		<b>TA 1535</b>		<b>TA 1537</b>		<b>TA 98</b>		<b>WP2 uvrA</b>	
<b>Metabol. activation</b>	<b>-S9</b>	<b>+S9</b>	<b>-S9</b>	<b>+S9</b>	<b>-S9</b>	<b>+S9</b>	<b>-S9</b>	<b>+S9</b>	<b>-S9</b>	<b>+S9</b>
<b>Historical negative control data</b>										
Based on Sorcerer Counter / Ames Study Manager System (Feb 2016 – Feb 2017)										
HCD										
Min	71	70	7	6	5	5	14	12	15	17
Max	132	147	16	18	13	16	34	38	34	36
Mean	100	107	10	10	8	9	21	28	24	25
SD	11.4	13.7	2.0	2.0	1.7	2.1	3.3	4.5	3.9	4.4
<b>Historical positive control data</b>										
Based on Sorcerer Counter / Ames Study Manager System (Feb 2016 – Feb 2017)										
HCD										
Min	1126	272	1541	105	253	50	324	493	164	61
Max	5557	3021	6171	520	2190	399	1746	3096	1721	537
Mean	3298	1748	3967	197	1044	141	863	1524	860	133
SD	1109.6	587.2	1253.9	56.3	404.3	50.3	206.8	529.1	431.9	61.8

Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant bacterial reverse mutation (Ames) test, cinmethylin (batch COD-002314, with new impurity) was not mutagenic, either in the presence or absence of metabolic activation, up to the limit concentration for this test.

(Woitkowiak, 2018b)

***In vitro forward mutation assay in mammalian cells***

<b>Author(s)</b>	Sokolowski A.
<b>Study title</b>	BAS 684 H - Cell mutation assay at the Thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells
<b>Study reference</b>	Sokolowski, 2018 BASF Doc ID: 2018/1066678 Study ID: 861794
<b>Laboratory</b>	Envigo CRD GmbH, In den Leppsteinswiesen 19, 64380 Rossdorf, Germany
<b>Dates of work</b>	12/03/18 – 16/04/18
<b>Test substance</b>	Cinmethylin (BAS 684 H)
<b>Purity (%)</b> <b>Batch no.</b>	93.5 COD-002038 (-) / (+) ratio = 48:52
<b>Test organisms</b>	L5178Y mouse lymphoma cells
<b>Test concentrations</b>	Preliminary toxicity assay: eight concentrations 3.9 to 500 µg/mL (solubility limit) Mutation assay : 1st experiment (4-h): +S9: 1.9, <b>3.8, 7.5, 15.0, 30.0, 45.0, 60.0</b> , 100.0, 125.0 µg/mL -S9: 1.9, <b>3.8, 7.5, 15.0, 30.0, 60.0, 80.0</b> , 100.0, 125.0 µg/mL 2nd experiment (24-h): 1.9, 3.8, <b>7.5, 15.0, 30.0, 45.0, 60.0</b> , 100.0, 125.0 µg/mL (evaluated concentrations indicated in bold)  Positive controls: -S9: Methyl methanesulfonate (MMS): 13.0 µg/mL (experiment II) and 19.5 µg/mL (experiment I). +S9: Cyclophosphamide (CPA): 3.0 µg/mL and 4.5 µg/mL.
<b>Solvent</b>	Acetone.

<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 490 (2016 – this is the current guideline), EPA 870.5300, Commission Regulation (EC) No 440/2008 - Part B No. B.17
<b>Deviation</b>	None.
<b>Impact of deviations</b>	N/A
<b>Acceptable</b>	Yes.
<b>Conclusion</b>	Cinmethylin was not mutagenic in the mouse lymphoma assay.

#### Methods

The mutagenic potential of cinmethylin in mammalian cells was assessed in a GLP and OECD test guideline compliant *in vitro* mammalian cell gene mutation assay in mouse lymphoma L5178Y cells (MLA assay). Two independent experiments were conducted, one each in the presence or absence of metabolic activation (S9-mix from the livers of phenobarbital/β-naphthoflavone induced rats) with two parallel cultures each.

In the preliminary cytotoxicity experiments, eight concentrations (3.9 – 500 µg/mL) were tested up to the solubility limit, in the presence (4-h) and absence of metabolic activation (4-h and 24-h). Based on the results of this preliminary cytotoxicity assay, concentrations of up to 125 µg/mL were used in the main experiment.

The exposure times for both experiments in the presence and absence of metabolic activation were 4-h, except in experiment II (in the absence of metabolic activation) where an exposure time of 24-h was employed. Methyl methanesulfonate (MMS) and Cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively. After the incubation period, treatment media were replaced by culture medium in both experiments and the cells were incubated for 48-h to allow for expression of mutant cells. This was followed by incubation of cells in selection medium containing TFT for about 10 - 15 days. A validated method of analysis for *in vitro* genotoxicity studies is not required.

#### Results

##### *Preliminary cytotoxicity assay:*

Relevant cytotoxic effects leading to RSG (Relative Suspension Growth; test culture vs. solvent control) values below 50 % were observed at 62.5 µg/mL and above (4-h in the absence of metabolic activation) and at 31.3 µg/mL and 125.0 µg/mL and above (4-h in the presence of metabolic activation). After 24-h cytotoxic effects were noted at 62.5 µg/mL and above. Additionally, phase separation (precipitation) was noticed at 125.0 µg/mL and above with and without metabolic activation at the end of the 4-h and 24-h treatment. Therefore, based on cytotoxicity and solubility, the top concentration of the main experiments was 125.0 µg/mL.

##### *Main experiments:*

##### *Solubility:*

Phase separation occurred in experiment I at 60.0 µg/mL and above (without metabolic activation) and at 80.0 µg/mL and above (with metabolic activation). In experiment II (only performed without metabolic activation) phase separation was noted at 100.0 µg/mL and above (Table 6.4-7).

##### *Cytotoxicity:*

Cytotoxic effects, defined as a mean relative total growth (RTG) below 50 %, occurred in the 4-h experiment (culture II) at 45.0 µg/mL and above in the absence of metabolic activation, and at 80.0 µg/mL and above in the presence of metabolic activation (Table 6.4-7). In the 24-h experiment (experiment II) without metabolic activation, cytotoxicity was noted from 45.0 µg/mL onwards, a RTG of <10 % was reported at 100 µg/mL (Table 6.4-7).

##### *Mutagenicity:*

No substantial and reproducible increase of the mutation frequency (MF) was noted in the main experiments either with or without metabolic activation. The threshold (GEF) of 126 above the mutation frequency of the solvent control was not exceeded at any experimental point. However, a linear regression analysis (least squares) was performed to assess a possible dose-dependent increase of mutant frequencies. A significant dose-dependent trend of the mutation frequency, indicated by a probability value of <0.05, was determined in 4-h experiment without metabolic activation. As the mutation frequency did not exceed the GEF under any conditions, there was no evidence of a genotoxic effect of the test.

In this study the range of the mean MF values of the solvent controls was from 70 - 164 mutant colonies per  $10^{-6}$  cells. The values are within the acceptable range of 50 – 170 mutant colonies per  $10^{-6}$  cells as well as within the range of the laboratory historical control data (HCD) (Table 6.4-8) and are therefore considered valid.

MMS (without metabolic activation) and CPA (with metabolic) were used as positive controls and produced a distinct increase in total mutant colonies within HCD (Table 6.4-8) and at acceptable levels of toxicity with at least one of the concentrations of the controls. Therefore, the ability of the test system to detect known mutagens was demonstrated.

Table 6.4-7. Gene mutation in mammalian cells - experiment I - culture I

Test group	Mutant frequency (MF per 10 <sup>-6</sup> cells)	Small mutant frequency (per 10 <sup>-6</sup> cells)	Toxicity data			Cloning efficiency (viability)	
			TSG	RSG	RTG	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (Acetone)	106	72	15.4	100.0	100.0	1.13	100.0
MF threshold <sup>§</sup>	232						
Test item							
1.9	#						
3.8	114	77	17.5	113.2	85.2	0.85	75.3
7.5	90	60	18.8	122.0	98.3	0.91	80.6
15.0	117	82	19.0	123.1	86.8	0.80	70.5
30.0	121	87	9.5	61.7	33.6	0.62	54.5
45.0	182	134	6.1	39.8	21.4	0.61	53.7
60.0 <sup>PS</sup>	173	109	4.6	30.0	15.0	0.57	50.0
100.0 <sup>PS</sup>	##						
125.0 <sup>PS</sup>	##						
MMS							
19.5 µg/mL	320	265	9.6	62.3	24.8	0.45	39.8
With metabolic activation; 4-hour exposure period							
Vehicle (Acetone)	152	130	19.8	100.0	100.0	0.88	100.0
MF threshold <sup>§</sup>	278						
Test item							
1.9	#						
3.8	233	190	15.4	77.6	67.0	0.76	86.3
7.5	210	167	16.8	85.0	91.2	0.95	107.3
15.0	225	189	14.3	72.2	72.2	0.88	100.0
30.0	113	82	12.8	64.4	84.6	1.16	131.3
60.0	120	107	10.4	52.5	65.9	1.11	125.7
80.0 <sup>PS</sup>	189	165	6.1	30.6	37.7	1.08	123.0
100.0 <sup>PS</sup>	##						
125.0 <sup>PS</sup>	##						
CPA							
3.0 µg/mL	287	224	17.4	87.6	57.9	0.58	66.1
4.5 µg/mL	499	423	12.7	64.0	37.7	0.44	50.4

TSG = total suspension growth; RSG = relative suspension growth; RTG = relative total growth

#: culture was not continued as only a minimum of four analysed concentration is required

##: culture was not continued due to exceedingly severe cytotoxic effects

§ = MF<sub>vehicle control corr</sub> + GEF (126 x  $10^{-6}$ ), rounded

<sup>PS</sup> = phase separation

Table 6.4-8. Historical control data (2014 – 2017) from the study report

Test group	Mutant frequency (MF per $10^{-6}$ cells)
<b>Without metabolic activation; 4-hour exposure period</b>	
HCD – negative/solvent control	
Range	31 – 204
Mean	94
<i>SD</i>	34
95 % confidence interval	27 – 162
No. studies	85
HCD – positive control (MMS)	
Range	191 – 1488
Mean	452
<i>SD</i>	210
95 % confidence interval	-
No. studies	85
<b>With metabolic activation; 4-hour exposure period</b>	
HCD – negative/solvent control	
Range	38 – 180
Mean	93
<i>SD</i>	31
95 % confidence interval	31 – 154
No. studies	105
HCD – positive control (CPA)	
Range	181 – 3658
Mean	489
<i>SD</i>	334
95 % confidence interval	--
No. studies	105
<b>Without metabolic activation; 24-hour exposure period</b>	
HCD – negative/solvent control	
Range	41 - 191
Mean	93
<i>SD</i>	32
95 % confidence interval	29 - 157
No. studies	64
HCD – positive control (MMS)	
Range	206 – 1904
Mean	528
<i>SD</i>	267
95 % confidence interval	--
No. studies	64

Table 6.4-9. Gene mutation in mammalian cells - experiment I - culture II

Test group	Mutant frequency (MF per 10 <sup>-6</sup> cells)	Small mutant frequency (per 10 <sup>-6</sup> cells)	Toxicity data			Cloning efficiency (viability)	
			TSG	RSG	RTG	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (Acetone)	70	51	24.5	100.0	100.0	1.24	100.0
MF threshold§	196						

Test group	Mutant frequency (MF per 10 <sup>-6</sup> cells)	Small mutant frequency (per 10 <sup>-6</sup> cells)	Toxicity data			Cloning efficiency (viability)	
			TSG	RSG	RTG	absolute	relative
Test item							
1.9	#						
3.8	134	92	34.1	139.2	93.7	0.84	67.4
7.5	95	55	27.6	112.8	110.0	1.21	97.6
15.0	90	63	23.3	95.1	67.4	0.88	70.9
30.0	105	71	29.1	118.8	125.2	1.31	105.4
45.0	87	58	8.5	34.5	28.3	1.02	82.0
60.0 <sup>PS</sup>	184	120	7.4	30.3	9.4	0.38	30.9
100.0 <sup>PS</sup>	##						
125.0 <sup>PS</sup>	##						
MMS							
19.5 µg/mL	321	242	13.3	54.1	24.8	0.56	44.8
With metabolic activation; 4-hour exposure period							
Vehicle (Acetone)	164	141	15.3	100.0	100.0	0.90	100.0
MF threshold <sup>§</sup>	290						
Test item							
1.9	#						
3.8	139	108	10.0	65.3	68.9	0.95	105.5
7.5	213	170	10.7	70.0	64.4	0.82	91.9
15.0	211	161	13.6	89.0	86.0	0.87	96.6
30.0	231	210	15.2	99.3	97.6	0.88	98.3
60.0	157	136	9.6	63.0	70.3	1.00	111.6
80.0 <sup>PS</sup>	247	225	4.0	26.1	25.6	0.88	98.3
100.0 <sup>PS</sup>	##						
125.0 <sup>PS</sup>	##						
CPA							
3.0 µg/mL	323	264	9.6	62.6	41.9	0.60	66.8
4.5 µg/mL	402	356	12.2	79.5	50.9	0.57	64.0

TSG = total suspension growth; RSG = relative suspension growth; RTG = relative total growth

#: culture was not continued as only a minimum of four analysed concentration is required

##: culture was not continued due to exceedingly severe cytotoxic effects

<sup>§</sup> = MF<sub>vehicle control corr</sub> + GEF (126 x 10<sup>-6</sup>), rounded

<sup>PS</sup> = phase separation

Table 6.4-10. Gene mutation in mammalian cells – experiment II - cultures I and II

Test group	Mutant frequency (MF per 10 <sup>-6</sup> cells)	Small mutant frequency (per 10 <sup>-6</sup> cells)	Toxicity data			Cloning efficiency (viability)	
			TSG	RSG	RTG	absolute	relative
Without metabolic activation; 24-hour exposure period							
Culture I							
Vehicle (Acetone)	94	79	47.5	100.0	100.0	1.18	100.0
MF threshold <sup>§</sup>	220						
Test item							
1.9	#						
3.8	#						
7.5	112	89	74.5	157.0	157.0	1.18	100.0

Test group	Mutant frequency (MF per 10 <sup>-6</sup> cells)	Small mutant frequency (per 10 <sup>-6</sup> cells)	Toxicity data			Cloning efficiency (viability)	
			TSG	RSG	RTG	absolute	relative
15.0	105	92	81.3	171.3	175.5	1.21	102.4
30.0	123	106	45.6	96.0	86.1	1.06	89.6
45.0	94	83	11.4	23.9	21.9	1.08	91.5
60.0	58	49	5.1	10.8	11.1	1.21	102.4
100.0 <sup>PS</sup>	n. r.		1.4	3.1	1.7	0.66	56.0
125.0 <sup>PS</sup>	##						
<b>MMS</b>							
13 µg/mL	322	265	53.2	112.2	47.1	0.50	42.0
<b>Culture II</b>							
<b>Vehicle (Acetone)</b>	127	114	85.0	100.0	100.0	1.02	100.0
MF threshold <sup>§</sup>	253						
<b>Test item</b>							
1.9	#						
3.8	#						
7.5	134	125	86.3	101.5	90.8	0.91	89.4
15.0	144	127	74.2	87.3	71.7	0.84	82.1
30.0	91	79	49.3	58.1	60.5	1.06	104.1
45.0	108	89	18.2	21.4	20.2	0.96	94.4
60.0	80	63	6.1	7.2	7.5	1.06	104.1
100.0 <sup>PS</sup>	n. r.		1.1	1.3	0.7	0.51	50.2
125.0 <sup>PS</sup>	##						
<b>MMS</b>							
13 µg/mL	390	356	48.3	56.9	29.0	0.52	50.9

TSG = total suspension growth; RSG = relative suspension growth; RTG = relative total growth

#: culture was not continued as only a minimum of four analysed concentration is required

##: culture was not continued due to exceedingly severe cytotoxic effects

n. r. = results not reported, since acceptance criteria (RTG ≥ 10%) not met

§ = MF<sub>vehicle control corr</sub> + GEF (126 x 10<sup>-6</sup>), rounded

PS = phase separation

### Conclusions

In conclusion, under the conditions of this GLP and OECD test guideline compliant study, cinnethylin did not cause an increase in mutant frequency meeting the criteria for a positive result either in the presence or absence of metabolic activation, up to concentrations causing precipitation and/or cytotoxicity. Therefore, cinnethylin is considered to be non-mutagenic in this mouse lymphoma assay.

(Sokolowski, 2018)

### In vitro micronucleus test in human lymphocytes

<b>Author(s)</b>	Naumann S.
<b>Study title</b>	BAS 684 H with 500 ppm [confidential impurity]: Micronucleus test in human lymphocytes <i>in vitro</i>
<b>Study reference</b>	Naumann, 2018 BASF Doc ID: 2018/1027282 Study ID: 1884300
<b>Laboratory</b>	Envigo CRD GmbH, In den Leppsteinswiesen 19, 64380 Rossdorf, Germany
<b>Dates of work</b>	10/01/18 – 28/02/18
<b>Test substance</b>	Cinnethylin (BAS 684 H) with 500 ppm [confidential impurity – see confidential section for more information]



<b>Purity (%)</b> <b>Batch no.</b>	93.2 COD-002038-[impurity] (-) / (+) ratio = 48:52
<b>Test organisms</b>	Human peripheral blood lymphocytes Experiment I: 31 years old female Experiment II: 21 years old male
<b>Activation</b>	S9 mix from the livers of rats, pre-treated with $\beta$ -naphthoflavone/phenobarbital
<b>Test concentrations</b>	Experiment I (4-h): +S9 : 11.2, 22.3, 44.7, 53.6, 64.3, <b>77.2, 92.6, 111</b> , 167, 500 $\mu\text{g/mL}$ -S9 : 11.2, <b>22.3, 44.7, 53.6</b> , 64.3, 77.2, 92.6, 111, 167, 500 $\mu\text{g/mL}$ Experiment II (20-h): -S9 : 10.9, <b>21.8, 43.7, 48.1</b> , 52.9, 58.1, 51.1, 64.1, 76.9, 100 $\mu\text{g/mL}$ (evaluated concentrations indicated in bold)  Positive controls: -S9: Mitomycin C (MMC), dissolved in deionized water (4 h): 0.8 $\mu\text{g/mL}$ ; pulse treatment Demecolcin, dissolved in deionized water (20 h): 100 ng/mL; continuous treatment +S9: Cyclophosphamide (CPA), dissolved in saline (0.9 % NaCl): 17.5 $\mu\text{g/mL}$
<b>Solvent</b>	Acetone (0.5% in culture medium).
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 487 (2016 – this is the current guideline), Commission Regulation (EU) 2017/735 B.49 No. L 193
<b>Deviation</b>	None.
<b>Impact of deviations</b>	N/A
<b>Acceptable</b>	Yes.
<b>Conclusion</b>	Cinmethylin was non-mutagenic in the <i>in vitro</i> micronucleus test.

#### Methods

Cinmethylin with 500 ppm [impurity] was tested for its potential to induce micronuclei in human lymphocytes *in vitro* in the absence and presence of metabolic activation (S9 mix from the livers of rats, pre-treated with  $\beta$ -naphthoflavone/phenobarbital) in an OECD test guideline and GLP compliant study.

Two independent experiments (experiments I and II) were performed in duplicate cultures where the cells were incubated for 4-hr (in the presence or absence of metabolic activation) or 20-hr (in the absence of metabolic activation) with the test substance at concentrations in the range of 10.9 – 500  $\mu\text{g/mL}$ ; three concentrations in the range of 21.8 to 111  $\mu\text{g/mL}$  were chosen for evaluation (see above table, test concentrations in bold and results Table 6.4-11). A preliminary cytotoxicity test (experiment I) was performed to determine the concentrations to be used in the main experiment. The pre-test was performed with 10 concentrations (11.2 - 500  $\mu\text{g/mL}$ ), in duplicate, with an exposure time of 4-hr (in the presence or absence of metabolic activation). Clear cytotoxic effects were observed after 4-hr treatment with 53.6  $\mu\text{g/mL}$  and above in the absence of metabolic activation. Considering the toxicity and phase separation (precipitation) data of experiment I, 100  $\mu\text{g/mL}$  (in the absence of metabolic activation) was chosen as the highest concentration in experiment II.

The vehicle acetone (0.5 % in culture medium) served as negative control, mitomycin C (4-hr) and demecolcin (20-hr) as positive controls (in the absence of metabolic activation) and cyclophosphamide as positive control (in the presence of metabolic activation). Treatment was started after a 48-hr stimulation period with phytohemagglutinine. Thereafter, cytochalasin B was added to the cultures to arrest cell cycle and the cultures were fixed and stained after another 20-hr. Cytokinesis-block proliferation index (CBPI) and cytostasis determined in 1000 binucleated cells served as cytotoxicity parameters and the number of micronucleated cells was determined in 2000 binucleated cells for evaluation of mutagenicity. A validated method of analysis for *in vitro* genotoxicity studies is not required.

### Results

#### *Cytotoxicity, precipitation and osmolarity:*

Phase separation (precipitation) of the test item in the culture medium was observed at the end of treatment in experiment I at 111 µg/mL and above (-S9 mix) and at 167 µg/mL and above (+S9 mix). In experiment II without metabolic activation, no phase separation was observed up to highest exposed concentration of 100 µg/mL. No relevant influence on osmolarity or pH was observed.

Cytotoxicity (where cytostasis is  $\geq 45 \pm 5$  %) was observed at the highest evaluated concentrations in experiments I (at 53.6 and 111.0 µg/mL) and II (at 48.1 µg/mL) in the absence and presence of metabolic activation (Table 6.4-11).

#### *Micronucleus assay:*

In both experiments, in the absence and presence of metabolic activation, no biologically-relevant increase in the number of cells carrying micronuclei was observed (Table 6.4-11). A statistically-significant increase of micronucleated cells was seen only in the two highest concentration groups of experiment II (43.7 and 48.1 µg/mL) after 20-hr exposure without metabolic activation; the increase was also positive in a trend test. However, both values (both 0.80 %) were well within the range of the historical control data for the solvent control (95 % ctrl limit: 0.00 - 1.11 %) and are therefore considered to be within normal biological variation. They were also below the 95 % ctrl limit of the positive control (1.47 – 5.89 %). No increase was seen with or without metabolic activation after 4-h exposure. Overall, no evidence of increased incidence of micronuclei was observed, following exposure to cinmethylin under any tested conditions.

All solvent control values were within the range of the laboratory historical negative control data (Table 6.4-11). In both experiments, either demecolcin (100 ng/mL), MMC (0.8 µg/mL) or CPA (17.5 µg/mL) were used as positive controls and showed distinct increases in cells with micronuclei, so demonstrating the sensitivity of the test system. All positive control values were within the range of the laboratory historical positive control data demonstrating the validity of the study.

Table 6.4-11. Summary of results of the *in vitro* micronucleus test in human lymphocytes with cinmethylin with 500 ppm [impurity].

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index (CBPI)	Cytostasis in % <sup>a</sup>	Micronucleated cells in % <sup>b</sup>	HCD	
Without S9 mix							
I	4	Solvent control <sup>1</sup>	1.87		0.75	No. experiments	61
						Mean	0.62
						95 % ctrl limit	0.09 – 1.19
						SD	0.28
						Min	0.00
		Max	1.18				
		22.3	1.92	n.c.	0.85		
		44.7	1.63	26.9	0.90		
		53.6	1.34	61.0	0.45		
		Positive control <sup>2</sup>	1.81	6.6	11.15*	No. experiments	62
						Mean	14.63
						95 % ctrl limit	3.92 – 25.34
						SD	5.35
						Min	2.60
		Max	28.50				
II	20	Solvent control <sup>1</sup>	1.81		0.30	No. experiments	65
						Mean	0.56
						95 % ctrl limit	0.00 – 1.11
						SD	0.28
						Min	0.05

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index (CBPI)	Cytostasis in % <sup>a</sup>	Micronucleated cells in % <sup>b</sup>	HCD		
						Max	1.10	
		21.8	1.73	10.2	0.50			
		43.7	1.47	41.7	<b>0.80*</b>			
		48.1	1.29	63.8	<b>0.80*</b>			
		Positive control <sup>3</sup>	1.40	51.2	<b>3.05*</b>	No. experiments	65	
						Mean	3.68	
						95 % ctrl limit	1.47 – 5.89	
						SD	1.11	
						Min	2.10	
							Max	8.80
	With S9 mix							
	I	4	Solvent control <sup>1</sup>	2.01		0.65	No. experiments	80
							Mean	0.73
95 % ctrl limit							0.08 – 1.38	
SD							0.33	
Min							0.10	
							Max	1.85
Positive control <sup>4</sup>		1.44	56.8	<b>8.85*</b>	No. experiments	86		
					Mean	5.45		
					95 % ctrl limit	0.70 – 10.20		
	SD				2.37			
	Min				2.25			
	Max				13.30			

a: For the positive control groups and the test item treatment groups the values are related to the solvent controls

b: The number of micronucleated cells was determined in a sample of 2000 binucleated cell

CBPI: cytokinesis-block proliferation index

HCD: historical control data of the performing laboratory: % of micronucleated cells in human lymphocyte cultures (2017)

n. c. Not calculated as the CBPI is equal or higher than the solvent control value

\*: The number of micronucleated cells is statistically significantly higher than corresponding control values ( $p \leq 0.05$ )

1 Acetone 0.5 % (v/v)

2 MMC 0.8 µg/mL

3 Demecolcin 100 ng/mL

4 CPA 17.5 µg/mL

### Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant study, cinmethylin with 500 ppm [impurity] did not induce micronuclei, as determined by the *in vitro* micronucleus test in human lymphocytes. Therefore, cinmethylin with 500 ppm [impurity] is considered to be non-mutagenic in this *in vitro* micronucleus test when tested up to cytotoxic concentrations with and without metabolic activation.

(Naumann, 2018)

**B.6.4.2. *In vivo* studies in somatic cells*****In vivo micronucleus test in mouse bone marrow***

<b>Author(s)</b>	
<b>Study title</b>	BAS 684 H - Micronucleus test in bone marrow cells of the mouse
<b>Study reference</b>	2018 BASF Doc ID: 2018/1048783
<b>Laboratory</b>	
<b>Dates of work</b>	10/11/2014 – 13/03/2018
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b>	96.3
<b>Batch no.</b>	COD-001950 (-) / (+) ratio = not specified.
<b>Test organisms</b>	Mice Crl:NMRI Male and female (preliminary study) 3/sex/dose Male only (main experiment and plasma analysis) 5/dose (main study), 2 males (bioavailability study)
<b>Route</b>	Oral, gavage Single administration
<b>Test concentrations</b>	Preliminary study: 2000 mg/kg bw, 10 mL/kg bw Main test: 0, 500, 1000 and 2000 mg/kg bw, 10 mL/kg bw Bioavailability study: 2000 mg/kg bw, 10 mL/kg bw Positive control: Cyclophosphamide (CPA) dissolved in deionized water: 20 mg/kg bw. Volume of all above doses: 10 mL/kg bw.
<b>Vehicle</b>	Corn oil.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 474 (2016 – the current test guideline), Commission Regulation (EC) No 440/2008 - Part B No. B.12, EPA 870.5395
<b>Deviation</b>	None.
<b>Impact of deviations</b>	N/A
<b>Acceptable</b>	Yes.
<b>Conclusion</b>	Cinmethylin did not increase the frequency of micronucleated immature erythrocytes.

**Methods**

In a GLP and OECD test guideline compliant study cinmethylin was tested for its potential to induce clastogenicity or aneugenicity in Crl:NMRI mice using the micronucleus test. Cinmethylin was administered once orally to groups of 5 male mice at dose levels of 500, 1000 and 2000 mg/kg bw. Dose selection was based on the results of a preliminary study in both sexes in which a single dose of 2000 mg/kg bw was tested. No sex difference was observed, therefore, only males were used in the main test. The vehicle (corn oil) served as negative and cyclophosphamide (CPA) as positive control. Animals were sacrificed 24-hr (all test substance concentrations, vehicle control and positive control) or 48-hr (an additional high dose group and vehicle control) after administration, the bone marrow of the two femora was prepared from each animal. After staining of the preparations, 4000 polychromatic erythrocytes (PCE) were evaluated per animal and examined for micronuclei. The number of PCE occurring per 500 normochromatic erythrocytes (NCE) were also recorded. Blood samples taken immediately after sacrifice were analysed to verify the bioavailability of the test substance. Blood samples of two additional Crl:NMRI mice exposed to 2000 mg/kg bw for 2-hr and 4-hr were also analysed. Methods for the analysis of cinmethylin in corn oil (Grauert & Hidding, 2017a; 2017/1067141), as well as cinmethylin and metabolites in rat plasma (Catchpole & Hidding, 2018; 2018/1037312), were evaluated and were considered validated (see Volume 3 CA B5, section B.5.1.2).

Results*Preliminary study*

The tested dose of 2000 mg/kg bw did not cause any deaths. Minor signs of toxicity (piloerection) were observed in all animals from administration of the test substance until sacrifice 48 hours later. There were no distinct differences in clinical observations between male and female animals. Therefore, only male animals were used in the main experiment. The maximum tolerated dose (MTD) could not be identified in this study, therefore a dose of 2000 mg/kg bw was defined as top dose (limit test) for the main test.

Table 6.4-12. Preliminary study - clinical observations

Clinical observations	Number of animals with signs (number examined: 3/sex)									
Dose:	2000 mg/kg bw									
Sex	M					F				
Time point of observation:	1h	2h	4h	1d	2d	1h	2h	4h	1d	2d
piloerection	3	3	3	3	3	3	3	3	3	3

*Micronucleus assay**Clinical examinations*

The single oral administration of the vehicle, the positive control (CPA) and the low dose group (500 mg/kg bw) was tolerated by all animals without any clinical observations indicative of toxicity. Administration of the mid and high doses (1000 and 2000 mg/kg bw) led to minor clinical signs of toxicity consisting of hunched posture and piloerection during the first 4 hours after application; piloerection persisted until termination of each dose group (Table 6.4-13).

Table 6.4-13. Main study - clinical observations

Clinical observations	Number of animals with signs (number examined: 5)												
Dose:	500 mg/kg bw				1000 mg/kg bw				2000 mg/kg bw				
Time point of observation:	1h	2h	4h	1d	1h	2h	4h	1d	1h	2h	4h	1d	2d
piloerection	0	0	0	0	5	5	5	5	5	5	5	5	5
hunched posture	0	0	0	0	5	5	5	0	5	5	5	0	0

*Micronucleus test results*

There was no treatment related or statistically-significant increase in the frequency of micronucleated immature erythrocytes (PCE), compared with the concurrent negative control, under any of the tested conditions. There was no evidence of cytotoxicity to the bone marrow.

*Acceptability of the test:*

The concurrent vehicle control (corn oil) data were considered acceptable (i.e. within the 95 % control limits of the distribution of the HCD, Table 6.4-15). Micronucleated PCE mean values of 1.6 % (at 24 hr sacrifice) and 1.2 % (at 48 hr sacrifice) were found in the study, compared to the HCD 95 % control limit of 0.5 – 1.9 % (at 24 hr sacrifice) and 0.6 – 1.9 % (at 48 hr sacrifice).

The concurrent positive control (CPA) data showed a statistically significant increase of induced micronucleus frequency (8.6 %) compared to the concurrent vehicle control; responses were compatible with those generated in the HCD (i.e. within the range of the HCD value, Table 6.4-15). The appropriate number of doses and cells were analysed (as required by the OECD test guideline). The selection of the high dose was appropriate (as required by the OECD test guideline).

Toxicity of the bone marrow (e.g. a reduction in the proportion of immature erythrocytes (NCE) among total erythrocytes compared to concurrent vehicle control), induced by treatment with cinmethylin, was not detected in the study (Table 6.4-14). However, bioavailability of the test item was demonstrated analytically (see below).

Table 6.4-14. Induction of micronuclei in bone marrow cells

Sampling time	Treatment groups	Dose (mg/kg Bw)	Micronucleated PCE (in 4000 PCE/animal)				PCE <sup>a</sup> [N]	Total erythrocytes <sup>b</sup> [N]	PCE among total erythrocytes [%]
			total [%] mean	range [N] min max		large MN [N] mean			
24 hours	Vehicle control (Corn oil)	0	1.6	2	14	0	243	743	33
	Cinmethylin	500	0.9	0	8	0	230	712	32
		1000	1.4	4	8	0	250	750	33
		2000	1.2	3	10	0.4	266	766	35
	Positive control (CPA)	20	8.6**	21	47	0.4	229	729	31
48 hours	Vehicle control (Corn oil)	0	1.2	3	7	0	223	723	31
	Cinmethylin	2000	0.8	1	4	0	306	806	38

Statistical analysis: \*\* =  $p \leq 0.01$  (Wilcoxon-test, 1-sided); N = number; MN = Micronucleated cells;

PCE = Polychromatic erythrocytes; NCE = normochromatic erythrocytes;

CPA = Cyclophosphamide

ery = erythrocytes

a = amount of PCE when scoring 500 NCE per animal

b = summary of PCE's and 500 NCE's per animal

N = number, absolute.

In male NMRI mice.

Single oral administration

24 h and 48 h sacrifice intervals

Table 6.4-15. Historical control data (HCD) - vehicle control and positive control data

	Vehicle control (all vehicles <sup>a</sup> )		Positive control (cyclophosphamide 20 mg/kg bw)
	Micronuclei in PCE		
	24 h sacrifice interval	48 h sacrifice interval	24 h sacrifice interval
Mean <sup>b</sup> (%)	1.2	1.3	14.4
Range mean group value (%)	0.7 – 2.0	0.6 – 2.0	11.7 – 18.8
Range individual animal data	0 – 13	1 – 11	19 - 126
Standard deviation	0.3	0.3	2.0
95 % lower control limit (%)	0.5	0.6	10.1
95 % upper control limit (%)	1.9	1.9	18.6
No. of test groups	20	20	20

a = deionised water, carboxy methylcellulose formulation, corn oil.

b = evaluation of 4000 PCE per animal in 5 animals per test group.

PCE = Polychromatic (i.e. immature) erythrocytes

4000 evaluated PCE per animal

Means of 5 animals per test group

Species: NMRI mice

Period Dec 2011 – Dec 2014

Route, oral

*Plasma analyses*

The bioavailability of the cinmethylin in mouse plasma samples was clearly demonstrated 2 and 4 hours after administration of 2000 mg/kg bw in either male or female Crl:NMRI mice. The plasma level was in a concentration range of 9.2 – 33.2 µg/mL. Cinmethylin was not detectable (LOQ < 100 ng/mL in plasma) 24 or 48 hours post-exposure.

Table 6.4-16. Results of plasma analysis

Dose (mg/kg bw)	Sampling time (hours)	n	Mean plasma levels (µg/mL)		
			Cinmethylin	M684H011 (Reg.No. 6055478)	M684H001 (Reg.No. 6055521)
0	24	5	<LOQ	n.d.	n.d.
500	24	5	<LOQ	<LOQ	<LOQ
1000	24	5	<LOQ	<LOQ	<LOQ
2000	2	2	26.65±9.3	2.28±1.39	2.14±0.21
	4	2	14.14±6.7	3.46±1.89	2.00±0.55
	24	5	<LOQ	0.76±0.06	0.23±0.06
	48	5	<LOQ	<LOQ	<LOQ

LOQ < 100 ng/mL plasma; n.d. not detectable

Metabolites of cinmethylin - M684H011 (Reg No. 6055478) and M684H001 (Reg. No. 6055521) were quantified in animals treated with 2000 mg/kg bw at 2, 4 and 24 h after administration. The plasma level of M684H011 (Reg No. 6055478) was in a concentration range of 1.3 – 4.8 µg/mL at the 2-4 h sampling interval and 0.2-1.4 µg/mL at the 24 h sampling interval. The plasma level of M684H001 (Reg No. 6055521) was in a concentration range of 1.6 – 2.4 µg/mL at the 2-4 h sampling interval and 0.1-0.4 µg/mL at the 24 h sampling interval. No cinmethylin metabolites were detectable 48 hours post-exposure.

Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant study, cinmethylin did not induce an increase in the frequency of micronucleated immature erythrocytes (PCEs) in the bone marrow of male mice administered the test substance up to the limit dose of 2000 mg/kg bw. Clinical signs of toxicity (hunched posture and piloerection) were noted at the mid- and top-dose (1000 and 2000 mg/kg bw). Bone marrow exposure was demonstrated in this assay directly by the presence of cinmethylin and/or its metabolites in plasma and indirectly by the systemic toxicity observed in the study from the mid dose.

(██████████, 2018)

***In vivo chromosome aberration assay in rat bone marrow******Old study***

<b>Author(s)</b>	██████████
<b>Study title</b>	<i>In vivo</i> chromosome aberration assay in rat bone marrow of SD95481 technical grade
<b>Study reference</b>	██████████, 1983 CI-435-004, ROT 43
<b>Laboratory</b>	████████████████████
<b>Dates of work</b>	10/03/1983 – 24-08/1983
<b>Test substance</b>	Cinmethylin (BAS 684 H) (SD 95481)
<b>Purity (%)</b>	92
<b>Batch no.</b>	513F (-) / (+) ratio = not specified.
<b>Test organisms</b>	Rat, Fischer 344 Male and female 6/sex/dose
<b>Route</b>	Oral, gavage Single administration
<b>Test</b>	Main experiment: 0.3, 1.0, and 3.0 mL/kg bw, equivalent to 304, 1014 and 3043 mg/kg bw

<b>concentrations</b>	Negative control: (Water) 3 mL/kg bw. Positive controls: Triethylenemelamine (TEM), 0.4 mg/kg bw
<b>Solvent</b>	None, undiluted test item was used
<b>GLP</b>	Compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	<p>Albeit not stated in the study report, the performance of the current study in line with OECD TG 475 (1984).</p> <p>According to the current OECD TG 475 (2016), intraperitoneal injection is generally not recommended. However, this way of exposure was used in the present study only for the positive control substance.</p> <p>According to the current guideline OECD TG 475 (2016), 200 metaphases per animal and 1000 metaphases per dose group should be evaluated. However, in the present study only 50 metaphases per animal and 600 metaphases per dose group were evaluated, since only “appropriate number of metaphases” was required for evaluation in the previous guideline OECD TG 475 (1984).</p> <p>Mitotic Index (MI) should be calculated based on metaphases of 1000 cells per animal and 5000 cells per dose group. In the current study, MI is based on 500 cells per animal. However, since 6 animals of both sexes were used in the current study instead of the required 5 animals of either sex, 6000 cells per dose group were evaluated for metaphases in this study and this is considered comparable to the requirement of the current OECD TG 475 (2016).</p> <p>Body weight prior to study-start and at termination was not recorded, since this was not a requirement of the previous versions of this test guideline (1984 and 1997).</p> <p>Blood samples were not taken in order to investigate plasma levels of the test chemical for demonstration of exposure of the bone marrow.</p> <p>Positive and negative historical control datasets were not available in the study report or applicant study summary; this impacted comparison with concurrent control data and treatment group results.</p>
<b>Impact of deviations</b>	The study is considered to have some deviations from the current test guideline. However, none of the deviations compromises the validity and the outcome of the study. Considering the fact, that the negative result was corroborated by the results of a recently conducted and guideline <i>in vivo</i> micronucleus study in mice (██████████, 2018) investigating the same endpoint, overall, the potential of the test item to induce <i>in vivo</i> cytogenetic damage was adequately assessed.
<b>Acceptable</b>	Yes, regarded as supplemental information.
<b>Conclusion</b>	Cinmethylin did not exhibit clastogenic activity in the bone marrow of rats.

#### Methods

In a GLP compliant study, cinmethylin was tested *in vivo* for the ability to induce chromosome aberrations in bone marrow cells of male and female Fischer rats. Six animals per sex were treated once with doses of 0.3, 1.0 or 3.0 mL/kg bw, equivalent to 304, 1014 and 3043 mg/kg bw (calculated based on the density of cinmethylin), by oral gavage. No preliminary range-finding study was conducted. Negative control (distilled water, 3 mL/kg bw by gavage) and positive control (triethylenemelamine (TEM), 0.4 mg/kg bw i.p.) were included. Sacrifice of the animals was performed 6, 16, and 24 hours after administration. Two to three hours prior to the scheduled sacrifice, the animals were injected intraperitoneally with colchicine (4.0 mg/kg bw) to arrest mitosis; at the time colchicine was administered clinical signs were observed and recorded. Cells were collected from the femoral bone marrow following sacrifice and processed to permit determination of the incidence of chromosome aberrations, with 50 cells for each rat being assessed. Cytotoxicity was determined by Mitotic Index (MI), by assessing the metaphases of 500 cells per animal. This study did not include analytical determination (see Volume 3 CA B5, section B.5.1.2), a validated method of analysis has not been provided.



Results*General toxicity:*

Clinical signs of toxicity were observed in the high dose animals only (Table 6.4-17). Whilst there was some indication of toxicity, it is questionable whether the maximum tolerated dose (MTD) was identified in the study. However, the highest dose tested (3043 mg/kg bw) exceeded the limit dose of 2000 mg/kg bw as specified in the current OECD test guidelines (No. 475, 2016).

Table 6.4-17. Clinical signs of toxicity in rats administered 3.0 mL/kg bw (equivalent to 3043 mg/kg bw), two hours prior to scheduled sacrifice

Clinical signs	Scheduled sacrifice					
	6 hours		16 hours		24 hours	
	Male	Female	Male	Female	Male	Female
Hypoactivity	3/6	1/6	1/6	2/6	2/6	0/6
Lacrimation	0/6	1/6	0/6	0/6	1/6	0/6
Polyurea	1/6	0/6	3/6	0/6	3/6	3/6
Chromadacryorrhea	1/6	0/6	0/6	0/6	0/6	0/6
Periocular swelling	1/6	0/6	0/6	0/6	0/6	0/6

First number / second number = number of animal affected / number of animals examined

*Cytotoxicity:*

No depression of bone marrow proliferation, as evidence of target tissue cytotoxicity, was observed in treated animals.

Table 6.4-18. Summary of chromosomal aberrations in bone marrow cells of rats of both sexes

Concentration (mL/kg bw) (mg/kg bw)	Parameter	MI		cells evaluated	Aberrations/Cell		% Aberrant cells	
		abs.	rel.		incl. gaps	excl. gaps	incl. gaps	excl. gaps
6-hour exposure								
Negative control (water) (3 mL/kg bw)	mean <sup>a</sup>	0.025	100.0	600	0.01	0.01	0.8	0.8
	<i>SD</i>	<i>0.012</i>	<i>49.3</i>		<i>0.01</i>	<i>0.01</i>	<i>1.0</i>	<i>1.0</i>
0.3 (304)	mean <sup>a</sup>	0.025	99.0	600	0.00	0.00	0.0	0.0
	<i>SD</i>	<i>0.009</i>	<i>34.7</i>		<i>0.00</i>	<i>0.00</i>	<i>0.0</i>	<i>0.0</i>
1.0 (1014)	mean <sup>a</sup>	0.034	134.9	598	0.01	0.01	0.7	0.5
	<i>SD</i>	<i>0.007</i>	<i>29.6</i>		<i>0.01</i>	<i>0.01</i>	<i>1.0</i>	<i>0.9</i>
3.0 (3043)	mean <sup>a</sup>	0.031	121.8	600	0.02	0.01	1.5	1.3
	<i>SD</i>	<i>0.007</i>	<i>32.2</i>		<i>0.02</i>	<i>0.02</i>	<i>1.7</i>	<i>1.6</i>
16-hour exposure								
Negative control (water) (3 mL/kg bw)	mean <sup>a</sup>	0.035	100.0	600	0.01	0.01	0.8	0.8
	<i>SD</i>	<i>0.010</i>	<i>29.5</i>		<i>0.01</i>	<i>0.01</i>	<i>1.3</i>	<i>1.3</i>
0.3 (304)	mean <sup>a</sup>	0.033	96.1	600	0.00	0.00	0.3	0.3
	<i>SD</i>	<i>0.014</i>	<i>40.3</i>		<i>0.01</i>	<i>0.01</i>	<i>0.8</i>	<i>0.8</i>
1.0 (1014)	mean <sup>a</sup>	0.031	90.8	600	0.00	0.00	0.3	0.3
	<i>SD</i>	<i>0.009</i>	<i>26.8</i>		<i>0.01</i>	<i>0.01</i>	<i>0.8</i>	<i>0.8</i>
3.0 (3043)	mean <sup>a</sup>	0.029	83.8	599	0.02	0.01	1.8	1.3
	<i>SD</i>	<i>0.010</i>	<i>27.5</i>		<i>0.06</i>	<i>0.04</i>	<i>5.7</i>	<i>4.0</i>
24-hour exposure								
Negative control (water) (3 mL/kg bw)	mean <sup>a</sup>	0.027	100.0	600	0.01	0.01	0.8	0.3
	<i>SD</i>	<i>0.011</i>	<i>33.8</i>		<i>0.02</i>	<i>0.01</i>	<i>1.3</i>	<i>0.8</i>
0.3	mean <sup>a</sup>	0.031	115.8	600	0.00	0.00	0.3	0.3

(304)	SD	0.015	53.0		0.01	0.01	0.8	0.8
1.0	mean <sup>a</sup>	0.023	92.9	600	0.01	0.01	0.7	0.7
(1014)	SD	0.009	45.9		0.03	0.03	1.3	1.3
3.0	mean <sup>a</sup>	0.029	108.3	599	0.01	0.01	1.3	1.3
(3043)	SD	0.013	37.3		0.02	0.02	1.6	1.6
Positive control (TEM)	mean <sup>a</sup>	0.010	39.5	359	3.56*	3.52*	53.0*	50.5*
(0.4 mg/kg bw)	SD	0.006	27.4		1.96	1.97	22.3	22.7

\*  $p \leq 0.05$  (Student's t-test, one-sided)

Number of animals = 12 per dose per exposure time period.

MI = Mitotic Index based on 500 cells

SD = standard deviation

a – mean of the means of the data on individual sexes.

#### Genotoxicity:

None of the treatment groups exhibited a statistically-significant increase in the frequency of cells with structural chromosomal aberrations ('aberrations per cell' and '% aberrant cells excluding gaps') compared with the concurrent negative control. This was the case at all timepoint (6, 16 and 24 h), in all dose groups (304, 1014 and 3043 mg/kg bw). There was a slight increase in '% aberrant cells excluding gaps' in animals treated with 3043 mg/kg bw at all timepoints, 1.3 % in treated animals compared to 0.3 – 0.8 % in the concurrent negative control. However, this was not statistically-significant and therefore does not represent a clear positive finding, in accordance with the criteria defined in the test guideline. In addition the values are well below those seen for the positive control group (50.5 % aberrant cells excluding gaps). The positive control (TEM) demonstrated a clear statistically-significant increase in the frequency of cells with structural chromosomal aberrations ('aberrations per cell' and '% aberrant cells excluding gaps'). Therefore, the test system was shown to be of appropriate sensitivity for detection of known clastogens. Overall, there was no evidence of a clastogenic effect of the test item, under the conditions of the study.

#### Conclusion

In conclusion, under the conditions of this GLP compliant study, cinmethylin did not induce an increase in the frequency of structural chromosomal aberrations in the bone marrow of rats administered the test substance up to the top dose of 3043 mg/kg bw, a dose at which systemic toxicity (clinical signs of toxicity) occurred. Cinmethylin did not exhibit clastogenic activity in the bone marrow of rats.

(██████████, 1983)

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

According to Commission Reg. (EU) 283/2013, an *in vivo* study in germ cells may be considered in some specific cases to investigate whether a somatic cell mutagen is or is not a germ cell mutagen. As cinmethylin was negative *in vivo* in a valid mutagenicity test in somatic cells, no *in vivo* mutagenicity studies in germ cells were conducted or are required.

#### B.6.4.4. Summary of genotoxicity

The genotoxic potential of cinmethylin has been investigated in a series of modern *in vitro* and *in vivo* studies. An old rat cytogenetics study is also available. A summary of the available genotoxicity studies is presented in the table below. The main findings are summarised in Table 6.4.19 below.

The following key conclusions were obtained from the evaluation of the genotoxic information:

- Cinmethylin is not genotoxic
- Classification for genotoxicity is not required. Further details are available in the aligned MCL dossier
- The data requirements of Regulation 283/2013 have been met.

Table 6.4-19. Summary of genotoxicity studies with cinmethylin

Test system and Acceptability	Concentration/ dose levels	Purity (%)	Results	Reference
<b><i>In vitro studies</i></b>				
Ames Test (Reverse mutation assay) <i>S. typhimurium</i> strains (TA 98, TA 100, TA 1535, TA 1537) <i>E. coli</i> strain (WP2/uvrA) +/- S9	33 - 5600 µg/plate  Batch COD-002345 - artificial batch with increased impurity levels. 89.6 % (-) / (+) ratio = not specified.	89.6	Negative	<a href="#">Woitkowiak, 2018a (2018/1029052)</a>
<i>Acceptable modern study</i>	33 - 5200 µg/plate  Batch COD-002314 - batch with new impurity. (-) / (+) ratio = 50:50	97.5	Negative	Woitkowiak, 2018b (2018/1029052)
<i>In vitro</i> forward mutation assay in mammalian cells (Mouse lymphoma assay). Mouse lymphoma L5178Y cells. +/- S9	3.8 – 80.0 µg/plate  Batch COD-002038. 93.5 % (-) / (+) ratio = 48:52	93.5	Negative	<a href="#">Sokolowski, 2018 (2018/1066678)</a>
<i>Acceptable modern study</i>				
<i>In vitro</i> micronucleus test in human lymphocytes +/- S9	21.8 - 111 µg/plate  Batch COD-002038-[impurity] (-) / (+) ratio = 48:52	93.2	Negative	<a href="#">Naumann, 2018 (2018/1027282)</a>
<i>Acceptable modern study</i>				
<b><i>In vivo studies</i></b>				
<i>In vivo</i> micronucleus test in mouse bone marrow (male and female NMRI mice) Oral, gavage	0, 500, 1000 and 2000 mg/kg bw  Batch COD-001950. 96.3 % (-) / (+) ratio = not specified.	96.3	Negative	<a href="#">[REDACTED], 2018 (2018/1048783)</a>
<i>Acceptable modern study</i>				
<i>In vivo</i> chromosome aberration assay in rat bone marrow (male and female Fischer 344 mice) Oral, gavage	0, 304, 1014 and 3043 mg/kg bw  Batch 513F. Purity (%): not specified. (-) / (+) ratio = not specified.	92	Negative	<a href="#">[REDACTED], 1983 (CI-435-004)</a>
<i>Old supplemental study</i>				

Cinmethylin was negative in these studies.

Cinmethylin was negative, when tested up to limit test concentrations, in two modern, GLP and guideline compliant Ames tests, using two different batches with different impurity profiles. Cinmethylin was non-mutagenic in a modern, GLP and guideline compliant mammalian cell gene mutation assay, in the presence and absence of metabolic activation up to cytotoxic concentrations. Cinmethylin was clearly not clastogenic or aneugenic to human lymphocytes in a modern, guideline compliant *in vitro* micronucleus assay conducted up to cytotoxic concentrations. Overall, there was no evidence of genotoxicity across these *in vitro* studies.

A modern, GLP and guideline compliant *in vivo* micronucleus test in mouse bone marrow, via oral administration, was available. No increase in the incidence of micronuclei was induced. Bone marrow exposure

was demonstrated this assay directly by the presence of cinmethylin and/or its metabolites in blood and indirectly by the systemic toxicity observed in the study from the mid dose. In an older, GLP but not strictly guideline compliant *in vivo* chromosome aberration assay in rat bone marrow, cinmethylin did not exhibit clastogenic activity. Overall, there was no evidence of genotoxicity across these *in vivo* studies.

According to Regulation (EU) 283/2013, photo-mutagenicity testing is not required for substances with a UV/VIS molar extinction/absorption coefficient less than  $1000 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ . There is no relevant absorption in the range 290 - 700 nm and the ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than  $10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$  (see chemistry evaluation section B.2.4). Photo-mutagenicity testing is therefore not required for cinmethylin.

Overall, HSE concludes that cinmethylin was not genotoxic *in vitro* or *in vivo* in a series of investigations that, together, meet the data requirements of Regulation 283/2013. Classification of cinmethylin for mutagenicity is not warranted.

### B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

The long-term toxicity and carcinogenic potential of cinmethylin have been investigated in rats and mice, via the oral (dietary) route of exposure, in 18-month and 24-month studies. For each species two studies are available - one new/modern standard guideline study and one older study not conducted according to GLP and OECD test guidelines.

#### B.6.5.1. Long-term toxicity and carcinogenicity in rats

The long-term toxicity and carcinogenicity of cinmethylin have been investigated in rats via the oral (dietary) route in one standard guideline 2-year study (new/modern) in Wistar rats and one older study with Fischer 344 rats.

##### 1) *New/modern study*

<b>Author(s)</b>	
<b>Study title</b>	BAS 684 H Combined Chronic Toxicity/Carcinogenicity Study in Wistar Rats Administration via the Diet up to 24 Months
<b>Study reference</b>	2018 BASF Doc ID: 2017/1093414
<b>Test facility</b>	
<b>Dates of work</b>	24/03/2015 – 18/04/2017
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b>	93.5
<b>Batch no.</b>	COD-002038 (-) / (+) ratio = 48:52
<b>Test organisms</b>	Rat Wistar, CrI :WI(Han) Males and females The same strain was used in the previous repeated dose toxicity studies.
<b>Groups</b>	50/sex/dose (carcinogenicity phase groups) 10/sex/dose (chronic toxicity phase groups).
<b>Dose/concentration</b>	0, 200, 1000 and 5000 ppm. Equivalent doses shown in Table 6.5-1
<b>Route</b>	Administered orally, via the diet, daily, for a period of 12 months (chronic phase) or 24 months (carcinogenicity phase).
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD Test Guideline No. 453 (2009 – the current test guideline was adopted in 2018), EPA 870.4300, JMAFF No 12 Nosan No 8147 (2000), Commission Regulation (EC) No 440/2008 - Part B No. B.33
<b>Deviation</b>	Clinical observations were not recorded for animals on study day 435.
<b>Impact of deviations</b>	The deviation identified is not considered to compromise the validity of the study.
<b>Acceptable</b>	Yes.
<b>Conclusion</b>	Cinmethylin induced a marginal increase in liver carcinomas in females at the top dose.
<b>NOAEL</b>	NOAEL for carcinogenicity: 1000 ppm, equivalent to 59 mg/kg bw/d in females. NOAEL for systemic chronic toxicity: 200 ppm, equivalent to 9 mg/kg bw/d in males.
<b>Effects at the LOAEL</b>	Carcinogenicity: Liver carcinomas in females at the top dose (5000 ppm, equivalent to 317 mg/kg bw/d) – equivocal evidence of carcinogenicity. Systemic chronic toxicity: Adverse nasal cavity histopathology from the mid dose in males (1,000 ppm, equivalent to 45 mg/kg bw/d).

#### Methods

In a GLP and OECD test guideline compliant study, cinmethylin was administered via the diet to male and female Wistar rats (the same strain was used in the previous repeated dose toxicity studies) over a period of either 12-months (10/sex/dose, chronic toxicity phase) or 24-months (50/sex/dose, carcinogenicity phase). Dietary concentrations of 0, 200, 1000 and 5000 ppm (see Table 6.5-1 for equivalent doses in mg/kg bw/d) were selected on the basis of previous 28- and 90-day studies in rats (see Section 6.3). No interim kills, satellite groups (to monitor reversibility) or sentinel animals (to monitor disease status) were used in this study. A

method for the detection of cinmethylin in rat/mouse diet (Catchpole & Hidding, 2017b; 2017/1123754), as well as cinmethylin and metabolites in rat plasma (Catchpole & Hidding, 2018; 2018/1037312), were evaluated and were considered validated (see Volume 3 CA B5, section B.5.1.2) was evaluated and was considered validated (see Volume 3 CA B5, section B.5.1.2).

Table 6.5-1. Mean test substance intake

Dose [ppm]	Males				Females			
	0	200	1000	5000	0	200	1000	5000
Dose [mg/kg bw/d]								
Chronic phase (12 months)	0	10	51	265	0	13	69	351
Carcinogenicity phase (24 months)	0	9	45	242	0	11	59	317

### Results

The stability and homogeneity of cinmethylin in the diet was confirmed in a separate study using a validated method of analysis. Plasma concentrations of cinmethylin and four of its metabolites (M684H001, M684H010, M684H011 and M684H026) were determined throughout the study (on days 22, 43, 64, 80, 172, 262 and 353) (Table 6.5-2). Analytical results demonstrated the clear presence of all four metabolites of cinmethylin in all plasma samples of treated animals from all days. Cinmethylin was regularly observed but only sporadically detected at levels above the LOQ at the highest dose level. In general, levels of metabolites increased in a dose-dependent manner, although in the top dose considerable variation was observed.

Table 6.5-2. Plasma concentration of cinmethylin and selected metabolites (chronic toxicity group, 10/dose)

Dose [ppm]		Males				Females			
		0	200	1000	5000	0	200	1000	5000
Cinmethylin	Day 22 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	105 <sup>1</sup>
	Day 43 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	95 <sup>1</sup>
	Day 64 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	94 <sup>1</sup>
	Day 80 [ng/mL] [RSD]	n. d.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	121 <sup>3</sup> 10.8
	Day 172 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	138 <sup>1</sup>
	Day 262 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	112 <sup>2</sup> 22.1
	Day 353 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	115 <sup>3</sup> 10.7
M684H001 Reg.No. 6055521	Day 22 [ng/mL] [RSD]	n. d.	500 20.5	1476 18.2	12811 18.8	n. d.	559 26.4	2265 16.1	11487 22.3
	Day 43 [ng/mL] [RSD]	n. d.	300 19.9	1273 26.9	8634 26.7	n. d.	616 24.8	2729 32.4	9652 46.1
	Day 64 [ng/mL] [RSD]	n. d.	304 15.5	1233 26.2	5962 35.4	n. d.	534 18.8	2340 32.5	16496 26.8
	Day 80 [ng/mL] [RSD]	n. d.	345 42.8	1108 22.8	10020 43.3	n. d.	416 30.2	2365 30.1	19439 36.4
	Day 172 [ng/mL] [RSD]	n. d.	249 32.2	945 22.0	11233 30.9	n. d.	410 30.9	1760 35.6	20743 32.8
	Day 262 [ng/mL] [RSD]	n. d.	229 24.1	1112 24.4	5400 32.8	n. d.	384 30.0	1538 26.1	8906 34.8
	Day 353 [ng/mL] [RSD]	n. d.	230 26.5	819 23.8	3271 30.0	n. d.	676 30.4	2971 19.2	13305 32.2

Dose [ppm]			Males				Females			
			0	200	1000	5000	0	200	1000	5000
M684H010 Reg.No. 111609	Day 22	[ng/mL] [RSD]	n. d.	<LOQ	277 20.3	1936 17.8	n. d.	<LOQ	129 <sup>8</sup> 19.0	1308.1 21.7
	Day 43	[ng/mL] [RSD]	n. d.	<LOQ	198 24.5	1393 19.7	n. d.	<LOQ	141 <sup>7</sup> 26.0	1204 18.0
	Day 64	[ng/mL] [RSD]	n. d.	<LOQ	193 13.7	1298 16.2	n. d.	<LOQ	122 <sup>5</sup> 9.5	1117 22.6
	Day 80	[ng/mL] [RSD]	n. d.	<LOQ	191 16.5	1191 22.0	n. d.	<LOQ	130 <sup>8</sup> 15.8	1247 24.2
	Day 172	[ng/mL] [RSD]	n. d.	<LOQ	192 12.5	1179 27.7	n. d.	<LOQ	121 18.3	1448 13.9
	Day 262	[ng/mL] [RSD]	n. d.	<LOQ	203 24.4	1058 16.0	n. d.	<LOQ	138 <sup>9</sup> 16.9	1430 25.8
	Day 353	[ng/mL] [RSD]	<LOQ	<LOQ	185 13.2	7816 20.8	n. d.	<LOQ	164 <sup>7</sup> 14.9	1407 24.3
M684H011 Reg.No. 6053478	Day 22	[ng/mL] [RSD]	n. d.	370 23.3	1278 14.4	8683 17.1	n. d.	258 28.1	1199 18.1	4255 25.2
	Day 43	[ng/mL] [RSD]	n. d.	269 8.1	1279 16.1	6179 19.2	n. d.	240 19.4	1039 34.6	3269 22.7
	Day 64	[ng/mL] [RSD]	n. d.	219 15.0	892 26.9	3353 22.6	<LOQ	182 16.7	816 25.0	2802 18.4
	Day 80	[ng/mL] [RSD]	<LOQ	247 19.3	920 20.1	3079 20.2	<LOQ	206 23.6	1031 15.4	3171 22.2
	Day 172	[ng/mL] [RSD]	<LOQ	189 23.2	884 22.9	3135 17.8	<LOQ	208 29.9	885 33.8	3241 23.4
	Day 262	[ng/mL] [RSD]	<LOQ	182 18.8	1127 13.2	3039 22.0	<LOQ	198 21.3	827 16.1	2876 25.4
	Day 353	[ng/mL] [RSD]	n. d.	176 20.1	727 17.7	2621 20.8	n. d.	191 25.2	890 25.3	2909 19.7
M684H026 Reg.No. 6059081	Day 22	[ng/mL] [RSD]	n. d.	329 15.7	1855 16.2	9282 33.5	n. d.	216 23.5	1207 18.6	12133 23.2
	Day 43	[ng/mL] [RSD]	n. d.	273 13.7	1601 13.3	8884 20.6	n. d.	240 24.2	1207 20.9	8570 19.1
	Day 64	[ng/mL] [RSD]	n. d.	303 12.0	1529 13.9	7755 26.7	n. d.	206 24.9	988 20.6	14632 23.9
	Day 80	[ng/mL] [RSD]	n. d.	295 12.8	1609 11.5	11327 16.1	n. d.	225 25.8	984 15.2	15323 24.5
	Day 172	[ng/mL] [RSD]	n. d.	297 12.5	1419 11.5	13185 19.4	n. d.	214 24.5	1021 14.8	16137 20.5
	Day 262	[ng/mL] [RSD]	n. d.	262 14.0	1431 11.9	6346 22.6	n. d.	211 30.4	1090 21.9	8849 28.7
	Day 353	[ng/mL] [RSD]	n. d.	248 20.0	1381 13.2	7816 20.8	n. d.	190 32.0	1033 25.6	10540 26.7

LOQ (limit of quantification) = 100 ng/mL

RSD = relative standard deviation in %

n. d. = not detectable

n<sup>x</sup> = superscript x is the number of animals used for plasma concentration measurement; if no x is depicted, mean is based on values of all 10 animals per dose group

**Mortality:** There were no treatment-related effects on mortality during both the chronic phase (12-months) and the carcinogenic phase (24-months). No animals of the chronic phase group were sacrificed moribund or found dead. In the carcinogenic phase groups a dose-related increase in mortality rates was observed in males (Table 6.5-3), however, mortality rates were within the HCD provided. No treatment-related histopathological findings were seen to have caused early deaths. Most of the deceased animals showed different kinds of tumours and/or inflammatory lesions in varying organs.

Table 6.5-3. Mortality of carcinogenicity phase group at 24-months (728 days)

Dose Level (ppm)	Mortality			
	Males		Females	
	No. animals	(%)	No. animals	(%)
0	6/50	12	11/50	22
200	8/50	16	10/50	20
1000	9/50	18	13/50	26
5000	12/50	24	9/50	18
HCD <sup>a</sup>	Mean ± SD	15.2 ± 12.8	Mean ± SD	23.6 ± 6.5
	Min	0	Min	16
	Max	32	Max	34

a: Historical control data (HCD) of mortality (%) at the end of the two-year exposure period based on 5 dietary Combined Chronic Toxicity/Carcinogenicity Studies with Wistar rats (supplier: [REDACTED]) performed at [REDACTED] according to the guideline OECD 453 as well as OPPTS 870.4300 during 2007 - 2017 under GLP conditions.

*Clinical signs of toxicity:* There were no treatment-related clinical findings in both the chronic and the carcinogenic phases. Findings (e.g. palpable masses through skin and poor general condition) occurred in treated and control animals at similar incidences or occurred incidentally in single animals.

*Ophthalmoscopy:* There were no treatment-related ophthalmological findings in both the chronic and the carcinogenic phases.

*Body weight, food and water consumption:* In the chronic phase group (12-months) treatment-related and adverse decreases in body weight (statistically-significant, maximum -12.6 %) and body weight gain (statistically-significant, overall -21.3 %) were seen in females of the top dose. No significant changes occurred in males of any dose group or in females of the low and mid dose group. In the carcinogenicity phase group (24-months) statistically-significant decreases in body weight and body weight gain were seen in males and females of the top dose (Table 6.5-4). A dose-response relationship was evident for males. A reduction in body weight gain of > 10 % compared to controls was seen for males and females of the top dose. This depression of body weight gain supports the selection of the top dose and indicates achievement of the MTD. There were no treatment-related effects on food and water consumption in both the chronic and the carcinogenic phases. Overall, there were treatment-related and adverse effects on body weight and body weight gain in males and females at the top dose (5000 ppm, equivalent to 242 and 317 mg/kg bw/d in males and females respectively).

Table 6.5-4. Body weight and body weight gain in the 24-month group

		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	9	45	242	0	11	59	317
Body weight [g]									
Day 0		157.0	155.8	155.6	153.6	121.7	120.2	120.8	121.6
Day 91		384.1	382.3	380.8	369.9*	236.3	233.5	235.7	226.4**
Day 371		513.4	505.9	504.9	488.7*	287.6	278.3	282.9	267.0**
Day 728		595.1	568.0	559.5	541.6**	360.4	333.9*	342.0	314.9**
Δ%#		-	-5	-6	-9	-	-7	-5	-13
Overall body weight gain (g)									
Day 91		227.1	226.5	225.1	216.3	114.6	113.3	114.8	104.7**
Day 371		356.2	349.8	349.1	335.1*	165.8	158.0	162.1	145.4**
Day 728		437.7	412.0	403.8	387.4**	237.6	213.8*	220.2	194.2**
Δ%#		-	-6	-8	-12	-	-10	-7	-18



Statistical evaluation: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Dunnett test (two-sided)

$\Delta\%$  – percent change compared to control.

# Values may not calculate exactly due to rounding of figures

*Haematology and clinical chemistry:* There were no treatment-related haematology findings in both the chronic and the carcinogenic phases.

Statistically-significant increases in  $\gamma$ -glutamyl transferase (GGT) activity were found in both sexes at the top dose. Although the mean GGT among individuals of the control, low and mid dose groups was below the lowest quantifiable value (25 nkat/L) for this parameter, a clear and toxicologically significant increase was noted at the top dose. Statistically significant changes in aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and triglycerides did not follow any dose-response or time-response relationship and therefore were not considered treatment-related. Glucose levels were statistically-significantly decreased at the top dose for both males (at 3- and 6-months) and females (at 3-months). However, changes compared to controls were not toxicologically-significant as they were small and did not show a temporal consistency. Albumin levels were statistically-significantly increased for males of the top dose at 6-months only (4.1 % change compared to control). As this increase was rather small, it was not considered toxicologically significant. Potassium levels (K) were statistically-significantly increased (by 14.8 %) for females of the top dose at 6 months. However, no dose-relationship or temporal consistency was observed. Overall, with the exception of an increase in GGT at the top dose in both sexes, there were no other treatment-related or adverse effect on clinical chemistry parameters.

Table 6.5-5. Selected clinical chemistry parameters

Dose [ppm]		Males				Females			
		0	200	1000	5000	0	200	1000	5000
Parameter	Day								
GGT [nkat/L]	92	0	0	0	<b>34**</b>	2	2	<b>9**</b>	<b>63**</b>
	183	1	0	<b>3*</b>	<b>56**</b>	4	4	8	<b>70**</b>
	365	0	0	1	<b>77**</b>	0	0	1	<b>47**</b>
AST [ $\mu$ kat/L]	92	2.43	1.64	1.74	1.74	1.72	1.75	1.49	1.75
	183	1.61	1.46	1.61	1.54	1.60	1.58	1.42	<b>1.71*</b>
	365	1.71	1.49	1.75	1.85	2.25	2.56	<b>1.77*</b>	<b>1.53**</b>
ALP [ $\mu$ kat/L]	92	1.31	1.16	1.25	1.33	0.65	0.62	<b>0.48**</b>	0.60
	183	1.08	<b>0.91*</b>	0.97	1.08	0.51	0.42	<b>0.36**</b>	<b>0.43**</b>
	365	1.15	1.00	1.01	1.18	0.45	0.44	<b>0.31**</b>	<b>0.36*</b>
ALT [ $\mu$ kat/L]	92	1.24	0.64	0.70	0.59	0.62	0.64	0.66	0.62
	183	0.63	0.57	0.58	0.69	0.62	0.63	0.62	0.61
	365	0.8	0.66	0.70	0.81	0.86	0.85	0.79	<b>0.63**</b>
Glucose [mmol/L] ( $\Delta\%$ )	92	5.19	5.47 (5.4)	<b>4.79*</b> (-7.7)	<b>4.35**</b> (-16.2)	5.81	5.73 (-1.4)	5.69 (-2.1)	<b>4.98*</b> (-14.3)
	183	6.57	6.80 (3.5)	6.11 (-7.0)	<b>5.85*</b> (-11.0)	5.75	5.74 (-0.2)	5.29 (-8.0)	5.20 (-9.6)
	365	5.92	<b>6.44*</b> (8.8)	5.97 (0.8)	5.51 (-6.9)	5.84	5.98 (2.4)	5.67 (-2.9)	5.07 (-13.2)
Albumin [g/L] ( $\Delta\%$ )	92	36.26	36.99 (2.0)	37.36 (3.0)	37.64 (3.8)	38.82	39.56	39.15	40.52
	183	34.20	34.78 (1.7)	35.07 (2.5)	<b>35.61**</b> (4.1)	35.92	37.31	36.50	38.19
	365	35.52	36.11 (1.7)	36.22 (2.0)	36.62 (3.1)	38.48	38.89	38.93	40.30
Triglycerides [mmol/L]	92	0.99	1.11	0.94	0.98	0.46	0.51	0.69	0.56
	183	1.09	1.10	1.07	0.94	0.54	0.79	0.90	0.83
	365	1.32	1.27	1.15	0.92	0.68	1.03	<b>1.31**</b>	<b>0.95**</b>
K [mmol/L] ( $\Delta\%$ )	92	5.04	4.88	5.08	5.12	4.17	4.27 (2.4)	4.32 (3.6)	4.26 (2.2)
	183	4.97	4.88	5.00	5.20	4.20	4.62 (10.0)	4.50 (7.1)	<b>4.82**</b> (14.8)

Dose [ppm]	Males				Females			
	0	200	1000	5000	0	200	1000	5000
365	4.91	4.79	4.86	4.94	4.31	4.69 (8.8)	4.41 (2.3)	4.54 (5.3)

Statistical evaluation: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$  (Kruskal-Wallis and Wilcoxon-test, two sided)

GGT – serum- $\gamma$ -glutamyltransferase.

AST – aspartate aminotransferase.

ALP – alkaline phosphatase.

ALT – alanine aminotransferase.

K – potassium.

$\Delta\%$  – percent change compared to control.

**Urinalysis:** There were no treatment-related urinalysis findings in both the chronic and the carcinogenic phases. After 3- and 6-months of administration, in males of the top dose, higher incidences of transitional epithelial cells as well as epithelial and granulated casts from the kidney were observed in urine. These findings were restricted to males and were likely due to  $\alpha_2\mu$ -globulin accumulation, as eosinophilic droplets were observed histopathologically in these animals. A similar finding was seen in the 90-day rat study [REDACTED], 2018a) where it was confirmed with immunohistochemistry that these observations were due to  $\alpha_2\mu$ -globulin accumulation. This response is regarded as specific to male rats and not relevant to humans.

**Organ weight:** At 12-months statistically-significant decreases in absolute adrenal gland weights (15 % change compared to control) were seen in females from the mid dose. However, neither a dose-response nor a histopathological correlate or a change in relative weight was evident, therefore this was not considered treatment-related. The reduced ovary weight in all treated groups at 12- and 24-months was due to a much higher mean ovary weight in control animals. At 12-months, the mean ovary weight for the control group (140 mg) was increased by one animal with a tumour and an individual ovary weight of 520 mg. This was much higher compared to the 12-month HCD absolute ovary weight mean of 84 mg and maximum of 104 mg (Table 6.5-7). At 24-months, the high mean ovary weight for the control group was caused by either tumours or cysts in some control animals. The control group mean at 24-months was far beyond the historical control values (mean absolute ovary weight of 1078 mg at 24-months in concurrent control compared to 134 mg in HCD, Table 6.5-9). Therefore, the change in ovary weight was not considered treatment-related. At 24-months statistically-significant increases were seen in relative heart weight from the mid dose; a dose-response was evident and change compared to control was  $\geq 10\%$  for the top dose in males and from the mid dose in females. In absence of effects at 12 months and on the absolute values and as no concomitant histopathological findings were observed in the heart, the increase in relative heart weight was not considered toxicologically-significant.

At 12-months a statistically-significant increase ( $> 10\%$  change compared to control) in absolute and relative kidney weights was seen in males of the top dose. In addition non-neoplastic histopathological findings (eosinophilic droplets) were also observed in males of the top dose (Table 6.5-11). At the end of the carcinogenicity phase relative kidney weights were statistically-significantly increased ( $> 10\%$  change compared to control) at the top dose in both sexes (by 18 % in males and 11 % in females). A dose-response was evident, and for males the increased weight was accompanied by histopathological findings (mineralisation). The increased kidney weight with associated histopathology observed in top dose males at 12- and 24-months was likely due to  $\alpha_2\mu$ -globulin accumulation (as demonstrated in the 90-day study) and hence not relevant to humans. The increased relative kidney weight in top dose females at 24-months only was just above 10 % and hence not considered adverse. Statistically-significant increases in liver weight were observed at both 12- and 24-months. At 12-months this was seen in the top dose, with a relative change compared to control of  $> 15\%$  seen in males (absolute) and females (relative). At 24-months this was seen in relative liver weight from the mid dose, with a relative change compared to control of  $> 15\%$  seen in females at the top dose only. A dose-response was evident in relative liver weights at 12- and 24-months in both sexes. Histopathological findings in the liver were observed in the top dose in both sexes at 12- and 24-months and in females of the mid dose at 24-months. Overall, adverse ( $> 15\%$ ) increases in liver weight were seen at the top dose in both sexes.

Large changes in absolute and relative uterus weights were seen at 12- and 24-months, however, no dose-response and/or statistical-significance was observed. The values reflect the large variance of uterus weights within the oestrous cycle and are not considered treatment-related. Histopathological findings of the uterus are discussed below. A statistically-significant increase in relative brain weight was seen in animals of the top dose at 24-months; relative change compared to control was  $> 10\%$  in females, however, no concomitant histopathology was found. These changes in relative brain weight were most likely the consequence of the

decreased body weights and body weight gains observed at the top dose. A statistically-significant decrease in absolute spleen weight was seen at 24-months in females of the top dose; however, change compared to controls was < 10 % and no concomitant histopathology was found. Statistically-significant increases in relative testes and epididymides weight was seen at 24-months in males of the top dose. A dose-response was evident, and change compared to controls was > 10 % in the top dose; however, no concomitant histopathology was found. Therefore, these increases in testis and epididymis relative weights were most likely the consequence of the decreased body weights and body weight gains observed at the top dose.

Overall, only changes in liver weights were considered treatment-related and adverse at the top dose in males and females.

Table 6.5-6. Organ weights (12 month chronic toxicity group)

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Terminal body weight [g]	0	457.51				279.19			
	200	494.12	8			277.53	-1		
	1000	446.68	-2			256.33	-8		
	5000	467.88	2			243.88	-13		
Adrenal glands (mg)	0	53.2		0.012		75.0		0.027	
	200	52.3	-2	0.011	-8	71.6	-5	0.026	-4
	1000	51.7	-3	0.012	0	<b>63.9**</b>	-15	0.025	-7
	5000	55.6	5	0.012	3	<b>64.1*</b>	-15	0.026	-4
Brain (g)	0	2.214		0.489		2.033		0.737	
	200	2.217	0	0.452	-8	2.022	-1	0.75	2
	1000	2.186	-1	0.491	0	2.048	1	0.805	9
	5000	2.165	-2	0.466	-5	2.044	1	0.844	14
Epididymides (g)	0	1.212		0.266					
	200	1.242	2	0.254	-5				
	1000	1.175	-3	0.264	-1				
	5000	1.253	3	0.27	1				
Ovaries (mg)	0					140.4		0.051	
	200					96.8	-31	0.035	-31
	1000					77.5	-45	0.03	-41
	5000					82.2	-41	0.034	-33
Heart (g)	0	1.081		0.237		0.843		0.304	
	200	1.168	8	0.237	0	0.795	-6	0.294	-3
	1000	1.137	5	<b>0.254*</b>	7	0.773	-8	0.303	0
	5000	1.177	9	0.252	6	0.801	-5	<b>0.329*</b>	8
Kidneys (g)	0	2.489		0.545		1.775		0.64	
	200	2.597	4	0.528	-3	1.735	-2	0.635	-1
	1000	2.504	1	0.561	3	1.721	-3	0.675	5
	5000	<b>2.871*</b>	15	<b>0.615**</b>	13	1.699	-4	0.698	9
Liver (g)	0	9.217		2.014		6.192		2.227	
	200	9.873	7	1.997	-1	5.891	-5	2.154	-3
	1000	9.396	2	2.106	5	5.853	-5	2.292	3
	5000	<b>10.725*</b>	16	<b>2.293**</b>	14	6.376	3	<b>2.603**</b>	17
Spleen (g)	0	0.696		0.153		0.542		0.195	
	200	0.764	10	0.156	2	0.508	-6	0.184	-6
	1000	0.698	0	0.156	2	0.535	-1	0.209	7
	5000	0.701	1	0.15	-2	0.47	-13	0.193	-1
Testes (g)	0	3.888		0.855					
	200	4.119	6	0.84	-2				
	1000	3.801	-2	0.853	0				
	5000	3.953	2	0.852	0				

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Uterus (g)	0					0.95		0.347	
	200					1.384	46	0.542	56
	1000					1.087	14	0.42	21
	5000					0.733	-23	0.303	-13
Thyroid (mg)	0	30.2		0.007		25.5		0.009	
	200	33.2	10	0.007	1	23.6	-7	0.009	-4
	1000	30.8	2	0.007	4	23.3	-9	0.009	-1
	5000	35.3	17	0.008	14	22.8	-11	0.009	1

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$  (Kruskal-Wallis and Wilcoxon-test, two sided)

# Values may not calculate exactly due to rounding of figures

Table 6.5-7. Historical Control Data - ovary weights, in female Wistar rats, duration 12 months

	Min	Max	Mean
<b>Ovary</b>			
Absolute weight (mg)	70.700	103.600	84.613
Relative weight (%)	0.026	0.037	0.032

Ovary HCD: Total number of animals: 80, Total number of studies: 8, Study dates: 2011 – 2018, [REDACTED] data.

Route of application: feeding. (Same lab, species, strain, concurrent studies).

Table 6.5-8. Organ weights (24 month carcinogenicity group)

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Terminal body Weight [g]	0	569.243				338.58			
	200	<b>547.195*</b>	-4			318.502	-6		
	1000	<b>536.742*</b>	-6			323.868	-4		
	5000	<b>520.839**</b>	-9			<b>297.193**</b>	-12		
Adrenal glands (mg)	0	61.341		0.011		65.718		0.02	
	200	143.488	134	0.028	161	62.275	-5	0.02	1
	1000	59.634	-3	0.011	6	83.649	27	0.027	38
	5000	61.184	0	0.012	9	64.488	-2	0.022	11
Brain (g)	0	2.276		0.404		2.11		0.633	
	200	2.248	-1	0.417	3	2.088	-1	0.667	5
	1000	2.246	-1	0.433	7	2.09	-1	0.655	4
	5000	2.261	-1	<b>0.437**</b>	8	2.109	0	<b>0.722**</b>	14
Heart (g)	0	1.37		0.241		0.991		0.295	
	200	1.336	-2	0.246	2	0.989	0	0.314	7
	1000	1.362	-1	<b>0.257*</b>	7	1.033	4	<b>0.323**</b>	10
	5000	1.4	2	<b>0.269**</b>	12	0.982	-1	<b>0.333**</b>	13
Kidneys (g)	0	3.053		0.538		2.075		0.619	
	200	3.109	2	<b>0.57*</b>	6	2.043	-2	0.649	5
	1000	3.048	0	<b>0.577*</b>	7	2.136	3	0.669	8
	5000	3.312	8	<b>0.635**</b>	18	2.016	-3	<b>0.685**</b>	11
Liver (g)	0	12.175		2.139		7.313		2.18	
	200	11.61	-5	2.121	-1	<b>6.189*</b>	-7	2.157	-1
	1000	12.018	-1	<b>2.242*</b>	5	7.749	6	<b>2.42*</b>	11
	5000	12.573	3	<b>2.406**</b>	13	<b>7.771*</b>	6	<b>2.62**</b>	20

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Spleen (g)	0	1.062		0.187		0.747		0.224	
	200	0.995	-6	0.182	-3	0.668	-11	0.211	-6
	1000	0.983	-7	0.185	-2	0.709	-5	0.222	-1
	5000	0.985	-7	0.189	1	<b>0.733*</b>	-2	0.243	9
Testes (g)	0	4.209		0.745					
	200	4.200	0	0.776	4				
	1000	4.276	2	0.806	8				
	5000	4.286	2	<b>0.828**</b>	11				
Ovaries (mg)	0					1077.564		0.291	
	200					122.15	-89	0.04	-86
	1000					114.243	-89	0.035	-88
	5000					162.575	-85	0.053	-82
Epididymides (g)	0	1.23		0.218					
	200	1.238	1	0.229	5				
	1000	1.206	-2	0.228	4				
	5000	<b>1.334**</b>	8	<b>0.258**</b>	18				
Uterus (g)	0					1.299		0.41	
	200					1.539	18	0.501	22
	1000					1.188	-9	0.383	-7
	5000					4.611	255	1.319	222

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$  (Kruskal-Wallis and Wilcoxon-test, two sided)

# Values may not calculate exactly due to rounding of figures

Table 6.5-9. Historical Control Data - selected organ weights, in female Wistar rats, duration 24 months

	Min	Max	Mean	Standard deviation
<b>Liver</b>				
Absolute weight (g)	6.674	7.835	7.284	0.450
Relative weight (%)	2.094	2.312	2.200	0.088
<b>Ovary</b>				
Absolute weight (mg)	85.179	244.225	133.636	-
Relative weight (%)	0.025	0.07	0.04	-

Liver HCD: Total number of animals: 239, Total number of studies: 6, Study dates: 2015 – 2017, Laboratory data presented in the study report. Route of application: dietary. (Same lab, species, strain, concurrent studies).

Ovary HCD: Total number of animals: 232, Total number of studies: 6, Study dates: 2011 – 2017, Laboratory data presented in the study report. Route of application: dietary. (Same lab, species, strain, concurrent studies).

*Gross pathology:* There were no treatment-related gross pathology findings in the chronic phase. At 24-months the following gross necropsy findings were noted (Table 6.5-10): the number of females with ovarian cysts was increased in the top dose group, however, in the histopathology exam there was no difference in the incidence of this lesion between control and females of the top dose group. In the uterus, masses were observed in a higher number of females in all treatment groups but with no dose-response relationship. In males of the top dose group there was a higher incidence of size reduction in the epididymides, prostate and seminal vesicle. All affected animals died on test or were sacrificed in a moribund state (mortality was 24 % in top dose males compared to 12 % in controls). Therefore, this finding was regarded to have been caused by the poor general status of these animals and not as a direct effect of cinmethylin. Overall, there were no treatment-related gross pathology findings.

Table 6.5-10. Gross pathology findings (carcinogenicity group)

		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	9	45	242	0	11	59	317
No. of animals		50	50	50	50	50	50	50	50
Epididymides									
Organ Size Reduced				1	4	-			
Prostate									
Organ Size Reduced				1	6	-			
Seminal Vesicle									
Organ Size Reduced				1	7	-			
Uterus									
Mass		-				5	11	7	9
Ovaries									
Cyst		-				5	3	4	10

*Histopathology:*

*Non-neoplastic findings:* Non-neoplastic findings were observed at 12-months at the top dose; in the kidneys of males (eosinophilic droplets), liver (cytoplasmic alterations in males and centrilobular hypertrophy in both sexes), nasal cavity (degeneration/regeneration of the olfactory epithelium and proteinaceous exudate in the lumen in both sexes) and thyroid glands (hypertrophy/hyperplasia in males and altered colloid in both sexes) (Table 6.5-11). Findings in the liver, nasal cavity and thyroids were considered treatment-related and adverse. Findings in the kidneys (eosinophilic droplets) were previously shown to be due to accumulation of  $\alpha 2\mu$ -globulin, a male rat specific phenomenon of no relevance to humans. All other findings were considered to be incidental and not treatment-related.

Table 6.5-11. Selected non-neoplastic findings (12-months) - incidences shown

		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	10	51	265	0	13	69	351
No. of animals examined		10	10	10	10	10	10	10	10
Kidneys									
Eosinophilic droplets		0	0	0	5	0	0	0	0
Grade 1		-	-	-	5	-	-	-	-
Liver									
Alteration, cytoplasmic		0	0	0	3	0	0	0	0
Grade 1		-	-	-	1	-	-	-	-
Grade 2		-	-	-	2	-	-	-	-
Hypertrophy, centrilobular		0	0	1	4	0	0	0	6
Grade 1		-	-	1	4	-	-	-	6
Nasal cavity, level III									
Degeneration/regeneration, olfactory epithelium		0	0	0	10	0	0	0	10
Grade 2		-	-	-	4	-	-	-	1
Grade 3		-	-	-	6	-	-	-	9
Exudate, proteinaceous		0	0	0	10	0	0	0	10
Grade 1		-	-	-	4	-	-	-	5
Grade 2		-	-	-	6	-	-	-	4
Grade 3		-	-	-	-	-	-	-	1

		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	10	51	265	0	13	69	351
Thyroid gland									
Hypertrophy / hyperplasia		0	0	0	3	0	0	0	0
Grade 1		-	-	-	2	-	-	-	-
Grade 2		-	-	-	1	-	-	-	-
Altered colloid		1	2	4	8	0	1	1	6
Grade 1		-	2	2	5	-	1	1	4
Grade 2		1	-	-	2	-	-	-	2
Grade 3		-	-	2	1	-	-	-	-

Similar non-neoplastic findings were observed at 24-months, mainly at the top dose but also, on occasion from the mid dose (Table 6.5-12). Findings in the kidneys (mineralisation) in males were considered related to  $\alpha_2\mu$ -globulin accumulation and hence not relevant to humans. Findings in the liver at the top dose - cytoplasmic alterations in males, centrilobular or periportal hypertrophy in females, pigment (found to be lipofuscin) in hepatocytes of the periportal region in both sexes and in the centrilobular area in females from 1000 ppm – were considered treatment-related. There was also an increase in the incidence of multinucleated hepatocytes in top dose males. Findings in the nasal cavity level III at the top dose in females and from the mid dose in males - degeneration/regeneration of the olfactory epithelium, proteinaceous exudate, respiratory epithelium (metaplasia) and inflammation - were considered treatment-related. Findings in the thyroid at the top dose - an increase in follicular cell hyperplasia in males and an increase in altered colloid in males and females - were also considered treatment-related. Other findings seen at the top dose - increase in Harderian gland alteration in the extraorbital lacrimal gland (36 versus 44), higher incidence of alveolar histiocytosis (22 versus 32), higher incidence of size reduction in the prostate and the seminal vesicle, higher incidence of cystic/papillary hyperplasia (24 versus 34) and increased number of parasites observed (1 versus 9) - were not considered treatment-related because they were either common age-related findings or caused by the generalised toxicity noted at the top dose. Overall, treatment-related and adverse non-neoplastic findings were observed in the liver, nasal cavity and thyroid in rats, at both 12- and 24-months, in females at the top dose and in males from the mid dose.

Table 6.5-12. Selected non-neoplastic findings (24 months) - incidences shown

		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	9	45	242	0	11	59	317
Kidneys									
No. of animals examined		50	10	11	50	50	12	18	50
Mineralisation, tubular		1	1	0	8*	3	0	1	2
Grade 1		-	-	-	8				
Mineralisation, papilla		0	0	1	7*	8	1	5	7
Grade 1		-	-		4				
Grade 2		-	-		3				
Liver									
No. of animals examined		50	50	50	50	50	50	50	50
Alteration, cytoplasmic		0	0	0	18*	0	0	0	0
Grade 1		-	-	-	6	-	-	-	-
Grade 2		-	-	-	9	-	-	-	-
Grade 3		-	-	-	3	-	-	-	-
Hypertrophy, centrilobular		0	0	0	1	1	1	2	12*
Grade 1		-	-	-	-	1	-	2	10
Grade 2		-	-	-	1	-	1	-	2

		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	9	45	242	0	11	59	317
Hypertrophy, periportal		0	0	0	0	1	0	1	7*
	Grade 1	-	-	-	-	1	-	1	5
	Grade 2	-	-	-	-	-	-	-	2
Pigment storage, periportal		0	0	0	24*	0	0	0	15*
	Grade 1	-	-	-	17	-	-	-	12
	Grade 2	-	-	-	7	-	-	-	3
Pigment storage, centrilobular		0	0	2	1	7	10	22**	27**
	Grade 1	-	-	-	1	6	9	20	21
	Grade 2	-	-	2	-	1	1	2	6
Multinucleated hepatocytes		8	8	5	17*	17	12	9	23
	Grade 1	6	7	5	13	12	7	6	17
	Grade 2	1	1	-	3	5	3	3	6
	Grade 3	1	-	-	1	-	1	-	-
	Grade 4	-	-	-	-	-	1	-	-
Nasal cavity, level III									
No. of animals examined		50	50	50	50	50	50	50	50
Degeneration/regeneration, olfactory epithelium		0	0	2	50**	0	0	0	50**
	Grade 1			1					3
	Grade 2			1	14				23
	Grade 3				23				23
	Grade 4				14				1
Exudate, proteinaceous		0	0	0	20**	0	0	0	30**
	Grade 1				7				10
	Grade 2				10				15
	Grade 3				3				3
	Grade 4				-				2
Metaplasia, respiratory epithelium		0	0	0	14**	0	0	0	12**
	Grade 1				5				7
	Grade 2				8				3
	Grade 3				1				2
Inflammation, (multi)focal		2	4	5	12**	1	3	0	8*
	Grade 1	1	1	2	3	-	1	-	-
	Grade 2	-	2	3	5	1	2	-	4
	Grade 3	-	1	-	2	-	-	-	4
	Grade 4	2	-	-	2	-	-	-	-
Thyroid gland									
No. of animals examined		50	50	50	50	50	49	50	49
Hyperplasia, follicular cell		2	2	4	10*	3	2	2	5
Altered colloid		7	4	12	24**	5	6	7	33**
	Grade 1	3	2	8	6	5	4	5	24
	Grade 2	4	1	4	18	-	2	2	8
	Grade 3	-	1	-	-	-	-	-	1



		Males				Females			
Dose level	[ppm]	0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	9	45	242	0	11	59	317

\*.  $p \leq 0.05$ ; \*\*.  $p \leq 0.01$

*Neoplastic findings:* Tumours were noted in the uterus and liver. An increase in the incidence of endometrial adenocarcinoma was seen in all treated animals, rising to a statistically-significant level (16 % vs 4 % in controls) in the top dose group (Table 6.5-13). HCD was submitted from the same species, strain and laboratory, showing that values lie within the range of the HCD (2 - 30 %). The incidence of endometrial adenocarcinoma in the control group was noted as being low. However, HSE notes that the dates of the HCD (2001 – 2015) go beyond what is considered contemporaneous (e.g. a period of 5 years centred as closely as possible on the date of the index study (conducted in 2015-2017)) and that incidence in the top dose group is just above the mean value (15.1 %) from the HCD. For this reason HSE acknowledges the lower relevance of these HCD. A re-evaluation of the HCD, using only studies dated between 2009 – 2015 was made and a new lower mean – 13.6 % was calculated (Table 6.5-13). HSE notes that the value obtained in the top dose (16 %) is higher than the time-relevant HCD mean but still well within the top end of the time-relevant HCD range (2 – 20 %). It is noted that the concurrent control incidences were low compared to the HCD (both submitted by the applicant and re-evaluated based on dates). Overall, an increase in uterus adenocarcinoma was seen at the top dose; however, as this was well within relevant and valid HCD range, it was not considered treatment-related, but part of normal variation. HSE also notes that no uterus tumours were observed in the older carcinogenicity study in Fisher F344 rats (although a lower dose was used) or in mice and that the uterus is not a target organ of toxicity of cinmethylin.

A similar increase, rising to a statistically-significant level (28 % vs 12 % in controls) was seen at the top dose for endometrial stromal polyps (Table 6.5-13). Again the dates of the HCD are wider than 5 years of the study. The incidence seen for the top dose group (28 %) was greater than the HCD mean (16 %) but still well within the HCD range (4 % - 38 %). However, the re-evaluated HCD mean (22 %), using studies only dated between 2009 – 2015, is closer to the incidence seen in the top dose group. Overall, an increase in endometrial stromal polyps was seen at the top dose; however, as this was well within relevant and valid HCD, it was not considered treatment-related, but part of normal variation.

The incidence of liver cell carcinoma in females of the top dose group was minimally increased, with 3 (6 %) occurrences vs 1 (2 %) in controls. This was within the HCD range of 0 - 6 % presented in the study report; however HSE notes these HCD (1999 – 2015) go beyond what is considered contemporaneous (e.g. a period of 5 years centred as closely as possible on the date of the study (conducted in 2015 – 2017)). Re-evaluation of the HCD, using only studies dated between 2009 - 2015 (5 years before the current study) was made and a new range, 0 – 4 % was calculated (Table 6.5-13). When questioned on the relevancy of the HCD between 1999 – 2015 the applicant responded stating: ‘This low number of studies [conducted in the period of 5 years before the study] is not appropriate to cover all rare findings. No further carcinogenicity study has been finalised and no further in house data are ready to be used.’. To support the in-house HCD the applicant submitted HCD from the Rita database which listed 9 studies (same species, strain and sex) conducted in the period of 10 years before the study date. These Rita database studies showed a hepatocellular carcinoma mean incidence of 1.9 % and max of 5.6 %, from 3 females with hepatocellular carcinomas, in a group of 54 control animals. HSE has considered data from the Rita database as part of a weight of evidence approach (rather than in isolation) and notes that it is collected from different laboratories. The Rita database supports the statement that liver carcinomas are rare findings in rats. Therefore a treatment-related increase (above appropriate HCD) in liver carcinoma in females at the top dose cannot be excluded.

Liver hyperplasia was not recorded at 12-months. Hyperplasia at 24-months (Table 6.5-15) was increased in females from the mid-dose but did not show a clear dose-response and is unlikely to be related to treatment with cinmethylin. It is noted that in females liver adenomas were present in controls (6 %) but not in treated animals. The liver is clearly a target organ of toxicity of cinmethylin in rats, however, there are inconsistencies in the evidence for carcinogenicity, including a lack of hyperplasia and adenomas.

The numbers of animals with neoplasms (benign, malignant, systemic and metastasised) and the total numbers of neoplasms (primary, benign, malignant, systemic and metastasised) were comparable between control and high dose groups (Table 6.5-14) and therefore not considered treatment-related.

Overall, with the exception of a marginal increase in liver carcinoma in females only at the top dose of 5000 ppm (317 mg/kg bw/d), there were no other treatment-related tumour findings in this study. To conclude, there is equivocal evidence of carcinogenicity in rats.

Table 6.5-13. Selected neoplastic findings (24-months) - incidence

Dose level	[ppm]	Females			
	[mg/kg bw/d]	0	200	1000	5000
No. of animals		50	50	50	50
UTERUS exam.		50	50	50	50
Adenocarcinoma, endometrial		2 (4 %)	6 (12 %)	6 (12 %)	8* (16 %)
HCD#		Mean (14 studies): 15.1% (2 - 30%) Mean (5 studies between 2009-2015): 13.6 % (2 - 20 %)			
Polyp, endometrial stromal		6 (12 %)	11 (22 %)	10 (20 %)	14* (28 %)
HCD#		Mean (11 studies): 16% (4% - 38%) Mean (5 studies between 2009-2015): 22 % (12 - 38 %)			
LIVER exam.		50	50	50	50
Carcinoma, hepatocellular		1 (2 %)	0 (0 %)	1 (2 %)	3 (6 %)
HCD&		Mean (13 studies between 1999-2015): 1.7 % (0 - 6 %) Mean (4 studies between 2009-2015): 1.5 % (0 - 4 %)			
Adenoma, hepatocellular		3 (6 %)	0 (0 %)	0 (0 %)	0 (0 %)
HCD&		Mean (13 studies between 1999-2015): 1.5 % (0 - 6 %) Mean (4 studies between 2009-2015): 3.5 % (0 - 6 %)			

Statistical analysis: \*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$  (Fisher's Exact test, 1-sided)

#: Historical control data (HCD) from 2 year studies in Wistar rats (supplier: [REDACTED]) run at the test facility between 2001-2015 for adenocarcinoma, and 2004-2015 for endometrial stromal polyp.

&Historical control data from 24-month studies in Wistar rats run at the test facility between 1999 – 2015 for hepatocellular carcinoma.

Liver carcinomas are rare findings in rats. HSE notes that the dates of HCD is greater than 5 years around the study dates (2015-2017). Therefore the HCD was re-analysed to cover a period of 5 years centred as closely as possible on the date of the study (as for this current (new) study HCD after the study dates is not available).

Table 6.5-14. Total incidence of neoplastic findings (24-months)

Dose level	[ppm]	Males				Females			
	[mg/kg bw/d]	0	200	1000	5000	0	200	1000	5000
No. of animals		50	50	50	50	50	50	50	50
Number of animals with:									
- neoplasms		37	26	32	42	42	40	37	44
- 1 primary neoplasm		20	16	17	20	15	18	22	14
- 2 and > primary neoplasms		17	10	15	22	27	22	15	30
Number of animals with:									
- benign neoplasms		27	20	25	35	39	37	31	40
- benign neoplasms only		22	15	19	25	27	25	19	27
- malignant neoplasms		15	11	13	17	15	15	18	17
- malignant neoplasms only		10	6	7	7	3	3	6	4
- systemic neoplasms		-	1	2	1	1	1	-	-
- metastasized neoplasms		1	2	3	3	7	4	5	6
Total number of:									
- primary neoplasms		69	38	50	82	96	77	59	99
- benign neoplasms		52	27	37	60	74	57	41	78

- malignant neoplasms	17	11	13	22	22	20	18	21
- systemic neoplasms	-	1	2	1	1	1	-	-
- metastasized neoplasms	1	2	3	3	7	4	5	7

Table 6.5-15. Incidence of liver hyperplasia (24-months)

Dose level	[ppm]	Males				Females			
		0	200	1000	5000	0	200	1000	5000
	[mg/kg bw/d]	0	9	45	242	0	11	59	317
No. of animals		50	50	50	50	50	50	50	50
Number of animals with:									
Hyperplasia, hep. regenerative		1	-	-	-	-	-	-	1
Hyperplasia, hep. non-regenerative		-	2	-	-	1	2	6	5
Hyperplasia, bile duct, dif		6	2	5	1	16	16	16	6
Hyperplasia, bile duct, (m)f		1	2	3	1	4	1	2	3
Hyperplasia, bile duct, cysti		1	-	1	-	1	3	2	4

Liver hyperplasia was not recorded in the satellite group (12-months).

(m)f: multi(focal)

### Conclusion

In a standard guideline chronic/carcinogenicity study, cinmethylin was administered via the diet to male and female Wistar rats for 2-years. Cinmethylin induced a marginal increase in liver carcinoma in females only at the top dose of 5000 ppm (317 mg/kg bw/d), providing equivocal evidence of carcinogenicity in the female rat. Therefore **the NOAEL for carcinogenicity in the rat is 1000 ppm (59 mg/kg bw/d)**. HSE notes that the carcinogenicity response observed is very weak, sex- and species-specific, and that although the liver is a target organ of toxicity in the rat, there was no clear evidence of pre-neoplastic lesions. It is also noted that the incidence of liver carcinoma was within the extended laboratory HCD range and the Rita database HCD. Overall, there is insufficient evidence to classify cinmethylin for carcinogenicity. Classification for carcinogenicity is considered further in the aligned MCL dossier.

Systemic toxicity was observed in both sexes at the top dose (5000 ppm, equivalent to 242 and 317 mg/kg bw/d in M/F respectively) which indicated that the MTD was reached. Body weight gain was reduced by > 10 % at 24-months. Consistent with the findings of repeated-dose studies, the liver was identified as a target organ of toxicity; relative liver weights were increased (> 15 %) at the top dose and concomitant histopathology (cytoplasmic alterations in males and centrilobular hypertrophy in both sexes) was found at 12- and 24-months. In addition, GGT was increased at the top dose in both sexes. Adverse thyroid histopathology (follicular cell hyperplasia and colloid alteration) was seen at the top dose in males and females. Nasal cavity findings were considered treatment-related and adverse at the top dose in females and from the mid dose in males. Kidney toxicity (increased weight and histopathology) of no relevance to humans was also seen in males at the top dose.

In conclusion, there were adverse effects on body weight gain, the liver and thyroid at the top dose in both sexes and effects on nasal cavities from the mid dose in males and at the top dose in females. Therefore **the NOAEL for systemic chronic toxicity in the rat is 200 ppm (9 mg/kg bw/d) in males.**

(██████████, 2018)

## 2) Old study

The following summarises a single study which was split into several reports (original and supplemental submissions) with several references.

<b>Author(s)</b>	<ul style="list-style-type: none"> <li>• [REDACTED]</li> <li>• [REDACTED]</li> <li>• [REDACTED]</li> <li>• [REDACTED]</li> </ul>
<b>Study title</b>	<ul style="list-style-type: none"> <li>• A 2 year feeding study with SD95481 in rats - [REDACTED], 1985</li> <li>• A 2 year feeding study with SD95481 in rats corrigendum – [REDACTED], 1991</li> <li>• Preparation of supplement for submission to the Japanese Ministry of Agriculture, Forestry and Fisheries from Shell Group research report SBGR.85.084 (a 2 year feeding study with SD 95481 in rats) - [REDACTED], 1991a</li> <li>• Preparation of supplement for submission to the Japanese Ministry of Agriculture, Forestry and Fisheries from Shell Group research report SBGR.85.084 (a 2 year feeding study with SD 95481 in rats) - [REDACTED], 1991b</li> </ul>
<b>Study reference</b>	<ul style="list-style-type: none"> <li>• [REDACTED], 1985 - CI-427-001</li> <li>• [REDACTED], 1991 - CI-427-007</li> <li>• [REDACTED], 1991a - CI-427-008</li> <li>• [REDACTED], 1991b - CI-427-006</li> </ul>
<b>Test facility</b>	[REDACTED]
<b>Dates of work</b>	04-Jan-1983 – 01-Feb-1985
<b>Test substance</b>	SD95481 (Cinmethylin)
<b>Purity (%)</b>	92
<b>Batch no.</b>	5-4-0-0, 513F; ST82/255
<b>Test organisms</b>	Rat Fischer 344 Males and females
<b>Groups</b>	50/sex/dose (2-year groups – carcinogenicity group) 15/sex/dose (18-month groups – chronic toxicity group) 10/sex/dose (6 and 12-month groups – chronic toxicity group).
<b>Dose/concentration</b>	0, 30, 100 and 3000 ppm. Equivalent to 0, 1.4, 4.7 and 144.2 mg/kg bw/d in males and 0, 1.7, 5.8 and 177.4 mg/kg bw/d in females.
<b>Route</b>	Administered orally, via the diet, daily, for a period of 6, 12, 18 or 24 months.
<b>Vehicle</b>	Acetone <sup>4</sup> .
<b>GLP</b>	Not compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	<p>Compared with the currently valid OECD TG 453 (2009):</p> <ul style="list-style-type: none"> <li>• The spacing for the top dose was greater than 10-fold, not matching the recommended 2-4-fold interval.</li> <li>• Detailed clinical observations outside the home cage, preferably in a standard arena, were not performed in this study.</li> <li>• For clinical pathology (haematology, clinical chemistry, urinalysis), no values were determined prior to treatment initiation.</li> <li>• The clinical chemistry parameter creatinine was not determined in this study.</li> <li>• At termination, epididymides, ovaries and thyroids weights were not determined in animals of chronic toxicity groups.</li> <li>• Generally, except for some neoplastic findings, laboratory historical control data are lacking.</li> </ul>
<b>Impact of</b>	For clinical pathology (haematology, clinical chemistry, urinalysis), as no values were

<sup>4</sup> Studies to determine the solubility of cinmethylin in DMSO and acetone have been submitted and are evaluated in detail in Volume 3 – B.2, section B.2.6. Cinmethylin was found to be readily soluble in acetone, as confirmed by a GLP study.

<b>deviations</b>	determined prior to treatment initiation and together with the absence of historical control data, the interpretation of whether an effect is induced by the test-item application is more difficult. Despite the above listed deviations, this study, together with the new/modern rat study (■■■■■, 2018), is considered to contribute in a WoE approach to the carcinogenic hazard assessment of cinmethylin.
<b>Acceptable</b>	Yes in a WoE approach.
<b>Conclusion</b>	Cinmethylin demonstrated no carcinogenic potential.
<b>NOAEL</b>	NOAEL for carcinogenicity: 3,000 ppm, equivalent to 144 and 177 mg/kg bw/d in males and females respectively. NOAEL for systemic chronic toxicity: 100 ppm, equivalent to 4.7 and 5.8 mg/kg bw/d in males and females respectively.
<b>Effects at the LOAEL</b>	Carcinogenicity: N/A - Cinmethylin demonstrated no carcinogenic potential. Systemic chronic toxicity: Increased mortality and clinical signs of toxicity in males, decrease in food consumption, body weight and body weight gain, haematological effects, increases in liver weights with concomitant clinical chemistry (increases in GGT) and histopathology at the top dose (3,000 ppm, equivalent to 144 and 177 mg/kg bw/d in males and females respectively). Kidney toxicity of no relevance to humans was also observed in males at the top dose.

### Methods

In a non-GLP compliant and non OECD test guideline compliant study, cinmethylin was administered to groups of male and female Fischer 344 rats at dietary dose levels of 0, 30, 100 and 3000 ppm for 6, 12, 18 months (chronic toxicity groups; 10/15 animals/sex/dose) and 2 years (carcinogenicity groups; 50 animals/sex/dose). Diets were fortified with 3 ppm vitamin K<sup>5</sup>. Dietary concentrations corresponded to mean substance intakes of 1.4, 4.7 and 144.2 mg/kg bw/d in males and 1.7, 5.8 and 177.4 mg/kg bw/d in females (Table 6.5-16). Clinical signs of toxicity were monitored at least daily, body weights and food consumption measured weekly for 14-weeks and thereafter every other week for 2-years. Ophthalmoscopic examinations were performed on the 2-year study groups on control and top dose animals at 0, 12 and 24 months, and on mid dose males at 24 months. Blood samples were taken at necropsy for haematology and clinical chemistry analyses, though blood glucose was assayed ~2 weeks before scheduled sacrifice. Urinalysis was performed on samples collected from designated animals of each sex and dose group over a 16-hour period at 2.5 - 4 weeks prior to necropsy. All rats were subjected to macroscopic necropsy and major organs were weighed and assessed histopathologically. The periportal areas of the livers of 2 rats/sex in the 12-month control and top dose groups were additionally examined by electron microscopy. A method for the analysis of cinmethylin in foodstuffs was evaluated and was regarded as fit for purpose (see Volume 3 CA B5, section B.5.1.2).

Table 6.5-16. Mean test substance intake

	Males				Females			
Dose [ppm]	0	30	100	3000	0	30	100	3000
Dose [mg/kg bw/d]								
Week 0-15, daily mean	0	1.99	6.60	198.94	0	2.36	7.90	234.47
Week 16-104, daily mean	0	1.24	4.12	127.40	0	1.55	5.19	160.46
Mean cinmethylin intake	0	1.4	4.7	144.2	0	1.7	5.8	177.4

\*Re-calculation based on weekly data for food consumption and body weight

### Results

The stability and homogeneity of cinmethylin in the diet was confirmed in a separate study using a validated method of analysis.

<sup>5</sup> The vitamin K1 requirement of rats is 50 mg/kg feed. Vitamin K supplementation is standard in feed. The amount of vitamin K in the used in the study diets was 10 ppm. However, it was thought that most of the vitamin K was lost during the manufacturing process, therefore, diets were fortified at mixing with additional 3 ppm vitamin K, to guard against vitamin K deficiency by less than recommended levels in the diet.

**Mortality:** No animals of the 6- and 12-month groups died during the administration period. No treatment-related increase in mortality was observed in the 18-month group (Table 6.5-17). At the end of the study (2 years), the mortality of males (54 – 64 %) was higher than that of the females (30 – 42 %), and an increase was seen in the top dose compared to controls (64 % in the top dose, compared to 56 % in controls). Survival of males of the top dose (36 %) was both lower than the control (44 %) and also outside the NTP HCD range (46 – 78 %, Table 6.5-18). Whereas survival of the high dose females (70 %) was higher than that of other groups (58 – 62 %). A clear dose-response relationship was lacking for both sexes, however, a treatment-related effect on mortality was seen in males at the top dose. Chronic nephropathy was a major cause of death for males during the study.

Table 6.5-17. Mortality and survival

		Males				Females			
Dose level	[ppm]	0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
18-months									
No. of animals		15	15	15	15	15	15	15	15
Survival [no. animals] [%]		12 (80)	14 (93)	13 (87)	12 (80)	12 (80)	14 (93)	12 (80)	12 (80)
Mortality [no. animals] [%]		3 (20)	1 (7)	2 (13)	3 (20)	3 (20)	1 (7)	3 (20)	3 (20)
Chronic renal disease		0	0	0	1	0	0	1	1
2-years									
No. of animals		50	50	50	50	50	50	50	50
Survival [no. animals] [%]		22 (44)	22 (44)	23 (46)	18 (36)	29 (58)	31 (62)	30 (60)	35 (70)
Mortality [no. animals] [%]		28 (56)	28 (56)	27 (54)	32 (64)	21 (42)	19 (38)	20 (40)	15 (30)
Chronic renal disease		12	8	11	22	3	3	4	3

Table 6.5-18. HCD - survival rate at the end of a 2-year exposure period

Sex	Mean [%]	Min [%]	Max [%]
Male	66 (1142/1729)	46	78
Females	73 (1295/1778)	50	84

N – number of individuals

NTP (Haseman *et al.*, 1985)

NTP = National Toxicology Program; HCD are based on 40 carcinogenicity feeding studies each using 50 F344 rats per dose group (in total, data on 1936 male and 1983 female rats), performed between 1977 and 1987 at the National Cancer Institute (NCI) and the National Toxicology Program in US.

HSE notes that while the same species and strain were used, and the date range (1977 – 1987) is more or less concurrent with the study under evaluation (conducted in 1983 – 1985), these data are from a wide number of laboratories.

**Clinical signs of toxicity:** At 2-years, particular males of the high dose group (3000 ppm) lost weight and developed a hunched appearance and pale eyes. Overall, treatment-related clinical signs (hunched appearance and pale eyes) were observed in males at the top dose.

**Ophthalmoscopy:** Despite a relatively high incidence of spontaneous or acquired ocular disease across all groups including controls, no ocular abnormality was detected which was considered treatment-related.

*Body weight, food consumption:* Body weights were statistically-significantly reduced in the top dose in males (during the first 2 weeks and from week 64 to the end of the administration period) and females (most weeks). Body weight gain was reduced at the top dose in males - with an overall reduction of 20 %, and females – with a consistent reduction by ~10 % and an overall reduction of 9 %. This depression of body weight gain supports the adequacy of the top dose. Food consumption followed the same pattern of reduction – lower than controls in males (during the first 2 weeks and from week 64 to the end of the administration period, occasionally statistically-significant) and females (statistically-significantly reduced for most weeks). There were no treatment-related effects on body weight, body weight gain and food consumption in the low and mid doses of both sexes. Overall, there was a treatment-related and adverse reduction in food consumption, body weight and body weight gain at the top dose in both sexes.

Table 6.5-19. Body weight and body weight gain - 24-month group

		Males				Females			
Dose level	[ppm]	0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
Body weight [g]									
Week 0		138.4	140.6	138.9	138.8	108.7	109.0	109.3	108.6
Week 1		170.3	170.4	170.9	168.6**	125.6	125.5	125.5	123.8**
Δ% <sup>#</sup>			+0.1	+0.4	-1.0		-0.1	-0.1	-1.4
Week 2		197.2	197	197.5	194.3**	138.3	138.0	137.7	135.6**
Δ% <sup>#</sup>			-0.1	+0.2	-1.5		-0.2	-0.4	-2.0
Week 4		235.7	234.9	235.9	233.0	155.2	154.9	155.2	151.0**
Δ% <sup>#</sup>			-0.3	+0.1	-1.1		-0.2	±0.0	-2.7
Week 13		317.1	315.4	316.4	315.9	187.5	187.3	186.8	179.8**
Δ% <sup>#</sup>			-0.5	-0.2	-0.4		-0.1	-0.4	-4.1
Week 26		374.5	374.1	373.0	372.0	214.5	215.6	214.4	203.0**
Δ% <sup>#</sup>			-0.1	-0.4	-0.6		+0.5	-0.1	-5.4
Week 52		413.0	413.0	410.0	408.4	251.7	254.0	254.5	233.9**
Δ% <sup>#</sup>			±0.0	-0.7	-1.1		+0.9	+1.1	-7.1
Week 64		418.1	420.2	415.0	410.9*	273.0	278.2	277.8	250.7**
Δ% <sup>#</sup>			+0.5	-0.7	-1.7		+1.9	+1.8	-8.2
Week 74		424.7	422.9	419.9	410.4**	285.6	289.2	288.3	263.9**
Δ% <sup>#</sup>			-0.4	-1.1	-3.4		+1.3	+0.9	-7.6
Week 104		356.4	367.3	362.0	313.5**	280.9	287.6	286.6	265.8**
Δ% <sup>#</sup>			+3.1	+1.6	-12.0		+2.4	+2.0	-5.4
Body weight gain [g]									
Week 0-4		97.6	94.3	97.0	94.2	46.5	45.9	45.9	42.4
Δ% <sup>#</sup>			-3.4	-0.6	-3.5		-1.3	-1.3	-8.8
Week 0-13		179.0	174.8	177.5	177.1	78.8	78.3	77.5	71.2
Δ% <sup>#</sup>			-2.3	-0.8	-1.1		-0.6	-1.6	-9.6
Week 0-26		236.4	233.5	234.1	233.2	105.8	106.6	105.1	94.4
Δ% <sup>#</sup>			-1.2	-1.0	-1.3		+0.8	-0.7	-10.8
Week 0-52		274.6	272.4	271.1	269.6	143.0	145.0	145.2	125.3
Δ% <sup>#</sup>			-0.9	-1.4	-1.9		+1.4	+1.5	-12.4
Week 0-74		286.6	282.3	281.0	271.6	176.9	180.2	179.0	155.3
Δ% <sup>#</sup>			-1.5	-2.0	-5.2		+1.9	+1.2	-12.2
Week 0-104		218.0	226.7	223.1	174.7	172.2	178.6	177.3	157.2
Δ% <sup>#</sup>			+4.0	+2.3	-19.9		+3.7	+3.0	-8.7

$\Delta\%$  – percent change compared to control.

$\#$  Difference to the control group in percent; values may not calculate exactly due to rounding of figures

Statistical evaluation: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Williams' test

*Haematology:* The statistically-significant increase in platelet counts observed throughout the study in females of the top dose points to mild thrombocytosis (Table 6.5-20). At 2 years this group had a greater blood coagulation efficiency. Since the thrombocytosis was mild it was not associated with alterations of the bone

marrow. Platelet counts were statistically-significantly increased also in males of the top dose at 6 months only. Signs of mild normochromic normocytic anaemia were also noted at 6 months at the top dose, evident in statistically-significant reductions in red blood cell count (RBC), haemoglobin (HGB) and haematocrit (HCT) concentrations. However, the anaemia was not considered to be of sufficient severity to induce reticulocytosis. Shortened coagulation time was observed at the top dose – decreased Activated Partial Thromboplastin Time (APPT) (both sexes) and Prothrombin Time (PT) (females only). Whilst these effects on coagulation time were considered treatment-related, they were likely to be secondary to induced enzyme activity in the liver triggering increased biosynthesis of coagulation factors. Polymorphic neutrophil/lymphocyte reversal, typical of ageing rats, was also evident at the top dose in both sexes, becoming more marked and gaining statistical significance at 24-months. Overall, there were adverse and treatment-related haematological effects indicative of mild anaemia and thrombocytosis at the top dose in both sexes.

Table 6.5-20. Selected clinical haematology parameters

Dose level	[ppm]	Males				Females			
		0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
Parameter	Months								
Platelets [10 <sup>9</sup> /L]	6	603.5	635.6	643.3	<b>820.4**</b>	624.5	620.6	617.2	<b>759.5**</b>
	12	713.5	668.7	756.2	677.5	605.1	601.9	606.7	<b>775.5**</b>
	18	777.8	712.8	765.3	818.3	598.4	640.3	607.2	<b>739.4**</b>
	24	890.8	908.4	814.7	943.3	664.5	710.9	709.3	<b>822.5**</b>
APPT [seconds]	6	16.94	17.12	16.87	16.32	15.71	17.13	15.79	17.57
	12	17.16	16.56	17.59	17.33	18.88	18.54	19.06	18.76
	18	20.48	20.15	19.91	<b>18.55**</b>	19.65	20.35	20.71	19.00
	24	18.85	19.78	18.98	17.80	20.50	20.39	19.83	<b>18.94**</b>
PT [seconds]	6	12.05	12.16	12.25	12.06	11.34	11.47	11.20	11.30
	12	12.81	12.79	12.79	12.71	11.69	11.42	11.55	11.49
	18	11.72	12.02	11.84	11.74	11.17	11.03	11.17	11.02
	24	10.96	10.92	10.92	10.92	10.71	10.50	10.63	<b>10.42*</b>
WBC [10 <sup>9</sup> /L]	6	2.78	2.70	2.73	2.61	2.16	2.33	2.08	2.17
	12	2.73	2.65	2.49	2.76	2.13	2.17	1.90	2.03
	18	3.60	<b>2.74*</b>	<b>2.75*</b>	3.68	1.72	1.84	1.93	2.07
	24	4.76	4.55	4.37	4.67	2.42	2.45	2.72	<b>2.99*</b>
Lymphocytes [%]	6	81.4	81.2	75.0	76.9	78.8	78.1	78.7	80.0
	12	57.7	62.2	59.8	53.3	68.0	62.2	68.2	67.8
	18	47.8	55.5	50.4	43.7	53.8	58.1	52.4	53.5
	24	35.1	32.9	35.9	<b>26.8**</b>	37.4	37.0	36.7	<b>30.4**</b>
Polymorphic neutrophils [%]	6	16.0	15.7	21.1	19.7	17.0	17.0	18.0	18.0
	12	37.4	30.8	33.5	40.3	26.2	31.0	25.4	26.5
	18	46.5	40.8	44.9	50.4	41.3	37.6	42.4	41.0
	24	55.1	58.0	55.6	<b>65.6*</b>	51.1	52.8	51.3	<b>57.9*</b>
Eosinophils [%]	6	0.5	0.8	1.0	0.6	1.3	1.6	<b>0.3*</b>	0.5
	12	1.1	1.2	1.2	0.6	1.1	1.1	0.8	1.6



Dose level	[ppm]	Males				Females			
		0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
Parameter	Months								
	18	1.5	1.1	1.5	0.9	0.8	0.8	1.0	1.0
	24	0.8	0.8	0.8	0.8	0.7	0.6	0.9	0.8
RBC [10 <sup>12</sup> /L]	6	8.22	8.17	8.03	<b>7.67**</b>	8.36	8.39	8.22	8.27
	12	8.24	8.50	8.00	8.49	8.08	7.96	8.14	7.98
	18	7.52	7.58	7.61	7.51	7.68	7.50	7.63	7.65
	24	6.80	6.90	7.18	6.66	7.48	7.52	7.24	7.56
Erythrocyte diameter [μm]	3	5.8	5.8	5.8	5.8	5.8	5.9	5.8	5.8
	6	5.7	5.7	5.7	5.7	5.7	5.8	5.8	5.7
	18	5.5	5.6	5.4	5.5	5.8	5.9	5.9	5.7
	24	5.6	5.6	5.5	5.5	5.9	5.9	5.9	<b>5.8**</b>
HGB [g/dL]	6	15.34	15.46	15.11	<b>14.35**</b>	15.80	15.92	15.59	15.52
	12	15.49	15.84	15.23	15.77	15.95	15.79	16.02	15.59
	18	14.61	14.84	14.85	14.40	15.26	15.07	15.25	15.01
	24	13.30	13.47	14.04	12.65	14.94	14.92	14.60	14.83
HCT [L/L]	3	0.416	0.415	0.409	<b>0.393**</b>	0.454	0.456	0.444	0.444
	6	0.429	0.440	0.422	0.438	0.45	0.44	0.45	0.44
	18	0.407	0.415	0.414	0.401	0.434	0.424	0.430	0.428
	24	0.380	0.387	0.401	0.360	0.425	0.424	0.416	0.427
MCV [fL]	6	50.6	50.7	51.0	51.3	54.3	54.3	54.0	<b>53.6**</b>
	12	52.1	51.7	52.7	51.6	55.6	56.3	55.0	55.3
	18	54.1	54.7	54.4	53.4	56.4	56.5	56.3	55.9
	24	56.0	56.0	55.8	<b>54.1**</b>	56.7	56.3	57.4	56.5
MCH [pg]	6	18.67	18.93	18.84	18.75	18.91	18.98	18.97	18.77
	12	18.82	18.64	19.07	18.59	19.74	20.03	19.69	<b>19.45<sup>#</sup></b>
	18	19.43	19.59	19.56	19.19	19.89	20.10	19.99	19.64
	24	19.68	19.56	19.66	<b>19.13*</b>	19.98	19.86	20.21	<b>19.64<sup>##</sup></b>
MCHC [g/dL]	6	36.87	37.28	36.93	36.54	34.81	34.94	35.10	34.97
	12	36.08	36.02	36.13	36.01	35.50	35.54	35.74	35.27
	18	35.88	35.79	35.93	35.89	35.20	35.55	35.46	35.13
	24	35.13	34.93	35.20	35.34	35.18	35.26	35.15	<b>34.73**</b>

Dose level	[ppm]	Males				Females			
	[mg/kg bw/d]	0	30	100	3000	0	30	100	3000
Parameter	Months	0	1.4	4.7	144.2	0	1.7	5.8	177.4

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$  (Williams' test);

#  $p \leq 0.05$ ; ##  $p \leq 0.01$  (Wilcoxon 2 sample rank sum test);

APPT: Activated Partial Thromboplastin Time;

PT: Prothrombin time;

WBC: White Blood Cells;

RBC: red blood cells;

HGB: haemoglobin;

HCT: haematocrit;

MCV: mean corpuscular/cell volume;

MCH: mean corpuscular/cellular hemoglobin;

MCHC: mean corpuscular/cellular hemoglobin concentration

*Clinical chemistry:* At the top dose in females statistically-significant decreases in ALP, AST and ALT (throughout the treatment period), as well as LDH (up to 18-month) were seen (Table 6.5-21). Top dose males also showed decreased activity of these enzymes, however, changes (excluding AST) were confined to the first 6 - 12 months. Due to consistency of these findings, the depressed activity of these enzymes is considered to be treatment-related. However, given the direction of the change (a reduction rather than an increase), these decreases are not considered toxicologically-significant. Gamma glutamyl transferase (GGT) was increased at the top dose in both sexes throughout the exposure period. This increase is considered treatment-related and adverse and indicative of liver toxicity.

Urea nitrogen was statistically-significantly elevated and total protein statistically-significantly decreased in males of the top dose at 24-months. In addition, inorganic phosphate was increased at the top dose in both sexes. In males these effects were considered to be treatment-related and adverse, indicative of nephropathy, which was confirmed by urinalysis and histopathologically. However, these findings in males are most likely due to  $\alpha_2\mu$ -globulin accumulation (as proven in the recent 90-day study) and hence not relevant to humans. In females, as no corroborative pathological findings indicative of kidney damage were observed, the increased serum phosphate level was considered to be treatment related but not adverse. All other findings gaining statistical-significance (in calcium, sodium and glucose levels) were not considered to be treatment-related or of toxicological-relevance due to temporal inconsistency of findings and/or lack of dose-response. Overall, treatment-related and adverse increases in GGT were seen at the top dose in both sexes.

Table 6.5-21. Selected clinical chemistry parameters

Dose level	[ppm]	Males				Females			
	[mg/kg bw/d]	0	30	100	3000	0	30	100	3000
Parameter	Months	0	1.4	4.7	144.2	0	1.7	5.8	177.4
LDH [IU/L]	6	677	548	536	<b>498*</b>	689	688	692	<b>538*</b>
	12	532	656	514	497	596	627	606	<b>434*</b>
	18	495	509	478	500	524	506	484	<b>387*</b>
	24	454	478	575	492	495	508	525	450
ALP [IU/L]	6	261	269	<b>244*</b>	<b>238**</b>	255	227	229	<b>182**</b>
	12	270	262	<b>237*</b>	<b>214**</b>	192	196	176	<b>154**</b>
	18	242	247	256	210	245	222	236	<b>158**</b>
	24	173	181	169	172	182	156	174	<b>119**</b>
AST [IU/L]	6	196.6	161.0	142.6	<b>129.6*</b>	191.7	208.3	189.3	<b>107.0**</b>
	12	182.6	195.8	153.2	<b>105.8*</b>	188.9	206.9	160.6	<b>68.0**</b>

Dose level	[ppm]	Males				Females			
		0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
Parameter	Months								
	18	112.8	131.1	113.4	108.9	134.5	120.6	141.8	<b>67.1**</b>
	24	95.5	92.7	105.2	<b>71.3##</b>	100.4	113.9	103.7	<b>69.1**</b>
ALT [IU/L]	6	144.9	114.1	100.8	<b>93.6*</b>	143.1	152.5	128.2	<b>59.0**</b>
	12	123.5	135.3	104.7	83.2	119.8	118.9	102.3	<b>37.1**</b>
	18	94.2	115.2	92.1	89.5	108.7	102.3	123.7	<b>46.2**</b>
	24	56.0	55.4	60.9	57.5	60.9	64.2	60.0	<b>49.1*</b>
GGT [IU/L]	6	1.1	2.1	1.7	<b>3.4##</b>	2.2	2.9	1.5	<b>5.5##</b>
	12	0.0	0.7	0.1	<b>2.2##</b>	1.2	1.3	1.0	<b>3.4##</b>
	18	4.6	2.0	1.4	<b>5.9#</b>	0.9	1.4	<b>2.0#</b>	<b>5.6##</b>
	24	2.0	2.7	2.5	<b>7.2**</b>	1.7	1.9	1.9	<b>4.8**</b>
Bilirubin [μmol/L]	6	3.4	3.2	3.0	3.3	2.8	2.6	2.6	2.9
	12	2.0	2.0	1.5	1.9	2.6	2.4	2.5	2.6
	18	3.3	2.8	<b>2.4*</b>	<b>2.5*</b>	2.6	2.5	2.4	2.7
	24	3.5	3.8	4.3	<b>5.1*</b>	2.5	2.3	2.1	2.6
Total protein [g/L]	6	64.9	65.8	64.4	<b>68.7**</b>	68.0	66.9	68.7	<b>71.9**</b>
	12	62.9	63.5	63.5	63.0	72.5	75.0	73.7	75.0
	18	64.3	63.2	62.4	63.3	68.0	69.6	68.8	70.1
	24	63.3	62.4	60.7	<b>57.6**</b>	68.6	68.9	68.0	69.0
Inorganic phosphate [mmol/L]	6	1.72	1.72	1.70	1.91	1.91	1.78	<b>1.59§</b>	1.98
	12	1.57	1.58	1.54	1.64	1.52	1.61	1.45	1.60
	18	1.53	1.45	1.43	1.59	1.55	1.33	1.37	1.62
	24	1.70	1.67	1.71	<b>2.36*</b>	1.14	1.18	1.14	<b>1.42**</b>
Calcium [mmol/L]	6	2.70	2.73	2.71	2.77	2.75	2.72	2.74	<b>2.83*</b>
	12	2.64	2.67	2.65	2.66	2.71	2.76	2.70	2.77
	18	2.80	2.73	2.77	2.82	2.73	2.74	2.71	2.78
	24	2.83	2.81	2.79	2.85	2.79	2.86	2.83	2.85
Sodium [mmol/L]	6	146.4	146.0	145.8	146.5	147.1	146.8	147.1	146.9
	12	151.7	151.1	151.9	152.1	147.4	148.1	147.4	147.4
	18	145.5	145.9	146.3	146.3	144.4	143.2	143.5	143.9
	24	150.6	<b>148.3*</b>	149.5	149.4	147.3	147.3	146.8	146.5
Glucose [mmol/L]	6	3.0	3.2	3.0	3.0	2.8	2.8	2.7	<b>2.6§</b>
	12	3.4	3.5	3.6	3.3	3.1	3.1	3.2	3.0
	18	2.8	2.7	2.7	3.0	2.2	2.1	1.9	2.2
	24	1.7	1.8	1.9	<b>2.4*</b>	2.1	2.3	2.3	2.3
Urea nitrogen	6	8.0	8.0	7.7	8.3	9.2	9.0	8.4	8.8

Dose level	[ppm]	Males				Females			
		0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
Parameter	Months								
[mmol/L]	12	7.1	7.0	7.0	7.4	7.3	7.9	7.3	7.3
	18	8.5	7.3	7.9	9.2	7.1	7.2	7.3	6.7
	24	10.6	12.1	12.0	<b>17.5**</b>	7.4	7.7	8.1	8.2

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$  (Williams' test);

§  $p \leq 0.05$ ; §§  $p \leq 0.01$  (Dunnett's test);

#  $p \leq 0.05$ ; ##  $p \leq 0.01$  (Wilcoxon 2 sample rank sum test);

LDH = Lactate dehydrogenase;

ALP = alkaline phosphatase;

ALT = alanine aminotransferase;

AST = aspartate aminotransferase;

GGT =  $\gamma$ -glutamyl transferase

*Urinalysis:* Statistically significant changes in urinary parameters occurred at the top dose, mainly in males, and were indicative of severe nephropathy (increase in casts, protein, leukocytes and erythrocytes). High dose males showed statistically-significant change in tubular epithelial cells (Table 6.5-22), especially after 6 and 12 months, accompanied by large numbers of mixed casts from the lumen of the nephrons. The colour of urine was observed to be paler after 1-year exposure onwards, the volume was increased, osmolality decreased, and fewer spermatozoa were present compared with the controls. The number of leukocytes was statistically significantly increased after 1- and 2-years. Due to the consistency of the findings, indicative of kidney damage (severe nephropathy), these alterations in urine parameters were considered treatment-related at the top dose in males. However, the 90-day study (■■■■■, 2018a) demonstrated that this was due to accumulation of  $\alpha 2\mu$ -globulin, a male rat specific phenomenon of no relevance to humans.

Table 6.5-22. *Urinalyses*

Dose level	[ppm]	Male				Female			
		0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
Parameter	Months								
Tubular epithelial cells [0-100]	6	3.4	2.5	4.3	<b>8.1##</b>	0.0	0.0	0.0	0.0
	12	1.2	1.6	1.1	<b>5.1##</b>	0.0	0.1	0.6	0.0
	18	0.0	0.0	0.2	0.2	0.0	0.5	0.0	0.0
	24	0.2	0.1	0.6	<b>1.0#</b>	0.1	0.1	0.1	0.1
Casts [0-100]	6	0.1	0.2	0.0	<b>3.3##</b>	0.0	0.0	0.0	0.0
	12	0.0	0.0	0.0	<b>1.3##</b>	0.0	0.0	0.0	0.0
	18	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0
	24	0.1	0.1	0.2	<b>0.3#</b>	0.2	0.5	0.2	0.3
Urine volume [mL]	6	2.4	2.5	2.7	2.8	1.8	1.6	1.6	1.9
	12	2.7	2.9	3.3	<b>3.7**</b>	2.2	1.9	2.3	2.3
	18	6.0	5.2	4.9	7.7	3.8	4.7	3.8	4.0
	24	9.7	9.0	7.2	<b>12.9*</b>	5.0	5.7	4.5	4.4
Colour [1-10]	6	5	5	5	5	5	5	5	5
	12	5	5	5	4	5	5	5	5
	18	5	5	5	<b>4##</b>	5	5	5	5
	24	5	5	5	<b>4##</b>	5	5	5	5
Osmolality [mOsm/L]	6	2538	2448	2390	2368	2811	2880	2634	2738
	12	2132	2184	2013	1907	2200	2266	1865	2305
	18	1333	1548	1515	1077	1383	1294	1222	1321
	24	891	973	892	<b>549**</b>	980	896	1064	1184
Leukocytes [0-100]	6	0.6	0.4	0.6	0.7	0.2	0.1	0.1	0.6
	12	0.4	0.5	0.9	<b>2.2#</b>	0.5	0.2	0.5	0.7

Dose level	[ppm]	Male				Female			
		0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
Parameter	Months								
	18	2.4	1.1	2.6	2.4	0.5	1.8	0.2	0.6
	24	4.2	13.6	24.2	17.0 <sup>#</sup>	2.5	2.7	2.5	2.5
Spermatozoa [0-100]	6	2.8	3.7	3.3	5.2				
	12	1.7	1.1	1.1	4.1				
	18	3.8	3.1	4.3	2.8				
	24	4.5	4.6	2.7	0.6 <sup>#</sup>				
Ketones [mmol/L]	6	1.3	1.2	1.4	1.8 <sup>#</sup>	1.0	1.1	0.8	1.2
	12	0.9	0.9	0.8	0.9	0.5	0.6	0.4	0.7
	18	0.2	0.1	0.2	0.1	0.0	0.0	0.0	0.1
	24	0.0	0.1	0.0	0.0	0.0	0.0	0.4	0.4 <sup>#</sup>
Bilirubin [mmol/L]	6	10	9	10	8 <sup>##</sup>	16	15	14	10 <sup>##</sup>
	12	13	11	11	7 <sup>##</sup>	14	16	11	7 <sup>##</sup>
	18	0	0	0	0	2	0	1	0
	24	0	0	0	0	0	0	0	0
Protein [g/L]	6	3.3	2.6	3.3	4.5	0.6	0.7	0.6	0.7
	12	5.3	5.7	5.1	6.0 <sup>#</sup>	2.9	3.8	4.2	5.0 <sup>#</sup>
	18	5.0	5.0	5.0	5.0	3.7	4.0	3.7	4.8
	24	5.0	5.0	4.8	5.0	5.0	5.0	4.8	4.8
Erythrocytes [0-100]	6	0.0	0.1	0.0	0.2	0.0	0.0	0.0	0.0
	12	0.0	0.0	0.1	0.4 <sup>##</sup>	0.6	0.0	0.0	1.0
	18	0.5	0.4	1.2	0.4	2.2	1.8	0.1	0.9
	24	0.1	0.1	1.1	0.4	2.0	0.5	0.5	0.0

\* p ≤ 0.05; \*\* p ≤ 0.01 (Williams' test);

<sup>#</sup> p ≤ 0.05; <sup>##</sup> p ≤ 0.01 (Wilcoxon 2 sample rank sum test)

**Organ weight:** Liver weights, both absolute and relative, were consistently increased at the top dose, in both males and females, throughout the study. Statistically-significant increases were seen in absolute liver weights, in males at 18-months and in females at 6- and 12-months. Change compared to controls exceeded 15 % for both males and females at several time points, reaching a maximum of 23 % for relative liver weight in males at 24-months, and 18 % for relative weight in females at 18-months. Comparable effects were noted in the new/modern combined chronic toxicity and carcinogenicity study (■■■■■■■■■■, 2018) as well as in other repeated dose toxicity studies in rats. Concomitant clinical chemistry findings (a statistically-significant increase in GGT, see Table 6.5-21) were observed at the top dose. Concomitant histopathology (Table 6.5-24) was also observed at the top dose. Overall, owing to the consistency in findings, accompanying clinical chemistry and histopathology, the effects on liver weights were considered adverse and treatment-related at the top dose.

Kidney weights did not show a consistent pattern of statistically-significant increases; statistical-significance was only reached at the top dose in males at 12-months. Relative kidney weights were increased at the top dose by 17 % for males and 13 % for females. In addition, urinalyses (Table 6.5-22) and pathology (Tables 6.5-24 and 6.5-25) findings in males indicative of kidney damage (nephropathy) were observed. However, the 90-day study (Flick *et al.*, 2018a) demonstrated that this was due to accumulation of α<sub>2</sub>μ-globulin, a male rat specific phenomenon of no relevance to humans. In the absence of any urinalysis and histopathology findings, the increase (by 13 %) in relative weight in top dose females is most likely the consequence of the reductions in body weight gains observed at this dose. Effects on adrenal gland and spleen weights did not show consistent changes which were either statistically-significant and/or ≥ 10 % change compared to control. Dose-response and/or time-response relationships were not evident. Adverse clinical chemistry and histopathology were also not observed. Overall, there were treatment-related and adverse increases (> 15%) in liver weights at the top dose.

Table 6.5-23. Selected organ weights

Sex		Males				Females			
Organ	Dose [ppm]	Absolute weight [g]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g]	Δ%&	Relative weight [% of bw]	Δ%&
Terminal weight [g]		6-month							
	0	362.3				204.5			
	30	353.9	-2.3			204.5	±0.0		
	100	359.1	-0.9			199.7	-2.3		
	3000	<b>344.4*</b>	-4.9			195.1	-4.6		
		12-month							
	0	392.6				235.5			
	30	391.7	-0.2			239.1	+1.5		
	100	385.7	-1.8			240.0	+1.9		
	3000	390.0	-0.7			224.5	-4.7		
		18-month							
	0	411.7				277.3			
	30	411.3	-0.1			282.4	+1.8		
	100	397.9	-3.4			283.5	+2.2		
	3000	397.3	-3.5			<b>255.1*</b>	-8.0		
		24-month							
	0	357.9				286.9			
	30	360.7	+0.8			284.9	-0.7		
	100	361.4	+1.0			288.4	+0.5		
	3000	<b>313.2**</b>	-12.5			<b>262.2**</b>	-8.6		
Liver [g]		6-month <sup>#</sup> for ♀							
	0	12.32		3.40		6.90		3.40	
	30	11.78	-4.4	3.33	-2.1	6.66	-3.5	3.29	-3.2
	100	12.01	-2.5	3.35	-1.5	6.83	-1.0	3.38	-0.6
	3000	13.15	+6.7	3.81	+12.1	<b>7.66**</b>	+11.0	3.91	+15.0
		12-month <sup>#</sup> for ♂							
	0	12.94		3.31		8.20		3.48	
	30	13.25	+2.4	3.44	+3.9	8.59	+4.8	3.60	+3.4
	100	12.76	-1.4	3.30	-0.3	8.46	+3.2	3.53	+1.4
	3000	13.90	+7.4	3.51	+6.0	<b>9.08**</b>	+10.7	4.05	+16.4
		18-month <sup>#</sup> for ♂							
	0	14.93		3.68		9.58		3.45	
	30	13.68	-8.4	3.36	-8.7	10.37	+8.2	3.72	+7.8
	100	14.99	+0.4	3.74	+1.6	10.11	+5.5	3.58	+3.8
	3000	16.54*	+10.8	4.15	+12.8	10.45	+9.1	4.08	+18.3
		24-month <sup>#</sup> for ♂							
	0	14.81		4.16		11.60		4.05	
	30	15.51	+4.7	4.30	+3.4	11.68	+0.7	4.13	+2.0
	100	15.27	+3.1	4.29	+3.1	11.99	+3.4	4.19	+3.5
	3000	15.86	+7.1	5.13	+23.3	12.06	+4.0	4.62	+14.1
Kidneys [g]		6-month <sup>#</sup>							
	0	2.30		0.64		1.43		0.71	
	30	2.24	-2.6	0.63	-1.6	1.42	-0.7	0.69	-2.8
	100	2.34	+1.7	0.65	+1.6	1.40	-2.1	0.70	-1.4
	3000	2.30	±0.0	0.66	+3.1	1.45	+1.4	0.74	+4.2
		12-month <sup>#</sup>							
	0	2.67		0.68		1.70		0.72	
	30	2.64	-1.1	0.69	+1.5	1.80	+5.9	0.75	+4.2
	100	2.67	±0.0	0.69	+1.5	1.80	+5.9	0.76	+5.6

Sex		Males				Females			
Organ	Dose [ppm]	Absolute weight [g]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g]	Δ%&	Relative weight [% of bw]	Δ%&
	3000	<b>2.85*</b>	+6.7	0.71	+4.4	1.77	+4.1	0.79	+9.7
	<b>18-month<sup>#</sup></b>								
	0	3.19		0.79		2.06		0.76	
	30	2.93	-8.2	0.72	-8.9	2.15	+4.4	0.77	+1.3
	100	3.09	-3.1	0.77	-2.5	2.11	-1.9	0.74	-2.6
	3000	3.27	+2.5	0.82	+3.8	2.10	-0.5	0.83	+9.2
	<b>24-month<sup>#</sup> for ♂</b>								
	0	3.34		0.95		2.39		0.84	
	30	3.45	+3.3	0.97	+2.1	2.50	+4.6	0.89	+6.0
	100	3.44	+3.0	0.97	+2.1	2.44	+2.1	0.86	+2.4
	3000	3.42	+2.4	1.11	+16.8	2.45	+2.5	0.95	+13.1
Adrenal glands [g]	<b>6-month</b>								
	0	0.042		0.011		0.056		0.028	
	30	0.040	-4.8	0.011	±0.0	0.055	-1.8	0.027	-3.6
	100	0.042	±0.0	0.012	+9.1	0.053	-5.4	0.026	-7.1
	3000	0.045	+7.1	0.013	+18.2	0.056	±0.0	0.029	+3.6
	<b>12-month<sup>#</sup> for ♂</b>								
	0	0.038		0.010		0.053		0.023	
	30	0.037	-2.6	0.010	±0.0	0.054	+1.9	0.023	±0.0
	100	0.038	±0.0	0.010	±0.0	0.056	+5.7	0.023	±0.0
	3000	<b>0.044*</b>	+15.8	0.011	+10.0	0.053	±0.0	0.024	+4.3
	<b>18-month</b>								
	0	0.055		0.013		0.063		0.023	
	30	0.052	-5.5	0.013	±0.0	0.059	-6.3	0.021	-8.7
	100	0.053	-3.6	0.013	±0.0	0.060	-4.8	0.021	-8.7
	3000	0.054	-1.8	0.014	+7.7	<b>0.056**</b>	-11.1	0.022	-4.3
	<b>24-month</b>								
	0	0.067		0.019		0.059		0.021	
	30	0.065	-3.0	0.018	-5.3	0.063	+6.8	0.022	+4.8
	100	0.067	±0.0	0.019	±0.0	0.060	+1.7	0.021	±0.0
	3000	0.075	+11.9	0.025	+31.6	0.058	-1.7	0.022	+4.8
Spleen [g]	<b>6-month</b>								
	0	0.64		0.18		0.42		0.21	
	30	0.67	+4.7	0.19	+5.6	0.42	±0.0	0.21	±0.0
	100	0.68	+6.3	0.19	+5.6	0.41	-2.4	0.21	±0.0
	3000	0.65	+1.6	0.19	+5.6	0.41	-2.4	0.21	±0.0
	<b>12-month</b>								
	0	0.69		0.18		0.47		0.20	
	30	0.69	±0.0	0.18	±0.0	0.48	+2.1	0.20	±0.0
	100	0.70	+1.4	0.18	±0.0	0.45	-4.3	0.19	-5.0
	3000	0.65	-5.8	0.17	-5.6	0.43	-8.5	0.19	-5.0
	<b>18-month<sup>#</sup> for ♂</b>								
	0	1.28		0.31		0.53		0.19	
	30	0.86	-32.8	0.22	-29.0	0.53	±0.0	0.19	±0.0
	100	1.11	-13.3	0.26	-16.1	0.56	+5.7	0.20	+5.3
	3000	0.94	-26.6	0.23	-25.8	0.53	±0.0	0.21	+10.5
	<b>24-month</b>								
	0	1.19		0.34		0.69		0.24	
	30	1.14	-4.2	0.31	-8.8	0.58	-15.9	0.20	-16.7
	100	1.09	-8.4	0.41	+20.6	0.71	+2.9	0.25	+4.2
	3000	<b>0.77**</b>	-35.3	0.24	-29.4	<b>0.48**</b>	-30.4	0.18	-25.0

Sex		Males				Females			
Organ	Dose [ppm]	Absolute weight [g]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g]	Δ%&	Relative weight [% of bw]	Δ%&

\* p ≤ 0.05. \*\* p ≤ 0.01; Williams' or Dunnett's test. Statistical analysis performed for the absolute values, only.

& : Values may not calculate exactly due to rounding of figures.

# : absolute organ weight values were adjusted to initial body weight

*Gross pathology:* At 6- and 12-month necropsies, there were no treatment-related macroscopic changes. At 18-months higher incidences of testicular oedema and subcapsular pale areas were seen at the low and high dose groups, and minor epididymal changes in all treated groups was seen in males. However, no dose-response was evident, therefore these findings were not considered treatment-related. At 24-months clear macroscopic findings were seen in males of the top dose (Table 6.5-24). This included an increased incidence of pale kidneys with a pitted/rough surface and reduced occurrences of exaggerated lobular patterns of the liver. There were several changes in the male reproductive tissues at the top dose (epididymal and seminal vesicular pathology), as a consequence of the testicular interstitial-cell tumours (not considered treatment-related - see below). In addition, changes to the renal lymph node, thickening of the stomach and aortic mucosa and enlargement of the parathyroid were also observed. Females of the top dose also showed reduced occurrences of exaggerated lobular patterns of the liver, as well as an increased in hepatic subcapsular dark depressed areas. Overall, there were treatment-related and adverse macroscopic effects (in the liver, kidney, lymph nodes, stomach, parathyroid gland and blood vessels) at the top dose at 24-months.

Table 6.5-24. Gross pathology findings - carcinogenicity group

		Males				Females			
Dose level	[ppm]	0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
No. of animals		50	50	50	50	50	50	50	50
<b>Kidneys</b>									
Subcapsular pitting/rough surface (severe/very severe)		12	8	10	27	1	0	3	0
Diffuse subcapsular pallor		14	8	10	23	3	4	5	0
<b>Liver</b>									
Exaggerated lobular patterns		18	20	20	3	32	33	33	14
Subcapsular dark depressed foci		7	13	11	9	29	31	26	40
<b>Testes</b>									
Subcapsular pale areas		26	34	35	37	-	-	-	-
<b>Epididymides</b>									
Atrophy, bilateral		0	6	5	9	-	-	-	-
<b>Prostate</b>									
Small size		2	2	2	7	-	-	-	-
<b>Seminal vesicles</b>									
Bilateral atrophy		6	10	7	14	-	-	-	-
<b>Lymph nodes</b>									
Renal node hemorrhage/ enlargement		0	0	1	4	-	-	-	-
<b>Stomach</b>									
Gastric mucosal thickening		8	6	7	18	1	4	3	3
<b>Parathyroid gland</b>									
Enlargement		7	8	4	16	3	2	3	1
<b>Blood vessels</b>									
Aortic mucosal thickening		11	5	12	18	3	4	4	4



*Histopathology:*

*Non-neoplastic findings:* A range of statistically-significant effects on the liver (biliary epithelial hyperplasia, cholangiofibrosis, glycogenic vacuolation, periportal acidophilia, basophilic parenchymal foci, periportal chromidial clumping) were seen in the top dose in males and females. In the kidney there was a statistically-significant increase in the incidence of very severe chronic nephropathy in the top dose in males only. However, the 90-day study in rats (■■■■■, 2018a) demonstrated that findings in the kidneys of males was due to accumulation of  $\alpha 2u$ -globulin, a male rat specific phenomenon of no relevance to humans.

At the top dose the following statistically-significant increased incidences were recorded in males: diffuse hyperplasia of the parathyroid, fundic glandular mineralisation of the stomach, luminal mineralised droplet in the prostate, accelerated haematopoiesis of bone marrow, excessive pigment deposits in the lacrimal gland, patchy tubular epithelial vacuolation of the epididymides. Many of these findings (in the parathyroid, stomach, prostate, aorta, bone and bone marrow and lacrimal glands) were considered to be secondary to the finding of chronic nephropathy, which was not considered to be human-relevant. Also at the top dose a statistically-significant increase in the incidence of patchy exocrine atrophy in the pancreas was recorded in females.

In the thyroid increases in focal colloidal basophilia / mineralisation were seen; incidences which were graded 'slight' were statistically-significantly increased from the mid dose of 100 ppm in females. In the absence of any follicular cell hypertrophy and/or hyperplasia, these very mild histopathological findings, although treatment-related, were not considered adverse. This is consistent with the adverse thyroid findings noted in the new/modern carcinogenicity rat study which occurred only at the top dose of 5000 ppm (265/351 mg/kg bw/d for males and females, respectively).

Overall, treatment-related and adverse histopathological findings were seen in the liver at the top dose in both sexes (3,000 ppm, equivalent to 144 and 177 mg/kg bw/d in M/F respectively). Other organs were also affected at the top dose, mainly in males. These effects were the possible consequence of the severe nephropathy (not relevant to humans) seen in males or the generalised toxicity occurring at the top dose.

Table 6.5-25. Non-neoplastic findings

Dose level		Males				Females			
		0	30	100	3000	0	30	100	3000
	[ppm] [mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
Number of animals		50	50	50	50	50	50	50	50
Number of animals examined		50	50	50	50	50	50	50	50
<b>Liver</b>									
Biliary epithelial hyperplasia		45	48	47	35	21	23	20	13
	very slight	1	2	0	2	2	0	0	2
	slight	22	17	21	28	19	22	19	11
	moderate	22	28	25	4**	0	1	1	0
	severe	0	1	1	1	0	0	0	0
	[mean] <sup>#</sup>	[2.5]	[2.6]	[2.6]	[2.1]	[1.9]	[2.0]	[2.1]	[1.8]
Cholangiofibrosis		39	48	46	28	22	23	20	7
	very slight	1	0	0	3	3	0	1	1
	slight	16	22	23	21	17	20	16	6*
	moderate	22	24	23	4**	2	3	3	0
	severe	0	2	0	0	0	0	0	0
	[mean] <sup>#</sup>	[2.5]	[2.6]	[2.5]	[2.0]	[2.0]	[2.1]	[2.1]	[1.9]
Glycogenic vacuolation		46	47	45	48	50	49	50	50
	very slight	8	16	13	13	3	8	8	8
	slight	13	12	12	17	22	15	15	10*
	moderate	23	19	20	18	25	26	27	31
	severe	2	0	0	0	0	0	0	1
	[mean] <sup>#</sup>	[2.4]	[2.1]	[2.2]	[2.1]	[2.4]	[2.4]	[2.4]	[2.5]
Periportal acidophilia		9	6	8	33**	2	2	2	22**
Basophilic parenchymal foci		23	19	23	19	33	30	31	15**
Periportal chromidial clumping		0	0	0	9**	0	0	0	1

		Males				Females			
Dose level	[ppm]	0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
<b>Kidney</b>									
Chronic nephropathy		50	40	50	50	48	49	50	50
	very slight	2	0	1	0	2	4	2	0
	slight	4	3	5	0	15	8	13	10
	moderate	27	21	28	17	26	34	26	35
	severe	13	13	11	19	5	3	9	5
	very severe	4	3	5	14*	0	0	0	0
	[mean] <sup>#</sup>	[3.3]	[3.4]	[3.3]	[3.9]	[2.7]	[2.7]	[2.8]	[2.9]
<b>Parathyroid</b>									
Diffuse hyperplasia		15	12	19	29**	7	6	11	4
<b>Stomach</b>									
Fundic glandular mineralisation		3	6	6	13*	1	1	1	1
Ulceration		12	6	11	7	4	6	7	9
<b>Prostate</b>									
Luminal mineralised droplets		7	14	10	17*				
<b>Aorta</b>									
Mural mineralisation		7	8	5	12	1	3	2	2
<b>Bones</b>									
Osteofibrosis of bone matrix		11	10	6	16	4	3	5	3
<b>Bone marrow</b>									
Accelerated haematopoiesis		7	8	8	18*	3	7	7	2
<b>Lacrimal gland</b>									
Excessive pigment deposits		6	6	13	16*	7	9	7	11
<b>Epididymides</b>									
Patchy tubular epithelial vacuolation		21	33*	29	34*				
<b>Thyroid</b>									
Parafollicular cell hyperplasia		2	4	3	0	5	4	3	4
Focal colloidal basophilia / mineralisation		29	22	22	33	8	8	18	21
	very slight	0	0	0	0	0	1	0	0
	slight	23	18	18	22	8	7	18*	21**
	moderate	6	4	4	11	0	0	0	0
	[mean] <sup>#</sup>	[2.2]	[2.2]	[2.2]	[2.3]	[2.0]	[1.9]	[2.0]	[2.0]
<b>Pancreas</b>									
Patchy exocrine atrophy		16	21	12	19	8	13	15	20*

\* p ≤ 0.05. \*\* p ≤ 0.01; Fischer's test

<sup>#</sup> = mean severity grading; histopathological findings were graded very slight/minimal (Grade 1); slight/few (Grade 2); moderate (Grade 3); marked/severe (Grade 4) and massive/very severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding.

*Neoplastic findings:* The numbers of animals with neoplasms and the total numbers of neoplasms (benign, malignant and metastasized) were comparable between control and treated groups (Table 6.5-26) and therefore not considered treatment-related.

Benign interstitial tumours of the testes (Leydig cell adenomas) were present at an increased incidence in treated groups (Table 6.5-27); a dose-response was evident and statistical-significance was reached in the top dose group at both 18- (80 %) and 24-months (76 %). It is well established that Leydig cell adenomas in male F344 rats have a high spontaneous tumour incidence (see Guidance on the application of the CLP criteria, version 5.0 – July 2017, e.g. 80 – 100 %). This is supported by time-relevant HCD for the same strain from the National Toxicology Program (NTP) in the US, which demonstrate that the concurrent control incidence (53 % at 18 months and 54 % at 24-months compared to a HCD mean of 89 %) was low and that even at the top dose the incidence of this tumour (80 % at 18-months and 76 % at 24-months) was below the HCD mean (Table 6.5-28). This finding is therefore considered unrelated to treatment and part of normal variation. There were no other treatment-related increases in tumours in any other tissues/organs.

Table 6.5-26. Selected neoplastic findings (18- and 24-months)

Sex		Males				Females			
Dose level	[ppm]	0	30	100	3000	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2	0	1.7	5.8	177.4
Number of animals									
Total (examined)		85	85	85	85	85	85	85	85
<b>Tumours</b>									
Total number of tumours		116	134	125	113	55	66	63	56
[N]		63	67	63	67	40	48	49	42
[%]		74	79	74	79	47	56	58	49
Single tumours		26	20	21	32	27	32	36	30
[N]		31	24	25	38	32	38	42	35
[%]		37	47	42	35	13	16	13	12
Multiple tumours		44	55	49	41	15	19	15	14
[N]									
[%]									
<b>Benign tumours</b>									
Total number of tumours		96	113	102	100	46	55	45	44
[N]		60	65	60	65	36	40	39	36
[%]		71	76	71	76	42	47	46	42
<b>Malignant tumours</b>									
Total number of tumours		20	21	23	13	9	11	18	12
[N]		19	20	19	11	9	11	17	12
[%]		22	24	22	13	11	13	20	14
<b>Metastasising tumours</b>									
Total number of tumours		0	0	0	1	1	0	1	1
[N]		0	0	0	1	1	0	1	1
[%]		0	0	0	1	1	0	1	1

N = total number of animals.

% = percent of animals with tumours.

Table 6.5-27. Selected neoplastic findings (24 months) - testes

		Males			
Dose level	[ppm]	0	30	100	3000
	[mg/kg bw/d]	0	1.4	4.7	144.2
# animals 18-month		15	15	15	15
# animals 24-month		50	50	50	50
<b>Testes</b>					
Interstitial cell tumours (B)					
18-month tumour incidence		8	11	10	12*
[N]		53	73	67	80
[%]					
2-year tumour incidence		27	39	34	38*
[N]		54	78	68	76
[%]					

Statistical analysis: \*:  $p \leq 0.01$  (Significance of trend statistic (Peto analysis); B = benign.

Table 6.5-28. HCD for ♂ testicular interstitial cell tumours

HCD source	Mean [%]	Min [%]	Max [%]
NTP (Haseman <i>et al.</i> , 1985)	89 (1511/1703)	68	98
Laboratory <sup>a</sup>	78	72	86

NTP = National Toxicology Program; HCD are based on 40 carcinogenicity feeding studies each using 50 F344 rats per dose group (in total, data on 1936 male and 1983 female rats), performed between 1977 and 1987 at the National Cancer Institute (NCI) and the National Toxicology Program in the US.

HSE notes that while the same species and strain were used, and the date range (1977 – 1987) is more or less concurrent with the study under evaluation (conducted in 1983 – 1985), these data is from a wide number of laboratories.

a: Laboratory historical control data for interstitial-cell tumours in F344 rats (n =2) based on 2-year feeding studies in F344 rats conducted at [REDACTED]. Study No. 2670, (time parallel to Study No 2604) 1983 –1985, 43/57 (75%); Study No. 2885, 1985 –1987, 36/50 (72%); Study No. 4102, 05/1989 - 05/1991, 43/50 (86%).

#### Conclusion:

In a relatively old chronic/carcinogenicity study, cinmethylin was administered via the diet to male and female Fischer 344 rats for 24-months. There were no treatment-related increases in the incidence, severity or onset of tumours in any tissue up to the top dose of 3,000 ppm at which systemic toxicity (increased mortality and clinical signs of toxicity in males, reduction in food consumption, body weight and body weight gain, haematological effects, liver toxicity in both sexes and nephropathy in males of no relevance to humans) occurred. Overall, a **NOAEL of 3,000 ppm (equivalent to 144 and 177 mg/kg bw/d in M/F respectively) can be identified for carcinogenicity** in rats from this study.

Systemic toxicity was observed in both sexes at the top dose, which indicated that the top dose was sufficiently high. Increased mortality and clinical signs of toxicity were seen in males; body weight gain was reduced by > 10 % at 24-months in both sexes. Consistent with the findings of repeated-dose studies and the new/modern study, the liver was identified as a target organ in rats; relative liver weights were increased at the top dose and concomitant clinical chemistry parameters (increased GGT) and histopathology (biliary epithelial hyperplasia, cholangiofibrosis, glycogenic vacuolation, periportal acidophilia, basophilic parenchymal foci, periportal chromidial clumping) were noted. In addition, haematological effects indicative of mild anaemia and thrombocytosis were seen in both sexes at the top dose. Findings (changes in serum and urine parameters, increased kidney weights and histopathology) indicative of kidney damage (severe nephropathy) were observed in males at the top dose; however, results of the 90-day study ([REDACTED], 2018a) demonstrated that these kidney finding were due to accumulation of  $\alpha_2\mu$ -globulin, a male rat-specific phenomenon of no relevance to humans. Nasal cavity findings were not observed.

Based on these findings, the **NOAEL for systemic chronic toxicity in the rat is 100 ppm (equivalent to 4.7 and 5.8 mg/kg bw/d in M/F respectively).**

([REDACTED], 1985)

#### **B.6.5.2. Long-term toxicity and carcinogenicity in mice**

The long-term toxicity and carcinogenicity of cinmethylin have been investigated in mice via the oral (dietary) route in one standard guideline 18-month study (new/modern) in C57BL/6J Rj mice and one older 24-month study with B6C3F1 mice.

*1) New/modern study*

<b>Author(s)</b>	
<b>Study title</b>	BAS 684 H - Carcinogenicity study in C57BL/6J Rj mice - Administration via the diet up to 18 months
<b>Study reference</b>	, 2018d BASF Doc ID: 2017/1094161
<b>Test facility</b>	
<b>Dates of work</b>	21/04/2015 – 21/10/2016
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b> <b>Batch no.</b>	93.5 COD-002038 (-) / (+) ratio = 48:52
<b>Test organisms</b>	Mice C57BL/6J Rj Males and females The same strain was used in the previous repeated dose toxicity studies.
<b>Groups</b>	50/sex/dose (main groups) 6/sex/dose (satellite groups).
<b>Dose/concentration</b>	0, 150, 1000 and 5000 ppm. Equivalent doses shown in Table 6.5-29
<b>Route</b>	Administered orally, via the diet, daily, for a period of 63-days (satellite groups) or 18-months (main groups).
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD Test Guideline No. 451 (2009 – the current test guideline was adopted in 2018), EPA 870.4200, JMAFF No 12 Nosan No 8147, Commission Regulation (EC) No 260/2014 - Part B B.32
<b>Deviation</b>	None.
<b>Impact of deviations</b>	Not applicable.
<b>Acceptable</b>	Yes.
<b>Conclusion</b>	Cinmethylin demonstrated no carcinogenic potential in mice.
<b>NOAEL</b>	NOAEL for carcinogenicity: 5000 ppm, equivalent to 904 and 939 mg/kg bw/d in males and females respectively. LOAEL for systemic chronic toxicity: 150 ppm, equivalent to 25/27 mg/kg bw/d in males/females. Lowest BMDL <sub>10</sub> (for body weight effects in females) = 37.5 mg/kg bw/d.
<b>Effects at the LOAEL</b>	Carcinogenicity: No increase in the incidence, severity or onset of tumours was observed up to and including the highest dose tested Systemic chronic toxicity: Reductions in terminal body weight and body weight gain in both sexes and food consumption in females from the low dose of 150 ppm.

**Methods**

In a GLP and OECD test guideline compliant study, cinmethylin was administered via the diet to male and females C57BL/6J Rj mice (the same strain was used in the previous repeated dose toxicity studies) over a period of either 63-days (6/sex/dose, satellite groups) or 18-months (50/sex/dose, main groups). Dietary concentrations of 0, 150, 1000 and 5000 ppm (see Table 6.5-29 for equivalent doses in mg/kg bw/d) were selected on the basis of previous 28- and 90-day studies in mice (see Section 6.3). Methods for the analysis of cinmethylin in corn oil (Grauert & Hidding, 2017a; 2017/1067141), as well as cinmethylin and metabolites in plasma (Catchpole & Hidding, 2018; 2018/1037312, which supports the mouse plasma analysis; 2018/1048783 and 2017/1094161), were evaluated and were considered validated (see Volume 3 CA B5, section B.5.1.2). Blood sampling for plasma concentration of cinmethylin and metabolites (M684H001, M684H010, M684H011 and M684H026) were determined for satellite group animals after 20, 41 and 62 days of administration.

Table 6.5-29. Mean test substance intake

Dose [ppm]	Males				Females			
	0	150	1000	5000	0	150	1000	5000
Dose [mg/kg bw/d]								
Satellite group (63-days)	0	32.1	223.1	1174.8	0	34.8	301.1	1224.5
Main group (18-months)	0	25.0	162.3	904.0	0	27.0	183.8	939.1

**Results**

The stability and homogeneity of cinmethylin in the diet was confirmed in a separate study using a validated method of analysis. Cinmethylin was observed in plasma analyses but most of the time below the limit of quantification. However, cinmethylin metabolites were detected and quantified in plasma samples from treated animals; the concentrations of metabolites increased (however not linearly) with dose.

Table 6.5-30. Plasma concentration of cinmethylin and selected metabolites (satellite group, 6 animals/dose)

Dose [ppm]		Males				Females			
		0	150	1000	5000	0	150	1000	5000
BAS 684 H	Day 20 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	173.9 <sup>2</sup> 53.2	<LOQ	<LOQ	<LOQ	<LOQ
	Day 41 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	101.7 <sup>1</sup>	<LOQ	<LOQ	<LOQ	<LOQ
	Day 62 [ng/mL] [RSD]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
M684H001 Reg.No. 6055521	Day 20 [ng/mL] [RSD]	n. d	<LOQ	<LOQ	186.4 <sup>4</sup> 9.8	n. d	<LOQ	<LOQ	230.6 <sup>5</sup> 26.6
	Day 41 [ng/mL] [RSD]	n. d	<LOQ	<LOQ	131.6 <sup>4</sup> 11.8	n. d	<LOQ	<LOQ	145.9 <sup>3</sup> 32.9
	Day 62 [ng/mL] [RSD]	n. d	<LOQ	<LOQ	157.2 <sup>5</sup> 18.7	n. d	<LOQ	<LOQ	171.0 <sup>4</sup> 49.3
M684H010 Reg.No. 111609	Day 20 [ng/mL] [RSD]	n. d.	116.3 <sup>1</sup>	532.5 <sup>6</sup> 26.2	2380.7 <sup>6</sup> 26.4	n. d.	<LOQ	382.6 <sup>6</sup> 17.9	1410.2 <sup>6</sup> 26.1
	Day 41 [ng/mL] [RSD]	n. d.	109.6 <sup>2</sup> 0.7	578.1 <sup>6</sup> 15.0	2041.3 <sup>6</sup> 21.2	n. d.	97.9 <sup>1</sup>	287.6 <sup>6</sup> 21.3	1149.9 <sup>6</sup> 31.2
	Day 62 [ng/mL] [RSD]	n. d.	99.9 <sup>2</sup> 5.4	538.3 <sup>6</sup> 19.9	2018.8 <sup>6</sup> 22.5	n. d.	<LOQ	287.7 <sup>6</sup> 16.7	1115.3 <sup>6</sup> 36.3
M684H011 Reg.No. 6055478	Day 20 [ng/mL] [RSD]	n. d.	<LOQ	142.9 <sup>1</sup>	399.0 <sup>6</sup> 55.4	n. d.	<LOQ	170.9 <sup>4</sup> 31.2	879.9 <sup>6</sup> 35.3
	Day 41 [ng/mL] [RSD]	n. d.	<LOQ	106.3 <sup>2</sup> 2.3	266.3 <sup>6</sup> 19.8	n. d.	<LOQ	108.6 <sup>1</sup>	414.9 <sup>6</sup> 67.7
	Day 62 [ng/mL] [RSD]	n. d.	<LOQ	107.2 <sup>1</sup>	366.5 <sup>6</sup> 32.7	n. d.	<LOQ	138.0 <sup>1</sup>	548.5 <sup>6</sup> 52.4
M684H026 Reg.No. 6059081	Day 20 [ng/mL] [RSD]	n. d.	102.9 <sup>6</sup> 9.4	406.0 <sup>6</sup> 12.2	964.6 <sup>6</sup> 28.6	n. d.	<LOQ	239.6 <sup>6</sup> 21.2	696.6 <sup>6</sup> 26.6
	Day 41 [ng/mL] [RSD]	n. d.	99.6 <sup>6</sup> 8.7	448.9 <sup>6</sup> 15.1	801.6 <sup>6</sup> 26.5	n. d.	<LOQ	130.2 <sup>6</sup> 28.9	400.8 <sup>6</sup> 66.6
	Day 62 [ng/mL] [RSD]	n. d.	100.4 <sup>5</sup> 10.5	413.7 <sup>6</sup> 14.9	824.7 <sup>6</sup> 29.7	n. d.	<LOQ	162.6 <sup>6</sup> 23.9	435.3 <sup>6</sup> 35.2

LOQ (limit of quantification) = 100 ng/mL

RSD = relative standard deviation in %

n. d. = not detectable

n<sup>x</sup> = superscript x is the number of animals used for plasma concentration measurement;

**Mortality:** There were no treatment-related effects on mortality in both the satellite group (63-days) and the main group (18-months). No animals of the satellite group died during the administration period. In the main group, the mortality rate in treated males and females was comparable to that observed in controls and no dose-response was evident (Table 6.5-31). Total mortality rates ranged from 10 - 14 % in males and 2 – 8 % in females. Most of the deceased animals showed different kinds of tumours, high grades of amyloidosis and/or inflammatory lesions in varying organs.

Table 6.5-31. Mortality - main group (18-months)

Dose level		Spontaneous death		Killed <i>in extremis</i>		Mortality total	
[ppm]	[mg/kg bw/d]	N	(%)	N	(%)	N	(%)
<b>Males</b>							
0	0	1	2	5	10	6	12
150	25.0	2	4	5	10	7 <sup>#</sup>	14
1000	162.3	2	4	5	10	7	14
5000	904.0	2	4	3	6	5 <sup>#</sup>	10
<b>Females</b>							
0	0	-	-	4	8	4	8
150	27.0	-	-	1	2	1	2
1000	183.8	-	-	1	2	1	2
5000	939.1	-	-	4	8	4	8

<sup>#</sup> = since the necropsy period started on study day 547, one male animal of low dose group (150 ppm; # 87) was sacrificed moribund on study day 553 and one male animal of the top dose group (5000 ppm; #158) died on study day 548 during the sacrifice period. Thus, increasing the mortality rate in the male low dose group (150ppm) from 12% to 14% and in the top dose group (5000 ppm) from 8% to 10%.

**Clinical findings:** There were no treatment-related clinical findings in both the satellite group and the main group.

**Nodules and masses:** There were no treatment-related increased incidence of nodules and masses (Tables 6.5-32). None were recorded for satellite group animals. Incidences and distributions in the main group did not show a dose-response and/or were higher in controls compared to treated groups.

Table 6.5-32. Incidence of nodules / masses at 18 months

		Males				Females			
Dose level	[ppm]	0	150	1000	5000	0	150	1000	5000
	[mg/kg bw/d]	0	25.0	162.3	904.0	0	27.0	183.8	939.1
Mass	[N]	9	8	8	8	6	5	1	1
	[%]	18	16	16	16	12	10	2	2
- palpable in abdomen	[N]	6	3	0	3	4	3	0	1
	[%]	12	6	0	6	8	6	0	2
- palpable through skin	[N]	4	7	8	5	2	2	1	0
	[%]	8	14	16	10	4	4	2	0

**Body weight and food and water consumption:** In the satellite group mean body weights were decreased at the top dose, in both sexes, at all time points (Table 6.5-33). A dose-response was observed in males but not females. Statistically-significant decreases were reached on day 56 for males (10 % lower than controls) and days 14 and 21 for females (6 – 7 % lower than controls). This resulted in overall (day 0 – 63) mean body weight gains which were 47 % lower than controls for males and 29 % lower than controls for females in the top dose group. Toxicologically-significant decreases in body weight gain were also seen in females during the first 21 days of treatment from the lowest dose. A dose-response in body weight gains was clear for both sexes. Statistically-significant decreases were observed at the top dose at all time points for males and several time points for females (days 0 – 14, 0 – 21 and 0 – 56). HCD for body weights at 63-days show that concurrent

control values were below the HCD range for males and at the HCD minimum for females, and that values for treated animals were mostly below the HCD range. Food consumption was generally lower compared to controls for both treated males and females, with some statistically-significant decreases, however, only effects at the top dose were considered treatment-related and adverse as only at this dose were impairments in body weight gain observed.

In the main group, mean body weights were decreased at all doses, in both sexes, at almost all time points (except for females at the low dose on day 28) (Table 6.5-34). Statistically-significant decreases were recorded in all dose groups for males (various time points) and females (at the end of the administration period), and at the top dose in males and females (all time points). Statistically-significant decreases in body weight seen in males in the low dose were outside (lower than) the HCD for body weight (study means). The statistically-significant decrease in body weight seen in females in the low dose on day 546 was within the HCD for body weight (study means). However, given the consistency of the effect at different time points, the statistical-significance and the dose-response, the effect is considered treatment-related. On day 546, decreases in terminal body weights were generally > 5 % for both sexes from the lowest dose. Therefore, treatment-related and adverse decreases in terminal body weights were seen in both sexes from the lowest dose. Overall (day 0 – 546) mean body weight gains were  $\geq 10$  % lower than controls in all dose groups for both sexes; 47 % lower than controls for males and 45 % lower than controls for females at the top dose. Although a clear dose-response was not evident for males, the reductions in body weight gain over the duration of the study were considered treatment-related and adverse in both sexes from the lowest dose. Food consumption was generally lower compared to controls for both males and females (for whom reductions were more pronounced) at all dose levels, with many statistically-significant decreases (Table 6.5-36). In males effects from the mid dose were considered treatment-related and adverse as statistically-significant decreases in food consumption were recorded on weeks 1, 4 and 13, and total mean food consumption was reduced by  $\geq 10$  % from day 0 to week 13. In females effects at all doses were considered treatment-related and adverse as reductions were marked (well above 10 %) and statistically-significant throughout the duration of the study. There were no treatment-related effects on water consumption. Overall, there were adverse reductions in terminal body weights and in body weight gains in both sexes from the lowest dose and adverse decreases in food consumption from the mid dose in males and from the lowest dose in females.

As only a LOAEL could be established for the effects on terminal body weights and overall body weight gains in both sexes and for decreases in food consumption in females, Benchmark Dose (BMD) analyses were performed by HSE (using PROAST) on terminal body weight and body weight gain data for both sexes (Table 6.5-35). A BMD analysis for food consumption in females was not performed. BMD analyses for body weight (on day 546) revealed a BMDL<sub>10</sub> (using a response level of 10 % and model averaging) of 301 mg/kg bw/d for males and 37.5 mg/kg bw/d for females. BMD analyses for body weight gain (ratio of body weight on day 546 / body weight on day 0) revealed a BMDL<sub>10</sub> (using a response level of 10 % and model averaging) of 319 mg/kg bw/d for males and 51.9 mg/kg bw/d for females. The lowest BMDL calculated for males (301 mg/kg bw/d, for body weight effects) was between the mid and high dose levels (ie above the low dose); this agrees with the values for  $\Delta\%$  of control, which was -4.6 % and -18.1 % for the mid and high doses respectively. The lowest BMDL calculated for females (37.5 mg/kg bw/d, for body weight effects) was between the low and mid dose levels (ie above the low dose); this also agrees with the values for  $\Delta\%$  of control, which was -7.7 % and -10.7 % for the low and mid doses respectively.

Table 6.5-33. Body weight and body weight gain - satellite group (63-days)

		Males				Females			
Dose level	[ppm]	0	150	1000	5000	0	150	1000	5000
	[mg/kg bw/d]	0	32.1	223.1	1174.8	0	34.8	301.1	1224.5
Body weight [g]									
Day 0	[mean]	22.3	22.7	22.2	22.0	18	17.9	18.2	18.1
	[SD]	1.2	0.8	1.3	0.8	0.7	0.8	0.4	0.6
	Δ% of control		+1.9	-0.2	-1.1		-0.7	+0.9	+0.4
Day 14	[mean]	23.5	24.2	23.4	22.0	19.4	18.6	18.6	18.1*
	[SD]	1.3	1.0	1.6	0.9	0.9	0.7	0.7	0.8
	Δ% of control		+2.8	-0.5	-6.2		-4.1	-3.9	-6.9
Day 21	[mean]	23.8	24.8	23.4	22.2	19.6	19.2	19.2	18.3*
	[SD]	1.5	1.0	1.4	1.0	0.9	0.8	0.7	0.9



Dose level		Males				Females			
	[ppm]	0	150	1000	5000	0	150	1000	5000
	[mg/kg bw/d]	0	32.1	223.1	1174.8	0	34.8	301.1	1224.5
	$\Delta\%$ of control		+4.4	-1.4	-6.7		-1.7	-1.8	-6.4
Day 28	[mean]	24.7	25.5	24.4	23.3	19.6	19.8	19.8	19.5
	[SD]	1.6	1.2	1.6	0.7	1.1	0.7	0.8	0.9
	$\Delta\%$ of control		+3.2	-1.1	-5.5		+0.9	+1.2	-0.4
Day 56	[mean]	26.5	27.4	25.8	<b>23.9*</b>	21.6	21.1	21.1	20.4
	[SD]	1.8	1.6	1.6	0.8	1.0	0.8	0.8	0.6
	$\Delta\%$ of control		+3.7	-2.4	-9.8		-2.0	-2.1	-5.5
Day 63	[mean]	26.0	27.5	26.0	24.0	21.5	20.8	21.4	20.6
	[SD]	1.8	1.7	1.7	0.9	1.2	1.1	1.1	0.7
	$\Delta\%$ of control		+5.8	-0.2	-7.8		-3.2	-0.5	-4.4
<b>Body weight gain [g]</b>									
Day 0-7	[mean]	0.9	1.1	0.5	<b>-0.2**</b>	0.7	0.5	0.4	0.4
	[SD]	0.2	0.4	0.4	0.5	0.5	0.5	0.5	0.7
	$\Delta\%$ of control		+23.6	-41.8	-118.2		-36.4	-50.0	-40.9
Day 0-14	[mean]	1.2	1.4	1.2	<b>0.0**</b>	1.4	0.7	<b>0.4*</b>	<b>0.0**</b>
	[SD]	0.4	0.6	0.5	0.9	0.4	0.7	0.5	0.4
	$\Delta\%$ of control		+17.6	-5.4	-97.3		-48.8	-67.1	-102.4
Day 0-21	[mean]	1.5	2.1	1.2	<b>0.1*</b>	1.5	1.3	1.0	<b>0.2**</b>
	[SD]	0.6	0.6	0.5	1.1	0.5	0.8	0.5	0.6
	$\Delta\%$ of control		+41.6	-19.1	-89.9		-12.9	-33.3	-84.9
Day 0-28	[mean]	2.4	2.8	2.2	<b>1.3*</b>	1.6	1.9	1.6	1.4
	[SD]	0.7	0.6	0.6	0.8	0.8	0.6	0.6	0.8
	$\Delta\%$ of control		+15.2	-9.0	-46.2		+20.4	+4.3	-9.7
Day 0-56	[mean]	4.2	4.8	3.6	<b>1.9**</b>	3.6	3.2	2.9	<b>2.3**</b>
	[SD]	0.9	1.1	0.8	1.0	0.8	0.6	0.5	0.4
	$\Delta\%$ of control		+12.6	-13.8	-55.7		-8.5	-17.4	-35.2
Day 0-63	[mean]	3.8	4.8	3.8	<b>2.0*</b>	3.5	3.0	3.3	2.5
	[SD]	0.9	1.3	1.0	1.1	1.0	0.7	0.8	0.4
	$\Delta\%$ of control		+28.3	$\pm 0.0$	-47.3		-16.0	-7.5	-28.8
<b>Historical control data for body weight (g)</b>									
Day 63	Mean		27.6				21.9		
	Min		27.1				21.5		
	Max		28.6				22.6		

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Dunnett test (two-sided)

HCD: 3 studies with start dates between May 2013 and Oct 2015 (concurrent to this study).

Range of study means shown.

Same species (mouse), strain (C57BL/6 J), laboratory and administration route (oral).

50/sex in each study.

Table 6.5-34. Body weight and body weight gain - main group (18-months)

Dose level		Males				Females			
	[ppm]	0	150	1000	5000	0	150	1000	5000
	[mg/kg bw/d]	0	25.0	162.3	904.0	0	27.0	183.8	939.1
<b>Body weight [g]</b>									
Day 0	[mean]	22.6	22.5	22.4	22.5	18.5	18.2	18.4	18.2
	[SD]	1.0	0.9	1.0	1.0	0.7	0.7	0.6	0.7
	$\Delta\%$ of control		-0.7	-0.9	-0.8		-1.6	-0.8	-1.7
Day 28	[mean]	25.5	25.1	25	<b>23.6**</b>	20.7	20.7	<b>20.2*</b>	<b>19.3**</b>
	[SD]	1.3	1.3	1.3	1.2	0.9	0.9	0.8	0.9
	$\Delta\%$ of control		-1.4	-1.9	-7.4		0.1	-2.2	-6.6
Day 63	[mean]	28.3	<b>27.5*</b>	<b>27.4**</b>	<b>25.5**</b>	22.3	22.2	21.9	<b>21.0**</b>
	[SD]	1.9	1.7	1.3	1.3	1.0	1.0	0.9	0.8

		Males				Females			
Dose level	[ppm]	0	150	1000	5000	0	150	1000	5000
	[mg/kg bw/d]	0	25.0	162.3	904.0	0	27.0	183.8	939.1
Δ% of control			-3.0	-3.3	-9.9		-0.4	-1.8	-6.0
Day 91	[mean]	30.6	29.4*	29.4*	26.8**	23.2	23.1	22.7	21.6**
	[SD]	2.4	2.1	1.9	1.5	1.3	1.3	1.3	0.8
	Δ% of control		-3.9	-3.8	-12.5		-0.6	-2.1	-7.0
Day 546	[mean]	36.4	33.6**	34.8	29.9**	35.2	32.5**	31.4**	27.3**
	[SD]	4.6	5.0	4.8	2.8	4.4	4.8	4.4	2.4
	Δ% of control		-7.7	-4.6	-18.1		-7.7	-10.7	-22.5
Historical control data for body weight (g) – range of study means									
Day 546	Mean	37.1				32.4			
	Min	35.0				31.0			
	Max	40.3				33.5			
Body weight gain [g]									
Day 0-28	[mean]	2.8	2.6	2.6	1.2**	2.1	2.5	1.8	1.1**
	[SD]	0.9	1.0	1.0	0.9	0.7	0.7	0.7	0.7
	Δ% of control		-7.5	-9.2	-59.4		+14.9	-14.3	-49.1
Day 0-63	[mean]	5.7	5.0*	5.0*	3.1**	3.8	4.40	3.5	2.7**
	[SD]	1.4	1.4	1.0	1.0	0.8	0.6	0.7	0.6
	Δ% of control		-12.1	-12.5	-46.4		+5.8	-6.6	-27.4
Day 0-91	[mean]	8.0	6.9**	7.0*	4.3**	4.7	4.8	4.3	3.3**
	[SD]	1.9	1.8	1.6	1.2	1.0	1.0	1.1	0.6
	Δ% of control		-12.8	-11.9	-45.7		+3.2	-7.3	-28.3
Day 0-546	[mean]	13.7	11.2*	12.4	7.3**	16.6	14.2*	13.0**	9.1**
	[SD]	4.2	4.7	4.5	2.6	4.3	4.5	4.3	2.2
	Δ% of control		-18.3	-9.9	-46.5		-14.4	-21.6	-45.4

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Dunnett test (two-sided)

HCD: 3 studies with start dates between May 2013 and Oct 2015 (concurrent to this study).

Same species (mouse), strain (C57BL/6 J), laboratory and administration route (oral).

50/sex in each study.

Table 6.5-35. Bench mark dose (BMD) analysis for terminal body weights and body weight gains

Parameter	BMDL [mg/kg bw/d]	BMDU [mg/kg bw/d]	BMDU/BMDL	Conclusions
Males				
Body weight on day 546 (BMR = 0.10 (10 %))	301	845	2.8	The BMDL <sub>10</sub> (301 mg/kg bw/d) lies between the mid (162.3 mg/kg bw/d) and high (904 mg/kg bw/d) dose.
Body weight gain day 0-546 (BMR = 0.10 (10 %))	319	876	2.7	The BMDL <sub>10</sub> (319 mg/kg bw/d) lies between the mid (162.3 mg/kg bw/d) and high (904 mg/kg bw/d) dose.
Females				
Body weight on day 546 (BMR = 0.10 (10 %))	37.5	232	6.2	The BMDL <sub>10</sub> (37.5 mg/kg bw/d) lies between the low (27 mg/kg bw/d) and mid (183.8 mg/kg bw/d) dose.
Body weight gain day 0 - 546 (BMR = 0.10 (10 %))	51.9	339	6.5	The BMDL <sub>10</sub> (51.9 mg/kg bw/d) lies between the low (27.0 mg/kg bw/d) and mid (183.8 mg/kg bw/d) dose.

BMD analysis for body weight on day 546:

Analysis generated on 11/08/2020; using PROAST version 69.0

Type of response: Continuous, individual data.

Settings used: Set of models (default), perform model averaging (default), 200 Bootstrap runs (default), max. difference for AIC acceptance of 2 (default), CES value of 0.10 (specified) and confidence level for the BMD confidence intervals of 0.9 (default).

Justification for the response level of 10 % (the Benchmark Response or BMR) for body weight effects: Based on the Coefficient of Variation (CV) (for body weight), based on 3 HCD studies, which ranged between 13.7 – 14.2 %. The CV was used (rather than the default BMR of 5 %) as this indicated relatively large within group variation.

BMD analysis for body weight gain day 0 - 546:

Analysis by the applicant; using PROAST version 65.5.

Type of response: Continuous, individual data.

Single model used, therefore BMDL and BMDU quoted are lowest and highest respectively.

Justification for the response level of 10 % (the Benchmark Response or BMR) for body weight gain effects: HSE considers a 10 % change as toxicologically-relevant, particularly where statistical-significance is calculated. HSE notes that the applicant proposed a BMR of 10 %.

Table 6.5-36. Food consumption - main group (18-months)

Dose	[ppm] [mg/kg bw/d]	Males				Females			
		0	150	1000	5000	0	150	1000	5000
		0	25.0	162.3	904.0	0	27.0	183.8	939.1
Food consumption [g/animal/day]									
Week 1	[mean]	5.7	5.2*	5.1**	4.7**	5.8	4.6**	4.6**	4.1**
	[SD]	1.3	0.9	0.8	0.8	1.0	1.2	1.2	0.7
	Δ% of control		-9.0	-10.5	-16.9		-20.5	-21.9	-29.3
Week 4	[mean]	6.3	5.7*	5.5**	5.1**	6.7	5.1**	5.2**	4.6**
	[SD]	1.4	1.0	0.7	1.7	0.6	1.0	1.0	1.3
	Δ% of control		-10.6	-13.8	-19.2		-23.7	-21.8	-30.8
Week 9	[mean]	6.1	5.7	5.6	4.8**	6.5	5.4**	5.2**	4.4**
	[SD]	1.3	0.8	0.8	1.0	1.1	1.0	1.3	0.9
	Δ% of control		-5.7	-7.3	-21.0		-16.3	-19.2	-32.7

Dose		Males				Females			
		0	150	1000	5000	0	150	1000	5000
		0	25.0	162.3	904.0	0	27.0	183.8	939.1
Week 13	[mean]	5.7	5.6	5.3*	4.8**	5.9	4.7**	4.8**	4.5**
	[SD]	0.9	0.9	0.6	1.0	1.0	0.7	0.9	1.2
	Δ% of control		-2.9	-8.3	-16.4		-20.6	-18.1	-24.4
Week 78	[mean]	5.1	5.0	4.8	5.1	5.8	4.5**	4.8*	4.9*
	[SD]	1.0	1.0	0.9	1.1	1.3	1.2	1.1	1.5
	Δ% of control		-2.1	-4.9	1.2		-23.1	-18.1	-15.7
<b>Total mean food consumption [g/animal/day]</b>									
Day 0 – week 4	[mean]	6.2	5.5	5.4	5.0	6.4	4.9	4.9	4.4
	[SD]	0.3	0.3	0.2	0.2	0.4	0.2	0.3	0.2
	Δ% of control		-11.0	-12.6	-18.3		-22.7	-23.1	-31.8
Day 0 – week 9	[mean]	6.2	5.6	5.5	5.0	6.5	5.1	5.2	4.4
	[SD]	0.2	0.2	0.2	0.2	0.3	0.3	0.4	0.2
	Δ% of control		-8.5	-10.5	-18.4		-21.1	-19.2	-31.7
Day 0 – week 13	[mean]	6.1	5.7	5.5	5.0	6.4	5.1	5.2	4.4
	[SD]	0.2	0.2	0.2	0.2	0.3	0.3	0.4	0.2
	Δ% of control		-7.1	-10.1	-18.1		-19.8	-18.2	-31.2
Day 0 – week 78	[mean]	5.6	5.4	5.3	5.1	5.9	4.9	5.0	4.5
	[SD]	0.5	0.3	0.3	0.3	0.7	0.4	0.5	0.4
	Δ% of control		-4.4	-7.0	-10.0		-17.4	-16.3	-24.9

\* p ≤ 0.05; \*\* p ≤ 0.01; Dunnett test (two-sided)

**Haematology:** No statistically-significant changes in differential blood counts and/or red cell morphology were observed up to the top dose of both sexes after 12-month (Tables 6.5-37 and 6.5-38). At 18-months statistically-significant changes in differential blood cell counts - decrease in lymphocyte counts and increase in neutrophil counts in males, and decrease in monocyte counts and eosinophil counts in both sexes – were recorded. However, the increased segmented neutrophils count was not corroborated by higher band or occurrence of the precursor cells (metamyelocytes, myelocytes, promyelocytes), consequently this finding was not considered to be of toxicological-relevance. The decreases in monocyte, eosinophil and lymphocyte counts were not considered to be biologically-relevant due to modest magnitude of these changes. At 18-months marginal but statistically-significant changes in red blood cell morphology (increased Howell-Jolly body (HJB) counts and polychromasia grade) were observed. HJBs represent nuclear remnants in immature erythrocytes and are normally increased in anaemic situations, however, no red blood cell precursor cells occurred and the red blood cell morphology was also not affected (cell size i.e. micro- / macrocytosis and haemoglobin content i.e. hyper- / hypochromasia) apart from the marginal higher polychromasia grade in some cells. Consequently these findings were not considered to be toxicologically-relevant. Additionally, no treatment-related, adverse effects were found in the blood smears of the animals killed *in extremis*. Overall, there were no treatment-related adverse effects on haematological parameters at either 12- or 18-months.

HSE notes that urinalysis and clinical chemistry were not conducted in this study.

Table 6.5-37. Haematology - selected differential blood cell count parameters

Sex		Males				Females			
Sampling time point		12 months		18 months		12 months		18 months	
Dose	[ppm]	0	5000	0	5000	0	5000	0	5000
Lymphocytes	[%]	82.0	79.9	72.3	65.2*	82.2	84.2	74.5	74.8
	SD	6.0	9.3	13.1	15.2	5.7	4.1	9.9	13.0
	Δ%	-	-3	-	-10	-	+2	-	±0
Neutrophils	[%]	10.6	12.4	21.7	31.1**	10.4	9.4	17.2	19.3
	SD	4.6	8.0	12.3	15.4	4.7	3.3	8.0	10.8
	Δ%	-	+17	-	+43	-	-10	-	+12
Monocytes	[%]	4.6	5.1	4.5	3.0**	4.7	3.9	6.1	4.7**
	SD	2.7	2.7	2.6	2.5	3.1	2.0	3.5	4.7

Sex		Males				Females			
Sampling time point		12 months		18 months		12 months		18 months	
Dose	[ppm]	0	5000	0	5000	0	5000	0	5000
	$\Delta\%$	-	+12	-	-33	-	-15	-	-24
Eosinophils	[%]	2.0	2.0	0.8	<b>0.3*</b>	1.9	1.4	1.3	<b>0.6*</b>
	<i>SD</i>	1.4	1.6	1.2	0.7	1.8	1.4	2.6	1.0
	$\Delta\%$	-	-3	-	-65	-	-28	-	-56
Promyelocytes	[%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>SD</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	$\Delta\%$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$
Myelocytes	[%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>SD</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	$\Delta\%$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$
Metamyelocytes	[%]	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
	<i>SD</i>	0.0	0.0	0.0	0.0	0.3	0.1	0.1	0.1
	$\Delta\%$	-	$\pm 0$	-	$\pm 0$	-	-74	-	$\pm 0$
Bands	[%]	0.2	0.3	0.1	0.1	0.2	0.3	0.0	0.0
	<i>SD</i>	0.6	0.7	0.3	0.4	0.4	0.6	0.1	0.0
	$\Delta\%$	-	+22	-	+63	-	+59	-	-100

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Wilcoxon test (one-sided).

$\Delta\%$  - percent change compared to control.

Table 6.5-38. Haematology - selected red blood cell morphology parameters

Sex		Males				Females			
Sampling time point		12 months		18 months		12 months		18 months	
Dose	[ppm]	0	5000	0	5000	0	5000	0	5000
Howell-Jolly bodies (HJB)	[%]	0.8	0.9	2.3	<b>3.0**</b>	0.9	1.1	1.4	1.4
	<i>SD</i>	0.7	0.8	0.9	1.3	0.9	0.8	0.8	0.7
	$\Delta\%$	-	+8	-	+28	-	+13	-	$\pm 0$
Polychromasia	[%]	0.3	0.4	0.6	<b>0.9*</b>	0.4	0.2	0.6	0.5
	<i>SD</i>	0.5	0.6	0.5	0.5	0.5	0.4	0.5	0.5
	$\Delta\%$	-	+29	-	+41	-	-47	-	-11
Hyperchromasia	[%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>SD</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	$\Delta\%$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$
Hypochromasia	[%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>SD</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	$\Delta\%$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$
Microcytosis	[%]	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
	<i>SD</i>	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
	$\Delta\%$	-	$\pm 0$	-	+29.1	-	$\pm 0$	-	$\pm 0$
Macrocytosis	[%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>SD</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	$\Delta\%$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$	-	$\pm 0$

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Wilcoxon test (one-sided)

$\Delta\%$  - percent change compared to control.

**Organ weights:** Liver weights were statistically-significantly increased in both males (absolute and relative) and females (relative) from the mid dose (Table 6.5-38). At the top dose changes in relative liver weights compared to controls were 38 % for males and 27 % for females; in addition values recorded were outside the HCD range for males (absolute and relative) and females (relative). At the mid dose, whilst relative liver weights were

statistically-significant and above the HCD range, changes compared to controls were 12 % for males and 7 % for females. Histopathology showed statistically-significantly increased liver findings (hypertrophy - both centrilobular and periportal, oval cell hyperplasia, and eosinophilic foci of cellular alterations) at the top dose in males and females but not at the mid dose. Therefore, only the increases seen at the top dose were considered treatment-related and adverse.

A statistically-significant increased in relative ovary weight was observed at the top dose; change compared to controls was 75 % for relative weight and 34 % for absolute weight. However, this was based on an extremely high individual ovarian weight (473 mg) in one female (# 372) and was not regarded as treatment-related due to its isolated occurrence. Other statistically-significant changes in organ weights, seen in adrenal glands, brain, heart, kidney, spleen, epididymides, testes and uterus, were not considered treatment-related due to a lack of dose-response and/or concomitant histopathological findings. Overall, treatment-related and adverse increases (> 15 %) in liver weight were seen at the top dose.

Table 6.5-39. Organ weights - main group (18-months)

Sex		Males				Females			
	Dose [ppm]	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
Terminal weight [g]	0	32.702				31.826			
	150	<b>30.307*</b>	-7.3			30.104	-5.4		
	1000	31.135	-4.8			<b>28.898**</b>	-9.2		
	5000	<b>26.464**</b>	-19.1			<b>24.770**</b>	-22.2		
Adrenal glands [mg]	0	3.977		0.012		7.630		0.024	
	150	4.326	+8.8	0.015	+25.0	7.510	-1.6	0.025	+4.2
	1000	4.279	+7.6	0.014	+16.7	8.020	+5.1	<b>0.028*</b>	+16.7
	5000	4.711	+18.5	<b>0.018**</b>	+50.0	8.043	+5.4	<b>0.033**</b>	+37.5
Brain [mg]	0	473.114		1.473		494.087		1.578	
	150	472.605	-0.1	<b>1.593*</b>	+8.1	490.388	-0.7	1.659	+5.1
	1000	472.279	-0.2	1.546	+5.0	488.592	-1.1	<b>1.721**</b>	+9.1
	5000	<b>464.200**</b>	-1.9	<b>1.770**</b>	+20.2	<b>481.891**</b>	-2.5	<b>1.957**</b>	+24.0
Heart [mg]	0	175.182		0.544		159.978		0.510	
	150	184.465	+5.3	<b>0.624**</b>	+14.7	<b>151.551**</b>	-5.3	0.510	±0.0
	1000	183.814	+4.9	<b>0.602*</b>	+10.7	<b>153.184**</b>	-4.2	<b>0.534*</b>	+4.7
	5000	170.911	-2.4	<b>0.650**</b>	+19.5	<b>144.957**</b>	-9.4	<b>0.587**</b>	+15.1
Kidneys [mg]	0	508.659		1.576		438.761		1.396	
	150	520.163	+2.3	<b>1.753**</b>	+11.2	<b>410.612**</b>	-6.4	1.379	-1.2
	1000	522.419	+2.7	<b>1.708*</b>	+8.4	<b>414.939*</b>	-5.4	<b>1.446*</b>	+3.6
	5000	<b>424.778**</b>	-16.5	1.610	+2.2	<b>391.413**</b>	-10.8	<b>1.584**</b>	+13.5
Spleen [mg]	0	92.568		0.296		165.565		0.546	
	150	164.674	+77.9	0.533	+80.1	161.408	-2.5	0.556	+1.8
	1000	104.047	+12.4	0.352	+18.9	<b>123.653**</b>	-25.3	0.426	-22.0
	5000	82.578	-10.8	0.322	+8.8	<b>99.109**</b>	-40.1	0.401	-26.6
Liver [mg]	0	1298.30		3.998		1440.70		4.586	
	150	1259.44	-3.0	4.215	+5.4	<b>1369.14**</b>	-5.0	4.605	+0.4
	1000	<b>1381.42**</b>	+6.4	<b>4.474**</b>	+11.9	1404.74	-2.5	<b>4.890**</b>	+6.6
	5000	<b>1458.27**</b>	+12.3	<b>5.530**</b>	+38.3	1442.83	+0.1	<b>5.836**</b>	+27.3
HCD#		Mean (mg): 1331.96		Mean (%): 3.912		Mean (mg): 1309.81		Mean (%): 4.569	
		Min (mg): 1270.24		Min (%): 3.622		Min (mg): 1209.46		Min (%): 4.293	
		Max (mg): 1424.71		Max (%): 4.187		Max (mg): 1443.74		Max (%): 4.887	
Epididymides [mg]	0	83.295		0.259					
	150	85.116	+2.2	<b>0.286**</b>	+10.4				
	1000	84.581	+1.5	<b>0.276*</b>	+6.6				
	5000	<b>77.889**</b>	-6.5	<b>0.296**</b>	+14.3				
Ovaries [mg]	0					19.826		0.063	

Sex		Males				Females			
	Dose [ppm]	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
	150					13.265	-33.1	0.045	-28.6
	1000					14.878	-25.0	0.052	-17.5
	5000					26.522	+33.8	<b>0.110**</b>	+74.6
Testes (♂) [mg]	0	192.023		0.598					
	150	192.372	+0.2	<b>0.644*</b>	+7.7				
	1000	191.279	-0.4	0.624	+4.3				
	5000	186.845	-2.7	<b>0.711**</b>	+18.9				
Uterus [mg]	0					154.630		0.491	
	150					142.633	-7.8	0.487	-0.8
	1000					143.041	-7.5	0.505	+2.9
	5000					148.478	-4.0	<b>0.605**</b>	+23.2

\* p ≤ 0.05. \*\* p ≤ 0.01; Kruskal-Wallis and Wilcoxon-test (two-sided)

& : Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means

# : historical control data (HCD) based on 4 carcinogenicity studies with C57BL/6J Rj mice (supplier: XXXXXXXXXX) performed at XXXXXXXXXX during 2008 – 2015 under GLP conditions.

HSE notes that HCD dates exceed ± 5 years from the study start date (2015).

*Gross pathology:* There were no treatment-related macroscopic findings in both the satellite group and the main group. Findings were incidental, occurring either individually or biologically equally distributed over control and treatment groups.

#### *Histopathology:*

*Non-neoplastic findings:* Statistically-significantly increased non-neoplastic findings were observed at 18-months in the liver, nasal cavity and kidney mainly at the top dose (Table 6.5-40). In the liver increased incidences were noted for hypertrophy - both centrilobular (5/50 for males) and periportal (34/50 for females), oval cell hyperplasia (38/50 for females), and eosinophilic foci of cellular alterations (6/50 for males). Liver findings correlated with the observed increase in liver weight at the top dose (> 15 % change compared to control) and were therefore considered treatment-related and adverse at the top dose. In the nasal cavity III increases were seen for metaplasia of olfactory epithelium in the ethmoid turbinates as well as of submucosal glands from the mid dose in males and at the top dose in females and for degeneration/regeneration of olfactory epithelium at the top dose in both sexes. Nasal cavity findings were considered treatment-related and adverse. The loss of vacuolation in the kidneys of males of the top dose was considered treatment-related but not adverse due to absence of any other corroborative kidney findings. All other findings were considered to be incidental and not treatment-related. Overall, there were treatment-related and adverse histopathological findings in the liver (hypertrophy, oval cell hyperplasia and eosinophilic foci of cellular alterations) at the top dose in both sexes and in the nasal cavity from the mid dose in males and at the top dose in females.

Table 6.5-40. Non-neoplastic findings (18-months) - incidences shown

		Males				Females			
Dose level	[ppm]	0	150	1000	5000	0	150	1000	5000
	[mg/kg bw/d]	0	25.0	162.3	904.0	0	27.0	183.8	939.1
Number of animals		50	50	50	50	50	50	50	50
<b>Liver</b>									
Hypertrophy, centrilobular		0	0	0	<b>5*</b>	0	0	0	0
	Grade 1	-	-	-	4	-	-	-	-
	Grade 2	-	-	-	1	-	-	-	-
	[mean] <sup>#</sup>	[0.0]	[0.0]	[0.0]	[1.2]	[0.0]	[0.0]	[0.0]	[0.0]
Hypertrophy, periportal		0	0	0	0	0	0	0	<b>34*</b>
	Grade 1	-	-	-	0	-	-	-	34
	[mean] <sup>#</sup>	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[1.0]

		Males				Females			
Dose level	[ppm]	0	150	1000	5000	0	150	1000	5000
	[mg/kg bw/d]	0	25.0	162.3	904.0	0	27.0	183.8	939.1
Hyperplasia, oval cell.		3	2	0	1	2	1	3	38*
	Grade 1	2	1	-	1	2	1	3	34
	Grade 2	1			-	-	-	-	4
	[mean] <sup>#</sup>	[1.3]	[1.0]	[0.0]	[1.0]	[1.0]	[1.0]	[1.0]	[1.1]
Foci of cellular alteration		4	1	1	8	1	1	0	2
	eosinophilic	0	0	0	6*	0	0	0	1
	basophilic	4	1	1	3	1	1	0	1
Fatty change, diffuse		41	38	40	34	47	48	48	47
	Grade 1	7	7	6	13	2	-	1	1
	Grade 2	25	22	14	14	7	13	6	15
	Grade 3	7	9	19	7	32	31	39	30
	Grade 4	2	-	1	-	6	4	2	1
	[mean] <sup>#</sup>	[2.1]	[2.1]	[2.4]	[1.8]	[2.9]	[2.8]	[2.9]	[2.7]
Fatty change, macrovesicular		15	12	12	4	39	35	25	16
	Grade 1	11	10	5	3	24	23	23	15
	Grade 2	3	2	6	1	14	12	2	1
	Grade 3	1	-	1	-	1	-	-	-
	[mean] <sup>#</sup>	[1.8]	[1.2]	[1.7]	[1.3]	[1.4]	[1.3]	[1.1]	[1.1]
Nasal cavity, level III									
Metaplasia, respiratory		8	11	19*	47**	8	19*	13	41**
	Grade 1	8	9	17	11	8	18	12	24
	Grade 2	-	2	2	20	-	1	1	15
	Grade 3	-	-	-	16	-	-	-	2
	[mean] <sup>#</sup>	[1.0]	[1.2]	[1.1]	[2.1]	[1.0]	[1.1]	[1.1]	[1.5]
Degeneration/regeneration olfactory epithelium		1	0	6	50**	0	0	1	27**
	Grade 1	1	-	5	7	-	-	1	27
	Grade 2	-	-	1	32	-	-	-	-
	Grade 3	-	-	-	11	-	-	-	-
	[mean] <sup>#</sup>	[1.0]	[0.0]	[1.2]	[2.1]	[0.0]	[0.0]	[1.0]	[1.0]
Eosinophil. subst., septum		31	27	45*	23	31	35	30	31
Kidney									
No. of animals examined		50	50	50	50	50	4	6	50
Vacuoles, tubules		47	44	49	22	0	0	0	0
	Grade 1	9	5	9	19	-	-	-	-
	Grade 2	15	22	16	2	-	-	-	-
	Grade 3	21	14	21	1	-	-	-	-
	Grade 4	2	3	3	-	-	-	-	-
	[mean] <sup>#</sup>	[2.3]	[2.3]	[2.4]	[1.2]	[0.0]	[0.0]	[0.0]	[0.0]

\*p <= 0.05; \*\*p <= 0.01 (Fisher's Exact test, one sided)

N = number of animals examined

<sup>#</sup> = mean severity grading; histopathological findings were graded minimal/very few (Grade 1); slight/few (Grade 2); moderate (Grade 3); marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding

*Neoplastic findings:* There were no treatment-related neoplastic findings. The numbers of animals with neoplasms (benign, malignant, systemic and metastasised) and the total numbers of neoplasms (primary, benign, malignant and systemic) were comparable between control and high dose groups (Table 6.5-41) and therefore not considered treatment-related. There were no increases in neoplastic findings observed in the liver up to and including the top dose (Table 6.5-42).



Table 6.5-41. Total incidence of neoplastic findings (18-months)

		Males				Females			
Dose level	[ppm]	0	150	1000	5000	0	150	1000	5000
	[mg/kg bw/d]	0	25.0	162.3	904.0	0	27.0	183.8	939.1
No. of animals		50	50	50	50	50	50	50	50
Number of animals with <sup>#</sup> :									
- neoplasms		14	9	10	13	31	29	32	28
- 1 primary neoplasm		13	7	8	13	20	21	25	19
- 2 and > primary neoplasms		1	2	2	0	11	8	7	9
Number of animals with:									
- benign neoplasms		7	0	1	6	22	23	30	24
- benign neoplasms only		6	0	1	6	14	15	23	16
- malignant neoplasms		8	9	9	7	17	14	9	12
- malignant neoplasms only		7	9	9	7	9	6	2	4
- systemic neoplasms		7	7	6	7	14	14	7	11
- metastasized neoplasms		0	0	0	0	0	0	1	0
Total number of <sup>§</sup> :									
- primary neoplasms		15	11	12	13	43	37	41	40
- benign neoplasms		7	0	1	6	26	23	31	27
- malignant neoplasms		7	11	11	7	17	14	10	13
- systemic neoplasms		7	8	7	7	14	14	7	11
- metastasized neoplasms		0	0	0	0	0	0	1	0

<sup>#</sup> = affected animals had one or more neoplasms, systemic neoplasms were all categorized as malignant neoplasms; therefore, they are shown twice in this table.

<sup>§</sup> = all systemic neoplasms were categorized as malignant neoplasms; therefore, they are shown twice in this table.

Table 6.5-42. Incidence of hepatic neoplastic findings (18-months)

		Males				Females			
Dose level	[ppm]	0	150	1000	5000	0	150	1000	5000
	[mg/kg bw/d]	0	25.0	162.3	904.0	0	27.0	183.8	939.1
No. of animals		50	50	50	50	50	50	50	50
Adenoma, hepatocellular		2	0	1	2	0	0	0	0
Carcinoma, hepatocellular		1	2	0	0	0	0	0	0
Haemangiosarcoma		0	0	1	0	0	0	0	0
Tumour, ito cell, benign		1	0	0	0	0	0	0	0
Infiltrates, lymphoma		1	1	0	0	3	4	1	2
Infiltrates, histiocytic sarcoma		0	0	0	2	0	2	0	1
Infiltration, leukemic		0	0	0	0	1	0	0	0
Metastasis		0	0	0	0	0	0	1	0

### Conclusion

Cinmethylin was administered via the diet to male and female C57BL/6J Rj mice for 18-months, in a standard guideline carcinogenicity study. No increase in the incidence, severity or onset of tumours was observed up to and including the highest dose tested (5000 ppm, equivalent to 904 and 939 mg/kg bw/d in M/F respectively), which caused significant toxicity. Therefore a **NOAEL for carcinogenicity in the mouse of 5000 ppm (904 and 939 mg/kg bw/d in M/F respectively)** is proposed by HSE.

Significant systemic toxicity was observed in both sexes at the top dose (5000 ppm, equivalent to 904 and 939 mg/kg bw/d in M/F respectively) which indicated that the top dose was sufficiently high. Body weight and body weight gain were adversely reduced in both sexes across all treatment groups. The lowest BMDL<sub>10</sub> of 37.5 mg/kg bw/d was identified for decreases in body weight in females. Consistent with the findings of repeated-dose studies, the liver was identified as a target organ in mice; relative liver weights were increased by 27/38 % in M/F of the top dose and concomitant histopathology (hypertrophy, oval cell hyperplasia and eosinophilic foci of cellular alterations) was found at 18-months. Nasal cavity findings were considered treatment-related and adverse at the top dose in females and from the mid dose in males.

In conclusion, there were adverse effects on the liver at the top dose in both sexes and on the nasal cavity from the mid dose. In addition there were adverse effects on body weight and body weight gain in both sexes and on food consumption in females from the lowest dose. **Therefore, a LOAEL for systemic chronic toxicity in the mouse of 150 ppm (25/27.0 mg/kg bw/d in M/F) was identified. Alternatively, the lowest BMDL<sub>10</sub> for effects on body weight in females – 37.5 mg/kg bw/d, can be considered as the reference point from this study.**

(██████████, 2018d)

## 2) Old study

The following summarises a single study which was split into two reports (original and supplemental submission) with two references.

<b>Author(s)</b>	<ul style="list-style-type: none"> <li>• ██████████</li> <li>• ██████████</li> </ul>
<b>Study title</b>	<ul style="list-style-type: none"> <li>• Oncogenicity study of SD95481 in the mouse</li> <li>• Preparation of supplement for submission to the Japanese Ministry of Agriculture, Forestry and Fisheries from regulatory information record No. WRC RIR-424 (Oncogenicity study with sd95481 in mice)</li> </ul>
<b>Study reference</b>	<ul style="list-style-type: none"> <li>• ██████████, 1986; CI-428-001</li> <li>• ██████████, 1991; CI-428-002</li> </ul>
<b>Test facility</b>	██
<b>Dates of work</b>	10/01/1983 – 20/01/1985
<b>Test substance</b>	BAS 684 H (900202) (Cinmethylin)
<b>Purity (%)</b>	92
<b>Batch no.</b>	5-4-0-0, 513F; ST82/255
<b>Test organisms</b>	Mouse B6C3F1 Males and females
<b>Groups</b>	120/sex controls; 20/sex for 12-months and 100/sex for 24-months. 10/sex/dose (12-month groups – satellite group) 50/sex/dose (24-month groups – main group)
<b>Dose/concentration</b>	0, 30, 100 and 1000 ppm. Equivalent to 0, 7.2, 22.1 and 231 mg/kg bw/d in males and 0, 8.3, 26.8 and 272 mg/kg bw/d in females.
<b>Route</b>	Administered orally, via the diet, daily, for a period of 12 or 24 months.
<b>Vehicle</b>	Acetone.
<b>GLP</b>	Not compliant.
<b>Guideline</b>	None.
<b>Deviation</b>	<ul style="list-style-type: none"> <li>• Water was stated to be available ad libitum. However, difficulties in adjusting the automatic watering system occurred at the start of this study, especially for females. Furthermore, inspections of the watering systems revealed inaccessibility of the water for the most low-dose males (40/44) at week 74 and most control males (61/73) at week 84 that resulted in thin appearance and mortalities of five control males due to dehydration.</li> <li>• Due to technical error, pre-necropsy (fasted weights) were not taken on animals sacrificed during the first 3 days of the scheduled terminal necropsy. Therefore, at</li> </ul>

	<p>terminal sacrifice (104 week) non-fasted weights were used to calculate relative organ weights (organ to body weights) for all animals.</p> <p>Compared with the currently valid OECD TG 453 (2009):</p> <ul style="list-style-type: none"> <li>• The spacing for the top dose was 10-fold, not matching the recommended 2-4-fold interval.</li> <li>• Detailed clinical observations outside the home cage, preferably in a standard arena, were not performed in this study.</li> <li>• Ophthalmoscopy were not performed in this study.</li> <li>• Urinalysis was not performed for animals of the chronic toxicity group.</li> <li>• For haematology, the following parameters were not determined in animals of chronic toxicity groups: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), prothrombin time, and activated partial thromboplastin time.</li> <li>• The clinical chemistry parameter creatinine was not determined in this study.</li> <li>• Due to insufficient blood volume collected at the time of the 52-week interim sacrifice, the following parameters were not determined for all animals: direct bilirubin, globulin, phosphorous, and sodium.</li> <li>• At termination, epididymides, ovaries and thyroids weights were not determined in animals of chronic toxicity groups.</li> <li>• Generally, except for some neoplastic findings, laboratory historical control data are lacking.</li> </ul> <p>Limitations:</p> <ul style="list-style-type: none"> <li>• Results of testing for mouse hepatitis virus (MHV) at week 52 revealed positive findings in 28/42 males (56 %, 44 %, 86 % and 90 % for 0, 30, 100 and 100 ppm groups, respectively) and 39/42 females (86 %, 100 %, 89 % and 100 % for 0, 30, 100 and 100 ppm groups, respectively). The data showed an active infection between weeks 40 to 52, seen in all treated groups, including the controls, without a dose-response.</li> <li>• The study lacked evidence which indicates the MTD was achieved - evidence of generalised toxicity, including a of depression of body weight gain, was lacking.</li> </ul>
<b>Impact of deviations</b>	Despite the above listed deviations and limitations, this study, together with the new/modern mouse study (■■■■■, 2018d), is considered to contribute in a WoE approach to the carcinogenic hazard assessment of cinmethylin.
<b>Acceptable</b>	This study is considered supplemental information only with regards to systemic toxicity but is inconclusive with regards to carcinogenicity.
<b>Conclusion</b>	Cannot conclude on carcinogenicity.
<b>NOAEL</b>	NOAEL for carcinogenicity: N/A. NOAEL for systemic chronic toxicity: 100 ppm, equivalent to 22.1 and 26.8 mg/kg bw/d in males and females respectively.
<b>Effects at the LOAEL</b>	Carcinogenicity: No conclusion can be drawn regarding hepatic neoplastic findings. Systemic chronic toxicity: Increase in liver weight at the top dose in both sexes.

### Methods

In a relatively old, non-GLP and non-OECD test guideline compliant study, cinmethylin was administered to groups of male and female B6C3F1 mice at dietary dose levels of 0, 30, 100 and 1000 ppm for 12-months (satellite groups; 10/sex/dose) and 24-months (main groups; 50/sex/dose). Dietary concentration corresponded to mean intakes of 0, 7.2, 22.1 and 231 mg/kg bw/d in males and 0, 8.3, 26.8 and 272 mg/kg bw/d in females. Clinical signs of toxicity were monitored daily, body weights and food consumption measured weekly for 26 weeks and thereafter every two weeks for 2 years. After 52 weeks, mice were necropsied for pathologic evaluation (interim sacrifice), and at the end of two years (104 weeks) remaining, surviving mice were sacrificed and necropsied. Evaluation of macroscopic and microscopic pathology of selected tissues and gross lesions, hematology and clinical chemistry analyses, and selected organ weight determinations were performed. A method for determination of cinmethylin in mouse diet was evaluated and was regarded as fit for purpose (see Volume 3 CA B5, section B.5.1.2).

**Results**

The stability and homogeneity of cinmethylin in the diet was confirmed in a separate study using a validated method of analysis. Plasma concentration of the test substance and/or its metabolites was not determined in this study.

*Mortality:* No treatment-related increase in mortality was observed. Total mortality rates ranged from 14 – 21 % (compared to 22 % in controls) in males and 20 – 31 % (compared to 34 % in controls) in females; slightly higher in females (Table 6.5-43). As expected in a lifetime toxicity study, the mortality rate increased during weeks 78 - 104, during which 49 % of all male deaths and 46 % of all female deaths occurred. Overall, mortality rates were acceptable.

Table 6.5-43. Mortality – main group (24-months)

Dose		Spontaneous death	Sacrificed <i>in extremis</i>	Mortality total	
[ppm]	[mg/kg bw/d]			[N]	[%]
Males					
0	0	22	-	22 / 100	22
30	7.2	8	-	8 / 57	14
100	22.1	10	1	11 / 55	20
1000	231	12	-	12 / 57	21
Females					
0	0	38	2	40 / 117	34
30	8.3	11	1	12 / 59	20
100	26.8	16	-	16 / 60	27
1000	272	18	-	18 / 59	31

*Clinical observations:* There were no treatment-related clinical findings. Other than hair loss (observed across all groups, from weeks 13 - 104, at highest frequency in the control, with no dose-response), the incidence of clinical signs was very low. At week 90, through to week 104, thinness and hunched posture were recorded – mainly in females, at all doses with no dose-response (Table 6.5-44). Due to the lack of dose-response and/or as highest incidences were recorded in controls, clinical observations were not considered treatment-related.

Table 6.5-44. Selected clinical observations - animals thin and with hunched posture

Dose [ppm]		Male				Female			
Week	Observation	0	30	100	1000	0	30	100	1000
90	Thin							3	
	Hunched							1	
92	Thin								
	Hunched					1		2	
94	Thin					1		2	
	Hunched								
96	Thin					1	1	2	
	Hunched						1		
98	Thin	1				2	1	1	
	Hunched						1		
100	Thin					1	1	1	1
	Hunched						1		1
102	Thin					1	1	1	
	Hunched						1		

Dose [ppm]		Male				Female			
		0	30	100	1000	0	30	100	1000
Week	Observation								
104	Thin		2		1	7	2	3	2
	Hunched				1	6	2	2	2

*Nodules and masses:* There were no treatment-related increase in the incidences of modules and masses. Incidences and distribution of nodules and masses remained within the normal background variation for mice of this strain in this type of study.

*Body weight, food and water consumption:* There were no treatment-related effects on body weight and body weight gain (Table 6.5-45). Statistically-significant changes in body weight were observed in males at top dose in week 5 and at low dose in week 74; however, due to the isolated occurrence of these findings they were not considered treatment-related. The absence of effects on body weights up to the top dose of 1000 ppm (231/272 mg/kg in M/F) is in contrast with the findings of the new/modern study which revealed treatment-related and adverse effects on body weight and body weight gain from the low dose (35/27 mg/kg bw/d). Statistically-significant differences in food consumption in treated animals compared to controls were recorded, however, a time-response and/or dose-response was not evident, and there was an absence in relevant body weight changes. Water consumption was not recorded. Overall, were no treatment-related effects on body weight and body weight gain.

Table 6.5-45. Overall body weight gain

		Males				Females			
Dose level	[ppm]	0	30	100	1000	0	30	100	1000
	[mg/kg bw/d]	0	7.2	22.1	231	0	8.3	26.8	272
Body weight gain (g) ± SD		16.8 ± 5.2	15.4 ± 5.4	17.9 ± 5.7	15.2 ± 5.7	18.8 ± 5.1	19.7 ± 5.6	19.7 ± 5.6	21.1 ± 5.5
Δ%		-	-8.3	6.5	-9.5	-	4.8	4.8	12.2

SD – standard deviation.

Δ% – percent change compared to control.

*Haematology and clinical chemistry:* After treatment for 12-months (interim sacrifice), blood samples were insufficient for the determination of serum phosphorus, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), total bilirubin, Na and K. Statistically-significant changes in certain haematological and clinical chemistry parameters (erythrocyte count, haemoglobin, haematocrit, platelets, glucose, cholesterol, blood urea nitrogen, bilirubin and phosphorus) were seen but with no does-response or temporal-response and no consistency between males and females (Table 6.5-46). Despite some limitations, the lack of treatment-related haematological effects concurs with findings of the new/modern study in mice (██████████, 2018d). Overall, there were no treatment-related adverse effects on haematological or clinical chemistry parameters at either 12- or 24-months.

Table 6.5-46. Haematology and clinical chemistry

Sex		Males				Females			
Dose level	[ppm]	0	30	100	1000	0	30	100	1000
	[mg/kg bw/d]	0	7.2	22.1	231	0	8.3	26.8	272
Time point (months)									
Haematological parameter									
RBC (x10 <sup>6</sup> /mm <sup>3</sup> )	12	10.30	9.49*	9.96	9.56*	9.94	9.92	9.72	9.85
	24	9.60	9.48	9.75	9.12	8.96	8.67	9.48	9.29

Sex		Males				Females			
Dose level	[ppm]	0	30	100	1000	0	30	100	1000
	[mg/kg bw/d]	0	7.2	22.1	231	0	8.3	26.8	272
Time point (months)									
Hb (g/dL)	12	17.7	<b>16.6*</b>	17.2	<b>16.7*</b>	17.0	17.3	17.2	17.5
	24	14.7	14.6	15.0	14.0	14.3	13.4	14.8	14.3
HCT (%)	12	49.1	48.9	49.8	50.1	48.8	49.8	49.0	<b>51.6*</b>
	24	41.2	40.3	42.1	39.9	39.8	38.1	41.2	40.1
Platelets (x10 <sup>3</sup> /mm <sup>3</sup> )	12	571	617	573	591	434	407	468	440
	24	1412	1572	1349	1570	850	<b>627*</b>	892	792
Clinical chemistry parameter									
Glucose (mg/dL)	12	115	<b>93*</b>	<b>107*</b>	<b>99*</b>	93	81	72	79
	24	136	136	127	117	132	115	132	151
Total cholesterol (mg/dL)	12	127	110	113	<b>105*</b>	-	1	1	1
	24	110	170	136	174	106	102	114	112
Blood urea nitrogen (mg/dL)	12	22	20	19	19	18	<b>13*</b>	<b>12*</b>	<b>12*</b>
	24	22	24	22	29	21	23	18	19
Total bilirubin (mg/dL)	24	0.2	0.2	<b>0.4*</b>	0.3	0.2	<b>0.2*</b>	<b>0.2*</b>	0.3
Phosphorus (mg/dL)	24	8.3	<b>7.1*</b>	8.9	8.5	6.7	7.2	7.1	7.6

Statistical analysis: Dunnett's t-test: \*: p≤0.05

RBC – Erythrocyte count

Hb – Haemoglobin

HCT - Haematocrit

*Organ weights:* The only statistically-significant changes in organ weight were seen at interim sacrifice (12-months) – where increases in testes weights at 100 and 1,000 ppm, increased relative liver weight at 1,000 ppm in males, and decreased absolute heart weight at 1,000 ppm in males were seen. However, liver and heart weight differences were found to be statistically different using the Student's t-test but not Dunnett's t-test. At 24-months, liver weights (absolute and relative) were adversely increased (> 15%) in both males and females at the top dose. There were no concomitant non-neoplastic histopathological findings in males and females; however, neoplastic findings showed an increase in hepatic tumours (adenomas and carcinomas) from the mid dose in males and at the top dose in females (Table 6.5-47).

The decrease of the cardiac weight (absolute and relative) in males did not show a dose-response and was not corroborated by any gross necropsy and histopathology findings. In females an increase in cardiac weight was seen which was comparable in magnitude to the decrease in males. Due to the lack of dose-response and inconsistency between sexes this effect was not considered treatment-related. The testes weights (absolute and relative) were increased at 12-months, a dose-response was evident and values reached statistical-significance for both absolute (from the mid dose) and relative (at the top dose) weights. However, a similar change was not evident at terminal sacrifice (24-months), and there was no supporting histopathology at either 12- or 24-months. Consequently, these findings were considered treatment-related but not adverse. This is further supported as no relevant testicular findings were observed in a new/modern carcinogenicity study in C57BL/6J Rj mice (■■■■■, 2018d). Overall, the only treatment-related and adverse changes in organ weights were increases (> 15 %) in liver weight at the top dose in both sexes.

Table 6.5-47. Organ weights

Sex		Month	Males				Females			
Dose level	[ppm]		0	30	100	1000	0	30	100	1000
	[mg/kg bw]k		0	7.2	22.1	231	0	8.3	26.8	272
No. animals examined		12	16	10	8	10	16	9	9	10
		24	62	39	36	36	61	38	35	31
Body weight <sup>a</sup> [g] (Δ%) <sup>#</sup>		12	36	34 (-5)	35 (-3)	33 (-8)	33.1	31.3 (-5)	33.4 (+1)	32.9 (±0)
		24	37.9	37.5 (-1)	39.6 (4.5)	37.0 (-2.4)	36.4	37.4 (2.7)	37.4 (2.7)	38.7 (6.3)
<b>Liver</b>										
Absolute weight [g] (Δ%) <sup>#</sup>		12	1.64	1.48 (-9.76)	1.57 (-4.27)	1.68 (2.44)	1.59	1.50 (-5.66)	1.49 (-6.29)	1.63 (2.52)
		24	1.68	1.75 (4.17)	1.89 (12.50)	2.05 (22.02)	1.77	1.88 (6.21)	1.81 (2.26)	2.10 (18.64)
Relative weight [% bw] (Δ%) <sup>#</sup>		12	4.64	4.38 (-5.60)	4.49 (-3.23)	5.12 <sup>b</sup> (10.34)	4.86	4.81 (-1.03)	4.49 (-7.61)	4.99 (2.67)
		24	4.48	4.75 (6.03)	4.90 (9.38)	5.69 (27.01)	4.97	5.08 (2.21)	4.89 (-1.61)	5.62 (13.08)
<b>Heart</b>										
Absolute weight [g] (Δ%) <sup>#</sup>		12	0.24	0.22 (-8.33)	0.23 (-4.17)	0.21 <sup>b</sup> (-12.50)	0.17	0.18 (5.88)	0.17 (±0)	0.19 (11.76)
Relative weight [% bw] (Δ%) <sup>#</sup>		12	0.70	0.66 (-5.71)	0.66 (-5.71)	0.65 (-7.14)	0.53	0.57 (7.55)	0.53 (±0)	0.57 (7.55)
<b>Testes</b>										
Absolute weight [g] (Δ%) <sup>#</sup>		12	0.38	0.41 (8)	<b>0.46*</b> (21)	<b>0.50*</b> (32)				
		24	0.36	0.39 (8)	0.36 (±0)	0.34 (-5.5)				
Relative weight [% bw] (Δ%) <sup>#</sup>		12	1.10	1.23 (12)	1.33 (21)	<b>1.52*</b> (38)				
		24	0.94	1.04 (11)	0.92 (-2)	0.92 (-2)				

\*  $p \leq 0.05$ , Dunnett's t-test, #  $p \leq 0.05$ , statistically significant by Student's t-test, but not Dunnett's t-test.

<sup>a</sup> The Relative organ weights in week 104 were calculated using the respective week non-fasted body weights instead of the terminal weights.

<sup>b</sup> The values were statistically significant by Student's t-test, however, these values were not statistically significant when compared by Dunnett's t-test.

Δ% - percent change compared to control

# Values may not calculate exactly due to rounding of figures;

*Gross pathology:* There were no treatment-related macroscopic findings in both the satellite group and the main group. Findings, some of which are commonly seen, were incidental, occurring either infrequently or biologically equally distributed over control and treatment groups. At 24-months hepatic lesions (nodules, masses, coloured foci and discolourations) were seen; in males lesions were seen at higher incidences at the mid and high doses compared to control and low doses. However, the slight differences in incidence were not considered treatment-related.

*Histopathology:*

*Non-neoplastic findings:* There were no treatment-related non-neoplastic findings both at 12- and 24-months. Findings were spontaneous and occurred with similar incidence in both treated and control groups (Table 6.5-48). At 24-months focal necrosis of the liver was observed in all groups, with the highest incidence in control females.

Table 6.5-48. Non-neoplastic findings in the liver (24-months)

Dose		Males				Females			
	[ppm]	0	30	100	1000	0	30	100	1000
	[mg/kg bw/d]	0	7.2	22.1	231	0	8.3	26.8	272
No. of animals		62	40	37	36	64	40	36	31
Clear cell focus		5	0	4	4	4	2	0	5
Focus of cellular alteration		5	5	2	2	4	5	2	6
<b>Focal necrosis</b>		<b>6</b>	2	1	5	<b>12</b>	2	3	1
Hepatocellular vacuolation		1	2	5	1	0	0	0	1

*Neoplastic findings:* The total numbers of mice with tumours of all types were slightly above control levels in males of all dose groups, and in females of the low dose group; no dose-response was evident (Table 6.5-49). The numbers of females with malignancies in all treatment groups were similar to or less than controls, while there was a slight increase (compared to control) in males of the mid and high dose. In the adrenals, cortical adenomas observed in mid dose males were statistically-significantly increased, however, based on the absence of dose-dependence, the effects were not considered treatment-related. Tumours from other tissues occurred at a greater frequency in treated mice relative to concurrent controls (Table 6.5-49), however, due to the isolated incidences and/or lack of dose-response these tumours were not considered treatment-related.

*Hepatic tumours:* The most common tumours observed at necropsy were of hepatic origin (Table 6.5-50). A higher incidence of adenomas and carcinomas was observed in treated animals compared to controls. A dose-response was not evident for adenomas but was for carcinomas. Two types of statistical analyses were conducted - prevalence analysis (death incidental to tumour) and fatal analysis (tumour considered fatal). Incidences in adenomas were statistically-significant for males from the mid dose, and for females at the low and high dose. Incidences in carcinomas were statistically-significant for females at the top dose. The applicant provided HCD, however, HSE notes that the HCD (whilst conducted in the same strain and species, and being contemporaneous) are taken from the National Toxicology Program and not directly from the same laboratory which conducted this study. For this reason HSE acknowledges the lower relevance of these HCD. In males, adenomas and carcinomas were well within these HCD ranges; however, in females, only the carcinomas (but not the adenomas) were well within the HCD ranges. Taking all of this into account, a relation to treatment of these tumours in males and females cannot be excluded. However, it is noted that the infection with MHV may have affected the hepatic response to cinmethylin in this study. In addition, this result does not agree with the findings of the new/modern study in C57BL/6J Rj mice (██████████, 2018d), in which no hepatic tumours were observed up to a higher dose of 5000 ppm. Overall, no firm conclusion can be drawn regarding the hepatic neoplastic findings seen in this study.

Table 6.5-49. Selected neoplastic findings

Dose		Males				Females			
	[ppm]	0	30	100	1000	0	30	100	1000
	[mg/kg bw/d]	0	7.2	22.1	231	0	8.3	26.8	272
No. of animals examined		108	57	55	58	116	60	60	60
Animals with tumours (%)		43	47	56	52	47	62	42	38
Animals with malignant tumours (%)		19	16	25	24	28	30	22	22
<b>Adrenals</b>									
cortical adenoma (B)		0	0	2*	1	0	1	0	0
<b>Colon</b>									



		Males				Females			
Dose	[ppm]	0	30	100	1000	0	30	100	1000
	[mg/kg bw/d]	0	7.2	22.1	231	0	8.3	26.8	272
No. of animals examined		108	57	55	58	116	60	60	60
adenocarcinoma (M)		0	0	0	0	0	0	0	1
Harderian gland									
adenocarcinoma (M)		0	1	0	0	1	2	0	0
Jejunum									
adenocarcinoma (M)		0	0	0	1	0	0	0	0
Kidney									
tumour unknown origin (M)		0	0	0	0	0	1	0	0
Lung									
tumour unknown origin (M)		0	0	0	0	0	0	0	1
Mesenteric lymph node									
tumour unknown origin (M)		0	0	0	0	0	0	1	0
Ovaries									
papillary cystadenoma (B)						0	1	0	0
granulosa cell tumour (B)						1	2	0	0
teratoma (M)						0	0	0	1
Pancreas									
islet adenoma (B)		0	0	0	0	0	1	0	1
islet adenocarcinoma (M)		0	0	0	0	0	0	0	1 <sup>s</sup>
Parathyroids									
adenoma (B)		0	0	1	0	0	0	0	0
Pituitary									
adenocarcinoma (M)		0	0	0	0	0	0	1	0
Sternum/rib									
chondroma (B)		0	0	0	1	0	0	0	0
Stomach									
tumour unknown origin (M)		0	1	0	0	0	0	0	0
Testes									
seminoma/disgerminoma (M)		0	1	0	0	-	-	-	-
Thyroids									
adenoma (B)		1	0	1	0	1	2	1	1
Uterus									
tumour unknown origin (M)		-	-	-	-	0	1	0	0
Vascular system									
haemangioma (B)		0	0	0	0	0	3	1	0

\*:  $p \leq 0.05$ , comparison against control, 1-tailed Hoel-Walburg test for 2 groups.

\$ :  $p \leq 0.05$ , test for trend;

Table 6.5-50. Frequencies of hepatic tumours (%)

Dose [ppm]	0	30	100	1000	Historical control <sup>1</sup>	
Males						
No. animals examined	98	54	53	55	Range	Mean
Adenoma §	15.3	18.5	26.4°	23.6°	0 - 44	10.0
Carcinoma	11.2	5.6	15.1	18.2	8 - 32	21.1
Combined <sup>2§§</sup>	24.5	24.1	37.7*,°	38.2°	14 - 58	30.8
Females						
No. animals examined	98	56	54	53		
Adenoma <sup>§§</sup>	9.2	21.4*,°	16.7	18.9*,°	0 - 18	3.8
Carcinoma	2.0	1.8	3.7	5.7*	0 - 15	4.6
Combined <sup>2§§</sup>	11.2	21.4 (23.6) <sup>3</sup>	20.4	22.6*,°	0 - 20	8.3

1: The historical control data from ~40 carcinogenicity studies conducted between 1977 and 1987 by the National Toxicology Program using B6C3F1 mice (Haseman, J. K., Huff, J. E., Rao, G. N., Arnold, J. E., Boorman, G. A. and McConnell, E. E. (1985) Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N x C3H/HeN)F1 (B6C3F1) mice. JNCI 73, 975-984, for reference see KCA 5.5/10 CI-905-002

2: Mice with 2 or more tumours are counted once only for the combined total.

\*  $p \leq 0.05$ , comparison against control, 1-tailed Hoel-Walburg test for 2 groups (prevalence analysis)

°  $p \leq 0.05$ , comparison against control, 1-tailed Cox's test for 2 groups (fatal analysis)

§  $p \leq 0.05$ , test for trend (prevalence analysis)

§  $p \leq 0.05$ , test for trend (death rate analysis)

3: Mice had uncharacterised hepatic neoplasms, when these were added to the combined total, the result is in parentheses.

### Conclusion

In this limited chronic/carcinogenicity study, cinmethylin was administered via the diet to male and female B6C3F1 mice for 24-months at 30, 100 or 1000 ppm. Liver tumours were increased above controls in both sexes at all doses. However, due to intercurrent MHV infection, no firm conclusion could be drawn with regards to the relation to treatment of these liver tumours.

No clear evidence of systemic toxicity was apparent up to the top dose of 1000 ppm (231/272 mg/kg bw/d in M/F) at which there was only an adverse increase ( $> 15\%$ ) in liver weight.

In conclusion, there were adverse effects on liver weight at the top dose in both sexes. Therefore a **NOAEL for systemic chronic toxicity in the mouse of 100 ppm (22.1 and 26.8 mg/kg bw/d in M/F respectively)** is proposed by HSE. In relation to carcinogenicity, the study is considered inconclusive.

(██████, 1986)

### B.6.5.3. Summary of long-term toxicity and carcinogenicity

The long-term toxicity and carcinogenic potential of cinmethylin have been investigated in rats (Wisatr and F-344) and mice (C57BL and B6C3F1), via the oral (dietary) route of exposure, in 18-month and 24-month studies. For each species two studies are available - one new/modern standard guideline study and one older study not conducted according to GLP and OECD test guidelines. The main findings are summarised (Table 6.5-51) below.



The following key conclusions were obtained from the evaluation of the long-term toxicity and carcinogenicity information:

- There is equivocal evidence of carcinogenicity in the female rat (liver carcinomas in female Wistar rats at 317 mg/kg bw/d) but not in the male rat or mice
- HSE notes that the carcinogenicity response observed is very weak, sex- and species-specific, and occurs in the presence of significant generalised toxicity (effects on body weight, body weight gain and histopathology of thyroid and nasal cavities). In addition, although the liver is a target organ of toxicity in the rat, there was no clear evidence of pre-neoplastic lesions and/or adenomas. It is also noted that the incidence of liver carcinoma was within the extended laboratory HCD range and the Rita database

HCD. Overall, there is insufficient evidence to classify cinmethylin for carcinogenicity. Further details on classification are available in the aligned MCL report

- The data requirements of Regulation 283/2013 have been met.

Table 6.5-51. Summary of long-term and carcinogenicity studies with cinmethylin

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
24-month oral (dietary)	Rat (Wistar)	0, 200, 1000 and 5000 ppm	Carcinogenicity: F: 59 [1,000]	Carcinogenicity: <u>5,000 ppm (317 mg/kg bw/d)</u> : ↑liver carcinomas (♀) – equivocal evidence of carcinogenicity.
GLP compliant. Guideline compliant.	Chronic phase: 10/sex/dose (12-months)	Equivalent to: Chronic phase: M: 0, 10, 51 and 265 mg/kg bw/d		
Cinmethylin Batch: COD- 002038 Purity (%): 93.5 (-) / (+) ratio = 48:52	Carcinogenicity phase: 50/sex/dose (24-months)	F: 0, 13, 69 and 351 mg/kg bw/d		
 <a href="#">2018</a> <a href="#">(2017/1093414)</a>		Carcinogenicity phase: M: 0, 9, 45 and 242 mg/kg bw/d	Systemic chronic toxicity: M: 9 [200]	Systemic chronic toxicity: <u>1,000 ppm (45 mg/kg bw/d)</u> : Histopathology of the nasal cavities - degeneration/regeneration of the olfactory epithelium and proteinaceous exudate (♂).
<i>Acceptable</i>		F: 0, 11, 59 and 317 mg/kg bw/d	F: 59 [1,000]	
24-month oral (dietary)	Rat (Fischer 344)	0, 30, 100 and 3000 ppm	Carcinogenicity: M: 144 F: 177 [3,000]	Carcinogenicity: N/A – cinmethylin demonstrated no carcinogenic potential.
Non-GLP. Non-guideline.	Chronic phase: 10/sex/dose (6- and 12-months)	Equivalent to: Chronic and carcinogenicity phases:	(highest tested dose).	
Cinmethylin Batch: 513F (5-4- 0-0)	15/sex/dose (18-months)	M: 0, 1.4, 4.7 and 144.2 mg/kg bw/d	Systemic chronic toxicity: M: 4.7	Systemic chronic toxicity: <u>3,000 ppm</u> <u>(144/177 mg/kg bw/d)</u> :
Purity (%): 92 (-) / (+) ratio = not specified.	Carcinogenicity phase: 50/sex/dose (24-months)	F: 0, 1.7, 5.8 and 177.4 mg/kg bw/d	F: 5.8 [100]	↑mortality (♂). ↑clinical signs of toxicity (hunched appearance and pale eyes) (♂). ↓food consumption (♂+♀). ↓body weight (♂+♀). ↓body weight gain (♂+♀). Changes in haematology parameters (indicative of mild anaemia and thrombocytosis) (♂+♀). ↑liver weight, relative (23 % in ♂, 14 % in ♀). ↑kidney weight (16 % in ♂) and kidney histopathology (severe chronic nephropathy) (not relevant to humans). ↑GGT (♂+♀). Histopathology of the
 <a href="#">1985</a> <a href="#">(CI-427-001)</a>				
<i>Supplementary</i>				

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
				liver (♂+♀).
18-month oral (dietary)  GLP compliant. Guideline compliant.  Cinmethylin Batch: COD- 002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  ██████████, 2018d (2017/1094161)  <i>Acceptable</i>	Mice (C57BL/6J Rj)  Chronic phase: 6/sex/dose (63- days)  Carcinogenicity phase: 50/sex/dose (18-months)	0, 150, 1000 and 5000 ppm  Equivalent to: Chronic phase: M: 0, 32.1, 223.1 and 1175 mg/kg bw/d  F: 0, 34.8, 301.1 and 1225 mg/kg bw/d  Carcinogenicity phase: M: 0, 25, 162.3 and 904 mg/kg bw/d  F: 0, 27, 183.8 and 939 mg/kg bw/d	Carcinogenicity: M: 904 F: 939 [5,000]  (highest tested dose).  Systemic chronic toxicity: M: N/A F: N/A  BMDL <sub>10</sub> (for body weight effects): 37.5	Carcinogenicity: N/A – cinmethylin demonstrated no carcinogenic potential.  Systemic chronic toxicity: <u>150 ppm (25/27 mg/kg bw/d) (lowest tested dose):</u> ↓terminal body weight (♂+♀). ↓body weight gain (♂+♀). ↓food consumption (♀).
24-month oral (dietary)  Non-GLP. Non-guideline.  Cinmethylin Batch: 513F (5-4- 0-0) Purity (%): 92 (-) / (+) ratio = not specified.  ██████████ 1986 (CI-428-001)  <i>Limited</i>	Mouse (B6C3F1)  Chronic phase: 10/sex/dose (12-months)  Carcinogenicity phase: 50/sex/dose (24-months)  120/sex controls; 20/sex for 12-months and 100/sex for 24-months.	0, 30, 100 and 1000 ppm  Equivalent to: Chronic and carcinogenicity phases: M: 0, 7.2, 22.1 and 231 mg/kg bw/d  F: 0, 8.3, 26.8 and 272 mg/kg bw/d	Carcinogenicity: N/A - no firm conclusion could be drawn.  Systemic chronic toxicity: M: 22.1 F: 26.8 [100]	Carcinogenicity: N/A - no firm conclusion could be drawn.  Systemic chronic toxicity: <u>1,000 ppm</u> <u>(231/272 mg/kg bw/d):</u> ↑liver weight, absolute (22 % in ♂, 19 % in ♀) and relative (27 % in ♂, 13 % in ♀).

In the new/modern study in rats, an increase in the incidence of liver carcinomas was observed in females at the top dose of 317 mg/kg bw/d at which systemic toxicity occurred. Systemic toxicity included: decreases in body weight and body weight gain, increases in GGT and liver weight, histopathology of the liver (cytoplasmic alterations and hypertrophy), thyroid (hypertrophy/hyperplasia and altered colloid) and nasal cavities (degeneration/regeneration of the olfactory epithelium and proteinaceous exudate). At a lower dose still - 45 mg/kg bw/d, adverse histopathology of the nasal cavities was observed.

In the older study in rats, there were no treatment-related increases in the incidence, severity or onset of tumours in any tissue up to the top dose of 177 mg/kg bw/d. At this dose systemic toxicity occurred; there was an

increase in mortality, clinical signs of toxicity, liver weight (with concomitant histopathology) and GGT. Decreases in food consumption, body weight and body weight gain, and changes in some haematology parameters were also observed.

Therefore, there is equivocal evidence of carcinogenicity in the female rat at 317 mg/kg bw/d. Overall, **the lowest relevant NOAEL for carcinogenicity in the rat was 59 mg/kg bw/d.** The lowest NOAEL for systemic chronic toxicity in the rat was 4.7 mg/kg bw/d from the older chronic study in the rat; the LOAEL in this study was 144 mg/kg bw/d. However, in the new/modern chronic study in the rat the highest NOAEL was 9 mg/kg bw/d, with a LOAEL of 45 mg/kg bw/d. Since the new/modern study provides the highest NOAEL which lies below the lowest LOAEL in this relevant species and study type, **the most reliable NOAEL for systemic chronic toxicity in the rat was 9 mg/kg bw/d.**

In the new/modern study in mice, there were no treatment-related increases in the incidence, severity or onset of tumours in any tissue up to the top dose 939 mg/kg bw/d. At this dose systemic toxicity occurred; there was a decrease in terminal body weight, body weight gain and food consumption (from the low dose of 25/27 mg/kg bw/d), adverse histopathology of the nasal cavities (degeneration/regeneration of the olfactory epithelium and proteinaceous exudate) from the mid dose of 162 mg/kg bw/d, and an increase in liver weight with concomitant histopathology at the top dose of 904 mg/kg bw/d.

The older study in mice was considered inconclusive with regards to carcinogenicity due to limitations in the study. No clear evidence of systemic toxicity was apparent up to the top dose of 272 mg/kg bw/d, at which there was only an adverse increase (> 15 %) in liver weight.

Overall, cinmethylin was not carcinogenic in mice up to the highest dose tested of 939 mg/kg bw/d. **A LOAEL for systemic chronic toxicity in the mouse of 25/27 mg/kg bw/d (M/F) was identified. Alternatively, the lowest BMDL<sub>10</sub> for effects on body weight in females – 37.5 mg/kg bw/d, can be considered.**

Overall, therefore, there is equivocal evidence of carcinogenicity in the female rat, but the evidence is insufficient for classification. Further details are available in the aligned MCL report.

### B.6.6. REPRODUCTIVE TOXICITY

The reproductive toxicity of cinmethylin has been investigated in a new/modern guideline dietary 2-generation study in rats and a new/modern guideline gavage pre-natal developmental toxicity study in rabbits. In addition, older studies are available – a second 2-generation study in rats, one pre-natal developmental toxicity study in rats and two further pre-natal developmental toxicity studies in rabbits.

#### B.6.6.1. Generational studies

Two dietary multi-generation studies in the rat are available. One new/modern, GLP and OECD test guideline compliant study and one older study, which was conducted according to GLP but not conducted according to OECD test guidelines.

##### *New/modern study*

<b>Author(s)</b>	
<b>Study title</b>	<ul style="list-style-type: none"> <li>BAS 684 H - Two-generation reproduction toxicity study in Wistar rats - Administration via the diet</li> <li>Amendment No. 1 - BAS 684 H - Two-generation reproduction toxicity study in Wistar rats - Administration via the diet</li> </ul>
<b>Study reference</b>	<ul style="list-style-type: none"> <li>, 2018 a - BASF DocID : 2017/1094504</li> <li>, 2018 - BASF DocID : 2018/1099151</li> </ul>
<b>Test facility</b>	
<b>Date</b>	01/12/2015 – 18/08/2016
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Batch no.</b>	COD-002038
<b>Purity (%)</b>	93.5 (-) / (+) ratio = 48:52
<b>Test animals</b>	Rat Wistar, CrI : WI(Han) (the same species used in previous 28-day and 90-day studies) Male and female
<b>Groups</b>	25/sex/dose (F <sub>0</sub> parental generation) 25/sex/dose (F <sub>1</sub> parental generation)
<b>Dose/concentrations</b>	0, 125/250, 500/1000 and 2500/5000 ppm (see Table 6.6-1 for equivalent mg/kg bw/d doses)
<b>Route</b>	Administered daily via the diet for 10 weeks prior to mating throughout mating, gestation and lactation.
<b>Vehicle</b>	None.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD 416 (2001) (this is the current test guideline) Commission Regulation (EC) No 440/2008 - Part B No. L 142, EPA 870.3800, JMAFF No 12 Nosan No 8147
<b>Deviation</b>	None. Reason for amendment : During the lactation periods, cinmethylin concentrations in the diet of the F <sub>0</sub> and F <sub>1</sub> females were reduced by 50 %. This dietary adjustment was not factored in the calculation of the test substance intake in the original report. The amendment corrects this error.
<b>Impact of deviations</b>	Not applicable.
<b>Acceptable</b>	Yes
<b>NOAEL</b>	Reproductive toxicity : 2500/5000 ppm (equivalent to 394 mg/kg bw/d) (highest dose tested). Parental toxicity : 500/1000 ppm (equivalent to 80 mg/kg bw/d). Developmental/Offspring toxicity : 2500/5000 ppm (equivalent to 394 mg/kg bw/d) (highest dose tested).
<b>Effects at the LOAEL</b>	Reproductive toxicity : N/A – no adverse treatment-related findings were observed up to the top dose. Parental toxicity : Based on decreases in food consumption, body weight and body weight gain, increases in liver and thyroid weights, thyroid and nasal cavity

	histopathology. Developmental/Offspring toxicity : N/A – no adverse treatment-related findings were observed up to the top dose.
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### Methods

The potential effects of cinmethylin on the integrity and performance of the reproductive system, and the growth and development of the offspring, have been investigated in a standard 2-generation study in the rat at dietary concentrations of 0, 250, 1000 and 5000 ppm (see Table 6.6-1 for equivalent mg/kg bw/d doses). The dietary concentrations were adjusted to 0, 125, 500 and 2500 ppm in females during lactation to ensure the achieved dose levels/intakes during this period were equivalent to those in the pre-mating phase. In this study, F<sub>0</sub> parental animals (25/sex/dose) were administered cinmethylin via the diet for a 10-week pre-mating period, throughout mating, gestation and lactation. Litters were standardised on PND 4 (postnatal day). After weaning of F<sub>1</sub>, pups the F<sub>0</sub> generation parental animals were sacrificed. Selected F<sub>1</sub> pups (25/sex/dose) were retained post weaning and mated to produce the F<sub>2</sub> generation. F<sub>1</sub> females were allowed to litter and rear pups (F<sub>2</sub>) until day 4. Shortly after weaning of F<sub>2</sub> pups, the F<sub>1</sub> parental animals were sacrificed. The stability and homogeneity of cinmethylin in the diet was acceptable. Calculated achieved intakes were as follows:

Table 6.6-1. Achieved intakes of cinmethylin

Phase	Generation	Dietary concentration of cinmethylin [ppm]					
		Males			Females		
		250	1000	5000	250 / 125 <sup>a)</sup>	1000 / 500 <sup>a)</sup>	5000 / 2500 <sup>a)</sup>
Pre-mating [mg/kg bw/d]	F <sub>0</sub>	19.7	79.4	412	21.4	82.2	417
	F <sub>1</sub>	21.8	87.7	450	22.8	90.1	460
Gestation [mg/kg bw/d]	F <sub>0</sub>				20.7	81.3	395
	F <sub>1</sub>				20.6	81.6	394
Lactation [mg/kg bw/d]	F <sub>0</sub>				23.8	93.8	473
	F <sub>1</sub>				23.5	96.9	481

<sup>a)</sup> Feed concentrations were halved to compensate the increased food intakes during the lactation period

A method for the detection of cinmethylin in rat/mouse diet (██████████, 2017b; 2017/1123754) was evaluated and was considered validated (see Volume 3 CA B5, section B.5.1.2).

### Results

Doses were selected on the basis of prior studies - the 28-day (██████████, 2015) and 90-day (██████████, 2018a) studies in the rat and the old two-generation study (Lu *et al.*, 1986). In the previous 28-day study in rats (section B.6.3.1, ██████████, 2015), dosed at 1500, 5000 and 15000 ppm, a LOAEL of 5000 ppm (equivalent to 477 mg/kg bw/d) was derived based on effects on the liver, thyroid and water consumption. In the previous 90-day rat study (section B.6.3.2, ██████████, 2018a), with animals dosed up to 10,000 ppm, a LOAEL of 3,000 ppm was derived based on liver, thyroid and nasal cavity findings. At 3,000 ppm (equivalent to 211/240 mg/kg bw/d in males and females respectively) body weight (-5/-2 % in males and females, respectively) and body weight gain (-8/-6 % in males and females respectively) started to be reduced. At 10,000 ppm (equivalent to 792/814 mg/kg bw/d in males and females respectively) body weight was statistically-significantly reduced by around -15 %, and body weight gain by -27/-33 % in males and females respectively. In the old two-generation study in Sprague Dawley rats (██████████, 1986), with animals dosed at 200, 2000 and 20000 ppm, severe toxicity including mortality during gestation/early lactation was induced at the top dose (1434 - 2893 mg/kg bw/day). Consequently, a concentration between 3000 and 10000 ppm was considered appropriate to induce sufficient toxicity; 5000 ppm was chosen as top dose, expected to correspond to intakes of approx. 400 mg/kg bw during gestation.

#### Parental and offspring toxicity

There were no effects of treatment on mortality and clinical signs. Food consumption was statistically-significantly decreased in F<sub>0</sub> females of the top dose during gestation, leading to an overall reduced food consumption of 8 % during gestation in this group (Table 6.6-2).

Statistically-significant changes in body weight and body weight gain were observed in high dose F<sub>0</sub> females (Table 6.6-3). Mean body weights of the high dose F<sub>0</sub> females were comparable to control values during the pre-

mating period but were statistically-significantly below control values over most of the gestation (up to 5 %) and lactation periods (up to 6%). Mean body weight gains of the high dose F<sub>0</sub> females was statistically-significantly below control values during GD (gestation day) 0-7 (20 %) and overall (10 % change compared to controls during GD 0-20). During lactation, both lower (during PND 7-14) and higher (during PND 4-7) body weight gains were noted in the high dose females, however, overall an increased body weight gain (+16 %) was seen. There were no treatment-related effects on food consumption, body weight and body weight gain in F<sub>0</sub> males and all F<sub>1</sub> animals.

Statistically-significant decreases in body weight gain were observed in high dose F<sub>1</sub> pups (Table 6.6-4), during PND 14 – 21, in both males and females. However, percent change compared to controls was < 10 %, overall (PND 1 – 21) body weight gain was not statistically-significant, and there were no statistically-significant effects on body weight gain in the F<sub>2</sub> generation.

Overall, treatment-related and adverse reductions in food consumption (in F<sub>0</sub> females during gestation), mean body weights (in F<sub>0</sub> females during gestation and lactation) and mean body weight gains (in F<sub>0</sub> females) were recorded at the top dose (5000/2500 ppm, equivalent to 395 - 481 mg/kg bw/d).

Table 6.6-2. Food consumption of F<sub>0</sub> females during pre-mating, gestation and lactation

Dose [ppm]	0		250 / 125 <sup>a)</sup>		1000 / 500 <sup>a)</sup>		5000 / 2500 <sup>a)</sup>	
Food consumption [g / day]	mean	SD	mean	SD	mean	SD	mean	SD
<b>Premating phase, F<sub>0</sub> females</b>	N = 5		N = 5		N = 5		N = 5	
Week 1 (d 0 - 7) [g]	12.5	0.3	12.9	0.3	12.4	0.4	13.2	0.7
[% of control]	100		103.0		99.7		105.9	
Week 10 (d 63 - 70) [g]	17.4	2.1	16.1	0.8	<b>15.1*</b>	0.8	<b>14.8**</b>	0.6
[% of control]	100		92.2		86.7		84.7	
Week 0 - 10 (d 0 - 70) [g]	15.2	0.7	15.2	0.7	14.4	0.6	14.6	0.6
[% of control]	100		99.7		94.9		96.1	
<b>Gestation phase, F<sub>0</sub> females</b>	N = 25		N = 25		N = 25		N = 24	
GD 0 - 7 [g]	20.3	1.8	20.1	1.5	19.3	1.7	<b>18.2**</b>	1.5
[% of control]	100		99.4		95.2		89.8	
GD 7 - 14 [g]	22.1	1.7	22.1	2.3	21.4	1.7	<b>20.1**</b>	1.5
[% of control]	100		100.0		96.6		91.1	
GD 14 - 20 [g]	23.7	1.5	23.7	2.0	23.6	1.8	22.6	1.8
[% of control]	100		99.9		99.4		95.2	
GD 0 - 20 [g]	21.9	1.5	21.9	1.8	21.3	1.5	<b>20.2**</b>	1.4
[% of control]	100		99.8		97.1		92.0	
<b>Lactation phase, F<sub>0</sub> females</b>	N = 25		N = 25		N = 25		N = 24	
PND 1 - 4 [g]	32.9	4.4	34.9	5.1	33.5	5.0	32.0	4.9
[% of control]	100		106.2		101.9		97.4	
PND 4 - 7 [g]	40.3	2.6	<b>43.6**</b>	4.9	41.9	3.0	42.1	3.8
[% of control]	100		108.3		104.0		104.6	
PND 10 - 14 [g]	59.0	6.4	59.4	6.0	59.1	4.1	58.0	5.0
[% of control]	100		100.7		100.2		98.4	
PND 18 - 21 [g]	71.0	5.3	71.1	6.1	70.2	3.8	69.7	7.2
[% of control]	100		100.3		98.9		98.2	
PND 1 - 21 [g]	33.4	2.0	34.3	2.8	33.7	1.9	33.2	3.1
[% of control]	100		102.7		100.7		99.3	

\* = p<0.05; \*\* = p<0.01; Dunnett test (two-sided);

GD = gestation day;

PND = postnatal day

<sup>a)</sup> Feed concentrations were halved to compensate the increased food intakes during the lactation period



Table 6.6-3. Body weight and body weight gain of F<sub>0</sub> females during premating, gestation and lactation

Dose [ppm]	0		250 / 125 <sup>a)</sup>		1000 / 500 <sup>a)</sup>		5000 / 2500 <sup>a)</sup>	
Body weight and body weight gain [g / day]	mean	SD	mean	SD	mean	SD	mean	SD
<b>Premating phase, F<sub>0</sub> females</b>	N = 5		N = 5		N = 5		N = 5	
Day 0 [g]	108.6	5.9	109.5	5.9	109.0	5.6	108.5	5.6
[% of control]	100		100.9		100.4		99.9	
Day 70 [g]	221.0	11.7	220.4	10.8	216.3	10.9	214.0	9.9
[% of control]	100		99.8		97.9		96.9	
Day 0 – 70 [g]	112.4	10.1	110.9	12.4	107.3	9.3	105.5	10.0
(Δ week 0-10) [% of control]	100		98.7		95.5		93.9	
<b>Gestation phase, F<sub>0</sub> females</b>	N = 25		N = 25		N = 25		N = 24	
GD 0 [g]	225.1	11.2	224.7	9.5	221.8	11.7	218.7	10.0
[% of control]	100		99.8		98.5		97.2	
GD 7 [g]	250.9	11.6	248.6	11.5	245.1	13.5	239.4**	12.5
[% of control]	100		99.1		97.7		95.4	
GD 14 [g]	275.3	13.5	272.4	15.2	270.0	15.1	262.2**	13.1
[% of control]	100		98.9		98.1		95.3	
GD 20 [g]	338.8	20.2	333.8	23.3	331.8	21.7	320.9*	20.5
[% of control]	100		98.5		97.9		94.7	
Δ GD 0 – 7 [g]	25.9	5.2	23.9	5.9	23.3	4.4	20.7**	4.8
[% of control]	100		92.4		90.3		80.0	
Δ GD 0 - 20 [g]	113.8	12.5	109.1	17.7	110.0	12.6	102.2*	12.9
[% of control]	100		95.9		96.7		89.8	
<b>Lactation phase, F<sub>0</sub> females</b>	N = 25		N = 25		N = 25		N = 24	
PND 1 [g]	243.3	10.4	239.9	14.8	242.3	13.4	229.4**	13.7
[% of control]	100		98.6		99.6		94.3	
PND 4 [g]	262.2	15.0	262.0	15.3	258.9	15.5	246.4**	14.9
[% of control]	100		99.9		98.8		94.0	
PND 7 [g]	264.3	14.4	264.9	16.5	263.2	11.8	259.5	14.3
[% of control]	100		100.3		99.6		98.2	
PND 14 [g]	294.7	16.8	289.7	18.4	288.2	15.9	282.4*	17.2
[% of control]	100		98.3		97.8		95.8	
PND 21 [g]	285.7	17.9	283.7	16.2	279.3	12.0	278.7	15.5
[% of control]	100		99.3		97.7		97.5	
Δ PND 4 - 7 [g]	2.1	13.9	2.9	12.3	4.2	12.7	13.1**	8.0
[% of control]	100		138.1		200.0		623.8	
Δ PND 7 - 14 [g]	30.4	11.7	24.8	11.9	25.0	10.7	22.9*	9.1
[% of control]	100		81.6		82.2		75.3	
Δ PND 1 - 21 [g]	42.4	13.7	43.8	13.2	36.9	11.8	49.3	16.9
[% of control]	100		103.3		87.0		116.3	

\* = p&lt;0.05; \* = p&lt;0.01; Dunnett test (two-sided);

Δ = body weight gain

GD = gestation day;

PND = postnatal day

<sup>a)</sup> Feed concentrations were halved to compensate the increased food intakes during the lactation period

Table 6.6-4. Pup weights

Pup generation	F <sub>1</sub> pups				F <sub>2</sub> pups			
Dose [ppm]	0	250	1000	5000	0	250	1000	5000
<b>Male pup weight [g]</b>								
- day 1 [HCR: 6.5 – 7.4]	6.6	6.6	6.7	6.5	6.9	7.2	7.2	7.1
- day 4 [HCR: 10.1 – 11.2]	10.0	10.3	10.1	9.9	10.4	11.0	10.9	10.9
- day 7 [HCR: 15.9 – 17.5]	16.1	16.6	16.3	16.0	16.6	17.6	17.5	17.2
- day 14 [HCR: 32.2 – 35.2]	33.0	33.8	32.8	32.5	33.4	35.0	34.3	33.2

Pup generation	F <sub>1</sub> pups				F <sub>2</sub> pups			
Dose [ppm]	0	250	1000	5000	0	250	1000	5000
- day 21 [HCR: 49.9 – 55.6]	52.5	53.3	51.7	50.8	52.7	54.7	54.4	51.8
<b>Male pup weight gain</b>								
- day 14 to 21 [g] [Δ% control]	19.5	19.5 ±0.0	18.8 -3.6	18.3* -6.2	19.3	19.7 +2.1	20.1 +4.1	18.6 -3.6
- day 1 to 21 [g] [Δ% control]	45.3	45.6 +1.5	44.6 -2.0	43.6 -3.5	45.7	47.5 +3.9	47.2 +3.3	44.6 -2.4
<b>Female pup weight [g]</b>								
- day 1 [HCR: 6.2 – 7.1]	6.2	6.3	6.3	6.2	6.6	6.7	6.8	6.7
- day 4 [HCR: 9.7 – 11.0]	9.6	9.8	9.8	9.6	10.0	10.5	10.6	10.4
- day 7 [HCR: 15.6 – 17.0]	15.6	16.0	15.9	15.6	16.2	17.0	16.9	16.5
- day 14 [HCR: 31.2 – 34.4]	32.4	32.7	32.2	31.6	32.7	34.2	33.4	32.1
- day 21 [HCR: 48.3 – 53.3]	51.0	51.1	50.4	49.0	51.0	52.8	52.2	49.3
<b>Female pup weight gain</b>								
- day 14 to 21 [g] [Δ% control]	18.6	18.4 -1.1	18.3 -1.6	17.4** -6.5	18.3	18.6 +1.6	18.8 +2.7	17.2 -6.0
- day 1 to 21 [g] [Δ% control]	44.7	44.8 +0.2	44.0 -1.6	42.8 -4.3	44.3	46.0 +3.8	45.3 +2.3	42.5 -4.1

Statistical analysis: \* p < 0.05; \*\* p < 0.01 (Dunnett-test, two-sided);

HCR: Historical control range

[HCR]: Historical control range from 18 studies (1-gen or 2-gen) with Wistar rats (supplier: [REDACTED]) run at test facility between 2011-2015

In relation to general toxicity, statistically-significant changes in organ weights were seen in the liver, thyroid, kidney, prostate, seminal vesicles, epididymidis and uterus, in one or more generations. Changes are reported and discussed below.

#### Adults

**Liver:** In F<sub>0</sub> parental males statistically-significant increases in liver weights (absolute and relative) were recorded from the mid dose. At the mid dose, however, percent change compared to controls was 8 and 10 % (absolute and relative, respectively) and statistically-significant values were within the HCD range. No concomitant gross pathology or histopathology was seen in mid dose group animals. At the high dose percent change compared to controls was 24 % and 26 % (absolute and relative, respectively) (> 15 %). In F<sub>1</sub> parental males statistically-significant increases in liver weights (absolute and relative) were recorded at the top dose, with changes of 19 % and 22 % compared to controls (> 15 %). At the top dose, statistically-significant values, both absolute and relative, in both generations were outside the HCD range. A dose response was evident in parental males. In both F<sub>0</sub> and F<sub>1</sub> parental females statistically-significant increases in liver weights (absolute and relative) were recorded at the top dose. At this dose all increases were > 15 % compared to controls and most statistically-significant values were outside the HCD range. A dose response was evident in parental females. A number of animals of the high dose group (both generations) showed enlarged livers (Table 6.6-8) without histopathologic correlate. Overall, treatment-related and adverse increases in liver weights were seen at the top dose in parental males and females of both generations.

**Thyroid:** Thyroid weights (absolute and relative) were consistently statistically-significantly increased at the top dose, in parental males and females of both generations. Change compared to controls was between 15 – 24 %. In parental males statistically-significant values were within the HCD but close to the HCD maximum; in parental females values were at the HCD maximum or outside the HCD range. Concomitant histopathology (hypertrophy/hyperplasia of follicular epithelial cells) was recorded at the top dose of both generations, in both males and females. Overall, treatment-related and adverse increases in thyroid weights were seen at the top dose in parental males and females of both generations.

**Kidney:** Kidney weights (absolute and relative) were statistically-significantly increased at the top dose, in parental males of both generations. A dose-response was evident in these groups. In the top dose change

compared to controls was between 12 – 14 % and values were outside the HCD. In parental females statistically-significant increases were only seen in the F<sub>0</sub> generation in the low (absolute and relative) and top dose (relative only), with no dose-response. Values were within the HCD range and no associated histopathology was noted. Consequently these findings in parental females were not considered treatment-related. Concomitant histopathology (chronic nephropathy as well as eosinophilic droplets) was recorded in males of the high dose group of both generations. Effects in the kidney corroborate findings of the repeated dose studies in the rat; the 90-day study in rats (██████████ 2018a) already identified a male rat-specific mode of action via  $\alpha$ -2 $\mu$ -globulin storage of no relevance to humans.

There were no treatment-related effects on epididymides, prostate, seminal vesicle, adrenal gland and/or uterus weights. Whilst some statistically-significant values were recorded, there was no dose-response and statistically-significant values were close to the HCD means (where data were available).

#### Pups

There were no treatment-related effects on organ weights in pups of both the F<sub>1</sub> and F<sub>2</sub> generations (Table 6.6-7). The only statistically-significant change was seen in relative brain weight of high dose F<sub>2</sub> pups, however, the increase seen was very close to the HCD mean, so not considered treatment-related. Decreases (not statistically-significant) in spleen weights were recorded, particularly in the top dose, however, a dose-response was not evident and values were close to the HCD means.

Overall, treatment-related, adverse and human-relevant increases in liver and thyroid weights (with concomitant thyroid histopathology) were seen at the top dose in parental males and females of both generations.

Table 6.6-5. Mean terminal body weight and organ weights – parental males

Generation	Dose [ppm]	F <sub>0</sub> Males				F <sub>1</sub> Males			
		Absolute weight [g or mg]	$\Delta\%$ <sup>&amp;</sup>	Relative weight [% of bw]	$\Delta\%$ <sup>&amp;</sup>	Absolute weight [g or mg]	$\Delta\%$ <sup>&amp;</sup>	Relative weight [% of bw]	$\Delta\%$ <sup>&amp;</sup>
Terminal weight [g]	0	397.12				406.60			
	250	392.74	-1.1			407.70	+0.3		
	1000	406.75	+2.4			413.06	+1.6		
	5000	388.43	-2.2			396.16	-2.7		
	HCD <sup>#</sup>								
Epididymides [g]	0	1.22		0.309		1.252		0.309	
	250	<b>1.15*</b>	-6.0	0.294	-4.9	1.202	-4.0	<b>0.296*</b>	-4.2
	1000	1.18	-3.8	0.291	-5.8	1.216	-2.9	0.295	-4.5
	5000	1.21	-1.2	0.312	+1.0	1.224	-2.2	0.309	$\pm 0.0$
	HCD <sup>#</sup>	Mean: 1.15 g Min: 1.03 g Max: 1.25 g		Mean: 0.3 % Min: 0.28 % Max: 0.33 %					
Kidneys [g]	0	2.53		0.639		2.523		0.622	
	250	2.45	-3.2	0.625	-2.2	2.545	+0.9	0.625	+0.5
	1000	2.66	+5.0	0.654	+2.3	2.632	+4.3	0.638	+2.6
	5000	<b>2.83**</b>	+11.7	<b>0.729**</b>	+14.1	<b>2.819**</b>	+11.7	<b>0.711**</b>	+14.3
	HCD <sup>#</sup>	Mean: 2.44 g Min: 2.19 g Max: 2.63 g		Mean: 0.63 % Min: 0.57 % Max: 0.7 %		Mean: 2.4 g Min: 2.06 g Max: 2.56 g		Mean: 0.63 % Min: 0.59 % Max: 0.71 %	
Liver [g]	0	8.80		2.217		9.914		2.435	
	250	8.85	+0.6	2.250	+1.5	9.862	-0.5	2.414	-0.9
	1000	<b>9.75**</b>	+10.8	<b>2.396**</b>	+8.1	9.963	+0.5	2.410	-1.0
	5000	<b>10.87**</b>	+23.5	<b>2.793**</b>	+26.0	<b>11.754**</b>	+18.6	<b>2.960**</b>	+21.6
	HCD <sup>#</sup>	Mean: 8.87 g Min: 8.02 g Max: 10.0 g		Mean: 2.29 % Min: 2.15 % Max: 2.51 %		Mean: 9.27 g Min: 7.78 g Max: 10.1 g		Mean: 2.41 % Min: 2.28 % Max: 2.69 %	
Prostate [g]	0	1.22		0.307		1.122		0.277	
	250	<b>1.09**</b>	-11.1	<b>0.277*</b>	-9.8	1.085	-3.3	0.267	-3.6
	1000	1.17	-4.2	0.288	-6.2	1.127	+0.4	0.274	-1.1
	5000	<b>1.08**</b>	-11.4	<b>0.278*</b>	-9.4	1.172	+4.5	0.296	+6.9

Generation	Dose [ppm]	F <sub>0</sub> Males				F <sub>1</sub> Males			
		Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
	HCD <sup>#</sup>	Mean: 1.06 g Min: 0.91 g Max: 1.26 g		Mean: 0.28 % Min: 0.23 % Max: 0.32 %					
Seminal vesicle [g]	0	1.45		0.365		1.365		0.336	
	250	<b>1.29*</b>	-10.8	<b>0.327*</b>	-10.4	1.318	-3.4	0.324	-3.6
	1000	<b>1.33*</b>	-8.2	<b>0.328**</b>	-10.1	1.343	-1.6	0.325	-3.3
	5000	<b>1.34*</b>	-7.4	0.347	-4.9	1.367	+0.1	0.345	+2.7
	HCD <sup>#</sup>	Mean: 1.30 g Min: 1.08 g Max: 1.57 g		Mean: 0.34 % Min: 0.30 % Max: 0.4 %					
Thyroid glands [mg]	0	24.00		0.006		22.64		0.006	
	250	24.88	+3.7	0.006	+4	23.84	+5.3	0.006	+5
	1000	25.32	+5.5	0.006	+3	24.04	+6.2	0.006	+4
	5000	<b>27.64**</b>	+15.2	<b>0.007**</b>	+17	<b>27.40**</b>	+21.0	<b>0.007**</b>	+23
	HCD <sup>#</sup>	Mean: 24.3 mg Min: 19.0 mg Max: 29.7 mg		Mean: 0.006 % Min: 0.005 % Max: 0.008 %		Mean: 24.1 mg Min: 17.3 mg Max: 28.9 mg		Mean: 0.006 % Min: 0.005 % Max: 0.007 %	

Statistical analysis: \* p ≤ 0.05, \*\* p ≤ 0.01 [Kruskal-Wallis and Wilcoxon-test (two-sided)]

& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means.

<sup>#</sup>: Historical control data (HCD) from 1- and 2-gen studies with Wistar rats (supplier: XXXXXXXXXX) run at the test facility between 2011-2017. The numbers of studies are as follows:

- F<sub>0</sub>: Thyroid/Liver/Kidney/Seminal vesicles/Prostate/Epididymides: 22/25/25/25/25/26 studies;

- F<sub>1</sub>: Thyroid/Liver/Kidney: for all 10 studies

Table 6.6-6. Mean terminal body weight and organ weights – parental females

Generation	Dose [ppm]	F <sub>0</sub> Females				F <sub>1</sub> Females			
		Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
Terminal weight [g]	0	236.02				235.46			
	250	233.22	-1.2			238.72	+1.4		
	1000	231.86	-1.8			241.02	+2.4		
	5000	227.98	-3.4			230.91	-1.9		
Adrenal gland [mg]	0	79.08		0.033		84.36		0.036	
	250	77.20	-2.4	0.033	-1	79.88	-5.3	<b>0.033*</b>	-7
	1000	75.68	-4.3	0.033	-3	82.56	-2.1	0.034	-4
	5000	80.24	+1.5	0.035	+5	87.80	+4.1	0.038	+6
Kidneys [g]	0	1.71		0.73		1.790		0.761	
	250	<b>1.82**</b>	+6.5	<b>0.78**</b>	+7.7	1.837	+2.6	0.77	+1.2
	1000	1.72	+0.6	0.74	+2.2	1.874	+4.7	0.778	+2.2
	5000	1.74	+1.9	<b>0.76**</b>	+5.4	1.766	-1.3	0.766	+0.7
	HCD <sup>#</sup>	Mean: 1.74 g Min: 1.59 g Max: 1.96 g		Mean: 0.76 % Min: 0.69 % Max: 0.81 %					
Liver [g]	0	6.23		2.64		6.846		2.907	
	250	6.24	+0.3	2.67	+1.4	6.862	+0.2	2.868	-1.3
	1000	6.34	+1.8	<b>2.73*</b>	+3.6	7.200	+5.2	2.982	+2.6
	5000	<b>7.48**</b>	+20.2	<b>3.28**</b>	+24.5	<b>8.142**</b>	+18.9	<b>3.526**</b>	+21.3
	HCD <sup>#</sup>	Mean: 6.5 g Min: 5.71 g Max: 8.06 g		Mean: 2.82 % Min: 2.48 % Max: 3.27 %		Mean: 6.6 g Min: 5.18 g Max: 7.6 g		Mean: 2.87 % Min: 2.57 % Max: 3.32 %	
Thyroid glands	0	18.04		0.008		17.84		0.008	

Generation	Dose [ppm]	F <sub>0</sub> Females				F <sub>1</sub> Females			
		Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [g or mg]	Δ%&	Relative weight [% of bw]	Δ%&
[mg]	250	18.52	+2.7	0.008	+4	17.92	+0.4	0.008	-1
	1000	18.24	+1.1	0.008	+3	19.04	+6.7	0.008	+4
	5000	<b>20.72**</b>	+14.9	<b>0.009**</b>	+19	<b>21.80**</b>	+22.2	<b>0.009**</b>	+24
	HCD <sup>#</sup>	Mean: 17.96 mg Min: 15.6 mg Max: 20.08 mg		Mean: 0.008 % Min: 0.007 % Max: 0.009 %		Mean: 19.19 mg Min: 16.3 mg Max: 20.64 mg		Mean: 0.008 % Min: 0.007 % Max: 0.009 %	
Uterus [g]	0	0.634		0.27		0.688		0.293	
	250	0.711	+12.1	<b>0.31*</b>	+13.4	0.756	+9.9	0.317	+8.2
	1000	0.770	+21.5	<b>0.33*</b>	+23.4	0.820	+19.2	0.342	+16.7
	5000	0.745	+17.5	<b>0.33*</b>	+21.6	0.708	+2.9	0.309	+5.5
	HCD <sup>#</sup>	Mean: 0.74 g Min: 0.59 g Max: 1.09 g		Mean: 0.32 % Min: 0.26 % Max: 0.51 %					

Statistical analysis: \* p ≤ 0.05, \*\* p ≤ 0.01 [Kruskal-Wallis and Wilcoxon-test (two-sided)]

& Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means.

<sup>#</sup>: historical control data (HCD) from 1- and 2-gen studies with Wistar rats (supplier: XXXXXXXXXX) run at the test facility between 2011-2017. The numbers of studies are as follows:

- F<sub>0</sub>: Thyroid/Liver/Kidney/Uterus: 21/25/26/25 studies

- F<sub>1</sub>: Thyroid/Liver: for both 10 studies;

Table 6.6-7. Mean organ weights – pups

Generation	Dose [ppm]	F <sub>1</sub> (males & females combined)				F <sub>2</sub> (males & females combined)			
		Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [mg]	Δ%	Relative weight [% of bw]	Δ%
Brain	0	1.515		2.941		1.489		2.864	
	250	1.529	+0.9	2.957	+0.5	1.493	+0.3	2.798	-2.3
	1000	1.501	-0.9	2.950	+0.3	1.488	±0.0	2.796	-2.4
	5000	1.507	-0.5	3.028	+2.9	1.496	+0.5	<b>2.961*<sup>a</sup></b>	+3.4
	HCD	Abs. mean: 1.47 g; spread: 1.35 – 1.60 g; Rel. mean: 2.97%; spread: 2.47 – 3.48%;							
Thymus	0	0.242		0.469		0.268		0.467	
	250	0.244	+0.9	0.470	+0.2	0.251	+3.6	0.467	-0.1
	1000	0.242	-0.2	0.473	+1.0	0.263	+2.0	0.465	-0.3
	5000	0.239	-1.2	0.478	+1.9	0.24	-2.3	0.470	+0.7
Spleen	0	0.263		0.510		0.268		0.511	
	250	0.256	-2.8	0.492	-3.5	0.251	-6.1	0.464	-9.1
	1000	0.254	-3.7	0.496	-2.8	0.263	-1.9	0.488	-4.3
	5000	0.236	-10.3	0.471	-7.5	0.240	-10.3	0.473	-7.3
	HCD	Abs. Mean: 0.24 g; spread: 0.15 – 0.33 g; Rel. mean: 0.48%; spread: 0.35 – 0.62%							

Statistical evaluation: Kruskal-Wallis and Wilcoxon-test (two-sided); \* p ≤ 0.05, \*\* p ≤ 0.01

<sup>a</sup> mean relative brain weight of females was statistically significantly increased (2.99%\*\*; Δ%: +4.6%, covered by HCD:

Mean: 2.97; range: 2.45-3.49%);

HCD: Historical control data are the Mean and 95% spread [16 studies run 2007-2015 at test facility with Wistar rats (supplier: XXXXXXXXXX)]; Values may not calculate exactly due to rounding of figures.

A number of animals of the high dose group (6 males and 5 females of the F<sub>0</sub> generation and 4 males of the F<sub>1</sub> generation) showed enlarged livers (Table 6.6-8) without histopathologic correlate. A number of males (8 of the F<sub>1</sub> generation) of the high dose group showed enlarged kidneys with corresponding microscopic findings (Table 6.6-10).

Treatment-related histopathological findings were observed in the kidney, nasal cavity and thyroid gland in adult animals. All other findings occurred either individually or were biologically equally distributed over control and treatment groups and were therefore not considered to be treatment-related. Males of the high dose group of

both generations showed higher incidences in chronic nephropathy as well as eosinophilic droplets stored in tubular epithelial cells and granular casts within tubules (Tables 6.6-9 and 6.6-10). The 90-day study in rats (■■■■■ 2018a) already identified a male rat-specific mode of action via  $\alpha$ -2 $\mu$ -globulin storage, of no relevance to humans. All examined animals of the high dose group of both generations showed degeneration/regeneration of the olfactory epithelium (minimal, slight (most commonly seen) or moderate) represented by: irregular epithelium, rosette-like formation, loss of sustentacular cell processes, eosinophilic homogeneous exudate on epithelium surface or in the nasal cavity. An increase in the incidence and severity of hypertrophy/hyperplasia of follicular epithelial cells in the thyroid was recorded at the top dose of both generations, in both males and females. Overall, treatment-related, adverse and human-relevant histopathological changes were seen in the nasal cavities and thyroid at the top dose (5,000 ppm equivalent to 412 – 450 and 394 – 481 mg/kg bw/d in males and females, respectively) in adult animals of both generations.

A few pups (in both F<sub>1</sub> and F<sub>2</sub> generations) showed spontaneous findings at gross necropsy (Table 6.6-11) (pups were subjected to only macroscopic examination and not histopathological examination). These findings occurred individually and/or without any dose-relationship. The higher incidence of dilated renal pelvis in F<sub>2</sub> pups (6 pups out of 233; pup incidence of 2.6 % and pup/litter incidence of 2.4 %) was above the HCD range (pup incidence: 0 - 2.3 %; pup/litter incidence: 0 - 2.1 %), but within the HCD range on a litter basis (4 of 24 litters; litter incidence of 16 %; HCD: 0 - 20%). The increased pup incidence was mainly caused by one litter (#376) with 11 pups in total, of which 3 were affected. Three further litters had single affected pups. As this was a single incident, not statistically-significantly, within the HCD range for litters and there was no similar effect on the F<sub>1</sub> pups, it was not considered treatment-related. Consequently no gross necropsy findings in pups were considered to be treatment-related.

Table 6.6-8. Incidence of gross necropsy observations – parental animals

Sex		Males				Females			
Dose	[ppm]	0	250	1000	5000	0	250	1000	5000
Animals examined		25	25	25	25	25	25	25	25
F <sub>0</sub> generation									
Animals with findings		1	2	0	7	1	3	0	7
No offspring or not pregnant					1				1
Glandular stomach	- focus	1	-	-	-	1	1	-	-
Kidney	- cyst(s)	-	1	-	2	-	1	-	-
Liver	- deformation	-	-	-	-	-	1	-	-
	- enlarged	-	-	-	6	-	-	-	5
	- focal constriction	-	1	-	-	-	-	-	-
	- focus	-	-	-	-	-	1	-	-
	- torsion	-	-	-	-	-	-	-	1
F <sub>1</sub> generation									
Animals with findings		0	1	0	12	0	1	0	1
Glandular stomach	- focus	-	-	-	1	-	-	-	-
Kidney	- enlarged	-	1	-	8	-	-	-	-
Liver	- discolouration	-	-	-	1	-	-	-	-
	- enlarged	-	1	-	4	-	-	-	-
	- focal constriction	-	-	-	-	-	1	-	-
	- focus	-	-	-	1	-	-	-	-
	- torsion	-	-	-	-	-	-	-	1
Epididymis (left)	- reduced size	-	-	-	1				
Testis (left)	- reduced size	-	-	-	1				

Statistical evaluation: Fischer's test (pair-wise); \* p ≤ 0.05; \*\* p ≤ 0.01

Table 6.6-9. Histopathological findings – parental F<sub>0</sub> generation (incidence and severity)

Sex		Male animals				Female animals			
Dose	[ppm]	0	250	1000	5000	0	250	1000	5000
Animals examined		25	25	25	25	25	25	25	25
Kidneys <sup>a</sup>									
Nephropathy, chronic		10	11	15	25				
Grade 1		8	11	15	9				
Grade 2		2			15				
Grade 3					1				
Eosinophilic droplets		0	0	1	22				
Grade 1				1	12				
Grade 2					10				
Casts, granular		0	0	0	17				
Grade 1					10				
Grade 2					4				
Grade 3					3				
Nasal cavity									
Degeneration/regeneration olfactory epithelium		0	0	0	25	0	0	0	25
Grade 1									8
Grade 2					19				17
Grade 3					6				
Thyroid glands									
Hypertrophy/hyperplasia, follicular cell epithelium		0	2	3	10	0	0	0	16
Grade 1			2	3	2				5
Grade 2					8				11

<sup>a</sup>) Kidneys were examined in males only, as the identified mode of action (a-2u-globulin) is male specific

Table 6.6-10. Histopathological findings – parental F<sub>1</sub> generation (incidence and severity)

Sex		Male animals				Female animals			
Dose	[ppm]	0	250	1000	5000	0	250	1000	5000
Animals examined		25	25	25	25	25	25	25	25
Kidneys <sup>a</sup>									
Nephropathy, chronic		11	9	11	25				
Grade 1		11	9	11	14				
Grade 2					6				
Grade 3					5				
Eosinophilic droplets		0	0	2	23				
Grade 1				2	13				
Grade 2					9				
Casts, granular		0	0	0	18				
Grade 1					7				
Grade 2					6				
Grade 3					2				
Grade 4					3				
Nasal cavity									
Degeneration/regeneration olfactory epithelium		0	0	0	25	0	0	0	25
Grade 1					7				8

Grade 2				15				14
Grade 3				3				3
Thyroid glands								
Hypertrophy/hyperplasia, follicular cell	0	1	1	15	0	0	0	8
Grade 1		1	1	4				4
Grade 2				11				4

<sup>a)</sup> Kidneys were examined in males only, as the identified mode of action (a-2u-globulin) is male specific

Table 6.6-11. Incidence of gross necropsy observations – pups

Sex		Male pups				Female pups			
Dose level	[ppm]	0	250	1000	5000	0	250	1000	5000
<b>F<sub>1</sub> pups</b>									
Animals examined		126	125	131	115	137	129	122	118
No. without signs		126	122	129	111	131	127	121	116
No. with signs		0	3	2	4	6	2	1	2
<i>post-mortem autolysis</i>					1		1		1
<i>partly cannibalized</i>			1						
<i>not assessed (missing/cannibalized)</i>			1			4			1
<i>new sign (lobus sinister lateralis small)</i>			1						
Thymus, discoloured					1	1		1	
Renal pelvis, dilated				2		1	1		
Renal pelvis, Hydronephrosis					1				
Ureter, dilated				1					
Hydroureter					1				
Diaphragm, hernia					1				
<b>F<sub>2</sub> pups</b>									
Animals examined		140	137	145	134	155	119	139	142
No. without signs		140	136	144	129	155	118	136	138
No. with signs		0	1	1	5	0	1	3	4
<i>post-mortem autolysis</i>								2	
<i>not assessed (= missing/cannibalized)</i>					2				
Liver lobe, discoloured								1	
Thymus, discoloured							1		
Renal pelvis, dilated					2				4
Testis, discoloured				1					
Testis, small					1				
Diaphragm, hernia			1						

HCD for dilated renal pelvis: pup incidence: 0-2.3 %; litter incidence: 0-20.0 %; pup/litter incidence: 0-2.1 %  
Wistar rat, study dates Jan 2007 to Nov 2017, 25 studies covering a range of routes of administration (diet, drinking water and gavage), 6390 pups evaluated, 6329 live, 61 dead.

#### Reproductive Toxicity

Cinmethylin did not adversely effect fertility and reproduction; oestrus cyclicity, mating performance and fertility, sperm parameters, differential ovarian follicle count, pup survival and sex ratio, nipple development, anogenital parameters and sexual maturation of pups (vaginal opening and preputial separation) were not affected by treatment. The majority of values were not statistically-significantly changed, were within the HCD range and/or no dose-response was evident. The number of F<sub>0</sub> females with stillborn pups in the high dose (5 out of 24 vs 1 out of 25 in controls) was slightly above the historical control range (Table 6.6-12), however, the increase was not statistically-significant and was only seen in the F<sub>0</sub> generation but not in the F<sub>1</sub> generation. In addition, the increased number of stillborn pups (10 out of 283 vs 1 out of 313 in controls) was mainly caused by one litter (#176) which contained 5 stillborn offspring. The 5 stillborn pups at the mid dose were also clustered in one litter (#170). As these were single incidents and there was no effect on the number of stillborns in the F<sub>2</sub> generation, this finding was not considered to be treatment-related. Although the mean day of puberty was marginally above the HCD range (day of vaginal opening: 29.5 - 31.9 days) in low and high dose female groups of the F<sub>1</sub> generation (32.0 and 32.2 days respectively vs 31.3 days in controls) (Table 6.6-17), as the difference



was less than half a day, not dose-related and lacked statistical-significance, this finding was not considered to be treatment-related.

Table 6.6-12. Oestrus cycle, male and females reproductive performance

Parental generation		F <sub>0</sub>				F <sub>1</sub>			
Dose [ppm]		0	250	1000	5000	0	250	1000	5000
Oestrus cycle (females)									
No. of cycles	[mean]	4.04	4.08	4.12	4.00	4.24	4.36	4.36	4.36
	[SD]	0.35	0.4	0.33	0.58	0.52	0.49	0.86	0.64
	HCD <sup>a</sup>	No. of oestrus cycles: 3.72 – 4.36							
Cycles length [day]	[mean]	4.04	4.06	4.04	4.09	4.00	3.98	3.94	4.06
	[SD]	0.17	0.22	0.11	0.61	0.09	0.14	0.10	0.36
	HCD <sup>a</sup>	Cycle length [days]: 3.99 – 4.46.							
Reproductive parameters (females)									
No. females placed with males <sup>f</sup>		25	25	25	25	25	25	25	25
No. females mated <sup>f</sup>		25	25	25	25	25	24	25	24
Female mating index <sup>f</sup> [%]		100	100	100	100	100	96	100	96
No. females pregnant <sup>f</sup>		25	25	25	24	25	23	24	24
Female fertility index <sup>f</sup> [%]		100	100	100	96	100	95.8	96.0	100
HCD <sup>b</sup>		Female fertility index [%]: 90 – 100%;							
Pre-coital interval <sup>w</sup> [mean days]		2.0	2.6**	2.4	2.2	2.6	2.3	2.9	2.3
HCD <sup>b</sup>		Pre-coital interval [mean days]: 1.9 – 3.7 days							
Duration of gestation <sup>d</sup> [mean days]		22.1	22.3	22.1	22.2	22.0	22.1	22.1	22.1
HCD <sup>b</sup>		Duration of gestation: 21.8 – 22.3 days							
Implantation sites, total <sup>w</sup>		330	323	318	303	320	269	306	295
- Mean per dam <sup>w</sup>		13.2	12.9	12.7	12.6	12.8	11.7	12.8	12.3
HCD <sup>b</sup>		Implantation sites/dam: 9.4 – 13.9							
Post-implantation loss <sup>w</sup> [mean %]		5.1	5.8	4.4	4.7	7.7	5.2	7.2	6.4
HCD <sup>b</sup>		Post-implantation loss [mean %]: 0.9 – 17.7%							
Females with liveborn <sup>f</sup>		25	25	25	24	25	23	24	24
- with stillborn pups <sup>f</sup> [N]		1	2	1	5	1	0	1	1
HCD <sup>b</sup>		Female with stillborn pups [N]: 0 – 4							
- with all stillborn <sup>f</sup>		0	0	0	0	0	0	0	0
Gestation index <sup>f</sup> [%]		100	100	100	100	100	100	100	100
No. pups delivered <sup>w</sup>		313	304	303	283	295	256	284	276
- per dam <sup>w</sup>		12.5	12.2	12.1	11.8	11.8	11.1	11.8	11.5
HCD <sup>b</sup>		Pups delivered/dam: 9.9 – 12.7							
- liveborn <sup>w</sup>		312	300	298	273	294	256	282	274
- stillborn <sup>w</sup>		1	4	5	10	1	0	2	2
- stillborn <sup>w</sup> [%]		0.3	1.3	1.7	3.5	0.3	0	0.7	0.7
HCD <sup>b</sup>		Pups stillborn [%]: 0- 4.2%							
-cannibalized / Dead		4	1	0	1	0	0	0	2
-cannibalized / Dead [%]		1.3	0.3	0	0.4	0	0	0	0.7
Live birth index <sup>w</sup> [%]		99.7	98.7	98.3	96.5	99.7	100.0	99.3	99.3
HCD <sup>b</sup>		Live birth index [mean %]: 95.8 – 100%							
Reproductive parameters (males)									
No. males placed with females		25	25	25	25	25	25	25	25
No. Males mated		25	25	25	25	25	24	25	24
Male mating index [%]		100	100	100	100	100	96	100	96
HCD <sup>c</sup>		Male mating index [%]: 90-100							
No. mated females pregnant		25	25	25	24	25	23	24	24
Male fertility index [%]		100	100	100	96	100	92	96	96
HCD <sup>c</sup>		Male fertility index [%]: 90-100							

Parental generation		F <sub>0</sub>				F <sub>1</sub>			
Dose [ppm]		0	250	1000	5000	0	250	1000	5000
Sperm parameters (males)									
No. animals per group		25	25	25	25	25	25	25	25
Sperm count [10 <sup>6</sup> / g]	- testis	107	N.A.	N.A.	107	106	N.A.	N.A.	108
	- cauda epididymis	532	N.A.	N.A.	535	605	N.A.	N.A.	614
Sperm motility [%]		86	86	87	86	90	89	88	90
Abnormal sperm [%]		6.2	N.A.	N.A.	6.4	6.1	N.A.	N.A.	6.1

\* p ≤ 0.05; \*\* p ≤ 0.01

Oestrus cycle: Kruskal-Wallis + Wilcoxon test, two-sided.

Female reproductive parameters: w: Wilcoxon with Bonferroni-Holmes test (one-sided), f: Fisher's exact test (one-sided); d Dunnett's test.

Males reproductive parameters: Fisher's Exact test (one-sided -).

Sperm parameters: Wilcoxon test (one-sided).

N.A.= not analysed

HCD:

a: Oestrus: [4 studies, run 2012-2014, at test facility, with Wistar rats (supplier: [REDACTED])]

b: Female reproductive parameters: [27 studies run 2008-2015 at the test facility with Wistar rats (supplier: [REDACTED])]

c: Male reproductive parameters: [27 studies run 2008-2015 at test facility with Wistar rats (supplier: [REDACTED])]

Table 6.6-13. Differential ovarian follicle count (DOFC)

Number of animals	Dose [ppm]	Absolute values			Relative values		
		Primordial	Growing	Primordial + growing	Primordial	Growing	Primordial + growing
25	0	8739	390	9129	349.56	15.60	365.16
25	5000	8389	379	8768	335.56	15.16	350.72

Statistical analysis: \* p ≤ 0.05, \*\* p ≤ 0.01, Wilcoxon-test (1-sided)

Table 6.6-14. Pup survival and sex ratio

Parental generation		F <sub>0</sub>				F <sub>1</sub>			
Dose [ppm]		0	250	1000	5000	0	250	1000	5000
Number of litters		25	25	25	24	25	23	24	24
- with liveborn pups		25	25	25	24	25	23	24	24
- with stillborn pups		1	2	1	5	1	0	1	1
Pups liveborn		312	300	298	273	294	256	282	274
Pups found dead (Day 1-4)		0	1	0	1	0	0	0	0
Pups cannibalized (Day 1-4)		4	1	0	1	0	0	0	2
Pups PND4 (pre-cull)		308	299	298	271	294	256	282	272
Viability index [%]		98.9	99.7	100	99.4	100	100	100	98.9
Pups culled day 4		108	101	98	83	94	82	92	82
Pups PND4 (post-cull)		200	198	200	188	200	174	190	190
Pups found dead (Day 5-21)		0	0	0	0	0	0	0	0
Pups cannibalized (Day 5-21)		0	0	0	0	0	0	0	0
Pups PND21		200	198	200	188	200	174	190	190
Lactation index [%]		100	100	100	100	100	100	100	100
Sex ratio [% live males], PND 0		48.7	49.3	51.3	48.9	47.8	53.2	51.8	48.3
Sex ratio [% live males], PND 21		50.5	48.9	50.0	48.4	50.5	51.1	51.2	45.7

Statistical analysis, viability and lactation indices: Wilcoxon with Bonferroni-Holm (one-sided -),

sex ratio: Wilcoxon test (two-sided), Fisher's exact test (one-sided); \* p ≤ 0.05, \*\* p ≤ 0.01

Table 6.6-15. Areola nipple development in male F<sub>1</sub> and F<sub>2</sub> pups

Pup generation		F <sub>1</sub>				F <sub>2</sub>			
Dose [ppm]		0	250	1000	5000	0	250	1000	5000
Nipple development		25	25	25	24	25	23	24	24

Pup generation	F <sub>1</sub>				F <sub>2</sub>			
Dose [ppm]	0	250	1000	5000	0	250	1000	5000
- Day 13 [no. examined]								
- Day 13 Mean [% ± SD]	73.1 ± 31.3	74.7 ± 34.7	76.0 ± 29.7	79.4 ± 28.6	49.0 ± 37.2	38.4 ± 39.4	42.6 ± 36.1	39.6 ± 33.3
- Day 13 Number [# ± SD]	1.4 ± 0.7	1.3 ± 0.7	1.4 ± 0.8	1.6 ± 0.8	0.93 ± 0.96	0.8 ± 1.04	0.87 ± 0.97	0.74 ± 0.75
Nipple development								
- Day 20 [no. examined]	25	25	25	24	25	23	24	24
- Day 20 Mean [% ± SD]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
- Day 20 Number [# ± SD]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Statistical analysis: Wilcoxon with Bonferroni-Holm (one-sided +); \* p < 0.05; \*\* p < 0.01

Historical control data [12 studies run 2007-2015 at test facility with Wistar rats (supplier: XXXXXXXXXX)]

- Males with areolae/nipples Day 12/13 [%]: 8.6-95%;

- Males with areolae/nipples Day 20/21 [%]: 0%;

Table 6.6-16. Anogenital distance and index

Pup generation	F <sub>1</sub>				F <sub>2</sub>			
Dose [ppm]	0	250	1000	5000	0	250	1000	5000
<b>Males</b> (No. examined)	25	25	25	24	25	23	24	24
- Anogenital distance [mm] (Day 1) [Δ% control]	3.03	3.09 +2.0	3.04 +0.3	3.03 ±0.0	3.11	3.15 +1.3	3.12 +0.3	3.14 +1.0
- Anogenital index (Day 1) [Δ% control]	1.62	1.65 +1.9	1.62 ±0.0	1.63 +0.6	1.63	1.64 +0.6	1.62 -0.6	1.64 +0.6
<b>Females</b> (No. examined)	25	25	25	24	25	23	24	24
- Anogenital distance [mm] (Day 1) [Δ% control]	1.49	1.51 +1.3	1.52 +2.0	1.50 +0.7	1.58	1.6 +1.3	1.59 +0.6	1.58 ±0.0
- Anogenital index (Day 1) [Δ% control]	0.81	0.82 +1.2	0.82 +1.2	0.82 +1.2	0.85	0.85 ±0.0	0.84 -1.2	0.84 -1.2

Statistical analysis: \* p < 0.05; \*\* p < 0.01 (Dunnett-test, two-sided)

Historical control data [12 studies run 2007-2015 at test facility with Wistar rats (supplier: XXXXXXXXXX)]

- AG distance [mm]: ♂: 2.99 – 3.15; ♀: 1.48-1.60;

- AG index: ♂: 1.58 – 1.67; ♀: 0.79- 0.86

Table 6.6-17. Sexual maturation of F<sub>1</sub> pups

F <sub>1</sub> parental generation	Vaginal opening in females				Preputial separation in males			
Dose [ppm]	0	250/125	1000/500	5000/2500	0	250	1000	5000
No. animals examined	25	25	25	25	25	25	25	25
Days to criterion	31.3	32.0	31.0	32.2	42.7	42.9	42.7	43.5
Body weight at criterion [g]	95.6	99.8	96.3	98.8	183.0	188.5	181.5	181.3

Statistical analysis: \* p < 0.05; \*\* p < 0.01 (Dunnett-test, two-sided)

Historical control data: 17 studies (2010-2015) from the test facility with Wistar rats (supplier: XXXXXXXXXX)

Day of vaginal opening: 29.5 – 31.9 days (body weight at criterion: 83.1 – 100.7 g)

Day of preputial separation: 40.5 – 45.2 days (body weight at criterion: 168.1 – 195.3 g)

Conclusion

The potential of cinmethylin to adversely affect reproduction has been well investigated in a standard 2-generation dietary study conducted in Wistar rats.

There was no effects of treatment on oestrus cycle, mating and gestation, pup survival and sex ratio, nipple development, anogenital distance and/or index, sexual maturation, and/or differential ovarian follicle counts up to the top dose of 5000/2500 ppm (394 mg/kg bw/d), a dose at which parental (but not offspring) toxicity occurred. In addition, examination of the reproductive organs did not reveal any treatment-related changes. Specific investigation of the spermatogenic cycle did not find any cell or stage-specific abnormalities at any dose level. Therefore a **NOAEL for reproductive toxicity of 2500/5000 ppm (394 mg/kg bw/d) (highest dose tested)** can be identified from this study.

In relation to general toxicity, in parental animals, reductions in food consumption (in F<sub>0</sub> females during gestation), mean body weights (in F<sub>0</sub> females during gestation and lactation) and mean body weight gains (in F<sub>0</sub> females) were recorded at the top dose. Increases in liver and thyroid weights (with concomitant thyroid histopathology) were seen at the top dose in parental males and females of both generations. In addition adverse histopathological changes were seen in the nasal cavities at the top dose. There were no treatment-related effects in parental animals at the low and mid doses. Therefore, a **NOAEL of 500/1000 ppm (80 mg/kg bw/d) can be identified for parental toxicity** from this study. As no **developmental/offspring toxicity was observed up to the top dose, a NOAEL of 2500/5000 ppm (394 mg/kg bw/d) (highest dose tested)** can be identified from this study.

(██████████, 2018 a ; ██████████ 2018)

*Old study*

<b>Author(s)</b>	██████████.
<b>Study title</b>	Two generation reproduction study of Cinch herbicide (SD 95481) in rats
<b>Study reference</b>	██████████, 1986 BASF DocID: CI-430-001
<b>Test facility</b>	██████████
<b>Date</b>	07/02/1984 – 10/04/1985
<b>Test substance</b>	BAS 684 H (Cinmethylin) Cinch herbicide (SD 95481)
<b>Batch no. Purity (%)</b>	513K and 513N 92.4 and 93 (-) / (+) ratio = not specified.
<b>Test animals</b>	Rat Sprague Dawley Male and female
<b>Groups</b>	F <sub>0</sub> (parental generation) : 20 male and 30 female/dose F <sub>1</sub> (parental generation) : 20 male and 25 - 30 female/dose
<b>Dose/concentrations</b>	0, 200, 2000 and 20000 ppm (see Table 6.6-18 for equivalent mg/kg bw/d doses)
<b>Route</b>	Administered daily via the diet for 10 weeks prior to mating, throughout mating, gestation and lactation (total exposure 167 days for males and 209 days for females). Administration to F <sub>1</sub> males was stopped at the end of week 24, 4-weeks prior to necropsy (4-week off treatment period).
<b>Vehicle</b>	Acetone <sup>6</sup> .
<b>GLP</b>	Compliant.
<b>Guideline</b>	EPA. Non-OECD test guideline compliant.
<b>Deviation</b>	A large number of deviations (see below) and examples of poor/insufficient reporting were noted. Importantly, F <sub>0</sub> female fertility index was low, across all groups and more so in controls. As a consequence in the F <sub>0</sub> generation, the minimum group size of 20

<sup>6</sup> Studies to determine the solubility of cinmethylin in DMSO and acetone have been submitted and are evaluated in detail in Volume 3 – B.2, section B.2.6. Cinmethylin was found to be readily soluble in acetone, as confirmed by a GLP study.

	pregnant females (required by the OECD test guidelines) was not reached for any group in the first mating (F <sub>1a</sub> )
<b>Impact of deviations</b>	The deficiencies (listed above and below) negatively impact the relevance and reliability of the study.
<b>Acceptable</b>	Not acceptable.
<b>NOAEL</b>	Not set due to study limitations.
<b>Effects at the LOAEL</b>	N/A - due to study limitations.

Due to the significant limitations of this study, no detailed assessment has been performed. The study is unreliable and should be discounted. The study has been reported for transparency but no robust NOAELs and LOAELs have been derived and the study is not relied upon.

Deviations to current OECD 416 (2001) and/or previous guideline (1983):

- Oestrus cycle data analysis was not performed.
- Assessment of sperm parameters were not performed.
- Differential ovarian follicle count was not performed in parental females.
- Organ weight was not measured for uterus, prostate, seminal vesicles, brain, spleen, thymus.
- Assessment of sexual maturation (vaginal opening / preputial separation) was not performed.
- Inappropriate high dose level (20000 ppm, corresponding to mean intakes in females of up to 2213 /1609 / 2893 mg/kg bw/d during premating / gestation / lactation, respectively) associated with mortality rate of 23 % and 17 % in parental females of the F<sub>0</sub> and F<sub>1</sub> generation respectively; exceeding the acceptable upper limit of 10 %.
- Dose levels were spaced by a factor of 10, exceeding guideline recommendations of factor 3 for dietary studies and reducing the sensitivity of the study to detect treatment-related effects and/or a dose-response relationship.
- Male mating index could not be determined.
- Gestation index calculations excluded potential females with only resorptions (not examined).
- Corpora lutea and implantation sites were not recorded for assessment of pre- and post-implantation loss.
- Due to malfunction of the automatic lighting system during the first mating of the F<sub>0</sub> generation animals were continuously exposed to light for an 8-day period. These conditions likely account for the unusually low female fertility indices in control test groups, in both matings of the F<sub>0</sub> generation (F<sub>1a</sub>: 42-61 %, F<sub>1b</sub>: 62-80 %).
- As a consequence, the required minimum group size of 20 pregnant females was not reached for control and test groups of the F<sub>0</sub> generation in both matings (except for the mid- and high-dose group of the 2nd mating).
- In addition there were numerous instances of poor/insufficient reporting of the study.

#### Methods

The potential effects of cinmethylin on the reproductive performance of Sprague Dawley rats and their offspring over two generations have been investigated. In this study, parental rats (F<sub>0</sub>, 20 males/dose and 30 females/dose) were administered cinmethylin via the diet for a 10-week pre-mating period, throughout mating, gestation and lactation at concentrations of 0, 200, 2000 or 20000 ppm (see Table 6.6-18 for equivalent mg/kg bw/d doses). Groups were mated to produce a first litter (F<sub>1a</sub>), and subsequently re-mated 10 - 14 days after weaning to produce a second litter (F<sub>1b</sub>). Following weaning of the F<sub>1b</sub> generation, groups of 20 males and 25 - 30 females were selected as F<sub>1</sub> breeding animals. These rats were mated to produce two F<sub>2</sub> litters. Treatment of F<sub>1</sub> males with cinmethylin was discontinued four weeks before necropsy (off-treatment). Fertility, gestation, lactation, pup growth and survival were monitored. Daily clinical observations and weekly body weight and food consumption measurements were performed. Gross necropsies and histopathology was performed on all F<sub>0</sub> and F<sub>1</sub> mated rats and the selected F<sub>2b</sub> weanlings. Liver, kidneys, testes and ovaries were weighed. The stability and homogeneity of cinmethylin in the diet was acceptable. A validated method of analysis has not been submitted (see Volume 3 CA B5, section B.5.1.2). Calculated achieved intakes were as follows:

Table 6.6-18. Mean cinmethylin intake - parental animals

Sex / generation / study phase	Dose [ppm]					
	F <sub>0</sub>			F <sub>1</sub>		
	200	2000	20000	200	2000	20000
	Mean cinmethylin intakes [mg/kg bw/d]					
Premating						
Males (up to 1 <sup>st</sup> mating)	12.0	122.5	1366.5	16.1	163.4	2125.4
Males (up to 2 <sup>nd</sup> mating)	11.3	114.6	1288.8	13.5	137.7	1791.5
Females	13.9	138.9	1450.0	17.3	169.9	2213.0
Gestation						
Females (1 <sup>st</sup> mating)	14.7	148.4	1520.7	14.4	142.2	1609.3
Females (2 <sup>nd</sup> mating)	13.7	133.7	1434.0	12.8	129.7	1575.1
Lactation						
Females (1 <sup>st</sup> mating)	34.0	352.8	2859.3	33.9	332.7	2893.3
Females (2 <sup>nd</sup> mating)	30.6	279.9	2283.3	30.4	307.3	2256.0

## Results

### *Parental and offspring toxicity*

There was an increased incidence of parental mortality at the top dose in females (Table 6.6-19). Mortality generally occurred at the end of pregnancy or shortly after birth. Dystocia was suggested as the most common cause of death; this was sometimes accompanied by fatal haemorrhage from the uterus. However, given there were multiple signs of toxicity, including severe body weight loss, dystocia was considered to be an expression of maternal toxicity. F<sub>0</sub> parental males of the top dose sporadically showed chromodacryorrhea (blood-stained tears), lacrimation and soft stool at incidences above the concurrent control (Table 6.6-20), however, it is not clear how many males were affected (whether the same or different individuals were effected at each time point). A wide range of clinical signs were noted in F<sub>0</sub> parental females at the top dose: rales (abnormal/crackling sounds in the lung), red vaginal discharge, urine-stained urogenital area and reduced defecation were more common in the top dose. F<sub>1</sub> parental males of the top dose showed: unilateral small testis, urine-stained urogenital area, an increased incidence of red discharge around penis and soft stool (Table 6.6-21). Again, the range of clinical signs noted in F<sub>1</sub> parental females was increased in the top dose: rales, urine-stained urogenital area, hypoactivity and hypersensitivity when touched were recorded. An increase in clinical signs was noted in pups of the F<sub>1</sub> generation at the top dose (Table 6.6-22). General toxicity of mothers in the top dose (seen in both generations), leading to reduced nursing of their pups, was suggested as a cause; however, an increase in clinical signs was not seen in the F<sub>2</sub> generation pups. The malfunction of the lighting system during the first mating of the F<sub>0</sub> generation (which lead to continuous light for 8-days) was suggested as a possible contributing factor; however, increased clinical signs were seen in both matings (1a and 1b). Overall, treatment-related and adverse effects on mortality (in parental females) and clinical signs (in parental males and females, and pups of the F<sub>1</sub> generation) were seen at the top dose. The top dose caused excessive toxicity and was well above the MTD.

Parental body weight was statistically-significantly reduced at the top dose, in both sexes and both generations (F<sub>0</sub> and F<sub>1</sub>), during pre-mating, gestation and lactation (Tables 6.6-23 to 6.6-26). Reductions were consistent throughout the study. Reductions in body weight gains were far greater than 10 % (change compared to control). Statistically-significant decreases in body weight were also seen in (F<sub>0</sub> and F<sub>1</sub>) females of the mid dose. In the first generation (F<sub>0</sub>), during the gestation and lactation period of the second litter (F<sub>1b</sub>), body weights were statistically-significantly reduced (Table 6.6-24) at the mid dose. Body weight gain was decreased in this group during gestation (> 10 %) but were increased during lactation. In the F<sub>1</sub> generation body weights were statistically-significantly reduced during the lactation phase of the first litter (F<sub>2a</sub>) and the gestation period of the second litter (F<sub>2b</sub>) at the mid dose (Table 6.6-26). However, body weight gains were increased in this group. Food consumption was statistically-significantly reduced in females of the top dose, in both generations, during pre-mating, gestation and lactation (F<sub>1</sub> only); with a change compared to control ranging from 10 – 51 % (Tables 6.6-27 and 6.6-28). Food consumption was not effected in males of the F<sub>0</sub> generation but was reduced in the F<sub>1</sub> generation (28 % change) at the top dose (Table 6.6-28). Pup body weights were statistically-significantly reduced, throughout the study period, at the top dose (both generations and matings) (Table 6.6-29), with a 47 – 59 % change compared to controls. A dose-response was evident in this parameter; statistically-significant

decreases in body weight were recorded at the mid dose (at later time points, in the first mating of both generation), resulting in an 11 % reduction in body weight in F<sub>1a</sub> and F<sub>2a</sub> pups. Overall, treatment-related and adverse decreases in body weight were seen in parental females and pups from the mid dose and in parental males at the top dose. In addition treatment-related and adverse effects on body weight gain were observed in parental males and females at the top dose.

**Liver:** In males of the F<sub>0</sub> generation, statistically-significant increases in liver (absolute and relative) weights were observed from the mid dose (Table 6.6-30). A dose-response was evident and increases > 15 % were recorded. In males of the F<sub>1</sub> generation, increases in relative liver weights were < 10 %; this difference compared to the F<sub>0</sub> generation was a result of the 4-week off-treatment period. In parental females liver weights were statistically-significantly increased from the mid dose (Table 6.6-31). A dose-response was evident. A change compared to controls of > 10 % but < 15 % was seen at the mid dose and a change of > 65 % was recorded at the top dose. In pups (F<sub>2b</sub> generation), statistically-significant increases in relative liver weights were seen in males from the mid dose; increases of > 15 % were observed in males from the mid dose and in females at the top dose (Table 6.6-34). A dose-response was evident. Enlargement of the liver was recorded at the mid dose in 5/30 F<sub>0</sub> females and at the top dose, in 15/20 F<sub>0</sub> males, 23/20 F<sub>0</sub> females and 20/25 F<sub>1</sub> females (Table 6.6-32). Histopathological examination of parental livers showed consistent effects in both males and females (Table 6.6-33). A dose-response was evident for certain findings (e.g. eosinophilic hepatocytes and cytoplasmic vacuolation) and observations support the liver weight increases from the mid dose. Histopathological examinations of pup livers (confined to control and top dose groups) confirmed increases in liver pathology in the top dose (Table 6.6-35). Overall, treatment-related and adverse effects on the liver (increase in weight with concomitant histopathology) were seen from the mid dose, in parental animals (males and females) and pups.

**Kidney:** In males of the F<sub>0</sub> generation, a statistically-significant increase in relative kidney weight was seen at the top dose (Table 6.6-30). A dose-response was evident and an increase of > 20 % was recorded at the top dose.

In males of the F<sub>1</sub> generation, increases in relative kidney weights were < 10 %; this difference compared to the F<sub>0</sub> generation was a result of the 4-week off-treatment period. In parental females and pups changes in kidney weights were not considered treatment-related as changes in relative weights were < 10 % (Table 6.6-31 and 6.6-34). Histopathological examination of the kidney in parental animals did not show consistent treatment-related effects (Table 6.6-33); most findings were seen at similar incidences in all groups including controls. Renal tuular mineralisation was statistically-significantly increased at 2000 ppm, however, no dose-response was observed. Overall, treatment-related and adverse effects on the kidney (increase in weight) were seen at the top dose, in parental males.

**Testes:** In males of the F<sub>0</sub> generation, a statistically-significant increase in relative testes weight was seen at the top dose (Table 6.6-30). A dose-response was evident and an increase ≥ 20 % was recorded at the top dose. In males of the F<sub>1</sub> generation statistical-significance was seen in the top dose. The increase in relative testes weight at the top dose (> 20 %) was similar to that seen in the F<sub>0</sub> generation. In pups a statistically-significant decrease in absolute testes weight (-60 %) was seen at the top dose, resulting in a toxicologically-significant decrease in relative testes weight (-15 %) (Table 6.6-34). Histopathological examinations of adult and pup testes did not show treatment-related findings. Overall, treatment-related and adverse effects on testes (changes in weight) were seen at the top dose, in parental males and pups.

**Ovary:** In parental females changes in ovary weights were not considered treatment-related as changes in relative weights were < 10 % (Table 6.6-31). In pups a statistically-significant decrease in absolute ovary weight (-66 %) was seen at the top dose, resulting in a toxicologically-significant decrease in relative ovary weight (-25 %) (Table 6.6-34). A dose-response was evident. Histopathological examinations of adults and pups did not show treatment-related findings. Overall, treatment-related and adverse effects on the ovary (decreases in weight) were seen at the top dose in pups.

**Uterus:** Gross necropsy observations in parental animals (haemorrhagic diathesis, dystocia and luminal dilatation) generally increased in incidence at the top dose (Table 6.6-32).

Haematological and clinical chemistry parameters were not analysed in this study.

Table 6.6-19. Mortality (parental)

Generation		F <sub>0</sub>				F <sub>1</sub>			
Dose [ppm]		0	200	2000	20000	0	200	2000	20000
Males	Group size	20	20	20	20	20	20	20	20
	Died	0	0	1	1	0	0	0	0
	time (cause of death)	-	-	w20 (no lesions&)	w24 (haemorrhagic shock&)	-	-	-	-
Females	Group size	30	30	30	30	29	30	30	25
	Died	0	1	0	7	0	0	2	5
	time (cause of death)			++	w2 (nephropathy&)				
			w16 (dystocia / acute peritonitis&)		2 x w16 (dystocia <sup>§</sup> )			w15 (moribund after birth&)	w15 (acute pneumonia&)
								w19 (hydro-nephrosis&)	w22 (no tissue lesions&)
					w24 (acute cystitis&) 2 x w25 (dystocia <sup>§</sup> ) w26 (dystocia <sup>§</sup> )				3 x w25 (dystocia <sup>§</sup> )

mortality rate x/y, where x = number of unscheduled deaths and y = number of animals in the dose group;

time = time of occurrence in weeks; <sup>§</sup> = considered to be treatment related; & = considered to be not treatment relatedTable 6.6-20. Clinical signs (F<sub>0</sub> parental)

Sex	Males				Females			
Dose [ppm]	0	200	2000	20000	0	200	2000	20000
Clinical sign [n / incidence]								
Unkempt						1/1		2/3
Chromodacryorrhea	1/1			7/13		2/2	1/3	
Viscous salivation			1/1*					
Periocular swelling				2/2			1/1	1/1
Decreased food consumption						2/2		1/3
Sneezing						1/1		1/1
Red nasal discharge						2/2		
Piloerection						2/2		2/2 <sup>#</sup> “
Pale					3/5	1/1	2/2	3/7 <sup>#</sup>
Red discharge around penis			1/1					
Red vaginal discharge					2/4	2/2	1/1	9/9
Rales						2/6	9/16	14/18 <sup>#</sup>
Urine-stained urogenital area				1/1				8/13 <sup>§</sup>
Hypoactivity			1/1*					1/1 <sup>#</sup>
Lacrimation				3/5				2/2
Hypopnea								1/1 <sup>“</sup>
Unsteady stance			1/1*					1/1 <sup>“</sup>
Hypothermia								3/3 <sup>#</sup> “



Sex	Males				Females			
Dose [ppm]	0	200	2000	20000	0	200	2000	20000
Soft stool				3/4	1/1	1/1		
Small amount faeces								6/6
Hypersensitive when touched								1/2
Difficult breathing								1/1
Cut on back								2/9
Squinting			1/1*					
Mass	2/7		1/1		10/57	20/124	7/34	

n = number of animals showing each clinical sign in each week interval (n>1 could be 1 animal showing sign during several weeks or several animals)

incidence = number of days during which clinical sign was observed

\* = observed in animal #126 that was found moribund and sent to necropsy at week 20

# = observed in animal #209 that was found moribund and sent to necropsy at week 26

“ = observed in animal #183 before her death at week 16

§ = observed in 4 animals #203, #223, #233 and #239

Due to poor reporting, it was not possible to clarify for all study groups the total number of animals without any clinical findings or the total number of animals that showed a specific clinical sign; only for some findings footnote information was given, clarifying which and how many rats were affected.

Table 6.6-21. Clinical signs (F<sub>1</sub> parental)

Sex	Males				Females			
Dose [ppm]	0	200	2000	20000	0	200	2000	20000
Clinical sign [n / incidence]								
Unkempt								8/19 <sup>B</sup>
Chromodacryorrhea	44/76	61/111	65/174	18/35	18/29	6/10	7/8	3/3
Periocular swelling	2/3	10/10	21/39	11/16	10/14	7/9	5/6	1/1
Decreased food consumption						1/1		
Red nasal discharge	1/1	3/3	3/3				2/2 <sup>α</sup>	3/8 <sup>B</sup>
Piloerection								1/1
Pale					2/4	0	2/2	3/3
Unilateral small testis				31/189 <sup>S</sup>				
Red discharge around penis		1/1	5/5*	33/41 <sup>#</sup>				
Red vaginal discharge								1/1
Rales		1/1	2/2	1/1			2/3	24/63
Urine-stained urogenital area				4/10 <sup>%</sup>			1/1	225/727 &
Hypoactivity								3/5
Lacrimation	11/21	21/29	23/43	9/13	8/8	9/12	6/11	1/1
Hunched posture								1/2 <sup>B</sup>
Unsteady stance								1/1
Soft stool		1/1	1/1	4/5 <sup>§</sup>				1/1
Decreased defecation						1/2	2/2	1/3
Hypersensitive when touched								5/11
Dyspnea							1/1	1/1
Hypopnea								1/2
Hypotonea	5/24	3/15	2/7	-	18/23	-	2/7	-

n = number of animals showing each clinical sign in each week interval (n>1 could be 1 animal showing the effect during several weeks or several animals during e.g. 1 week)

incidence = number of days during which clinical sign was observed

\* = observed in 4 animals

# = observed in 11 animals

§ = observed in 3 animals

S = observed in 3 animals #422, #428 and #440 since week 12

% = observed in 2 animals #450 and #452

& = observed in 21 animals

α = observed in animal #411 that was found moribund and sent to necropsy at week 15

B = observed in animal #451

Due to poor reporting, it was not possible to clarify for all study groups the total number of animals without any clinical findings or the total number of animals that showed a specific clinical sign; only for some findings footnote information was given, clarifying which and how many rats were affected.

Table 6.6-22. Clinical signs - pups

Generation	F <sub>1</sub>				F <sub>2</sub>			
Dose level [ppm]	0	200	2000	20000	0	200	2000	20000
	F <sub>1a</sub>				F <sub>2a</sub>			
Pups tested	107	133	110	36	290	327	296	24
Litter tested	12	16	12	7	26	29	28	5
Clinical signs [n]	0	2	0	<b>16</b>	9	4	6	1
l (L)	0 (0.0)	2 (1.4)	0 (0.0)	<b>4 (35.7)</b>	7 (6.8)	3 (1.1)	4 (1.9)	1 (3.3)
	F <sub>1b</sub>				F <sub>2b</sub>			
Pups tested	167	215	172	76	280	297	293	40
Litter tested	18	19	21	17	25	24	27	9
Clinical signs [n]	5	14	4	<b>26</b>	12	8	2	0
l (L)	5 (3.6)	5 (7.2)	4 (1.6)	<b>7 (36.4)</b>	7 (8.5)	6 (2.9)	2 (1.5)	0 (0.0)

n = total number of pups showing clinical sign;

l = total number of litters affected; L = mean litter incidence of clinical signs in %

Table 6.6-23. Body weight - F<sub>0</sub> males and females (pre-mating)

Dose [ppm]	0	200	2000	20000
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
<b>F<sub>0</sub> males, Body weight [g]</b>	N = 20	N = 20	N = 20/19	N = 20/19
Week 1 (Day 0)	250.9 ± 13.0	249.7 ± 11.3	251.6 ± 12.6	250.2 ± 11.3
Week 2 (Day 7)	287.4 ± 15.3	284.5 ± 14.3	289.0 ± 15.3	<b>253.1 ± 10.3*</b>
Week 5 (Day 28)	345.2 ± 23.6	343.2 ± 23.1	346.8 ± 23.3	<b>305.6 ± 15.9*</b>
Week 11 (Day 70)	406.6 ± 25.9	402.1 ± 30.0	407.0 ± 28.4	<b>354.5 ± 23.6*</b>
Week 14 (Day 91)	409.3 ± 28.6	405.0 ± 34.0	410.1 ± 28.6	<b>353.0 ± 23.3*</b>
Week 21 (Day 140)	437.9 ± 30.7	432.3 ± 38.4	439.0 ± 34.7	<b>368.4 ± 27.0*</b>
Week 25 (Day 168)	448.3 ± 34.9	446.1 ± 39.6	452.6 ± 34.0	<b>379.3 ± 29.2*</b>
<b>F<sub>0</sub> males, Body weight gain [g]</b>				
Δ week 1 – 11	155.7 ± 17.6	152.4 ± 24.0	155.4 ± 20.9	<b>104.3 ± 19.6</b>
[% of control]	100	98	100	<b>67</b>
Δ week 1 – 21	187.0 ± 23.9	182.5 ± 32.7	187.3 ± 27.8	<b>118.2 ± 22.2</b>
[% of control]	100	98	100	<b>63</b>
Δ week 1 – 25	197.4 ± 29.0	196.4 ± 33.7	200.9 ± 26.9	<b>128.9 ± 23.7</b>
[% of control]	100	100	102	<b>65</b>
<b>F<sub>0</sub> Females, Body weight [g]</b>	N = 30	N = 30	N = 30	N = 30/29
Week 1 (Day 0)	174.8 ± 7.7	175.5 ± 8.7	173.5 ± 10.0	176.2 ± 6.6
Week 2 (Day 7)	194.6 ± 13.9	193.1 ± 12.4	191.3 ± 11.8	<b>186.9 ± 8.3*</b>
Week 5 (Day 28)	212.6 ± 15.9	214.4 ± 12.3	210.9 ± 16.7	206.1 ± 10.3
Week 11 (Day 70)	234.6 ± 16.8	233.8 ± 13.8	227.4 ± 18.6	<b>214.5 ± 10.8*</b>
<b>F<sub>0</sub> Females, Body weight gain [g]</b>				
Δ week 1 – 11	59.8 ± 14.3	58.3 ± 11.0	53.9 ± 15.5	<b>38.4 ± 10.5</b>
[% of control]	100	98	90	<b>64</b>

Statistics performed for body weight: \* = p&lt;0.05; Dunnett test (one-sided); Δ = body weight gain

Table 6.6-24. Body weight data of F<sub>0</sub> females – producing litters F<sub>1a</sub> and F<sub>1b</sub> (gestation and lactation)

Dose [ppm]		0		200		2000		20000	
		mean	SD	mean	SD	mean	SD	mean	SD
<b>F<sub>0</sub> producing litter F<sub>1a</sub></b>		N = 13		N = 14		N = 13		N = 9	
<b>Gestation phase</b>									
Body weight [g]	GD 0	246.3	21.2	245.0	11.9	236.1	15.3	<b>211.0*</b>	11.6
	GD 7	270.2	19.5	271.5	13.7	261.8	16.0	<b>229.9*</b>	12.5
	GD 14	294.3	20.3	293.5	15.4	284.4	17.6	<b>242.6*</b>	11.6
	GD 20	354.7	41.0	356.4	32.8	349.5	28.7	<b>282.1*</b>	22.8

Dose [ppm]		0		200		2000		20000	
		mean	SD	mean	SD	mean	SD	mean	SD
Δ GD 0 – 20	bw gain [g]	108.4	33.9	111.4	26.3	113.4	27.4	71.1	19.1
	[% of control]	100		103		105		66	
<b>F<sub>0</sub> producing litter F<sub>1a</sub></b> <b>Lactation phase</b>		N = 12		N = 17		N = 13		N = 10	
Body weight [g]	LD 1	287.2	15.2	283.7	19.1	271.5*	19.0	228.8*	15.0
	LD 7	294.3	17.7	293.8	19.1	287.9	16.7	238.1*	10.5
	LD 14	307.7	26.3	303.5	18.5	302.0	18.9	247.1*	7.4
	LD 21	296.6	25.8	293.2	17.8	297.1	18.0	247.5*	21.0
Δ LD 1 – 21	bw gain [g]	17.2	11.8	9.5	16.5	25.6	18.6	18.7	18.7
	[% of control]	100		55		149		109	
<b>F<sub>0</sub> producing litter F<sub>1b</sub></b> <b>Gestation phase</b>		N = 17		N = 19		N = 19		N = 13	
Body weight [g]	GD 0	277.7	20.3	271.5	15.6	262.5*	19.5	232.4*	10.5
	GD 7	302.1	20.5	296.0	17.4	283.3*	18.0	248.7*	11.8
	GD 14	329.2	22.7	321.8	17.9	305.8*	17.6	259.5*	11.7
	GD 20	401.5	28.2	396.1	24.8	369.8*	31.0	301.8*	21.6
Δ GD 0 – 20	bw gain [g]	123.8	18.4	124.5	20.3	107.3	29.6	68.7	17.2
	[% of control]	100		101		87		56	
<b>F<sub>0</sub> producing litter F<sub>1b</sub></b> <b>Lactation phase</b>		N = 18		N = 19		N = 21		N = 18/17	
Body weight [g]	LD 1	312.3	21.3	309.1	20.6	284.8*	27.7	240.2*	12.4
	LD 7	331.6	18.1	328.1	19.3	305.1*	18.7	251.9*	12.5
	LD 14	341.5	22.4	338.8	21.1	215.5*	18.9	261.0*	13.7
	LD 21	333.3	21.8	328.4	25.8	313.7*	17.5	269.8*	16.0
Δ LD 1 – 21	bw gain [g]	21.0	12.3	19.2	14.5	28.9	25.5	29.5	15.1
	[% of control]	100		92		138		141	

Statistics performed for body weight: \* = p<0.05; Dunnett test (one-sided); Δ = body weight gain  
GD = gestation day; LD = lactation day

Table 6.6-25. Body weight - F<sub>1</sub> male and female rats (pre-mating)

Dose [ppm]		0		200		2000		20000	
		mean	SD	mean	SD	mean	SD	Mean	SD
<b>F<sub>1</sub> males, Body weight [g]</b>		N = 20		N = 20		N = 20		N = 20	
Week 1 (Day 0)		155.0	15.0	150.4	25.3	142.4	21.8	69.9*	22.1
Week 5 (Day 28)		313.4	20.2	311.7	28.9	300.6	21.2	179.0*	37.1
Week 11 (Day 70)		415.5	31.1	415.7	33.1	404.3	22.6	259.1*	32.3
Week 14 (Day 91)		438.7	30.4	440.6	36.2	433.2	24.0	277.5*	31.4
Week 21 (Day 140)		480.9	33.5	487.8	42.7	481.6	26.6	318.0*	32.4
Week 25 (Day 168) #		490.5	35.0	496.8	43.9	501.9	27.3	342.1*	33.7
Necropsy (Day 188) #		501.6	7.6 <sup>b</sup>	506.9	10.4 <sup>b</sup>	505.9	6.6 <sup>b</sup>	359.9*	7.7 <sup>b</sup>
<b>F<sub>1</sub> males, Body weight gain [g]</b>									
Δ week 1 – 11 (end 1 <sup>st</sup> premating)		260.5	28.4	265.3	22.7	261.8	21.4	189.2	17.0
[% of control]		100		102		101		73	
Δ week 1 – 21 (end 2 <sup>nd</sup> premating)		325.9	31.0	337.4	32.1	341.2	28.0	248.1	24.7
[% of control]		100		104		105		76	
Δ week 1 – 25		334.6	32.2	346.4	34.1	359.5	28.7	272.2	26.1
[% of control]		100		104		107		81	
Δ week 25 – 28 <sup>#</sup>		12.0		10.1		4.1		17.8	
[% of control]		100		84		33		148	
<b>F<sub>1</sub> females, Body weight [g]</b>		N = 29		N = 30		N = 30		N = 25	
Week 1 (Day 0)		127.3	10.7	125.8	17.8	117.1*	17.5	58.4*	15.2
Week 2 (Day 7)		153.5	11.6	153.6	17.4	144.0*	20.1	81.8*	18.6
Week 3 (Day 14)		169.1	11.3	169.7	18.7	164.6	15.2	102.6*	18.5
Week 5 (Day 28)		201.3	13.5	204.5	22.2	195.3	13.9	139.8*	17.6

Dose [ppm]	0		200		2000		20000	
	mean	SD	mean	SD	mean	SD	Mean	SD
Week 11 (Day 70)	244.8	18.0	250.7	28.8	238.1	13.8	192.6*	15.6
<b>F<sub>1</sub> females, Body weight gain [g]</b>								
Δ week 1 – 11	113.6	27.4	124.9	24.5	121.0	15.5	134.2	15.3
[% of control]	100		110		107		118	

Statistics performed for body weight: \* = p<0.05; Dunnett test (one-sided); Δ = body weight gain

# = from week 24 onwards until the scheduled sacrifice, F<sub>1</sub> males were fed the basal diet.

Table 6.6-26. Body weight data of F<sub>1</sub> females – producing litters F<sub>2a</sub> and F<sub>2b</sub> (gestation and lactation)

Dose [ppm]		0		200		2000		20000	
		mean	SD	mean	SD	mean	SD	mean	SD
<b>F<sub>1</sub> producing litter F<sub>2a</sub></b>		N = 22/20		N = 29		N = 25		N = 6/3	
<b>Gestation phase</b>									
Body weight [g]	GD 0	243.5	17.4	248.1	23.1	235.5	14.1	197.1*	13.3
	GD 7	269.1	16.1	272.4	24.2	258.0	16.4	212.0*	8.2
	GD 14	296.3	16.3	301.8	25.8	282.9	19.0	224.4*	5.7
	GD 20	370.2	26.2	376.2	38.3	356.2	29.1	261.6*	8.8
Δ GD 0 – 20	bw gain [g]	126.7	13.2	128.1	22.9	120.8	18.9	64.6	20.1
	[% of control]	100		101		95		51	
<b>F<sub>1</sub> producing litter F<sub>2a</sub></b>		N = 26/25		N = 29/28		N = 27		N = 8/5	
<b>Lactation phase</b>									
Body weight [g]	LD 1	293.0	17.2	289.2	25.1	273.6*	15.9	207.7*	16.3
	LD 7	304.9	15.8	303.0	24.7	288.0*	16.4	211.7*	14.8
	LD 14	317.8	22.1	315.9	25.2	306.4	24.6	214.1*	11.0
	LD 21	308.2	15.9	305.2	23.8	293.3*	20.1	218.4*	16.1
Δ LD 1 – 21	bw gain [g]	15.1	11.5	15.9	16.8	19.7	16.3	12.3	12.1
	[% of control]	100		105		130		82	
<b>F<sub>1</sub> producing litter F<sub>2b</sub></b>		N = 20		N = 23		N = 23		N = 5	
<b>Gestation phase</b>									
Body weight [g]	GD 0	291.1	18.2	291.1	24.5	265.8*	14.0	221.0*	15.9
	GD 7	309.5	15.5	310.3	26.6	288.3*	16.6	231.0*	15.3
	GD 14	337.1	19.2	339.4	31.0	314.7*	15.4	244.8*	15.2
	GD 20	412.1	34.9	425.2	41.3	389.6*	24.8	287.0*	14.2
Δ GD 0 – 20	bw gain [g]	121.0	23.1	134.1	21.4	123.8	19.2	65.9	14.7
	[% of control]	100		111		102		55	
<b>F<sub>1</sub> producing litter F<sub>2b</sub></b>		N = 25		N = 24		N = 27		N = 8	
<b>Lactation phase</b>									
Body weight [g]	LD 1	326.0	18.4	328.8	30.8	306.0*	22.6	240.8*	10.5
	LD 7	338.6	17.0	342.5	28.2	328.8	22.6	254.1*	11.8
	LD 14	352.3	18.6	352.7	29.9	343.1	19.5	257.5*	14.
	LD 21	333.5	19.8	334.6	27.6	325.3	18.0	260.2*	16.8
Δ LD 1 – 21	bw gain [g]	7.5	7.3	5.7	12.8	19.3	17.1	19.4	11.7
	[% of control]	100		76		256		258	

Statistics performed for body weight: \* = p<0.05; Dunnett test (one-sided); Δ = body weight gain

GD = gestation day; LD = lactation day

Table 6.6-27. Food consumption - F<sub>0</sub> animals

Dose [ppm]	0		200		2000		20000	
	g / rat / wk	[% ctrl]	g / rat / wk	[% ctrl]	g / rat / wk	[% ctrl]	g / rat / wk	[% ctrl]
<b>F<sub>0</sub> males, pre-mating</b>								
Group size	N = 20		N = 20		N = 20/19		N = 20/19	
Overall mean Week 1-10	144.9	[100]	142.9	[99]	147.3	[102]	147.2	[102]
<b>F<sub>0</sub> females, pre-mating</b>								

Dose [ppm]	0	200	2000	20000
Group size	N = 30	N = 30	N = 30	N = 30/29
Overall mean Week 1-10	104.7 [100]	103.6 [99]	101.7 [97]	103.1 [98]
<b>F<sub>0</sub> females, F1a gestation</b>				
Group size	N = 12	N = 14	N = 13	N = 9
GD 0-7	139.75 [100]	142.64 [102]	137.52 [98]	<b>107.77*</b> [77]
GD 7-14	146.58 [100]	146.86 [100]	140.36 [96]	<b>131.78*</b> [90]
GD 14-20	128.75 [100]	129.93 [101]	132.63 [103]	123.76 [96]
<b>F<sub>0</sub> females, F1a lactation</b>				
Group size	N = 12	N = 16	N = 12	N = 7
LD 1-7	239.6 [100]	245.0 [102]	250.5 [105]	194.4 [81]
LD 7-14	374.7 [100]	361.2 [96]	372.9 [98]	<b>252.3*</b> [67]
LD 14-21	476.7 [100]	458.1 [96]	467.4 [98]	<b>275.9*</b> [58]
<b>F<sub>0</sub> females, F1b gestation</b>				
Group size	N = 17	N = 19	N = 19	N = 13
GD 0-7	146.1 [100]	145.1 [99]	<b>133.0*</b> [91]	<b>117.9*</b> [81]
GD 7-14	153.6 [100]	151.9 [99]	140.4 [91]	<b>132.6*</b> [86]
GD 14-20	133.4 [100]	132.8 [100]	126.8 [95]	<b>119.4*</b> [89]
<b>F<sub>0</sub> females, F1b lactation</b>				
Group size	N = 17	N = 19	N = 21	N = 17
LD 1-7	218.9 [100]	234.5 [107]	195.7 [89]	<b>167.9*</b> [77]
LD 7-14	363.1 [100]	353.1 [97]	<b>313.9*</b> [86]	<b>216.3*</b> [60]
LD 14-21	462.5 [100]	466.3 [101]	399.8 [86]	<b>236.2*</b> [51]

Statistics: \* = p<0.05; Dunnett test (one-sided); GD = gestation day; LD = lactation day

Table 6.6-28. Food consumption – F<sub>1</sub> animals

Dose [ppm]	0		200		2000		20000	
	g / rat /wk	[% ctrl]	g / rat /wk	[% ctrl]	g / rat /wk	[% ctrl]	g / rat /wk	[% ctrl]
<b>F<sub>1</sub> males, pre-mating</b>								
Group size	N = 20		N = 20		N = 20/19		N = 20	
Overall mean Week 1-10	172.0 [100]		168.8 [98]		165.3 [96]		<b>124.2</b> [72]	
<b>F<sub>1</sub> females, pre-mating</b>								
Group size	N = 29		N = 30		N = 30		N = 25	
Overall mean Week 1-10	121.0 [100]		121.2 [100]		<b>113.1</b> [93]		<b>102.8</b> [85]	
<b>F<sub>1</sub> females, F1a gestation</b>								
Group size	N = 20		N = 29		N = 25		N = 3	
GD 0-7	137.2 [100]		136.8 [100]		<b>127.9*</b> [93]		<b>116.7*</b> [85]	
GD 7-14	144.9 [100]		147.0 [101]		<b>138.8*</b> [96]		<b>123.6*</b> [85]	
GD 14-20	132.6 [100]		135.6 [102]		126.1 [95]		114.4 [86]	
<b>F<sub>1</sub> females, F1a lactation</b>								
Group size	N = 25		N = 29		N = 26		N = 5 / 4	
LD 1-7	249.9 [100]		231.5 [93]		<b>230.3*</b> [92]		<b>176.0*</b> [70]	
LD 7-14	377.8 [100]		374.6 [99]		<b>348.2*</b> [92]		<b>185.1*</b> [49]	
LD 14-21	510.2 [100]		486.8* [95]		<b>447.1*</b> [88]		<b>288.3*</b> [57]	
<b>F<sub>1</sub> females, F1b gestation</b>								
Group size	N = 20		N = 23		N = 23		N = 5	
GD 0-7	140.0 [100]		138.0 [99]		<b>130.9*</b> [94]		143.5* [102]	
GD 7-14	143.9 [100]		148.8 [103]		138.4 [96]		<b>121.7*</b> [85]	
GD 14-20	133.7 [100]		138.4 [104]		130.0 [97]		<b>116.1*</b> [87]	
<b>F<sub>1</sub> females, F1b lactation</b>								
Group size	N = 25		N = 23		N = 27		N = 8	
LD 1-7	225.8 [100]		241.6 [107]		248.0 [110]		<b>179.2*</b> [79]	
LD 7-14	361.8 [100]		365.7 [101]		362.8 [100]		<b>190.6*</b> [53]	

Dose [ppm]	0	200	2000	20000
LD 14-21	457.1 [100]	487.3 [107]	452.5 [99]	232.0* [51]

Statistics: \* =  $p < 0.05$ ; Dunnett test (one-sided); GD = gestation day; LD = lactation day

Table 6.6-29. Pup body weights

Dose level [ppm]	0	200	2000	20000	0	200	2000	20000
Pup body weight	F <sub>1a</sub> pups				F <sub>2a</sub> pups			
Day 1 [g]	7.51	7.37	7.38	<b>6.38*</b>	7.15	7.31	7.12	<b>6.07*</b>
[Δ% control]	-	-1.9	-1.7	<b>-15.1</b>	-	+2.4	-0.3	<b>-15.0</b>
Day 4 [g]	11.40	11.40	10.98	<b>8.99*</b>	10.49	10.85	10.53	<b>8.45*</b>
Day 7 [g]	17.31	17.74	16.50	<b>11.37*</b>	16.52	16.62	16.06	<b>10.84*</b>
Day 14 [g]	35.34	34.84	32.69	<b>18.37*</b>	34.10	33.62	<b>31.76*</b>	<b>19.09*</b>
Day 21 [g]	58.36	56.39	<b>52.03*</b>	<b>24.05*</b>	56.53	55.69	<b>50.29*</b>	<b>25.06*</b>
[Δ% control]	-	-3.4	<b>-11</b>	<b>-59</b>	-	-1.5	<b>-11.0</b>	<b>-55.7</b>
Pup body weight [g]	F <sub>1b</sub> pups				F <sub>2b</sub> pups			
Day 1 [g]	6.84	7.16	6.91	<b>6.14*</b>	7.25	7.17	7.38	<b>6.55*</b>
[Δ% control]	-	+4.6	+0.9	<b>-10.3</b>	-	-1.2	+1.7	<b>-9.8</b>
Day 4 [g]	10.16	9.83	10.75	<b>8.87*</b>	10.61	10.08	10.71	<b>8.73*</b>
Day 7 [g]	15.55	14.95	16.06	<b>11.27*</b>	16.50	15.59	15.93	<b>11.31*</b>
Day 14 [g]	32.49	31.81	32.14	<b>17.98*</b>	34.64	33.34	<b>32.53*</b>	<b>20.55*</b>
Day 21 [g]	52.79	51.76	50.97	<b>24.81*</b>	56.29	54.66	<b>52.29*</b>	<b>30.06*</b>
[Δ% control]	-	-2.0	-3.4	<b>-53.0</b>	-	-2.9	-7.1	<b>-46.6</b>

Statistical analysis: \*  $p < 0.05$  (Dunnett-test, one-sided)

Δ% control – percent change compared to control

Table 6.6-30. Organ weights – parental males

Generation	Dose [ppm]	F <sub>0</sub> Males (Study Day 169)				F <sub>1</sub> Males (Study Day 188) <sup>a)</sup>			
		Absolute weight	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [mg]	Δ%&	Relative weight [% of bw]	Δ%&
Body weight [g]	0	452.8				501.6			
	200	449.5	-0.7			506.9	+1.1		
	2000	455.4	+0.6			505.9	+0.9		
	20000	<b>383.2*</b>	<b>-16</b>			<b>359.9*</b>	<b>-28</b>		
Liver [g]	0	17.42		3.853		22.57		4.496	
	200	18.35	+5.3	4.080*	+5.9	23.42	+3.8	4.620	+2.8
	2000	<b>20.91*</b>	<b>+20</b>	<b>4.592*</b>	<b>+19</b>	24.07	+6.6	4.746	+5.6
	20000	<b>28.54*</b>	<b>+64</b>	<b>7.465*</b>	<b>+94</b>	<b>17.57*</b>	<b>-22</b>	<b>4.876*</b>	<b>+8.5</b>
Kidneys [g]	0	3.366		0.744		4.029		0.8036	
	200	3.329	-1.1	0.743	-0.2	4.060	+0.8	0.8031	-0.1
	2000	3.587	+6.6	0.790	+6.1	4.124	+2.4	0.8144	+1.3
	20000	3.471	+3.1	<b>0.907*</b>	<b>+22</b>	<b>2.914*</b>	<b>-28</b>	0.8108	+0.9
Testes [g]	0	3.839		0.851		4.026		0.8036	
	200	3.705	-3.5	0.827	-2.8	4.023	-0.1	0.7980	-0.7
	2000	3.832	-0.2	0.844	-0.8	4.058	+0.8	0.8032	-0.1
	20000	3.916	+2.0	<b>1.023*</b>	<b>+20</b>	<b>3.476*</b>	<b>-14</b>	<b>0.9705*</b>	<b>+21</b>

Statistical evaluation: Dunnett's t-test (one-sided); \*  $p \leq 0.05$ ;

& = difference to the control group in percent.

<sup>a)</sup> during the last 4 weeks before necropsy, F<sub>1</sub> males did not receive cinmethylin.

Table 6.6-31. Organ weights – parental females

Generation	Dose [mg/kg]	F <sub>0</sub> Females (Study Day 211)				F <sub>1</sub> Females (Study Day 209)			
		Absolute weight	Δ%&	Relative weight [% of bw]	Δ%&	Absolute weight [mg]	Δ%&	Relative weight [% of bw]	Δ%&
Body weight [g]	0	295.2				307.1			
	200	295.3	0.0			309.9	+0.9		
	2000	<b>275.7*</b>	-6.6			299.2	-2.6		
	20000	<b>258.0*</b>	<b>-12.6</b>			<b>249.7*</b>	<b>-18.7</b>		
Liver [g]	0	12.18		4.119		13.38		4.350	
	200	12.45	+2.2	4.206	+2.1	13.96	+4.3	4.504	+3.5
	2000	12.92	+6.1	<b>4.683*</b>	<b>+13.7</b>	<b>14.93*</b>	<b>+11.6</b>	<b>4.988*</b>	<b>+14.7</b>
	20000	<b>21.86*</b>	<b>+79.5</b>	<b>8.444*</b>	<b>+105.0</b>	<b>22.15*</b>	<b>+65.5</b>	<b>8.851*</b>	<b>+103.5</b>
Kidneys [g]	0	2.217		0.752		2.348		0.766	
	200	2.367	+6.8	0.770	+2.4	2.398	+2.1	0.774	+1.0
	2000	2.239	+1.0	<b>0.813*</b>	<b>+8.1</b>	2.296	-2.2	0.767	+0.2
	20000	<b>2.036*</b>	<b>-8.2</b>	<b>0.789*</b>	<b>+4.9</b>	<b>2.003*</b>	<b>-14.7</b>	0.803	+4.8
Ovaries [g]	0	0.164		0.055		0.180		0.058	
	200	0.177	+7.9	0.060	+9.1	0.174	-3.2	0.056	-3.8
	2000	0.160	-2.4	0.057	+3.8	0.179	-0.8	0.060	-2.2
	20000	0.138	-15.7	0.054	-2.7	<b>0.136*</b>	<b>-24.7</b>	0.054	-6.9

Statistical evaluation: Dunnett's t-test (one-sided); \* p ≤ 0.05; & = difference to the control group in percent

Table 6.6-32. Gross necropsy - parental rats

MALES		F <sub>0</sub> males				F <sub>1</sub> males			
Dose [ppm]		0	200	2000	20000	0	200	2000	20000
Animals examined		20	20	20	20	20	20	20	20
Hydration	- minimal/slight dehydration			1	1				
Hair coat / Skin	- discolouration			1					
Liver	- minimal enlargement				<b>15</b>	1			1
FEMALES		F <sub>0</sub> females				F <sub>1</sub> females			
Dose [ppm]		0	200	2000	20000	0	200	2000	20000
Animals examined		30	30	30	30	29	30	30	25
Hydration	- minimal/slight dehydration				4				
Hair coat / Skin	- discolouration		1		<b>6</b>				<b>5</b>
Liver	- minimal/slight enlargement			<b>5</b>	<b>23</b>			1	<b>20</b>
Abdominal cavity	- acute fibrinous peritonitis		1						
Uterus	- hemorrhagic diathesis				<b>4</b>				<b>1</b>
	- dystocia		1		1				<b>2</b>
	- luminal dilatation	2	6	5	7	1	1	1	

Numbers indicate total number of animals with specified lesion. The absence of a number indicates that the lesion specified was not identified.

Table 6.6-33. Selected histopathology - liver and kidney

F <sub>0</sub> generation	F <sub>0</sub> males				F <sub>0</sub> females			
Dose [ppm]	0	200	2000	20000	0	200	2000	20000
Animals examined	20	20	20	20	30	30	30	29
<b>Liver</b>	<b>20</b>	<b>20</b>	<b>20</b>	<b>19</b>	<b>30</b>	<b>30</b>	<b>30</b>	<b>26</b>
- periportal increased hepatocell. cytoplasmic eosinophilic density			8**	18**	6	14	14	20**

<b>F<sub>0</sub> generation</b>		<b>F<sub>0</sub> males</b>				<b>F<sub>0</sub> females</b>			
<b>Dose [ppm]</b>		<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>	<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>
- periportal hepatocell. cytoplasmic pigment					19**				23**
- hepatocellular cytoplasmic vacuolation		10	12	16	14	2	10*	13**	24**
- parenchymal mononuclear-cell focus		4	15**	13**	13**	16	24	29**	15
- acute periportal necrosis (single cell)									6**
- acute parenchymal necrosis (single cell)					2			1	2
- acute parenchymal necrosis (coagulative)					2				2
<b>Kidney</b>		<b>20</b>	<b>20</b>	<b>20</b>	<b>20</b>	<b>30</b>	<b>30</b>	<b>30</b>	<b>26</b>
- cortical tubular dilatation		20	15*	20	17	17	18	15	18
- cortical tubular protein cast(s)		20	15*	20	15*	16	20	15	18
- corticomedullary tubular protein cast(s)		17	10*	10*	3**	5	5	12	2
- corticomedullary tubular dilatation		18	10*	10*	4*	5	5	12	2
- medullary tubular dilatation			19**	20**	16**	26	21	26	22
- medullary tubular protein cast(s)			19**	20**	16**	26	20	26	22
- renal tubular epithelial pigment deposit(s)					1	3			22*
- lymphocytic interstitial nephritis		5	12	16**	16**	23	11**	12**	17
- renal tubular epithelial degeneration					3				1
- renal tubular epithelial regeneration		13			2**				
- glomerulonephrosis		10			1**				
- renal tubular mineralization		4	1	2	3	6	8	20**	8
<b>F<sub>1</sub> generation</b>		<b>F<sub>1</sub> males</b>				<b>F<sub>1</sub> females</b>			
<b>Dose [ppm]</b>		<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>	<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>
Animals examined		20	20	20	20	29	30	30	25
<b>Liver</b>		<b>20</b>	<b>20</b>	<b>20</b>	<b>20</b>	<b>29</b>	<b>30</b>	<b>28</b>	<b>22</b>
- periportal increased hepatocell. cytoplasmic eosinophilic density		3		1	2	4	1	1	12*
- periportal hepatocell. cytoplasmic pigment					20**				21**
- hepatocellular cytoplasmic vacuolation		5	11	13*	13*	2	6	11*	11*
- parenchymal mononuclear-cell focus		12	18	17	8	25	26	24	15
- acute periportal necrosis (single cell)					4				
- acute parenchymal necrosis (coagulative)			1	2					2
<b>Kidney</b>		<b>20</b>	<b>20</b>	<b>20</b>	<b>20</b>	<b>29</b>	<b>30</b>	<b>29</b>	<b>20</b>
- cortical tubular dilatation		20	20	20	20	28	26	22*	19*
- cortical tubular protein cast(s)		20	20	20	20	28	26	22*	19*
- corticomedullary tubular protein cast(s)		8	18**	20**	3	11	20	14	7
- corticomedullary tubular dilatation		8	18**	20**	3	11	20	14	7
- medullary tubular dilatation		20	20	20	19	29	29	24*	20*
- medullary tubular protein cast(s)		19	20	20	19	29	29	24*	19**
- cortical tubular pigment deposit(s)					8**				17**
- lymphocytic interstitial nephritis		18	18	19	18	18	19	21	15
- renal tubular mineralization		0	2	4	4	10	19	16	7

Statistical evaluation: Fischer's test (pair-wise); \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$

Numbers indicate total number of animals with specified lesion. The absence of a number indicates that the lesion specified was not identified.

Table 6.6-34. Organ weights - selected F<sub>2b</sub> pups

Sex		Males				Females			
Dose [ppm]		Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [mg]	Δ%	Relative weight [% of bw]	Δ%
Carcass	0	145.4				124.6			
	200	162.6	+11.8			132.8	+6.6		
	2000	150.5	+3.5			120.7	-3.1		
	20000	<b>65.5*</b>	<b>-55.0</b>			<b>57.7*</b>	<b>-53.7</b>		
Liver	0	8.26		5.640		7.310		5.842	



Sex		Males				Females			
	Dose [ppm]	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [mg]	Δ%	Relative weight [% of bw]	Δ%
	200	9.90	+19.9	6.067	+7.6	7.714	+5.5	5.823	-0.3
	2000	10.18	+23.2	<b>6.756*</b>	<b>+19.8</b>	7.357	+0.6	6.095	+4.3
	20000	6.30	-23.7	<b>9.031*</b>	<b>+60.1</b>	5.244	-28.26	8.435	+44.4
Kidney	0	1.448		1.015		1.257		1.010	
	200	1.585	+9.5	0.976	-3.8	1.302	+3.6	0.985	-2.4
	2000	1.547	+6.8	1.026	+1.1	1.190	+5.3	0.988	-2.2
	20000	<b>0.658*</b>	<b>-54.6</b>	1.028	+1.3	<b>0.584*</b>	<b>-53.4</b>	1.023	+1.4
Testes	0	1.440		0.947					
	200	1.696	+17.8	1.040	+9.9				
	2000	1.615	+12.2	1.073	+13.3				
	20000	<b>0.581*</b>	<b>-59.7</b>	0.809	-14.6				
Ovary	0					0.064		0.053	
	200					0.068	+6.3	0.054	-1.3
	2000					0.057	-10.9	0.047	-11.5
	20000					<b>0.022*</b>	<b>-65.6</b>	0.040	-24.8

Statistical evaluation: Dunnett's t-test (one-sided); \* p ≤ 0.05

Pup organ weights were examined in 10 male and 10 female F<sub>2b</sub> weanlings per group, randomly selected, and confined to liver, kidney, testes and ovaries. The weight determinations were carried out on "Day 10" (10 days after weaning?).

Table 6.6-35. Histopathology - selected F<sub>2b</sub> pups

Sex	Male pups		Female pups	
Dose [ppm]	0	20000	0	20000
F <sub>2b</sub> pups examined	10	10	10	10
<b>Liver</b>				
- periportal increased hepatocell. cytoplasmic eosinophilic density	0	4	0	<b>6*</b>
- parenchymal mononuclear-cell focus	1	5	3	2
- parenchymal hepatocell. cytoplasmic vacuolation	9	10	5	<b>10*</b>
- focal sinusoidal hematopoiesis	10	<b>5*</b>	9	8
- focal acute subcapsular necrosis (coagulative)	0	1	0	0
- focal pericapsular scarring	0	0	0	1
<b>Epididymides</b>				
- bi-lateral immaturity	10	9		
- focal interstitial perivascular lymphocytic infiltration	1	1		
<b>Prostate</b>				
Tissues not processed for histology	1	2		
- Immaturity	9	8		
<b>Seminal vesicles</b>				
- bi-lateral immaturity	1	<b>9**</b>		
<b>Testes</b>				
- bi-lateral immaturity	10	9		
- interstitial oedema	10	10		

Statistical evaluation: Fischer's test (pair-wise); \* p ≤ 0.05; \*\* p ≤ 0.01

#### *Reproductive Toxicity*

In parental F<sub>1</sub> males, the male fertility index was statistically-significantly reduced at the top dose; this was not seen in F<sub>0</sub> males (Table 6.6-36). In the F<sub>0</sub> generation, the minimum group size of 20 pregnant females (required by the OECD test guidelines) was not reached for any group in the first mating (F<sub>1a</sub>) (Table 6.6-37). In the F<sub>1</sub> generation, the mating index (F<sub>2a</sub> and F<sub>2b</sub>) and fertility index (F<sub>2a</sub> only) were statistically-significantly reduced. The birth litter size was statistically-significantly decreased in both generations and both litters at the top dose

(Table 6.6-37 and 6.6-38). It is not clear if this observation resulted from pre- or post-implantation loss, since these parameters were not investigated.

In F<sub>1</sub> pups, live birth index and pup survival were reduced at the top dose (Table 6.6-39). Pups died mainly between the 1<sup>st</sup> and 2<sup>nd</sup> lactation weeks, resulting in a reduced lactation index at the top dose. Deaths were associated with generalised toxicity of mothers, leading to reduced nursing of their pups (see parental and offspring toxicity section above). In F<sub>2</sub> pups, live birth, viability and lactation (in F<sub>2a</sub> only) indices were reduced at the top dose, although statistical-significance was not reached. Histopathological examinations of pups showed a statistically-significant increase in bi-lateral immaturity of seminal vesicles, however, no treatment-related increases in immaturity of epididymides, prostate or testes were recorded (Table 6.6-35). The eye opening of pups was slightly delayed in the top dose group (Table 6.6-40); this was associated with the lower body weight in this group compared to controls. Sexual maturation (vaginal opening and preputial separation) was not analysed in this study.

Overall, treatment-related and adverse effects on fertility and mating indices, litter size, live birth index, pup survival and lactation index were seen at the top dose.

Table 6.6-36. Reproduction parameters - males

Parental generation	F <sub>0</sub>				F <sub>1</sub>			
Dose [ppm]	0	200	2000	20000	0	200	2000	20000
Males placed with 30 females [n]	20	20	20	20	20	20	20	20
- Male Mating index [%]	<i>Could not be determined</i> <sup>#</sup>				<i>Could not be determined</i> <sup>#</sup>			
- sired at least 1 litter§ [n]	19	20	20	19	20	20	20	13
- Male Fertility index [%]	95.0	100.0	100.0	100.0	100.0	100.0	100.0	<b>65.0*</b>

\* p ≤ 0.05 (one-sided Fisher's Exact Chi-Square test)

# Male mating index: Due to study design (unequal number of males and females, two subsequent matings for each generation to breed two litters), as well as raw data deficiencies, the number of males that mated females (defined by females with implant in utero) was not available. Therefore, the male mating index was could not be calculated.

§ Male fertility index: For mating of the 30 female rats/group, some of the 20 males/group had to be paired with more than one female during the two mating periods, therefore each male had the chance to prove fertility at least twice (during 1<sup>st</sup> and 2<sup>nd</sup> mating) and some even more than twice.

Table 6.6-37. Reproduction and gestational parameters – F<sub>0</sub> females

Parental generation	F <sub>0</sub> → F <sub>1a</sub>				F <sub>0</sub> → F <sub>1b</sub>			
Dose [ppm]	0	200	2000	20000	0	200	2000	20000
<b>Female fertility</b>								
- placed with males	30	30	30	29	30	29	30	27
- mated [n]	28	28	28	26	29	29	30	26
<b>Mating index</b> [%]	93.3	93.3	93.3	89.7	96.7	100	100	96.3
- pregnant [n]	<b>15</b>	<b>17</b>	<b>13</b>	<b>11</b>	<b>18</b>	<b>19</b>	21	21
<b>Fertility index</b> [%]	53.6	60.7	46.4	42.3	62.1	65.5	70.0	80.8
Pre-coital interval [d]	<i>No data</i>				<i>No data</i>			
Duration of gestation [d]	22.8	22.9	22.5	22.4	22.1	22.2	22.1	22.1
Females with live-born [n]	12 <sup>a</sup>	16 <sup>b</sup>	12 <sup>c</sup>	7 <sup>d</sup>	18	19	21	17
<b>Gestation index</b> [%]	80.0 <sup>a</sup>	94.1 <sup>b</sup>	92.3 <sup>c</sup>	63.6 <sup>d</sup>	100	100	100	80.9
- with all stillborn [n]	2	1	1	4	0	0	0	1
Implantation sites [mean n / dam]	<i>No data</i>				<i>No data</i>			
Pups delivered [mean n / dam]	8.57	8.41	9.54	4.73	11.4	11.95	10.10	<b>6.11*</b>
- alive [mean n / dam]	7.64	7.82	8.46	<b>3.27*</b>	9.28	11.32	8.19	<b>4.22*</b>
<b>Post-implantation loss</b> [man %]	<i>No data</i>				<i>No data</i>			

Statistical evaluation, mating, fertility and gestation indices: \* p ≤ 0.05 (one-sided Fisher's Exact Chi-Square test)

Statistical evaluation, pups delivered: \* p ≤ 0.05 (one-sided Dunnett's t-test)

a) report: gestation index: 93.3% (incorrectly considered ... two 2 further dams (#45 & #47) with 0 liveborn pups at birth)

b) report: gestation index: 100% (... 1 further dam (#89) with 0 liveborn pups at birth)

c) report: gestation index: 100% (... 1 further dam (#) with 0 liveborn pups at birth)

Parental generation	F <sub>0</sub> → F <sub>1a</sub>				F <sub>0</sub> → F <sub>1b</sub>			
Dose [ppm]	0	200	2000	20000	0	200	2000	20000

d) report: gestation index: 100% (... 4 further dams (#183, 201, 213, 233) with 0 liveborn pups at birth)

Table 6.6-38. Reproduction and gestational parameters – F<sub>1</sub> females

Parental generation	F <sub>1</sub> → F <sub>2a</sub>				F <sub>1</sub> → F <sub>2b</sub>			
Dose [ppm]	0	200	2000	20000	0	200	2000	20000
<b>Female fertility</b>								
- placed with males	29	30	30	25	29	30	28	23
- mated [n]	28	29	28	12	29	29	28	12
<b>Mating index</b> [%]	96.6	96.7	93.3	<b>48.0*</b>	100	96.7	100	<b>52.2*</b>
- pregnant [n]	26	29	28	8	25	24	27	11
<b>Fertility index</b> [%]	92.9	100	100	<b>66.7<sup>#</sup></b>	86.2	82.8	96.4	91.7
Pre-coital interval [d]	No data				No data			
Duration of gestation [d]	22.1	22.2	22.0	21.5	22.2	22.1	22.1	20.7
Females with live-born [n]	26	29	28	5	25	24	27	9
<b>Gestation index</b> [%]	100	100	100	62.5 <sup>a)</sup>	100	100	100	81.8 <sup>b)</sup>
- with all stillborn [n]	0	0	0	3	0	0	0	0
Implantation sites [mean n / dam]	No data				No data			
Pups delivered [mean n / dam]	11.77	12.41	12.07	<b>5.33*</b>	11.84	13.38	12.19	<b>6.33*</b>
- alive [mean n / dam]	11.15	11.28	10.587	<b>3.00*</b>	11.16	12.38	10.85	<b>4.44*</b>
<b>Post-implantation loss</b> [man %]	No data				No data			

Statistical evaluation, mating, fertility and gestation indices:

\* p ≤ 0.05 (one-sided Fisher's Exact Chi-Square test) ; # p = 0.055

Statistical evaluation, pups delivered: \* p ≤ 0.05 (one-sided Dunnett's t-test)

a) report: gestation index: 100% (incorrectly considered 3 further dams (#433, #449, #457) with 0 liveborn pups at birth)

b) report: gestation index: 90.9% (incorrectly considered 10 instead of 9 dams with liveborn pups)

Table 6.6-39. Survival and sex ratio

F <sub>1</sub> pups	F <sub>0</sub> → F <sub>1a</sub> pups				F <sub>0</sub> → F <sub>1b</sub> pups			
Dose level [ppm]	0	200	2000	20000	0	200	2000	20000
Number of litters delivered	14	17	13	11	18	19	21	18
- with liveborn pups	12	16	12	7	18	19	21	17
- with all stillborn	2	1	1	4	0	0	0	1
- with stillborn pups	7	8	8	8	15	7	14	14
Pups delivered [n]	120	143	124	52	205	227	212	110
- liveborn [n]	107	133	110	36	167	215	172	76
- stillborn [n]	13	10	14	16	38	12	40	34
<b>Live birth index</b> [%]	78.5	88.4	82.7	53.6	79.7	95.4	78.7	64.9
Pups found dead (day 1-4)	6	1	2	2	9	5	8	10
Pups PND 4 (pre-cull)	101	132	108	34	158	210	163	66
<b>Viability index</b> [%]	93.1	99.6	97.6	89.3	90.2	97.1	91.4	80.4
Pups culled day 4	23	29	26	1	37	70	34	0
Pups PND 4 (post-cull)	78	103	82	33	121	140	129	66
Pups found dead (day 5-21)	0	1	0	<b>5</b>	1	8	1	<b>16</b>
Pups PND 21	78	102	82	28	120	132	128	50
Lactation index [%]	100	99.22	100	<b>87.07<sup>#</sup></b>	99.26	94.74	98.75	<b>79.68*</b>
Sex ratio [% live males], PND 0	37.9	50.9	37.8	43.7	46.9	44.0	45.5	46.9
Sex ratio [% live males], PND 21	No data				No data			
<b>F<sub>2</sub> pups</b>	<b>F<sub>1</sub> → F<sub>2a</sub> pups</b>				<b>F<sub>1</sub> → F<sub>2b</sub> pups</b>			

<b>F<sub>1</sub> pups</b>	<b>F<sub>0</sub> → F<sub>1a</sub> pups</b>				<b>F<sub>0</sub> → F<sub>1b</sub> pups</b>			
<b>Dose level [ppm]</b>	<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>	<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>
<b>Dose level [ppm]</b>	<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>	<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>
Number of litters	26	29	28	8	25	24	27	9
- with liveborn pups	26	29	28	5	25	24	27	9
- with all stillborn pups	0	0	0	3	0	0	0	0
- with stillborn pups	9	16	17	6	11	12	16	7
Pups delivered [n]	306	360	338	43	299	321	329	57
- liveborn [n]	290	327	296	24	280	297	293	40
- stillborn [n]	16	33	42	19	19	24	36	17
<b>Live birth index [%]</b>	95.2	90.6	87.6	51.0	93.4	90.7	89.5	74.3
Pups found dead (day 1-4)	4	6	14	7	15	7	7	13
Pups PND 4 (pre-cull)	286	321	282	17	265	290	286	27
<b>Viability index [%]</b>	95.0	97.9	97.7	71.4	94.1	94.2	96.9	67.5
Pups culled day 4	91	100	76	0	92	107	80	1
Pups PND 4 (post-cull)	195	221	206	17	173	183	206	26
Pups found dead (day 5-21)	0	2	0	1	2	3	1	0
Pups PND 21	195	219	206	16	171	180	205	26
<b>Lactation index [%]</b>	100	99.14	100	80.00 <sup>s</sup>	99.00	98.29	99.54	100
Sex ratio [% live males], PND 0	48.4	45.2	55.0	38.0	50.0	45.1	44.6	48.5
Sex ratio [% live males], PND 21	<i>No data</i>				<i>No data</i>			

Statistical analysis: one-tailed Dunnett's t-test, \* p ≤ 0.05

<sup>#</sup> report: mean lactation index: 94.44%, data from #207 (litter size 7 on LD 1 and 7, 3 on LD21 → LI= 42.9%) disregarded

<sup>s</sup> report: mean lactation index: 100%, data from #459 (litter size 1 on LD4, 0 on LD21 → LI 100%) disregarded

Table 6.6-40. Eye opening of pups

<b>Dose level [ppm]</b>	<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>	<b>0</b>	<b>200</b>	<b>2000</b>	<b>20000</b>
	<b>F<sub>1a</sub></b>				<b>F<sub>2a</sub></b>			
Time of eye opening Mean	14.5	15.1	14.6	17.0	15.4	15.2	15.7	15.8
in days SD	0.8	0.9	0.5	1.0	0.6	0.8	0.5	1.0
	<b>F<sub>1b</sub></b>				<b>F<sub>2b</sub></b>			
Time of eye opening Mean	15.1	15.4	15.4	16.9	15.1	15.5	15.4	17.0
in days SD	0.8	0.9	0.8	1.1	0.8	0.7	0.9	0.9

n = total number of pups showing clinical sign;

l = total number of litters affected; L = mean litter incidence of clinical signs in %

### Conclusion

The potential of cinmethylin to adversely affect reproduction has been investigated in a limited two-generation dietary study in Sprague Dawley rats.

There were a number of effects on reproduction parameters (decreases in fertility and mating indices, litter size, live birth index, pup survival and lactation index) at the top dose of 20,000 ppm (1289 mg/kg bw/d). It should be noted however, that these effects on reproduction occurred at a dose causing excessive parental toxicity (mortality, clinical signs of toxicity, reductions in body weights, body weight gains and food consumption, liver histopathology, effects on kidney and testis weight) and are therefore the unspecific secondary consequence of the severe generalised toxicity noted at the top dose of 20,000 ppm.

In relation to general toxicity in parental animals, decreases in body weight (in females) and liver effects (increases in liver weight with concomitant histopathology) were recorded from the mid dose of 2,000 ppm (115 mg/kg bw/d). At the top dose effects on mortality (in females), body weight (in males), body weight gain, clinical signs of toxicity, kidney and testes weights were observed. In relation to general toxicity in pups, decreases in body weights and increases in liver weights (in male pups) were recorded from the mid dose of

2,000 ppm (115 mg/kg bw/d). At the top dose effects on clinical signs of toxicity, liver histopathology, liver weights (in female pups), testes and ovary weights were observed. There were no treatment-related effects in parental animals and pups at the low dose.

However, due to a number of significant limitations (in particular a low female fertility index in controls) and deviations from the OECD test guideline, these conclusions are not robust and hence, no reliable NOAELs have been set from this study.

(██████, 1986)

#### B.6.6.2. Developmental toxicity studies

Four developmental toxicity studies are available, one in rats and three in rabbits. An older study, which was conducted according to GLP but not conducted according to OECD test guidelines, is available for the rat. For the rabbit three studies are available, one new/modern, GLP and OECD test guideline compliant study, and two older limited studies, which were conducted according to GLP but were not conducted according OECD test guidelines.

### *Study in the rat (old study)*

<b>Author(s)</b>	[REDACTED]
<b>Study title</b>	CINCH Herbicide (SD 95481) Teratology Study in Sprague Dawley Rats
<b>Study reference</b>	[REDACTED], 1984 BASF DocID: CI-432-001
<b>Test facility</b>	[REDACTED]
<b>Date</b>	04/10/1983 – 10/11/1983
<b>Test substance</b>	BAS 684 H (Cinmethylin) (CINCH Herbicide SD 95481)
<b>Batch no.</b>	513H
<b>Purity (%)</b>	92.4 (-) / (+) ratio = not specified.
<b>Test animals</b>	Rat Sprague Dawley (CrI:COBS CD (SD) BR) Male and female
<b>Groups</b>	25/pregnant females/dose
<b>Dose/concentrations</b>	0, 30, 300, 1000 and 2000 mg/kg bw/d. Dosage volume: 5.0 mL/kg bw/day.
<b>Route</b>	Administered daily via oral gavage from gestation day (GD) 6 – 15.
<b>Vehicle</b>	Corn oil.
<b>GLP</b>	Compliant.
<b>Guideline</b>	None mentioned in the study report.
<b>Deviation</b>	The following deviations from the current OECD test guideline no. 414 (2018) occurred: <ul style="list-style-type: none"> <li>• Cinmethylin was only administered during the period of organogenesis (for rats: days 6 - 15). This dosing regime was acceptable according to the old OECD test guideline No. 414 (1981).</li> <li>• Food consumption was not recorded.</li> <li>• Gravid uteri including the cervix was not weighed.</li> <li>• The top dose of 2,000 mg/kg bw/d was much higher than the limit dose (of 1,000 mg/kg bw/d) for this test.</li> </ul>
<b>Impact of deviations</b>	The deviations identified were not considered to compromise the validity of the study.
<b>Acceptable</b>	Yes in a WoE approach with the new/modern rat 2-generation study
<b>NOAEL</b>	Maternal toxicity: 30 mg/kg bw/d. Developmental/Offspring toxicity: 300 mg/kg bw/day.
<b>Effects at the LOAEL</b>	Maternal toxicity: Based on clinical observations and decreases in body weight gain from 300 mg/kg bw/d. Developmental toxicity: Based on increases in the incidence of anomalies (predominantly variations) observed from 1000 mg/kg bw/d.

### Methods

In a relatively old, GLP compliant but non-guideline study, cinmethylin was administered to five groups of pregnant female rats (Sprague-Dawley, 25/dose) by oral gavage on days 6 - 15 of gestation at doses of 0, 30, 300, 1000 and 2000 mg/kg bw/d, in corn oil. Cinmethylin was stable in the chosen vehicle. No information was available on the basis for dose selection.

The health of animals and mortality was checked several times each day during administration then once daily until scheduled sacrifice. Body weights were recorded prior to mating, on GD 0, during administration and at study termination. Food consumption was not recorded. On GD 20, all females were sacrificed and assessed by gross pathology (spleen and liver weights were determined). For each dam, corpora lutea were counted and number and distribution of implantation sites, early and late resorptions, and live and dead fetuses were determined. The fetuses were removed, sexed, weighed and further investigated for external findings. Half of the fetuses of each litter was examined for soft tissue findings and the remaining fetuses for skeletal findings.

This study contained limited information on methods of analysis, as the archiving of raw data has expired and no further information is available. A fully validated method of analysis was not available (see Volume 3 CA B5, section B.5.1.2).

### Results

#### Maternal toxicity

At the top dose 2/25 females were found dead on GD 15; both were still pregnant. A range of clinical signs (alopecia, excess salivation, urine-stained abdominal fur, chromorrhinorrhea, vocalisation, hypersensitivity, thin appearance, tip-toe walk) were statistically-significantly increased at the top dose; additional clinical signs (not statistically different from controls) were recorded in this dose group only (Table 6.6-41). At the mid doses (300 and 1000 mg/kg bw/d) certain clinical signs were statistically-significantly increased: excess salivation from 300 mg/kg bw/d (seen in 25/25 at both dose levels) and urine-stained abdominal fur from 1000 mg/kg bw/d. At the low dose there was no treatment-related increase in clinical signs.

At the top dose the body weight was reduced by ~10 % (compared to control) from GD 12, however, statistical-significance was not seen (Table 6.6-42). There were no treatment-related effects on body weight findings at the mid and low doses. At the top dose statistically-significant decreases in body weight gain were seen throughout the study period (Table 6.6-43). Body weight gains were decreased by 58 % (compared to control) during the administration period (GD 6 – 16) and by 26 % over the whole study period. At the mid doses (300 and 1000 mg/kg bw/d) statistically-significant decreases in body weight gain were identified at several time points during the administration period. Body weight gains were reduced by 13 % and 16 % (compared to control, at 300 and 1000 mg/kg bw/d, respectively) over the administration period, although with no statistical-significance. There were no treatment-related effects on body weight gain at the low dose.

Absolute and relative liver weights were statistically-significantly increased at 1000 and 2000 mg/kg bw/d; relative liver weight of these two doses saw increases of > 15 % (change compared to control) (Table 6.6-44). There were no treatment-related effects on liver weight at the lowest two doses (30 and 300 mg/kg bw/d). The two rats from the top dose which died on GD 15 had stomach lesions, enlargement of the liver and decrease in spleen size. At scheduled necropsy, there were no treatment-related gross necropsy findings in pregnant dams.

Overall, maternal toxicity (clinical signs of toxicity and decreases in body weight gain) was seen from 300 mg/kg bw/d, with adverse increases in liver weight occurring from 1000 mg/kg bw/d; in addition two deaths and effects on body weights were noted at the top dose of 2000 mg/kg bw/d.

Table 6.6-41. Clinical signs

Dose [mg/kg bw/d]	0	30	300	1000	2000
Animals examined	25	25	25	25	25
No. animals which died					2
Alopecia	5	8	6	6	16**
Excess salivation		3	25**	25**	25**
Urine-stained abdominal fur			1	16**	24**
Chromorrhinorrhea				3	19**
Vocalisation					9**
Hypersensitivity				2	9**

Dose [mg/kg bw/d]	0	30	300	1000	2000
Animals examined	25	25	25	25	25
Thin appearance					4**
Tip-toe walk					3**
Decreased motor activity					2
Chromodacryorrhea					2
Ptosis					2
Exsudate from vagina					2
Excess lacrimation					1
Rales					1
Red-brown material on abd. fur					1
Soft or liquid faeces					1
Ungroomed coat					1

\*  $p \leq 0.05$ ; \*\*  $p < 0.01$

Table 6.6-42. Body weight

Dose [mg/kg bw/d]		Day of Gestation						
		0	6	7	9	12	16	20
0	Mean [g]	246.1	275.9	278.1	283.2	300.6	327.5	389.2
	SD	13.7	15.6	16.9	17.6	19.0	22.2	28.2
30	Mean [g]	246.0	273.4	274.6	280.3	294.5	322.6	386.2
	SD	13.4	13.8	15.2	14.5	15.8	19.3	22.0
	[% control]	100	99	99	99	98	99	99
300	Mean [g]	245.7	273.8	273.3	279.2	292.4	318.6	379.4
	SD	13.4	15.4	16.1	15.8	16.4	17.6	21.0
	[% control]	100	99	98	99	97	97	98
1000	Mean [g]	245.8	272.3	271.4	275.3	291.1	315.7	378.9
	SD	12.8	16.2	17.0	16.1	19.2	20.3	25.7
	[% control]	100	99	98	97	97	96	97
2000	Mean [g]	246.7	272.3	268.0	263.7	271.8	294.3 <sup>a)</sup>	353.1 <sup>a)</sup>
	SD	17.4	18.3	19.8	20.1	20.8	27.9	38.5
	[% control]	100	99	96	93	90	90	91

\*  $p < 0.05$ , \*\*  $p < 0.01$ ;

a) two females died intercurrently on GD 15, thus the mean is based on data of 23 females

Table 6.6-43. Body weight gain

Dose [mg/kg bw/d]		Day of Gestation						
		0–6	6–7	6–9	6–12	6–16	6–20	0–20
0	Mean [g]	29.8	2.2	7.3	24.7	51.6	113.3	143.1
	<i>SD</i>	8.2	4.4	4.5	6.9	9.5	15.8	19.7
30	Mean [g]	27.4	1.2	6.9	21.1	49.2	112.8	140.2
	<i>SD</i>	6.6	3.4	4.2	5.5	9.2	14.6	18.4
	[% control]	91.9	54.5	94.5	85.4	95.3	99.6	98.0
300	Mean [g]	28.2	<b>-0.5*</b>	5.4	<b>18.6*</b>	44.8	105.6	133.8
	<i>SD</i>	6.8	3.5	5.0	6.6	9.0	12.5	15.3
	[% control]	94.6	-22.7	74.0	75.3	86.8	93.2	93.5
1000	Mean [g]	26.5	<b>-0.9*</b>	<b>3.0*</b>	<b>18.8*</b>	43.4	106.6	133.1
	<i>SD</i>	7.9	4.3	4.5	6.8	8.8	14.4	18.9
	[% control]	88.9	-40.9	41.1	76.1	84.1	94.1	93.0
2000	Mean [g]	25.6	<b>-4.3**</b>	<b>-8.6**</b>	<b>-0.5**</b>	<b>21.5<sup>a</sup> **</b>	<b>80.4<sup>a</sup> **</b>	<b>106.1<sup>a</sup> **</b>
	<i>SD</i>	6.3	5.6	10.5	18.0	33.2	39.2	39.7
	[% control]	85.9	-195.5	-117.8	-2	41.6	71.0	74.1

\*  $p < 0.05$ , \*\*  $p < 0.01$ ;

a) two females died intercurrently on GD 15, thus the mean is based on data of 23 females

Table 6.6-44. Liver weight

Dose [mg/kg bw/d]	Absolute weight [g]	Δ%	Relative weight [% of bw <sup>#</sup> ]	Δ%
0	17.73		4.55	
30	17.61	-0.7	4.56	+0.2
300	17.80	+0.4	4.69	+3.1
1000	<b>19.92**</b>	+12	<b>5.26**</b>	+16
2000	<b>21.90**</b>	+24	<b>6.24**</b>	+37

\* p ≤ 0.05, \*\* p < 0.01

#: since no gravid uterus weight was determined in this study, organ weights were related to terminal body weight.

Δ% - percent change compared to control.

#### *Developmental toxicity*

Statistically-significant decreases in mean foetal weight were observed at the top dose (Table 6.6-45). In addition, at the top dose, two dams suffered total resorptions, consequently post-implantation loss and resorptions were increased and number of live foetuses were decreased compared to controls (with no statistical-significance). The increase in the number of early resorptions at the top dose (51 compared to 17 in the control group) was due to one of the dams which suffered total resorptions with 20 implantation sites. No treatment-related effects on foetal deaths and sex distribution were observed at any dose. There were no treatment-related effects on caesarean section findings at the mid and low doses (30, 300 and 1000 mg/kg bw/d). Overall, there was a decrease in foetal weight and a marginal increase in post-implantation loss due to two whole litter resorptions at the top dose.

One foetus in each of the two highest doses (1000 and 2000 mg/kg bw/d) showed multiple external malformations and variations, however, incidences were isolated and lacked a dose-response. Three foetuses of the top dose showed visceral malformations, compared with one foetus in the 1000 mg/kg bw/d group and two foetuses in the control group (Table 6.6-46). This was not considered treatment-related due to the isolated occurrence of each malformation and lack of dose-response.

Soft tissue variations and malformations were recorded in the top two doses, in four foetuses at 1000 mg/kg bw/d, raising to 18 foetuses at 2000 mg/kg bw/d (Table 6.6-47). This was predominantly caused by a statistically-significant increase in the foetal (11.7 % vs 0 % in controls) and litter incidence (28.6 % vs 0 % in controls) of (slight to moderate) lateral ventricles dilation in brain at the top dose; incidence of this finding was well above the HCD mean. Body weights of dams (on day 16 and 20) which produced foetuses with the finding of slight to moderate lateral ventricle dilation were reduced compared to controls (Table 6.6-49), a clear indication of maternal toxicity. In addition, in litters with the finding of slight to moderate lateral ventricle dilation, mean foetal body weight was also reduced compared to controls (Table 6.6-49). Where the severity of the finding was increased (i.e. moderate) the reduction in body weight of dams and mean foetal body weight (i.e. an indication of maternal toxicity and delayed foetal development) were greater. Overall, slight to moderate dilation of cerebral ventricles appeared to occur in foetuses with retarded development.

The applicant proposed that the effect of slight to moderate lateral ventricle dilation is a consequence of a delay in development. To supported this statement the applicant cited the mild severity, lack of other structural abnormalities and reference to data on humans. The applicant provided the following additional justification:

- The severity of findings seen in this rat developmental study is much lower than that reported by Kalter (1968)<sup>7</sup>. Kalter (1968) identified animals with an hydrocephalus like bolbous frontal region of the cranium, concave profile of the face, domed shape head, flattened cerebral tissues and further affected structures in the brain. Clinical symptoms related to hydrocephalus are described as enlargement of the head during the post-natal phase that progress in combination with uncertain movements and locomotion until death. The applicant argues that similar (severe) hydrocephalus related findings were not seen with cinmethylin in either the rat developmental toxicity study (██████████, 1984) or the old

<sup>7</sup> Kalter, H. (1968) Teratology of the Central Nervous System: Induced and Spontaneous Malformations of Laboratory, Agricultural, and Domestic Mammals, Univ of Chicago; First Edition edition (1968), ISBN 10: 0226422682/ISBN 13: 9780226422688



2-generation study (Lu, 1986). HSE agrees that severe dilation of lateral ventricles was not seen with cinmethylin.

- Solecki *et al.* (2013)<sup>8</sup> note that skeletal and visceral grey zone anomalies can be upgraded (to malformation) or downgraded (to variation) depending on their severities. HSE agrees with this but notes that the study report (and applicant) has not provided '*full descriptions to regulators, including, wherever possible, photographs, severity gradings and any other descriptive information that will help them to understand what has been observed in the laboratory, in particular for grey zone observations*' as recommended by Solecki *et al.* (2013).
- A study by Fox *et al.* (2018)<sup>9</sup> concluded that, in humans, the isolated finding of mild enlargement of the lateral ventricles is found to correlate with a normal postnatal evaluation in > 90 % of cases and moderate enlargement is found to correlate with a normal postnatal evaluation in 75 – 93 %. The authors also concluded that mild ventriculomegaly is likely to represent a normal variant if no other structural abnormalities are noted. In a recent meta-analysis, the rate of neurodevelopmental delay in truly isolated mild ventriculomegaly was 7.9 %, which is similar to the background rate. HSE considers this as the most persuasive element of the applicant's supporting information. The effect of slight to moderate dilation of lateral brain ventricles can be considered a consequence of a delay in development, which is reversible rather than detrimental to the fetuses.

No treatment-related skeletal malformations were observed. Incidences of skeletal variations were increased across all treated groups but in particular in the two highest doses (1000 and 2000 mg/kg bw/d) (Table 6.6-48). No statistical-significance was reached and no clear dose-response was evident, however, consideration of affected fetuses per litter and comparison against HCD revealed subtle differences. At the top dose, incidences of wavy ribs (foetal and litter) exceeded the HCD mean. Affected fetuses per litter was highest in the top dose for both wavy ribs and not/incompletely ossified skeletal structures. This was corroborated by the statistically-significant decrease of fetuses per litter with completely ossified cauda vertebrae or metacarpals at the top dose (Table 6.6-50). Assessment of all foetal observations revealed a statistically-significant increase in total foetal incidence of anomalies (malformations and variations) from 1000 mg/kg bw/d (Table 6.6-51).

Overall, there were treatment-related increases in the incidence of total alterations (predominantly skeletal variations) from 1000 mg/kg bw/d. In addition, increased incidences of wavy ribs, incompletely ossified structures and slight to moderate dilation of lateral ventricles in the brain were noted at the top dose. There was also a statistically-significant decrease in mean foetal weight and a marginal increase in post-implantation loss at the top dose.

Table 6.6-45. Pregnancy status and caesarean section data

Dose [mg/kg bw/d]	0	30	300	1000	2000
<b>Pregnancy status</b>					
Females					
- pregnant [n]	25	25	25	25	25
- died pre-scheduled [n]					2
- pregnant at scheduled kill [n]	25	25	25	25	23
<b>Caesarean section data<sup>a</sup></b>					
Litters examined GD 20	25	25	25	25	23
Corpora lutea [mean]	15.9 ± 2.1	16.8 ± 2.4	16.6 ± 1.9	15.9 ± 2.6	17.1 ± 3.2
Pre-implantation loss [%]	10.3 ± 11.2	10.8 ± 10.0	7.1 ± 6.6	9.0 ± 6.3	12.7 ± 19.3
Implantation sites [mean]	14.2 ± 2.4	15.0 ± 2.4	15.3 ± 1.5	14.4 ± 1.8	15.0 ± 3.6
- Litters with viable fetuses [n]	25	25	25	25	21
- Litters with only resorptions [n]					2
Live litter size [mean]	13.6 ± 2.4	14.1 ± 2.5	13.8 ± 2.5	13.4 ± 1.9	12.8 ± 4.5
- Post-implantation loss [%]	4.7 ± 6.4	5.7 ± 7.5	10.2 ± 13.8	6.9 ± 7.1	17.1 ± 27.5

<sup>8</sup> Solecki *et al.* (2013) Harmonization of description and classification of fetal observations: achievements and problems still unresolved: report of the 7th Workshop on the Terminology in Developmental Toxicology Berlin, 4-6 May 2011, Reprod Toxicol. 2013 Jan; 35:48-55.

<sup>9</sup> Fox, N.S., Monteagudo, A., Kuller, J.A., Craigo, S. and Norton, M.E. (2018) Mild fetal ventriculomegaly: diagnosis, evaluation, and management, Am J Obstet Gynecol. 2018 Jul;219(1):B2-B9.

Dose [mg/kg bw/d]	0	30	300	1000	2000
- Resorptions [mean]	0.7 ± 0.9	0.9 ± 1.2	1.6 ± 2.1	1.0 ± 1.0	2.2 ± 4.1
Total [n]	17	22	38	24	51
Early [n]	17	22	38	24	51
Late [n]			1	1	
- Live foetuses [n]	339	353	344	334	295
- Dead foetuses [n]	0	0	0	0	0
- Total live female foetuses [n]	174	170	170	168	157
- Total live male foetuses [n]	165	183	174	166	138
- Percent live females	48	52	50	49	51
- Percent live males	52	48	50	51	49
Mean foetal weight	3.51	3.46	3.57	3.55	3.20**
- males	3.61	3.55	3.67	3.65	3.26**
- females	3.41	3.37	3.46	3.45	3.12**

a: Means on litter basis;

\* p ≤ 0.05; \*\* p &lt; 0.01 ((Bartlett's test followed by Dunnett's test or Kruskal-Wallis test or Dunn's method for multiple comparison).

Table 6.6-46. Soft tissue (visceral) malformations

Dose levels [mg/kg bw/d]		0	30	300	1000	2000
Litters evaluated		25	25	25	25	21
Foetuses evaluated		164	171	166	161 <sup>a</sup>	145 <sup>a</sup>
Total visceral malformations						
Foetal incidence	# (%)	2 (1.22)			1 (0.62)	3 (2.07)
Litter incidence	# (%)	2 (8.0)			1 (4.0)	3 (14.3)
Affected foetuses / litter	%	1.14 ± 3.96			0.57 ± 2.86	1.96 ± 4.92
Foetus with multiple visceral malformations (and variations)						
Foetal incidence	# (%)				1 (0.62) <sup>§</sup>	1 (0.69) <sup>§</sup>
Litter incidence	# (%)				1 (4.0)	1 (4.76)
Affected foetuses / litter	%				0.57 ± 2.86	0.60 ± 2.73
Hydronephrosis						
Foetal incidence	# (%)	1 (0.61)				
Litter incidence	# (%)	1 (4.0)				
Affected foetuses / litter	%	0.57 ± 2.86				
Microphthalmia (associated with small orbit observed by skeletal examination)						
Foetal incidence	# (%)	1 (0.61)				
Litter incidence	# (%)	1 (4.0)				
Affected foetuses / litter	%	0.57 ± 2.86				
Malpositioned heart						
Foetal incidence	# (%)					1 (0.69)
Litter incidence	# (%)					1 (4.76)
Affected foetuses / litter	%					0.68 ± 3.12
HCD (malformed heart)	# (%)	Foetal incidence: 1 (0.02); Litter incidence: 1 (0.28)				
Situs inversus						
Foetal incidence	# (%)					1 (0.69)
Litter incidence	# (%)					1 (4.76)
Affected foetuses / litter	%					0.68 ± 3.12
HCD	# (%)	Foetal incidence: 1 (0.05); Litter incidence: 1 (0.29)				

\* p ≤ 0.05; \*\* p < 0.01 <sup>a</sup> = one foetus was examined for both, the visceral and skeletal observations<sup>§</sup> = foetus L1 from dam #14979; <sup>§</sup> = foetus L6 from dam #15017

HCD = historical control data (1980 – 1982) based on 343 litters and 1871 foetuses

Table 6.6-47. Soft tissue (visceral) abnormalities

Dose levels [mg/kg bw/d])	0	30	300	1000	2000
Litters evaluated	25	25	25	25	21
Foetuses evaluated	164	171	166	161 <sup>a</sup>	145 <sup>a</sup>
Total visceral variations					
Foetal incidence # (%)				4 (2.48)	18 (12.4)
Litter incidence # (%)				4 (16.0)	7 (33.3)
Affected foetuses / litter %				2.61 ± 6.18	13.66 ± 27.63
Foetus with multiple visceral variations (and malformations)					
Foetal incidence # (%)				1 (0.62) <sup>§</sup>	1 (0.69) <sup>§</sup>
Litter incidence # (%)				1 (4.0)	1 (4.76)
Affected foetuses / litter %				0.57 ± 2.86	0.60 ± 2.73
Dilated renal pelvis					
Foetal incidence # (%)				2 (1.24)	
Litter incidence # (%)				2 (8.0)	
Affected foetuses / litter %				1.24 ± 4.30	
HCD mean # (%)	Foetal incidence: 27 (1.44); Litter incidence: 25 (7.29)				
Lateral ventricles dilation in brain (slight to moderate)					
Foetal incidence # (%)				1 (0.62)	17 (11.7) **
Litter incidence # (%)				1 (4.0)	6 (28.6) **
Affected foetuses / litter %				0.80 ± 4.0	13.07 ± 27.79
HCD mean # (%)	Foetal incidence: 36/1871 (1.92); Litter incidence: 26/343 (7.58)				

\* p ≤ 0.05; \*\* p < 0.01 <sup>a</sup> = one foetus was examined for both, the visceral and skeletal observations

<sup>§</sup> = foetus L1 from dam #14979; <sup>s</sup> = foetus L6 from dam #15017

HCD = historical control data (1980 – 1982) based on 343 litters and 1871 foetuses

Table 6.6-48. Skeletal variations

Dose levels [mg/kg bw/d]		0	30	300	1000	2000
Litters evaluated		25	25	25	25	21
Foetuses evaluated		175	182	178	174 <sup>a</sup>	151 <sup>a</sup>
Total skeletal variations						
Foetal incidence	# (%)	9 (5.14)	11 (6.04)	11 (6.18)	17 (9.77)	13 (8.61)
Litter incidence	# (%)	6 (24.0)	6 (24.0)	8 (32.0)	10 (40.0)	7 (33.3)
Affected foetuses / litter	%	6.2 ± 12.2	5.7 ± 12.2	5.8 ± 9.5	9.4 ± 15.4	9.5 ± 16.7
Foetus with multiple skeletal variations (i.e. also malformations)						
Foetal incidence	# (%)				1 (0.57) <sup>§</sup>	1 (0.66) <sup>§</sup>
Litter incidence	# (%)				1 (4.0)	1 (4.8)
Affected foetuses / litter	%				0.67 ± 3.33	0.68 ± 3.12
Wavy ribs						
Foetal incidence	# (%)	1 (0.57)	4 (2.20)		3 (1.72)	4 (2.65)
Litter incidence	# (%)	1 (4.0)	1 (4.0)		1 (4.0)	3 (14.3)
Affected foetuses / litter	%	0.57 ± 2.86	2.0 ± 10.0		1.71 ± 8.57	2.57 ± 7.09
HCD		Foetal incidence: 2 (0.06); Litter incidence: 2 (0.55)				
Pelvis pubis: not or incompletely ossified						
Foetal incidence	# (%)	1 (0.57)	3 (1.65)	4 (2.25)	3 (1.72)	1 (0.66)
Litter incidence	# (%)	1 (4.0)	2 (8.0)	3 (12.0)	3 (12.0)	1 (4.8)
Affected foetuses / litter	%	0.67 ± 3.33	1.44 ± 5.39	2.03 ± 5.77	1.71 ± 4.74	0.68 ± 3.12
HCD		Foetal incidence: 21 (0.67); Litter incidence: 14 (3.87)				
Total findings: not or incompletely ossified skeletal structures						
Foetal incidence	# (%)	6 (3.43)	9 (4.95)	7 (3.93)	8 (4.60)	8 (5.30)
Litter incidence	# (%)	4 (16.0)	5 (20.0)	5 (20.0)	5 (20.0)	5 (23.8)
Affected foetuses / litter	%	3.64 ± 9.20	4.68 ± 11.5	3.75 ± 8.09	4.36 ± 9.92	6.10 ± 12.1
Total findings: bipartite skeletal structures						
Foetal incidence	# (%)	4 (2.29)	2 (1.10)	4 (2.25)	6 (3.45)	2 (1.32)
Litter incidence	# (%)	4 (16.0)	1 (4.0)	3 (12.0)	4 (16.0)	1 (4.76)
Affected foetuses / litter	%	3.24 ± 8.19	1.0 ± 5.0	2.09 ± 6.41	3.29 ± 9.32)	1.19 ± 5.46

\* p ≤ 0.05; \*\* p < 0.01; <sup>a</sup> = one foetus was examined for both, the visceral and skeletal observations

<sup>§</sup> = foetus L1 from dam #14979; <sup>§</sup> = foetus L6 from dam #15017

HCD = historical control data (1980 – 1982) based on 362 litters and 3150 foetuses

Table 6.6-49. Individual data of litters with lateral ventricles dilation

Dam					Litter / Foetuses			
Animal #	BW on GD 16		BW on GD 20		affected/total # of foetuses	severity of the finding	mean foetal body weight / litter	
	[g]	Δ% of control mean <sup>§</sup>	[g]	Δ% of control mean <sup>§</sup>			mean [g]	Δ% of control mean <sup>&amp;</sup>
14990	295	-9.9	350	-10.1	1/11	slight	3.46	-1.4
15000	270	-17.6	326	-16.2	5/16	slight	2.60	-25.9
15005	317	-3.2	369	-5.2	1/14	slight	3.26	-7.1
15006	300	-8.4	364	-6.5	1/13	slight	3.45	-1.7
15016	276	-15.7	325	-16.5	4/12	moderate	2.59	-26.2
15018	273	-16.6	319	-18.0	5/10	moderate	2.23	-36.5
15021	299	-8.7	359	-7.8	1/15	slight	3.43	-2.3

<sup>§</sup> Body weight of dams in the control group on GD 16 was 327.5 ± 22.2 g

<sup>§</sup> Body weight of dams in the control group on GD 20 was 389.2 ± 28.2 g

<sup>&</sup> Body weight of foetuses in the control group was 3.51 ± 0.20 g

Table 6.6-50. Statistically significant decline of foetal ossification sites

<b>Dose [mg/kg bw/d]</b>	<b>0</b>	<b>30</b>	<b>300</b>	<b>1000</b>	<b>2000</b>
Litters evaluated	25	25	25	25	21
Foetuses evaluated	175	182	178	174	151
<b>Caudal vertebrae</b>					
mean <sup>a</sup>	5.23	4.98	4.96	5.06	<b>4.35**</b>
SD	0.48	0.53	0.76	0.43	0.87
<b>Metacarpals</b>					
mean <sup>a</sup>	3.67	3.54	3.66	3.58	<b>3.34**</b>
SD	0.29	0.32	0.37	0.30	0.33

\* p ≤ 0.05; \*\* p &lt; 0.01;

a = per foetus per litter

Table 6.6-51. Total malformations and variations

<b>Dose levels [mg/kg bw/d]</b>	<b>0</b>	<b>30</b>	<b>300</b>	<b>1000</b>	<b>2000</b>
Litters evaluated	25	25	25	25	21
Foetuses evaluated	339	353	344	334	295
<b>Total alterations (malformations and variations)</b>					
Foetal incidence # (%)	11 (3.24)	12 (3.40)	13 (3.78)	<b>20 (5.99)*</b>	<b>31 (10.51)**</b>
Litter incidence # (%)	8 (32.0)	7 (28.0)	9 (36.0)	13 (52.0)	10 (47.6)
Affected foetuses/litter %	3.83 ± 6.34	3.05 ± 6.07	3.82 ± 5.58	5.84 ± 7.79	11.58 ± 19.62
<b>Total malformations</b>					
Foetal incidence # (%)	3 (0.88)	1 (0.28)	3 (0.87)	1 (0.30)	3 (1.02)
Litter incidence # (%)	3 (12.0)	1 (4.0)	3 (12.0)	1 (4.0)	3 (14.3)
Affected foetuses/litter %	0.87 ± 2.41	0.20 ± 1.0	0.99 ± 2.83	0.33 ± 1.67	1.00 ± 2.51
<b>Total variations</b>					
Foetal incidence # (%)	9 (2.65)	11 (3.12)	11 (3.20)	<b>20 (5.99)</b>	<b>30 (10.17)</b>
Litter incidence # (%)	6 (24.0)	6 (24.0)	8 (32.0)	<b>13 (52.0)</b>	<b>10 (47.6)</b>
Affected foetuses/litter %	3.23 ± 6.32	2.85 ± 6.09	2.99 ± 4.84	<b>5.84 ± 7.79</b>	<b>11.3 ± 19.54</b>

\* p ≤ 0.05; \*\* p &lt; 0.01

Conclusion

In conclusion, under the conditions of this developmental toxicity study in rats, no treatment-related external, visceral or skeletal malformations were observed at any dose. From 1000 mg/kg bw/d an increase in the incidence of total alterations (mainly skeletal), indicative of delayed development, was recorded. At the top dose of 2000 mg/kg bw/d, the incidences of (slight to moderate) dilated ventricles in the brain and skeletal variations such as wavy ribs and incompletely ossified structures were also significantly increased. In addition, at this dose, there was a statistically-significant decrease in mean foetal weight and a marginal increase in post-implantation loss (due to two whole resorptions). With the exception of dilated ventricles in the brain, the other developmental effects observed (skeletal variations, incomplete ossification, decreased foetal weight and post-implantation loss) were considered the unspecific, secondary consequence of the maternal toxicity recorded from 300 mg/kg bw/d (limited number of clinical signs of toxicity and decreases in body weight gain). It is noted that at the top dose of 2000 mg/kg bw/d maternal toxicity was particularly severe, with deaths, significant reductions in body weights, numerous clinical signs of toxicity and liver effects. No maternal toxicity was observed at the low dose (30 mg/kg bw/d). The increased incidence of (slight to moderate) dilated ventricles in the brain at 2000 mg/kg bw/d (a dose much higher than the limit dose) was associated with severe maternal toxicity. Slight to moderate dilation of brain ventricles is considered to be a variation and to represent a developmental delay with no detrimental or irreversible consequences for the foetus. Therefore, it is most likely that this abnormality was the secondary consequence of the excessive maternal toxicity occurring at the high dose of 2000 mg/kg bw/d. Overall, there was no evidence of specific developmental toxicity in the rat.

Based on these findings, the NOAELs proposed by HSE for developmental toxicity in the rat is 300 mg/kg bw/d and for maternal toxicity is 30 mg/kg bw/d, based on the lack of relevant effects at these dose levels.

(██████████, 1984)

*Studies in the rabbit*

Three studies are available, one new/modern, GLP and OECD test guideline compliant study, and two older studies, which were conducted according to GLP but were not conducted according to OECD test guidelines.

*New/modern study*

<b>Author(s)</b>	
<b>Study title</b>	BAS 684 H - Prenatal developmental toxicity study in New Zealand White rabbits oral administration (Gavage)
<b>Study reference</b>	██████████, 2018b BASF DocID: 2015/1158053
<b>Test facility</b>	██
<b>Date</b>	01/09/2014 – 07/12/2017
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Batch no.</b> <b>Purity (%)</b>	COD-001950 96.3 (-) / (+) ratio = not specified.
<b>Test animals</b>	Rabbit New Zealand White / CrI:KBL(NZW) Female – pregnant (inseminated)
<b>Groups</b>	25 pregnant females/dose
<b>Dose/concentrations</b>	0, 25, 80, 250 and extended to 320 mg/kg bw/d. Standard dose volume of 10 ml/kg bw.
<b>Route</b>	Administered daily via by gavage during gestation days 6 - 28 post insemination (p.i.)
<b>Vehicle</b>	0.5 - 1 % Sodium carboxymethyl cellulose suspension in drinking water (0.5 - 1% CMC) with 3 drops Tween 80/1000 mL.
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD test guideline no. 414 (Jan 2001 – update proposal) (the current test guideline was adopted in 2018) EEC 1907/2006, (EC) No 440/2008 of 30 May 2008 - Part B No. L 142, EPA 870.3700, JMAFF No 12 Nosan No 8147 (2-1-18 2000)
<b>Deviation</b>	None.
<b>Impact of deviations</b>	N/A.
<b>Acceptable</b>	Yes.
<b>NOAEL</b>	Maternal toxicity: 80 mg/kg bw/d. Developmental toxicity: 80 mg/kg bw/d.
<b>Effects at the LOAEL</b>	Maternal toxicity: Reduced body weight gain, increased liver weight and clinical chemistry (increased $\gamma$ -glutamyltransferase (GGT) activity) was observed from 250 mg/kg bw/d. Developmental toxicity: Foetal weight was reduced from 250 mg/kg bw/d.

Methods

In a GLP and OECD test guideline compliant study, cinmethylin was administered to groups of female rabbits (New Zealand White, 25/dose) by oral gavage on days 6 - 28 of gestation, initially at doses of 0, 25, 80 and 250 mg/kg bw/d, in 0.5% / 1% CMC. At the initial top dose of 250 mg/kg bw/d, slight maternal toxicity was observed and marginally increased incidences of skeletal findings (misshapen thoracic vertebrae) were noted. This finding was not observed in any previous rabbit prenatal developmental toxicity studies (██████████ 1985; ██████████, 1987), tested at higher doses (up to 750 mg/kg bw/d). Therefore, an additional higher dose of 320 mg/kg bw/d was tested and compared to an additional control group, with the aim of clarifying the relevance of the observed skeletal findings at 250 mg/kg bw/d. The test substance was stable in the chosen vehicle. A method for the analysis of cinmethylin in the gavage vehicle (██████████ 2017a; 2017/1166508) was evaluated and was considered validated (see Volume 3 CA B5, section B.5.1.2).

ResultsMaternal toxicity

There were no treatment-related deaths or clinical signs of toxicity. Food consumption was statistically-significantly decreased at 250 mg/kg bw/d, during GD 19 - 20, with a -30 % change compared to control (Table 6.6-52). However, no other statistically-significant changes were observed in the study, in particular during administration (GD 6 – 28) or at 320 mg/kg bw/d; no time- or dose-relationship was evident. Therefore the effects on food consumption at 250 mg/kg bw/d are considered to be unrelated to treatment. Mean body weights of pregnant animals was not affected by treatment (Table 6.6-53). Body weight gain was consistently decreased at 250 mg/kg bw/d, with an overall body weight gain decrease of 22 % compared to controls; a statistically-significant decrease was recorded during GD 6 – 9 and 19 - 20, with a -69 and -200 % change compared to control respectively (Table 6.6-54). A similar decrease in body weight gain was seen at 320 mg/kg bw/d (-22 % overall), however, the change compared to control was not as marked during GD 6 – 9 and 16 – 19 (did not reach statistical-significance). Body weight gain was also consistently decreased in the 25 mg/kg bw/d group (11 %) but not in the 80 mg/kg bw/d group (8 % increase). Overall, there was a treatment-related and adverse effect on body weight gain from 250 mg/kg bw/d. This finding, indicative of maternal toxicity, demonstrates that the top dose of 320 mg/kg bw/d was appropriate.

Table 6.6-52. Food consumption

Dose level [mg/kg bw/d]	Day of Gestation			
	0 - 6	6 - 28	0 - 29	19 - 20
<b>0</b>				
Mean fc [g]	168.6	133.3	139.9	134.7
SD	13.12	25.11	27.20	26.28
<b>25</b>				
Mean fc [g]	166.2	130.2	136.7	131.3
SD	9.57	30.08	30.88	46.7
[Δ% control]	-1.4	-2.3	-2.3	-2.5
<b>80</b>				
Mean fc [g]	169.7	139.3	144.9	130.4
SD	10.52	26.07	26.61	33.53
[Δ% control]	+0.7	+4.5	+3.6	-3.2
<b>250</b>				
Mean fc [g]	174.4	120.6	130.9	<b>95.0*</b>
SD	10.70	28.63	34.13	37.68
[Δ% control]	+3.4	-9.5	-6.4	<b>-29.5</b>
<b>0</b>				
Mean fc [g]	176.2	131.9	140.0	130.3
SD	4.42	27.78	31.20	45.47
<b>320</b>				
Mean fc [g]	178.2	121.3	132.3	119.6
SD	4.14	30.89	36.12	34.66
[Δ% control]	+1.1	-8.1	-5.5	-8.2

\* p &lt; 0.05, \*\* p &lt; 0.01 (Dunnett-test, two-sided)

fc – food consumption

Table 6.6-53. Mean body weight

Dose level [mg/kg bw/d]	Day of Gestation			
	0	6	28	29
<b>0</b>				
Mean bw [g]	3704	3868	4120	4144
SD	189.8	183.5	181.7	199.3
<b>25</b>				
Mean bw [g]	3697	3841	4064	4089
SD	158.8	193.5	231.6	227.8
[Δ% control]	-0.2	-0.7	-1.4	-1.3
<b>80</b>				
Mean bw [g]	3724	3877	4166	4200

Dose level [mg/kg bw/d]	Day of Gestation			
	0	6	28	29
<i>SD</i> [Δ% control]	156.1 +0.5	183.3 +0.2	183.4 +1.1	173.0 +1.4
<b>250</b> Mean bw [g] <i>SD</i> [Δ% control]	3711 176.1 +0.2	3875 208.2 +0.2	4021 233.3 -2.4	4049 246.7 -2.3
<b>0</b> Mean bw [g] <i>SD</i>	3675 163.6	3835 154.6	4104 235.3	4137 209.1
<b>320</b> Mean bw [g] <i>SD</i> [Δ% control]	3666 151.0 -0.2	3815 142.2 -0.5	4000 166.3 -2.5	4026 171.3 -2.7

\* p < 0.05, \*\* p < 0.01 (Dunnett-test, two-sided)

Table 6.6-54. Body weight gain

Dose level [mg/kg bw/d]	Day of Gestation				
	0 - 6	6 - 28	0 - 29	6 - 9	16 - 19
<b>0</b> Mean bwg [g] <i>SD</i>	164.0 49.18	252.1 125.44	440.6 130.63	56.0 45.80	-22.9 62.80
<b>25</b> Mean bwg [g] <i>SD</i> [Δ% control]	143.3 66.42 -12.6	223.4 159.78 -11.4	391.7 194.87 -11.1	63.6 44.45 +13.6	-9.4 46.58 +59.0
<b>80</b> Mean bwg [g] <i>SD</i> [Δ% control]	153.3 68.44 -6.5	288.3 126.63 +14.4	476.3 121.93 +8.1	55.1 28.76 +1.6	-17.8 64.44 +22.3
<b>250</b> Mean bwg [g] <i>SD</i> [Δ% control]	164.0 84.95 ±0.0	148.9 195.02 -40.9	342.3 203.53 -22.3	<b>17.5*</b> 68.73 -68.8	<b>-68.8*</b> 58.10 -200
<b>0</b> Mean bwg [g] <i>SD</i>	160.3 57.24	268.6 189.04	463.8 188.89	85.7 38.23	-25.6 55.46
<b>320</b> Mean bwg [g] <i>SD</i> [Δ% control]	149.2 62.04 -6.9	184.8 148.88 -31.2	360.0 176.94 -22.4	67.3 55.38 -21.5	-34.3 71.89 -34

\* p < 0.05, \*\* p < 0.01 (Dunnett-test, two-sided)

A statistically-significant reduction in reticulocytes was recorded at 320 mg/kg bw/d (Table 6.6-55). However, the values recorded were within the HCD (range and 25 – 75 percentile). A statistically-significant reduction in neutrophil counts was recorded at 250 mg/kg bw/d, with a 23 % change compared to controls. This value was within the HCD range but outside the 25 – 75 percentile range. A dose-response was not evident once the study was extended to 320 mg/kg bw/d. Other statistically-significant values (haemoglobin and haematocrit) were not considered treatment-related and/or adverse as percent change compared to controls was small. Overall, there were no treatment-related effects on haemology parameters.

Statistically-significant changes in various clinical chemistry parameters were recorded, mainly at the top two doses (250 and 320 mg/kg bw/d) (Table 6.6-56). Serum-γ-glutamyltransferase (GGT) was significantly increased, both statistically and biologically, at 250 and 320 mg/kg bw/d; values were outside the HCD range. This finding is indicative of liver toxicity. Dose-dependency was not evident for other clinical chemistry parameters and whilst values were within the HCD range they were not within the 25 – 75 percentile range.



Nevertheless, due to the lack of dose-response and consistency these parameters were not considered treatment-related. Overall, treatment-related and adverse increases in GGT were observed from 250 mg/kg bw/d.

Table 6.6-55. Haematology findings (day 29) - red and white blood cells

Dose level [mg/kg bw/day]	0	25	80	250	0	320
Animal number	21	25	24	19	23	25
HGB [mmol/L]						
mean ± SD	7.8 ± 0.5	7.8 ± 0.5	7.8 ± 0.6	7.4 ± 0.4	7.8 ± 0.5	7.5* ± 0.5
Δ%	-	0	0	-5.1	-	-3.8
Historical control	Mean: 7.8, range: 7.3 - 8.3, 25 & 75 percentile : 7.6 – 8.0.					
HCT [L/L]						
mean ± SD	0.38 ± 0.025	0.38 ± 0.029	0.38 ± 0.03	0.36 ± 0.023	0.38 ± 0.026	0.36* ± 0.024
Δ%	-	0	0	-5.3	-	-5.3
Historical control	Mean: 0.375, range: 0.351 - 0.393, 25 & 75 percentile : 0.368 – 0.385.					
RET [%]						
mean ± SD	1.0 ± 0.6	0.9 ± 0.5	0.9 ± 0.8	0.8 ± 0.7	1.2 ± 0.6	0.9* ± 0.4
Δ%	-	-10	-10	-20	-	-25
Historical control	Mean: 1.1, range: 0.6 - 1.9, 25 & 75 percentile : 0.9 – 1.3.					
NEUTA [Giga/L]						
mean ± SD	1.24 ± 0.56	1.30 ± 0.52	1.05 ± 0.32	0.96* ± 0.36	1.19 ± 0.59	1.01 ± 0.26
Δ%	-	4.8	-15.3	-22.6	-	-15.1
Historical control	Mean: 1.27, range: 0.89 - 1.84, 25 & 75 percentile : 1.06 – 1.43.					

\* p < 0.05, \*\* p < 0.01 (Kruskal-Wallis + Wilcoxon-test, two-sided).

Δ% - percent change compared to control.

HGB – haemoglobin

HCT – haematocrit

RET – reticulocytes

NEUTA – polymorphonuclear neutrophils (absolute)

HCD: Studies conducted in the same laboratory, species and stain. Studies dated March 2010 to July 2017 (concurrent). 36 studies.

Table 6.6-56. Clinical chemistry findings (day 29)

Dose level [mg/kg bw/day]	0	25	80	250	0	320
Animal number	21	25	24	19	23	25
GGT [nkat/L]						
mean ± SD	75 ± 48	72 ± 49	69 ± 30	113* ± 54	52 ± 21	97** ± 43
Δ%		-4.0	-8.0	+50.7		+86.5
Historical control	Mean: 57, range: 32 – 96, 25 & 75 percentile: 41 – 69.					
ALT [μkat/L]						
mean ± SD	0.49 ± 0.28	0.46 ± 0.27	0.39 ± 0.10	0.29** ± 0.008	0.37 ± 0.12	0.28** ± 0.08
Δ%		-6.1	-20.4	-40.8		-24.3
Historical control	Mean: 0.50, range: 0.28 - 0.72, 25 & 75 percentile: 0.40 – 0.61.					
AST [μkat/L]						
mean ± SD	0.48 ± 0.27	0.60 ± 0.95	0.33** ± 0.10	0.33* ± 0.13	0.33 ± 0.11	0.3 ± 0.1
Δ%		+25.0	-31.3	-31.3		-10
Historical control	Mean: 0.44, range: 0.22 - 0.74, 25 & 75 percentile: 0.36 – 0.51.					
CREA [μmol/L]						
mean ± SD	89.1 ± 12.5	88.1 ± 10.6	80.6* ± 9.6	85.3 ± 7.4	90 ± 11.9	79.7** ± 11.6
Δ%		-1.1	-9.5	-4.3		-11.4
Historical control	Mean: 97.2, range: 74.3 – 113.0, 25 & 75 percentile: 99.1 – 104.2.					
TRIG [mmol/L]						
mean ± SD	0.45 ± 0.14	0.51 ± 0.24	0.44 ± 0.11	0.60* ± 0.18	0.51 ± 0.16	0.68 ± 0.32
Δ%		+13.3	-2.2	+33.3		+33.3

Dose level [mg/kg bw/day]	0	25	80	250	0	320
Animal number	21	25	24	19	23	25
<i>Historical control</i>	<i>Mean: 0.45, range: 0.27 - 0.72, 25 &amp; 75 percentile: 0.37 – 0.52.</i>					

\* p < 0.05, \*\* p < 0.01 (Kruskal-Wallis + Wilcoxon-test, two-sided)

Δ% - percent change compared to control.

GGT – serum-γ-glutamyltransferase.

ALT – alanine aminotransferase.

AST – aspartate aminotransferase.

CREA – creatinine.

TRIG – triglycerides.

HCD: Studies conducted in the same laboratory, species and stain. Studies dated March 2010 to July 2017 (concurrent). 36 studies.

Liver weights (both absolute and relative) were statistically-significantly increased from 80 mg/kg bw/d (Table 6.6-57). Changes compared to control were >15 % from 250 mg/kg bw/d. Values recorded at 250 and 320 mg/kg bw/d were outside of the HCD range. Weights of all other organs (adrenal glands, kidneys and spleen) did not show significant differences and were therefore not considered to be effected by treatment. Overall, treatment-related and adverse increases in liver weight were seen from 250 mg/kg bw/d.

Table 6.6-57. Organ weights

Organ weight	Dose [mg/kg bw/day]	Absolute weight mean ± SD	Δ%	Relative weight [% of bw ± SD]	Δ% #
Terminal weight [g]	0	3667.8 ± 203.7			
	25	3640.4 ± 249.4	(-1)		
	80	3731.8 ± 167.6	(+2)		
	250	3599.2 ± 282.1	(-2)		
	0	3663.3 ± 169.5			
	320	3558.2 ± 147.8			
Liver [g]	0	79.25 ± 11.69		2.155 ± 0.252	
	25	83.36 ± 12.47	(+5)	2.283 ± 0.249	(+6)
	80	<b>89.58 ± 9.43**</b>	<b>(+13)</b>	<b>2.405 ± 0.279**</b>	<b>(+12)</b>
	250	<b>93.88 ± 10.34**</b>	<b>(+18)</b>	<b>2.609 ± 0.215**</b>	<b>(+21)</b>
	0	78.63 ± 8.86		2.144 ± 0.199	
	320	<b>96.66 ± 11.27**</b>	<b>(+23)</b>	<b>2.711 ± 0.242**</b>	<b>(+26)</b>
	<i>Historical control</i>	<i>Mean: 78.28</i> <i>Min: 73.12</i> <i>Max: 90.68</i>		<i>Mean: 2.13</i> <i>Min: 1.89</i> <i>Max: 2.36</i>	

\*: p ≤ 0.05, \*\*: p ≤ 0.01; Kruskal-Wallis H and Wilcoxon test, two-sided

HCD: Studies conducted in the same laboratory, species and stain. Studies dated June 2015 to July 2017 (concurrent). 13 studies. 89 animals.

#### Developmental toxicity

There were no treatment-related effects on the mean gravid uteri weights (Table 6.6-58). Mean carcass weights and the corrected body weight gain were statistically-significantly reduced (-3 % and -50 %, respectively) at 320 mg/kg bw/d.

Table 6.6-58. Uterus weight, carcass weight and corrected (net) body weight gain

Weight [g]	Dose [mg/kg bw/day]					
	0 (Group 0)	25 (Group 1)	80 (Group 2)	250 (Group 3)	0 (Group 4)	320 (Group 5)
<b>Gravid uterus</b>						
Mean [g]	476.5	448.8	468.3	450.1	473.2	467.6
SD	111.0	146.0	98.4	138.0	131.93	67.69
[Δ% control]	-	-5.8	-1.7	-5.5	-	-1.2

Carcass						
Mean [g]	3667.8	3640.4	3731.8	3599.2	3663.3	<b>3558.2*</b>
SD	203.7	249.4	167.7	282.1	169.46	147.8
[Δ% control]	-	-0.7	+1.7	-1.9	-	-2.9
Net weight change from GD 6						
Mean [g]	-199.9	-200.3	-145.4	-273.1	-171.3	<b>-256.8*</b>
SD	155.2	212.7	132.2	224.0	116.84	137.58
[Δ% control]	-	-0.2	+27.3	-36.6	-	-49.9

\* p < 0.05, \*\* p < 0.01 (Dunnett test, two-sided);

Carcass weight = terminal body weight minus uterine weight (values are rounded)

Net weight change from GD 6 = carcass weight minus GD 6 body weight

Δ% control – percent change compared to control.

A sufficient number (at least 20 per dose) of pregnant females was available in the study. At caesarean section there were no treatment-related and/or toxicologically-significant effects on resorptions, subsequent post-implantation losses, and the number of viable foetuses.

There were no treatment-related effects on sex distribution of foetuses, placental weights and/or mean litter weight at any dose (Table 6.6-59). The statistically-significant decrease in live females foetuses at 80 mg/kg bw/d and increase at 320 mg/kg bw/d were not considered treatment-related due to a lack of dose response. Statistically-significant decreases in mean foetal weight were seen at 250 and 320 mg/kg bw/d (-14 % and -11 %, respectively). Overall, treatment-related and adverse reductions in foetal weight were seen from 250 mg/kg bw/d.

Table 6.6-59. Sex distribution, weight of placentae, foetuses and litters

Dose level [mg/kg bw/d]	0	25	80	250	0	320
Litter size [mean ± SD]	8.6 ± 2.2	8.6 ± 2.6	8.0 ± 1.9	9.6 ± 1.7	8.9 ± 2.9	9.4 ± 1.8
Female foetuses [mean ± SD]	5.0 ± 2.0	4.9 ± 2.1	<b>3.5 ± 1.7*</b>	5.1 ± 1.8	3.9 ± 1.8	<b>5.2 ± 1.5**</b>
- total number [n]	104	117	85	97	90	131
- mean [%]	55.8	51.4	42.4	50.9	42.9	51.6
Male foetuses [mean ± SD]	3.7 ± 1.7	3.7 ± 1.7	4.5 ± 2.3	4.5 ± 1.7	5.0 ± 2.4	4.2 ± 1.6
- total number [n]	77	89	108	85	114	104
- mean [%]	41.1	40.1	49.8	44.7	50.1	40.5
- Percent live females	57.5	56.8	44.0	53.3	44.1	55.7
- Percent live males	42.5	43.2	56.0	46.7	55.9	44.3
Placental weights [g]	5.4 ± 1.0	5.4 ± 1.2	5.2 ± 0.7	4.9 ± 0.8	5.1 ± 0.8	5.0 ± 0.8
- male foetuses [g]	5.5 ± 1.1	5.5 ± 1.2	5.4 ± 0.7	4.9 ± 1.0	5.1 ± 0.8	5.1 ± 0.7
- female foetuses [g]	5.4 ± 1.0	5.2 ± 1.0	5.1 ± 0.8	4.8 ± 0.8	5.0 ± 0.9	5.0 ± 0.8
Mean litter weight [g]	334 ± 77.8	321 ± 80.4	316 ± 62.0	322 ± 70.4	329 ± 89.8	314 ± 43.7
[Δ% control]				-3		-4
Mean foetal weight [g]	39.5 ± 5.2	39.0 ± 6.9	39.8 ± 3.6	<b>33.8 ± 5.0**</b>	38.2 ± 5.1	<b>33.9 ± 4.3**</b>
[Δ% control]		-0.5	0.3	<b>-14.4</b>		<b>-11.2</b>
- males [g]	39.8 ± 5.9	40.1 ± 7.2	40.3 ± 4.5	<b>34.1 ± 5.4**</b>	37.7 ± 5.2	<b>34.4 ± 4.5*</b>
[Δ% control]		0.8	1.3	<b>-14.3</b>		<b>-8.8</b>
- females [g]	39.7 ± 5.0	37.3 ± 4.9	39.0 ± 4.4	<b>33.6 ± 5.2**</b>	38.2 ± 5.3	<b>33.7 ± 4.7**</b>
[Δ% control]		-6.0	-1.8	<b>-15.4</b>		<b>-11.8</b>

Δ% control – percent change compared to control.

Table 6.6-60. Historical control data – foetal data

Historical control data	Mean	±	SD	Range (per study)	
				Minimum	Maximum
- Resorptions [% ± SD]	7.1	±	14.42	2.4	12.6
- number [mean ± SD]	0.6	±	1.0	0.3	1.1
- Live foetuses [mean ± SD]	8.0	±	2.9	6.6	9.7
Mean foetal weight [g]	39.6			18.5	58.8
- males [g]	39.7			17.9	58.8

- females	[g]	38.9	19.1	56.3
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12 studies performed at (Jan 2010– Apr 2015) with New Zealand White rabbits ( ) (274 pregnant dams; 264 litters with 2140 viable foetuses)

There were no effects of treatment on external or visceral malformations and variations. Skeletal malformations were noted in foetuses of all groups including controls (Table 6.6-61). There were 3 foetuses with misshapen thoracic vertebra in 3 litters at 250 mg/kg bw/d (none in concurrent control) which caused a statistically-significant increase in the percent of affected foetuses per litter; values were also above the HCD range (Table 6.6-62). However, at the top dose no malformations in thoracic (or other) vertebrae were seen, therefore, this finding was not considered treatment-related. In addition, the total incidence of skeletal malformations were not effected by treatment with cinmethylin (no statistical-significance or dose-response seen). The incidences of all other skeletal malformations were not statistically-significant and/or no dose-response was evident, therefore, not considered treatment-related. Statistically-significant increases in certain skeletal variations were noted (Table 6.6-63). However, a clear and consistent dose-response was not evident and/or values were within the HCD range (Table 6.6-64). Overall, incidences of unclassified cartilage observations did not show statistical-significance and/or no dose-response was evident (Table 6.6-65). The statistically-significant increase in bipartite processus xiphoideus seen at 320 mg/kg bw/d was not considered treatment-related due to the lack of dose-response in this observation. There were no treatment-related effects on total foetal malformations and variations (Table 6.6-66 and 6.6-67). Overall, there were no treatment-related effects on external, skeletal and soft tissue alterations (malformations and variations) up to the top dose.

Table 6.6-61. Skeletal malformations

Dose [mg/kg bw/d]		0	25	80	250	0	320
Litters evaluated		21	24	24	19	23	25
Foetuses evaluated		181	206	194	182	205	235
Live		181	206	193	182	204	235
Dead		0	0	1	0	1	0
<b>Total skeletal malformations</b>							
Foetal incidence	# (%)	4 (2.2)	2 (1.0)	1 (0.5)	5 (2.7)	1 (0.5)	1 (0.4)
Litter incidence	# (%)	3 (14)	2 (8.3)	1 (4.2)	3 (16)	1 (4.3)	1 (4.0)
Affected foetuses / litter	%	1.8	0.7	0.5	2.9	0.4	0.4
<b>Individual skeletal malformations</b>							
<b>Misshapen thoracic vertebra</b>							
Foetal incidence	# (%)				3 (1.6)		
Litter incidence	# (%)				3 (16)		
Affected foetuses / litter	%				1.7*		
<b>Fused skull bone</b>							
Foetal incidence	# (%)	1 (0.6)					
Litter incidence	# (%)	1 (4.8)					
Affected foetuses / litter	%	0.5					
<b>Sutures fused</b>							
Foetal incidence	# (%)	1 (0.6)					
Litter incidence	# (%)	1 (4.8)					
Affected foetuses / litter	%	0.4					
<b>Misshapen interparietal</b>							
Foetal incidence	# (%)	1 (0.6)					1 (0.4)
Litter incidence	# (%)	1 (4.8)					1 (4.0)
Affected foetuses / litter	%	0.4					0.4
<b>Absent palatine bones</b>							
Foetal incidence	# (%)	1 (0.6)					
Litter incidence	# (%)	1 (4.8)					
Affected foetuses / litter	%	0.4					
<b>Severely malformed vertebral column and /or ribs</b>							
Foetal incidence	# (%)		2 (1.0)				
Litter incidence	# (%)		2 (8.3)				
Affected foetuses / litter	%		0.7				

Dose [mg/kg bw/d]		0	25	80	250	0	320
<b>Misshapen supraoccipital</b>							
Foetal incidence	# (%)						1 (0.4)
Litter incidence	# (%)						1 (4.0)
Affected foetuses / litter	%						0.4
<b>Thoracic hemivertebra</b>							
Foetal incidence	# (%)				1 (0.5)		
Litter incidence	# (%)				1 (5.3)		
Affected foetuses / litter	%				0.5		
<b>Absent lumbar vertebra</b>							
Foetal incidence	# (%)				2 (1.1)		
Litter incidence	# (%)				2 (11)		
Affected foetuses / litter	%				1.1		
<b>Branched rib. Cartilage present</b>							
Foetal incidence	# (%)				1 (0.5)		
Litter incidence	# (%)				1 (5.3)		
Affected foetuses / litter	%				0.7		
<b>Fused rib. Cartilage present</b>							
Foetal incidence	# (%)	1 (0.6)			1 (0.5)		
Litter incidence	# (%)	1 (4.8)			1 (5.3)		
Affected foetuses / litter	%	0.4			0.5		
<b>Absent rib</b>							
Foetal incidence	# (%)			1 (0.5)			
Litter incidence	# (%)			1 (4.2)			
Affected foetuses / litter	%			0.5			
<b>Intercostal rib; cartilage present</b>							
Foetal incidence	# (%)				2 (1.1)		
Litter incidence	# (%)				2 (11)		
Affected foetuses / litter	%				1.1		
<b>Acromion long; cartilage present</b>							
Foetal incidence	# (%)	1 (0.6)					
Litter incidence	# (%)	1 (4.8)					
Affected foetuses / litter	%	0.4					
<b>Short rib</b>							
Foetal incidence	# (%)			1 (0.5)			
Litter incidence	# (%)			1 (4.2)			
Affected foetuses / litter	%			0.5			
<b>Sternebrae severely fused (bony plate)</b>							
Foetal incidence	# (%)					1 (0.5)	
Litter incidence	# (%)					1 (4.3)	
Affected foetuses / litter	%					0.4	

Statistics. litter incidence: Fisher's Exact Test (1-sided); affected foetuses/litter: Wilcoxon-Test (1-sided)

\* p < 0.05. \*\* p < 0.01

Table 6.6-62. Historical control data – foetal skeletal malformations

Historical control data	Foetuses (2140)			Litters (264)			Affected foetuses / litter	
	No.	%	Range	No.	%	Range	No.	%
Misshapen thoracic vertebra - deformed cartilage	1	0.05	0.0 – 0.8	1	0.4	0.0 – 5.6	0.0	0.0 – 0.6
Severely malformed vertebral column and/or ribs	1	0.05	0.0 – 0.5	1	0.4	0.0 – 4.3	0.1	0.0 – 0.6
Thoracic hemivertebra	1	0.05	0.0 – 0.8	1	0.4	0.0 – 5.6	0.1	0.0 – 0.6
Absent lumbar vertebra	1	0.05	0.0 – 0.5	1	0.4	0.0 – 4.3	0.0	0.0 – 0.4

12 studies performed at [REDACTED] (Jan 2010– Apr 2015) with New Zealand White rabbits ([REDACTED])

Table 6.6-63. Skeletal variations

<b>Dose [mg/kg bw/d]</b>		<b>0</b>	<b>25</b>	<b>80</b>	<b>250</b>	<b>0</b>	<b>320</b>
Litters evaluated		21	24	24	19	23	25
Foetuses evaluated		181	206	194	182	205	235
	Live	181	206	193	182	204	235
	Dead	0	0	1	0	1	0
<b>Total skeletal variations</b>							
Foetal incidence	# (%)	173 (96)	199 (97)	188 (97)	173 (95)	182 (89)	223 (95)
Litter incidence	# (%)	21 (100)	24 (100)	24 (100)	19 (100)	23 (100)	25 (100)
Affected foetuses / litter	%	95.8	96.6	97.0	95.1	89.4	94.7
<b>Selected individual skeletal variations</b>							
<b>Incomplete ossification of cervical centrum; unchanged cartilage</b>							
Foetal incidence	# (%)	28 (15)	40 (19)	31 (16)	44 (24)	7 (3.4)	25 (11)
Litter incidence	# (%)	12 (57)	16 (67)	14 (58)	15 (79)	5 (22)	14* (56)
Affected foetuses / litter	%	14.0	17.0	15.4	23.1	3.4	10.8*
<b>Supernumerary thoracic vertebra</b>							
Foetal incidence	# (%)	23 (13)	30 (15)	48 (25)	23 (13)	37 (18)	40 (17)
Litter incidence	# (%)	9 (43)	13 (54)	16 (67)	9 (47)	16 (70)	17 (68)
Affected foetuses / litter	%	12.6	13.3	25.7*	13.3	18.4	17.7
<b>Incomplete ossification of thoracic centrum; unchanged cartilage</b>							
Foetal incidence	# (%)		1 (0.5)		4 (2.2)	1 (0.5)	1 (0.4)
Litter incidence	# (%)		1(4.2)		3 (16)	1 (4.3)	1 (4)
Affected foetuses / litter	%		0.4		2.2*	0.5	0.4
<b>Dumbbell ossification of thoracic centrum; dumbbell-shaped cartilage of centrum</b>							
Foetal incidence	# (%)	25 (14)	24 (12)	23 (12)	10 (5.5)	1 (0.5)	10 (4.3)
Litter incidence	# (%)	12 (57)	12 (50)	11 (46)	7 (37)	1 (4.3)	6 (24)
Affected foetuses / litter	%	13.6	11.6	11.7	5.5	0.3	4.5*
<b>Unilateral ossification of sternebra; unchanged cartilage</b>							
Foetal incidence	# (%)	6 (3.3)	6 (2.9)	7 (3.6)	14 (7.7)	4 (2.0)	4 (1.7)
Litter incidence	# (%)	5 (24)	6 (25)	6 (25)	10 (53)	4 (17)	4 (16)
Affected foetuses / litter	%	2.8	2.6	3.0	8.3*	1.8	1.8

\* p < 0.05. \*\* p < 0.01 (litter incidence: Fisher's Exact Test (1-sided); affected foetuses/litter: Wilcoxon-Test (1-sided))

Table 6.6-64. Historical control data – foetal skeletal variations

<b>Historical control data</b>	<b>Foetuses (2140)</b>			<b>Litters (264)</b>			<b>Affected foetuses / litter</b>	
	<b>No.</b>	<b>%</b>	<b>Range</b>	<b>No.</b>	<b>%</b>	<b>Range</b>	<b>No.</b>	<b>%</b>
Incomplete ossification of cervical centrum; unchanged cartilage	371	17.3	2.7-37.6	151	57.2	13.6-77.8	17.0	1.9-33.4
Supernumerary thoracic vertebra	508	23.7	16.1 - 32.8	169	64.0	52.2 – 85.0	22.7	14.9 – 35.3
Incomplete ossification of thoracic centrum; unchanged cartilage	10	0.5	0.0 - 1.6	7	2.7	0.0 – 8.7	0.5	0.0 – 2.2
Dumbbell ossification of thoracic centrum; dumbbell-shaped cartilage of centrum	168	7.9	0.6 - 16.4	94	35.6	5.0 - 53.8	8.2	0.6 - 15.8
Unilateral ossification of sternebra; unchanged cartilage	50	2.3	0.0 - 4.5	38	14.4	0.0 - 30.0	2.2	0.0 - 5.8

12 studies performed at [REDACTED] (Jan 2010– Apr 2015) with New Zealand White rabbits ([REDACTED])

Table 6.6-65. Skeletal unclassified cartilage observations

Dose [mg/kg bw/d]		0	25	80	250	0	320
Litters evaluated		21	24	24	19	23	25
Foetuses evaluated		181	206	194	182	205	235
	Live	181	206	193	182	204	235
	Dead	0	0	1	0	1	0
<b>Total skeletal unclassified cartilage observations</b>							
Foetal incidence	# (%)	26 (14)	22 (11)	20 (10)	19 (10)	20 (9.8)	22 (9.4)
Litter incidence	# (%)	12 (57)	13 (54)	12 (50)	12 (63)	9 (39)	10 (40)
Affected foetuses / litter	%	13.7	10.0	11.6	11.0	9.3	10.0
<b>Selected individual skeletal unclassified cartilage observations</b>							
<b>Bipartite processus xiphoideus</b>							
Foetal incidence	# (%)	8 (4.4)	7 (3.4)	6 (3.1)	3 (1.6)	4 (2.0)	11 (4.7)
Litter incidence	# (%)	6 (29)	7 (29)	4 (17)	3 (16)	3 (13)	8 (32)
Affected foetuses / litter	%	4.7	3.1	3.9	1.6	1.6	<b>4.9*</b>

Statistics. litter incidence: Fisher's Exact Test (1-sided); affected foetuses/litter: Wilcoxon-Test (1-sided)

\* p < 0.05. \*\* p < 0.01

Table 6.6-66. Total foetal malformations

Dose [mg/kg bw/d]		0	0	25	80	250	320
Litters evaluated		21	23	24	24	19	25
Foetuses evaluated		181	205	206	194	182	235
	Live	181	204	206	193	182	235
	Dead	0	1	0	1	0	0
<b>Total foetal malformations</b>							
Foetal incidence	# (%)	7 (3.9)	7 (3.4)	5 (2.4)	2 (1.0)	6 (3.3)	2 (0.9)
Litter incidence	# (%)	6 (29)	5 (22)	5 (21)	2 (8.3)	4 (21)	2 (8.0)
Affected foetuses / litter	%	3.4	2.8	2.6	1.0	3.9	0.8

Table 6.6-67. Total foetal variations

Dose [mg/kg bw/d]		0	0	25	80	250	320
Litters evaluated		21	23	24	24	19	25
Foetuses evaluated		181	205	206	194	182	235
	Live	181	204	206	193	182	235
	Dead	0	1	0	1	0	0
<b>Total foetal malformations</b>							
Foetal incidence	# (%)	173 (96)	184 (90)	199 (97)	188 (97)	173 (95)	224 (95)
Litter incidence	# (%)	21 (100)	23 (100)	24 (100)	24 (100)	19 (100)	25 (100)
Affected foetuses / litter	%	95.8	90.3	96.6	97.0	95.1	95.1

### Conclusion

In conclusion, under the conditions of this GLP and OECD test guideline compliant development toxicity study in the rabbit, no malformations or variations (external, skeletal or visceral) were observed up to 320 mg/kg bw/d (the highest dose tested). However, foetal weight was reduced from 250 mg/kg bw/d. Therefore a **NOAEL for developmental toxicity of 80 mg/kg bw/d** was identified.

Maternal toxicity - reduced body weight gain, increased liver weight with associated changes in clinical chemistry (increased  $\gamma$ -glutamyltransferase (GGT) activity) - was also observed from 250 mg/kg bw/d. Therefore, a **NOAEL for maternal toxicity of 80 mg/kg bw/d** was established. It is clear that the reduced foetal weight is the unspecific secondary consequence of the maternal toxicity elicited by the substance.

( [REDACTED] 2018 b)

*Old studies*

The developmental toxicity of cinmethylin has been investigated in two older studies which were conducted according to GLP but not according to OECD test guidelines.

*1)*

<b>Author(s)</b>	██████████
<b>Study title</b>	Teratology study of CINCH herbicide (Technical SD 95481) administered orally via stomach tube to New Zealand White (NZW) rabbits
<b>Study reference</b>	██████████ 1985 BASF DocID: CI-432-002
<b>Test facility</b>	██
<b>Date</b>	25/03/1985 – 26/04/1985
<b>Test substance</b>	SD 95481 BAS 684 H (Cinmethylin)
<b>Batch no. Purity (%)</b>	513N 93 (-) / (+) ratio = not specified.
<b>Test animals</b>	Rabbit New Zealand White (NZW) / (Dla Hra:(NZW) SPF) Female
<b>Groups</b>	Four groups of 19 - 20 females per dose. 19 in the control group and 20/dose in the treatment groups.
<b>Dose/concentrations</b>	0, 3, 30 and 100 mg/kg bw/day
<b>Route</b>	Administered daily via stomach tube (oral gavage) to artificially inseminated rabbits, on days 6 – 18 post-implantation (presumed gestation).
<b>Vehicle</b>	Corn oil.
<b>GLP</b>	Compliant.
<b>Guideline</b>	Non-OECD test guideline compliant. EPA guidelines followed.
<b>Deviation</b>	The following deviations from the current OECD test guideline no. 414 (2018) occurred: <ul style="list-style-type: none"> <li>• Cinmethylin was administered during the period of organogenesis (GD 6 - 18) only (rather than GD 6-29).</li> <li>• Maternal mortality exceeded 10 % in the study, even in the control (16 %). The high mortality rate in this study, equally distributed over all groups, was attributed to effects of the vehicle (corn oil) and improper administration procedure.</li> <li>• The historical control data (HCD) are of limited use, as only total incidences and the mean values are provided; but the range of values are not provided.</li> <li>• Premature or natural delivery was defined in this study as expulsion of conceptuses on GD 28 or 29, while abortion was defined as expulsion on GD 27 or earlier. This differentiation is outdated and differs from the OECD test guideline which defines abortion as the premature expulsion from the uterus of the products of conception, irrespective of date.</li> <li>• There was a high incidences of spontaneous malformations and variations; incidences were highest in control animals. This may mask the effects of treatment-related findings.</li> </ul>
<b>Impact of deviations</b>	The listed deviations impact the relevance and reliability of the study.
<b>Acceptable</b>	Not acceptable.
<b>NOAEL</b>	Not set due to study limitations.
<b>Effects at the LOAEL</b>	N/A - due to study limitations.

Due to the significant limitations of this study described above, no detailed assessment has been performed. The study is unreliable and should be discounted. The study has been reported for transparency but not relied upon.

Methods

In a relatively old, GLP compliant but non-guideline study, cinmethylin was administered daily to groups of 19 - 20 inseminated (presumed pregnant) New Zealand White rabbits, by stomach tube, during gestation days



6 - 18 post insemination (p.i.), at daily dose levels of 0, 3, 30 and 100 mg/kg bw/d (in corn oil), using an application volume of 2 mL/kg bw. Caesarean section was performed on gestation day (GD) 29, followed by gross necropsy including determinations of maternal liver and uterus weight, number of corpora lutea, number and location of implantation, resorptions as well as the number of live and dead fetuses. Live fetuses were sexed, weighed and external, visceral and skeletal anomalies were determined. The study report stated that the stability of cinmethylin preparations in corn oil for the study duration was verified analytically after study end. However, this study did not include analytical determination (see Volume 3 CA B5, section B.5.1.2), therefore methods of this aforementioned analysis was not verified.

The doses employed were selected based on a maternal toxicity dose range-finding study. Dose levels of 0, 30, 100, 300, 1000 and 2000 mg/kg bw/d cinmethylin in corn oil were tested in groups of four females. Pregnancy rates were low and mortalities occurred in some animals, however, no dose-response was evident in these parameters. All decedents, except one of top dose female, had stomach ulceration and/or white spots on stomach mucosa, however, mostly as a single incidence only, lacking a dose-response. Administration of cinmethylin resulted in generally reduced maternal body weight gain and feed consumption. A slight increase in the incidence of resorptions was reported for the top two dose groups. Based on these data dose levels of 3, 30 and 100 mg/kg bw/d were selected for the main study.

## Results

### Maternal toxicity

No treatment-related effects on mortality and clinical signs (Table 6.6-68), food consumption (Tables 6.6-69), body weight (Table 6.6-70), body weight gain (Tables 6.6-71 and 6.6-72), gravid uterus weight, carcass weight and net weight changes (Table 6.6-73) and liver weight (Table 6.6-74) were seen.

The high mortality rate seen (16 % in the control group, 10 % in the low and mid dose group and 20 % at the top dose) (Table 6.6-68) was attributed to the vehicle (corn oil) and the exposure route (gavage). Due to the lack of dose-response and high mortality seen in the control group, this effect was regarded as treatment- but not substance-related. However, it is noted that a similar finding was not observed in the pre-natal development toxicity study in the rat (██████████, 1984) which also used corn oil as a vehicle and gavage as the route of administration. Effects on food consumption and body weight gain (across all groups including the control, with no dose-response) also point to general maternal toxicity which was not related to treatment with cinmethylin.

Stomach ulceration / haemorrhagic area in mucosa were seen in decedents of all doses (Table 6.6-75). Gastric irritation was considered treatment-related, based on these findings and findings of the acute dermal irritation studies in the rabbit (██████████, 2016d; ██████████ 1981b). However, this finding differs from that of the new/modern developmental study in rabbits, where doses up to 320 mg/kg bw/day resulted in no stomach ulceration or irritation (██████████, 2018). In a second pre-natal developmental toxicity study in rabbits (██████████, 1987), stomach ulceration was observed in the dose range-finding study (at 1000 mg/kg bw/d) but not the main study (up to 750 mg/kg bw/d).

Table 6.6-68. Mortality and clinical signs

Dose [mg/kg bw/d]		0		3		30		100	
		N	%	N	%	N	%	N	%
Animals examined		N = 19		N = 20		N = 20		N = 20	
Mortality		3 (GD 25 (x2); GD 26)	16	2 (GD 22; GD 26)	10	2 (GD 18; GD 20)	10	4 (GD 19; GD 22; GD 26; GD 28)	20
Alopecia		9	47	7	35	11	55	9	45
Abrasions		0	0	0	0	0	0	1	5
Anorexia		9	47	5	25	12	60	8	40
Decreased motor activity		0	0	0	0	0	0	2	10
Red substance in cage pan		2	11	2	10	0	0	1	5
Mucoid discharge		0	0	0	0	1	5	2	10
Faeces	overall	15	79	15	75	17	85	18	90
	dried	12	63	10	50	15	75	16	80

Dose [mg/kg bw/d]		0		3		30		100	
		N	%	N	%	N	%	N	%
	soft/liquid	7	37	12	60	11	55	12	60
	white	0	0	1	5	0	0	0	0
	not present	4	21	3	15	4	20	5	25

N = number of incidences; GD = gestation day of occurrence

Table 6.6-69. Food consumption

Dose [mg/kg bw/d]		Day of Gestation			
		0 - 6	6 - 19	0 - 29	19 - 29
0	mean $\pm$ SD [g/animal/d]	164.3 $\pm$ 17.8	58.5 $\pm$ 45.4	93.0 $\pm$ 36.7	90.8 $\pm$ 60.1
3	mean $\pm$ SD [g/animal/d] [ $\Delta$ % control]	159.8 $\pm$ 26.9 -2.7	68.5 $\pm$ 45.6 16.9	96.5 $\pm$ 42.4 3.8	90.6 $\pm$ 66.1 -0.3
30	mean $\pm$ SD [g/animal/d] [ $\Delta$ % control]	158.3 $\pm$ 26.2 -3.6	33.3 $\pm$ 41.5 -43.1	77.7 $\pm$ 35.0 -16.4	86.3 $\pm$ 58.7 -5.0
100	mean $\pm$ SD [g/animal/d] [ $\Delta$ % control]	162.4 $\pm$ 25.3 -1.1	49.9 $\pm$ 43.8 -14.7	84.9 $\pm$ 37.2 -8.7	82.2 $\pm$ 65.0 -9.5

\* p < 0.05, \*\* p < 0.01

Only pregnant does were used for the calculations of mean maternal food consumption, body weight and body weight change.

Table 6.6-70. Body weight

Dose [mg/kg bw/d]		Day of Gestation			
		0	6	19	29
0	mean $\pm$ SD [kg]	4.13 $\pm$ 0.34	4.25 $\pm$ 0.34	3.98 $\pm$ 0.44	4.23 $\pm$ 0.44
3	mean $\pm$ SD [kg] [ $\Delta$ % control]	3.98 $\pm$ 0.3 -3.6	4.07 $\pm$ 0.3 -4.2	3.86 $\pm$ 0.36 -3.0	4.02 $\pm$ 0.35 -5.0
30	mean $\pm$ SD [kg] [ $\Delta$ % control]	3.96 $\pm$ 0.26 -4.1	4.09 $\pm$ 0.28 -3.8	3.71 $\pm$ 0.41 -6.8	4.07 $\pm$ 0.4 -3.8
100	mean $\pm$ SD [kg] [ $\Delta$ % control]	4.16 $\pm$ 0.39 +0.7	4.28 $\pm$ 0.42 +0.7	3.96 $\pm$ 0.38 -0.5	4.18 $\pm$ 0.43 -1.2

\* p < 0.05, \*\* p < 0.01

Only pregnant does were used for the calculations of mean maternal food consumption, body weight and body weight change.

Table 6.6-71. Body weight gain

Dose [mg/kg bw/d]		Day of Gestation			
		0	6	19	29
0	mean $\pm$ SD [kg]	0.11 $\pm$ 0.08	-0.27 $\pm$ 0.33	0.09 $\pm$ 0.39	0.12 $\pm$ 0.28
3	mean $\pm$ SD [kg] [ $\Delta$ % control]	0.08 $\pm$ 0.09 -27.3	-0.21 $\pm$ 0.31 +22.2	0.1 $\pm$ 0.22 +15.4	0.09 $\pm$ 0.13 -25.0
30	mean $\pm$ SD [kg]	0.12 $\pm$ 0.06	-0.37 $\pm$ 0.33	-0.23 $\pm$ 1.25	0.25 $\pm$ 0.16

Dose [mg/kg bw/d]		Day of Gestation			
		0	6	19	29
	[Δ% control]	+9.1	-37.0	-368.7	+108.3
100	mean ± SD [kg]	0.11 ± 0.1	-0.32 ± 0.36	0.13 ± 0.28	0.17 ± 0.16
	[Δ% control]	±0.0	-18.5	+48.2	+41.7

\* p < 0.05, \*\* p < 0.01

Only pregnant does were used for the calculations of mean maternal food consumption, body weight and body weight change.

Table 6.6-72. Body weight gain and food consumption - females that delivered naturally (early, on GD 28 or 29).

Animal #	Food consumption [g/kg bw/day]				Body weight gain [g]			
	Day of Gestation							
	0 - 6	6 - 19	19 – 28/29	0 – 28/29	0 - 6	6 - 19	19 – 28/29	0 – 28/29
0 mg/kg bw/d								
mean	39.4	14.6	27.3	25.6	113	-267	127	85
SD	3.9	10.3	10.8	6.1	80	326	276	393
30 mg/kg bw/d								
#9544	33.4	4.0	0.6	7.3	10	-600	-220	-810
#9558	39.0	0.6	0.4	6.1	110	-760	-20	-670
100 mg/kg bw/d								
#9566	38.6	5.9	0.6	9.3	110	-390	-340	-620
#9567	43.7	4.5	1.0	9.1	119	-880	-260	-950

Table 6.6-73. Uterus weight, carcass weight and corrected (net) body weight gain

Parameter [kg]		Dose [mg/kg bw/d]			
		0	3	30	100
Gravid uterus	[mean ± SD]	0.393 ± 0.164	0.337 ± 0.147	0.421 ± 0.113	0.384 ± 0.202
	[Δ% control]	-	-14.1	+6.9	-2.4
Carcass	[mean ± SD]	3.835 ± 0.405	3.688 ± 0.435	3.646 ± 0.376	3.800 ± 0.335
	[Δ% control]	-	-3.8	-4.9	-0.9
Net weight change from GD 0	[mean ± SD]	-0.309 ± 0.367	-0.239 ± 0.304	-0.295 ± 0.205	-0.255 ± 0.313
	[Δ% control]	-	-22.6	-4.4	-17.3
Net weight change from GD 6	[mean ± SD]	-0.425 ± 0.360	-0.340 ± 0.283	-0.442 ± 0.176	-0.372 ± 0.330
	[Δ% control]	-	-20.1	+4.0	-12.6

\* p < 0.05, \*\* p < 0.01

Carcass weight = terminal body weight minus uterine weight (values are rounded)

Net weight change from GD 0/6 = carcass weight minus GD 0/6 body weight

Table 6.6-74. Carcass and liver weights

Organ weight		Dose [mg/kg bw/d]			
		0	3	30	100
Carcass weight	[g]	3835.3	3688.0	3645.9	3799.7
	[Δ% control]	-	-3.8	-4.9	-0.9
Liver weight,	Absolute [g]	112.2	105.4	110.7	109.3
	[Δ% control]	-	-6.1	-1.3	-2.6
# Liver weight	Relative to bw [g]	2.931	2.828	3.046	2.889
	[Δ% control]	-	-3.5	+3.9	-1.5

\* p < 0.05, \*\* p < 0.01; Carcass weight = terminal body weight minus uterine weight (values are rounded)

Organ weight	Dose [mg/kg bw/d]			
	0	3	30	100

# Note: the relative liver weight was re-calculated based on carcass weight (terminal body weight minus uterus weight). In the study report, the rel. liver weight was calculated based on terminal body weight at sacrifice.

Table 6.6-75. Necropsy findings - pregnant females

Necropsy findings	Dose [mg/kg bw/d]			
	0	3	30	100
Females examined	19	20	20	20
Nothing abnormal detected [No. (%)]	11 (58)	9 (45)	6 (30)	8 (40)
Ovaries				
- Parovarian cyst(s)	7	10	12	7
Gall bladder				
- cystic area presented	-	-	-	1 <sup>\$</sup>
Liver				
- enlarged	-	-	-	1 <sup>\$</sup>
Lung				
- haemorrhagic and fluid filled	1 <sup>#</sup>	-	-	-
Head				
- discharge from mouth and nose	1 <sup>#</sup>	-	-	-
Stomach				
- ulceration in mucosa	-	1	-	3
- haemorrhagic area in mucosa	-	-	1	1 <sup>\$</sup>
- hair ball	-	-	-	1 <sup>\$</sup>

#, \$, \$ findings were observed in the same animal(s); <sup>\$</sup> animal (# 9579) aborted and was sacrificed on GD 26

#### Developmental toxicity

At caesarean section, percentage of pre- and post-implantation loss, mean number of early and late resorptions, mean number of fetuses per litter, sex ratio and mean foetal body weight were comparable between treated and control groups (Table 6.6-76). There were no dead fetuses in any group.

There were no treatment-related external, visceral or skeletal malformations (Tables 6.6-77, 6.6-78 and 6.6-79) or variations (Tables 6.6-80 and 6.6-81). There was a high incidences of spontaneous malformations and variations; incidences were highest in control animals.

Table 6.6-76. Pregnancy status and caesarean section data

Dose [mg/kg bw/d]		0	3	30	100
<b>Pregnancy status</b>					
Females					
- inseminated	[n]	19	20	20	20
- pregnant	[n]	19	16	17	20
- Conception rate	[%]	100	80	85	100
- aborted	[n]	2	1	2	1
- premature birth	[n]	0	0	2	2
- Does with viable fetuses	[n]	14	13	11	13
- Does with all resorptions	[n]	1	0	0	1
- Mortality	[n]	3	2	2	4
- Pregnant terminal sacrifice	[n]	15	13	11	14
<b>Caesarean section data<sup>a</sup></b>					
Corpora lutea	[mean/litter]	10.3 ± 1.73	10.9 ± 2.5	11.4 ± 1.6	11.7 ± 3.4
- total number	[n]	155	142	126	164
Implantation sites	[mean/litter]	7.9 ± 3.0	6.5 ± 3.2	7.7 ± 2.2	7.1 ± 3.8
- total number	[n]	120	85	85	100
Pre-implantation loss	[%]	25.2 ± 26.1	40.6 ± 27.2	32.3 ± 18.9	40.5 ± 26.8
Post-implantation loss	[%]	20.2 ± 27.1	8.0 ± 11.5	12.9 ± 18.5	11.1 ± 26.5

Dose [mg/kg bw/d]		0	3	30	100
Pregnancy status					
Females					
- inseminated	[n]	19	20	20	20
- pregnant	[n]	19	16	17	20
- Conception rate	[%]	100	80	85	100
- aborted	[n]	2	1	2	1
- premature birth	[n]	0	0	2	2
- Does with viable foetuses	[n]	14	13	11	13
- Does with all resorptions	[n]	1	0	0	1
- Mortality	[n]	3	2	2	4
- Pregnant terminal sacrifice	[n]	15	13	11	14
Caesarean section data <sup>a</sup>					
Resorptions	[mean/litter]	1.2 ± 1.5	0.5 ± 0.8	1.0 ± 1.4	0.8 ± 2.1
- total number	[n]	18	7	11	12
Early resorptions	[%]	11.6 ± 26.5	4.4 ± 9.8	5.3 ± 11.5	6.0 ± 11.3
- number	[mean/litter]	0.5 ± 0.8	0.2 ± 0.4	0.4 ± 0.9	0.4 ± 0.8
- total number	[n]	7	3	5	6
Late resorptions	[%]	7.9 ± 15.5	3.6 ± 7.3	7.6 ± 17.3	5.1 ± 16.7
- number	[mean/litter]	0.7 ± 1.4	0.3 ± 0.6	0.5 ± 1.3	0.4 ± 1.3
- total number	[n]	11	4	6	6
Dead foetuses	[n]	0	0	0	0
Live foetuses	[mean/litter]	6.7 ± 3.1	6.0 ± 3.0	6.7 ± 2.5	6.3 ± 4.2
- total number	[n]	101	78	74	88
- mean	[%]	80.6	92.0	87.1	88.9
Total live female foetuses	[mean]	3.9 ± 2.3	3.0 ± 1.5	3.6 ± 2.0	3.1 ± 2.4
- total number	[n]	59	39	39	43
Total live male foetuses	[mean]	2.8 ± 1.3	3.0 ± 2.4	3.2 ± 2.0	3.2 ± 2.5
- total number	[n]	42	39	35	45
Percent live females	[%]	58.4	50.0	52.7	48.9
Percent live males	[%]	41.6	50.0	47.3	51.1
Mean foetal weight	[g]	39.1 ± 10.2	42.1 ± 10.6	41.7 ± 6.5	43.2 ± 9.9
- males	[g]	39.6 ± 10.1	40.6 ± 10.3 <sup>b</sup>	41.9 ± 7.3 <sup>b</sup>	43.1 ± 10.6 <sup>b</sup>
- females	[g]	37.0 ± 8.5 <sup>c</sup>	41.1 ± 11.3	40.6 ± 6.8	41.5 ± 8.6 <sup>c</sup>

<sup>a</sup> Mean ± SD on litter basis;

Statistical evaluation: \* p ≤ 0.05; \*\* p < 0.01;

<sup>b</sup> Two low dose group litters, one mid dose group litter and one high dose group litter had no male foetuses;

<sup>c</sup> One vehicle control dose group litter and one high dose group litter had no female foetuses.

Table 6.6-77. External malformations

Dose [mg/kg bw/d]		0	3	30	100
Litters evaluated		14	13	11	13
Foetuses evaluated		101	78	74	88 <sup>a</sup>
Total external malformations					
Foetal incidence	# (%)	8 (7.9)	0 (0.0)	1 (1.4)	0 (0.0)
Litter incidence	# (%)	2 (14.3)	0 (0.0)	1 (9.1)	0 (0.0)
Affected foetuses / litter	%	8.0	0.0	2.3	0.0
Individual external malformations					
Multiple malformation					
Foetal incidence	# (%)	7 <sup>&amp;</sup> (6.9)			
Litter incidence	# (%)	1 (7.1)			
Affected foetuses / litter	%	7.1			
Short tail					
Foetal incidence	# (%)	1 <sup>#</sup> (1.0)			
Litter incidence	# (%)	1 (7.1)			
Affected foetuses / litter	%	0.9			

Dose [mg/kg bw/d]	0	3	30	100
Litters evaluated	14	13	11	13
Foetuses evaluated	101	78	74	88 <sup>a</sup>
<b>Umbilical hernia</b>				
Foetal incidence # (%)			1 <sup>s</sup> (1.4)	
Litter incidence # (%)			1 (9.1)	
Affected foetuses / litter %			2.3	

<sup>&</sup> = All 7 foetuses from 1 litter revealed multiple external and skeletal malformation and multiple skeletal variations

<sup>#</sup> with corresponding skeletal malformation – fused caudal vertebrae

<sup>s</sup> finding was confirmed visceraally

<sup>a</sup> Excluding 10 foetuses present in the uterus of the naturally delivered doe (#9566)

Table 6.6-78. Soft tissue malformations

Dose [mg/kg bw/d]	0	3	30	100
Litters evaluated	14	13	11	13
Foetuses evaluated	101	78	74	88
<b>Total soft tissue malformations</b>				
Foetal incidence # (%)		1 (1.3)		
Litter incidence # (%)		1 (7.7)		
Affected foetuses / litter %		0.8		
<b>Individual soft tissue malformations</b>				
<b>Ectopic kidney</b>				
Foetal incidence # (%)		1 <sup>%</sup> (1.3)		
Litter incidence # (%)		1 (7.7)		
Affected foetuses / litter %		0.8		

<sup>%</sup> = same foetus had also several skeletal malformations: fused frontals and wavy scapula, and several skeletal variations: flattened ribs and intraparietal extra ossification

Table 6.6-79. Skeletal malformations

Dose [mg/kg bw/d]	0	3	30	100
Litters evaluated	14	13	11	13
Foetuses evaluated	101	78	74	88
Live	101	78	74	88
Dead	0	0	0	0
<b>Total skeletal malformations</b>				
Foetal incidence # (%)	9 (8.9)	2 (2.6)	0 (0.0)	0 (0.0)
Litter incidence # (%)	2 (14.3)	2 (15.4)	0 (0.0)	0 (0.0)
Affected foetuses / litter %	8.9	2.1	0.0	0.0
<b>Individual skeletal malformations</b>				
<b>Multiple malformations</b>				
Foetal incidence # (%)	7 <sup>&amp;</sup> (6.9)			
Litter incidence # (%)	1 (7.1)			
Affected foetuses / litter %	7.1			
<b>Frontals fused</b>				
Foetal incidence # (%)		1 <sup>%</sup> (1.3)		
Litter incidence # (%)		1 (7.7)		
Affected foetuses / litter %		0.8		
<b>Wavy scapula</b>				
Foetal incidence # (%)		1 <sup>%</sup> (1.3)		
Litter incidence # (%)		1 (7.7)		
Affected foetuses / litter %		0.8		
<b>Caudal vertebrae fused (1-15)</b>				
Foetal incidence # (%)	1 <sup>#</sup> (1.0)			
Litter incidence # (%)	1 (7.1)			
Affected foetuses / litter %	0.9			

Dose [mg/kg bw/d]		0	3	30	100
Litters evaluated		14	13	11	13
Foetuses evaluated		101	78	74	88
Live		101	78	74	88
Dead		0	0	0	0
<b>Thoracic hemivertebra</b>					
Foetal incidence	# (%)	1 (1.0)			
Litter incidence	# (%)	1 (7.1)			
Affected foetuses / litter	%	0.9			
<b>Fused ribs</b>					
Foetal incidence	# (%)		1 (1.3)		
Litter incidence	# (%)		1 (7.7)		
Affected foetuses / litter	%		1.3		

& = a complete litter consisting of 7 foetuses with multiple external & skeletal malformations and multiple skeletal variations

# with corresponding external malformation – short tail

% = findings of the same foetus that also had a visceral malformation: ectopic kidney, and several skeletal variations: flattened ribs and intraparietal extra ossification

Table 6.6-80. Skeletal variations

Dose [mg/kg bw/d]		0	3	30	100
Litters evaluated		14	13	11	13
Foetuses evaluated		101	78	74	88
Total skeletal variations					
Foetal incidence	# (%)	35 (34.7)	15 (19.2)	12 (16.2)	13 (14.8)
Litter incidence	# (%)	8 (57.1)	6 (46.2)	6 (54.6)	5 (38.5)
Affected foetuses / litter	%	34.0	18.3	20.2	12.5
Selected individual skeletal variations					
Multiple variations					
Foetal incidence	# (%)	7 (6.9)			1 (1.1)
Litter incidence	# (%)	1 (7.1)			1 (7.1)
Affected foetuses / litter	%	7.1			0.8
Frontals and/or parietals contain hole(s)					
Foetal incidence	# (%)		2 (2.6)		7 (8.0)“
Litter incidence	# (%)		1 (7.7)		1 (7.7)
Affected foetuses / litter	%		1.7		5.4
HCD <sup>§</sup> – frontals contain holes		Foetal incidence # (%):6 (0.32)		Litter incidence# (%):3 (1.2)	
HCD <sup>§</sup> – parietals contain holes		Foetal incidence # (%):17 (0.9)		Litter incidence# (%):8 (3.2)	
Parietals contain extra ossification sites (intraparietals)					
Foetal incidence	# (%)		4* (5.1)	2 (2.7)	
Litter incidence	# (%)		4* (30.8)	1 (9.1)	
Affected foetuses / litter	%		3.5	4.6	
Fontanelle, irregularly shaped					
Foetal incidence	# (%)	24 <sup>γ</sup> (23.8)	8** (10.3)	8** (10.8)	4** (4.6)
Litter incidence	# (%)	4 <sup>γ</sup> (28.6)	3 (23.1)	3 (27.3)	3 (23.1)
Affected foetuses / litter	%	22.8	12.3	12.3	5.5
HCD <sup>§</sup>		Foetal incidence # (%):6 (0.3)		Litter incidence# (%):5 (2.0)	
Thoracic centra, asymmetric ossification					
Foetal incidence	# (%)			1 <sup>α</sup> (1.4)	
Litter incidence	# (%)			1 (9.1)	
Affected foetuses / litter	%			2.3	
HCD <sup>§</sup>		Foetal incidence # (%):6 (0.32)		Litter incidence# (%):6 (2.4)	
Thoracic centra, unilateral ossification					
Foetal incidence	# (%)			2 <sup>α, β</sup> (2.7)	
Litter incidence	# (%)			2 (18.2)	
Affected foetuses / litter	%			4.1	

Dose [mg/kg bw/d]	0	3	30	100
Litters evaluated	14	13	11	13
Foetuses evaluated	101	78	74	88
<i>HCD<sup>§</sup></i>	<i>Foetal incidence # (%):5 (0.27)</i>		<i>Litter incidence# (%):3 (1.2)</i>	
<b>Lumbar centrum, asymmetric ossification</b>				
Foetal incidence # (%)			1 <sup>B</sup> (1.4)	
Litter incidence # (%)			1 (9.1)	
Affected foetuses / litter %			1.8	
<b>Sternebrae fused</b>				
Foetal incidence # (%)	1 (1.0)	1 (1.3)	1 (1.4)	1 (1.1)
Litter incidence # (%)	1 (7.1)	1 (7.7)	1 (9.1)	1 (7.7)
Affected foetuses / litter %	0.9	0.8	1.1	0.8

\* p < 0.05, \*\* p < 0.01

“ = To note: in the study report, statistically significantly increased incidence of frontals (6\*\* foetuses of 1 litter) and of parietals (3 foetuses of 1 litter) containing holes is listed separately. However, during study re-evaluation it was noted that both developmental findings are of the same origin and thus, were assessed together. The most relevant parameter – the rate of affected foetus per liter was calculated and subjected to one-sided Wilcoxon test, revealing no statistical significance. Albeit, the combined foetal incidence is slightly exceeding HCD of frontals containing holes, if the foetal findings are compared with the individual HCD separately, both are within each HCD.

<sup>a</sup> = foetus #2 delivered by dam #9545 revealed asymmetric and unilateral ossification of thoracic vertebrae centra

<sup>B</sup> = foetus #8 delivered by dam #9546 revealed unilateral ossification of thoracic vertebra centrum, asymmetric ossification of lumbar vertebra centrum and irregularly shaped fontanelle

<sup>γ</sup> = To note: in study report, the foetal incidence is 31 and the litter incidence is 5. However, during re-evaluation 1 litter consisting of 7 foetuses revealing multiple malformations/variations was considered as a separate finding and the individual findings of those foetuses were not assessed separately. Thus, the incidence of the irregularly shaped fontanelle deviates from Table 12 of the study report.

<sup>§</sup> = historical control data of the test facility in the time-period 1982 – 1984 consisting of 250 litter and 1879 foetal findings

Table 6.6-81. Total anomalies, malformations and variations

Dose [mg/kg bw/d]	0	3	30	100
Litters evaluated	14	13	11	13
Foetuses evaluated	101	78	74	88
<b>Total anomalies</b>				
Foetal incidence # (%)	37 (36.6)	16 (20.5)	13 (17.6)	13 (14.8)
Litter incidence # (%)	8 (57.1)	7 (53.9)	7 (63.6)	5 (38.5)
Affected foetuses / litter %	35.8 ± 42.9	19.6 ± 20.0	22.5 ± 30.1	12.5 ± 23.0
<b>Total malformations</b>				
Foetal incidence # (%)	9 (8.9)	2 (2.6)	1 (1.4)	0 (0.0)
Litter incidence # (%)	2 (14.3)	2 (15.4)	1 (9.1)	0 (0.0)
Affected foetuses / litter %	9.3 ± 27.1	2.05 ± 5.2	2.3 ± 7.5	0.0
<b>Total variations</b>				
Foetal incidence # (%)	35 (34.7)	15 (19.2)	12 (16.2)	13 (14.8)
Litter incidence # (%)	8 (57.1)	6 (46.2)	6 (54.6)	5 (38.5)
Affected foetuses / litter %	34.0 ± 42.8	18.3 ± 29.5	20.2 ± 30.8	12.5 ± 23.0

### Conclusion

In conclusion, under the conditions of this limited developmental toxicity study in the rabbit, no treatment-related malformations or variations (external, skeletal or visceral) were observed up to 100 mg/kg bw/d (the highest dose tested).

With regards to maternal toxicity, findings were limited to increased incidence of stomach ulceration at the top dose. No maternal toxicity was observed at lower dose levels. Due to the study limitations, no robust conclusions have been drawn and no robust NOAELs set.

(██████, 1985)



2)

<b>Author(s)</b>	██████████
<b>Study title</b>	Teratogenicity study of IN-YA168 in rabbits
<b>Study reference</b>	██████████, 1987 BASF DocID: CL-432-003
<b>Test facility</b>	██ ██
<b>Date</b>	01/03/1987 – 03/04/1987
<b>Test substance</b>	BAS 684 H (IN-YA168; IN-42326; N.B. 5103-156) (Cinmethylin)
<b>Batch no. Purity (%)</b>	Batch not specified. 92.4 (-) / (+) ratio = not reported.
<b>Test animals</b>	Rabbit New Zealand White / Hra:(NZW)SPF Female
<b>Groups</b>	20 inseminated females/dose
<b>Dose/concentrations</b>	0, 30, 200, 500 and 750 mg/kg bw/d. Dose volume : 4 mL/kg bw.
<b>Route</b>	Administered daily via stomach tube (oral gavage) to artificially inseminated rabbits, on days 7 – 19 post-implantation (presumed gestation).
<b>Vehicle</b>	0.5 % aqueous methylcellulose (MC).
<b>GLP</b>	Compliant.
<b>Guideline</b>	Non-OECD test guideline compliant. EPA guidelines followed.
<b>Deviation</b>	The following deviations from the current OECD test guideline no. 414 (2018) occurred: <ul style="list-style-type: none"> <li>• Cinmethylin was administered during the period of organogenesis (days 7-19) only (rather than GD 6-28).</li> <li>• Room temperature was set to maintain a temperature range of 66-74 °F (i.e., up to 23°C); housing at elevated temperatures can induce stress to rabbits leading to pregnancy impairment and is associated with increased occurrence of abortions/early deliveries and decreased ovulation and nidation rates.</li> <li>• The present study consisted of 7 - 15 females with verified implantation sites per dose group, and 6 - 13 does with live litters at termination (rather than 20 females with implantation sites at necropsy).</li> <li>• Historical control data was not included in this study.</li> </ul>
<b>Impact of deviations</b>	The listed deviations impact the relevance and reliability of the study.
<b>Acceptable</b>	Not acceptable.
<b>NOAEL</b>	Not set due to study limitations.
<b>Effects at the LOAEL</b>	NA - due to study limitations.

Due to the significant limitations of this study (described above), no detailed assessment has been performed. The study is unreliable and should be discounted. The study has been reported for transparency but not relied upon.

#### Methods

In a relatively old, GLP compliant but non-OECD test guideline study, cinmethylin was administered daily to groups of 20 inseminated (presumed pregnant) New Zealand White rabbits, by oral gavage, during gestation days 7 - 19 post insemination (p.i.), at daily dose levels of 0, 30, 200, 500 and 750 mg/kg bw/d (in 0.5 % aqueous methylcellulose), using an application volume of 4 mL/kg bw. Caesarean section was performed on GD 29 followed by gross necropsy including determinations of maternal liver and uterus weight, number of corpora lutea, number and location of implantation, resorptions as well as the number of live and dead fetuses. Live fetuses were sexed, weighed and external, visceral and skeletal anomalies were determined. The stability of cinmethylin in the vehicle was verified analytically.

Doses were selected based on a maternal toxicity dose range-finding study. Dose levels of 0, 100, 500, 1000 and 2000 mg/kg bw/d cinmethylin (in 0.5 % MC) were tested in groups of 8 female artificially inseminated NZW rabbits by oral gavage from GD 7 - 19. On GD 21 the study was terminated. The gravid uterus was opened and the types of implantations (live and dead fetuses, and resorptions) and their relative positions were recorded. Foetuses were examined for external alterations only. Pregnancy rate was 7/8, 7/8, 8/8, 7/8, and 8/8 respectively. Deaths were seen in the two highest dosage groups. By day 18 of gestation, 7/8 females in the 2000 mg/kg group died or were sacrificed *in extremis*. The only survivor at 2000 mg/kg had total resorptions. At 1000 mg/kg, one female died spontaneously and one was sacrificed *in extremis*. Both top dose groups showed significant body weight loss and significantly reduced feed consumption. All eight top dose females and four 1000 mg/kg bw/d females revealed stomach ulcerations. Surviving 1000 mg/kg bw/d females showed an increase in resorptions. Food consumption and weight gains were reduced at 500 mg/kg bw/d, due primarily to effects in one animal. The litter sizes were reduced at 500 mg/kg bw/d due primarily to a lower number of corpora lutea (an observation independent of treatment). Other than reductions in foetal weights in the 500 and 1000 mg/kg bw/d dose groups, no alterations were seen in foetuses during external examinations. Based on these findings, the maximum tolerated dose was considered to lie between 500 and 1000 mg/kg bw/d.

A validated method of analysis has not been submitted (see Volume 3 CA B5, section B.5.1.2).

### Results

The number of females with implantation sites at necropsy was insufficient for all groups (13 in the control, 15 in 30, 200 and 500 mg/kg bw/d dose groups and 7 in the top dose group); this limited the reliability and validity of the study.

#### *Maternal toxicity*

Two females of the low dose (30 mg/kg bw/d) and one in the top dose (750 mg/kg bw/d) died during the study, however, due to the lack of dose-response, mortalities were not considered to be treatment-related. There were no treatment-related clinical signs observed throughout the study.

Food consumption of pregnant females over the administration period (GD 7 – 20) was reduced in a dose-dependent manner; a reduction of 10 % and 24 % were recorded at 500 and 750 mg/kg bw/d, respectively, however, statistical-significance was not reached (Table 6.6-82). Food consumption in treated animals was comparable to controls during the pre- and post-treatment periods. Statistically-significant reductions in food consumption were seen in non-pregnant females from a dose of 500 mg/kg bw/d, during the administration period (GD 13 – 16 and 16 – 20) (Table 6.6-83); changes compared to controls were between 26 – 43 %.

Small reductions in body weight of treated animals compared to controls (< 10 %) were seen in pregnant females from 500 mg/kg bw/d; however, no statistical-significance was seen (Table 6.6-84). Statistically-significant reductions in body weight gain (losses in body weight) were seen in pregnant females at the top dose during the administration period (Table 6.6-85). During the administration period (GD 7 – 20) reductions in body weight gain were > 10 % from 200 mg/kg bw/d (however, this group recovered during the post-administration period and showed an overall increase compared to controls) and body weight losses were recorded from 500 mg/kg bw/d. The pattern of effects on body weight gain was confirmed by (and was more dramatic in) non-pregnant females (Table 6.6-86). Statistically-significant reductions in body weight gain (losses in body weight) were seen in from 500 mg/kg bw/d during the administration period.

There were no treatment-related effects on mean carcass weight and corrected body weight gain (Table 6.6-87). Mean gravid uterus weight was reduced in the top two doses (-29 and -25 %, respectively), although without a dose-response and/or statistical-significance. This reduction was based on the lower litter size from 500 mg/kg bw/d (Table 6.6-89). This finding was not considered treatment-related, as it was based on events which occurred prior to the administration period (see developmental toxicity section below). There was no treatment-related effects on liver weight (absolute or relative) (Table 6.6-88).

Overall, treatment-related and adverse reductions in food consumption and body weight gain (body weight losses) were seen from 500 mg/kg bw/d.

Table 6.6-82. Food consumption - pregnant females

Dose [mg/kg bw/d]	Animals examined <sup>a)</sup>	Day of Gestation		
		0 - 7	7 - 20	20 - 29
0	N = 13 Mean ± SD [g/animal/d]	143.8 ± 12.19	126.5 ± 32.46	80.0 ± 41.53
30	N = 12 Mean ± SD [g/animal/d] [Δ% control]	146.7 ± 8.18 +2.0	138.3 ± 12.66 +9.3	84.3 ± 26.04 +5.4
200	N = 13 Mean ± SD [g/animal/d] [Δ% control]	145.4 ± 10.93 +1.1	126.1 ± 29.69 -0.3	89.6 ± 39.90 +12.0
500	N = 10 Mean ± SD [g/animal/d] [Δ% control]	150.0 ± 3.21 +4.3	114.3 ± 30.31 <b>-9.6</b>	78.7 ± 55.92 -1.6
750	N = 16 Mean ± SD [g/animal/d] [Δ% control]	148.4 ± 7.71 +3.2	96.0 ± 25.50 <b>-24.1</b>	82.20 ± 19.08 +2.8

\* p &lt; 0.05, \*\* p &lt; 0.01

<sup>a)</sup> Data from females that were non-pregnant, aborted, delivered early, had total resorptions, or died prior to scheduled sacrifice were excluded.Table 6.6-83. Food consumption – non-pregnant females

Dose [mg/kg bw/d]	Animals examined <sup>a)</sup>	Day of Gestation				
		0 - 7	7 - 20	20 - 29	13 - 16	16 - 20
0	N = 7 Mean [g/animal/d]	149.1	144.9	145	146.0	141.5
30	N = 5 Mean [g/animal/d] [Δ% control]	150.1 +0.7	138.3 -4.6	128.8 -11.2	134.9 -7.6	133.6 -5.6
200	N = 5 Mean [g/animal/d] [Δ% control]	149.7 +0.4	142.3 -1.8	149.5 +3.1	141.5 -3.1	140.2 -0.9
500	N = 4/5 <sup>a)</sup> Mean [g/animal/d] [Δ% control]	148.0 -0.7	107.0 -26.2	119.0 -17.9	<b>97.0*</b> -33.6	<b>91.1*</b> -35.6
750	N = 13 Mean [g/animal/d] [Δ% control]	146.3 -1.9	<b>95.3*</b> -34.2	131.0 -9.7	<b>99.5*</b> -31.8	<b>81.3*</b> -42.5

\* p &lt; 0.05, \*\* p &lt; 0.01

<sup>a)</sup> Feeder weights were not collected for doe #21083 on GD16; therefore, 4 data points are available for periods GD 13-16, 16-20 and 7-20

Δ% control – percent change compared to control.

Table 6.6-84. Body weight - pregnant females

Dose [mg/kg bw/d]		Day of Gestation					
		0	7	10	16	20	29
0	Mean [g]	3939.0	4072.9	4116.8	4155.7	4174.1	4174.6
	SD	281.0	271.1	346.2	292.6	297.4	337.6
30	Mean [g]	4020.5	4119.6	4087.1	4240.8	4247.9	4199.3
	SD	206.7	219.5	239.9	221.9	232.1	259.4
	[Δ% control]	+2.1	+1.1	-0.7	+2.0	+1.8	+0.6
200	Mean [g]	4000.6	4112.2	4125.8	4186.1	4198.7	4250.1
	SD	214.5	213.5	250.4	280.3	323.2	328.4
	[Δ% control]	+1.6	+1.0	+0.2	+0.7	+0.6	+1.8
500	Mean [g]	4005.8	4117.6	4101.3	4159.8	4084.1	4067.9
	SD	200.1	236.6	240.6	290.3	317.1	490.6
	[Δ% control]	+1.7	+1.1	-0.4	+0.1	-2.2	-2.6
750	Mean [g]	3999.0	4165.4	4071.1	4109.2	4029.9	4161.9
	SD	277.2	262.6	283.8	267.1	313.1	310.9
	[Δ% control]	+1.5	+2.3	-1.1	-1.1	-3.5	-0.3

\* p &lt; 0.05, \*\* p &lt; 0.01;

Δ% control – percent change compared to control.

Table 6.6-85. Body weight gain - pregnant females

Dose [mg/kg bw/d]		Day of Gestation					
		0 - 7	7 - 20	0 - 29	7 - 10	16-20	20- 29
0	Mean [g]	133.9	101.2	235.6	44.0	18.4	0.4
	SD	107.7	128.2	243.2	117.1	64.5	167.9
30	Mean [g]	99.1	128.3	178.8	-32.5	7.1	-48.7
	SD	71.8	69.6	158.4	93.4	53.3	164.8
	[Δ% control]	-26.0	+26.8	-24.1	-174.0	-61.5	-11004.3
200	Mean [g]	111.5	86.5	249.5	13.6	12.6	51.4
	SD	69.1	136.6	206.4	58.7	64.7	147.8
	[Δ% control]	-16.7	-14.5	+5.9	-69.1	-31.6	11419.0
500	Mean [g]	111.8	-33.5	62.1	-16.3	-75.7	-16.2
	SD	83.7	191.6	396.9	48.4	143.6	231.5
	[Δ% control]	-16.5	-133.1	-73.7	-137.1	-510.7	-3731.0
750	Mean [g]	166.4	<b>-135.5*</b>	162.9	<b>-94.3*</b>	<b>-79.3*</b>	132.0
	SD	36.2	76.2	69.7	44.3	60.0	59.4
	[Δ% control]	+24.3	-233.8	-30.9	-314.4	-530.2	29482.5

\* p &lt; 0.05, \*\* p &lt; 0.01;

Δ% control – percent change compared to control.

Table 6.6-86. Body weight gain – non-pregnant females

Dose [mg/kg bw/d]		Day of Gestation			
		0 - 7	7 - 20	0 - 29	7 - 10
0	Mean [g]	84.1	82.7	55.2	138.1
30	Mean [g]	46.4	31.9	17.9	52.4
	[Δ% control]	-44.8	-61.4	-67.6	-62.1
200	Mean [g]	79.0	50.7	4.7	119.2
	[Δ% control]	-6.1	-38.7	-91.5	-13.7
500	Mean [g]	70.6	<b>-129.7*</b>	-23.6	124.5
	[Δ% control]	-16.1	-256.8	-142.8	-9.8
750	Mean [g]	118.3	<b>-171.0*</b>	<b>-75.2*</b>	173.5
	[Δ% control]	40.7	-306.8	-236.2	25.6

Dose [mg/kg bw/d]	Day of Gestation			
	0 - 7	7 - 20	0 - 29	7 - 10

\* p < 0.05, \*\* p < 0.01;

Δ% control – percent change compared to control.

Table 6.6-87. Uterus weight, carcass weight and corrected (net) body weight gain

Parameter [g]		Dose [mg/kg bw/d]				
		0	30	200	500	750
Gravid uterus	[mean]	396.1	380.2	391.1	280.4	297.8
	[SD]	80.8	91.7	104.9	116.3	158.0
	[Δ% control]		-4.0	-1.3	-29.2	-24.8
Carcass	[mean]	3778.4	3819.0	3859.0	3787.5	3864.1
	[SD]	360.4	255.8	342.5	477.9	280.9
	[Δ% control]		+1.1	+2.1	+0.2	+2.3
Net change from GD 6	[mean]	-294.4	-300.6	-253.2	-330.1	-301.3
	[SD]	252.6	181.7	210.9	387.9	129.1
	[Δ% control]		+2.1	-14.0	+12.1	+2.3

\* p < 0.05, \*\* p < 0.01

Carcass weight = terminal body weight minus uterine weight (values are rounded)

Net weight change from GD 6 = carcass weight minus GD 6 body weight

Δ% control – percent change compared to control.

Table 6.6-88. Liver weights - pregnant females

Organs	Dose [mg/kg bw/d]	Absolute weight		Relative weight <sup>#</sup>	
		[g]	Δ%	[% of bw]	Δ%
Carcass	0	3778.4			
	30	3819.0	+1.1		
	200	3859.0	+2.1		
	500	3787.5	+0.2		
	750	3864.1	+2.3		
Liver	0	120.2		3.2	
	30	107.2	-10.9	2.8	-11.8
	200	116.1	-3.5	3.0	-5.8
	500	122.8	+2.1	3.2	+1.8
	750	117.2	-2.5	3.0	-4.8

\* p ≤ 0.05, \*\* p < 0.01; <sup>#</sup> = relative to carcass weight

Δ% - percent change compared to control.

#### *Developmental toxicity*

Whilst not considered treatment-related (as administration began after artificial insemination), low conception rates (65 % for the control, 75 % for 30, 200 and 500 mg/kg bw/d dose groups and 35 % for the top dose group) (Table 6.6-89), raised concerns regarding the insemination protocol. The number of females with implantation sites at necropsy was insufficient for all groups (13 in the control, 15 in 30, 200 and 500 mg/kg bw/d dose groups and 7 in the top dose group); this limited the reliability and validity of the study.

There were no treatment-related effects on abortions and/or premature deliveries. The mean number of corpora lutea and implantation sites were slightly but not statistically-significantly reduced from 500 mg/kg bw/d, resulting in an increase in pre-implantation loss. However, this effect occurred prior to administration and was therefore not considered treatment-related. Post-implantation loss was not affected by treatment. The increase in early resorptions at the top dose was mainly due to one litter with only four implantation sites, of which two were resorbed. The sex ratio of foetuses was variable but not affected by treatment (no statistical-significance). A statistically-significant increase in mean foetal weight, as a consequence of a reduction in litter size, was seen in the top dose; this was not considered to be treatment-related.

There were no treatment-related external, visceral or skeletal malformations (Tables 6.6-90, 6.6-91 and 6.6-93), variations or retardations (Tables 6.6-92 and 6.6-94 and 6.6-95).

Table 6.6-89. Pregnancy status and caesarean section data

Dose [mg/kg bw/d]	0	30	200	500	750
<b>Pregnancy status</b>					
Females					
- inseminated [n]	20	20	20	20	20
- pregnant [n]	13	15	15	15	7
conception rate [%]	65	75	75	75	35
- aborted [n]	0	0	0	1	1
- premature birth [n]	0	1	2	3	0
- Does with viable fetuses [n]	13	12	13	10	6
- Does with all resorptions [n]	0	1 <sup>#</sup>	0	1	0
- Mortality	0	2 <sup>#</sup>	0	0	1
- Pregnant terminal sacrifice [n]	13	12	13	12	6
<b>Caesarean section data<sup>a</sup></b>					
- Pregnant terminal sacrifice [n]	13	12	13	12	6
- Corpora lutea [mean]	11.5 ± 3.5	11.3 ± 3.5	12.6 ± 3.1	10.3 ± 2.7	9.3 <sup>s</sup> ± 2.5
total number [n]	150	135	164	103	56 <sup>s</sup>
- Implantation sites [mean]	8.3 ± 2.6	8.1 ± 2.1	8.0 ± 2.9	6.2 ± 2.7	5.5 ± 2.8
total number [n]	108	97	104	62	33
- Pre-implantation loss [%]	24.6 ± 23.3	25.4 ± 18.8	33.6 ± 24.0	40.6 ± 25.7	39.8 ± 29.9
- Post-implantation loss [%]	7.2 ± 10.1	0.0 ± 0.0	4.9 ± 7.9	6.8 ± 12.0	10.0 ± 20.0
- Resorptions [mean]	0.6 ± 0.8	0.0 ± 0.0	0.5 ± 0.9	0.5 ± 0.9	0.5 ± 0.8
total number [n]	8	0	7	5	3
- Early resorptions [%]	1.0 ± 3.5	0.0 ± 0.0	2.1 ± 5.3	4.0 ± 9.3	8.3 ± 20.4
number [mean]	0.1 ± 0.3	0.0 ± 0.0	0.2 ± 0.6	0.3 ± 0.7	0.3 ± 0.8
total number [n]	1	0	3	3	2
- Late resorptions [%]	6.2 ± 10.2	0.0 ± 0.0	2.8 ± 6.8	2.9 ± 9.0	1.7 ± 4.1
number [mean]	0.5 ± 0.8	0.0 ± 0.0	0.3 ± 0.8	0.2 ± 0.6	0.2 ± 0.4
total number [n]	7	0	4	2	1
- Dead fetuses [n]	0	0	0	0	0
- Live fetuses [mean]	7.7 ± 2.4	8.1 ± 2.1	7.5 ± 2.4	5.7 ± 2.6	5.0 ± 2.8
total number [n]	100	97	97	57	30
mean [%]	92.8 ± 10.1	100.0 ± 0.0	95.1 ± 7.9	93.2 ± 12.0	90.0 ± 20.0
- Total live female fetuses [mean]	4.3 ± 2.2	5.2 ± 2.0	2.8 ± 1.2	3.4 ± 2.4	3.3 ± 2.3
total number [n]	56	62	36	34	20
- Total live male fetuses [mean]	3.4 ± 1.6	2.9 ± 1.3	4.7 ± 1.9	2.3 ± 1.3	1.7 ± 1.6
total number [n]	44	35	61	23	10
- Percent live females [%]	56.0	63.9	37.1	59.6	66.7
- Percent live males [%]	44.0	36.1	62.9	40.4	33.3
Mean foetal weight [g]	41.2 ± 6.8	40.2 ± 5.6	43.5 ± 7.5	40.4 ± 10.3	48.3* ± 4.9
- males [g]	42.8	41.1	43.2	42.2	49.8
- females [g]	40.1	39.8	44.0	36.3	47.5

<sup>a</sup> Mean ± SD on litter basis; Statistical evaluation: \* p ≤ 0.05; \*\* p < 0.01

<sup>#</sup> = animal #21025 died on GD 24 due to a heart defect and had resorbed its complete litter (13 corpora lutea, 5 implantations, 5 late resorptions)

<sup>s</sup> = mean value deviate from study report due to an obvious typo for animal #21095 that is reported to have 9 corpora lutea and 10 implantations (Appendix I, p. 153) that is physiologically not possible. Thus, this animal was assumed to have 10 corpora lutea.

Table 6.6-90. External malformations

Dose [mg/kg bw/d]	0	30	200	500	750
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Dose [mg/kg bw/d]		0	30	200	500	750
Litters evaluated		13	12	13	10	6
Foetuses evaluated		100	97	97	57	30
Live		100	97	97	57	30
Dead		0	0	0	0	0
<b>Total external malformations</b>						
Foetal incidence	# (%)	1 (1.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)
Litter incidence	# (%)	1 (7.7)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)
Affected foetuses / litter	%	1.10	0.0	0.8	0.0	0.0
<b>Individual external malformations</b>						
<b>Malformed rump</b>						
Foetal incidence	# (%)	1 (1.0)				
Litter incidence	# (%)	1 (7.7)				
Affected foetuses / litter	%	1.10				
<b>Short tail</b>						
Foetal incidence	# (%)			1 <sup>§</sup> (1.0)		
Litter incidence	# (%)			1 (7.7)		
Affected foetuses / litter	%			0.8		

\* p &lt; 0.05, \*\* p &lt; 0.01

§ = finding with corresponding skeletal malformation – malformed caudal vertebrae

Table 6.6-91. Visceral malformations

Dose [mg/kg bw/d]		0	30	200	500	750
Litters evaluated		13	12	13	10	6
Foetuses evaluated		100	97	97	57	30
Live		100	97	97	57	30
Dead		0	0	0	0	0
<b>Total visceral malformations</b>						
Foetal incidence	# (%)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Litter incidence	# (%)	0 (0.0)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
Affected foetuses / litter	%	0.0	1.0	0.0	0.0	0.0
<b>Individual visceral malformations</b>						
<b>Liver fissure</b>						
Foetal incidence	# (%)		1 (1.0)			
Litter incidence	# (%)		1 (8.3)			
Affected foetuses / litter	%		1.0			

\* p &lt; 0.05, \*\* p &lt; 0.01

Table 6.6-92. Visceral variations / retardations

Dose [mg/kg bw/d]		0	30	200	500	750
Litters evaluated		13	12	13	10	6
Foetuses evaluated		100	97	97	57	30
<b>Total visceral variations / retardations</b>						
Foetal incidence	# (%)	56 (56.0)	56 (57.7)	59 (60.8)	43 (75.4)	17 (56.7)
Litter incidence	# (%)	12 (92.3)	12 (100)	13 (100)	10 (100)	5 (83.3)
Affected foetuses / litter	%	55.7	58.1	59.8	82.5	53.5
<b>Individual visceral variations / retardations</b>						
<b>Bladder, haemorrhage</b>						
Foetal incidence	# (%)	11 (11.0)	7 (7.2)	14 (14.4)	3 (5.3)	3 (10.0)
Litter incidence	# (%)	5 (38.5)	5 (41.7)	6 (46.2)	2 (20.0)	1 (16.7)
Affected foetuses / litter	%	13.6	7.8	14.2	14.0	7.1
<b>Gallbladder, small</b>						
Foetal incidence	# (%)	2 (2.0)		1 (1.0)	1 (1.8)	1 (3.3)
Litter incidence	# (%)	1 (7.7)		1 (7.7)	1 (10.0)	1 (16.7)
Affected foetuses / litter	%	1.9		0.8	2.0	1.9

Dose [mg/kg bw/d]		0	30	200	500	750
Litters evaluated		13	12	13	10	6
Foetuses evaluated		100	97	97	57	30
<b>Great heart vessels, left carotid off innominate</b>						
Foetal incidence	# (%)	37 (37.0)	42 (43.3)	47 (48.5)	31 (54.4)	6 (20.0)
Litter incidence	# (%)	10 (76.9)	9 (75.0)	12 (92.3)	9 (90.0)	3 (50.0)
Affected foetuses / litter	%	34.4	43.7	48.2	64.5	21.3
<b>Kidney, dilated renal pelvis</b>						
Foetal incidence	# (%)	1 (2.0)	1 (1.0)			
Litter incidence	# (%)	1 (7.7)	1 (8.3)			
Affected foetuses / litter	%	1.9	0.9			
<b>Supernumerary vessel, extra vessel originating from either innominate, left carotid, right carotid, or right subclavian</b>						
Foetal incidence	# (%)	31 (31.0)	23 (23.7)	33 (34.0)	22 (38.6)	12 (40.0)
Litter incidence	# (%)	12 (92.3)	11 (91.7)	10 (76.9)	8 (80.0)	5 (83.3)
Affected foetuses / litter	%	33.0	24.2	33.4	43.2	40.9
<b>Subcutis, hematoma</b>						
Foetal incidence	# (%)				2 (3.5)	
Litter incidence	# (%)				1 (10.0)	
Affected foetuses / litter	%				4.0	

\* p < 0.05, \*\* p < 0.01

Table 6.6-93. Skeletal malformations

Dose [mg/kg bw/d]		0	30	200	500	750
Litters evaluated		13	12	13	10	6
Foetuses evaluated		100	97	97	57	30
<b>Total skeletal malformations</b>						
Foetal incidence	# (%)	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.8)	0 (0.0)
Litter incidence	# (%)	0 (0.0)	0 (0.0)	1 (7.7)	1 (10.0)	0 (0.0)
Affected foetuses / litter	%	0.0	0.0	0.8	5.0 ± 15.8	0.0
<b>Individual skeletal malformations</b>						
<b>Caudal vertebrae malformations (hemivertebra, misshapen, absent)</b>						
Foetal incidence	# (%)			1 <sup>§</sup> (1.0)		
Litter incidence	# (%)			1 (7.7)		
Affected foetuses / litter	%			0.8		
<b>Lumbar vertebra, hemivertebra</b>						
Foetal incidence	# (%)				1 (1.8)	
Litter incidence	# (%)				1 (10.0)	
Affected foetuses / litter	%				5.0 ± 15.8	

\* p < 0.05, \*\* p < 0.01

§ = finding with corresponding external malformation – short tail

Table 6.6-94. Skeletal variations / retardations

Dose [mg/kg bw/d]		0	30	200	500	750
Litters evaluated		13	12	13	10	6
Foetuses evaluated		100	97	97	57	30
<b>Total skeletal variations / retardations</b>						
Foetal incidence	# (%)	73 (73.0)	67 (69.1)	65 (67.0)	29 (50.9)	18 (60.0)
Litter incidence	# (%)	13 (100)	12 (100)	13 (100)	7 (70.0)	5 (83.3)
Affected foetuses / litter	%	68.5	67.0	69.6	43.9	57.1
<b>Individual skeletal variations / retardations</b>						
<b>Hyoid, bent</b>						
Foetal incidence	# (%)	3 (3.0)	2 (2.1)	2 (2.1)	1 (1.8)	
Litter incidence	# (%)	3 (23.1)	2 (16.7)	1 (7.7)	1 (10.0)	
Affected foetuses / litter	%	3.9	2.2	7.7	2.0	



Dose [mg/kg bw/d]		0	30	200	500	750
Litters evaluated		13	12	13	10	6
Foetuses evaluated		100	97	97	57	30
<b>Hyoid, partially ossified</b>						
Foetal incidence	# (%)	38 (38.0)	43 (44.3)	35 (36.1)	13 (22.8)	2 (6.7)
Litter incidence	# (%)	9 (69.2)	11 (91.7)	11 (84.6)	4 (40.0)	2 (33.3)
Affected foetuses / litter	%	35.3	41.4	32.3	20.0	4.2
<b>Hyoid, unossified</b>						
Foetal incidence	# (%)	2 (2.0)	1 (1.0)	4 (4.1)		
Litter incidence	# (%)	1 (7.7)	1 (8.3)	3 (23.1)		
Affected foetuses / litter	%	1.7	1.2	3.4		
<b>Skull / nasal bone, bone island</b>						
Foetal incidence	# (%)		1 <sup>#</sup> (1.0)		1 (1.8)	
Litter incidence	# (%)		1 (8.3)		1 (10.0)	
Affected foetuses / litter	%		1.2		2.0	
<b>Skull, frontal / maxilla / interparietal partially ossified</b>						
Foetal incidence	# (%)		1 <sup>#</sup> (1.0)	1 (1.0)		1 (3.3)
Litter incidence	# (%)		1 (8.3)	1 (7.7)		1 (16.7)
Affected foetuses / litter	%		1.2	1.0		1.9
<b>Rib, rudimentary, 1<sup>st</sup> lumbar</b>						
Foetal incidence	# (%)	7 (7.0)	21 (21.7)	8 (8.3)	4 (7.0)	6 (20.0)
Litter incidence	# (%)	5 (38.5)	10 (83.3)	6 (46.2)	4 (38.5)	2 (33.3)
Affected foetuses / litter	%	5.8	22.4	11.8	5.8	12.2
<b>Rib, extra ossification site, 1<sup>st</sup> lumbar</b>						
Foetal incidence	# (%)	3 (3.0)		4 (4.1)		1 (3.3)
Litter incidence	# (%)	2 (15.4)		3 (23.1)		1 (16.7)
Affected foetuses / litter	%	3.3		4.6		4.2
<b>Rib, extra lumbar (bi or unilateral)</b>						
Foetal incidence	# (%)	43 (43.0)	25 (25.8)	28 (28.9)	20 (35.1)	11 (36.7)
Litter incidence	# (%)	11 (84.6)	10 (83.3)	11 (84.6)	7 (70.0)	4 (66.7)
Affected foetuses / litter	%	40.2	25.5	30.4	29.6	42.6
<b>Vertebra, extra lumbar</b>						
Foetal incidence	# (%)			1 (1.0)		
Litter incidence	# (%)			1 (7.7)		
Affected foetuses / litter	%			1.3		
<b>Sternebrae, fused and/or bipartite</b>						
Foetal incidence	# (%)		1 (1.0)	2 (2.1)		
Litter incidence	# (%)		1 (8.3)	2 (15.4)		
Affected foetuses / litter	%		1.0	1.8		
<b>Sternebrae, partially ossified</b>						
Foetal incidence	# (%)	8 (8.0)	12 (12.4)	12 (12.4)	4 (7.0)	4 (13.3)
Litter incidence	# (%)	5 (38.5)	6 (50.0)	5 (38.5)	2 (20.0)	2 (33.3)
Affected foetuses / litter	%	7.7	12.0	12.4	8.0	13.9
<b>Sternebrae, unossified</b>						
Foetal incidence	# (%)	3 (3.0)	1 (1.0)	3 (3.1)	1 (1.8)	
Litter incidence	# (%)	2 (15.4)	1 (8.3)	2 (15.4)	1 (10.0)	
Affected foetuses / litter	%	3.0	0.9	2.7	2.0	
<b>Fore / Hindlimb bones, unossified</b>						
Foetal incidence	# (%)		1 (1.0)			
Litter incidence	# (%)		1 (8.3)			
Affected foetuses / litter	%		1.0			

\* p < 0.05, \*\* p < 0.01 ; # = findings of the same foetus

Table 6.6-95. Total anomalies, malformations and variations

Dose [mg/kg bw/d]	0	30	200	500	750
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Dose [mg/kg bw/d]		0	30	200	500	750
Litters evaluated		13	12	13	10	6
Foetuses evaluated		100	97	97	57	30
Live		100	97	97	57	30
Dead		0	0	0	0	0
<b>Total anomalies</b>						
Foetal incidence	# (%)	89 (89.0)	80 (82.5)	85 (87.6)	53 (93.0)	24 (80.0)
Litter incidence	# (%)	13 (100)	12 (100)	13 (100)	10 (100)	5 (83.3)
Affected foetuses / litter	%	87.5 ± 16.5	80.9 ± 17.0	87.5 ± 17.8	95.0 ± 12.1	72.6 ± 37.5
<b>Total malformations</b>						
Foetal incidence	# (%)	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.8)	0 (0.0)
Litter incidence	# (%)	1 (7.7)	1 (8.3)	1 (7.7)	1 (10.0)	0 (0.0)
Affected foetuses / litter	%	1.1 ± 4.0	1.0 ± 3.6	0.8 ± 2.8	5.0 ± 15.8	0.0
<b>Total variations</b>						
Foetal incidence	# (%)	89 (89.0)	80 (82.5)	85 (87.6)	53 (93.0)	24 (80.0)
Litter incidence	# (%)	13 (100)	12 (100)	13 (100)	10 (100)	5 (83.3)
Affected foetuses / litter	%	87.5 ± 16.5	80.9 ± 17.0	87.5 ± 17.8	95.0 ± 12.1	72.6 ± 37.5

#### Conclusions

In conclusion, under the conditions of this limited development toxicity study in the rabbit, no treatment-related malformations or variations (external, skeletal or visceral) were observed up to 750 mg/kg bw/d (the highest dose tested).

Maternal toxicity - reduced food consumption and body weight gain (body weight losses) - was observed from 500 mg/kg bw/d. No maternal toxicity was observed at lower dose levels. Due to the study limitations, no robust conclusions have been drawn and no robust NOAELs set.

(██████, 1987)

#### B.6.6.3. Summary of reproductive toxicity

The reproductive toxicity of cinmethylin has been investigated in a new/modern guideline dietary 2-generation study in rats and a new/modern guideline gavage pre-natal developmental toxicity study in rabbits. A relatively old pre-natal developmental toxicity study in rats is considered to be of sufficient quality to meet the data requirements. An older 2-generation study in rats and the two older pre-natal developmental toxicity studies in rabbits have been discounted due to significant limitations, which compromise their reliability. The main findings are summarised (Table 6.6-96) below.

The following key conclusions were obtained from the evaluation of the reproductive toxicity information:

- There were no effects on fertility and reproductive performance up to dose levels causing generalised toxicity
- There were no specific developmental effects in rats or rabbits up to doses causing maternal toxicity
- Classification for reproductive toxicity is not required. Further details are available in the aligned MCL dossier
- The data requirements of Regulation 283/2013 have been met.

Table 6.6-96. Summary of reproductive toxicity studies with cinmethylin

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
2-generation study (dietary).  GLP-compliant. OECD test guideline (No. 416) compliant.  Cinmethylin Batch: COD- 002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  [REDACTED] <a href="#">2018a</a> <a href="#">2017/1094504</a> and [REDACTED]. <a href="#">2018</a> <a href="#">2018/1099151</a>  <i>Acceptable.</i>	Rat (Wistar).  Male and female.  25/sex/dose.	0, 125/250, 500/1000 and 2500/5000 ppm.  Equivalent to: 0, 19.7-21.8, 79.4- 87.7 and 412-450 mg/kg bw/d in males.  0, 21.4-22.8, 82.2- 90.1 and 417-460 in females (pre- mating)  0, 20.6-20.7, 81.3- 81.6 and 394- 395 mg/kg bw/d in females (during gestation).  0, 23.5-23.8, 93.8- 96.9 and 473- 481 mg/kg bw/d in females (during lactation).	Reproductive toxicity: <b>394</b> [2500/5000]  (the highest dose tested)	Reproductive toxicity: N/A – no adverse treatment-related findings were observed up to the top dose.
			Parental toxicity: <b>80</b> [500/1000]	Parental toxicity: <u>2,500/5,000 ppm</u> <u>(394 - 481 mg/kg</u> <u>bw/d):</u> ↓food consumption (♀) ↓body weight (♀) ↓body weight gain (♀) ↑liver weight, absolute (19-24 % in ♂, 19-20 % in ♀) and relative (22- 26 % in ♂, 21-25 % in ♀). ↑thyroid weight, absolute (15-21 % in ♂, 15-22 % in ♀) and relative (17- 23 % in ♂, 19-24 % in ♀). Histopathology of the thyroid – hypertrophy/hyperp lasia of follicular epithelial cells. Histopathology of the nasal cavity – degeneration/regen eration of the olfactory epithelium.
			Developmental/Off spring toxicity: <b>394</b> [2500/5000]  (the highest dose tested)	Developmental/Off spring toxicity: N/A – no adverse treatment-related findings were observed up to the top dose.
2-generation study (dietary).  GLP-compliant. Non-OECD test guideline	Rat (Sprague Dawley).  Male and female.  20-30/sex/dose.	0, 200, 2000 and 20000 ppm  Equivalent to: 0, 11.3-16.1, 115- 163 and 1289-2125	Reproductive toxicity: Not set due to study limitations.	Reproductive toxicity: N/A - due to study limitations.
			Parental and offspring toxicity:	Parental and offspring toxicity:

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
compliant.  Cinmethylin Batch: 513K Purity (%): 92.4 (-) / (+) ratio = not specified. and Batch: 513N Purity (%): 93 (-) / (+) ratio = not specified.  <a href="#">[REDACTED], 1986</a> <a href="#">CI-430-001</a>  <i>Not acceptable</i>		mg/kg bw/d in males.  0, 13.9-17.3, 139-170 and 1450-2213 mg/kg bw/d in females (during pre-mating).  0, 12.8-14.7, 130-148 and 1434-1609 mg/kg bw/d in females (during gestation).  0, 30.4-34.0, 280-353 and 2256-2893 mg/kg bw/d in females (during lactation).	Not set due to study limitations.	N/A - due to study limitations.
Pre-natal developmental toxicity study (oral gavage)  GLP compliant. Non-OECD test guideline compliant.  Cinmethylin Batch: 513H Purity (%): 92.4 (-) / (+) ratio = not specified.  <a href="#">[REDACTED], 1984</a> <a href="#">CI-432-001</a>  <i>Acceptable</i>	Rat (Sprague Dawley).  Male and female.  25 pregnant females/dose.	0, 30, 300, 1000 and 2000 mg/kg bw/d.	Maternal toxicity: <b>30</b>	Maternal toxicity: <u>300 mg/kg bw/d</u> : Clinical observations (excess salivation and urine-stained abdominal fur). ↓body weight gain (seen in the first few days of the study and over the administration period).
			Developmental toxicity: <b>300</b>	Developmental toxicity: <u>1000 mg/kg bw/d</u> : ↑incidence of anomalies (predominantly variations) – indicative of delayed development.
Pre-natal developmental toxicity study (oral gavage)  GLP compliant. OECD test guideline No. 414 (2001) compliant.  Cinmethylin Batch: COD-	Rabbit (New Zealand White).  Female.  25 inseminated females/dose.	0, 25, 80, 250 and 320 mg/kg bw/d.	Maternal toxicity: <b>80</b>	Maternal toxicity: <u>250 mg/kg bw/d</u> : ↓body weight gain (22 %) (over the duration of the study, including the first few days of administration (GD 6 – 9)). ↑liver weight, absolute (18 %) and relative (21 %).

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw/d) [ppm]	Effects at the LOAEL
001950 Purity (%): 96.3 (-) / (+) ratio = not specified.  ██████████. <a href="#">2018 b</a> <a href="#">2015/1158053</a>  <i>Acceptable</i>				Clinical chemistry (↑GGT, 51 %).
			Developmental toxicity: <b>80</b>	Developmental toxicity: <u>250 mg/kg bw/d</u> : ↓foetal weight (14 %).
Pre-natal developmental toxicity study (oral gavage)  GLP compliant. Non-OECD test guideline compliant.  Cinmethylin Batch: 513N Purity (%): 93 (-) / (+) ratio = not specified.  <i>Not acceptable.</i>  ██████████, 1985 <a href="#">CI-432-002</a>	Rabbit (New Zealand White).  Female.  19-20 inseminated females/dose.	0, 3, 30 and 100 mg/kg bw/d.	Not set due to study limitations.	N/A - due to study limitations.
Pre-natal developmental toxicity study (oral gavage)  GLP compliant. Non-OECD test guideline compliant.  Cinmethylin Batch: not specified. Purity (%): 92.4 (-) / (+) ratio = not specified.  <i>Not acceptable.</i>  ██████████, 1987 CL-432-003	Rabbit (New Zealand White).  Female.  20 inseminated females/dose.	0, 30, 200, 500 and 750 mg/kg bw/d.	Not set due to study limitations.	N/A - due to study limitations.

#### Effects on Sexual Function and Fertility

The potential of cinmethylin to adversely affect sexual function and fertility has been well investigated in a standard 2-generational dietary study conducted in rats.

Cinmethylin did not adversely affect fertility and reproduction; oestrus cyclicity, mating performance and fertility, differential ovarian follicle count, pup survival and sex ratio were not affected by treatment up to the top dose of 394 - 481 mg/kg bw/d. At this dose parental but not offspring toxicity occurred. In addition, examination of the reproductive organs and specific investigations of sperm parameters did not reveal any treatment-related changes. Therefore, **a NOAEL for reproductive toxicity of 394 mg/kg bw/d** (highest dose tested) can be identified from this study.

In relation to general toxicity in parental animals, reductions in food consumption, body weights and body weight gains were recorded at the top dose of 394 – 481 mg/kg bw/d. Increases in liver and thyroid weights (with concomitant thyroid histopathology) were also seen at the top dose of 394 – 481 mg/kg bw/d. In addition, adverse histopathological changes were seen in the nasal cavities at the top dose of 394 – 481 mg/kg bw/d. There were no treatment-related effects in parental animals at the low and mid doses. Therefore, **a NOAEL of 80 mg/kg bw/d can be identified for parental toxicity** from this study. **As no offspring toxicity was observed up to the top dose, a NOAEL of 394 mg/kg bw/d** (highest dose tested) can be identified from this study.

#### Developmental Toxicity

The developmental toxicity of cinmethylin has been investigated in gavage pre-natal developmental toxicity studies, conducted in rats (an older study) and rabbits (a guideline new/modern study). Additional information on the developmental toxicity potential of cinmethylin is also available from the new/modern 2-generation study.

In the rat developmental study, there were no effects of treatment on malformations (external, visceral and skeletal) up to the top dose of 2,000 mg/kg bw/d. Foetal weight was reduced at 2000 mg/kg bw/d in males and females; in addition, there was a marginal increase in post-implantation loss (due to two whole litter resorptions) at the top dose of 2,000 mg/kg bw/d. Incidences of skeletal and visceral variations were increased in the top two doses, from 1,000 mg/kg bw/d. At the top dose of 2000 mg/kg bw/d, the incidences of visceral (slight to moderate dilated ventricles in the brain) and skeletal variations such as wavy ribs and incompletely ossified structures were also significantly increased. Most developmental effects (skeletal variations, incomplete ossification, decreased foetal weight and post-implantation loss) were considered the unspecific, secondary consequence of the maternal toxicity recorded from 300 mg/kg bw/d (limited number of clinical signs of toxicity and decreases in body weight gain). No maternal toxicity was observed at the low dose (30 mg/kg bw/d). The increased incidence of slight to moderate dilated ventricles in the brain at 2,000 mg/kg bw/d (a dose much higher than the limit dose) was associated with severe maternal toxicity (deaths, significant reductions in body weights, numerous clinical signs of toxicity and liver effects). Slight to moderate dilation of brain ventricles is considered to be a variation and to represent a developmental delay with no detrimental or irreversible consequences for the foetus. Therefore, it is most likely that this abnormality was the secondary consequence of the excessive maternal toxicity occurring at the high dose of 2000 mg/kg bw/d. Overall, there was no evidence of specific developmental toxicity in the rat. Based on these findings, **the NOAELs proposed by HSE for maternal and developmental toxicity in the rat are 30 and 300 mg/kg bw/d, respectively**, based on the lack of relevant effects at these dose levels.

In the rabbit developmental study, there were no effects of treatment on external, skeletal and soft tissue alterations (malformations and variations) up to the top dose of 320 mg/kg bw/d. Foetal weight was reduced from 250 mg/kg bw/d, at which maternal toxicity (reduced body weight gain, increased liver weight with associated changes in clinical chemistry (GGT)) occurred. Based on these findings, **the NOAELs proposed by HSE for developmental and maternal toxicity in the rabbit are 80 mg/kg bw/d**, based on the lack of relevant effects at these dose levels.

In addition, in the rat 2-generation study, there were no effects of treatment on pup survival, sex ratio, pup bodyweight, nipple development, anogenital parameters and sexual maturation of pups (vaginal opening and preputial separation) up to the top dose of 394 – 481 mg/kg bw/d. At this dose parental (reduced food consumption, body weight, body weight gain, increased liver and thyroid weight, histopathology of the thyroid and nasal cavity) but not offspring toxicity occurred.

Classification for reproductive toxicity (either fertility or development) is therefore not required. Further details are available in the aligned MCL dossier.

**B.6.7. NEUROTOXICITY**

The neurotoxic potential of cinmethylin has been investigated in Wistar rats in an oral (gavage) acute neurotoxicity study.

**B.6.7.1. Neurotoxicity studies in rodents***New/modern study*

<b>Author(s)</b>	
<b>Study title</b>	BAS 684 H - Acute oral neurotoxicity study in Wistar rats - Administration by gavage
<b>Study reference</b>	, 2018e BASF Doc ID: 2016/1345328
<b>Test facility</b>	
<b>Dates of work</b>	28/11/2016 – 16/12/2016
<b>Test substance</b>	BAS 684 H (Cinmethylin)
<b>Purity (%)</b>	93.5
<b>Batch no.</b>	COD-002038 (-) / (+) ratio = 48:52
<b>Test organisms</b>	Rat Wistar, Crl:WI(Han) Males and females
<b>Groups</b>	10/sex/dose.
<b>Dose/concentration</b>	0, 300, 1000 and 2000 mg/kg bw/d. Volume: 10 mL/kg bw.
<b>Route</b>	Oral, gavage Single administration
<b>Vehicle</b>	0.5 % aqueous carboxymethylcellulose (CMC) and 3 drops of Tween® 80/ 1000 mL
<b>GLP</b>	Compliant.
<b>Guideline</b>	OECD TG No. 424 (1997 – the current guideline), EPA 870.6200, Commission Regulation (EC) No 440/2008 - Part B No. B.43
<b>Deviation</b>	A technical error occurred during the transfer of animals into the measuring boxes for the performance of the first Motor Activity examination (study day -7; 21 Nov 2016). The measurement was stopped and restarted about one hour later on the same day.
<b>Impact of deviations</b>	The deviation identified is not considered to compromise the validity of the study.
<b>Acceptable</b>	Yes.
<b>Conclusion</b>	Cinmethylin demonstrated some potential for acute neurotoxicity.
<b>NOAEL</b>	Acute neurotoxicity: 300 mg/kg bw. Systemic toxicity: 300 mg/kg bw.
<b>Effects at the LOAEL</b>	Acute neurotoxicity: Alterations in FOB and MA parameters (e.g. retarded righting response, reduced number of rearings and decreased motor activity) were recorded in females from 1,000 mg/kg bw (and in males at 2,000 mg/kg bw) on the day of administration only. In addition, axonal degeneration of the tibial and sciatic nerves was seen at the top dose in both sexes (more marked in females). Systemic toxicity: Salivation and clinical signs of toxicity were recorded in females from 1,000 mg/kg bw (and in males at 2,000 mg/kg bw) on the day of administration only.

**Methods**

In a GLP and OECD test guideline compliant study, cinmethylin was administered once orally (gavage) to male and female Wistar rats (10/sex/dose) at dose levels of 0, 300, 1000, and 2000 mg/kg bw in 0.5 % aqueous CMC solution. Animals were observed up to 2 weeks after dosing. General state of health was examined daily, body weight, functional observation battery (FOB) and motor activity (MA) were examined 7 days prior to and on the day of administration, then weekly thereafter until day 14. At termination, 5 animals per sex were fixed by *in situ* perfusion and subjected to brain weight determination as well as to neuropathological examinations.

### Results

**Mortality and general clinical observations:** There were no treatment-related effects on mortality. Slight, transient salivation was observed in the 1,000 (in 2/10 males and 1/20 females) and 2,000 mg/kg bw (in 1/10 males and 2/10 females) dose groups immediately after dosing.

**Body weight and body weight gain:** There were no statistically-significant alterations in body weight and/or body weight gain. Alterations in body weight in both males and females and body weight gain in males were generally < 5 % (change compared to control). Increases in body weight gain in females were > 10 % compared to controls, however, no clear dose-response was evident. Overall, there were no treatment-related adverse effects on body weight or body weight gain in males and females at any dose.

Table 6.7-1. Body weight and body weight gain

Dose [mg/kg bw]		Males				Females			
		0	300	1000	2000	0	300	1000	2000
<b>Body weight [g]</b>									
Day -7	[mean]	163.5	164.6	164.0	160.9	129.1	131.0	128.8	131.1
	[SD]	6.1	6.8	5.9	5.9	4.4	5.3	5.0	9.3
Day 0	[mean]	207.6	209.4	208.1	203.9	149.0	151.3	151.4	148.2
	[SD]	7.7	8.4	7.0	10.2	5.9	7.8	5.9	8.6
	Δ%	-	+0.9	+0.2	-1.8	-	+1.5	+1.6	-0.6
Day 7	[mean]	245.5	250.9	247.2	240.5	164.8	171.4	169.2	170.0
	[SD]	10.7	12.1	9.5	13.5	6.3	9.3	6.0	14.1
	Δ%	-	+2.2	+0.7	-2.0	-	+4.0	+2.7	+3.2
Day 14	[mean]	277.0	283.5	278.5	274.3	177.8	183.2	182.2	184.8
	[SD]	13.4	15.5	11.6	14.6	9.0	10.2	7.5	16.9
	Δ%	-	+2.3	+0.5	-1.0	-	+3.0	+2.5	+3.9
<b>Overall body weight gain [g]</b>									
Day 0 - 7	[mean]	37.8	41.5	39.1	36.7	15.8	20.1	17.8	21.9
	[SD]	3.7	4.7	4.4	5.0	4.6	5.9	5.6	10.4
	Δ%	-	+9.7	+3.5	-3.0	-	+27.7	+13.0	+38.8
Day 0 - 14	[mean]	69.4	74.1	70.4	70.4	28.8	31.9	30.8	36.6
	[SD]	8.0	8.9	7.1	7.0	6.8	6.8	6.8	10.3
	Δ%	-	+6.8	+1.5	+1.5	-	+10.7	+6.8	+27.0

Statistical evaluation: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; Dunnett test (two-sided)

Δ% - percent change compared to control.

**Clinical signs, Functional Observation Battery (FOB) and Motor Activity (MA):** At the top dose (2,000 mg/kg bw) a range of clinical signs of toxicity and FOB parameter findings were recorded on day 0. Laboured respiration was observed in 3 males and 2 females, compared to 0 in controls. Both affected females showed unsteady gait, one female showed reduced exploration of the area, another female showed piloerection and slightly closed eyelids. Retarded righting response was observed in 2 males and 5 females. During handling, six males were recorded as 'limply hanging in hand'.

The number of rearings was statistically-significantly reduced in females (Table 6.7-2). Statistically-significant decreases in motor activity were recorded in females (Table 6.7-3). At the mid dose (1,000 mg/kg bw) selected FOB parameter findings were recorded on day 0. Retarded righting response was observed in 5 females. These findings were considered to be treatment-related and adverse.

The statistically-significant decreases in motor activity at interval 2, at the mid dose in males and at the low dose in females, were not considered treatment-related as statistically-significant decreases were not seen at other time points or overall. The statistically-significant alterations in motor activity on day 14 were not considered treatment-related due to the lack of dose-response and as overall motor activity was not statistically-significantly changed.

Overall, treatment-related and adverse alterations in clinical observations, functional observation battery and motor activity parameters (e.g. retarded righting response, reduced number of rearings and decreased motor



activity) were recorded in females from the mid dose (1,000 mg/kg bw) and in males at the top dose (2,000 mg/kg bw) on the day of administration only.

Table 6.7-2. Number of rearings

Dose [mg/kg bw]		Males				Females			
		0	300	1000	2000	0	300	1000	2000
<b>Rearing [N]</b>									
Day -7	[mean]	5	4	5	5	10	10	12	11
	[SD]	3	3	5	5	6	6	8	6
Day 0	[mean]	7	4	4	4	14	12	7*	7
	[SD]	6	4	4	3	7	8	6	5
Day 7	[mean]	3	2	4	4	10	12	14	12
	[SD]	3	2	4	3	4	5	7	2
Day 14	[mean]	7	6	5	6	13	13	13	13
	[SD]	4	4	4	3	3	3	3	4

Statistical evaluation: \* p ≤ 0.05; \*\* p ≤ 0.01; Kruskal-Wallis + Wilcoxon test (two-sided)

Table 6.7-3. Selected individual and overall mean motor activity (MA)

Dose [mg/kg bw]		Males				Females			
		0	300	1000	2000	0	300	1000	2000
<b>Day -7</b>									
Interval 11	[mean]	80.8	25.4	64.6	23.0	74.8	49.3	8.0**	39.5
	[SD]	95.9	10.3	78.5	28.6	122.6	46.4	9.4	27.0
Sum intervals 1-12	[mean]	2427.1	2105.9	2365.8	1969.4	3330.2	3083.9	3193.9	2713.7
	[SD]	891.8	807.9	985.7	695.3	860.6	849.1	1208.1	561.8
<b>Day 0</b>									
Interval 1	[mean]	944.7	751.3	720.2	614.9	1466.1	1216.1	937.3*	802.3**
	[SD]	363.9	308.5	181.0	164.0	317.3	307.0	425.3	388.6
Interval 2	[mean]	810.1	700.8	592.6*	371.5**	1084.7	744.7*	447.8**	435.0**
	[SD]	201.0	188.2	218.8	87.9	332.7	320.8	184.2	196.7
Interval 3	[mean]	391.1	420.3	342.2	213.1**	668.9	507.8	210.0**	285.1*
	[SD]	141.2	195.8	75.0	107.0	450.9	250.8	140.0	215.5
Interval 4	[mean]	148.4	167.8	118.8	64.7*	372.3	175.2	76.2**	99.2**
	[SD]	89.7	91.5	70.8	55.2	295.0	127.8	93.6	81.0
Sum intervals 1-12	[mean]	2625.1	2371.4	2149.8	1582.7**	4379.1	3248.6	2247.1**	2229.9**
	[SD]	686.9	592.8	616.2	251.2	2193.6	969.3	899.2	916.7
<b>Day 7</b>									
Sum intervals 1-12	[mean]	2470.6	2589.9	2989.0	2694.3	4180.5	3133.1	3719.3	3698.8
	[SD]	872.3	757.9	853.8	529.4	1360.7	769.5	969.3	997.4
<b>Day 14</b>									
Interval 5	[mean]	60.9	132.3*	141.6*	59.7	92.4	73.3	209.6	123.5
	[SD]	58.9	84.0	104.0	54.0	104.7	75.0	365.1	105.4
Interval 6	[mean]	41.5	35.8	63.3	13.0*	17.6	74.3	48.7	49.3
	[SD]	32.7	63.6	33.0	16.0	16.7	88.3	75.2	44.1
Sum intervals 1-12	[mean]	2937.0	3035.0	3253.2	2757.0	3940.4	3741.1	3864.8	3965.1
	[SD]	944.0	1112.2	818.1	760.4	1305.1	938.1	1230.7	1151.0

Statistical evaluation: \* p ≤ 0.05; \*\* p ≤ 0.01; Kruskal-Wallis + Wilcoxon test (two-sided).

The number of beam interrupts was counted over 12 intervals for 5 minutes per interval.

*Brain weight:* There were no statistically-significant or toxicologically-significant alterations in brain weights at any dose (Table 6.7-4).

Table 6.7-4. Organ weights

Sex		Males				Females			
Organ weight	Dose [mg/kg bw]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Terminal weight [g]	0	262.90				162.92			
	300	263.08	+0.1			171.62	+5.3		
	1000	254.06	-3.4			166.88	+2.4		
	2000	250.86	-4.6			166.80	+2.4		
Brain [g]	0	1.902		0.725		1.810		1.112	
	300	1.946	+2.3	0.742	+2.3	1.728	-4.5	1.007	-9.4
	1000	1.898	-0.2	0.748	+3.2	1.746	-3.5	1.045	-6.0
	2000	1.882	-1.1	0.752	+3.7	1.756	-3.0	1.055	-5.1

no statistical significances were observed ( $\alpha = 0.01$ ) (Kruskal-Wallis and Wilcoxon-test, two sided)

# Values may not calculate exactly due to rounding of figures

*Gross pathology:* There were no treatment-related macroscopic findings.

*Histopathology:* An increased incidence of minimal axonal degeneration in proximal and distal tibial nerves and proximal sciatic nerve was observed in females at the top dose (Table 6.7-5). The incidence of minimal axonal degeneration of the proximal sciatic nerve was also increased in top dose males. Laboratory HCD indicate that this finding is rare. Therefore, a relation to treatment cannot be excluded.

Table 6.7-5. Selected neuro-histopathological lesions

Sex		Males				Females			
Dose [mg/kg]		0	300	1000	2000	0	300	1000	2000
Tibial nerve, distal - Axonal degeneration	# examined	5	5	5	5	5	5	5	5
	Grade 1	1	1	-	1	-	-	-	1 <sup>#</sup>
	[mean] <sup>§</sup>	[1.0]	[1.0]	[0.0]	[1.0]	[0.0]	[0.0]	[0.0]	[1.0]
		HCD <sup>§</sup> 1/40 (0 – 1); [2.0]				-			
Tibial nerve, proximal - Axonal degeneration	# examined	5	5	5	5	5	5	5	5
	Grade 1	-	-	-	-	-	-	-	1 <sup>#</sup>
	[mean] <sup>§</sup>	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[1.0]
		HCD <sup>§</sup> 1/40 (0 – 1); [1.0]				-			
Sciatic nerve, proximal - Axonal degeneration	# examined	5	5	5	5	5	5	5	5
	Grade 1	-	-	-	1	-	-	-	1
	Grade 2	-	-	-	-	-	-	-	1 <sup>#</sup>
	[mean] <sup>§</sup>	[0.0]	[0.0]	[0.0]	[1.0]	[0.0]	[0.0]	[0.0]	[1.5]
		HCD <sup>§</sup> 1/40 (0 – 1); [1.0]				2/40 (0 – 1); [1.0]			

<sup>§</sup> [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

<sup>#</sup> finding of the same animal #48

<sup>§</sup> historical control data (HCD) = total incidence, study range in brackets and the mean severity in square bracket. HCD based on 8 acute oral neurotoxicity studies in Wistar rats performed at [REDACTED] in a period between 2011 – 2016 under GLP conditions.

### Conclusions

Overall, in a GLP and guideline oral study, cinmethylin was acutely neurotoxic (changes in FOB and MA parameters) to rats from the mid dose of 1,000 mg/kg bw in females and at the top dose of 2,000 mg/kg bw in males. In addition, minimal axonal degeneration of the sciatic nerve was seen at the top dose in both sexes (more pronounced in females). Generalised toxicity (clinical signs of toxicity) was also evident from the mid dose (1,000 mg/kg bw) in females and at the top dose in males. Therefore, a **NOAEL of 300 mg/kg bw was identified for both acute neurotoxicity and systemic toxicity** from this study.

([REDACTED], 2018e)

### B.6.7.2. Delayed polyneuropathy studies

According to Commission Reg. (EU) 283/2013, these studies shall be performed for active substances of similar or related structures to those capable of inducing delayed polyneuropathy such as organophosphorus compounds. Cinmethylin does not belong to the chemical classes suspected to cause delayed neurotoxicity. Therefore, no acute delayed neurotoxicity study was required or performed.

### B.6.7.3. Summary of Neurotoxicity

The neurotoxic potential of cinmethylin has been investigated in Wistar rats in an oral (gavage) acute neurotoxicity study. Neurobehavioural parameters and histopathological examinations of neuronal tissues (i.e. sciatic nerve) were also part of the examinations of the 28-day and 90-day dietary toxicity studies in Wistar rats.

The following key conclusions were obtained from the evaluation of the neurotoxicity information:

- Cinmethylin is acutely neurotoxic from a dose of 1,000 mg/kg bw. An acute neurotoxicity NOAEL of 300 mg/kg bw was established.
- However, no neurotoxicity or neuropathology was observed on repeated exposure.

Table 6.7-6. Summary of neurotoxicity studies with cinmethylin

Study and Acceptability	Species/ Strain/ Groups	Doses	NOAEL (mg/kg bw) [ppm]	Effects at the LOAEL
Acute oral (gavage) neurotoxicity  GLP compliant Guideline compliant  Cinmethylin Batch: COD- 002038 Purity (%): 93.5 (-) / (+) ratio = 48:52  [REDACTED], 2018e (2016/1345328)  <i>Acceptable</i>	Rat (Wistar)  10/sex/dose	0, 300, 1000 and 2000 mg/kg bw	Acute neurotoxicity: M: 1000 F: 300	Acute neurotoxicity: <u>1,000 mg/kg bw</u> Alterations in FOB and MA parameters (e.g. retarded righting response, reduced number of rearings and decreased motor activity) (♀), on the day of administration only.
			Systemic toxicity: M: 1000 F: 300	Systemic toxicity: <u>1,000 mg/kg bw</u> Salivation and clinical signs of toxicity, on the day of administration only.

In the acute oral (gavage) neurotoxicity study in the rat, cinmethylin was acutely neurotoxic (changes in FOB and MA parameters) from the mid dose of 1,000 mg/kg bw in females and at the top dose of 2,000 mg/kg bw in males. In addition, minimal axonal degeneration of the sciatic nerve was seen at the top dose in both sexes (more pronounced in females). These acute neurotoxic effects were observed in the presence of some generalised toxicity (clinical signs of toxicity), which occurred from the mid dose (1,000 mg/kg bw) in females and at the top dose in males. Based on these findings, **a NOAEL of 300 mg/kg bw was identified for both acute neurotoxicity and generalised toxicity in the rat.**

In rats, clinical findings potentially relating to neurotoxicity (e.g. ataxia, hypoactivity, loss of righting reflex, hypersensitive to touch and depression of myotactic placing reflex) were observed in the old acute oral toxicity study ([REDACTED], 1982) at doses of 1014 – 5680 mg/kg bw. Similarly, clinical findings potentially relating to neurotoxicity (e.g. decreased activity and tip-toe gait) were observed in the old acute inhalation toxicity study ([REDACTED], 1986) at 3.5 mg/L. However, such effects were not seen up to 2,000 mg/kg bw or 5 mg/L in the more reliable modern oral and inhalation acute toxicity studies in the rat. No clinical signs of toxicity were seen by the dermal route up to 5,000 mg/kg bw.

In mice, clinical findings potentially related to neurotoxicity (e.g. hypoactivity and unsteady stance) were observed only in a relatively old acute oral toxicity study (██████████, 1982) at 5072 mg/kg bw. These effects were observed sporadically and animals recovered within 4 days.

Overall, there was no clear evidence of neurotoxicity in the acute toxicity studies; however, it should be noted that no specific neurobehavioural or neuropathology investigations are generally performed in these studies.

There were no neurotoxic effects of cinmethylin observed across all functional observations (FOB) and motor activity (MA) investigations and no neuropathology findings following repeated exposure in the new/modern 28- and 90-day oral studies in rats and mice, as well as the 28-day dermal study in rats up to doses ranging from 700 to 1,000 mg/kg bw/d.

It is most likely that the minimal axonal degeneration of the sciatic nerve and associated FOB and MA findings noted at 2,000 mg/kg bw/d in a specific rat neurotoxicity study are the acute consequences of high gavage doses of cinmethylin, possibly related to a Cmax, bolus effect.

Overall, cinmethylin is acutely neurotoxic from a dose of 1,000 mg/kg bw (NOAEL = 300 mg/kg bw); however, no neurotoxicity or neuropathology was observed on repeated exposure.

**B.6.8. OTHER TOXICOLOGICAL STUDIES****B.6.8.1. Toxicity studies on metabolites and relevant impurities**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.8.2. Supplementary studies on the active substance**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.8.3. Studies on endocrine disruption**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.9. MEDICAL DATA AND INFORMATION**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.9.2. Data collected on humans**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.9.3. Direct observation**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.9.4. Epidemiological studies**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment**

Refer to Volume 3 – B.6 (AS) – Part II

**B.6.10. REFERENCES RELIED ON**

Refer to Volume 3 – B.6 (AS) – Part II