Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Dimethomorph (ISO) (E,Z) 4-(3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl)morpholine

EC Number: **404-200-2**

CAS Number: **110488-70-5**

Index Number: **613-102-00-0**

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CONTENTS

1 PHYSICAL HAZARDS

1.1 Explosives

1.2 Flammable gases (including chemically unstable gases)

1.3 Oxidising gases

1.4 Gases under pressure

1.5 Flammable liquid

1.6 Flammable solids

1.7 Self-reactive substances

1.8 Pyrophoric liquids

1.9 Pyrophoric solid

1.10 Self-heating substances

1.11 Substances which in contact with water emit flammable gases

1.12 Oxidising liquids

1.13 Oxidising solids

1.14 Organic peroxides

1.15 Corrosive to metals

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 ADME by oral route Study 1

Reference:

B.6.1.1.1a - Anonymous 1990, The Biokinetics and Metabolism of ^{14}C-Dimethomorph in the Rat, BASF Document No. DK-440-001 /

B.6.1.1.1b - Anonymous. 1991, ^{14}C-Dimethomorph: Investigation on the Nature of the Metabolites Occurring in the Rat, BASF Document No. DK-440-006 [complementary data supporting study report DK-440-001]

Test type

Guideline: Directive 96/54/EC B 36; OECD Guideline 417 (EPA Pesticide Assessment Guidelines, subdivision F: Hazard Evaluation: Human and Domestic Animal, § 85-1 (October 1982) claimed by the author)

Deviations: None

GLP: No, at the time the study was conducted GLP was not compulsory. However the study was conducted according to the principle of Good Laboratory Practises.

Acceptability: The study is considered to be acceptable.

Test substance

Radiolabeled dimethomorph (chlorophenyl-ring), batch 2271-040, specific activity 45.12 mCi/g with chemical purity > 98 % and E/Z ratio 44-47/53-56. Unlabelled dimethomorph, batch H7879 with purity 99.2 % and E/Z ratio 49.5/50.5.

Detailed study summary and results:

Material and methods

Test animals: Male and Female rat (strain: Sprague-Dawley CD)

Male and Female rat were orally dosed with the test substance as follows:

Table 1: Summary of biokinetic experiments performed with 14C-dimethomorph in the rat

Test group	No of rat	Dose, mg/kg body weight (b.w.)	Dosing type	Sampling
A	1♂, 1♀	500	Single	Expired air
B	5♂, 5♀	10	Single	Urine and feces: 8 (urine only), 24, 72, 96, 120 and 144 hours after dosing Sacrifice at end of urine/feces collection, sampling of liver kidneys, fat and carcass
C	5♂, 5♀	10	Multiple (15 days)	
D	5♂, 5♀	500	Single	
E	5♂, 5♀	10	Multiple (7 days)	Urine and feces: 1, 6, 24, 48, and 120 after dosing Sacrifice at end of urine/feces collection, sampling of liver, adrenals, heart, brain, spleen, thyroid, bone marrow, stomach, kidneys, fat, uterus, lungs, gonads, pancreas, muscle, bone, blood, intestine and carcass

Samples were analysed for radioactivity level and identification of components when feasible.

Results

Group A:

No radioactivity (< 0.01 % dose) was detected in the air expired during a period of 24 hours after administration.

Group B, C, D:

Within 2 days after treatment, about 90 % of applied dose was excreted in the three test groups. Majority of the applied radioactivity was excreted via the feces for male and female rats. In Group D, excretion in the feces was delayed due to constipation occurring with some animals.

Table 2: Mean rate of daily excretion of radiolabelled ^{14}C-dimethomorph expressed as % total radioactivity administered

Day	Group B (single, low dose)		Group C (multiple, low dose)		Group D (single, high dose)	
	Feces	Urine	Feces	Urine	Feces	Urine
1	75.09/72.41[1]	4.93/12.66	65.64/52.35	5.83/12.53	58.91/33.14	4.57/6.15
2	13.10/12.40	0.51/0.75	16.36/22.31	1.48/3.11	25.44/39.39	1.45/2.67
Subtotal 0-48 hrs	**88.19/84.81**	**5.44/13.41**	**82/74.66**	**7.31/15.64**	**84.35/72.53**	**6.02/8.82**
3	1.43/1.16	0.07/0.13	6.32/5.12	0.33/0.51	1.98/13.62	0.13/1.37
4	0.24/0.21	0.03/0.04	0.56/0.58	0.05/0.08	0.27/2.36	0.04/0.20
5	0.10/0.10	0.01/0.02	0.16/0.15	0.02/0.04	0.09/0.32	0.02/0.03
6	0.06/0.06	0.01/0.02	0.07/0.07	0.02/0.02	0.06/0.04	0.01/0.01
7	0.05/0.04	0.01/0.01	0.05/0.03	0.01/0.02	0.03/0.04	0.01/0.01
Total	**90.07/86.38**	**5.57/13.62**	**89.17/80.61**	**7.74/16.31**	**86.79/88.91**	**6.23/10.44**

[1] x/y = values for Male and female

The mean distribution of applied radioactivity 7 days after last treatment is given for test group B,C and D in Table 3

Table 3: Distribution of radiolabelled ^{14}C-dimethomorph expressed as % total radioactivity administered

Distribution	Group B (single, low dose)	Group C (multiple, low dose)	Group D (single, high dose)
Feces	90.07/86.38	89.17/80.61	86.79/88.91
Urine	5.57/13.62	7.74/16.31	6.23/10.44
Total Carcass	0.10/0.08	0.425/0.360	0.129/0.189
Liver	0.088/0.050	0.111/0.064	0.020/0.043
Kidneys	0.002/0.002	0.002/0.002	0.001/0.002
Total fat	0.001/0.003	0.002/0.009	0.002/0.014

Metabolite profiles/degradation patterns were determined in urine and feces from Group D (Single high dose). No differences were found between metabolic profile of both sexes neither in urine nor in feces. From urine samples, dimethomorph (< 5 % of applied dose), polar fractions formed by demethylation of the dimethoxyphenyl ring and trace of compound formed by oxidation of morpholine ring were identified. From feces samples, the greatest portion of radioactivity was identified as the parent compound. Trace of demethylated metabolites from dimethoxyphenyl ring was also found.

Further investigation were conducted on urine sampled 24 hours after dosing from Group B (single, low dose). Trace of metabolites, CUR 7117, CUR 7586, CUR 7216 and Z43 was found confirming the oxidation of the morpholine ring.

Group E:

Following 7 dosing day of dimethomorph at 10 mg/kg bw, the highest residue level were found in the excretion organ with a decrease of about 70 % after 24-hours. Except for stomach, intestine and liver, the residue level in organs and tissues were very low. Based on the result presented inTable 4, it is clear, that neither dimethomorph nor its degradation products accumulated in any of the organs of tissues of rats.

Table 4: Concentration of radioactivity in the organs and tissues of rats after 7 dosing day of ^{14}C-dimethomorph at low dose level (values expressed as % total dose in organ and tissue)

Time of sacrifice after 7-dosing day	1h	6h	24h	48h	120h
Liver	1.44/1.26[1]	0.43/0.57	0.27/0.26	0.15/0.10	0.09/0.06
Kidneys	0.04/0.06	0.01/0.02	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Heart	0.01/0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Lungs	0.01/0.02	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Brain	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Gonads	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Spleen	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Pancreas	0.03/0.04	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Adrenals	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Thyroid	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01	<0.01/<0.01
Uterus	-/0.01	-/<0.01	-/<0.01	-/<0.01	-/<0.01
Muscle	0.39/0.76	0.14/0.31	0.02/0.04	0.01/0.01	0.01/0.02
Fat	0.15/0.50	0.07/0.11	0.02/0.03	<0.01/0.01	<0.01/0.01
Stomach (+content)	4.17/4.23	0.07/0.08	0.05/<0.01	<0.01/<0.01	<0.01/<0.01
Intestine (+content)	8.64/10.58	11.47/12.23	1.29/1.79	0.09/0.16	0.01/0.02

[1] x/y = values for male and female

Conclusion:

Dimethomorph is rapidly absorbed in the gastrointestinal tract following oral administration to rats. The amount absorbed is limited at high dose levels. Absorbed dimethomorph is efficiently metabolised and rapidly excreted mainly via the feces. Accumulation of dimethomorph in organs and tissues did not occur.

The main degradation pathway was found to be the demethylation of the dimethoxyphenyl ring. To a smaller extent, degradation also occurred by oxidation of the morpholine ring. Degradation pathway is given atFigure 1.

2.1.2 ADME by oral route Study 2.

Reference:

B.6.1.1.2 - Anonymous 1990, 14C-Dimethomorph (CME 151): Absorption, Distribution, Metabolism and Excretion after Bile Cannulation and Single Oral Administration to the Rat. BASF Document No. DK-440-002

Test type

Guideline: Directive 96/54/EC B 36; OECD Guideline 417 (EPA Guidelines, 40 CFR, Part 158,85-1 (October 1982) claimed by the author)

Deviations: None

GLP: Yes, Département Fédéral de L'intérieur, Suisse

Acceptability: The study is considered to be acceptable.

Test substance

Radiolabeled dimethomorph (chlorophenyl-ring), batch S 1050, specific activity 23.6 mCi/g with chemical purity > 99 % and E/Z ratio 50/50. Unlabelled dimethomorph, batch H7879; chemical purity: 99.2 % (E/Z ratio: 49.5/50.5)

Detailed study summary and results:

Material and methods

Male and female rats (Strain: Sprague-Dawley CD) were orally administered with ^{14}C-dimethomorph at nominal dose level of 10 (low dose) and 500 (high dose) mg/ kg body weight. Bile was collected in 3-hour intervals throughout the study. Urine and feces were collected 24 and 48 hours after dosing. At sacrifice (48-h after dosing), carcass and digestive tract were sampled. Cage wash were collected at the end of the study. Samples were analysed by liquid scintillation counter for the radioactivity level. Characterisation of fractions was conducted by TLC and HPLC.

Results

Excretion balance

At low dose, the total amount of radioactivity absorbed (bile, urine and residua; carcass) is about 100 % for both male and female rats. At high dose, the total amount of radioactivity absorbed is 65.5 % for male and 50 % for the female.

Table 5: Balance of radioactivity administered as total radioactivity administered 48-hour after dosing (mean value)

Distribution	Group 1 Low dose male	Group 2 Low dose female	Group 3 High dose male	Group 4 High dose female
Urine	6.6	6.9	14.8	8.6
Feces	7.6	4	21.8	3.5
Bile	95.1	92.6	49.1	31.2
Residual Carcass	0.6	1.2	1.6	10.1
Digestive tract	0.4	1.1	3.3	44.1
Cage wash	0.5	0.3	0.4	0.9
Total	110.8	106.1	91	98.4
Standard Deviation	± 5.6	± 3.6	± 4.3	± 2.7

Biliary excretion

At low dose (single administration, group 1 and 2), radioactivity was rapidly eliminated during the 1st 6-hour following administration (74.3 % and 85 % in group 1 and 2, respectively). At high dose (single administration, group 3 and 4), elimination of radioactivity via the bile was described by 1st order kinetics with a half-life of 6 -11 hours considering time interval of 6 to 24 hours.

Table 6: Excretion pattern via the bile after administration of a single dose (mean values expressed as % total radioactivity administration)

Time (hours)	Group 1 Low dose Male	Group 2 Low dose Female	Group 3 High dose Male	Group 4 High dose Female
0-3	65.2	64.4	11.5	12.8
6	19.9	9.9	7.3	5.4
9	8	6.2	6.9	3.4
12	3	4.4	6.5	2.5
15	1.4	3.1	4.6	1.6
18	0.9	1.5	3.5	1.3
21	1.2	1.0	3.7	1.4
24	0.3	0.6	3	1.4
27	0.2	0.4	0.3	0.8
30	0.2	0.3	0.7	0.5
33	0.2	0.2	0.3	0.4
36	0.1	0.2		0.3
39	0.1	0.2	0.5	0.2
42	0.1	0.1	0.1	-
45	0.1	0.1	0.2	-
Total	**100.9**	**92.6**	**49.1**	**31.2**

Metabolite pattern in bile

For both, male and female rats, the degradation pattern of the untreated and treated bile were similar as shown in Table 7andTable 8.

Table 7: Pattern of metabolites in the untreated bile after single oral administration of ^{14}C-dimethomorph (Total value expressed in percent of the radioactivity administered for sampling intervals 0-27 h after dosing)

Fraction	Group 1 Low dose male	Group 2 Low dose female	Group 3 High dose male	Group 4 High dose female
B1, unknown	1.2	0.1	ND	ND
B2, Z67/69 (not confirmed)	0.5	0.5	ND	ND
B3, unknown	0.2	9.1	ND	0.8
B4, unknown	10.1		3.3	1.7
B5, unknown	13.6	8.9	10.9	8.2
B6, unknown	ND	ND		
B7, unknown	44.4	57.2	16.4	12.7
B8, unknown	3.8	ND	2.2	1.8
B9, unknown	17.3	10.7	11.2	1.3
B10, unknown	4.6	3.3	2.3	3.1
B11, unknown	4.2	1.3	0.7	0.2

ND=not detectable

Table 8: Pattern of metabolites in the treated bile1 after single oral administration of ^{14}C-dimethomorph (total value expressed in percent of the radioactivity administered)

Fraction	Group 1/2 Low dose male/female	Group 3/4 High dose male/female	Group 1/2 Low dose male/female	Group 3/4 High dose male/female
Treated bile by	*HCl, in ethyl acetate partition*		*Glucuronidase, in ethyl acetate partition*	
B4, unknown	14.9/8.9	4.8/2.5	18.3/18.3	9.2/6.5
B12 (=B1)	7.1/5.7	2.4/2.0		
B13 (=B1)		ND		
B14, Z67/69	22.7/34.1	11.5/11.3	28.0/46.6	21.0/19.4
B15, unknown	9.5/6.6	2.2/3.3		
B16, unknown	3.2/4.3	1.0/0.9		
B17, unknown			2.1/1.0	1.5/0.6
B18, unknown			14.1/8.2	3.0/ND
B19, unknown			6.9/ND	2.2/ND
B20, unknown			5.5/0.6	2.3/ND
B21, unknown			5.8/3.8	1.6/1.1

Based on the characterisation, it can be stated that:

Fraction B4: unknown metabolite, acid-stable metabolite of dimethomorph, probably non-conjugated

Fraction B3-B10: glucuronide conjugates

[1] HCL treated bile: summary data for 0-6 hour sample for group 1 and 2, and 0-12 hour sample for group 3 and 4; Glucuronidase treated bile: summary data for 0-15 hour sample for group 1 and 2, and 0-27 hour sample for group 3 and 4.

Fraction B11: conjugate without glucuronide moiety

Fraction B14: Z67/Z69

Conclusion:

Dimethomorph is efficiently absorbed and metabolised in the rat. Dimethomorph is mainly excreted via the bile after conjugation to glucuronides. The main aglycone was Z67 and/or Z69.

2.1.3 ADME by oral route Study 3.

Reference:

B.6.1.1.3 - Anonymous 1993, ^{14}C-Dimethomorph (CME 151): Investigation of the Metabolite Profiles in the Rat Following Treatment with two Different Labels, BASF Document No. DK-440-011

Test type

Guideline: Directive 96/54/EC B 36; OECD Guideline 417 (USEPA Pesticide Assessment Guideline 85-1 claimed by the author)

Deviations: None

GLP: Yes, Ministerium für Umwelt und Gesundheit, Rheinland-Pfalz, Germany

Acceptability: The study is considered to be acceptable.

Test substance

^{14}C-dimethomorph: chlorophenyl-ring labelled, batch: 2271-040; radiochemical purity: 98 % (E/Z ratio: 45.3/54.7), specific activity: 45.12 mCi/g and morpholine-ring labeled, batch: S 1041, 1043; radiochemical purity: > 98 % (E/Z ratio: 49 %/51 %), specific activity: 18.7 mCi/g, dimethomorph, unlabelled: batch: H7879, chemical purity: 99.2 % (E/Z ratio: 49.5/50.5)

Detailed study summary and results:

Material and methods

5 Male and 5 female rats (Strain: Sprague Dawley CD) were orally administered one single dose of 500 mg/kg body weight per test substance. The test substance received were either labelled on the chlorophenyl or morpholine ring. Sample: urine and faeces were collected 8 (urine only), 24, 48 and 72 hours after single oral administration. Urine and Faeces were analysed by LSC for radioactivity level and by TLC for identification.

Results

Excretion balance

The mean daily excretion of radioactivity by male and female rats after single administration of 500 mg test substance/kg body weight is given inTable 9.

The results showed that the rate and route of degradation is similar for male and female rat with about 95 % and > 80 % excretion of administered dose in 48 hours, respectively.

Feces was the major route of excretion in both sexes and labelled compounds.

Table 9: Cumulative excretion 3 days after single dosing of 500 mg dimethomorph/kg bw [results expressed as % of dose administered]

Day	Chlorophenyl labelled				Morpholine labelled			
	Male		Female		Male		Female	
	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine
1	63.71	4.41	34.08	6.13	76.71	3.24	44.35	6.01
2	25.87	1.3	36.21	6.26	13.89	0.86	33.5	3.22
3	2.37	0.15	11.63	0.87	1.09	0.17	4.49	0.34
Total[2]	97.8		95.2		96		91.9	
SD	± 1.91		± 2.92		± 1.54		± 1.6	

SD: Standard deviation

Metabolite profile

The analysis of urine and feces by TLC revealed no difference between metabolic profile for rats treated with the ^{14}C-chlorophenyl and ^{14}C-morpholine ring. Cleavage of the amide bond is very unlikely to be a major pathway of metabolism of dimethomorph.

Conclusion:

Based on the results of this study, it was concluded that ^{14}C-label in the chlorophenyl ring of dimethomorph was the most favorable position for the investigation of its metabolism in rat.

2.1.4 ADME by oral route Study 4.

Reference:

B.6.1.1.4 - Anonymous 1994, ^{14}C-Dimethomorph: Additional Investigation on the Nature of the Metabolites Occurring in Rats, BASF Document No. DK-440-010

Test type

Guideline: Directive 96/54/EC B 36; OECD Guideline 417 (EPA Pesticide Assessment Guideline 85-1 (October 1982) claimed by the author)

Deviations: Mass spectroscopic investigations were not conducted in compliance with GLP.

GLP: Yes, Ministerium für Arbeit, Soziales, Familie und Gesundheit, Rheinland-Pfalz, Germany.

Acceptability: The study is considered to be acceptable.

[2] Rounded values

Test substance

^{14}C-dimethomorph, chlorophenyl-ring labeled: batch: S 1050; radiochemical purity: 98 % (E/Z ratio: 51/49), specific activity: 23.6 μCi/mg and dimethomorph, unlabelled, batch No: Re 8/5, chemical purity: 99.1 % (E/Z ratio: 48/52)

Detailed study summary and results:

Material and methods

10 Male (group 1) and 11 female (group 2) rats (strain: BRL-Man, Wistar) were orally administered one single dose of 50 mg/kg body weight.

Sample: urine and faeces were collected 8 (urine only), 24, 48 and 72 hours after single oral administration. After 72 hours, animals were sacrificed and carcass sent for waste combustion.

Analysis: Urine and feces were analysed by LSC for radioactivity level. Feces were further investigated using TLC and HPLC-UV or MS for identification.

Results

Excretion balance

Similar urine and feces excretion patterns were found in male and female rats after single oral administration of dimethomorph at 50 mg/kg bw Table 10.

Table 10: Cumulative excretion 3 days after single dosing of 50 mg dimethomorph/kg bw [results expressed as % of dose administered]

Day	Chlorophenyl labelled			
	Male		Female	
	Feces	Urine	Feces	Urine
1	63.0	13.3	65.4	11.1
2	19.4	3.2	31.0	1.8
3	2.8	0.2	2.7	0.7
Total	85.2	17.1	99.1	13.6
SD		5.4		3.7

Metabolite profile

The distribution of radioactivity after partitioning of extractable faecal residues, expressed as % dose, is as follow:

Table 11: Distribution of radioactivity after partitioning of extractable faecal residues – results are expressed as % dose

Sample period	0-24 h			24-48 h			48-72h		
Distribution	DCM	DCM, pH 1-2	H2O	DCM	DCM, pH 1-2	H_2O	DCM	DCM, pH 1-2	H_2O
Males	74.02	9.11	16.87	69.92	10.33	10.75	68.44	8.75	22.81
Females	72.95	12.10	14.95	72.42	10.71	16.86	75.72	7.34	16.94

DCM = dichloromethane

The above results showed that the sample radioactivity partitioned into dichloromethane. This fraction was further investigated for identification. In addition, the identification of metabolite nature in feces was focused during the first 24-hour after dosing as amount of radioactivity was >60 % total dose administered. The isolation and repeated purification of selected radioactive faecal residue fractions by HPLC and TLC facilitated the chromatographic and mass spectrometric identification of metabolite fractions as Z67 (6-8 % of dose), Z69 (2 % of dose), Z98 (trace-3 % dose), and trace of Z93 and parent.

Conclusion:

The findings of this study confirmed the fact that the demethylation of one of the methoxy groups of the dimethoxyphenyl ring is the major metabolism pathway of dimethomorph. The presence of Z98 and Z93 confirmed also the fact that there is a second minor metabolism pathway resulting in a degradation of the morpholine ring.

2.1.5 ADME by oral route Study 5.

Reference:

B.6.1.1.5 - Anonymous 1995, Dimethomorph (CL 336,379): Blood Pharmacokinetics of C-14 CL 336,379 Derived Residues in the Rat, BASF Document No. DK-452-008

Test type

Guideline: Directive 96/54/EC B 36; OECD Guideline 417 (JMAFF Testing Guidelines for Toxicology Studies: Metabolism Study (59 NohSan No. 4200, Jan. 28, 1985) claimed by the author)

Deviations: Feed and water analyses were not conducted under GLP. 0.1 % Tween 80 served as a control substance and was not characterised under GLP.

GLP: Yes. This laboratory was inspected by United States Environmental Protection Agency (US EPA) Office of Enforcement and Compliance Assurance

Acceptability: The study is considered to be acceptable

Test substance

^{14}C-dimethomorph; chlorophenyl-ring labeled: Lot Number: AC 10011:71, radiochemical purity: > 96 %, (E/Z ratio: about 50/50), specific activity: 25.3 µCi/mg, dimethomorph, unlabelled: Lot No.: AC 7467.004, chemical purity: 98.8 % (E/Z ratio: about 50/50)

Detailed study summary and results:

Material and methods

Adult male and female rats (strain: Sprague-Dawley, Crl:CD BR) were orally administered single dose of test substance at 10 (low dose) and 500 (high dose) mg/kg body weight.

Blood were sampled at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 72, 96, 120 and 144 hours after dosing. At 168 hours post-dosing, rats were sacrificed and blood collected for analysis. Radioactivity levels were determined in plasma and red blood cell (RBC).

Results

Signs of toxicity

Control and low dose: No signs of toxicity were observed.

High dose: 8-hour post-dosing signs of toxicity were observed. Those signs were diarrhea, excess salivation, irregular breathing, closed eyes, weakness and hunched stature. Total recovery occurred 24-hour post-dosing.

Pharmacokinetic paramater and concentration versus time profile

At low dose, radioactivity was rapidly absorbed and eliminated within 72 hours as indicated by the pharmacokinetic parameters (Table 12). At high dose, absorption occurred later than at low dose.

Table 12*: Pharmacokinetic parameter and concentration versus time profile*

Test group		T_{max} [hour]	C_{max} [µg/g]	$T^{3}_{1/2}$ [hour]	$AUC^{4}_{0-\infty}$
Group A Low dose	Male	2.8 ± 3.6	0.76 ± 0.56	59.2 ± 23.5	10.56 ± 1.36
	Female	1.4 ± 0.8	0.96 ± 0.26	68 ± 20.2	14.96 ± 6.24
Group B High dose	Male	11.0 ± 2.0	25.02 ± 4.35	65.4 ± 9.4	673.56 ± 89.1
	Female	14.7 ± 8.3	39.46 ± 8.4	75.8 ± 1.8	1211.4 ± 35.25

Limit of detection: 0.022 in plasma and 0.023 in RBC ppm

Conclusion:

[3] T1/2 is terminal elimination half-life

[4] AUC is area under curve

Based on the pharmacokinetic parameters, it can be concluded that at low dose absorption is quicker than at high dose. At both dose levels, the elimination occurred within 72 hours post-dosing.

2.1.6 ADME by oral route Study 6.

Reference:

B.6.1.1.6 - Anonymous, Dimethomorph (CL 336,379): Tissue distribution of C-14 CL 336,379 derived residues in the rat, BASF document No. DK-440-013 unpublished

Test type

Guideline: JMAFF Testing Guideline for Toxicity Studies: Metabolism Study (59 NohSan No. 4200, Jan. 28, 1985) claimed by the author

Deviations: Minor alterations were described in the protocol.

GLP: The author claimed that the study was conducted and reported in compliance with existing GLP regulations set forth in Titel 40, Part 160, of the Code of Federal Regulation of the United States of America

Acceptability: The study is considered to be as supplementary information.

Test substance

Radiolabeled dimethomorph (chlorophenyl-ring), (CL 336,379), (Lot No. AC:10011:71) radiochemical purity of 96 %; non-radiolabeled dimethomorph (CL 336,379), (Lot No. AC:7467.004) chemical purity of 98.8 %.

Detailed study summary and results:

Material and methods

Dimethomorph was administered as a single oral dose to rats (Sprague-Dawley) at nominal doses of 10 mg/kg bw (group A, low dose) and 500 mg/kg bw (group B, high dose). Each treated group consisted of 9 male and 9 female rats. Another 6 rats (3/sex) were dosed with dosing vehicle and served as the control group. For the treated groups, three male and three female rats were sacrificed and tissues were collected at about Tmax, 24 hour and 168 hour post dose, respectively. Control animals were sacrificed and tissues collected at 168 hour post dose. The T_{max} values were determined empirically from a previously conducted study (see B.6.1.1.5). Tissues and organs were collected and radioactive residues determined in plasma, red blood cells, adrenals, bone marrow, brain, fat, gastrointestinal tract, gastrointestinal contents, heart, kidneys, liver, lungs, muscle, ovaries, pancreas, pituitary, spleen, testes, thymus, uterus, and residual carcass.

Results

In the low dose group the Tmax for male rats ranged from 0.5 to 8 hours. In the low dose group the Tmax for female rats ranged from 0.5 to 2 hours. Similarly, the Tmax for female rats in the high dose group ranged

from 8 to 12 hours and from 8 to 24 hours, respectively. At the low dose (10 mg/kg bw) radioactivity was the highest at Tmax. The gastrointestinal tract, liver, kidneys, and certain endocrine organs such as the pancreas, pituitary, thyroid, adrenals and ovaries contained the highest amount of residues, but the compound was quickly eliminated from the body. At 24 hours most of the residues were depleted to very low levels. At 168 hours, only liver had detectable residue. At the high dose (500 mg/kg bw), dimethomorph was eliminated at much slower rate, which was reflected in higher residue levels at 24 hours post dose. Female rats retained the compound longer than male rats. In the high dose group, tissues and organs showed similar patterns of residues when compared to the low dose group. At 168 hours post dose, tissues from the high dose rats showed similar patterns of depletion as those in the low dose group.

Table 13: Total radioactive residues in tissues of the low dose group rats, expressed as percent of dose administered

	T_{max}[a]		24 hours		168 hours	
Tissue	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Plasma	0.06	0.10	0.01	0.01	0.00	0.00
RBC	0.06	0.09	0.01	0.01	0.00	0.00
Adrenals	0.00	0.01	0.00	0.00	0.00	0.00
Bone	0.01	0.02	0.00	0.00	0.00	0.00
Bone Marrow	0.00	0.00	0.00	0.00	0.00	0.00
Brain	0.01	0.02	0.00	0.00	0.00	0.00
Fat	0.04	0.09	0.00	0.00	0.00	0.00
Intestine	13.91	11.87	0.43	1.06	0.00	0.00
Intestine (contents)	67.67	67.52	4.68	9.03	0.01	0.02
Heart	0.06	0.04	0.00	0.00	0.00	0.00
Kidneys	0.25	0.20	0.02	0.02	0.00	0.00
Liver	5.36	3.29	0.90	0.75	0.10	0.07
Lungs	0.06	0.06	0.00	0.00	0.00	0.00
Muscle	0.03	0.06	0.00	0.00	0.00	0.00
Ovaries	NA	0.01	NA	0.00	NA	0.00
Pancreas	0.10	0.07	0.00	0.00	0.00	0.00
Pituitary	0.00	0.00	0.00	0.00	0.00	0.00
Spleen	0.02	0.02	0.00	0.00	0.00	0.00
Testes	0.02	NA	0.00	NA	0.00	NA
Thymus	0.01	0.02	0.00	0.00	0.00	0.00
Thyroid	0.00	0.00	0.00	0.00	0.00	0.00
Uterus	NA	0.03	NA	0.00	NA	0.00
Residual carcass	7.47	6.89	0.33	0.26	0.04	0.01
Total	95.16	90.39	6.37	11.14	0.15	0.10

a T_{max} male = 0.5 hours postdose, T_{max} female = 1.5 hours postdose
NA = not applicable

Table 14: Total radioactive residues in tissues of the high dose group rats, expressed as percent of dose administered

	T_{max}[a]		24 hours		168 hours	
Tissue	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Plasma	0.05	0.03	0.04	0.04	0.00	0.00
RBC	0.06	0.02	0.03	0.03	0.01	0.00
Adrenals	0.00	0.00	0.00	0.00	0.00	0.00
Bone	0.01	0.00	0.00	0.01	0.00	0.00
Bone Marrow	0.00	0.00	0.00	0.00	0.00	0.00
Brain	0.00	0.01	0.00	0.01	0.00	0.00
Fat	0.02	0.06	0.00	0.04	0.00	0.00
Intestine	5.30	3.87	2.19	3.35	0.00	0.00
Intestine (contents)	74.40	72.33	22.16	21.76	0.01	0.01
Heart	0.02	0.02	0.01	0.02	0.00	0.00
Kidneys	0.08	0.07	0.04	0.08	0.00	0.00
Liver	1.14	0.82	0.85	1.05	0.08	0.04
Lungs	0.03	0.03	0.01	0.03	0.00	0.00
Muscle	0.01	0.02	0.00	0.02	0.00	0.00
Ovaries	NA	0.00	NA	0.00	NA	0.00
Pancreas	0.02	0.02	0.01	0.03	0.00	0.00
Pituitary	0.00	0.00	0.00	0.00	0.00	0.00
Spleen	0.01	0.01	0.00	0.01	0.00	0.00
Testes	0.02	NA	0.01	NA	0.00	NA
Thymus	0.01	0.01	0.00	0.01	0.00	0.00
Thyroid	0.00	0.00	0.00	0.00	0.00	0.00
Uterus	NA	0.02	NA	0.01	NA	0.00
Residual carcass	4.61	3.62	0.96	3.86	0.04	0.04
Total	95.16	90.39	6.37	11.14	0.15	0.10

a T_{max} for both male and female rats = 8 hours
NA = not applicable

Conclusion:

Dimethomorph is rapidly absorbed in the gastrointestinal tract following oral administration to rats. Absorbed dimethomorph is rapidly excreted. Accumulation of dimethomorph in organs and tissues did not occur.

2.1.7 ADME by oral route Study 7 (new).

Reference:

B.6.1.1.7 - Anonymous, 2015 BAS 550 F: Excretion and metabolism in the rat after oral administration 2015/1000602

Test type

Guidelines: OECD 417, 2004/10/EC of 11 February 2004, EPA 870.7485

GLP: yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Test substance

Description: Dimethomorph (BAS 550 F)

Lot/Batch #: 1068-0101 (p-chlorophenyl-U-14C)
858-0101 (morpholine-2,3-14C)
1070-0101 (acrolyl-13C)
1071-0105 (morpholine-15N)
AC9978-68A (unlabeled)

Purity /
E:Z isomer ratio: 96.4% (p-chlorophenyl-U-14C) / 45:55 (HPLC UV), 43:57 (NMR)
98.6% (morpholine-2,3-14C) / 46.5:53.5 (HPLC UV), 45:55 (NMR)
91.3% (acrolyl-13C) / 37.5:62.5 (UV), 45:55 (NMR)
97.3% (morpholine-15N) / 44:56 (HPLC UV)
97.6% (unlabeled) / 44:56

CAS#: 110488-70-5

Development code: 247723 (Reg. No)

Stability of test compound: Stable during dosing period

Detailed study summary and results:

A new study was submitted with the aim to investigate the metabolic fate of dimethomorph radiolabeled in the morpholine moiety after a single oral low and high dose. A further aim was to thoroughly identify metabolites in bile and tissues, for which the previous studies provide only limited information. For a better comparability, both radiolabeled forms (chlorophenyl label and morpholine label) were investigated in parallel within the new study. This study also tries to provide information on the E and Z isomer ratio for parent and main metabolites as far as possible.

After a single oral dose application of [^{14}C]-BAS 550 F (250 mg/kg bw, labelled either in the p-chlorophenyl or morpholine ring) to male and female rats, the excretion of radioactivity during the 168 hours post-dose was similar for both sexes and both labels (>95% of the dose). Excretion of radioactivity was rapid (most was excreted within the first 48 hours after administration), with the majority excreted via faeces (ca. 84-92% of the dose) and smaller amounts via urine (ca. 6-12 % of the dose).

Following a single oral dose application of [^{14}C]-BAS 550 F at two dose levels (10 and 250 mg/kg bw, labelled in two separate locations: p-chlorophenyl or morpholine ring) to male and female bile duct cannulated rats, the excretion of radioactivity during the 72 hours post-dose (p-chlorophenyl label) or 48 hours post dose (morpholine label) was generally similar for both sexes, both dose levels and both labels (>83 % dose in the low dose group and >70 % of the dose in the high dose group). The majority of the

radioactivity was excreted via bile (ca. 61-88% of the dose for the low dose group and 46-60% of the dose for the high dose group), with smaller amounts via urine (ca. 3-5 % dose in males and 10-23 % of the dose in females) and faeces (ca. 5-7 % dose in the low dose group and 13 39% of the dose in the high dose group). The slightly lower recoveries in the bile from the high dose group in comparison to the low dose suggest a possible saturation of absorption at the high dose level (250 mg/kg).

Following a single oral dose application of [^{14}C]-BAS 550 F at two dose levels (10 and 500 mg/kg bw, labelled in two separate locations: p-chlorophenyl or morpholine ring) to male and female rats, radioactivity in the investigated tissues (kidney, liver, pancreas, whole blood and plasma) at the sampling times used (1 hour at the low dose level, 12 hours at the high dose level) were in the same range for both sexes and both labels. Highest proportions of administered radioactivity were observed in liver (3-5 % dose in the low dose group and 0.5 0.9 % dose in the high dose group). The radioactivity residues in kidney were considerably lower (ca. 0.2-0.3 % dose in the low dose group and 0.05-0.07 % dose in the high dose group) and even less in the pancreas. Residue levels in whole blood ranged from 0.3-0.5 % dose at the low dose level or 0.1-0.2 % dose at the high dose level, plasma residue levels were similar to whole blood.

BAS 550 F was extensively metabolised, all analysed matrices (urine, faeces, bile, plasma, kidney and liver) showed HPLC patterns with a multitude of peaks, each often containing multiple metabolites (due to the possibility of E/Z isomerism and also because of very similar polarity of many metabolites due to their structural similarity).

As a conclusion, the metabolism of Dimethomorph in rat was shown to be very extensive. The main transformation steps observed in this study confirm and complement the metabolic pathway known from the previous studies. The main metabolic steps were identified as:

- hydroxylation of either the dimethoxy or chlorophenyl ring and subsequent glucuronidation
- demethylation of the dimethoxy ring and subsequent glucuronidation
- hydroxylation and oxidative opening of the morpholine ring and subsequent conjugation
- cleavage and release of the intact morpholine ring

Material and methods

Test animals

Species: Rat

Strain: Sprague Dawley (Crl:CD(SD)

Age: Phase I: 8 weeks (males) / 11 weeks (females)
Phase II: 9-11 weeks (males) / 12- >15 weeks (females)
Phase III: 8-10 weeks (males) / 11- >15 weeks (females)

Sex: Male and female

Number of animals: 52 males and 52 females in total
Phase I: 16 males and 16 females
Phase II: 20 males and 20 females
Phase III: 16 males and 16 females

Weight at dosing: Phase I: means of 248-250 g (males) means of 244-246 g (females)
Phase II: means of 294-337 g (males), means of 255-293 (females)
Phase III: means of 254-320 g (males), means of 246-286 (females)

Acclimation period: minimum 3 days

Diet: Pelleted diet RM1 (E) SQC, Special Diets Services, Witham, Essex, UK, ad libitum

Water: Tap water, ad libitum

Housing: During acclimatization (all groups) and experiments (D4, D5, D9, D10) in groups of up to four animals in polypropylene cages. During experiments individually in metabolism cages (D1, D2, D3, D6, D7, D8).

Environmental conditions:

Temperature: 19-23°C
Humidity: 45-65%
Air changes: Air-conditioned
Photoperiod: Alternating 12-hour light and dark cycles

Dates of work: May 09, 2013 - April 10, 2015

The excretion and metabolism of BAS 550 F (dimethomorph) was investigated in male and female rats after oral application of a single oral dose of 10, 250 and 500 mg/kg bw BAS 550 F, respectively. The study comprised three experiments:

Phase 1 (excretion balance, investigation of urine and faeces)

Phase 2 (biliary elimination; investigation of bile)

Phase 3 (tissue depletion; investigation of plasma, liver, and kidney)

Since for dimethomorph already sufficient and valid peer reviewed data on the excretion behavior after oral administration is available, the main purpose of this study was to generate sample matrices for further investigation of metabolites.

Dosing

For all dose groups, animals were dosed with mixtures of ^{13}C- and ^{14}C-radiolabeled, ^{15}N-labeled and unlabeled test item in order to facilitate metabolite identification by mass spectrometry and quantitative analysis using HPLC. The dose formulations for the p-chlorophenyl label were prepared by mixing non-labelled BAS 550 F, [acrolyl-13C]-BAS 550 F and [p-chlorophenyl-^{14}C]-BAS 550 F in a ratio of 9:3:4 (for

administration at 10 mg/kg, dose groups 2 and 4) or in a ratio of 30:10:1 (for administration at 250 or 500 mg/kg, dose groups 1, 3 and 5), leading to target specific activities of ca. 1.925 MBq/mg at 10 mg/kg and ca. 0.1878 MBq/mg at 250 or 500 mg/kg.

The dose formulations for the morpholine label were prepared by mixing non-labelled BAS 550 F, [morpholine-15N]-BAS 550 F and [morpholine-^{14}C]-BAS 550 F in a ratio of 1:1:1 (for administration at 10 mg/kg, dose groups 7 and 9) or in a ratio of 22:11:1 for administration at 250 or 500 mg/kg, dose groups 6, 8 and 10), leading to target specific activities of ca. 1.913 MBq/mg at 10 mg/kg and ca. 0.1688 MBq/mg at 250 or 500 mg/kg.

Each formulation was prepared on the day of actual use by suspending/dissolving the batches of appropriately blended [^{14}C]-BAS 550 F in the required amount of 0.1% Tween 80. Animals were dosed by means of a syringe fitted with a gavage tube directly in to the stomach.

The following table summarize the dose groups and dosing parameters.

Table 15*: Summary of Dose Groups and Dosing Parameters*

Dose group	**Phase 1 Excretion balance**		**Phase 2 Biliary excretion**				**Phase 3 Tissue depletion**			
No. of doses and route of administration	Single oral high		Single oral low		Single oral high		Single oral low		Single oral high	
Dose group designation	D1	D6	D2	D7	D3	D8	D4	D9	D5	D10
Radiolabel*	C	M	C	M	C	M	C	M	C	M
Number of animals per Label	8♂, 8♀		5♂, 5♀		5♂, 5♀		4♂, 4♀		4♂, 4♀	
Nominal dose (mg/kg bw)	250		10		250		10		500	
Sampling	**Urine** **Faeces** Cage wash Carcass Blood Kidney Liver		**Bile** Urine Faeces Cage wash Carcass Blood Stomach Gut				**Liver** **Kidney** Pancreas **Blood** **Plasma** at T_{max}=1h		**Liver** **Kidney** Pancreas **Blood** **Plasma** at T_{max}=12h	

In bold print: matrices that were further investigated for metabolites; other matrices only sampled for balance purposes
*C=Chlorophenyl label, M= Morpholine label

Sampling

Phase 1 (excretion balance: groups D1M, D1F, D6M and D6F)

Urine and faeces were sampled at pre-dose, 6 (urine only), 12 (urine only) and 24 hours post application and thereafter in daily intervals until 168 hours. At 168 hours, a terminal whole blood sample was collected. The liver and kidneys were removed from each carcass. Prior to radioactivity analysis, the separate sample types (except carcass) were pooled per label, gender, sample type and collection period/sampling time. Faeces pools were homogenised to a paste with water. Kidney and liver pools were homogenised. Aliquots of each sample pool were taken for radioactivity measurement.

Phase 2 (biliary elimination: groups D2M, D2F, D3M, D3F, D7M, D7F, D8M and D8F)

Bile was sampled at pre-dose, 3, 6, 12, 24, 36, 48, 60 and 72 hours (for some dose groups only) post application. Urine and faeces were sampled at pre-dose, 6 (only urine) 24, 48 and 72 hours (for some dose groups only) post application. At 48 hours (D7M, D7F, D8M and D8F) or 72 hours (D2M, D2F, D3M and D3F), a terminal whole blood sample was collected. The stomach and gut were removed from each carcass. Faeces samples were homogenised to a paste with water. Stomach, stomach contents, gut and gut contents were homogenised. Aliquots of each sample were taken for radioactivity measurement.

Phase 3 (tissue depletion: groups D4M, D4F, D5M, D5F, D9M, D9F, D10M and D10F)

A terminal whole blood sample was collected at the Tmax (based on the data from study DK-452-008), which was at 1 hour post-dose (for the low dose groups) or at 12 hour post-dose (for the high dose groups) via cardiac puncture. The pancreas, kidney and liver were removed from each carcass. Prior to radioactivity analysis, the separate sample types were pooled per label, gender and sample type. Pancreas, kidney and liver pools were homogenised. Aliquots of each sample pool were taken for radioactivity measurement.

Analysis

TRR: For all sampled materials, radioactive residues were determined either by direct combustion or liquid scintillation counting.

HPLC: Selected matrices (Phase 1: urine, faeces; Phase 2 bile; Phase 3: liver, kidney, plasma) were further investigated by radio-HPLC for their metabolite patterns (using HPLC method LC02). The selection of tissues to be analyzed from phase 3 was based on the overall radioactivity content.

Extraction: For radio-HPLC measurement, faeces, plasma and tissue samples were sequentially extracted with acetonitrile, acetonitrile:water (90:10, v:v), acetonitrile:water (10:90, v:v), methanol, and 1% aqueous formic acid, if required. Urine were directly analysed without any further work-up. Bile samples were centrifuged and diluted with water, where necessary.

Non-extractable residues: Where considered necessary (the sample residue after extraction contained a suitable portion of the TRR), the sample residue after extraction was dried and treated with protease enzyme. The selection of samples depended on the overall % TRR, the % dose in the RRR, and the level of already achieved extractability (in cases of high overall recovery).

Metabolite identification: Structure elucidation of metabolites was achieved by LC-MS/MS. Extracts of selected faeces samples (0-24h and 24-48h timepoint) as well as extracts from all tissue and plasma samples were subjected to LC-MS/MS analysis. Urine and bile samples from selected timepoints were investigated by LC-MS/MS directly without further work-up. Selection criteria were the overall %dose contained in the respective time intervals, the presence of all major metabolites and the %dose in the individual peaks, with the aim to investigate all peaks >1% dose and provide identification as far as possible. The LC system used with MS/MS (LC02) was identical to the one used for generation of metabolite patterns, ensuring a good comparability.

Identification of polar region: For identification of metabolites in the polar region (unretained by LC02) observed in bile, urine, plasma and tissues, this region was isolated from those sample matrices where it contained enough radioactivity (urine, bile). The isolated regions were chromatographed on a second HPLC system (LC05), providing better retention. In order to identify free morpholine in the polar region, retention times of the isolated polar peaks were compared with the morpholine radioactive reference standard (on LC05). Moreover, MS identification of morpholine in the polar region was achieved by using a HILIC chromatographic system on a urine sample. For quantification of morpholine in the samples, the ratio of the morpholine peak to the rest of the polar fraction resulting from analysis on LC05 was employed to the amount of the entire polar region as observed in LC02.

Results

Excretion balance/radioactive residues

Phase 1: Excretion balance

As shown in Table 15, after a single oral dose application of [^{14}C]-BAS 550 F (250 mg/kg bw, labelled either in the p-chlorophenyl or morpholine ring) to male and female rats, the excretion of radioactivity during the 168 hours post-dose was similar for both sexes and both labels (>95% of the dose). Excretion of radioactivity was rapid (most was excreted within the first 48 hours after administration), with the majority excreted via faeces (ca. 84-92% of the dose) and smaller amounts via urine (ca. 6-12 % of the dose).

The excretion pattern was similar for both sexes with the females showing slightly higher urinary excretion than males. No difference was observed between the two radiolabelled forms.

The observed excretion pattern is in good agreement with the previous (already peer reviewed) data from study DK-440-001.

Phase 2: Biliary elimination

Following a single oral dose application of [^{14}C]-BAS 550 F at two dose levels (10 and 250 mg/kg bw, labelled either in the p-chlorophenyl or morpholine ring) to male and female bile duct cannulated rats, the excretion of radioactivity during the 72 hours post-dose (p-chlorophenyl label) or 48 hours post dose (morpholine label) was generally similar for both sexes, both dose levels and both labels (>83 % dose in the low dose group and >70 % of the dose in the high dose group). The majority of the radioactivity was

excreted via bile (ca. 61-88% of the dose for the low dose group and 46-60% of the dose for the high dose group), with smaller amounts via urine (ca. 3-5 % dose in males and 10-23 % of the dose in females) and faeces (ca. 5-7 % dose in the low dose group and 13 39% of the dose in the high dose group). The slightly lower biliary excretion in the high dose group in comparison to the low dose animals suggest a possible saturation of absorption at the high dose level (250 mg/kg).

This data clearly shows that the majority of the radioactivity excreted via faeces in the excretion balance phase (phase 1) was due to bile and that biliary excretion is an important route for the excretion of dimethomorph in rats. Biliary excretion in females was slightly less than that observed for males, while urinary excretion in females was slightly more than that observed for males, which is congruent with the results observed in the phase 1 experiments. At the higher dose level, biliary excretion was lower than observed at the low dose level suggesting a saturation of absorption. No major differences were observed between the two radiolabelled forms.

Again, these results are in good agreement with the already peer reviewed data from study DK-440-002.

Table 16*: Excretion balance (phase 1)*

Matrix	Time interval [h]	Dose Group (nominal dose)			
		D1 (250 mg/kg)		D6 (250 mg/kg)	
		p-Chlorophenyl label		Morpholine label	
		Male	Female	Male	Female
		% of the dose		% of the dose	
Urine	0-6	0.90	1.14	0.73	0.96
	6-12	1.34	1.67	1.53	1.54
	12-24	2.68	3.40	2.20	2.99
	24-48	1.41	4.20	0.95	4.00
	48-72	0.20	0.86	0.20	1.34
	72-96	0.13	0.22	0.12	0.31
	96-120	0.06	0.13	0.09	0.15
	120-144	0.06	0.12	0.04	0.09
	144-168	0.03	0.08	0.03	0.07
Total urine	*0-168*	***6.81***	***11.82***	***5.89***	***11.45***
Faeces	0-24	57.30	40.02	71.39	36.10
	24-48	29.63	40.12	19.03	36.58
	48-72	1.45	6.87	0.93	9.61
	72-96	0.24	1.55	0.22	1.26
	96-120	0.31	0.90	0.09	0.34
	120-144	0.06	0.07	0.16	0.09
	144-168	0.05	0.20	0.05	0.06
Total faeces	*0-168*	***89.04***	***89.73***	***91.87***	***84.04***
Total excreted	*0-168*	***95.85***	***101.55***	***97.76***	***95.49***
Cage wash	168	0.44	0.93	0.13	0.45
Whole Blood	168	0.01	0.01	0.06	0.06

Matrix	Time interval [h]	Dose Group (nominal dose)			
		D1 (250 mg/kg)		D6 (250 mg/kg)	
		p-Chlorophenyl label		Morpholine label	
		Male	Female	Male	Female
		% of the dose		% of the dose	
Kidney	168	0.00	0.00	0.02	0.02
Liver	168	0.07	0.05	0.23	0.22
Total	**0-168**	**96.37**	**102.54**	**98.20**	**96.24**

Table 17*: Biliary excretion (Phase 2)*

Matrix	Time interval [h]	Dose Group (nominal dose)							
		D2 (10 mg/kg)		D3 (250 mg/kg)		D7 (10 mg/kg)		D8 (250 mg/kg)	
		p-Chlorophenyl label				Morpholine label			
		Male	Female	Male	Female	Male	Female	Male	Female
		% of the dose				% of the dose			
Bile	0-3	55.06	40.08	8.56	8.85	34.17	30.76	12.81	11.87
	3-6	22.25	13.53	12.95	9.14	13.69	19.82	11.98	6.87
	6-12	8.97	6.05	18.00	11.20	10.16	17.48	18.81	14.98
	12-24	1.30	0.52	16.71	10.48	15.66	2.98	15.35	14.32
	24-36	0.16	0.16	2.82	4.83	0.61	0.31	0.90	7.92
	36-48	0.08	0.10	0.95	1.44	0.13	0.13	0.10	0.55
	48-60	0.06	0.04	0.19	0.33	-	-	-	-
	60-72	0.06	0.02	0.03/	0.00	-	-	-	-
Total bile	*0-Terminal*	***87.94***	***60.50***	***60.20***	***46.27***	***74.41***	***71.47***	***59.94***	***51.51***
Urine	0-6	1.42	17.91	0.61	1.19	0.83	1.44	0.99	2.92
	6-24	1.68	4.86	2.18	7.60	2.47	10.83	3.93	8.97
	24-48	0.15	0.28	0.28	1.65	0.36	1.50	0.35	5.36
	48-72	0.06	0.15	0.01	0.00	-	-	-	-
Total urine	*0-Terminal*	***3.31***	***23.20***	***3.08***	***10.44***	***3.66***	***13.76***	***5.27***	***17.26***
Faeces	0-24	4.85	6.63	17.30	12.59	3.66	5.01	37.17	14.37

Matrix	Time interval [h]	Dose Group (nominal dose)							
		D2 (10 mg/kg)		D3 (250 mg/kg)		D7 (10 mg/kg)		D8 (250 mg/kg)	
		p-Chlorophenyl label				Morpholine label			
		Male	Female	Male	Female	Male	Female	Male	Female
		% of the dose				% of the dose			
	24-48	0.31	0.51	3.20	0.35	1.09	0.86	2.22	7.26
	48-72	0.01	0.21	0.01	0.00	-		-	-
Total faeces	*0-Terminal*	***5.17***	***7.35***	***20.51***	***12.94***	***4.74***	***5.86***	***39.39***	***21.63***
Total excreted	*0-Terminal*	***96.42***	***91.05***	***83.79***	***69.64****	***82.81***	***91.09***	***104.60***	***90.40***

Terminal sampling: D2, D3 = 72 hours post dose, D7, D8 = 48 hours post dose
*for this dose group 18.96% dose was additionally recovered in stomach content

Phase 3: Tissue depletion

The data shows that the highest levels of radioactivity were found in liver, followed by blood, plasma and kidney while residue levels in pancreas were low (see Table 18 andTable 19). Residue levels in liver were marginally lower in females than males. Plasma residue levels were similar to whole blood. No differences were observed between the two radiolabelled forms.

Table 18: Radioactivity in blood and tissues of the low dose groups (Phase 3)

Matrix	Time interval [h]	Dose Group (nominal dose)			
		D4 (10 mg/kg)		D9 (10 mg/kg)	
		p-Chlorophenyl label		Morpholine label	
		Male	Female	Male	Female
		% of the dose/conc in µg/g		% of the dose/conc in µg/g	
Liver	1	4.81/11.37	3.58/10.18	4.02/8.679	2.74/7.486
Kidney	1	0.30/3.758	0.29/3.791	0.19/2.392	0.21/2.840
Pancreas	1	0.06/2.327	0.06/2.785	0.03/1.641	0.05/2.256
Whole blood	1	0.43/0.865	0.53/1.078	0.33/0.641	0.42/0.811
Plasma	1	0.30/1.105	0.37/1.398	0.24/0.836	0.31/1.114

Table 19: Radioactivity in blood and tissues of the high dose groups (Phase 3)

Matrix	Time interval [h]	Dose Group (nominal dose)			
		D5 (500 mg/kg)		D10 (500 mg/kg)	
		p-Chlorophenyl label		Morpholine label	
		Male	Female	Male	Female
		% of the dose/conc in µg/g		% of the dose/conc in µg/g	
Liver	12	0.80/130.2	0.53/87.56	0.86/149.2	0.55/87.07
Kidney	12	0.07/47.13	0.06/46.76	0.06/49.07	0.05/37.28
Pancreas	12	0.03/48.21	0.02/43.96	0.01/46.86	0.01/40.14
Whole blood	12	0.17/17.43	0.14/14.17	0.18/18.23	0.13/13.57
Plasma	12	0.11/21.53	0.10/18.54	0.13/24.93	0.09/17.81

Extractability

Faeces (Phase 1)

Pooled faeces samples from the chlorophenyl label were extracted with acetonitrile followed by acetonitrile:water (90:10, v:v). The achieved extractabilities (ERR) were 87-102% and 95-100% for male and female faeces, respectively.

Pooled faeces samples from the morpholine label were serially extracted, first with acetonitrile and then with acetonitrile:water (90:10, v:v). Some samples (with less than 90% recovery) were extracted a third time using acetonitrile:water (10:90, v:v) or a fourth time using methanol. The achieved extractabilities (ERR) were 83-94% and 90-94% for male and female faeces, respectively.

Tissues (Phase 3)

Pooled samples of plasma, liver, and kidney were sequentially extracted with acetonitrile, acetonitrile:water (90:10, v:v), acetonitrile:water (10:90, v:v), methanol, and 1% aqueous formic acid.

Achieved extractabilities ranged from 73-90% dose for the males and 78-95% dose for the females of the low dose group (chlorophenyl label), respectively. For the morpholine label low dose group, the ERR ranged from 75-99% dose for males and from 83-96% dose for females.

For the high dose group of the chlorophenyl label, extractabilities were 83-96% and 94-99% dose for males and females, respectively. For the high dose group of the morpholine label, the ERR were in the range of 63-90% dose and 77-88% dose for males and females, respectively.

The RRRs of some faeces, liver and plasma samples were treated with protease enzyme, which released between 38 and 41% of the bound radioactivity in faeces, between 39 and 54% of the bound radioactivity in liver and 30% of the bound radioactivity in plasma.

Identification of Metabolites

BAS 550 F was extensively metabolised, all analysed matrices (urine, faeces, bile, plasma, kidney and liver) showed HPLC patterns with a multitude of peaks, each often containing multiple metabolites (due to the possibility of E/Z isomerism and also because of very similar polarity of many metabolites due to their structural similarity). A detailed list of the structures of all identified metabolites with their molecular weight and structure can be found in Table 24 at the end of this section.

Urine and faeces (Phase 1)

The metabolite patterns in faeces extracts were largely comparable for both sexes and both labels. BAS 550 F was a major metabolite in faeces and was observed in both isomeric forms (BAS 550 F (E): up to 3% of dose, BAS 550 F (Z): up to 26% of dose). BAS 550 F (Z) was generally the most abundant component, with the following metabolites present in significant amounts: M550F016_37.8min (up to 18% of dose), M550F007E (up to 13% of dose) and M550F006E (up to 6 % of the dose).

The metabolite patterns in urine were also complex despite being a more minor route of excretion than faeces and were largely comparable for both sexes. BAS 550 F was not observed in urine. The following metabolites were present in urine in significant amounts: M550F013_31.4min (up to 2% of dose), M550F016_37.8min (up to 1% of dose), M550F027_38.4min (up to 1% of dose), M550F028_43.1min (up to 2% of dose) and M550F007E (up to 2% of dose).

The morpholine label samples generally contained an additional polar radioactive region which contained the metabolite M550F021 (morpholine, up to 1% of the dose). This metabolite was not observed in the p-chlorophenyl label samples because of the positioning of the [^{14}C]-label.

Table 20: Identified metabolites in urine and faeces of rats

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
BAS550F(E)	-	1.96	-	2.83	-	2.54	-	1.66
BAS550F(Z)	-	16.87	-	26.35	-	18.14	-	15.5
M357_36.2min*	-	-	-	1.82	-	-	-	-
M357_44.9min*	-	3.55	-	5.8	-	3.78	-	4.44
M361	-	-	-	-	0.26	-	-	-

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
M371_51.4min*	-	16.87	-	26.35	-	18.14	-	15.5
M371_52.6min*	-	-	-		-	-	-	
M377_40.9min	-	-	-	-	0.26	-	-	-
M385*	-	-	-	3.33	-	-	-	-
M391	-	4.16	-	-	-	-	-	-
M405	-	-	-	-	0.26	-	-	-
M405	-	-	-	-	0.26	-	-	-
M419	-	3.39	-	-	-	-	-	-
M419	-	-	-	-	-	4.49	-	-
M421	-	1.16	-	-	-	-	-	-
M435	-	17.96	-	-	-	-	-	-
M435	-	-	-	-	-	12.72	-	-
M471	-	-	-	-	-	4.49	-	-
M547_31.1min*	0.65	-	0.88	-	-	-	-	-
M547_37.5min*	-	-	0.99	-	-	-	1.07	-
M547_38.1min*	-	-	-	-	-	-		-
M550F006E	-	4.13	-	5.8	-	3.78	-	4.44
M550F006Z	-	1.08	-	1.35	-	-	-	1.06
M550F007E	-	5.91	1.66	9.52	-	12.72	1.83	9.57
M550F007Z	-	3.49	-	3.75	-	3.31	-	3.77
M550F008	-	1.08	-	-	-	-	-	-
M550F009_38.0min	-	17.96	0.99	17.25	-	13.7	1.07	13.7
M550F009_40.6min	-	3.55	-	2.23	0.26	3.52	-	2.28
M550F011_41.0min	-	3.85	-	1.41	-	3.43	-	2.55
M550F011_43.4min	-	5.91	1.66	9.52	-	-	1.83	9.57
M550F012_44.1min	-	4.13	-	5.8	-	-	-	-
M550F012_47.2min	-	1.08	-	1.35	-	-	-	-
M550F013_31.4min	0.7	-	2.37	-	0.32	-	1.3	-
M550F015_32.9min	0.74	-	0.98	-	0.56	-	-	-
M550F015_34.5min	-	-	-	-	-	2.86	-	-
M550F016_37.8min	-	17.96	0.99	17.25	-	13.7	1.07	13.7
M550F016_41.1min	-	3.85	-	1.41	-	-	-	-
M550F018_44.6min	-	4.13	-	5.8	-	-	-	-

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
M550F018_45.0min	-	3.55	-	3.33	-	-	-	-
M550F018_45.6min	-	3.49	-		-	3.04	-	3.53
M550F021 (Morpholine)	-	-	-	-	0.95	-	0.59	-
M550F022_36.2min	-	1.16	-	-	-	2.15	-	-
M550F022_39.3min	-	-	-	1.19	-	4.49	-	-
M550F023_33.5min	-	3.39	-	-	-	2.72	-	1.94
M550F023_36.2min	-	1.16	-	-	-	-	-	-
M550F024_28.6min	-	-	-	-	-	1.27	-	1.22
M550F024_32.9min	-	-	-	-	-	-	-	1.27
M550F025_36.1min	-	-	-	-	-	2.15	-	-
M550F025_37.3min	-	1.6	-	17.25	-	-	-	0.98
M550F025_38.3min	-	-	0.99		-	4.49	-	3.29
M550F025_40.3min	-	3.55	-	2.23	0.26	3.52	-	2.28
M550F027_34.5min	-	-	-	1.53	0.15	2.86	-	-
M550F027_38.4min	-	17.96	0.99	17.25	-	4.49	1.07	3.29
M550F027_41.4min	-	3.85	-	-	-	-	-	-
M550F028_40.5min	-	3.55	-	2.23	0.26	3.52	-	2.28
M550F028_41.7min	-	3.85	-	-		-	-	-
M550F028_43.1min	-	5.91	1.66	9.52	-	12.72	1.83	9.57
M550F029_30.4min	0.65	-	0.88	-	-	-	-	-
M550F029_31.3min	0.7	-	2.37	-	-	-	1.3	-
M550F030_41.2min	-	3.85	-	1.41	-	-	-	-
M550F030_43.4min	-	5.91	1.66	9.52	-	12.72	1.83	9.57
M550F031_45.1min	-	3.55	-	9.13	-	3.78	-	4.44

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
M550F033_34.6min	-	1.66	-	1.53	-	2.86	-	-
M550F033_38.6min	-	17.96	0.99	17.25	-	4.49	1.07	3.29
M550F034_37.9min	-	17.96	0.99	-	-	-	-	-
M550F034_41.0min	-	3.85	-	1.41	0.26	3.43	-	2.55
M550F035_36.5min	-	1.16	-	1.82	-	2.15	-	1.97
M550F035_38.8min	-	-	-	-	-	4.49	1.07	3.29
M550F036	-	1.6	-	-	-	-	-	-
M550F037	-	3.39	-	-	-	-	-	-
M550F038	-	3.85	-	1.2	-	-	-	-
M550F039_31.1min	-	-	0.88	-	-	-	-	-
M550F039_40.5min	-	3.55	-	-	-	-	-	-
M550F040_38.6min	-	-	0.99	-	-	-	-	-
M550F040_41.3min	-	3.85	-	-	-	-	-	-
M550F041_30.1min	-	0.98	-	0.72	-	-	-	-
M550F041_34.4min	-	1.66	-	1.53	-	2.86	-	-
M550F042	0.74	-	-	-	-	-	-	-
M550F043	-	1.16	-	-	-	-	-	-
M550F044	-	1.6	-	-	-	-	-	-
M550F045	-	3.85	-	-	-	-	-	-
M550F046	-	-	-	1.82	-	2.15	-	1.97
M550F047_30.7min	-	4.16	-	2.61	0.34	4.08	-	3.03
M550F047_35.1min	-	1.66	-	1.53	-	-	-	-
M550F049_35.4min	-	-	-	-	-	2.86	-	-
M550F049_39.0min	-	17.96	0.99	17.25	-	-	-	-
M550F050_37.1min	-	1.6	-	17.25	-	-	-	0.98
M550F050_39.5min	-	-	-	1.19	-	4.49	-	-
M550F051_49.8min	-	1.96	-	-	-	-	-	-

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
M550F051_51.2min	-	16.87	-	26.35	-	18.14	-	15.5
M550F052	-	4.13	-	5.8	-	-	-	-
M550F053_43.3min	-	5.91	1.66	-	-	12.72	-	-
M550F053_51.4min	-	16.87	-	-	-	18.14	-	-
M550F055_30.8min	0.65	4.16	0.88	-	0.34	-	-	-
M550F055_32.0min	0.7	-	-	-	-	1.12	-	0.28
M550F056_30.5min	-	4.16	-	0.72	-	-	-	-
M550F056_34.5min	-	1.66	-	-	-	-	-	-
M550F057_38.3min	-	-	-	-	-	4.49	-	3.29
M550F057_41.3min	-	3.85	-	1.41	-	-	-	-
M550F058_34.5min	-	1.66	-	-	-	2.86	-	-
M550F058_38.0min	-	17.96	-	-	-	13.7	-	-
M550F060	-	3.49	-	-	-	-	-	-
M550F061	-	3.55	-	-	-	3.52	-	-
M550F062_31.4min	0.7	-	2.37	-	-	-	-	-
M550F062_34.6min	-	1.66	-	-	-	-	-	-
M550F063_31.8min	0.7	-	2.37	-	-	-	-	-
M550F063_35.1min	-	1.66	-	-	-	-	-	-
M550F064_30.9min	0.65	-	-	-	-	-	-	-
M550F065	0.7	-	-	-	-	-	-	-
M550F066	-	16.87	-	26.35	-	18.14	-	-
M550F067_25.5min	-	-	0.96	-	-	-	-	-
M550F068_36.1min	-	1.16	-	-	-	-	-	-
M550F069_30.3min	0.65	-	0.88	-	0.34	-	-	-
M550F069_35.5min	-	-	-	-	-	2.86	-	-
M550F070_25.5min	0.64	-	-	-	-	-	-	-

Designation	Chlorophenyl label				Morpholine label			
	Male		Female		Male		Female	
	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]	Urine [%dose]	Faeces [%dose]
M550F070_32.2min	0.74	-	-	-	-	-	-	-
M550F071	-	-	0.99	-	-	-	-	-
M550F072_26.5min	0.64	-	-	-	-	-	-	-
M550F073_32.1min	0.7	-	-	-	-	-	-	-
M550F074_25.4min	0.64	-	0.96	-	-	-	-	-
M550F074_27.7min	-	-	-	-	0.16	-	-	-
M550F074_31.0min	-	-	-	-	0.34	-	-	-
M550F075_32.1min	-	-	-	-	-	-	1.3	-
M550F076_30.8min	0.65	-	-	-	0.34	4.08	-	-
M550F076_31.3min		-	0.88	-		-	-	-
M550F078_30.5min	0.65	-	0.88	-	-	-	-	-
M550F079_30.4min	-	-	-	-	0.34	-	-	-
M550F081_32.1min	0.7	-	-	-	-	-	1.3	-
M550F083_37.4min	-	-	-	17.25	-	-	-	13.7
M550F083_37.9min	-	-	0.99	-	-	13.7	1.07	13.7
M550F084	0.64	-	-	-	-	-	-	-
M550F087	0.74	-	0.98	-	-	-	-	-
M550F088	-	-	-	-	0.16	-	-	-
M550F090_31.9min	0.7	-	-	-	0.32	1.12	1.3	0.28
M550F090_34.6min	-	-	-	1.53	-	2.86	-	-
M563	0.07	-	-	-	-	-	-	-
M565	0.64	-	-	-	-	-	-	-
M567	0.64	-	-	-	-	-	-	-
M567_25.4min	-	-	0.96	-	-	-	-	-
M567_25.4min	-	-	0.42	-	-	-	-	-
M581	-	-	-	-	0.16	-	-	-
M595	-	-	-	1.53	-	-	-	-

* This metabolite was not assigned a metabolite code name (designation contains the M550F prefix) as it is considered to be a degradation product of an original metabolite

Bold type face in the metabolite identity indicates that the metabolite was considered a main metabolite present based on MS response

Bile (Phase 2)

The metabolite patterns in bile were again complex and were largely comparable for both sexes and both dose levels. BAS 550 F was not observed in bile. The most abundant metabolite in bile was M550F013, in amounts from 22-40% dose (sum of isomers at 31.4min and 34.7min). The following metabolites were also present in bile in significant amounts: M550F069_30.3min (up to 12% of dose), M550F076_30.8min (up to 6% of dose), M547_31.1min (up to 6% of dose), M550F075_32.1min (up to 8% of dose), M547_37.5min (up to 9% of dose), M550F083_37.9min (up to 9% of the dose), M550F027_38.4min (up to 9% of dose) and M550F007E (up to 7% of dose).

Although the morpholine label bile samples generally contained an additional polar radioactive region which contained the metabolite M550F021 (morpholine), this metabolite was not present in significant amounts in bile.

Table 21. Identified metabolites in bile from rats

Metabolite / Component[#]	**Low dose Male**	**Low dose Female**	**High dose male**	**High dose Female**	**Low dose Male**	**Low dose Female**	**High dose male**	**High dose Female**
	Chlorophenyl label				**Morpholine label**			
	Composition of radioactive residues in % of the dose (Sum 0-72 h)							
M550F021 (Morpholine)	-	-	-	-	0.34	-	-	-
M550F069_23.5min / M550F086_24.0min / M550F062_24.3min	1.03	-	-	-	-	-	-	-
M550F080_25.4min / M550F074_25.4min / M550F070_25.5min / M550F082_25.9min / M550F084	4.00	1.36	2.29	1.11	3.21	1.79	2.00	1.44
M550F089_27.6min / M550F074_27.7min / M550F064_27.7min / M550F088	2.20	1.43	2.84	1.13	3.38	1.91	2.46	1.08
M550F076_28.6 / M550F077 M550F067_29.8min M550F068_29.7min /	-	-	-	-	-	-	3.10	0.99

Metabolite / Component[#]	Low dose Male	Low dose Female	High dose male	High dose Female	Low dose Male	Low dose Female	High dose male	High dose Female
	Chlorophenyl label				Morpholine label			
	Composition of radioactive residues in % of the dose (Sum 0-72 h)							
M550F086_30.0min / M550F069_30.3min / M550F029_30.4min M550F055_30.8min M550F072_29.9min M550F079_30.2min ;M623 M550F086_30.0min M550F079_30.4min								
M550F085 / M550F078_28.7	-	1.19	-	-	-	1.47	-	0.41
M550F077 / M550F068_29.7min / M550F086_30.0min	2.79	-	-	-	2.27	-	-	-
M550F069_30.3min / M550F079_30.4min / M550F029_30.4/ M550F078_30.5 / **M550F076_30.8min /** M550F055_30.8min / M550F064_30.9min / M550F074_31.0min / M579 **M547_31.1min /** M550F076_31.3min / M480 M547_31.5min	11.53	5.46	5.97	2.65	8.65	6.42	3.94	2.27
M550F029_31.3 M550F062_31.4min / M550F081_31.4min / M550F082_31.5min / **M550F013_31.4min** / **M550F063_31.8min**	32.52	24.97	20.77	17.90	25.13	27.54	26.40	23.90
M550F073_32.1min M550F081_32.1min / **M550F075_32.1min** /	7.86	5.33		1.45	3.84	5.37		

Metabolite / Component[#]	Low dose Male	Low dose Female	High dose male	High dose Female	Low dose Male	Low dose Female	High dose male	High dose Female
	Chlorophenyl label				Morpholine label			
	Composition of radioactive residues in % of the dose (Sum 0-72 h)							
M550F070_32.2min M550F089_32.6								
M550F090_31.9min / M550F055_32.0min / M550F081_32.1min / M550F073_32.1min / M550F075_32.1min / M550F070_32.2min / M623_32.2min / M550F086_32.6min / M550F089_32.6min	-	-	-	-	-	-	-	1.43
M550F015_32.9min	1.52	2.41	3.60	2.61	2.41	2.17	4.02	3.43
M550F074_33.7min	1.98	-	-	-	-	-	-	-
M550F027_34.5min / M550F015_34.5min / M550F073_34.6min/ M550F090_34.6min / **M550F013_34.7min /** M550F033_34.6min	7.55	7.35	5.55	4.37	8.02	7.77	5.41	8.15
M547_37.5min / **M550F083_37.9min /** M550F009_38.0min/ **M550F027_38.4min** M550F059/ M547_38.1min / M550F071 M550F040_38.6min M550F033_38.6min	9.17	6.11	6.32	6.01	6.97	7.64	7.18	7.48
M550F025_40.3min / M550F028_40.5min / M550F009_40.6min / M577_41.6min	-	-	-	-	-	-	-	0.64
M577_42.6 / M550F018_42.6min M550F028_43.1min /	4.31	3.51	2.62	2.44	5.54	6.82	3.19	4.25

Metabolite / Component[#]	Low dose Male	Low dose Female	High dose male	High dose Female	Low dose Male	Low dose Female	High dose male	High dose Female
	Chlorophenyl label				Morpholine label			
	Composition of radioactive residues in % of the dose (Sum 0-72 h)							
M550F030_43.4min M550F053_43.3 M550F011_43.4min / **M550F007E**								
M550F006E / M417 / M550F031_45.1min	-	-	-	-	-	-	-	0.48

#: Bold type face in the metabolite identity indicates that the metabolite was considered a main metabolite present based on MS response. Not all metabolites were identified during every sample period.

Plasma and tissues (Phase 3)

The metabolite patterns in plasma were complex despite containing relatively small amounts of radioactivity and were similar for both sexes and both dose levels. BAS 550 F was a major metabolite in plasma and was observed in the both isomeric forms (BAS 550 F (E): up to 0.01% of the dose, BAS 550 F (Z): up to 0.15% of the dose). The following metabolites were also present in the major radioactive regions in plasma: M550F013_31.4min (up to 0.02% of dose), M550F022_39.3min (up to 0.02% of dose), M550F018_45.0min (up to 0.10% of dose, sum of isomers at 45.0 and 45.6 min).

The metabolite patterns in kidney were complex and largely comparable for both sexes and both dose levels. BAS 550 F was a major metabolite in kidney and was observed in the both isomeric forms (BAS 550 F (E): up to 0.01% of the dose, BAS 550 F (Z): up to 0.06% of the dose). The following metabolites were also present in the major radioactive regions in kidney: M550F027_38.4min (up to 0.04% of dose), M550F011_41.0min (up to 0.01% of dose), M550F028_43.1min (up to 0.07% of dose), M550F007E (up to 0.02% of dose), M550F031_45.1min (up to 0.03% of dose), M550F011_43.4min (up to 0.01% of dose) and M550F018_45.6min (up to 0.04% of dose).

The metabolite patterns in liver were complex and largely comparable for both sexes and both dose levels. BAS 550 F (Z) was present in the major radioactive regions in liver (up to 0.43% of dose). The following metabolites were also present in the major radioactive regions in liver: M550F076_28.6min (up to 0.22% of dose), M550F009_38.0min (up to 0.10% of dose), M550F027_38.4min (up to 0.41% of dose), M550F028_40.5min (up to 0.06% of dose), M550F028_43.1min (up to 0.63% of dose), M550F007E (up to 0.63% of dose), M550F011_43.4min (up to 0.63% of dose), M550F031_45.1min (up to 0.21% of dose) and M550F018_45.6min (up to 0.41% of dose).

The morpholine label samples of plasma, kidney and liver generally contained an additional polar radioactive region (up to 0.03%, 0.05% and 0.74% of dose in plasma, kidney and liver, respectively). In matrices which contained sufficient radioactivity to allow additional analysis (urine and bile), M550F021 (morpholine) was identified as one of the radioactive components present in that region, thus it is very likely that the polar regions in plasma, kidney and liver partly contain this metabolite as well.

Table 22: Identified metabolites in plasma, kidney, and liver (p-chlorophenyl label)

Metabolite / Component	Plasma male low	Plasma female low	Plasma male high	Plasma female high	Kidney male low	Kidney female low	Kidney male high	Kidney female high	Liver male low	Liver female low	Liver male high	Liver female high
	Composition of radioactive residues in % of the dose											
BAS 550 F (Z)	0.03	0.15	0.02	0.05	-	0.04	0.01	0.02	-	0.43	-	0.10
BAS 550 F (E)	-	-	0.01	0.01	-	-		0.01	-	-	-	-
M550F009_38.0	-	-	-	-	-	-	-	-	-	-	0.10	-
M550F009_40.6											0.06	
M550F007E					0.03				0.28		0.05	0.08
M550F011_41.0	-	-	-	-			0.01	-	-	-	0.06	-
M550F011_43.4	-	-	-	-	0.03	0.07	-	-	0.28	0.42	0.05	0.08
M550F018_45.0	0.02	0.04	-	-	0.03	-	-	-	-	-	-	0.08
M550F018_45.6	0.04	0.06	0.01	0.01		-	0.01	0.01		0.41	-	0.07
M550F027_38.4	-	-	-	-	0.04	0.04	-	-	0.38	-	-	-
M550F028_40.5	-	-	-	-	-	-	0.01	-	-	-	0.06	-
M550F028_43.1	-	-	-	-	0.03	0.07	-	-	0.28	0.42	-	0.08
M550F030_41.2							0.01					
M550F030_43.4						0.07				0.42		0.08
M550F031_45.1	-	-	-	-	0.03	-	0.01	-	-	-	-	0.07
M550F031_43.4												0.08
M550F034_41							0.01					
M550F076_28.6	-	-	-	-	-	-	-	-	0.22	-	-	-
M550F083_37.9											0.10	

Table 23: Identified metabolites in plasma, kidney, and liver (morpholine label)

Metabolite / Component	Plasma male low	Plasma female low	Plasma male high	Plasma female high	Kidney male low	Kidney female low	Kidney male high	Kidney female high	Liver male low	Liver female low	Liver male high	Liver female high
	Composition of radioactive residues in % of the dose											
BAS 550 F (Z)	0.02	0.09	0.01	0.03	-	0.06	0.01	0.01	-	0.22	-	0.04
BAS 550 F (E)	-	-	-	-	-	-	0.00	-	-	-	-	-

Metabolite / Component	Plasma male low	Plasma female low	Plasma male high	Plasma female high	Kidney male low	Kidney female low	Kidney male high	Kidney female high	Liver male low	Liver female low	Liver male high	Liver female high
	Composition of radioactive residues in % of the dose											
M550F007E	-	-	-	-	0.02	-	0.00	-	0.53	0.63	0.04	0.12
M550F009_38.0	-	-	-	-	-	-	-	-	-	-	0.05	0.04
M550F009_40.6											0.05	
M550F011_43.4	-	-	-	-	0.02	0.04	0.00	0.01	0.53	0.63	0.04	0.12
M550F013_31.4	0.02	0.01	0.01	0.00	-	-	-	-	-	-	-	-
M550F018_45.0	-	0.03	-	-	0.02	0.04	-	-	0.21	-	-	0.12
M550F018_45.6	0.02	0.03	0.01	0.01			-	-		-	-	-
M550F022_39.3	0.02	-	-	-	-	-	-	-	-	-	-	-
M550F027_38.4	-	-	-	-	0.03	-	-	0.00	0.41	0.29	0.05	0.04
M550F028_40.5	-	-	-	-	-	-	-	-	-	-	0.05	-
M550F028_43.1	-	-	-	-	0.02	0.04	0.00	0.01	0.53	0.63	0.04	0.12
M550F029_31.3			0.01	0.01								
M550F030_43.4						0.04	0.00	0.01		0.63	0.04	0.12
M550F031_45.1	-	-	-	0.01	0.02	-	0.00	-	0.21	-	0.04	0.12
M550F083_37.9											0.05	0.04

E/Z isomer ratio of dimethomorph

The E/Z isomer ratio for BAS 550 F in the application formulations were approx. 44:52.

The E and Z isomers of BAS 550 F were identified in most faeces samples and some plasma/tissue samples.

In faeces, the E/Z isomer ratio was relatively stable at ca. 13:87 in the first sampling period (0-24 hours), regardless of sex and label. In the later sampling periods (24 48 and 48-72 hours), only smaller amounts of the Z isomer were observed.

In plasma, kidney and liver, the E/Z isomer ratio (where calculable) ranged between 57:43 and 7:93 with most samples containing more Z isomer.

Storage stability

Preliminary HPLC analysis (method LC02) was performed on selected samples of urine (male and female, both labels) and bile (male and female, both labels, 10 mg/kg) ca. 3 months after sampling. Additional preliminary HPLC analysis (method LC02) was performed on selected samples of urine (male, p-chlorophenyl label), bile (male, p-chlorophenyl label, 10 mg/kg) and faeces (male, p-chlorophenyl label) ca. 6 months after sampling (within 1 month of extraction for faeces).

All quantitative HPLC runs (method LC02) of urine, faeces and bile were accomplished within ca. 9 months after sampling. Comparison of preliminary HPLC analysis and actual analysis showed that the metabolic patterns obtained were similar, demonstrating stability during storage.

The quantitative HPLC runs (method LC02) of plasma/kidney/liver extracts were accomplished with the LC-MS analysis ca. 6 months after extraction. The extraction of the plasma/kidney/liver samples was generally accomplished within ca. 6 months after sampling.

Metabolic pathway

The proposed metabolic pathway in rats is depicted in Figure 1: Proposed metabolic pathway of BAS 550 F in rats (representing already peer reviewed and new data)Figure 1.

The observed transformation steps in the metabolism of dimethomorph are:

- hydroxylation of either the dimethoxy or chlorophenyl ring and subsequent glucuronidation (Metabolic steps 1 and 2)
- demethylation of the dimethoxy ring and subsequent hydroxylation and/or glucuronidation (Metabolic step 3)
- hydroxylation of the morpholine ring and subsequent modification (further hydroxylation, ring opening, degradation, conjugation) (Metabolic step 5)
- cleavage and release of the intact morpholine ring (Metabolic step 4)

Generally, many observed metabolites combine different metabolic steps with their sequence not being able to be exactly stated.

The metabolic step 3 leads to a considerable number of different metabolites, combining single or double demethylation, hydroxylation/opening of the morpholine ring and glucuronide conjugation.

Metabolic step 5 comprises many metabolic structures arising from several subsequent metabolic steps following the hydroxylation of the morpholine ring.

Substep 5a includes double hydroxylation of the morpholine ring, its opening, demethylation and glucuronidation.

Substep 5b includes metabolites with a reduction at the morpholine ring

Substep 5c includes metabolites with an additional hydroxylation of the dimethoxy ring.

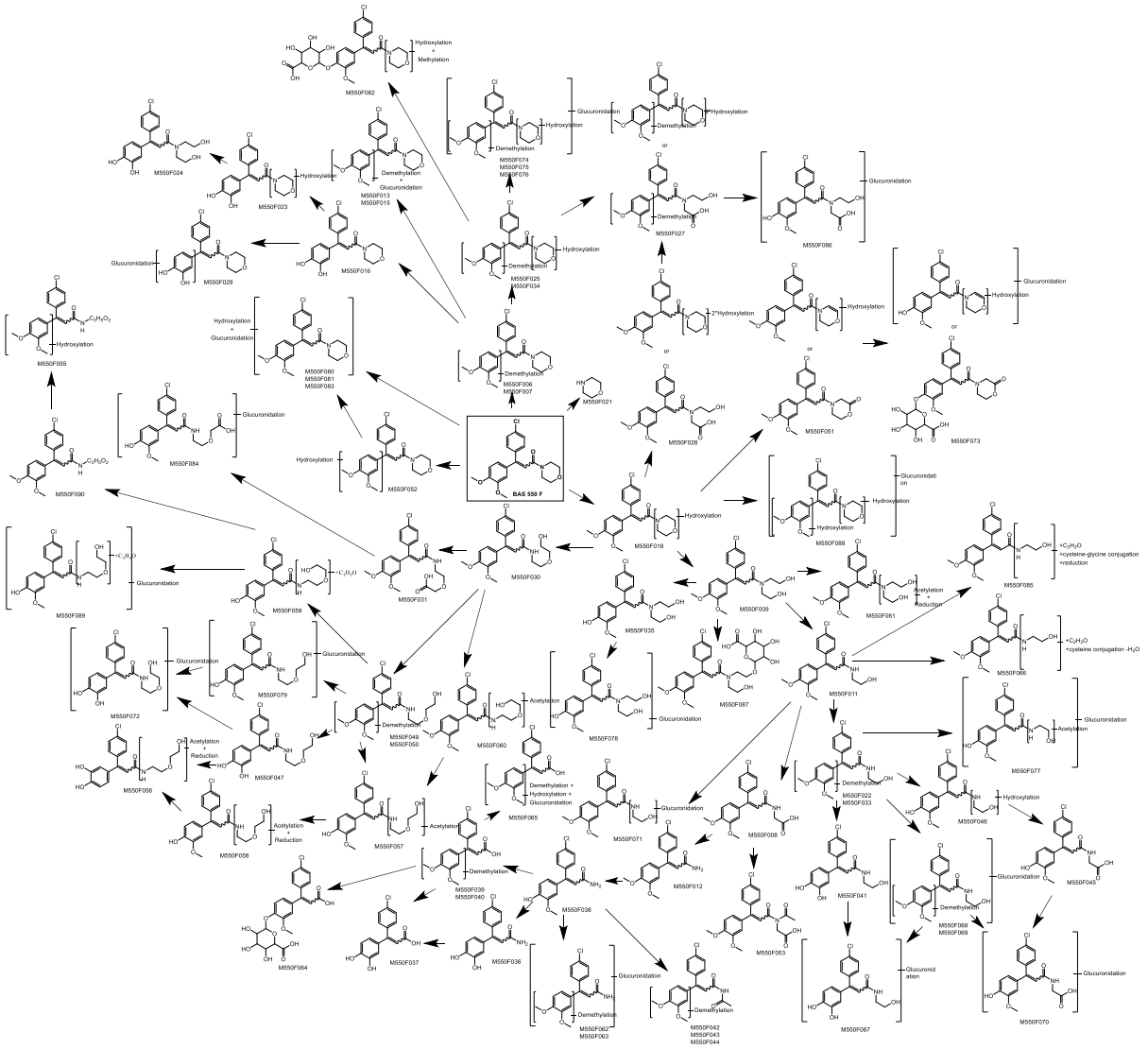
Substep 5d summarizes all structures with an opening of the morpholine ring at the oxygen atom and subsequent degradation as well as glucuronidation/acetylation.

Substep 5e summarizes all structures with an opening of the morpholine ring at the nitrogen atom and subsequent degradation as well as glucuronidation/acetylation

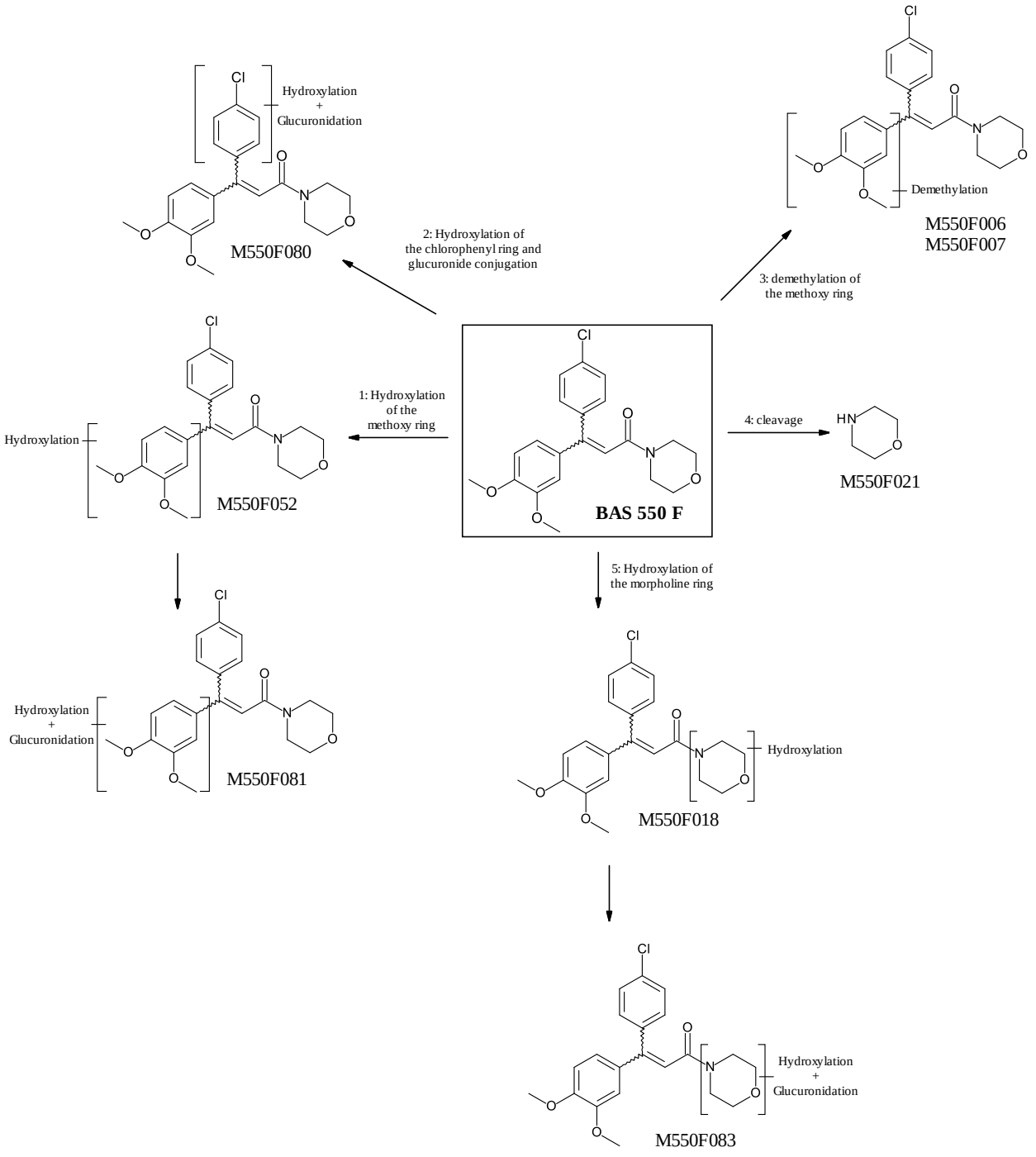
Although difficult to say due to the complexity of the metabolic patterns, it seems that metabolic steps 3 and 5 are the predominant ones also in quantitative terms. Step 4 (Cleavage and free morpholine) was clearly found to be a minor pathway in the rat.

The metabolic pathway observed in the new study is in good agreement with the information coming from the already peer reviewed studies (DK-440-001 and DK-440-002). Metabolite CUR 7586 (=M550F010), depicted in the pathway based on previous data, was found in its other isomeric form as M550F051. Metabolite Z43 (=M550F005) from the previous pathway was observed in its single or double demethylated form as M550F039, M550F040 and M550F037, respectively. This confirms that all metabolic steps observed in the previous studies are observed in the new study as well.

Figure 1: Proposed metabolic pathway of BAS 550 F in rats (representing already peer reviewed and new data)

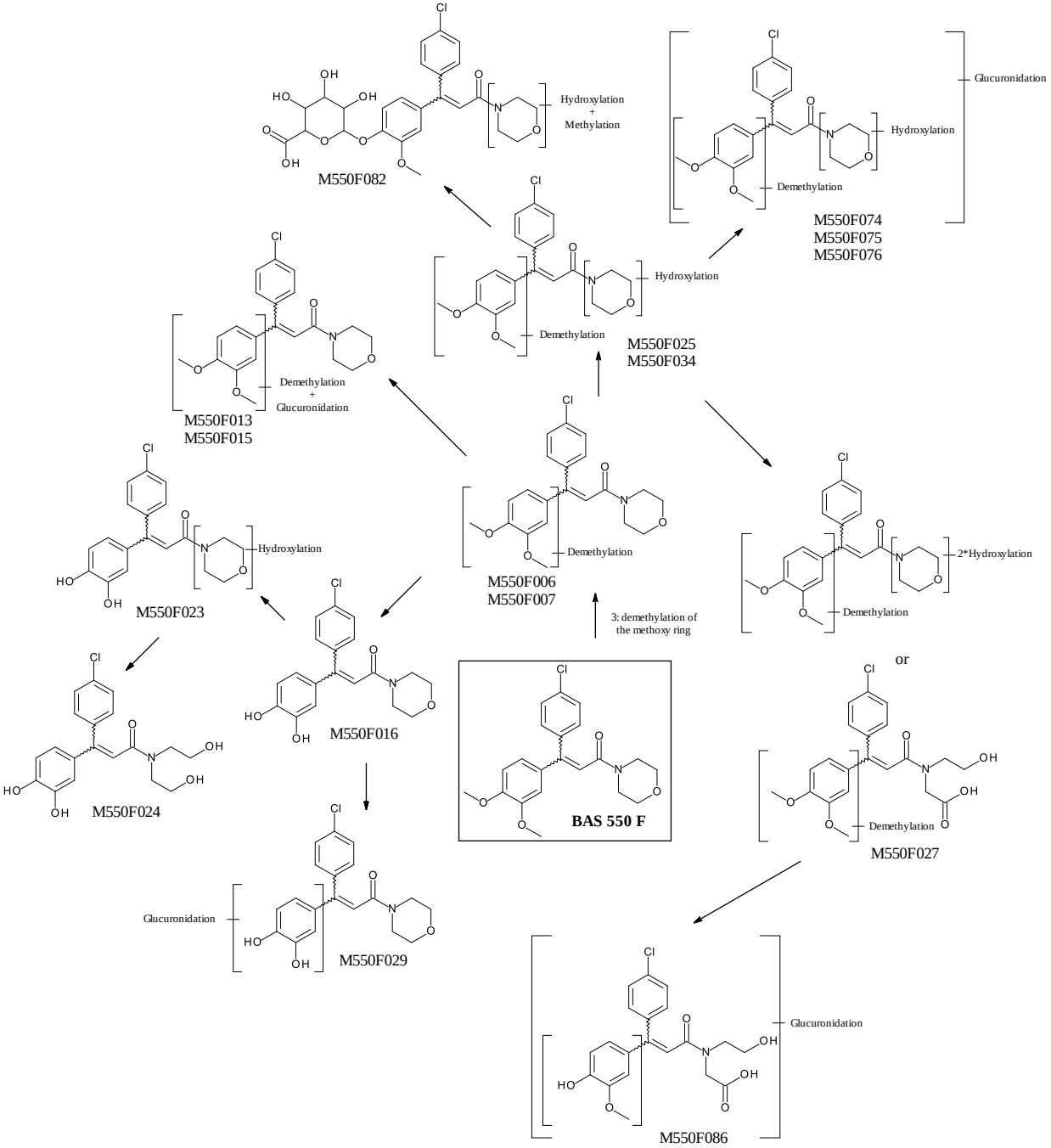


Initial 5 metabolic steps

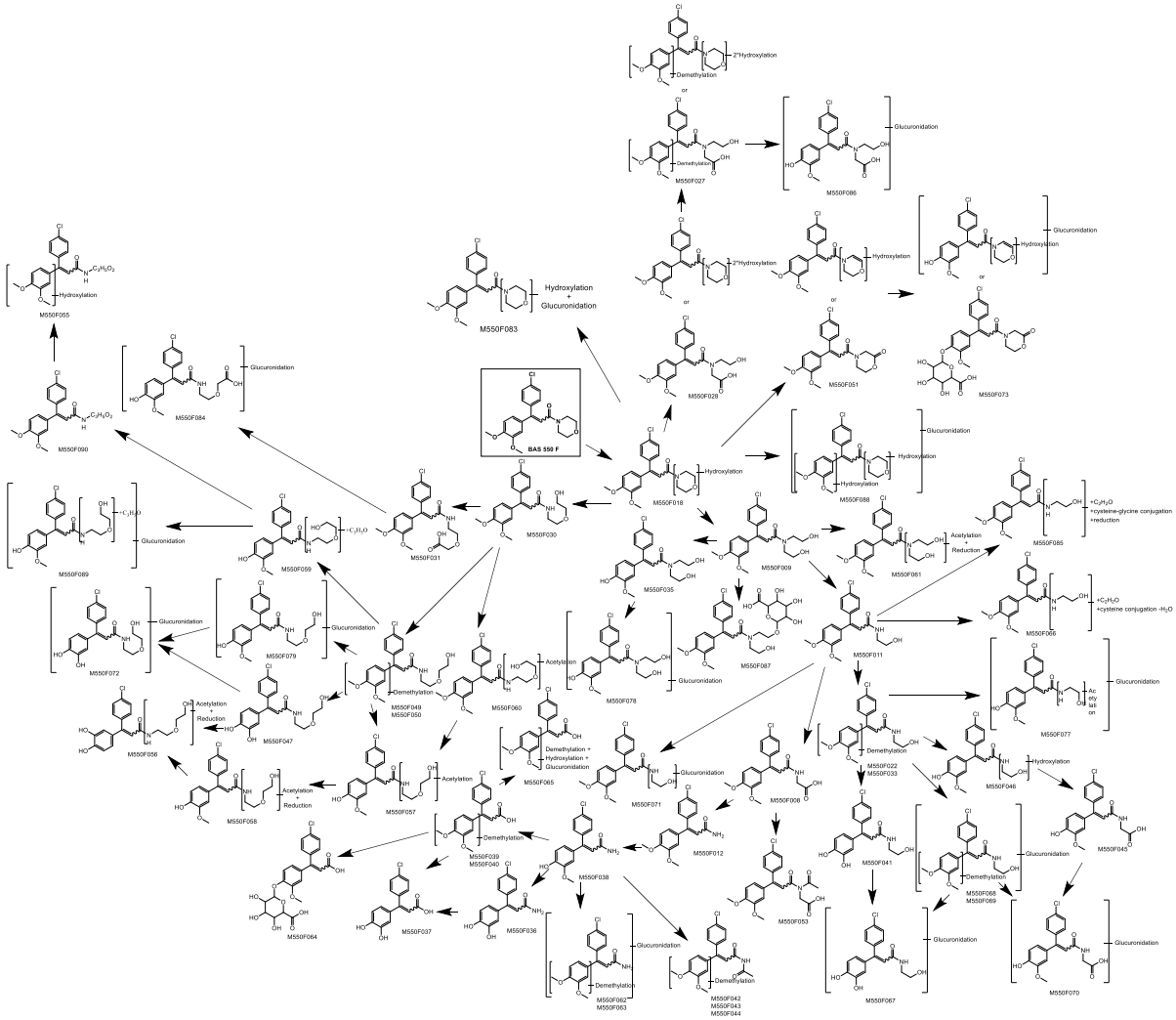


Steps 3 and 5 have further metabolic steps, as shown in the following figures.

Initial metabolic step 3: Demethylation of the dimethoxy ring

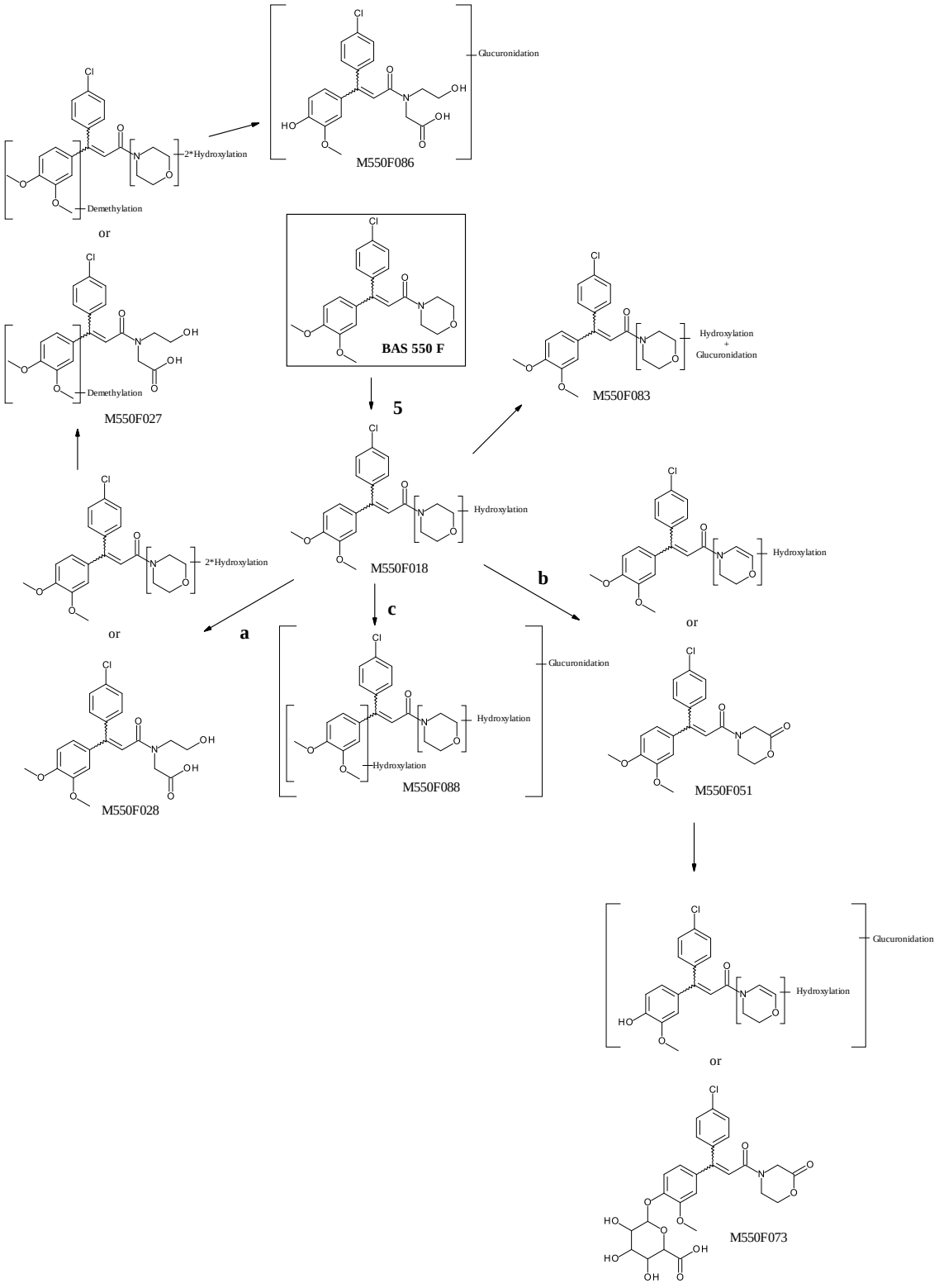


Initial metabolic step 5: Hydroxylation and further modification of the morpholine ring (opening, degradation, conjugation)

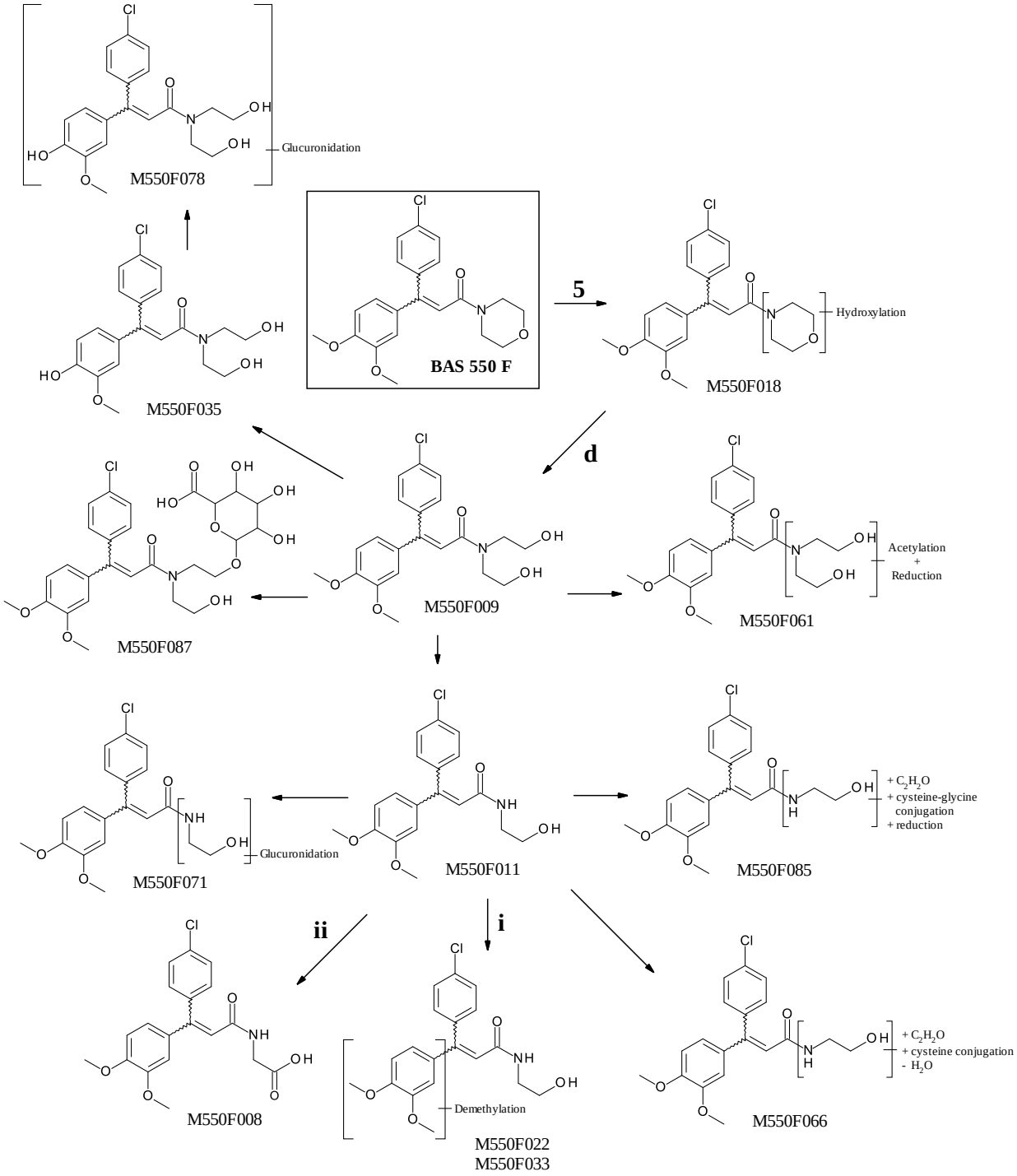


Initial metabolic step 5: Hydroxylation and further modification of the morpholine ring,

sub-steps a, b and c:



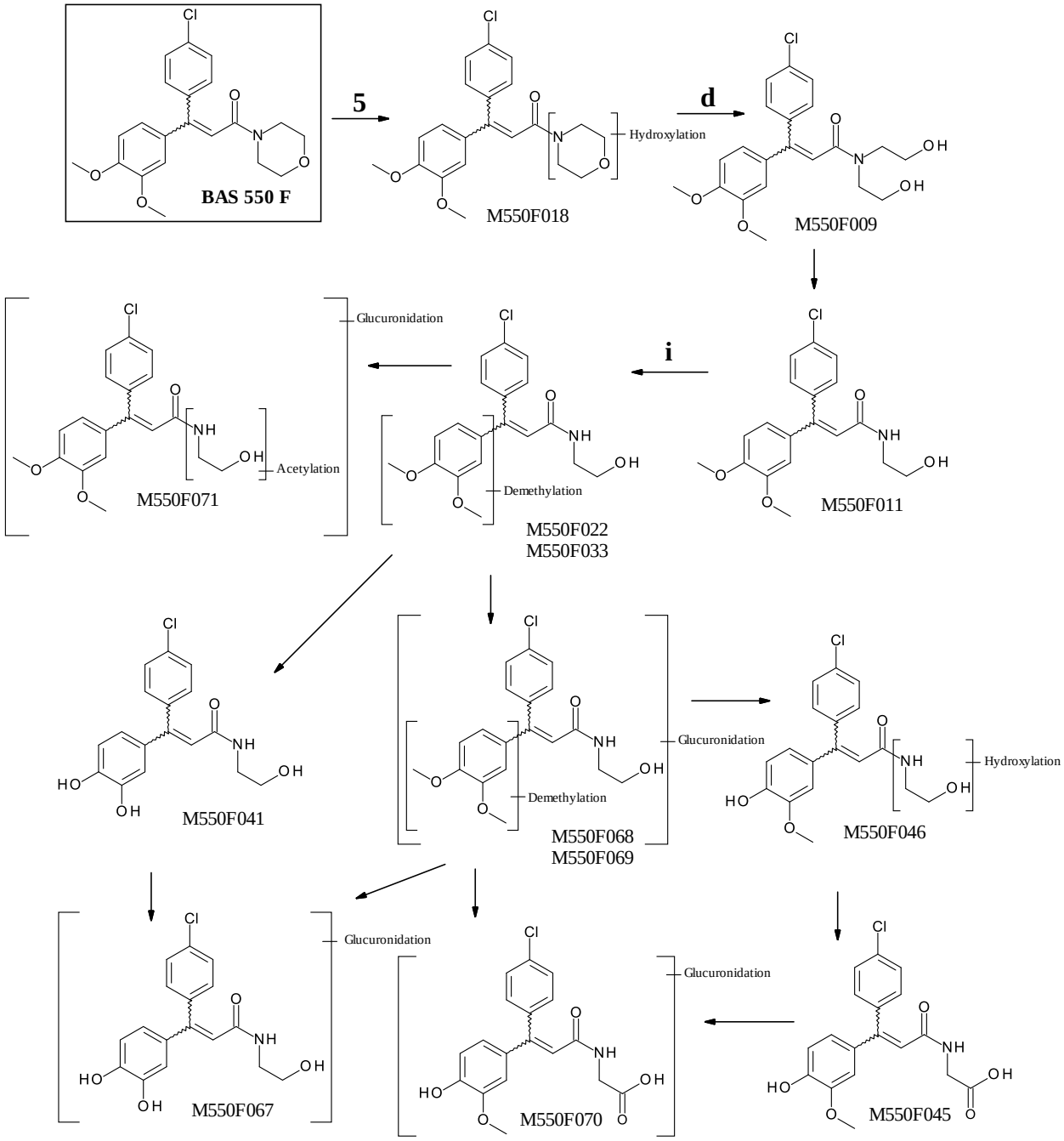
Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step d (ring opening at the oxygen and degradation)



Steps 5 d i and 5 d ii have further metabolic steps, as shown in the following figures.

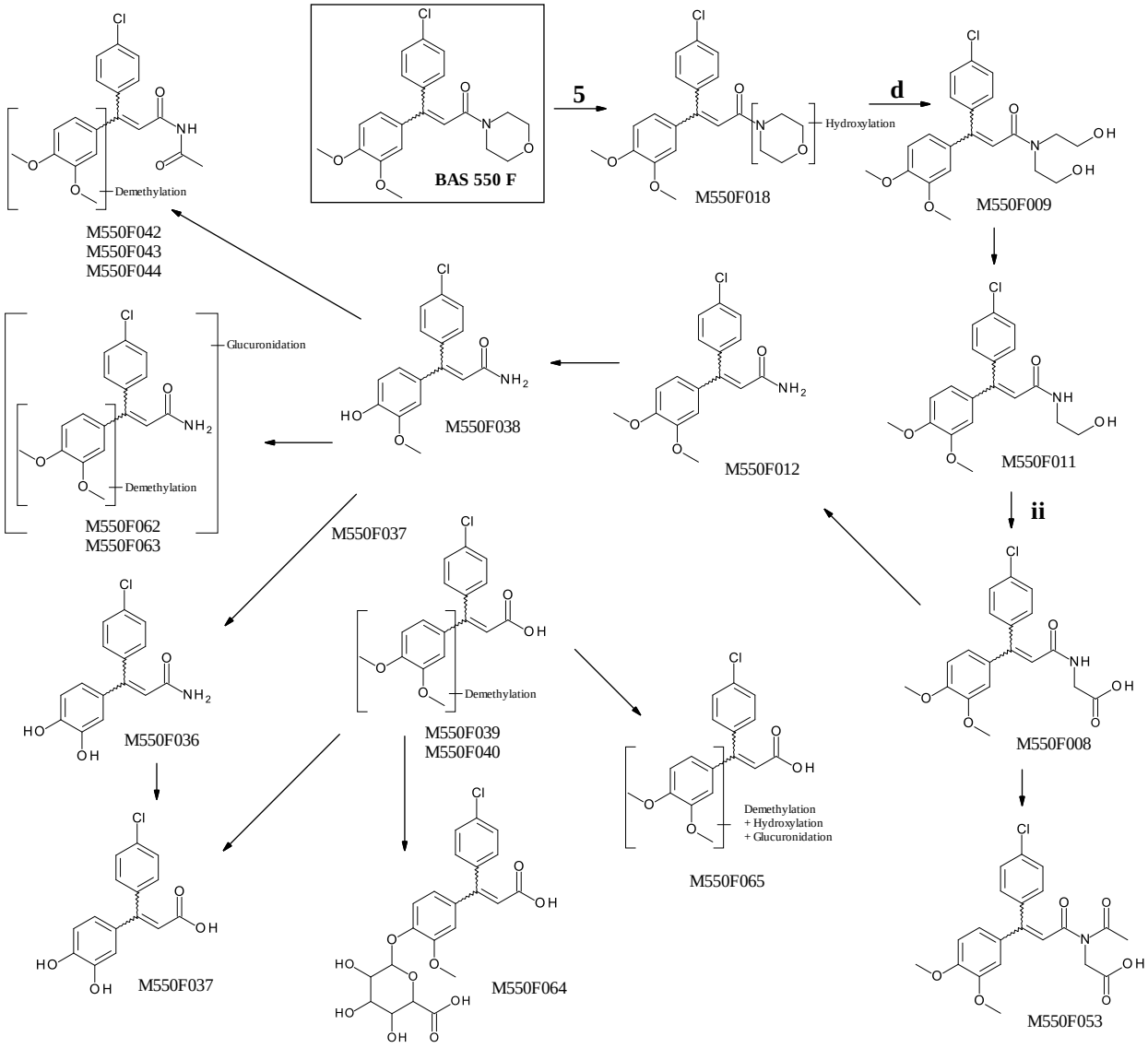
Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring,

sub-step d i



Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step d ii[5]

[5] Please note the metabolites M550F039, M550F040, M550F037, M550F064 and M550F065 could also occur as products of the Cleavage pathway (Step 4)



Initial metabolic step 5: Hydroxylation and oxidative opening of the morpholine ring, sub-step e (ring opening at the nitrogen and degradation)

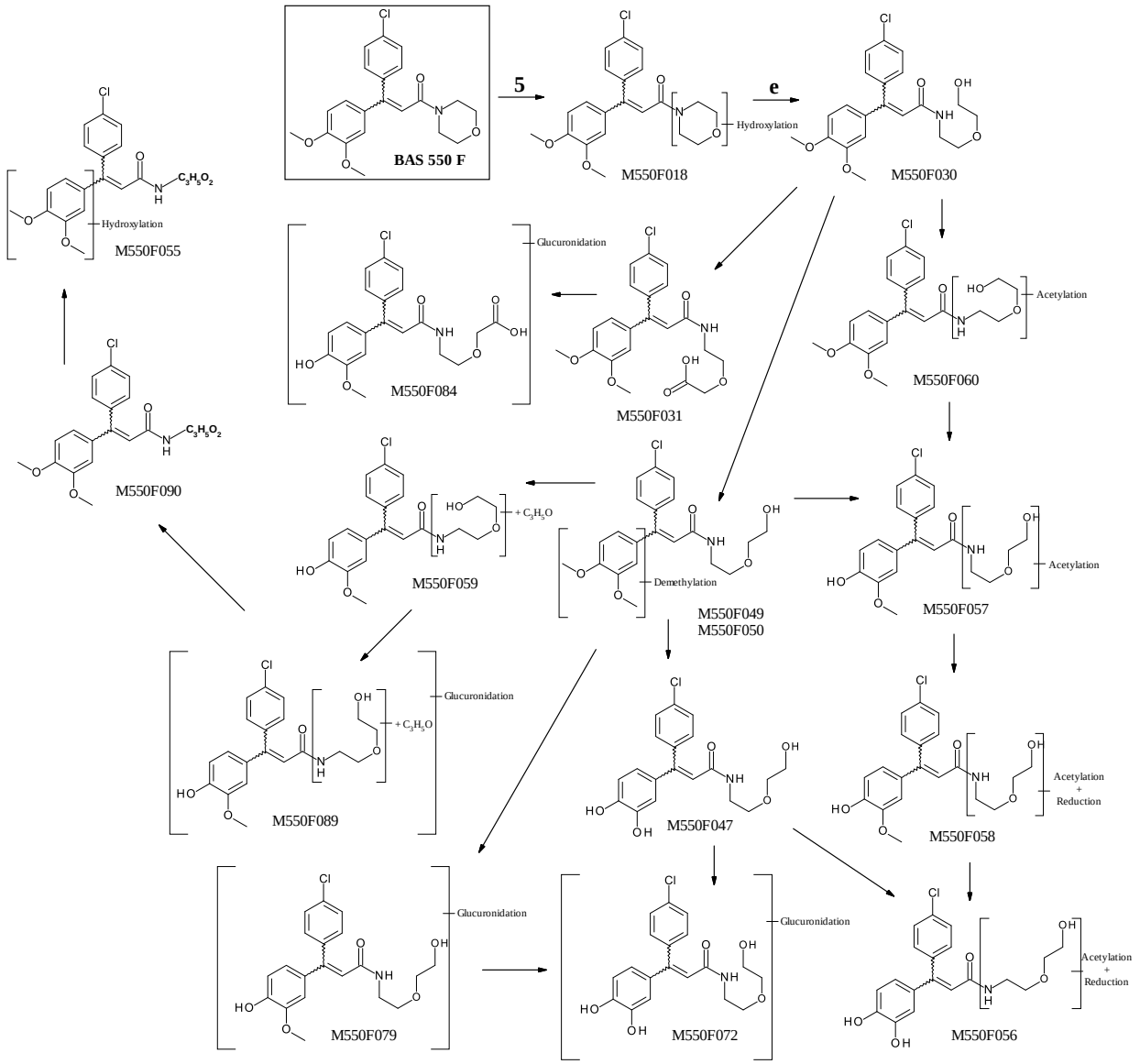
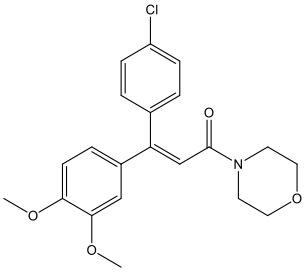
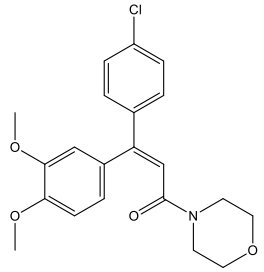
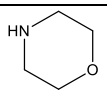
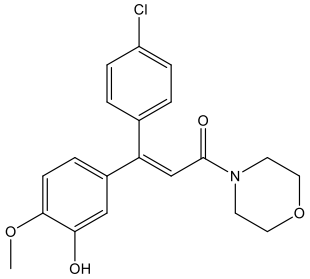
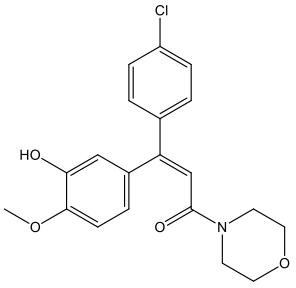
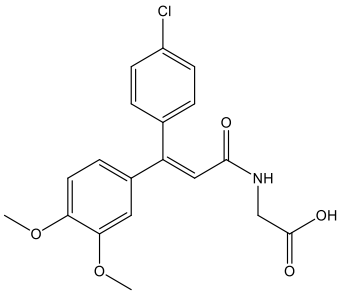
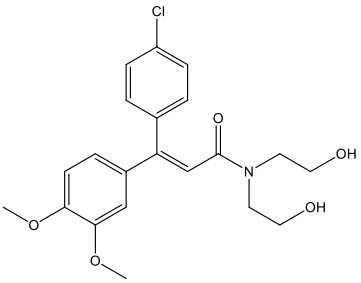
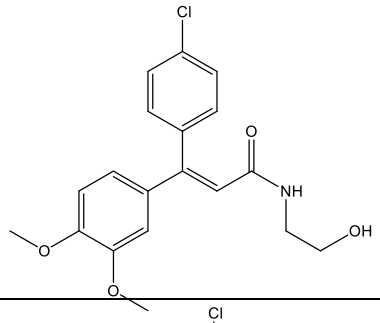
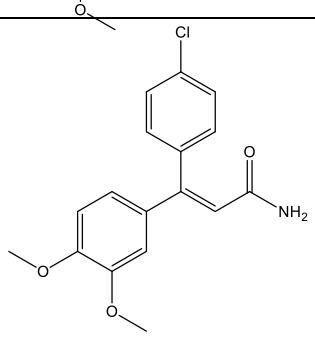
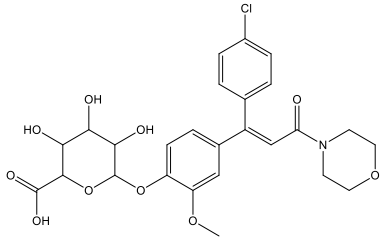
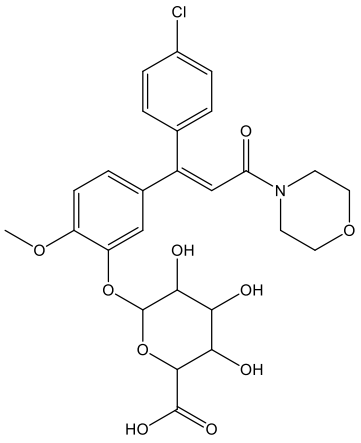
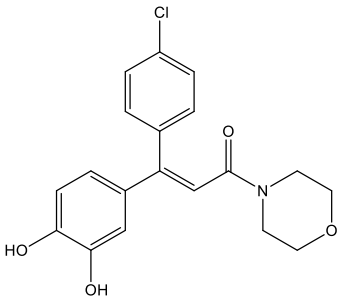
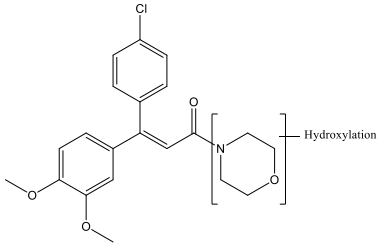
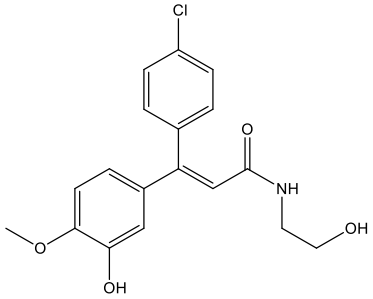
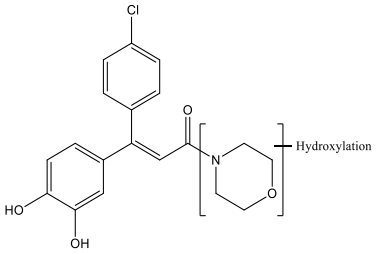
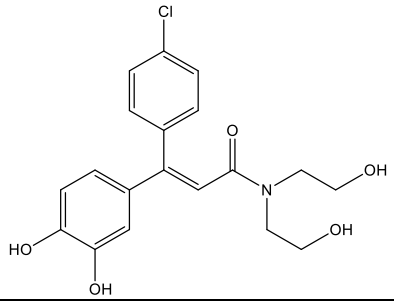
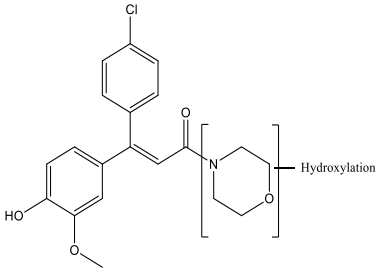


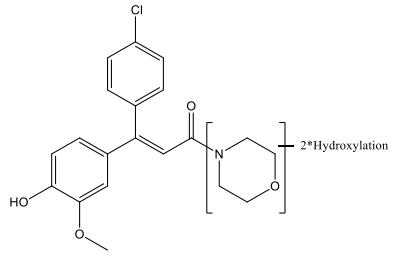
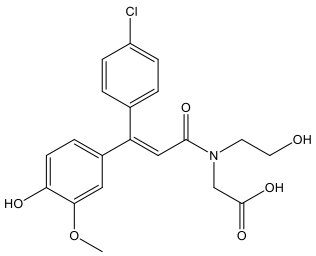
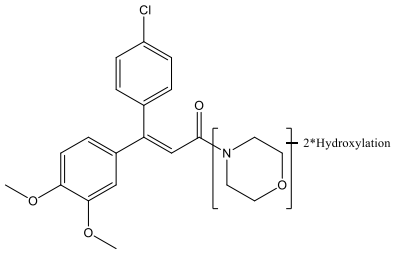
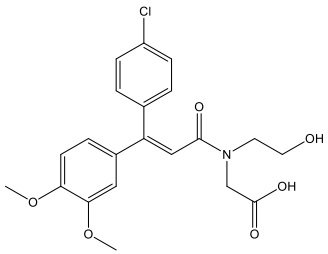
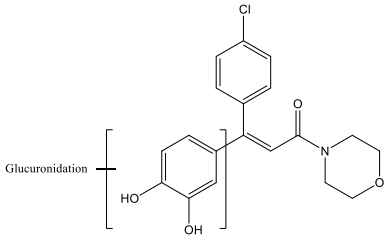
Table 24: List of identified metabolites in rats

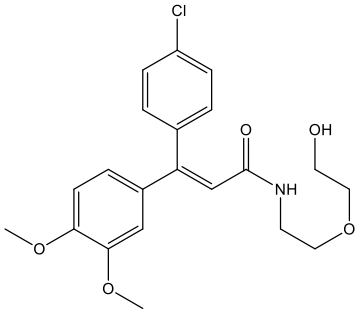
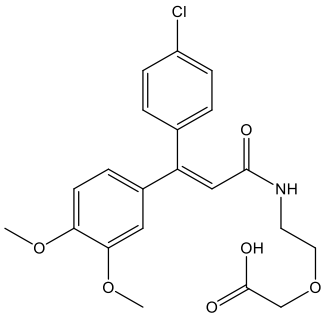
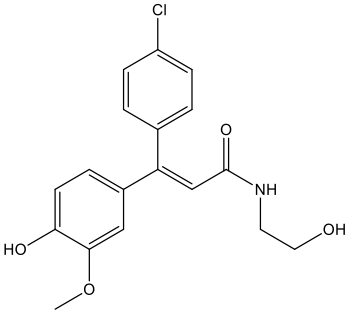
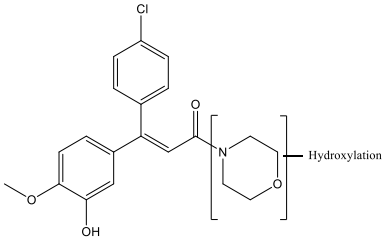
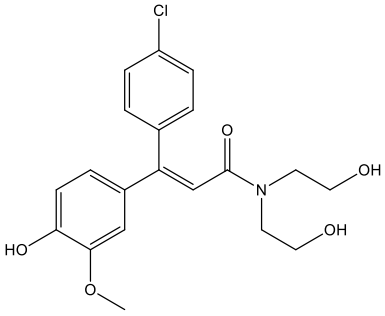
Metabolite code	MW	Proposed structure
BAS550F(E)	387	

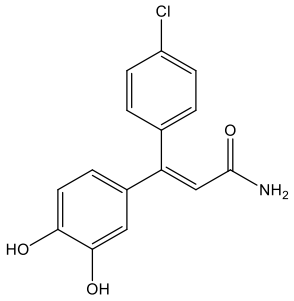
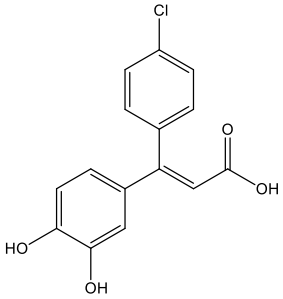
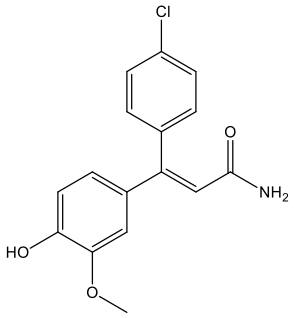
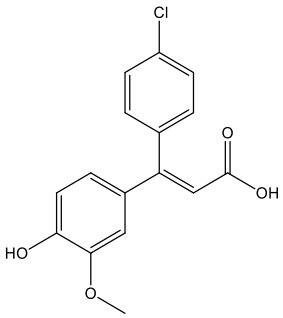
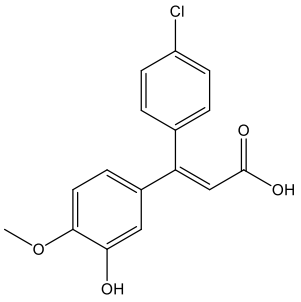
Metabolite code	MW	Proposed structure
BAS550F(Z)	387	
M550F021	87	
M550F006E	373	
M550F006Z	373	
M550F008	375	
M550F009	405	

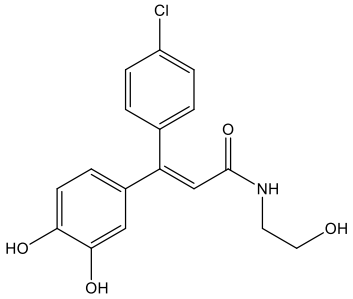
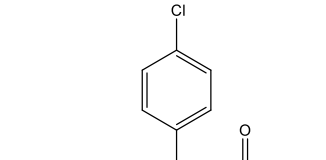
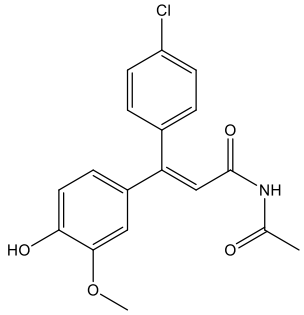
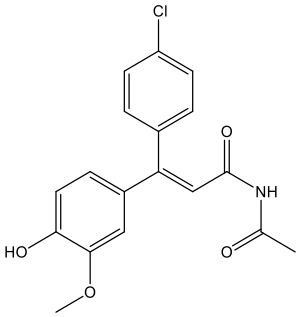
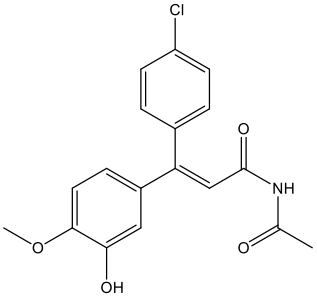
Metabolite code	MW	Proposed structure
M550F011	361	
M550F012	317	
M550F013	549	
M550F015	549	
M550F016	359	

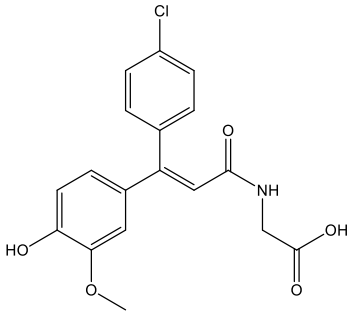
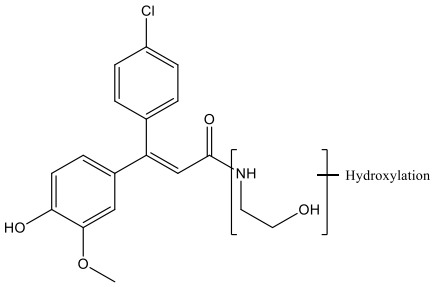
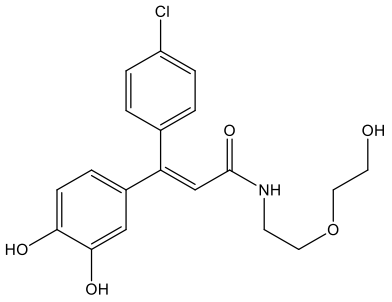
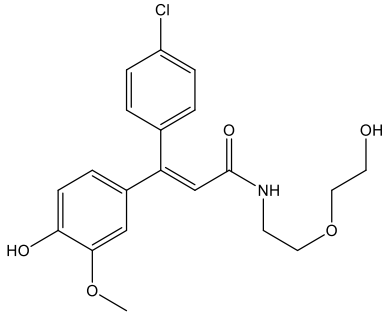
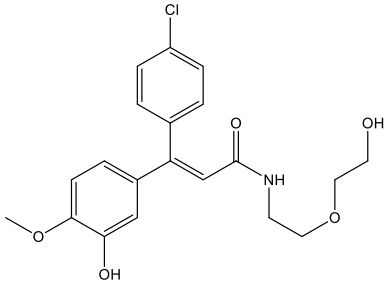
Metabolite code	MW	Proposed structure
M550F018	403	
M550F022	347	
M550F023	375	
M550F024	377	
M550F025	389	

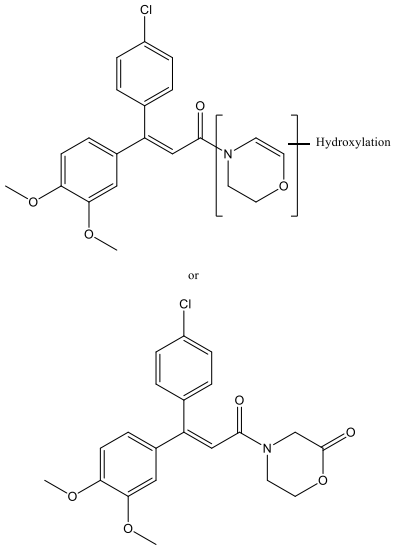
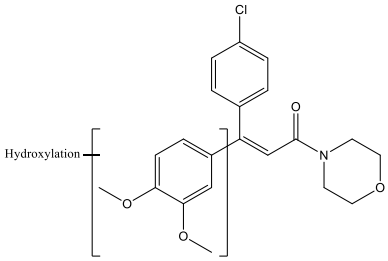
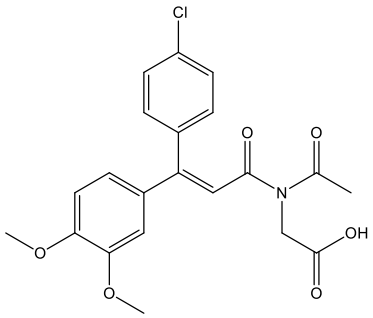
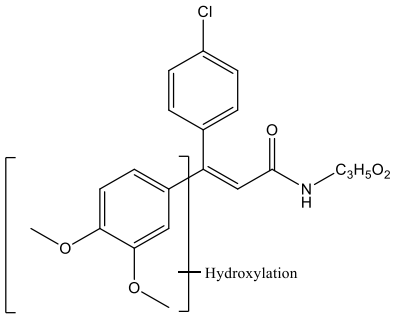
Metabolite code	MW	Proposed structure
M550F027	405	 or 
M550F028	419	 or 
M550F029	535	

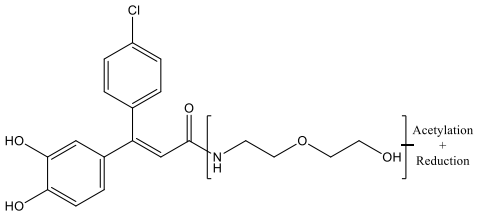
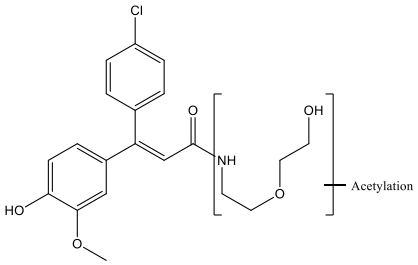
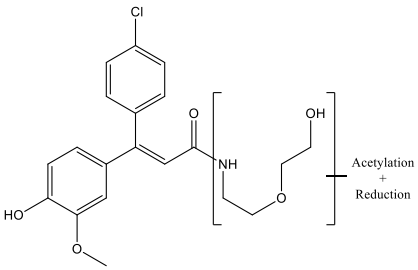
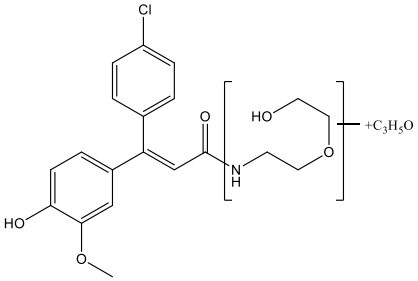
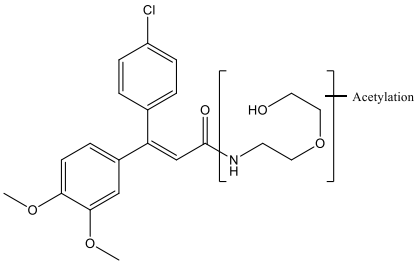
Metabolite code	MW	Proposed structure
M550F030	405	
M550F031	419	
M550F033	347	
M550F034	389	
M550F035	391	

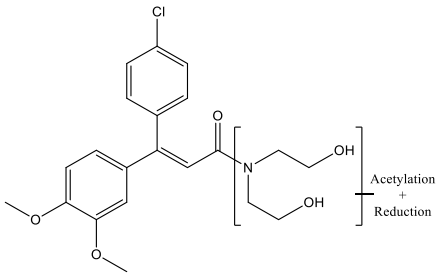
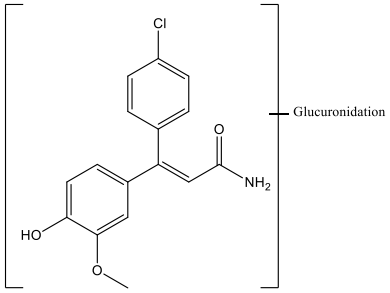
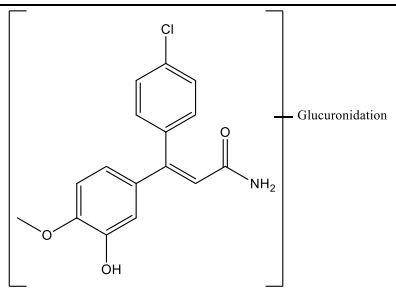
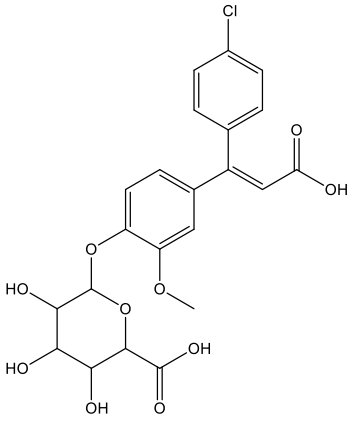
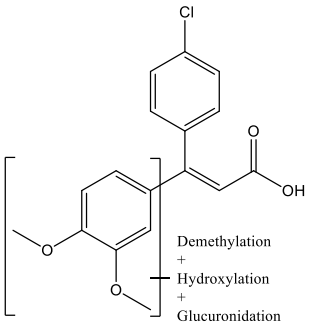
Metabolite code	MW	Proposed structure
M550F036	289	
M550F037	290	
M550F038	303	
M550F039	304	
M550F040	304	

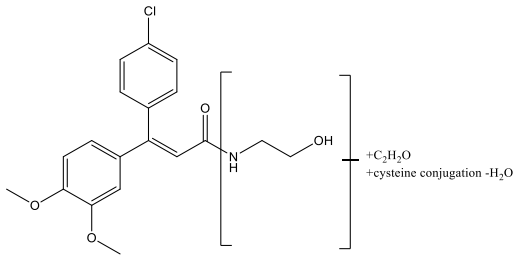
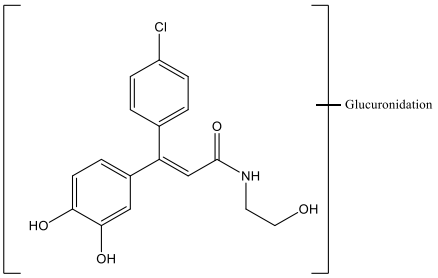
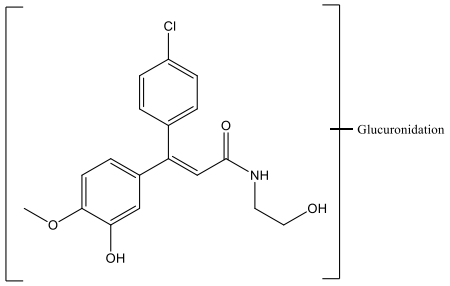
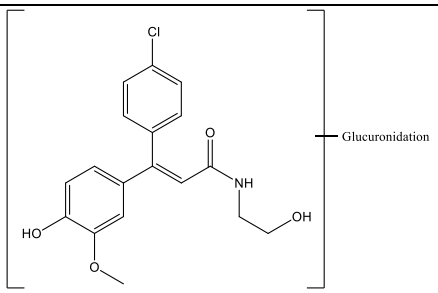
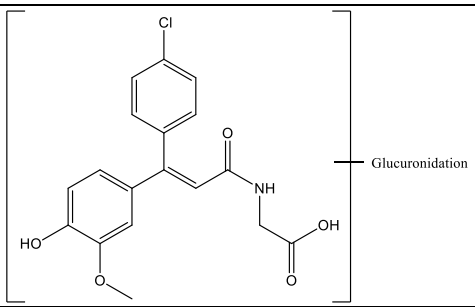
Metabolite code	MW	Proposed structure
M550F041	333	
M550F042	345	 or 
M550F043	345	
M550F044	345	

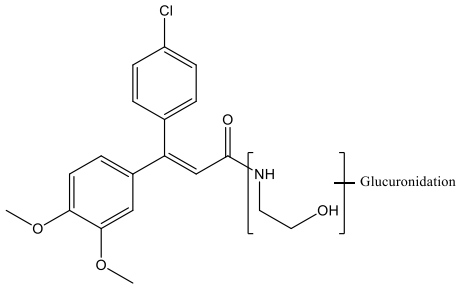
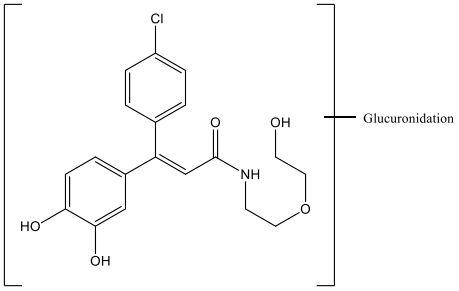
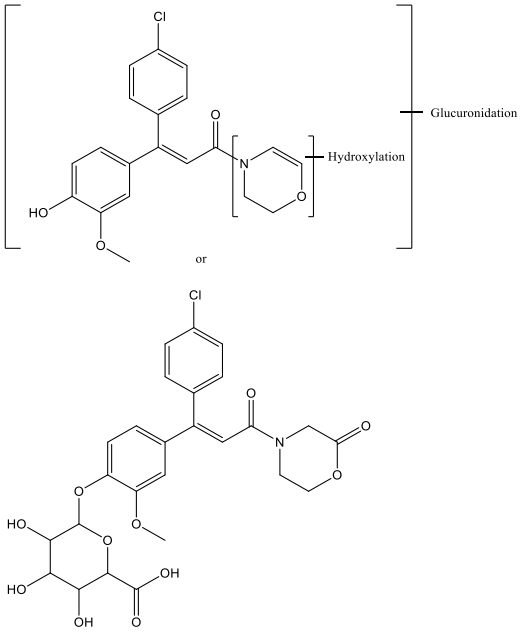
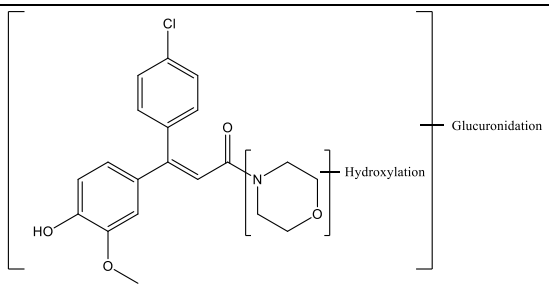
Metabolite code	MW	Proposed structure
M550F045	361	
M550F046	363	
M550F047	377	
M550F049	391	
M550F050	391	

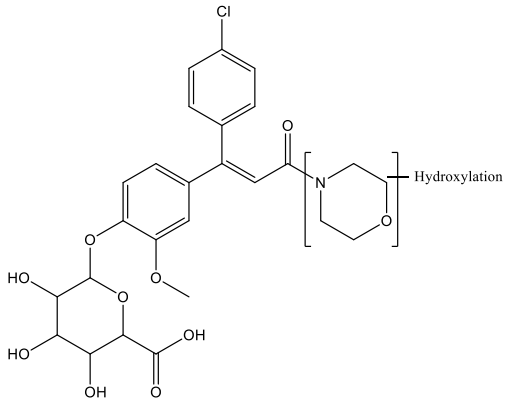
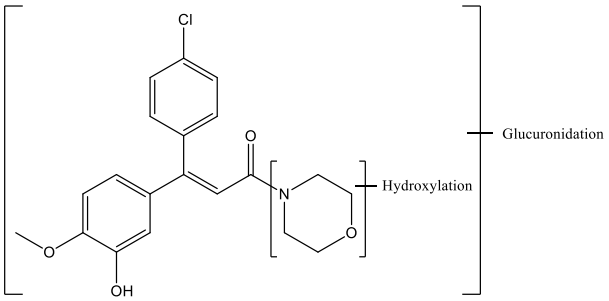
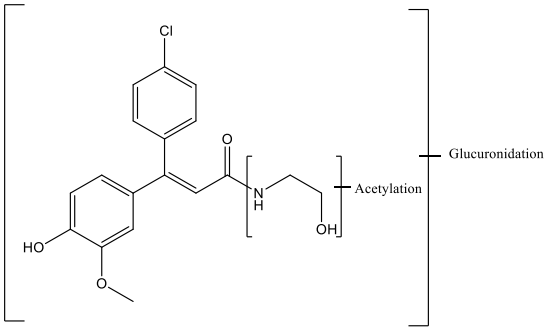
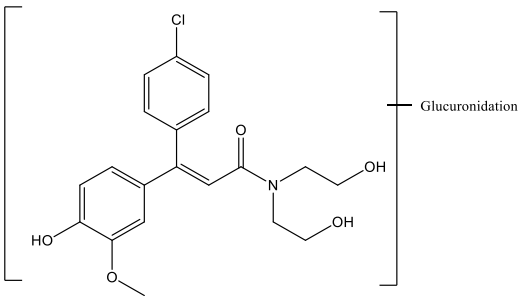
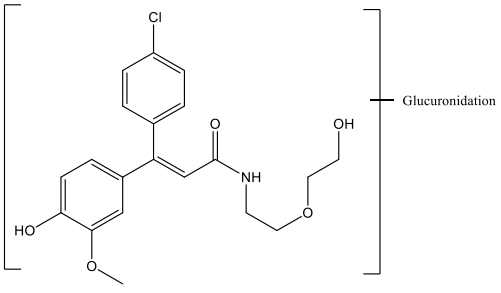
Metabolite code	MW	Proposed structure
M550F051	401	
M550F052	403	
M550F053	417	
M550F055	405	

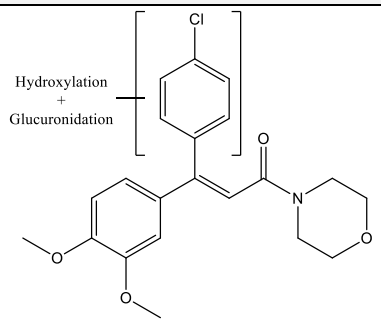
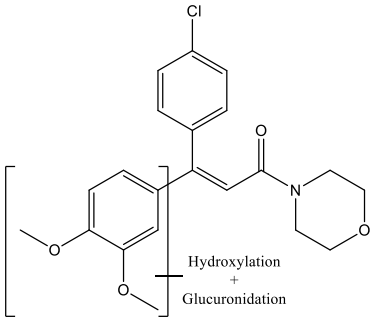
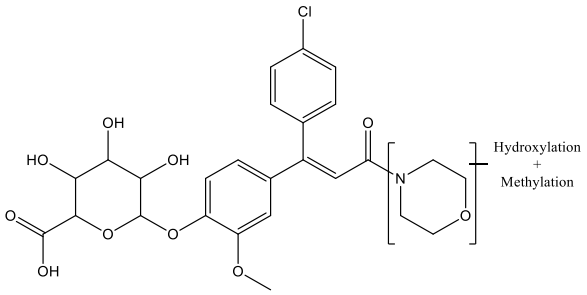
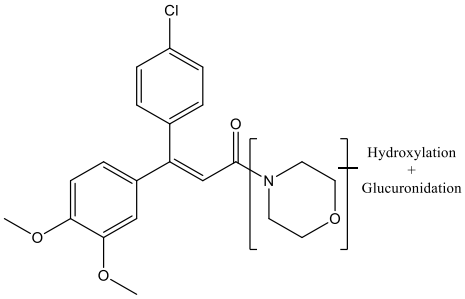
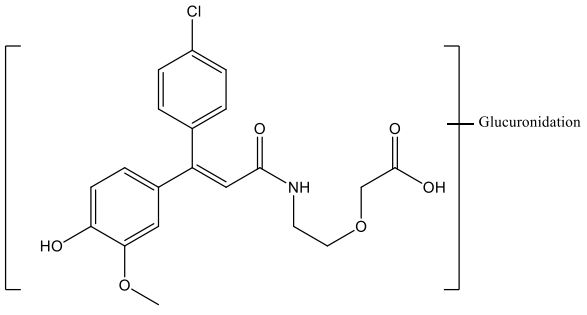
Metabolite code	MW	Proposed structure
M550F056	421	
M550F057	433	
M550F058	435	
M550F059	447	
M550F060	447	

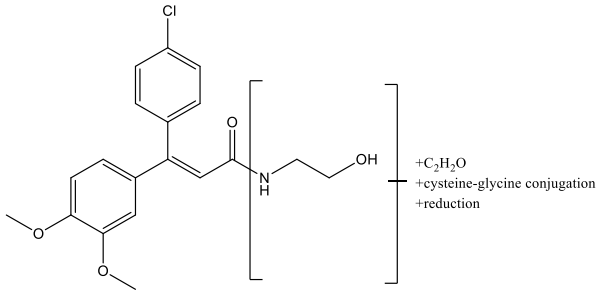
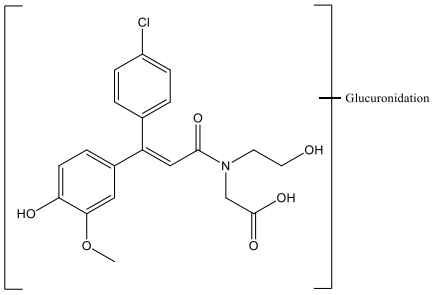
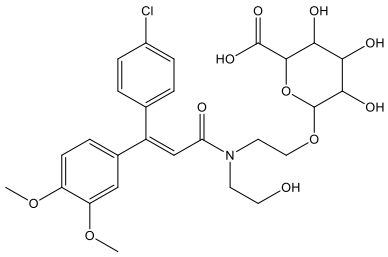
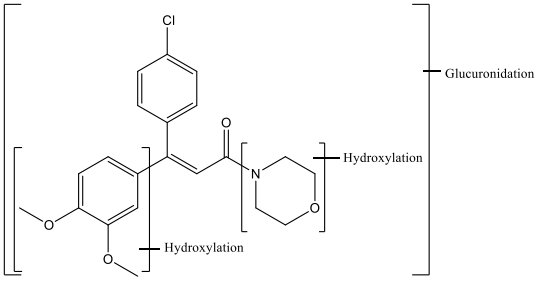
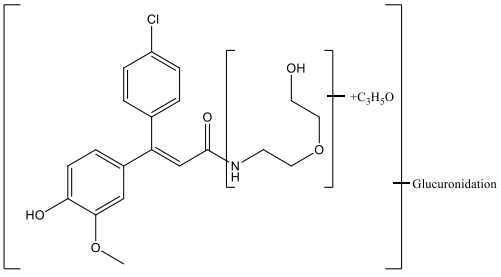
Metabolite code	MW	Proposed structure
M550F061	449	
M550F062	479	
M550F063	479	
M550F064	480	
M550F065	496	

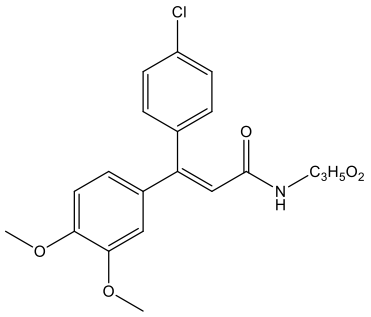
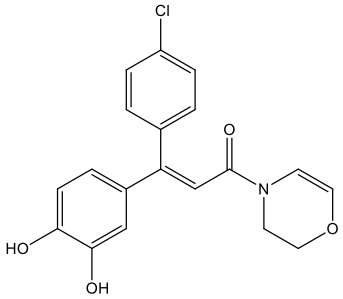
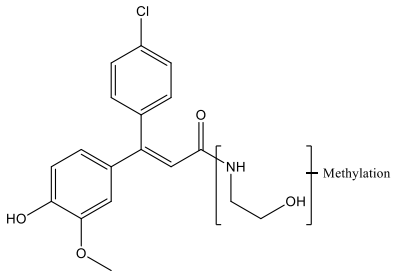
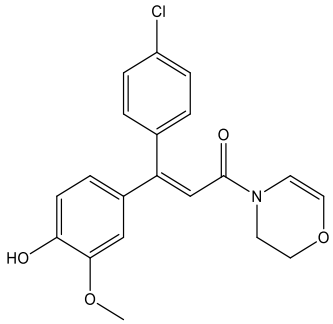
Metabolite code	MW	Proposed structure
M550F066	504	
M550F067_25.5min	509	
M550F068	523	
M550F069	523	
M550F070	537	

Metabolite code	MW	Proposed structure
M550F071	537	
M550F072	553	
M550F073	563	
M550F074	565	

Metabolite code	MW	Proposed structure
M550F075	565	
M550F076	565	
M550F077	565	
M550F078	567	
M550F079	567	

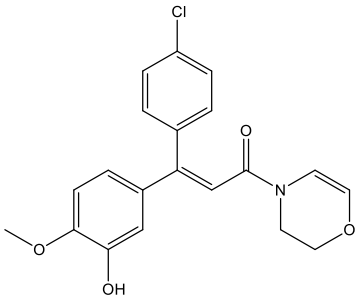
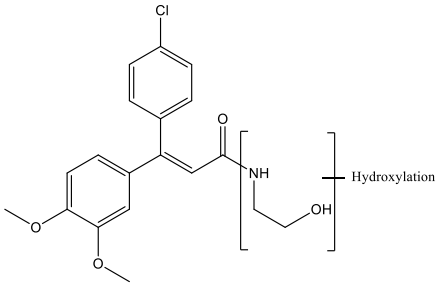
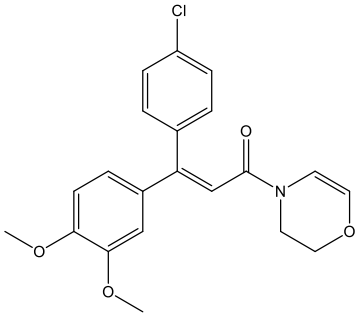
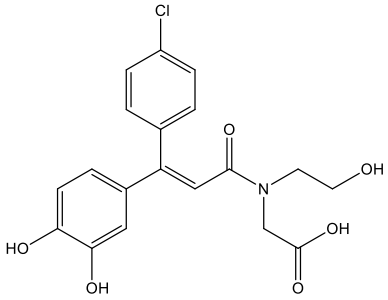
Metabolite code	MW	Proposed structure
M550F080	579	
M550F081	579	
M550F082	579	
M550F083	579	
M550F084	581	

Metabolite code	MW	Proposed structure
M550F085	581	
M550F086	581	
M550F087	581	
M550F088	595	
M550F089	623	

Metabolite code	MW	Proposed structure
M550F090	389	
M357*	357	
M361	361	
M371*	371	

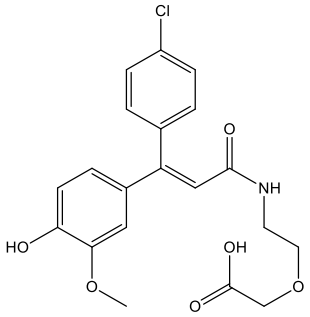
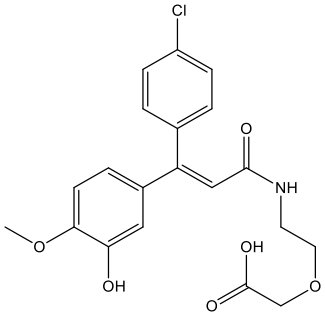
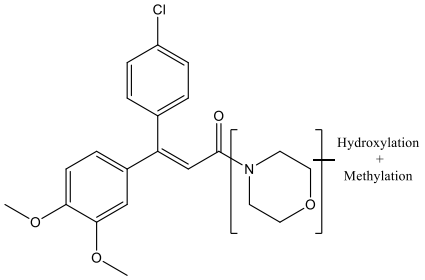
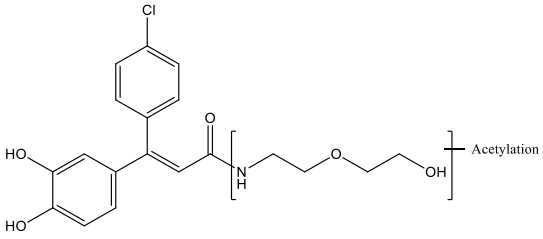
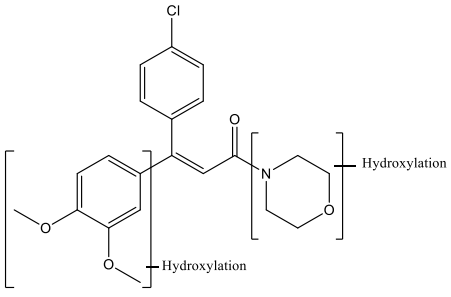
* This metabolite is considered to be a degradation product of an original metabolite.

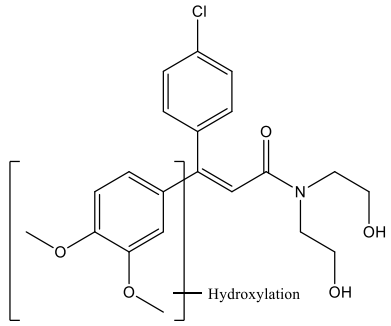
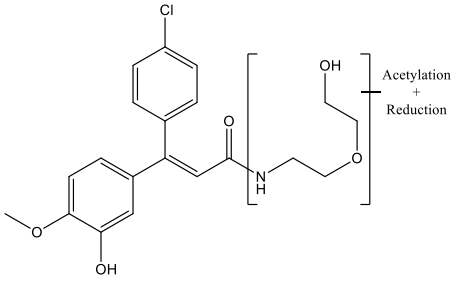
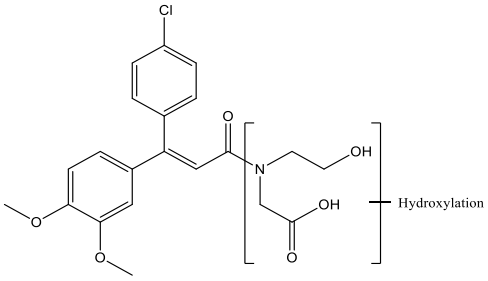
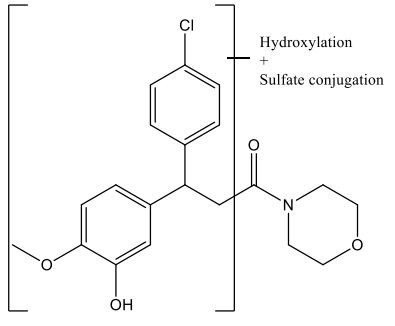
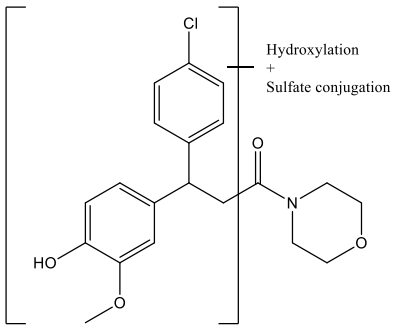
Metabolites with designation “Mxxx” (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

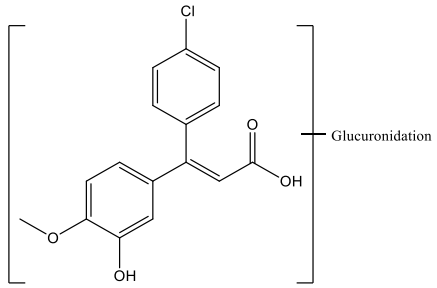
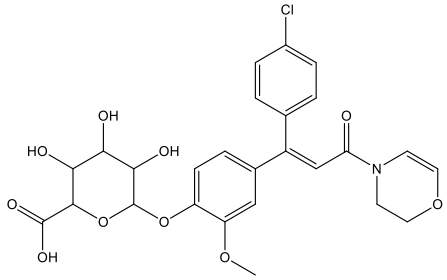
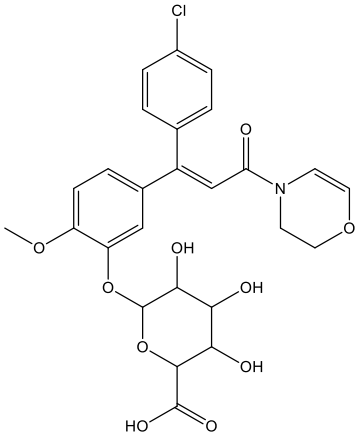
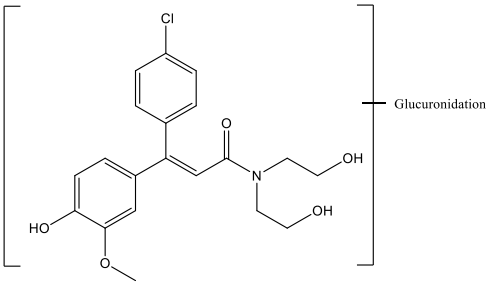
Metabolite code	MW	Proposed structure
M371*	371	
M377	377	
M385*	385	
M391	391	

* This metabolite is considered to be a degradation product of an original metabolite.

Metabolites with designation “Mxxx” (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

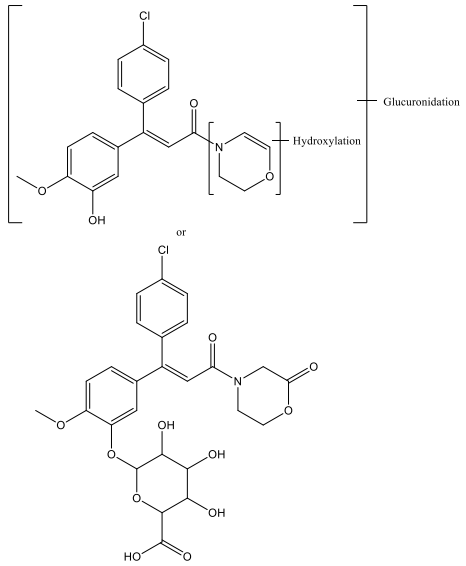
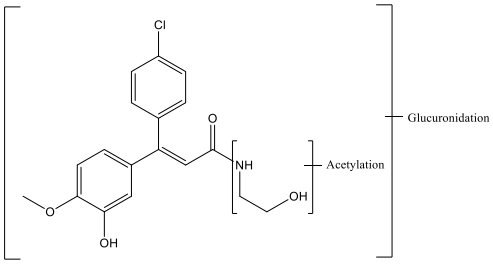
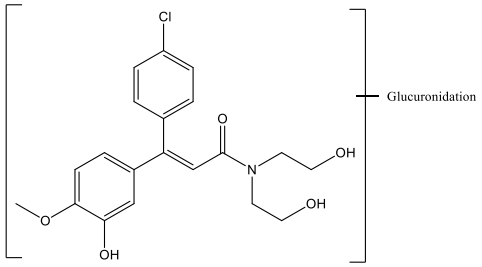
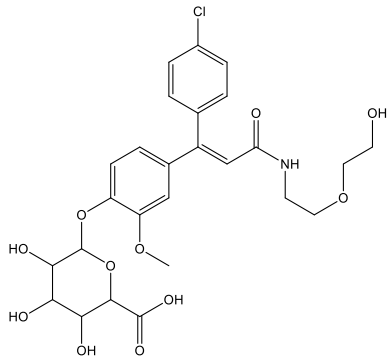
Metabolite code	MW	Proposed structure
M405	405	
M405	405	
M417	417	
M419	419	
M419	419	

Metabolite code	MW	Proposed structure
M421	421	
M435	435	
M435	435	
M471	471	 or 

Metabolite code	MW	Proposed structure
M480	480	
M547*	547	
M547*	547	
M553	553	

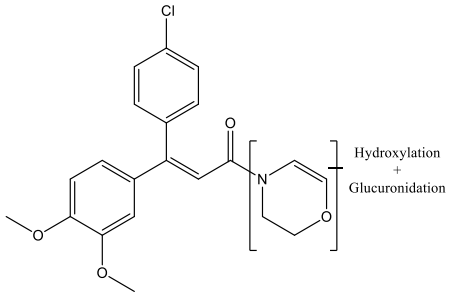
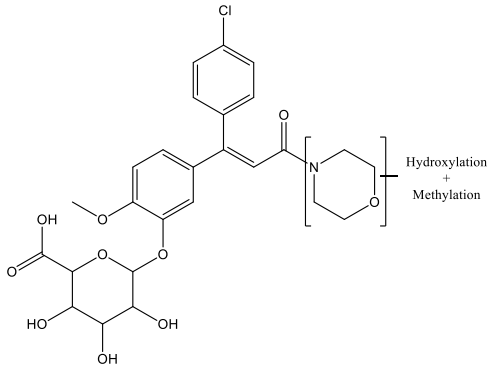
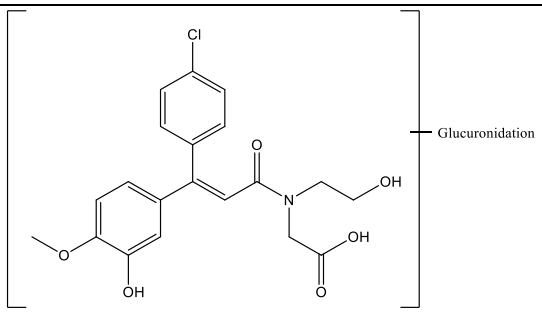
* This metabolite is considered to be a degradation product of an original metabolite.

Metabolites with designation “Mxxx” (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

Metabolite code	MW	Proposed structure
M563*	563	
M565	565	
M567	567	
M567	567	

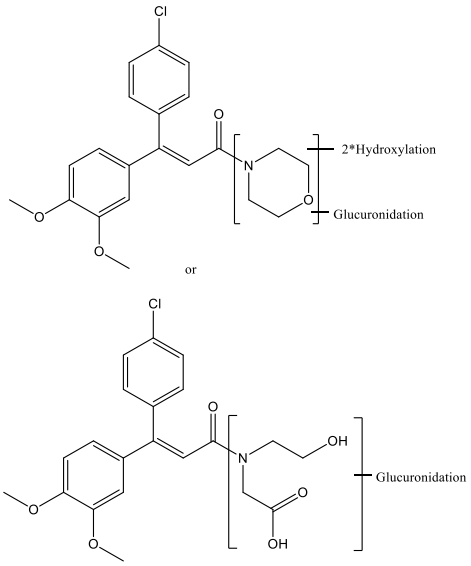
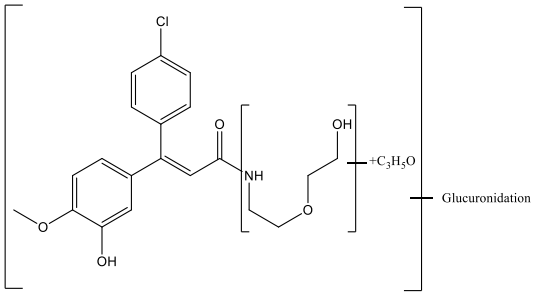
* This metabolite is considered to be a degradation product of an original metabolite.

Metabolites with designation “Mxxx” (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

Metabolite code	MW	Proposed structure
M577*	577	
M579	579	
M581	581	

* This metabolite is considered to be a degradation product of an original metabolite.

Metabolites with designation “Mxxx” (where xxx is the molecular weight) were not assigned a classical code (M550Fxx) since they occurred only in minor amounts.

Metabolite code	MW	Proposed structure
M595	595	
M623	623	

2.1.8 In vitro metabolism study 1 (new)

Reference:

Birks V., 2015. 14C-BAS 550 F: Comparative in-vitro metabolism studies with rat, dog and human cryopreserved hepatocytes. 2015/1245078

Test type

Guidelines: No guideline available

GLP: yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Test substance

Test Material:

Description:	[morpholine-2,3-C14]-BAS 550 F (morpholine label)	
	[p-chlorophenyl-U-C14]-BAS 550 F (chlorophenyl label)	
	[acrolyl-2-C13]-BAS 550 F (^{13}C- label)	
	[morpholine-N15]-BAS 550 F (^{15}N label)	
	Unlabeled BAS 550 F	
Lot/Batch #:	858-1101 (morpholine label)	
	1068-1101 (chlorophenyl label)	
	1070-0101 (^{13}C label)	
	1071-0105 (^{15}N label)	
	AC9978-68A (unlabeled)	
Purity:	97.6% (unlabeled)	
	91.3% (^{13}C label)	
	97.3% (^{15}N label)	
	Radiochemical purity:	95.4% (morpholine label)
		99.1% (chlorophenyl label)
	Specific activity:	4.39 MBq/mg (morpholine label)
		7.4 MBq/mg (chlorophenyl label)
CAS#:	110488-70-5	

Stability of test compound:

The test item was stable over the test period. Stability controls without cells showed nearly identical HPLC profiles that contained only peaks corresponding to the unchanged active substance BAS 550 F.

Vehicle and/or positive control:

Vehicle: hepatocytes

Positive control: testosterone or 7-ethoxycoumarin instead of the test substances were incubated with hepatocytes from the three different species to indicate the metabolic activity of the different hepatocytes.

Test animals:	Mammals
Species:	Human, rat, dog
Strain:	-, Wistar, Beagle

Detailed study summary and results:

Material and methods

Dates of work: 28-Apr-2015 - 16-June-2015

This study was carried out at Quotient Bioresearch (Rushden) Ltd., United Kingdom.

Test procedure

The objective of this study was to compare the in vitro metabolism in hepatocytes of animal species used in toxicological testing of dimethomorph (BAS 550 F, Reg. No. 247723) to the metabolism in human hepatocyte samples and to determine whether metabolic profiles are similar and whether unique human metabolites occur.

To address this question, the radiolabeled test item was incubated with hepatocytes from human, rat and dog (all mixed gender) at a final concentration of 10 µM. The concentration was chosen after a cell viability pre-test. Two differently ^{14}C labeled test items (p-chlorophenyl-U-14C-BAS 550 F and morpholine-2,3-^{14}C-BAS 550 F) were used. Hepatocytes were incubated with 10 µM BAS 550 F of both labels, respectively. The viability of the hepatocytes was determined after 180 min of incubation using the AQ_{ueous} One Solution cell viability assay by Promega.

After incubation for 0, 10, 30, 60 or 180 min, the reaction was terminated by addition of acetonitrile (25% of incubation sample volume) and the resulting supernatant was analysed by HPLC. Selected samples were additionally investigated by LC-HR-MS.

All the supernatants contained at least >88% of the applied radioactivity (% AR), therefore pellet extraction was not required.

Negative and positive controls were run in parallel to demonstrate the absence of non metabolic degradation and the metabolic activity of the hepatocytes (Phase I and Phase II metabolic reactions), respectively. The control experiments yielded the expected results.

Test design and analytical procedures

Test solutions

Stock solutions of BAS 550 F were prepared in DMSO. The radioactive concentration of the solutions was confirmed by LSC. Radiochemical purity was confirmed on each experimental day by HPLC.

For the unlabeled test item a 2 mM solution of ^{12}C-BAS 550 F (unlabeled) was prepared by dissolving an aliquot of ^{12}C-BAS 550 F in DMSO and subsequent dilutions.

For the preparation of the application solutions (either morpholine or chlorophenyl label), specific amounts of labeled test items were reduced to dryness under N_2 gas and reconstituted in DMSO. Then, 2 mM solutions of ^{14}C/^{15}N/^{12}C-BAS 550 F (morpholine label) and ^{1}4C/^{13}C/^{12}C-BAS 550 F (chlorophenyl label) were prepared by combining suitable amounts of the prepared solutions to give the final stocks. The final stock solutions were analyzed by LC-HR-MS in order to confirm the identity of BAS 550 F and the initial isotope pattern.

Positive control incubations were performed with ^{14}C-testosterone and ^{14}C-7-ethoxycoumarin, respectively. The final concentration of ^{14}C-testosterone in incubations was 150 µM at a target of 1% (v/v). Therefore, a stock solution of 15 mM ^{14}C-testosterone was prepared. Initially, unlabeled testosterone was weighed and dissolved in methanol to give a 15 mM solution. An aliquot of ^{14}C-testosterone was reduced to dryness under N2 gas and reconstituted in the unlabeled testosterone. The final concentration of ^{14}C-7-ethoxycoumarin in incubations was 25 µM at a target of 1% (v/v). Therefore, a stock solution of 2.5 mM ^{14}C-7-ethoxycoumarin was prepared. An aliquot of ^{14}C-7-ethoxycoumarin in toluene was reduced to dryness under N_2 gas and reconstituted in methanol.

Negative controls:

In the negative controls no metabolism should occur. For the “stability control”, the application solution was mixed with incubation medium instead of the cell suspension. For the “zero incubation control” (t = 0 min), the reaction was stopped immediately after addition of the cell suspension.

Preparation of hepatocytes

Mixed gender rat and dog hepatocytes were purchased from Bioreclamation IVT. Mixed gender Hepatosure™ Pooled Cryopreserved Human Hepatocytes (100 donors) were sourced from Xenotech. Hepatocytes were resuscitated as per the instruction provided by the supplier.

Viability tests and non-specific binding tests

The viability of human, rat and dog hepatocytes after incubation with 1, 5 and 10 µM BAS 550 F (non-radiolabeled) was tested using the cell proliferation assay AQ_{ueous} One Solution (Promega) in order to select the appropriate concentration of the test item in the in vitro assays.

Prior to the species comparison of ^{14}C-BAS 550 F metabolism, an assessment of the non-specific binding to the incubation plate was performed at 10 µM ^{14}C-BAS 550 F (2 radiolabeled forms, separately).

In vitro assays

On each incubation day, the application solutions in DMSO were diluted with hepatocyte incubation medium (WME) by a factor of 200 to prepare the respective application medium. Aliquots of the application media were analyzed by LSC to calculate the amounts of applied radioactivity per well (representing 100% AR). The application media were incubated at a final concentration of 10 µM BAS 550 F (both labels) with human, rat or dog hepatocytes. In the case of testosterone and 7-ethoxycoumarin, the incubations were performed at 150 µM and 25 µM, respectively and were diluted with hepatocyte incubation medium (WME) by a factor of 100 to prepare the respective application medium.

Each sample (4.02 mL total incubation volume) comprised 0.02 mL of application medium and 4 mL of hepatocyte cell suspension in one of the wells of a 24 well plate. The final cell concentration was approximately 1 x 10^6 cells/mL. The reactions were performed for 0, 10, 30, 60 or 180 min at 37 °C and at 5% CO_2 in an incubator.

Two negative controls (stability and zero incubation control), two positive controls (testosterone and 7-ethoxycoumarin) and a blank control (application medium with DMSO instead of the test item) were performed for each analyzed species and label.

In each experimental setup, the incubation of BAS 550 F as well as the test compound control assays were performed in triplicates. Incubations with testosterone or 7-ethoxycoumarin were performed in singlicate. In parallel, the viability of the human, rat and dog hepatocytes after 180 minutes incubation with 10 µM BAS 550 F (chlorophenyl label) was tested using a cell proliferation assay.

Sampling and sample storage

The incubation was terminated by pipetting the incubation mixture into a tube containing cold acetonitrile to adjust the sample to an acetonitrile concentration of approximately 20% (v/v). All incubations were sonicated for a few seconds, vortex mixed and centrifuged for 5 min at 16,000 x g at 4°C prior to analysis. The radioactive residues in the supernatants were determined by LSC analysis of aliquots pre and post centrifugation. The remaining pellet was then redissolved in water and the percentage of radioactivity remaining determined by LSC analysis. All samples were stored in a freezer at 20 °C.

Work-up of the residual pellet

In the supernatant the radioactive residues were quantified and ranged from 88.0% to 112%. Therefore, pellet extraction was not required. The remaining pellet was redissolved in water and aliquots were measured via LSC.

Evaluation of the data by HPLC and MS

Each *in vitro* assay was analyzed in triplicates by HPLC and one sample per triplicate was further investigated by LC-HR-MS for the qualitative evaluation of the masses of peaks representing more than 5% AR in human hepatocytes.

Samples were removed from the freezer and allowed to thaw in the refrigerator. Samples were sonicated for 10 minutes and centrifuged at 13,000 rpm at 10°C for 5 minutes in order to sediment out any particulate matter. An aliquot was transferred to a polypropylene vial, capped and transferred to the autosampler for LC-MS analysis. The mass spectra of those ^{14}C peaks were evaluated to obtain the m/z values of prominent ions corresponding to the test item or its conversion products.

The m/z values were assigned to prominent peaks in the supernatant and are listed together with the retention times and the % AR values.

Results

The radio HPLC analyses of human, rat and dog hepatocyte samples were compared in order to determine whether a unique human metabolite occurred or not. Selected human, rat and dog supernatant samples were also analysed by LC-HR-MS to assign m/z values to prominent peaks representing more than 5% AR in human samples.

For the triplicates of each negative control (stability controls without cells and zero incubation controls), all replicates showed an HPLC profile that contained only peaks corresponding to the unchanged active substance BAS 550 F. Therefore no significant metabolism or degradation of BAS 550 F occurred without the influence of hepatocytes.

The positive controls with testosterone showed that the metabolic activity of the hepatocytes with respect to Phase I metabolic reactions was sufficiently high. Testosterone was metabolized (>40%) after incubation with human, rat and dog hepatocytes.

HPLC analyses of the positive controls with 7-ethoxycoumarin revealed mean portions of the metabolised 7-ethoxycoumarin reaching values of 29.9% AR after incubation with human hepatocytes and 27.9% and 83.3% AR after incubation with rat and dog hepatocytes respectively. Hydroxylated, sulphated and glucuronidated metabolites of 7-ethoxycoumarin were detected in human, rat and dog with similar levels of 7-hydroxycoumarin (7HC) sulphate and 7HC glucuronide formed in dog (30-34% AR). In rat, 7HC sulphate

was the major metabolite (12% AR). However, in human both 7HC sulphate and 7HC glucuronide were formed at lower levels (4.4-7.4% AR).

After the incubation of BAS 550 F with human hepatocytes, four 14C peaks were detected that represented more than 5% AR on at least one time point in one label. Two of these signals represented the E- and Z-isomers of unchanged active substance BAS 550F. The other peaks, named Region 9 and Region 12, corresponded to metabolites of BAS 550 F.

The peak at 42.0 min (Region 9) was detected after 60 min in the chlorophenyl and morpholine label with 1.58% AR and 1.45% AR, respectively. After 180 min, the mean % AR for Region 9 increased to a maximum of 4.97% AR (chlorophenyl label) and 4.85% AR (morpholine label).
The peak at 44.0 min (Region 12) was detected after 10 min in the chlorophenyl and morpholine label with 2.46% AR and 1.76% AR, respectively. After 60 min, the mean % AR for Region 12 increased to 13.76% AR (chlorophenyl label) and 11.64% AR (morpholine label) and further to 16.78% AR (chlorophenyl label) and 16.13% AR (morpholine label) after 180 min of incubation.

Both regions were found to be consisting of multiple components, following LC-MS/MS analysis.
Region 9 comprised components with retention times of 40.6 min (m/z 406), 40.8 min (m/z 362), 41.4 min (m/z 374), 41.0 min (m/z 420), 41.3 min (m/z 420) and 41.4 min (m/z 420).
Region 12 comprised components retention times of 43.0 min (m/z 420), 43.1 min (m/z 404) and 43.6 min (m/z 374) and 43.7 min (m/z 374).

The identified components were similar for both labels, with the exception of an additional component detected in the region 9 of the morpholine label, with a retention time of 41.4 min (m/z 404). The results are shown in Table 25 andTable 26.

Table 25: Comparison of the relevant metabolites of BAS 550 F (morpholine label) after incubation with human, rat and dog hepatocytes

Relevant Peak		**Region 9** [a]	**Region 12** [a]	**BAS 550 F *E*-isomer**	**BAS 550 F *Z*-isomer**
		[% AR] [b]			
0 min	Human	n.d.	n.d.	41.94	55.47
	Rat	n.d.	n.d.	42.19	55.72
	Dog	n.d.	n.d.	42.33	55.37
10 min	Human	n.d.	1.76	41.05	53.25

	Rat	5.27	1.36	35.92	53.28
	Dog	n.d.	2.60	41.35	51.26
30 min	Human	1.02	6.35	36.49	45.52
	Rat	8.84	5.22	25.61	46.76
	Dog	0.95	7.74	38.76	45.22
60 min	Human	1.45	11.64	31.28	38.20
	Rat	7.63	9.81	15.99	36.93
	Dog	1.89	11.28	35.09	36.30
180 min	Human	4.85	16.13	21.75	24.85
	Rat	5.24	15.41	5.70	17.66
	Dog	3.75	13.11	31.48	25.88

[a] Found to be multicomponent following LC-MS/MS analysis;

Region 9: 40.6 min (m/z 406), 40.8 min (m/z 362), 41.4 min (m/z 374), 41.0 min (m/z 420), 41.3 min (m/z 420), 41.4 min (m/z 420) and 41.4 min (m/z 404, morpholine label only)

Region 12: 43.0 min (m/z 420), 43.1 min (m/z 404) and 43.6 min (m/z 374) and 43.7 min (m/z 374)

[b] % AR of supernatant

n.d. not detected

Table 26: Comparison of the relevant metabolites of BAS 550 F (chlorophenyl label) after incubation with human, rat and dog hepatocytes

Relevant Peak		**Region 9 [a]**	**Region 12 [a]**	**BAS 550 F *E*-isomer**	**BAS 550 F *Z*-isomer**
		[% AR] [b]			
0 min	Human	n.d.	n.d.	39.10	60.90
	Rat	n.d.	n.d.	38.77	61.00
	Dog	n.d.	n.d.	38.40	59.67
10 min	Human	n.d.	2.46	37.20	56.58
	Rat	5.80	1.98	32.60	58.57
	Dog	n.d.	3.14	38.33	57.34
30 min	Human	n.d.	8.05	33.94	50.55
	Rat	8.52	6.97	21.73	51.17
	Dog	n.d.	9.29	35.54	48.05
60 min	Human	1.58	13.76	28.61	40.91
	Rat	6.99	12.41	12.77	38.74
	Dog	1.13	13.19	32.91	39.73
180 min	Human	4.97	16.78	18.29	24.81

	Rat	5.91	18.13	4.12	17.87
	Dog	2.64	14.69	28.84	27.86

[a] Found to be multicomponent following LC-MS/MS analysis;

Region 9: 40.6 min (m/z 406), 40.8 min (m/z 362), 41.4 min (m/z 374), 41.0 min (m/z 420), 41.3 min (m/z 420) 41.4 min (m/z 420) and 41.4 min (m/z 404, morpholine label only)

Region 12: 43.0 min (m/z 420), 43.1 min (m/z 404) and 43.6 min (m/z 374) and 43.7 min (m/z 374)

[b] % AR of supernatant

n.d. not detected

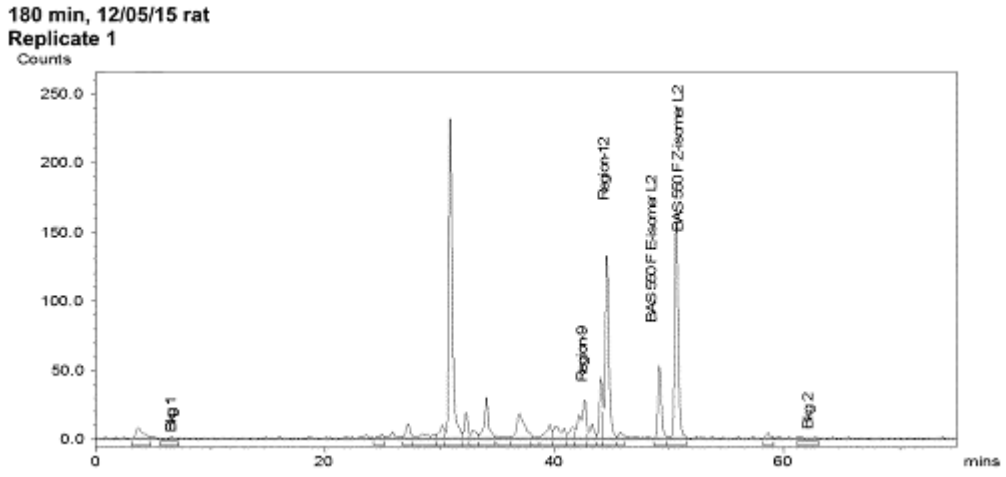
Conclusion

In this comparative *in vitro* metabolism study with dimethomorph (BAS 550 F, Reg. No. 247723) no significant differences were noted between the two different radiolabels (p-chlorophenyl-U-14C and morpholine-2,3-14C).

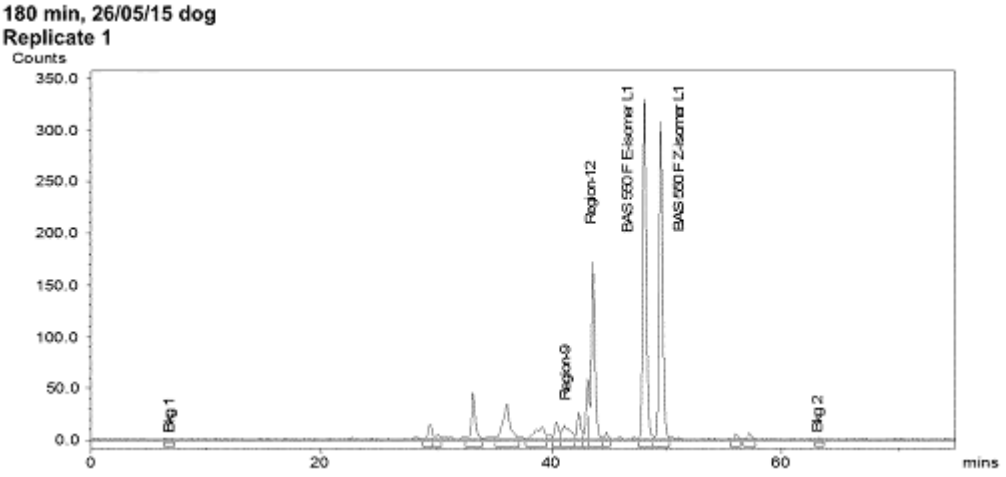
After the incubation of human hepatocytes with the active substance, four 14C peaks were detected that represented more than 5% AR on at least one time point in one label. These were the E- and Z-isomers of the unchanged active substance BAS 550 F plus two regions (Region 9 and Region 12) that comprised several metabolites. The components identified were generally similar for both labels and all tested species.

All the components identified in human hepatocytes were also detected in rat and dog hepatocyte samples. Therefore it can be concluded that no human-specific metabolites were found and that the metabolic degradation in the tested species was similar. It was however noted in the HPLC chromoatograms that a large peak was present in the rat at 180 minutes which was not present in the dog or human hepatocyte samples (see figures below).

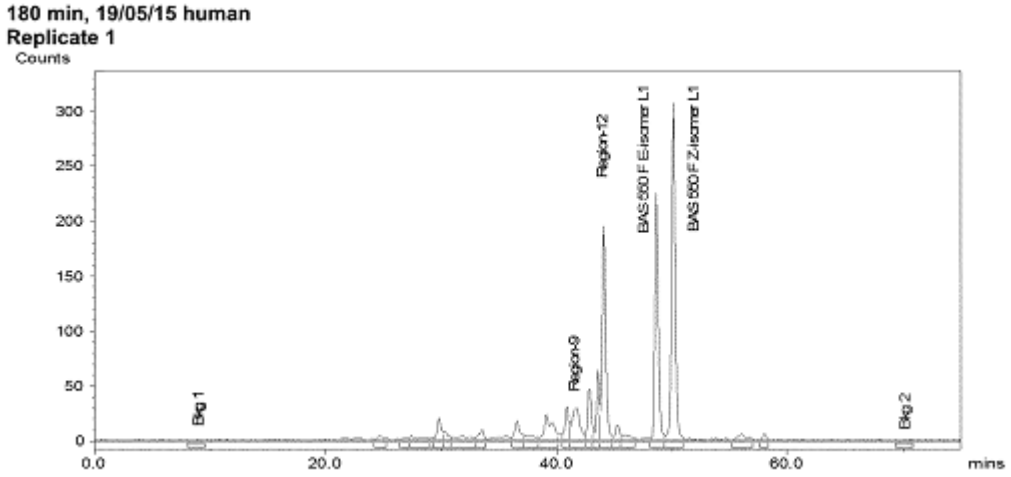
HPLC Chromatograms of the Replicates of 10 µM $^{14}C/^{15}N/^{12}C$ BAS 550 F (morpholine-2,3-^{14}C Label) Incubated with Rat Hepatocytes for 180 min (Supernatants) Using HPLC Method LC01



HPLC Chromatograms of the Replicates of 10 µM $^{14}C/^{13}C/^{12}C$ BAS 550 F (p-chlorophenyl-U-^{14}C Label) Incubated with Dog Hepatocytes for 180 min (Supernatants) Using HPLC Method LC01



HPLC Chromatograms of the Replicates of 10 µM $^{14}C/^{13}C/^{12}C$ BAS 550 F (p-chlorophenyl-U-^{14}C Label) Incubated with Human Hepatocytes for 180 min (Supernatants) Using HPLC Method LC01



3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

3.2 Acute toxicity - dermal route

3.3 Acute toxicity - inhalation route

3.4 Skin corrosion/irritation

3.5 Serious eye damage/eye irritation

3.6 Respiratory sensitisation

3.7 Skin sensitisation

3.8 Germ cell mutagenicity

3.9 Carcinogenicity

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Two generation reproduction study

Study reference:

B.6.6.1.1. - Anonymous 1990, SAG 151: Two generation oral (dietary administration) reproduction toxicity study in the rat (two litters in the F1 generation), unpublished, BASF Document No. DK-430-001

Detailed study summary and results:

Dimethomorph was fed daily to four groups of Sprague-Dawley rats at dietary concentrations of 0, 100, 300 and 1000 ppm. The NOAEL for parental toxicity was 300 ppm (equivalent to 21 mg/kg bw/day), based on reductions in pre-mating body weight gains for P1 and F1 females in the 1000 ppm group. Because the delay in incisor eruption at 1000 ppm was not considered adverse, the NOAEL for the growth and development of offspring was 1000 ppm (equivalent to 69 mg/kg bw/day). The NOAEL for fertility and reproductive function was 1000 ppm (equivalent to 69/79 mg/kg bw/day), based on a shortened pregnancy duration for P1 and F1 females in the 1000 ppm group.

Test type

Guideline: Directive 96/54/EC B 34, OECD Guideline 416; (US EPA Guideline 83-4; Japanese MAFF 59 NohSan 4200 claimed by the author)

Deviations: No deviations from the original guideline.. Deviations from current OECD guideline 416:

- Sperm parameters were not evaluated.
- Oestrous cycle length was not evaluated.
- Age of vaginal opening, preputial separation and anogenital distance not evaluated.
- No organ weights were measured.

GLP: Yes (This laboratory certified by Ministerium für Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz.)

Acceptability: The study is considered to be acceptable (with reservations because of unusually high pup losses in all three rearing phases).

Test substance

Dimethomorph (SAG 151); Batch No. DW 11/86; purity 96.6 %

Test animals

Male and female Sprague-Dawley rats (~6 weeks, males 160-225 g, females 135-170 g)

The P1 generation consisted of 30 male and 30 female rats per group while the F1 generation comprised 25 pairs per group.

Administration/exposure

Dimethomorph was fed daily to four groups of Sprague-Dawley rats at dietary concentrations of 0, 100, 300 and 1000 ppm.

Description of test design:

Male and female Sprague-Dawley rats (~6 weeks, males 160-225 g, females 135-170 g)

Dimethomorph was fed daily to four groups of Sprague-Dawley rats at dietary concentrations of 0, 100, 300 and 1000 ppm. The P1 generation consisted of 30 male and 30 female rats per group while the F1 generation comprised 25 pairs per group. The pre-mating treatment periods for the P1 and F1 parental animals were 100 days. Treatment for both parental generations continued during the 21-day mating periods and post-mating periods until scheduled sacrifice. Following weaning of the F2a litters, the F1 parents were paired for a second 21-day mating period to produce the F2b litters. Mated P1 and F1 females continued to be treated during the ensuing gestation and lactation periods. All litters were culled to eight pups/litter on postnatal day 4.

All animals were examined twice daily for morbidity and mortality. Clinical signs were observed at least once daily. Body weight and food consumption was recorded once weekly during the premating treatment period and in females on days 0, 6, 10, 15, 20 of gestation and days 1, 4, 7, 14 and 21 of lactation.

Results and discussion

The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis.

Table 27*:* ***Test substance intake (mg/kg bw/day)***

Dose level (ppm)		**M**				**F**			
		0	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
Intake (mg/kg bw/d)									
F0	**Pre-mate**	-	6.9	20.8	69.0	-	8.0	24.0	79.3
	Gestation					-	6.8	20.8	71.4
	Lactation					-	13.5	40.7	140.2
F1 first mating round	**Pre-mate**	-	7.9	23.7	78.6	-	8.9	27.0	89.2
	Gestation					-	7.0	21.5	74.1
	Lactation					-	14.6	46.2	151.8
F1 second mating round	**Pre-mate**	-	4.9	15.0	50.0	-	6.7	21.2	68.5
	Gestation					-	6.5	19.7	66.4
	Lactation					-	13.3	41.6	138.1

Test substance intake values were similar for P1 and F1 females during gestation and increased during lactation. Corresponding mean test substance intake values, for the P1 and F1 parents, are 6.9, 21 and 69 mg/kg bw/day for the 100, 300 and 1000 ppm groups, respectively, based on nominal concentrations.

P1 and F1 parental animals

In the P generation, 1 control female died on day 1 post partum and 1 female of the 300 ppm group was killed moribund on day 1 post partum. The observed mortalities are not considered to be treatment-related. No clinical signs of toxicity were observed at any dietary level.

Food consumption, body weights and body weight gains for P1 and F1 males were unaffected by treatment with dimethomorph. Dose-related reductions in body weights and body weight gains were observed during the pre-mating treatment period for P1 females in the 1000 group. Body weight gains during the pre-mating treatment period for P1 females in the 1000 ppm group were reduced 14.7 % as compared to controls. Statistically significant reductions in food consumption, relative to controls, were only noted during the first five weeks of the pre-mating treatment period for P1 females in the 1000 ppm group. Although not statistically significant, a decrease in body weight gain of 6.8 % was observed during the pre-mating treatment period for F1 females in the 1000 ppm group as compared to controls. No toxicologically significant changes in food consumption patterns were noted for F1 females in treated groups when compared to controls.

No adverse effects of treatment were indicated from the macroscopic postmortem evaluations of the P1 of F1 parental animals and no treatment-related microscopic changes were observed in any of the parental reproductive organs and tissues or pituitary glands.

Table 28*: Two-generation oral reproduction toxicity study in the rat - Mean body weight and mean body weight gain*

Dose level (ppm)		M				F			
		0	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
F0	**Bodyweight** (g)								
	Week 5	362.5	360.7	356.2	368.2	222.5	221.0	217.0	**206.8***
	Week 15	526.5	525.8	511.0	543.0	292.3	291.2	280.8	**267.2***
	Weight gain (g)								
	Week 1-15	332.6	334.3	320.2	352.2	138.0	137.5	128.3	**117.7***
	Food consumption (g)								
	Week 1	24.9	24.5	24.6	24.5	18.1	17.8	18.1	**16.3***
	Week 2	26.7	25.8	25.6	26.2	18.2	18.2	17.9	**17.0***
	Week 3	27.4	26.9	26.8	27.3	19.0	19.1	18.9	**17.7***
	Week 4	28.6	27.4	27.5	28.0	19.4	19.5	19.2	**18.0***
	Week 5	28.2	27.1	26.8	27.8	19.0	19.2	18.7	**17.3***
	Week 1-14	27.4	26.3	26.0	27.0	18.3	18.5	18.3	17.5
F1	**Bodyweight** (g)								
	Week 15	521.6	520.0	507.2	520.2	292.4	285.2	281.0	277.4
	Weight gain (g)								
	Week 1-15	397.8	399.6	385.0	398.6	183.4	178.2	174.4	171.0

* = significantly different $p < 0.5$; analysis of variance with one factor treatment followed by Student Newman-Keuls test

No adverse effects at the 100, 300 or 1000 ppm dietary levels were evident for mating, fertility or gestation indices, or for the parturition data during either litter interval (see Table 29). However, the number of females with a duration of pregnancy of 21 days was increased (borderline statistical significance) in both the P1 and F1 generation in the 1000 ppm group (see Table 30).

Table 29: Summary of fertility parameters

Parameter	Dose level (ppm)			
	0	**100**	**300**	**1000**

Number of mated females	P	30	30	30	30
Number of inseminated females		29[a]	27[b]	28[c]	30[d]
Number of pregnant females		23	26	27	24
Number of females which littered		23[e]	26	27[f]	24
Number of females with live pups on day 1 post partum		22	26	26	24
Number of females with live pups on day 21 post partum		21	24	23	22
Insemination index (%)		96.7	90.0	93.3	100.0
Fecundity index (%)		79.3	96.3	96.4	80.0
Fertility index (%)		76.7	86.7	90.0	80.0
Gestation index (%)		95.7	100.0	96.3	100.0
Mating performance (in days)[#]		2.8 ± 1.6	3.0 ± 2.2	2.6 ±1.4	3.0 ± 3.0
Number of mated females	F1 first mating	25	25	25	25
Number of inseminated females		21	23	21	25
Number of females which littered		19	21	20	24
Number of females with live pups on day 1 post partum		19	21	20	24
Number of females with live pups on day 21 post partum		17	19	18	20
Insemination index (%)		84.0	92.0	84.0	100.0
Fecundity index (%)		90.5	91.3	95.2	96.0
Fertility index (%)		76.0	84.0	80.0	96.0
Gestation index (%)		100.0	100.0	100.0	100.0
Mating performance (in days)[##]		2.7 ± 1.5	2.9 ± 1.5	3.6 ± 3.5	2.8 ± 1.3
Number of mated females	F1 second mating	25	25	25	25
Number of inseminated females		21	21	18	25
Number of females which littered		19	17	12	18
Number of females with live pups on day 1 post partum		19	17	12	18
Number of females with live pups on day 21 post partum		18	17	12	17
Insemination index (%)		84.0	84.0	72.0	100.0

Fecundity index (%)		90.5	81.0	66.7	72.0
Fertility index (%)		76.0	68.0	48.0	72.0
Gestation index (%)		100.0	10.0	100.0	100.0
Mating performance (in days)[###]		3.2 ± 1.6	2.8 ± 1.3	2.9 ±1.7	3.3 ±3.3

[a] for one additional animal sperm could not be detected, apparently not pregnant
[b] for one of these animals sperm could not be detected, but animal littered; for three additional animals sperm could not be detected, apparently not pregnant
[c] for one of these animals sperm could not be detected, but animal littered
[d] for three of these animals sperm could not be detected, but animals littered; for two additional animals sperm could not be detected, apparently not pregnant
[e] one of these animals died on day 1 post-partum
[f] one of these animals was killed moribund on day 1 post-partum
[#] ANOVA F-Probability (based on taking the ranks of the variables): 0.8952
[##] ANOVA F-Probability (based on taking the ranks of the variables): 0.8958
[###] ANOVA F-Probability (based on taking the ranks of the variables): 0.9332

Table 30*: Summary of pregnancy duration.*

Parameter		**Dose level (ppm)**			
		0	**100**	**300**	**1000**
Gestation duration (d)	F1	22.0 +/- 0.3	22.1 +/- 0.3	21.9 +/- 0.4	**21.8 +/- 0.4***
ND (#)		-	1	3	1
21 days (#)		1	-	3	4
22 days (#)		21	23	20	18
23 days (#)		1	2	1	-
Gestation duration (d)	F2A	21.9 +/1- 0.4	21.9 +/- 0.3	22.1 +/- 0.3	**21.7 +/- 0.5***
ND (#)		-	-	-	-
21 days (#)		2	2	-	7
22 days (#)		16	19	18	17
23 days (#)		1	-	2	-
Gestation duration (d)	F2B	21.9 +/- 0.2	22.2 +/- 0.5	21.9 +/- 0.3	21.8 +/- 0.4
ND (#)		-	-	-	-
21 days (#)		1	1	1	3
22 days (#)		18	12	11	15
23 days (#)		-	4	-	-

*Statistically significant $p<0.05$ (ANOVA)

ND = sperm could not be detected, therefore day 0 not determined.

The study report reported the effect on duration of pregnancy as statistically significant using an Analysis of Variance (ANOVA) with on factor TREATMENT, based on taking the ranks of the variables – and followed

by the Student-Newman-Keuls test for multiple group comparisons. For the purpose of the renewal the notifier provide a re-evaluation using the Dunnett test (two sided).

Table 31*: Re-evaluation of the duration of pregnancy (Dunnett test (two-sided))*

Statistical analysis		**Dose level (ppm)**			
		0	**100**	**300**	**1000**
Mean	F1	22.00	22.08	21.92	21.82
SD		0.30	0.28	0.41	0.39
N		23	25	24	22
%DEV		0	0	-0	-1
SIG					
pvalue			0.7653	0.7489	0.1999
	F2A				
Mean		21.95	21.90	22.10	21.70
SD		0.40	0.30	0.31	0.47
N		19	21	20	23
%DEV		0	-0	1	-1
SIG					
pvalue			0.9696	0.4491	0.0900
	F2B				
Mean		21.95	22.18	21.92	21.83
SD		0.23	0.53	0.29	0.38
N		19	17	12	18
%DEV		0	1	-0	-1
SIG					
pvalue			0.1832	0.9930	0.6947

Based on this re-evaluation the notifier concluded the effect to be not treatment-related. The RMS notes that a similar effect on gestation length was observed in the extended one-generation study at a higher dose level of 1600 ppm. However, considering that the effect was only very slight and not statistically significant based on the Dunnett test the re-evaluation of the notifier is agreed with.

F1 and F2 pups

Pup survival and mean pup weights for the F1, F2a and F2b offspring were comparable among all groups. However, in all groups, including controls, the pup mortality after postnatal day 4 was higher than it is

considered normal for well-conducted studies. It must be assumed that the environmental conditions in this study were sub-optimal.

In the 1000 ppm group, the percentage of pups in the F1, F2a and F2b generations which achieved incisor eruption was reduced on one or more days from postnatal days 9 - 11 when compared to controls and the differences were statistically significant. There were no treatment-related effects observed for any other developmental landmark (i.e., pinna unfolding, hair growth, or eye opening).

The mean days of incisor eruption for the F1, F2a and F2b litters in the 1000 ppm group were 10.5, 10.5 and 10.7 days, respectively while the mean days of incisor eruption for control litters were 9.6, 9.6 and 9.7 days, respectively, for the same generations. Thus, incisor eruption in the 1000 ppm group was delayed by approximately one day. Rat pups begin to eat solid food in addition to milk at around postnatal day 15. By this time incisor eruption was complete in all groups. Thus, the delay in incisor eruption did not interfere with the development of feeding ability and is not considered an adverse finding.

Figure 2: F1a Generation – Incisor eruption

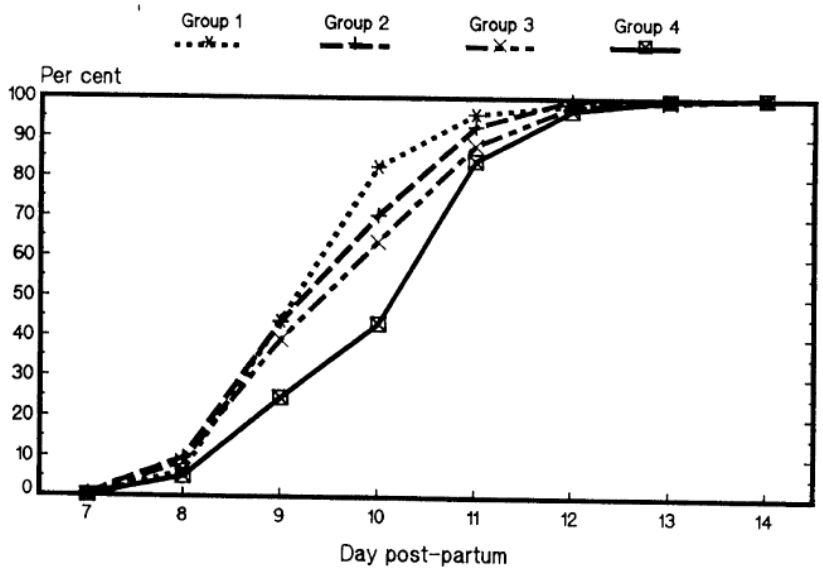


Figure 3: F2a Generation – Incisor eruption

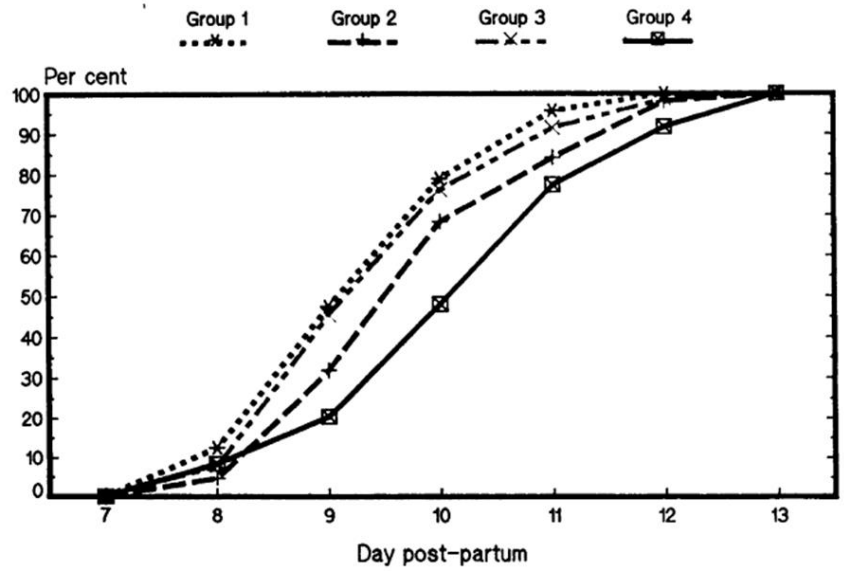
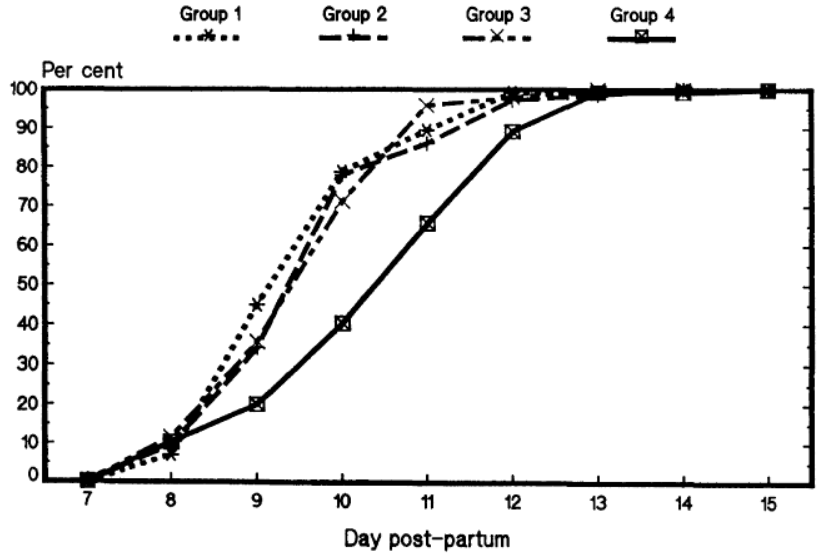


Figure 4: F2b Generation – Incisor eruption



All other physical development and reflex assessments were unaffected by prenatal exposure to dimethomorph at dietary concentrations up to and including 1000 ppm. No adverse effects of treatment with dimethomorph were indicated from the macroscopic postmortem evaluations of the F1, F2a or F2b offspring.

3.10.1.2 Extended one generation study

Study reference:

B.6.6.1.2 - Anonymous 2014. Extended one-generation reproduction toxicity study in wistar rats - Administration via the diet. 2014/1181670

Detailed study summary and results:

Dimethomorph (BAS 550 F; Batch: COD-001646; Purity 99.7%) was administered in the diet to groups of 25 male and 25 female Crl:WI(Han) Wistar rats at nominal dose levels of 0, 300, 800, and 1600 ppm. The dietary concentrations of dimethomorph were adjusted to 0, 150, 400, and 800 ppm during lactation, thereby maintaining constant dose-levels during this period of increased food intake. The overall mean dose of dimethomorph administered to the rats during the entire study period was approx. 26 mg/kg bw/day in the 300 ppm group, approx. 70 mg/kg bw/day in the 800 ppm group and approx. 144 mg/kg bw/day in the 1600 ppm group.

There were no test substance-related mortalities or adverse clinical observations noted in any of the groups. In particular, regularly conducted detailed clinical observations revealed no effects at all.

Reduced food consumption was observed in the 1600 ppm dose groups in F0 females during lactation and in cohort 1A males throughout the study (800 and 1600 ppm). Body weights and weight changes were impaired in the F0 high dose animals during several stages of the study (1600 ppm) and in cohort 1A animals (800 and 1600 ppm). Treatment-related and adverse effects on liver weight together with centrilobular hepatocellular hypertrophy (females of high dose group) were observed in F0 and cohort 1A animals from 800 ppm onwards. Decreased absolute and relative seminal vesicle weight was observed in the cohort 1A (1600 ppm) and 1B males (800 and 1600 ppm). Relative prostate weight was decreased in the cohort 1B males (800 and 1600 ppm).

In the 1600 ppm group, gestation length was significantly decreased. Pup body weight development was affected in the 1600 ppm dose group (-13% after birth) but had no effect on pup survival.

A slight effect on anogenital distance/index in mid and high dose males (based on pup-data) as well as a significant delay in vaginal opening (female F1 offspring of 1600 ppm group) and preputial separation (male F1 offspring of 800 and 1600 ppm group) were observed. The effect on vaginal opening and preputial separation in males at 800 ppm appear to be due to the decreased body weight leading to slow general development. The effect on preputial separation at 1600 ppm however, could not be fully explained by a delay in general development and a specific effect on preputial separation cannot be excluded. Hormone measurements (luteinizing hormone and testosterone) in male animals of cohort 1A did not reveal any changes.

Under the conditions of the present extended one-generation reproduction toxicity study the NOAEL (no observed adverse effect level) for general systemic toxicity is 300 ppm, based on decreased food consumption and body weight/body weight gain, decreased seminal vesicle weight, as well as clinical-

chemical changes and pathological evidence of liver toxicity at 800 and/or 1600 ppm, in the F0 parental animals and adult F1 offspring.

The NOAEL for fertility and reproductive performance for the parental rats is 800 ppm, based on reduced gestational length at 1600 ppm.

The NOAEL for developmental toxicity in the F1 progeny is 300 ppm, due to the decrease in the pre-weaning pup body weights/pup weight gains, as well as decreased anogenital distance/index (pup-based data) in males at 800 and 1600 ppm.

Test type

Guidelines: OECD 443

Deviations: No

GLP: yes

Acceptability: The study is considered to be acceptable

Test substance

Dimethomorph (BAS 550 F; Batch: COD-001646; Purity 99.7%). The test substance was stable over the study period at room temperature; (Expiry date: 31.01.2016)

Test animals

Species:	Rat
Strain:	Wistar, Crl:WI(Han)
Sex:	Male and female
Age:	F0 parental animals: 28 ± 1 days at delivery; 35 ± 1 days at beginning of treatment
Weight at dosing:	♂: 97.1 - 133.5 g, ♀: 89.8 - 112.5 g
Acclimation period:	6 days
Diet:	Kliba maintenance diet rat/mouse "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	drinking water from water bottles, ad libitum
Housing:	Groups of 5 animals/sex/group housed in Polysulfonate cages (H-Temp, supplied by TECHNIPLAST, Hohenpeßenberg, Germany) having a floor area of about 2065 cm², with the following exceptions: During the mating, gestation and lactation period the rats were housed individually in Makrolon type M III cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area of about 800 cm²), with the following exceptions: During overnight mating male and female mating pairs were housed together in Makrolon type M III cages overnight pregnant animals and their litters housed together until PND 21 (end of lactation)

bedding: wooden, dust-free

enrichment: wooden gnawing blocks (Typ NGM E-022 supplied by Abedd® Lab. and Vet. Service GmbH, Vienna, Austria);

nesting material: cellulose wadding

Environmental conditions:

Temperature: 20 - 24°C (central air-conditioned rooms)

Humidity: 30 - 70% (central air-conditioned rooms)

Air changes: 15 times per hour

Photo period: 12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

Administration/exposure

Dimethomorph (BAS 550 F; Batch: COD-001646; Purity 99.7%) was administered in the diet to groups of 25 male and 25 female Crl:WI(Han) Wistar rats at nominal dose levels of 0, 300, 800, and 1600 ppm. The dietary concentrations of dimethomorph were adjusted to 0, 150, 400, and 800 ppm during lactation, thereby maintaining constant dose-levels during this period of increased food intake. The overall mean dose of dimethomorph administered to the rats during the entire study period was approx. 26 mg/kg bw/day in the 300 ppm group, approx. 70 mg/kg bw/day in the 800 ppm group and approx. 144 mg/kg bw/day in the 1600 ppm group.

Description of test design:

Dates of experimental work: 25-Jun-2013 - 28-Jul-2015

(In-life dates: 02-Jul-2013 (start of administration of F0 parental animals) to 11-Nov-2013 (sacrifice of F0 parental animals) and 05 Nov-2013 (start of administration of F1 animals) to 14-Jan-2014 (sacrifice of F1 animals))

Animal assignment and treatment:

Dimethomorph was administered to groups of 25 male and 25 female rats at nominal dietary dose levels of 0 (ground diet), 300 (low dose), 800 (mid dose), and 1600 ppm (high dose). The animals used as F0 parental animals were derived from different litters according to a written statement from the breeder. By this, sibling mating was avoided. The animals were randomly assigned to the test groups by means of computer generated randomization list based on body weights.

After the acclimatization period F0 parental animals continuously received the test-substance throughout the entire study. Prior to sacrifice food was withdrawn overnight (approx. 16 hours).

At least 75 days after the beginning of treatment, male and female F0 rats of the same dose groups were mated overnight (details see below).

Females were allowed to deliver and rear their pups (F1 generation pups) until day PND 4 (standardization; see below) or PND 21. After weaning of F1 pups the F0 generation parental animals were sacrificed.

Mating procedure: Males and females were mated overnight at a 1:1 ratio for a maximum of 2 weeks. Throughout the mating period, each female was paired with a predetermined male from the same dose group. A vaginal smear was prepared after each mating and examined for the presence of sperm. If sperm was detected, pairing of the animals was discontinued. The day on which sperm were detected was denoted "gestation day (GD) 0" and the following day "gestation day (GD) 1".

Standardization of litters (F1 generation pups): On PND 4, the individual litters were standardized in such a way that, where possible, each litter contained 4 male and 4 female pups (always the first 4 pups/sex and litter were taken for further rearing). If individual litters did not have 4 pups/sex it was proceeded in such a way that the most evenly distributed 8 pups per litter were taken for further rearing (e.g., 5 male and 3 female pups). Standardization of litters was not performed in litters with ≤ 8 pups.
With the exception of those F1 generation pups, which were chosen as F1 rearing animals, all pups were sacrificed by decapitation under isoflurane anaesthesia after standardization or weaning. All culled pups, including stillborn pups and those that died during their rearing period, were subjected to a macroscopic (external and visceral) examination.
All pups without any notable findings or abnormalities were discarded after their macroscopic evaluation. Animals with notable findings or abnormalities were further evaluated on a case-by-case basis (e.g., histopathological evaluation or special staining), depending on the findings noted.

F1 rearing animals (Cohort 1A and Cohort 1B): After weaning, 40 male and 40 female F1 pups in test groups 00, 01, 02, and 03 (0, 300, 800 and 1600 ppm), became F1 rearing animals in test groups 10, 11, 12, and 13. These animals were assigned to 2 different cohorts (Cohort 1A [one male and one female/litter (20/sex/group)] and Cohort 1B [one male and one female/litter (20/sex/group)]). These animals were chosen by lot and each litter was taken into account as far as technically feasible. If fewer than 25 litters were available in a group or if one sex was missing in a litter, more animals were taken from the other litters of the respective test group to obtain the required number of paired animals.
All selected rearing animals were treated with the test substance at the same dose level as their parents, from post-weaning through adulthood.
The F1 rearing animals were sacrificed about 70 days after weaning.
Since there were no indications for neurohistopathological alterations or immunotoxicity of Dimethomorph in previous studies (acute and subchronic neurotoxicity, and 28-day immunotoxicity studies), the inclusion of cohorts for developmental neurotoxicity or immunotoxicity into the present extended 1-generation study is not required.

Test substance preparation and analysis:

The required quantity of test substance was weighed in a beaker depending on the dose group and thoroughly mixed with a small amount of food. Then further amounts of food were added to this premix and thoroughly mixed for 1 minute. Afterwards, further amounts of food, depending on the dose group, were added to this premix in order to obtain the desired concentrations. Mixing of this final mix was carried out for about 10 minutes in a laboratory mixer.

Dimethomorph concentrations in the diet of the F0 females were reduced to 50% during the lactation period. This dietary adjustment, derived from historical body weight and food consumption data, maintained the dams at constant dose-levels of Dimethomorph during this period of increased food intake.

Analytical verifications of the stability of the test substance in the diet for a period of 49 days at room temperature were carried out via HPLC-UV before the study was initiated with a comparable batch (AC 9978-131, purity: 98.3%).

Homogeneity and concentration control analyses were carried out at the beginning of the premating phase, lactation period and towards the end of the post-weaning period. For homogeneity analysis of the diet preparations 3 samples were taken from the top, middle and bottom of the beaker for the low (300 ppm) and high dose level (1600 ppm). Concentration control analyses were carried out towards the end of the premating phase. Duplicate samples were kept in reserve and will be discarded after report finalization.
The sample preparation and analysis of the test substance in feed was carried out according to the valid control procedure 01/0271_02-01 and 01/0271_02-02, respectively.

Table 32: Analysis of diet preparations for homogeneity and test-item content

Nominal Dose level [ppm]	Sampling	Concentration [ppm]	% of nominal concentration	% of mean nominal concentration[#]	Relative standard deviation [%]
300	02.06.13	314.40 ± 3.85		104.8	1.2
	24.09.13	309.07	103.0		
	*01.10.13**	*147.07 ± 8.33*		*98.0*	*5.7*
	18.10.13	307.26 ± 3.03		102.4	1.0
	28.11.13	300.53	100.2		
	06.01.14	316.59 ± 7.05		105.5	2.2
	average[§]	**309.57** ± 6.32		**103.2**	**2.0**
800	02.06.13	863.37	107.9		
	24.09.13	795.77	99.5		
	*01.10.13**	*414.85*	*103.7*		
	18.10.13	795.53	99.4		

	28.11.13	834.82	104.4		
	06.01.14	879.95	110.0		
	average[§]	**833.89** ± 38.46		**104.2**	**4.6**
1600	02.06.13	1590.08 ± 41.60		99.4	2.6
	24.09.13	1639.59	102.5		
	*01.10.13**	*806.04 ± 26.62*		*100.8*	*3.3*
	18.10.13	1575.99 ± 39.54		98.5	2.5
	28.11.13	1632.77	102.0		
	06.01.14	1648.34 ± 53.41		103.0	3.2
	average[§]	**1617.35** ± 32.20		**101.1**	**2.0**

[#] based on mean values of the three individual samples

* concentrations in the diet of the F_0 females were reduced to 50% during the lactation period due to increased food consumption.

[§] except the sample form 01.10.13 with reduced concentration

No test substance could be detected in the control feed samples.

The relative standard deviation in the range of 1.0% to 2.2% and of 2.6% to 3.2% for the 300 and 1600 ppm dose levels, respectively, reveals the homogeneous distribution of the test item in the diet preparations.

The mean and single values of BAS 550 F (Dimethomorph) in Ground Kliba maintenance diet mouse/rat „GLP“ meal were found to be in the acceptable range of 90 % – 110 % of the nominal concentrations, thus demonstrating the correctness of the concentration of the test item in the diet preparations.

Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Parameter	Statistical test
Statistics of clinical examinations	
Food consumption (parental animals), body weight and body weight change (parental animals and pups; for the pup weights, the litter means were used), number of mating days, duration of gestation, number of implantation sites, post-implantation loss and % post-implantation loss, number of pups delivered per litter	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means
Male and female mating indices, male and female fertility indices, gestation index, females delivering, females with live-born pups, females with stillborn pups, females with all stillborn pups	Pair-wise comparison of each dose group with the control group using FISHER'S EXACT test for the hypothesis of equal proportions
Mating days until day 0 pc, % post-implantation loss	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided+) with BONFERRONI-HOLM adjustment for the hypothesis of equal medians
Implantation sites, pups delivered, live pups day x, viability index, lactation index	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided-) with BONFERRONI-HOLM adjustment for the hypothesis of equal medians
Number of cycles and Cycle Length (days 54 -74)	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair-wise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians
Statistics of clinical pathology	
Blood parameters	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians. For parameters with unidirectional changes: Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided) with BONFERRONI-HOLM adjustment for the hypothesis of equal medians
Urinalysis parameters (apart from pH, urine volume, specific gravity, colour and turbidity)	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided) for the hypothesis of equal medians
Urine pH, urine volume, specific gravity, colour and turbidity	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians. Urine colour and turbidity are not evaluated statistically
Sperm parameters	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided) with BONFERRONI-HOLM adjustment for the hypothesis of equal medians. If only the control and one dose group are measured,

Parameter	Statistical test
Statistics of clinical examinations	
	WILCOXON test (one-sided) without adjustment were used. For the percentage of abnormal sperms values < 6% were set to 6% (cut off 6%).
Statistics of pathology	
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians.
DOFC (differential ovarian follicular count)	Pair-wise comparison of each dose group with the control group using WILCOXON test (one-sided) for the hypothesis of equal medians

Observations:

The animals, i.e. parental animals and pups, were examined for mortality twice daily on working days and once daily on weekends and public holidays. If animals were in a moribund state, they were sacrificed and necropsied.

Observations for evident signs of toxicity were performed at least once daily.

The parturition and lactation behaviour of the dams was generally evaluated in the mornings in combination with the daily clinical inspection of the dams. Only special findings (e.g., disability to deliver) were documented on an individual dam basis. Except on weekends and public holidays, the parturition behaviour was additionally checked in the afternoons.

The live pups were examined daily for clinical symptoms (including gross morphological findings) during the clinical inspection of the dams. If pups showed particular findings, these were documented with the dam concerned.

All F0 parental animals and F1 animals in Cohorts 1A and 1B were subjected to detailed clinical observations (including palpation) outside their cages once per week (as a rule in the mornings), by the same trained technicians, whenever possible. The scope of examinations and the scoring of the findings were lexicon-based. Because not all potential observations were contained in the lexicon, free-field descriptions were also allowed. Examinations include but were not limited to the following parameters

Detailed clinical observation (DCO) parameters	
1. abnormal behaviour during “handling”	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation

4. posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. faeces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

Body weight:

Body weight of parental F0 animals and F1 rearing animals was determined on the first day of the premating period and weekly thereafter at the same time of the day (in the morning), with following exceptions:

a. During pregnancy, body weight of the F0 females with evidence of sperm was determined weekly for GD 0-7, 7-14, and 14-20.

b. F0 females were not weighed during mating until there was a positive evidence of sperm in vaginal smears

c. Females without litter were not weighed during the lactation phase.

d. During lactation, body weight of the F0 females, which gave birth to a litter was determined for PND 1-4, 4-7, 7-14, and 14-21.

Pup body weights were determined on the day after birth (PND 1) and on PND 4 (before standardization), 7, 14, and 21.

Food consumption, food efficiency and compound intake:

Food consumption was determined once a week (for a period of 6 days) for parental F0 animals and F1 rearing animals, with the following exceptions:

a. Food consumption was not determined after the 10th premating week (male F0 animals) and during the mating period (male and female F0 animals).

b. Food consumption of F0 females during pregnancy was determined weekly for GD 0-7, 7-14, and 14-20

c. During the lactation period, food consumption of the F0 females which gave birth to a litter was determined for PND 1 4, 4-7, 7-14, and 14-21.

d. No food consumption was determined during the mating period and for females without positive evidence of sperm and females without litter.

The mean daily intake of test substance (group means in mg/kg bw/day) was calculated based upon individual values for body weight and food consumption.

Oestrous cycle determination:

Oestrous cycle length was evaluated by daily analysis of vaginal smear for all F0 female parental rats for a minimum of 3 weeks prior to mating. Determination was continued throughout the pairing period until the female exhibited evidence of copulation. At necropsy, an additional vaginal smear was examined to determine the stage of oestrous cycle for each F0 female with scheduled sacrifice.
For all cohort 1B females, oestrous cycle length and normality was evaluated by preparing vaginal smears during a minimum of 3 weeks prior to necropsy.
In all cohort 1A females, vaginal smears were collected after vaginal opening until the first cornified smear (oestrous) was recorded. The oestrous cycle also was evaluated in cohort 1A females for 2 weeks around PND 75.

Male and female reproduction data:

Male reproduction data

The pairing partners, the number of mating days until vaginal sperm was detected in the female animals, and the gestational status of the females were recorded for F0 breeding pairs.
For the F0 males, mating and fertility indices were calculated.

Female reproduction and delivery data

The pairing partners, the number of mating days until vaginal sperm were detected, and gestational status were recorded for F0 females.
For the F0 females, mating, fertility and gestation indices were calculated.
The total number of pups delivered and the number of live-born and stillborn pups were noted, and the live birth index was calculated for F1 litters
The implantations were counted and the post-implantation loss (in %) was calculated. To determine the number of implantation sites, the apparently non-pregnant uteri were stained for about 5 minutes in 1% ammonium sulphide solution according to the method of SALEWSKI.

Litter data

All F1 pups were examined as soon as possible on the day of birth to determine the total number of pups, the sex and the number of live-born and stillborn members of each litter. At the same time, the pups were also being examined for macroscopically evident changes. Pups, which died before the first examination on the day of birth, were designated as stillborn pups.
The number and percentage of dead pups on the day of birth (PND 0) and of pups dying between PND 1-4, 5-7, 8-14 and 15-21 (lactation period) were determined; however, pups, which died accidentally or had to be sacrificed due to maternal death, were not included in these calculations. The number of live pups/litter was calculated on the day after birth, and on lactation days 4, 7, 14, and 21. Furthermore, viability and lactation indices were calculated.

One the day after birth (PND 1) the sex of the pups was determined by observing the distance between the anus and the base of the genital tubercle; normally, the anogenital distance is considerably greater in male than in female pups. The anogenital index was also calculated

Later, during the course of lactation, this initial sex determination was followed up by surveying the external appearance of the anogenital region and the mammary line. The sex of the pups was finally confirmed at necropsy. The sex ratio was calculated at PND 0 and PND 21 after birth .

All surviving male pups were examined for the presence of nipple/areola complex on PND 12 and were re-examined on PND 20 before necropsy.

Date of sexual maturation of all F1 pups was recorded.
All female F1 pups selected to become the F1 rearing females for Cohort 1A and Cohort 1B (20/group/cohort) were evaluated daily for vaginal patency beginning on PND 27. On the day of vaginal opening the body weights of the respective animals were determined.
All male F1 pups selected to become the F1 rearing males for Cohort 1A and Cohort 1B (20/group/cohort) were evaluated daily for preputial separation beginning on PND 38. On the day of preputial separation the body weights of the respective animals were determined.
To differentiate between effects on sexual maturation and impaired general growth that can alter the onset of puberty an analysis was performed to graphically compare the ages and weights at puberty of the individual animals, using the change in body weight as a marker for general animal development.

Clinical pathology

Samples were withdrawn from 10 F0 parental and Cohort 1A males and females per group at termination.

Haematology and clinical chemistry:
Blood samples were taken from animals by puncturing the retro-bulbar venous plexus following isoflurane anaesthesia. Blood sampling and blood examinations were carried out in a randomized sequence. The list of randomization instructions was compiled with a computer.

Haematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time
✓	Haemoglobin (HBG)	✓	Neutrophils (differential)	✓	Platelet count
✓	Haematocrit (HCT)	✓	Eosinophils (differential)		Partial thromboplastin time
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		Thromboplastin time
✓	Mean corp. haemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		

✓	Reticulocytes	✓	Large unstained cells (differential)		
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Creatinine	✓	γ-glutamyltransferase (GGT)
✓	Potassium	✓	Globulin (by calculation)		
✓	Sodium	✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Hormones:

Additionally to F0 parental and Cohort 1A males and females, blood samples for hormone determination were withdrawn from 10 surplus (culled) PND 4 offspring (as far as possible of different litters) per sex and group. PND 4 samples were pooled per sex and litter if the available amount is not sufficient for a hormone analysis. Furthermore, blood samples were withdrawn from 10 surplus PND 22 offspring (as far as possible of different litters) per sex and group. The blood samples was collected after decapitation (following isoflurane anaesthesia) from the Vena cava cranialis.

The concentrations of TSH and T4 were determined with a radioimmunoassay (RIA) on a Gamma-Counter and ELISA, respectively.

Three days before sacrifice, in 10 male animals of cohort 1A blood samples has been taken from animals by puncturing the retrolobular venous plexus following isoflurane anesthesia. Blood sampling and blood examinations has been carried out in a randomized sequence. Animals were not fasted before blood sampling. The following hormones have been measured in the prepared serum samples:

- LH
- Testosterone

Urinalyses:

In the afternoon preceding the day of urinalysis, the animals were individually transferred into metabolism cages (no food or drinking water provided); on the following morning, the individual urine specimens were examined in a randomized sequence for the following parameters:

Urinalysis					
	Quantitative parameters:		*Semi-quantitative parameters*		

✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Colour and turbidity (visual exam.)	✓	Urobilinogen
		✓	Glucose	✓	Sediment (microscop.exam.)
		✓	Ketones		Nitrite

Sperm parameter:

After the organ weight determination, the following parameters were determined in the right testis or right epididymis of all male F0 parental animals and cohort 1A males sacrificed on schedule:

- sperm motility
- sperm morphology
- sperm head count (cauda epididymis)
- sperm head count (testis)

Sperm motility examinations were carried out in a randomized sequence. Sperm head count (testis and cauda epididymis) and sperm morphology were evaluated in control and high dose animals, only.

Sacrifice and pathology:

All F0 parental animals and Cohorts 1A and 1B (PND 90) rearing animals were sacrificed by decapitation under isoflurane anaesthesia. The exsanguinated animals were necropsied and assessed by gross pathology with special attention given to the reproductive organs. A quantitative assessment of primordial and growing follicles in the ovaries was performed for all control and high-dose F1A rearing females. As there were no effects on oestrous cyclicity evident, no histopathological investigation was performed in cohort 1B animals.

Female # 101 (F0, 0 ppm) had unscheduled mated with male no. 40 (F0, 300 ppm). It was sacrificed prematurely and no further investigations were performed. Female animal (# 317; Cohort 1A, 0 ppm) died inter-currently. It was necropsied and assessed by gross pathology as soon as possible after its death.

On PND 4, all surplus F1 pups as a result of standardization were sacrificed by decapitation under isoflurane anaesthesia and blood was sampled for determination of serum thyroid hormone concentrations. After sacrifice, these pups were examined externally, eviscerated and their organs were assessed macroscopically.

On PND 22, the surplus F1 generation pups that were not used for the formation of the cohorts were sacrificed by decapitation under isoflurane anaesthesia in the pathology lab and blood was sampled for thyroid hormone analyses. Anaesthetised animals, brain, spleen and thymus were weighed. All gross lesions, brain, mammary gland, spleen, thymus and thyroid glands were fixed in 4% formaldehyde solution, but no histopathological investigation was performed.

All stillborn pups and all pups that died before weaning were examined externally, eviscerated and their organs were assessed macroscopically.

All pups without notable findings or abnormalities were discarded after their macroscopic evaluation. Animals with notable findings or abnormalities were evaluated on a case-by-case basis, depending on the type of finding noted.

Pathology:

The following organs were collected (column C), weighed (W: ✓: F_0 and Cohort 1A and 1B, #: F_0 and Cohort 1A) and examined histopathologically (H: ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
			adipose tissue	✓	#	#	kidneys	✓	✓	✓	seminal vesicles**
✓	#	✓	adrenals				lachrymal glands				skin
			aorta	✓	#	✓	liver	✓		#	spinal cord[$]
✓		#	bone marrow (femur)	✓		#	lungs	✓	#	#	spleen
✓	#	#	brain	✓	#	#	lymph nodes**				sternum w. marrow
✓		#	caecum	✓		#	mammary gland	✓		#	stomach[§]
✓		#	colon					✓	✓	✓	testes[##]
	✓	#	cauda epididymis	✓		#	muscle, skeletal	✓	#	#	thymus
✓		#	duodenum	✓		#	nerve (sciatic)	✓	#	#	thyroid/ parathyroid glands
✓	✓	✓	epididymides[##]	✓		#	oesophagus				tongue
✓		#	eyes with optic nerve	✓	✓	✓	ovaries / oviduct[#]				tonsils
			femur (with knee joint)	✓		#	pancreas	✓		#	trachea
			gall bladder	✓	✓	✓	pituitary	✓		#	urinary bladder
✓		✓	gross lesions	✓	✓	✓	prostate	✓	✓	✓	uterus with cervix
✓	#	#	heart	✓		#	rectum	✓		✓	vagina
✓		#	ileum				salivary glands	✓		✓	vas deferens
✓		#	jejunum (with Peyer's patches)				hind-limb (one)		✓		body (fasted animals)

* mesenteric and axillaris, Cohort 1A animals only; ** including coagulation glands; [#] oviduct not weighted, ovaries fixed in modified Davidson's solution; [##] left epididymis and testis was fixed in modified Davidson's solution; [§] fore-stomach and glandular stomach; [$] cervical-, thoracic- and lumbar cord;

The ovaries of animal no. 317 (Cohort 1A, 300 ppm) that died were fixed in 4% buffered formaldehyde solution. The left testis and left epididymis of all male F0 parental and Cohort 1A animals sacrificed at scheduled dates were fixed in modified Davidson's solution, whereas the right testis and epididymis were used for sperm parameters.

The uteri of all cohabited female F0 parental animals were stained according to SALEWSKI's method.

Special attention was given to stages of spermatogenesis in the male gonads.

Special attention was given to the synchrony of the morphology in ovaries, uterus, cervix, and vagina to the oestrous cycle status.

Auto-fluorescence examination of the liver was performed in animals No 102, 177, 302, and 362 to determine the presence of lipofuscin.
Reproductive organs of all low- and mid-dose F0 parental animals suspected of reduced fertility were subjected to histopathological investigation.

A differential ovarian follicle count (DOFC) was conducted in test groups 10 and 13 (Cohort 1A females, control and high dose group) according to Plowchalk et.al. 1993. Therefore, both ovaries from each female were embedded in paraffin blocks. Each block was sectioned serially until the total ovary was laminated. Sections were prepared with 2 µm thickness and every 100 µm three serial sections (1A, 1B, 1C / 2A, 2B, 2C / 3A, 3B, 3C /…) were taken and mounted on glass slides:

- the first of the serial sections 1A, 2A, 3A…was stained with haematoxylin and eosin,
- the second section (e.g. 1B, 2B, 3B…) was used for immunohistochemistry (using MVH antibody [mouse vasa homolog], a protein expressed in all oocyte stages),
- the third section (e.g. 1C, 2C, 3C …) was taken as possible negative control for immunohistochemistry (the third serial section of the 14th serial sections (14C) was used as negative control).

The immunohistochemically stained slides were used for counting of primordial and growing follicles. Starting with "section 2B", each second section (section Nos. 2B, 4B, 6B …) was evaluated of both ovaries of each female. Primordial follicles and growing follicles were counted by light microscope (magnification: 100x), according to the definitions given by Plowchalk et al., 1993.
To prevent multiple counting on serial sections, especially of the growing follicles, only follicles with an oocyte with visible chromatin were counted. The number of each type of follicle was recorded individually for both ovaries of every animal, giving in summary the incidence of each type of follicle. Finally, the results of all types of follicles were summarized for all animals per group in test groups 10 and 13. As primordial follicles continuously develop into growing follicles, the assessment of the follicles was extended to the combined incidence of primordial plus growing follicles.
In general, the 10th section stained with haematoxylin and eosin from all females of test groups 10 and 13 was evaluated for histological findings.

Results and discussion

The historical control data reported in the following results section was based on studies (n= 24) carried out in the same lab between January 2007 to February 2012 with Wistar rats.

CLINICAL EXAMINATIONS AND EXAMINATION OF REPRODUCTIVE PERFORMANCE

F0 GENERATION PARENTAL ANIMALS

OBSERVATIONS

Clinical signs of toxicity

No clinical signs of toxicity or changes of general behaviour, which may be attributed to the test substance, were detected in any of the male F0 parental animals in any of the groups.

There were no test substance-related clinical findings in all F0 females of all dose groups during premating, gestation and lactation periods for F1 litter. Three sperm positive females of 1600 ppm dose group (# 182, 196 and 197) and one sperm positive female of 300 ppm dose group (# 133) did not deliver F1 pups. One further sperm positive female of 300 ppm dose group (# 146) did not deliver pups but had implants in the uterus.

F0 animals in any Dimethomorph treated dose group did not show any abnormalities detectable during detailed clinical observations.

Mortality

There were no test substance-related or spontaneous mortalities in any of the groups.

PARENTAL BODY WEIGHT AND BODY WEIGHT GAIN

Body weights of all parental F0 males were comparable to the concurrent control group throughout the entire study period.

Body weights of all parental F0 females were comparable to the concurrent control group throughout the entire premating period.
Body weights of the high-dose F0 females were statistically significantly below the concurrent control values during GD 14-20 (up to 6%) and during PND 14-21 (up to 5%) as shown inTable 33.
Body weights of the mid- and low-dose parental females were comparable to the concurrent control group throughout the entire study period.

Table 33: Body weight development of F0 females during gestation and lactation phases

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight [g]								
Gestation phase	N = 24		N = 24		N = 25		N = 22	
GD 0	223.8	13.5	223.0	15.7	223.5	13.6	216.5	13.0
Δ% (compared to control)			-0.4		-0.1		-3.3	
GD 7	245.9	16.0	240.5	16.6	244.6	13.5	235.6	12.0
Δ% (compared to control)			-2.2		-0.5		-4.2	
GD 14	269.6	18.1	262.6	18.1	267.6	13.9	**256.4***	13.0
Δ% (compared to control)			-2.6		-0.8		-4.9	
GD 20	332.6	23.7	323.8	27.7	328.7	19.9	**313.7***	19.0
Δ% (compared to control)			-2.7		-1.2		-5.7	
Lactation phase	N = 24		N = 23		N = 25		N = 22	
LD 1	252.8	17.0	247.2	16.4	250.5	13.1	245.8	16.9
Δ% (compared to control)			-2.2		-0.9		-2.8	
LD 4	260.7	19.0	259.5	20.0	265.4	13.8	256.0	15.5
Δ% (compared to control)			-0.5		1.8		-1.8	
LD 7	262.7	16.1	264.3	11.2	265.1	13.0	257.0	9.2
Δ% (compared to control)			0.6		0.9		-2.2	
LD 14	289.9	19.1	282.8	19.1	288.6	12.1	**276.1***	14.2
Δ% (compared to control)			-2.4		-0.5		-4.8	
LD 21	279.7	14.4	278.2	18.4	277.6	10.5	**269.0***	15.0
Δ% (compared to control)			-0.5		-0.7		-3.8	
* = p<0.05; Dunnett test (two-sided) GD = gestation day; LD = lactation day								

The body weight change of the high-dose parental F0 males was below control during premating and mating at several timepoint, the difference gaining statistical significance during premating days 35 - 49 (up to 25% below control) [see Table 34]. Overall body weight gain (day 0-70) was only marginally affected (-3.9%).
The body weight change of the low- and mid-dose males was comparable to the concurrent control group throughout the entire study period.
Any other body weight changes in males, like the decreased body weight change of the mid-dose parental males during premating days 35 - 42 and 49 – 56, the statistically significantly increased body weight change in the mid- and low-dose males during premating days 56 - 63 and 21 - 28, respectively, and in the high-dose males during post-mating days 1 - 8 were considered to be spontaneous in nature and not treatment-related.

The body weight change of the high-dose parental F0 females was below the concurrent control values during gestation, the difference to the control gained statistical significance on GD 7 - 14 and GD 0 - 20 (about 12% and 11%, respectively) [see Table 34].
The body weight change of the low- and mid-dose females was generally comparable to the concurrent control group throughout the entire study.
Any other changes in females, like the decreased body weight change of the low-dose parental females during GD 0 - 7 and PND 7 – 14, and of the mid-dose parental females during premating days 7 – 14, as well

as the increased body weight change in the mid-dose females during PND 1 – 4 were considered to be spontaneous in nature and not treatment related.

Table 34: Body weight development of F0 animals during pre-mating and gestation phases

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight gain [g]								
F0 males								
Premating phase	N =25		N =25		N =25		N =25	
Day 0-7	47.2	6.9	46.7	5.7	47.4	4.6	47.7	5.5
Δ%			-1.1		0.2		1.1	
Day 7-14	40.4	4.7	41.3	4.5	40.2	4.0	38.8	4.9
Δ%			2.2		-0.5		-3.7	
Day 14-21	36.9	5.1	37.0	5.2	37.4	5.4	36.2	5.8
Δ%			0.3		1.4		-2.2	
Day 21-28	21.6	12.5	**27.4***	4.6	26.6	5.4	25.0	6.7
Δ%			27.4		23.7		16.7	
Day 28-35	23.5	5.9	22.9	4.6	22.8	5.3	20.0	5.2
Δ%			-2.1		-3.0		-14.9	
Day 35-42	22.3	8.3	18.7	5.1	**16.9****	3.9	**17.7***	
Δ%			-16.6		-24.2		-20.6	
Day 42-49	15.7	4.6	14.1	2.8	13.2	4.3	**11.7****	4.4
Δ%			-10.2		-15.9		-25.5	
Day 49-56	15.9	4.7	14.8	3.9	**11.2****	4.2	15.8	5.1
Δ%			-7.5		-30.0		-1.2	
Day 56-63	8.6	6.6	11.2	4.5	**13.6****	5.0	10.3	5.2
Δ%			30.2		59.3		18.6	
Day 63-70	9.7	12.5	8.8	3.6	8.8	3.6	9.0	3.9
Δ%			-8.3		-8.3		-3.9	
Day 0-70	241.7	32.1	242.9	31.8	238.1	30.3	232.2	36.1
Δ%			0.5		-1.5		-3.9	
F0 females								
GD 0-7	22.2	5.3	**17.9***	4.3	21.1	5.9	19.2	4.1
Δ%			-20.8		-4.5		-13.6	
GD 7-14	23.7	3.6	21.5	4.2	23.0	4.5	**20.8***	3.3

Δ%			-6.8		-3.0		-12.2	
GD14-20	62.9	12.8	61.2	14..2	61.2	10.5	57.3	9.7
Δ%			-2.9		-3.0		-9.0	
GD 0-20	108.8	15.3	100.8	18.0	105.2	13.6	**97.2***	9.9
Δ%			-7.4		-3.3		-10.7	

* = p<0.05; * = p<0.01; Dunnett test (two-sided)

GD = gestation day

PARENTAL FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption of all male F0 rats in all dose groups was comparable to the concurrent control throughout the entire study period.

Food consumption of the high-dose female F0 rats was comparable to the concurrent control values throughout the premating and gestation periods. From PND 7 onwards throughout remaining lactation period, food consumption of the high-dose F0 females was statistically significantly below (up to 8%) the concurrent control values [seeTable 35]. Food consumption of the female F0 rats in the mid- and low-dose groups was comparable to the concurrent control values throughout the entire study.

Table 35: Food consumption of parental F0 females during lactation period

Dose level [ppm]	**0**		**300**		**800**		**1600**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Food consumption [g/rat/day]								
Lactation phase	N = 24		N = 23		N = 25		N = 22	
LD 1-4	32.0	4.0	32.2	4.8	33.9	4.4	31.2	5.3
Δ% (compared to control)			0.6		6.0		-2.4	
LD 4-7	38.4	2.9	39.2	3.1	39.7	3.2	37.0	2.7
Δ% (compared to control)			2.1		3.3		-3.7	
LD 7-14	54.1	4.5	53.1	5.2	54.3	3.4	**50.7***	5.6
Δ% (compared to control)			-2.0		0.2		-6.3	
LD 14-21	66.4	4.8	63.5	10.1	64.8	3.1	**61.3***	5.3
Δ% (compared to control)			-4.3		-2.3		-7.6	
LD 1-21	52.7	3.6	51.5	5.3	52.7	2.8	**49.4***	4.5
Δ% (compared to control)			-2.3		0.0		-6.2	

* = p<0.05; Dunnett test (two-sided)

LD = lactation day

For all test groups the intake of Dimethomorph correlated well with the desired target doses. The actual test substance intake was calculated on the basis of interpolated mean body weights of each test group. With regard to the target dose levels, the mean values and the minimum/maximum deviations of the actual test substance intake per time interval examined are shown in Table 36.

Table 36: Average Dimethomorph intake (mg/kg bw/day) in F0 parental animals

Dose	300 / 150 ppm		800 / 400 ppm		1600 / 800 ppm	
Group & sex	average	min/max	average	min / max	average	min / max
F_0 males	23.4	16.5 / 37.7	63.0	44.3 / 100.3	128.4	93.9 / 199.8
F_0 females						
- premating	25.0	20.0 / 33.7	66.8	54.4 / 89.2	136.5	116.2 / 178.0
- gestation period	21.9	20.5 / 22.4	58.9	53.8 / 61.1	118.4	111.3 / 119.9
- lactation period	27.5	18.5 / 34.3	74.5	51.0 / 93.5	144.7	97.3 / 182.4

OESTROUS CYCLE DETERMINATIONS

Oestrous cycle data of the F0 females, generated during the last 3 weeks prior to mating, revealed regular cycles in the females of all test groups including the control. The mean estrous cycle duration in the different test groups was comparable: 4.4 days in control, 4.4 in the low-dose group, 5.6 in the mid-dose group and 4.4 in the high-dose group.

MATING AND GESTATION DATA

Male reproductive performance

Copulation was confirmed for all F0 parental males, which were paired with females to generate F1 pups. Thus, the male mating index was 100% in all test groups.

Fertility was proven for most of the F0 parental males within the scheduled mating interval for F1 litter.

Three high-dose males (#. 82, 96 and 97) and one low-dose male (# 33) did not generate F1 pups. One low-dose male (# 46) did not generate F1 pups but implants in the uterus of low-dose female # 146. Thus, the male fertility index ranged between 88% (1600 ppm test group), 96% (300 ppm test group) and 100% (control and 800 ppm test group) without showing any relation to dosing. This reflects the normal range of biological variation inherent in the strain of rats used for this study. All respective values are within the range of the historical control data of the test facility.

Table 37: Reproductive data of F0 males administered Dimethomorph

Parental generation	F_0 males			
Dose [ppm]	0	300	800	1600
Males placed with females	24	25	25	25
Mated [n]	24	25	25	25
Male mating index [%]	100	100	100	100
did not mate [n]	0	0	0	0

with females pregnant [n]	24	24	25	22
Male fertility index [%]	100	96	100	88
without females pregnant [n]	0	1	0	2
without females pregnant [%]	0	4	0	8

Sperm analysis

Concerning the motility of the sperms and the incidence of abnormal sperms in the cauda epididymis as well as the sperm head counts in the testis and in the cauda epididymis of F0 males, no treatment-related effects were observed.

Table 38: Sperm parameters of F0 males administered Dimethomorph

Parental generation		**F_0 males**			
Dose	**[ppm]**	**0**	**300***	**800***	**1600**
Sperm count	[10^6 / g]				
Testis		120			122
Cauda epididymis		676			641
Normal sperm	[%]	93.5			92.9
Abnormal sperm	[%]	6.5			7.1
Sperm motility	[%]	85	87	85	86

*low and mid dose have not been evaluated.

Female reproductive performance

The female mating index calculated after the mating period for F1 litter was 100% in all test groups.

The mean duration until sperm was detected (GD 0) varied between 2.2 and 2.7 days without any relation to the doses applied.

All female rats delivered pups or had implants in utero with the following exception:

- High-dose females # 182, 196 and 197 (mated with males # 82, 96 and 97, respectively) did not become pregnant.
- Low-dose female # 133 (mated with male # 33) did not become pregnant.

The fertility index varied between 88% in 1600 ppm test group, 96% in 300 ppm test group and 100% in control and 800 ppm test group. These values reflect the normal range of biological variation inherent in the strain of rats used for this study (HCD range: 84-100%, 36 studies, year 2000-2012, strain: Wistar.

The mean duration of gestation values varied between 22.0 and 22.3 days in control, 300 and 800 ppm test groups without any relation to dosing. The mean duration of gestation was statistically significantly decreased in comparison to the concurrent control group in 1600 ppm test group ($p \leq 0.01$). The value (21.4

days) was marginally below the historical control range (21.5 to 22.3 days), while the concurrent control (22.3 days) was at the upper end of the historical control range.

The gestation index was 100% in the control, 800 and 1600 ppm test groups and 96% in 300 ppm test group. These values reflect the normal range of biological variation inherent in the strain of rats used for this study. All respective values are within the range of the historical control data of the test facility (91 - 100%).

Implantation was not affected by the treatment since the mean number of implantation sites was comparable between all test substance-treated groups and the controls, taking normal biological variation into account (12.1 / 11.5 / 12.2 and 11.7 implants/dam in control, 300, 800 and 1600 ppm groups, respectively). Furthermore, there were no indications for test substance-induced intrauterine embryo-/fetolethality since the post-implantation loss did not show any significant differences between the groups, and the mean number of F1 pups delivered per dam remained unaffected (11.4 / 11.4 / 11.7 and 11.1 pups/dam in control, 300, 800 and 1600 ppm groups, respectively).
The rate of live-born pups was also not affected by the test substance, as indicated by live birth indices of 100% (high-, mid- and low-dose group) and 98.5% (control). Moreover, the number of stillborn pups was comparable between the groups.
Thus, Dimethomorph did not adversely affect reproduction and delivery of the F0 generation parental females.

Table 39: Summary of F0 female reproduction and delivery data

Parental generation		**F_0 females**			
Dose	**[ppm]**	**0**	**300 / 150**	**800 / 400**	**1600 / 800**
Animals per dose	[n]	25	25	25	25
Female fertility					
- placed with males	[n]	24	25	25	25
- mated	[n]	24	25	25	25
- mating index	[%]	100	100	100	100
- pregnant	[n]	24	24	25	22
- **Fertility index**	[%]	100	96	100	88
Mating days until Day 0 pc		2.2	2.7	2.2	2.4
Duration of gestation	[days]	22.3	22.2	22.0	**21.4****
Implantation sites, total	[n]	290	276	305	258
- per dam	[n]	12.1	11.5	12.2	11.7
Post implantation loss	[n]	14	22	11	15
- per dam	[n]	0.6	0.9	0.4	0.7

Parental generation	**F_0 females**			
Dose [ppm]	**0**	**300 / 150**	**800 / 400**	**1600 / 800**
- per litter [mean %]	4.7	8.0	3.6	5.7
Females with live-born	24	23	25	22
- **Gestation index** [%]	100	95.8	100	100
- with stillborn pups [n]	2	0	0	0
- with all stillborn [n]	0	0	0	0
Pups delivered [n]	273	263	293	245
- per dam [mean n]	11.4	11.4	11.7	11.1
- live-born [n]	269	263	293	245
- stillborn [n]	4	0	0	0
- **Live birth index** [%]	98.5	100	100	100

** = p<0.01; Dunnett test (two-sided)

LITTER AND PUP DATA (F1 GENERATION)

Survival

The mean number of delivered F1 pups per dam and the rates of live-born and stillborn F1 pups were evenly distributed about the groups. The respective values reflect the normal range of biological variation inherent in the strain used in this study.

The viability index indicating pup mortality during early lactation (PND 0-4) varied between 97.6% (1600 ppm), 98.7% (800 ppm), 99.1% (300 ppm) and 100% (control).
The lactation index indicating pup mortality on PND 4-21 varied between 98.9% (1600 ppm), 99.6% (800 ppm), 100% (300 ppm) and 99.5% (control) without showing any association with the treatment.
Thus, the test substance did not influence pre-weaning pup survival in any of the treated groups.

Table 40: Summary of litter data

Pup generation	**F_1**			
Dose [ppm]	**0**	**300 / 150**	**800 / 400**	**1600 / 800**
Number of litters	24	23	25	22
- with liveborn pups	24	23	25	22
- with stillborn pups	2	0	0	0
Pups live-born [n]	269	263	293	245
Pups stillborn [a] [n]	4	0	0	0
Pups died [n]	0	2	2	1

Pups cannibalized [n]	0	1	3	7
Pups culled day 4 [n]	77	76	91	67
Pups day 4 - pre-cull [n]	269	260	289	239
Viability index [%]	100	99.1	98.7	97.6
Pups day 4 - post cull [n]	192	184	198	172
Pups day 21 [n]	191	184	197	170
Lactation index [%]	99.5	100	99.6	98.9
Sex ratio [% live males]				
Day 0	53.8	50.5	50.5	46.1
Day 21	51.8	51.1	51.1	47.7
Male pup weight [g]				
PND 1 [g]	7.0	7.0	6.7	**6.2****
PND 4 [g]	10.8	10.3	10.3	**9.5****
PND 7 [g]	16.9	16.3	16.5	**15.3****
PND 14 [g]	34.0	32.8	33.7	**31.9****
PND 21 [g]	54.6	52.6	53.1	**49.5****
Male body weight gain				
PND 1 to 21 [g]	47.6	45.7	46.3	**43.3****
Female pup weight [g]				
PND 1 [g]	6.6	6.6	6.4	**5.8****
PND 4 [g]	10.2	10.0	10.0	**9.0****
PND 7 [g]	16.0	15.7	16.0	**14.6****
PND 14 [g]	32.7	32.0	32.9	30.9
PND 21 [g]	52.2	50.8	51.4	**47.8****
Female body weight gain				
PND 1 to 21 [g]	45.5	44.2	45.0	**42.0****

** = p<0.01; Dunnett test (two-sided)

Sex ratio

The sex distribution and sex ratios of live F1 pups on the day of birth and on PND 21 did not show substantial differences between the control and the test substance-treated groups; slight differences were regarded to be spontaneous in nature [seeTable 40].

Pup clinical observations

The F1 generation pups did not display any clinical signs until weaning.

Nipple/areola complex

The apparent number and percentage of male pups having areolae was not influenced by the test substance when examined on PND 12. Owing to the high background rate in control animals on this day, a recheck of all animals for nipples/areolae was performed on PND 20, one day prior to weaning. During the re-examination no areolae were detected in all male pups of all test groups [seeTable 41].

Table 41: Nipple development in male lactation pup

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Nipple development [%]								
Lactation male pup	N = 24		N = 23		N = 25		N = 22	
Day 12	95	10	95	13	95	13	99	4
Day 20	0	0	0	0	0	0	0	0

Body weight

At 1600 ppm statistically significantly lower mean pup body weights were noted on postnatal day 1 (about 13% below control). High-dose pup weights remained lower until weaning (about 9% below control at PND 21).

No test compound-related influence on F1 pup body weights was noted in the mid- and low-dose groups (800 and 300 ppm).

Mean body weight change of high-dose male and female pups was statistically significantly below the concurrent control towards the end of lactation (during PND 14–21, about 14% below control). This also had an impact on body weight change throughout lactation, which was decreased by 8% below control.

A very minor decrease of pup body weight change was also noted for the mid-dose pups during PND 14-21 (about 6% below control).

No test compound-related influence on F1 pup body weight change was noted in the low-dose group.

Anogenital distance/ anogenital index

Pup-based evaluation; Anogenital (AG) distance of male pups was statistically significantly reduced at all dose levels; 2, 6 and 10% below control, respectively. When corrected for body weight (AG index) the reduction was 2, 4 and 5%, respectively.

For the low-dose male offspring, the AG distance and index are well covered by the historical control range of the test facility. In addition the AG distance in the control group was at the max of the historical control range. Thus, these changes are considered to be incidental and not treatment-related. The AG distance of the mid- and high-dose males is below the range of historical control data. The AG index of the mid-dose males is in the lower range of historical control data, and of the high-dose males below the historical data range. Thus, these changes are considered to be treatment-related.

AG distance of all female treated pups was also statistically significantly decreased, by 2, 3 and 6%, respectively. When corrected for body weight (AG index) only the low and mid-dose pups were significantly below control; the reduction was 2% in both groups.

For the low-dose female offspring the AG distance is well covered by the historical control range of the test facility. Considering AG index, the apparent changes lack a dose-response and are all covered by the historical control range of the test. Thus, none of the apparent changes in females is considered to be treatment-related.

Litter-based evaluation: An additional evaluation of these data, based on litter data (as provided by the applicant upon a request by EFSA during the renewal application; see part B of the table below), showed that for males only the changes at the top dose are statistically significant and slightly outside the historical controls. For females, the analysis based on litter data confirmed that no treatment-related effects are noticed.

Table 42:

A. Anogenital distance and index of lactation pup on PND1 - pup based

Dose level [ppm]	**0**		**300**		**800**		**1600**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
PND 1								
male pup	N = 146		N = 129		N = 145		N = 112	
AG distance [mm]	3.15	0.19	**3.09***	0.23	**2.95****	0.19	**2.85****	0.25
historical control [mm]	2.99–3.15							
AG index	1.65	0.09	**1.62***	0.11	**1.58****	0.08	**1.57****	0.12
historical control	1.58–1.67							
female pup	N = 123		N = 131		N = 145		N = 131	
AG distance [mm]	1.53	0.13	**1.50***	0.08	**1.48****	0.11	**1.44****	0.12
historical control [mm]	1.48-1.67							
AG index	0.82	0.07	**0.80****	0.05	**0.80****	0.06	0.81	0.06
historical control	0.79-0.86							
* = p<0.05; ** = p<0.01; Dunnett test (two-sided)								

B. Anogenital distance and index op lactation pup on PND1 - litter based [#]

Dose level [ppm]	**0**		**300**		**800**		**1600**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
PND 1								
male pup	N = 24		N = 23		N = 25		N = 22	
AG distance [mm]	3.16	0.13	3.10	0.19	**2.99****	0.16	**2.87****	0.21
historical control [mm]	2.99–3.15 (mean 3.06, sd 0.04)							
AG index	1.65	0.04	1.62	0.09	**1.59****	0.05	**1.57****	0.09
historical control	1.58–1.67 (mean 1.62, sd 0.03)							
female pup	N = 24		N = 23		N = 25		N = 22	
AG distance [mm]	1.53	0.10	1.50	0.06	1.49	0.09	**1.45****	0.10
historical control [mm]	1.48-1.67 (mean 1.52, sd 0.04)							
AG index	0.82	0.05	0.80	0.03	0.80	0.05	0.81	0.05
historical control	0.79-0.86 (mean 0.82, sd 0.02)							

* = p<0.05; ** = p<0.01; Dunnett test (two-sided)
[#] additional analysis based on litter-data as provided by the applicant upon request by EFSA during the renewal application

Sexual maturation

Male and female F1 pups selected to become F1 rearing animals for Cohort 1A and Cohort 1B were examined for sexual maturation. Delay of puberty at 800 ppm (males) and 1600 ppm (both sexes) was observed.

Table 43: Sexual maturation of F1 pups

Sex & parameter	Females / Vaginal opening				Males / Preputial separation			
Dose [mg/kg]	**0**	**300/150**	**800/400**	**1600/800**	**0**	**300/150**	**800/400**	**1600/800**
pups examined	40	40	40	40	38	40	39	39
Days to criterion	31.4	31.9	32.0	**33.4****	42.0	41.8	**43.7****	**47.9****
Historical control range	30.0 - 32.1[#]				39.7 - 42.5[##]			
Body weight at criterion [g]	96.4	96.9	95.9	96.9	176.6	174.0	180.5	**199.5****
Historical control range	86.4 - 99.6[#]				156.5 - 181.0[##]			

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

additional historical control data (2010-2015) for vaginal opening as provided by the applicant upon request by EFSA during the renewal application: 29.5-31.9 days, 83.1-100.7 g

additional historical control data (2010-2015) for preputial separation as provided by the applicant upon request by EFSA during the renewal application: 40.5-45.2 days, 168.1-195.3 g

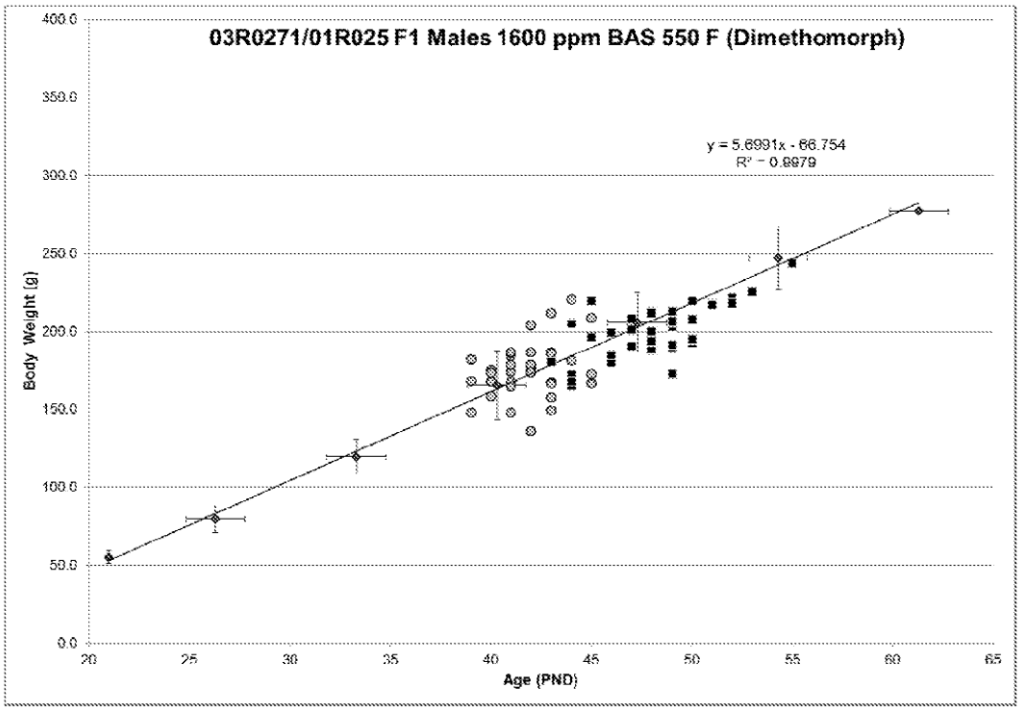
Vaginal opening

The first day when vaginal opening was observed was PND 27, the last was PND 37. There was a statistically significant increase in the high-dose group for the days to criterion value that exceeded the historical control range [seeTable 43]. In order to evaluate whether the day of vaginal opening might be seconday to alterations inbody weight, the individual results of body weight and age at vaginal opening were

compared to the mean body weight development of the control animals. This analysis showed that the delay in vaginal opening is due to a decrease in body weight (slowed general development) in this dose level. This is reflected in the mean body weight at vaginal opening which does not differ between the control and high dose females. The data indicate that the delay in vaginal opening reflects the general toxicity in the pups at this dose, rather than a specific mechanism.

Preputial separation

The first day when preputial separation was observed was PND 39, the last was PND 47. There was a statistically significant increase in the mid- and high-dose groups for the days to criterion value that exceeded the historical control range [see Table 43]. Additionally, a statistically significant increase of the body weight at criterion that exceeded the historical control range was observed in the high-dose group. In order to evaluate whether the day of preputial seperation might be seconday to alterations in body weight, the individual results of body weight and age at preputial separation were compared to the mean body weight development of the control animals. For the mid dose group the data indicated that the observed effect was due to a decrease in body weight (slowed general development). However, for the high dose level distribution of the male animals showed a slight shift to the right with respect to controls (see figure below), and a corresponding increase in body weight is observed in the older animals. This data indicate that for the high dose group a specific effect on preputial separation cannot be excluded.



F1 REARING ANIMALS

Observations

No clinical signs of toxicity, changes of general behaviour or other abnormalities, which may be attributed to the test substance, were detected in any of the male and female F1 rearing animals in any of the groups during daily observations as well as during the detailed clinical observations.

One high-dose Cohort B1 male (#468) showed a reddish encrusted right eye during in-life days 22-33, days 35-48 and days 51-59. This findings was not considered to be associated witht the test substance.

Mortality

There were no test substance-related mortalities in any of the Cohort 1A groups.
On control female (# 317) died accidentally on in-life day 50.

There were no test substance-related or spontaneous mortalities in any of the Cohort 1B groups.

Body weight and body weight gain

Body weights of 800 ppm and 1600 ppm Cohort 1A males were consistently slightly below the concurrent control throughout the in-life period (up to 8% and 10%, respectively). The difference gained statistical significance from day 28 and 14 onwards, respectively. No significant differences to the control were noted for 300 ppm Cohort 1A males [seeTable 44].

Body weights of all Cohort 1A females of all dose groups were basically comparable to the concurrent control values throughout the entire study period.

Body weights of all Cohort 1B males and females of all dose groups (300, 800, and 1600 ppm) were generally comparable to the concurrent control values throughout the entire study period.

Table 44: Body weight development of Cohort 1A males during in-life

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Body weight [g]								
Cohort 1A males								
in-life phase	N = 20		N = 20		N = 20		N = 20	
Day 0	77.7	8.3	74.8	7.8	78.0	8.7	74.1	10.0
Δ% (compared to control)			-3.7		0.3		-4.6	
Day 7	123.4	10.1	119.9	10.5	121.7	14.1	115.7	12.6
Δ% (compared to control)			-2.9		-1.4		-6.3	
Day 14	171.3	12.8	166.2	11.9	165.4	16.5	**159.6***	12.3
Δ% (compared to control)			-3.0		-3.4		-6.8	
Day 21	211.1	14.6	205.5	14.0	202.3	18.0	**196.3****	13.9
Δ% (compared to control)			-2.7		-4.2		-7.0	
Day 28	252.6	17.9	244.8	16.3	**238.6***	21.7	**232.2****	14.5
Δ% (compared to control)			-3.1		-5.5		-8.1	
Day 35	279.8	19.3	275.1	20.1	**263.4***	23.0	**260.4****	15.3
Δ% (compared to control)			-1.7		-5.9		-6.9	
Day 42	299.3	22.7	294.5	23.9	**282.0***	23.0	**276.0****	18.4
Δ% (compared to control)			-1.6		-5.8		-7.8	
Day 49	325.4	25.1	312.9	26.0	**298.9****	24.6	**293.7****	22.1
Δ% (compared to control)			-3.9		-8.1		-9.7	
Day 56	344.1	27.2	328.6	27.2	**316.9****	25.9	**309.2****	23.0
Δ% (compared to control)			-4.5		-7.9		-10.1	

* = p<0.05; * = p<0.01; Dunnett test (two-sided)
GD = gestation day

The body weight change of 800 ppm and 1600 ppm Cohort 1A males was statistically significantly below the concurrent control values during major parts of the in-life period (about 35% and 32%, respectively). The males of these groups generally gained 10 and 12% less weight than the control throughout in-life, respectively

The body weight change of the low-dose Cohort 1A males was statistically significantly below the concurrent control values just on one occasion (in-life days 42 – 49, about 30%). The males of this group generally gained 5% less weight than the control throughout in-life, however this difference was not statistically significant [seeTable 45].

The body weight change of 1600 ppm Cohort 1A females was statistically significantly below the concurrent control values during in-life days 0 - 14 and 21 - 28 (up to 17% and 29%, respectively). The females of this group generally gained 4% less weight than the control throughout in-life, however this difference was not statistically significant.

The body weight change of 800 ppm Cohort 1A females was statistically significantly below the concurrent control values during in-life days 14 - 21 and 49 - 56 (about 27% and 68%, respectively). The females of this group generally gained 8% less weight than the control throughout in-life, however this difference was not statistically significant.

The body weight change of the 300 ppm Cohort 1A females was comparable to the concurrent control values throughout the entire study period [see Table 45].

Body weight change of all Cohort 1B males and females of all dose groups (300, 800, and 1600 ppm) were generally comparable to the concurrent control values throughout the entire study period. Occasional statistically significant decreases or increases of body weight change were not considered to be treatment-related.

Table 45: Body weight change of Cohort 1A rearing F1 animals during in-life

Dose level [ppm]	**0**		**300**		**800**		**1600**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Body weight gain [g]								
Cohort1A males								
in-life phase	N =20		N =20		N =20		N =20	
Day 0-7	45.7	4.1	45.1	3.8	43.8	5.7	**41.6****	3.3
Δ%			-1.3		-4.2		-9.0	
Day 7-14	47.9	4.6	46.2	4.2	**43.7****	4.5	**43.9****	3.4
Δ%			-3.5		-8.8		-8.4	
Day 14-21	39.8	3.7	39.3	4.0	**36.9***	3.1	**36.7***	4.0
Δ%			-1.3		-7.3		-7.8	
Day 21-28	41.5	4.6	39.4	4.7	**36.3****	5.4	**35.9****	3.1
Δ%			-5.1		-12.5		-13.5	
Day 28-35	27.2	7.1	30.2	7.1	24.8	5.5	28.2	4.8
Δ%			11.0		-8.8		3.7	
Day 35-42	19.6	6.3	19.4	6.5	18.7	4.3	**15.5**	5.4
Δ%			-1.0		-4.6		-20.9	
Day 42-49	26.1	6.9	18.4**	5.0	**16.9****	4.7	**17.8****	5.5
Δ%			-29.5		-35.2		-31.8	
Day 49-56	18.7	4.8	15.7	4.3	**18.0**	3.9	15.5	4.1
Δ%			-16.0		-3.7		-17.1	
Day 0-56	266.4	23.1	253.8	25.8	**238.9****	20.7	**235.1****	18.0
Δ%			-4.7		-10.3		-11.7	
Cohort1A females								
gestation phase	N =20		N =20		N =20		N =20	
GD 0-7	34.0	2.9	33.5	3.0	31.8	3.7	**31.0***	3.0
Δ%			-1.5		-6.5		-8.8	

GD 7-14 Δ%	28.4	5.8	28.4 0.0	5.8	29.0 2.1	5.6	**33.2**** 16.9	6.9
GD14-21 Δ%	17.1	6.0	16.8 -1.8	3.7	**12.4*** -27.5	7.1	17.8 4.1	4.6
GD 21-28 Δ%	19.4	4.4	17.4 -10.3	4.8	18.4 -5.3	6.6	**13.8**** -28.9	4.8
Day 28-35 Δ%	11.5	5.6	10.3 -10.4	6.9	11.4 -0.9	4.6	11.9 3.5	3.8
Day 35-42 Δ%	10.6	5.7	10.7 0.9	5.8	10.7 0.9	4.0	9.0 -15.1	3.7
Day 42-49 Δ%	11.0	7.1	9.7 -11.8	7.9	10.9 -0.9	5.8	8.0 -27.3	5.9
Day 49-56 Δ%	7.5	4.7	7.0 -6.7	6.0	**2.4*** -68.0	6.5	7.6 1.3	3.5
Day 0-56 Δ%	138.3	13.0	133.7 -3.3	15.0	127.0 -8.2	13.3	132.3 -4.3	17.6

Food consumption and substance intake

Food consumption of all treated Cohort 1A males was below the concurrent control values during in-life period, the difference to the control gained statistical significance during a number of study sections. The most consistent decrease was, however, noted towards the end of in-life. When summarized for the entire in-life period, the differences to the control were -5%, -9% and -7% in at the 300, 800 and 1600 ppm dose levels, respectively. However, as the reduction of food consumption did not correlate to body weight effects in the low-dose group, this was considered as a potentially treatment-related but non-adverse finding [seeTable 46].

Table 46: Food consumption of Cohort 1A rearing F1 males during in-life

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Food consumption [g/animal/day]								
Cohort 1A males								
in-life phase	N = 20		N = 20		N = 20		N = 20	
Day 0-7	14.8	2.1	13.1	0.8	13.1	1.4	12.8	0.7
Δ% (compared to control)			-12.1		-11.8		-13.8	
Day 7-14	18.9	0.5	18.2	0.4	**17.6***	0.8	**17.7***	0.3
Δ% (compared to control)			-3.3		-6.8		-6.0	
Day 14-21	19.9	0.7	19.3	0.4	**18.8***	0.3	19.3	0.5
Δ% (compared to control)			-2.6		-5.4		-2.8	
Day 21-28	21.5	0.8	20.9	0.7	**20.2***	0.9	20.4	0.3
Δ% (compared to control)			-2.7		-6.2		-5.2	
Day 28-35	22.1	0.9	21.5	1.1	**20.6***	0.7	20.9	0.2
Δ% (compared to control)			-2.8		-7.0		-5.8	
Day 35-42	21.9	1.1	21.1	1.7	20.1	0.8	20.6	0.4
Δ% (compared to control)			-4.0		-8.1		-6.2	
Day 42-49	22.9	1.1	21.2	1.1	**19.8****	0.5	**20.4****	0.6
Δ% (compared to control)			-7.1		-13.7		-10.7	
Day 49-56	22.3	0.3	**20.9***	0.9	**20.1****	0.6	**20.2****	0.6
Δ% (compared to control)			-6.4		-9.6		-9.5	
Day 0-56	20.6	0.3	**19.5***	0.7	**18.8****	0.7	**19.0****	0.3
Δ% (compared to control)			-5.1		-8.5		-7.4	
* = p<0.05; * = p<0.01; Dunnett test (two-sided)								

Food consumption of the Cohort 1A females in all treated groups was basically comparable to the concurrent control.

Food consumption of all Cohort 1B males and females of all dose groups was generally comparable to the concurrent control values throughout the entire study period.

For all test groups the intake of Dimethomorph correlated well with the desired target doses. The actual test substance intake was calculated on the basis of interpolated mean body weights of each test group. With regard to the target dose levels, the mean values and the minimum/maximum deviations of the actual test substance intake per time interval examined are shown in Table 47.

Table 47: Average Dimethomorph intake (mg/kg bw/day) in F1 rearing animals

Dose	300 ppm		800 ppm		1600 ppm	
Group & sex	average	min/max	average	min / max	average	min / max
F_1 males (in-life)						
Cohort 1A	28.1	19.5 / 40.2	73.7	52.4 / 104.7	153.8	107.2 / 216.5
Cohort 1B	27.8	19.2 / 38.8	75.8	53.1 / 106.8	151.7	108.8 / 208.1

Dose	300 ppm		800 ppm		1600 ppm	
Group & sex	average	min/max	average	min / max	average	min / max
F_1 females (in-life)						
Cohort 1A	28.0	22.3 / 38.2	73.4	59.0 / 98.0	159.3	135.2 / 203.2
Cohort 1B	28.7	23.6 / 38.3	74.1	60.7 / 97.6	158.3	137.8 / 199.2

Oestrous cycle data

Oestrous cycle data revealed regular cycles in the rearing F1 Cohort 1A females of all test groups including the control. The mean oestrous cycle duration in the different test groups was similar: 4.6 days in control, 5.0 days in the low-dose group, 5.4 days in the mid-dose group and 4.1 days in the high-dose groups.

Table 48: Oestrous cycle data of rearing F1 females

Dose level [ppm]	0		300		800		1600	
	mean	SD	mean	SD	mean	SD	mean	SD
Cohort 1A females								
	N = 20		N = 20		N = 20		N = 20	
No. cycles	2.35	0.99	2.15	0.88	2.20	0.77	2.40	0.82
Cycles length (days)	4.67	2.13	5.00	2.53	5.43	2.55	4.10	0.63
Cohort 1B females								
	N = 20		N = 20		N = 20		N = 20	
No. cycles	4.15	0.67	3.75	1.12	3.85	0.93	4.00	0.92
Cycles length (days)	4.11	0.44	5.38	3.13	4.75	1.65	4.59	1.25

* = $p<0.05$; * = $p<0.01$; Dunnett test (two-sided)

Oestrous cycle data revealed regular cycles also in the rearing F1 Cohort 1B females of all test groups including the control. The mean oestrous cycle duration in the different test groups was similar: 4.1 days in control, 5.4 days in the low-dose group, 4.8 days in the mid-dose group and 4.6 days in the high-dose groups.

Sperm analysis

Concerning the incidence of abnormal sperms in the cauda epididymidis as well as the sperm head counts in the testis and in the cauda epididymidis no treatment-related effects were observed.

At the end of the administration period in males of the 800 ppm test group, motility of the sperms was marginally reduced, but the effect was not dose-dependent. Therefore, this finding was regarded as incidental and not treatment-related.

Table 49: Sperm parameters of F1 rearing males Cohort 1A on Day 90

F_1 rearing males	Cohort 1A			
Dose [ppm]	0	300	800	1600

F_1 rearing males		**Cohort 1A**			
Dose	**[ppm]**	**0**	**300**	**800**	**1600**
Sperm count	[10^6 / g]				
Testis		118	-	-	127
Cauda epididymis		685	-	-	703
Normal sperm	[%]	93.2			91.9
Abnormal sperm	[%]	6.8	-	-	8.1
Sperm motility	[%]	87	86	**81***	85

Urinalysis

No treatment-related changes of urinalysis parameters were observed.

In females of the 300 ppm test group urine pH value and urine volume (not statistically significant) were higher compared to controls and urine specific gravity was decreased. All mentioned parameters were not dose-dependently changed and therefore the alterations were regarded as incidental and not treatment-related.

Table 50: Urinalyses parameters of F0 parental females

Parameter	**Dose level [ppm]**							
	0		**300**		**800**		**1600**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
pH value	5.4	0.5	**6.3****	0.5	5.8	0.8	5.6	0.7
Vol. [mL]	3.6	1.2	4.8	1.8	4.3	2.8	4.1	1.7
Spec. gravity [g/L]	1069	24	**1045****	8	1062	21	1063	19

* = $p<0.05$; * = $p<0.01$

CLINICAL PATHOLOGY

Clinical pathology parameters in F0 parental animals

Hematology

At the end of the administration period in males of 800 and 1600 ppm test groups, relative neutrophil cell counts were lower and relative lymphocyte cell counts were higher compared to controls without any change of the total white blood cell counts.

In males of the 800 ppm test group, relative monocyte counts were decreased and in males of the 300 ppm test platelet counts were increased, but both parameter were not dose dependently changed and therefore the alterations were regarded as incidental and not treatment-related.

Table 51: Haematology parameters of F0 parental animals

Parameter	**sex**	**specification**	**Dose level [ppm]**

			0		300		800		1600	
			mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
WBC	♂	absolute	5.98	1.09	6.03	1.42	5.96	1.57	6.30	0.87
[giga/L]	♀	absolute	3.25	0.36	**3.98***	0.66	3.75	1.07	**5.08****	1.20
NEUT	♂	absolute	1.42	0.56	1.11	0.32	0.96	0.27	1.02	0.18
[giga/L]		relative	23.4	7.0	18.6	4.2	**16.4***	3.7	**16.3****	3.0
	HC	relative	15.0 - 25.4							
	♀	absolute	0.60	0.18	**0.76***	0.18	0.67	0.27	**0.92***	0.28
		relative	18.5	5.2	19.3	4.2	18.0	5.0	18.5	6.2
	HC	absolute	0.37 - 0.93							
LYMPH	♂	absolute	4.26	0.84	4.62	1.17	4.75	1.30	5.00	0.79
[giga/L]		relative	71.5	7.1	76.5	4.5	**79.5****	3.7	**79.2****	3.3
	HC	relative	69.8 - 81.2							
	♀	absolute	2.37	0.34	2.88	0.57	2.77	0.88	**3.83****	1.13
		relative	72.8	5.3	72.0	4.6	73.8	4.5	74.8	6.0
MONO	♂	absolute	0.14	0.03	0.14	0.05	0.10	0.05	0.12	0.04
[giga/L]		relative	2.3	0.4	2.4	0.8	**1.6****	0.4	1.9	0.5
	♀	absolute	0.09	0.02	0.10	0.04	0.09	0.04	0.12	0.04
		relative	2.7	0.7	2.5	1.1	2.5	0.8	2.4	0.8
PLT	♂	absolute	728	43	**836****	82	768	62	770	93
[giga/L]	♀	absolute	795	123	794	91	802	196	876	154

* = p<0.05; * = p<0.01

HC = historical control data

At the end of the administration period in females of the 1600 ppm test group, total white blood cell (WBC) counts and absolute lymphocyte and neutrophil cell counts were increased. The neutrophil cell counts were within the historical control range and therefore this change was regarded as incidental and not treatment-related.

In females of the 300 ppm test group total white blood cell (WBC) and absolute neutrophil cell counts were higher and relative basophil cell counts were lower compared to controls, but the changes were not dose-dependent and therefore these alterations were regarded as incidental and not treatment-related.

Clinical chemistry

No treatment-related, adverse changes of clinical chemistry parameters were observed.

At the end of the administration period in females of the 1600 ppm test group, total bilirubin values were decreased and calcium levels were increased. Without any signs of anaemia, a decrease of total bilirubin levels was most probably due to an increased conjugation rate and a subsequent accelerated excretion of bilirubin via the bile. This mechanism is regarded as adaptive and not adverse. The calcium mean of females in the 1600 ppm test group was within the historical control range and therefore, this alteration was regarded as incidental and not treatment-related.

Table 52: Clinical chemistry parameters of F0 parental females

Parameter	Dose level [ppm]

	0		300		800		1600	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Bilirubin (total) [µmol/L]	2.18	0.37	2.07	0.26	1.94	0.55	**1.63****	0.29
Calcium [mmol/L]	2.51	0.06	2.51	0.07	2.51	0.07	**2.59****	0.04
	HC: 2.50 - 2.74							

* = p<0.05; * = p<0.01

HC = historical control data

Clinical pathology parameters in F1 rearing animals

At the end of the administration period in males and females of the 1600 ppm test group, WBC counts and absolute lymphocyte counts were increased.

Additionally, in males of the mentioned test group relative lymphocyte counts were increased and relative neutrophil and eosinophil cell counts were decreased.

Table 53*: Haematology parameters of F1 Cohort 1A animals*

Parameter	sex	specification	Dose level [ppm]							
			0		300		800		1600	
			mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
WBC	♂	absolute	5.83	0.99	6.00	1.11	6.21	1.21	**7.16****	1.03
[giga/L]	♀	absolute	4.53	0.96	4.35	0.72	4.71	0.80	**6.75****	1.64
NEUT	♂	absolute	1.14	0.38	1.16	0.27	0.98	0.31	0.93	0.18
[giga/L]		relative	19.4	5.2	20.0	6.2	15.7	3.5	**13.2****	3.2
	HC	relative	15.0 - 25.4							
	♀	absolute	0.81	0.28	0.68	0.20	0.62	0.10	0.96	0.34
		relative	17.8	4.4	16.2	5.5	13.4	0.01	14.0	2.9
LYMPH	♂	absolute	4.42	0.78	4.56	1.09	4.97	0.97	**5.97****	1.10
[giga/L]		relative	75.9	5.2	75.4	6.4	80.1	4.2	**83.0****	3.7
	HC	relative	69.8 - 81.2							
	♀	absolute	3.50	0.73	3.44	0.69	3.88	0.76	**5.54****	1.31
		relative	77.5	4.8	78.9	5.0	82.2	2.8	82.3	2.9
EOS	♂	absolute	0.09	0.02	0.10	0.03	0.08	0.03	0.07	0.02
[giga/L]		relative	1.6	0.4	1.6	0.4	1.4	0.4	**1.0****	0.3
	♀	absolute	0.07	0.02	0.07	0.02	0.07	0.02	0.07	0.02
		relative	1.5	0.4	1.7	0.4	1.5	0.5	1.2	0.5
MCH	♂	absolute	1.06	0.04	1.05	0.04	1.07	0.04	1.04	0.03
[fmol]	♀	absolute	1.10	0.04	1.10	0.03	1.10	0.04	**1.06****	0.03
MCHC	♂	absolute	21.14	0.29	21.48	0.34	21.58	0.69	21.17	0.25
[mmol/L]	♀	absolute	21.59	0.37	21.51	0.36	21.21	0.35	**21.06****	0.37
HCT	♂	absolute	0.417	0.01	0.405	0.01	0.417	0.01	0.410	0.01
[L/L]	♀	absolute	0.392	0.01	0.394	0.01	**0.407***	0.01	0.394	0.01

* = p<0.05; * = p<0.01

HC = historical control data

In females of the 1600 ppm test group, mean corpuscular haemoglobin content (MCH) and mean corpuscular haemoglobin concentration (MCHC) were lower compared to controls. Only these calculated red blood cell indices were altered without any change of the measured red blood cell parameters (i.e. haematocrit and

haemoglobin values and red blood cell (RBC) counts. In females of the 800 ppm test group haematocrit levels were higher compared to controls, but the change was not dose-dependent. Therefore, the mentioned alterations were regarded as incidental and not treatment-related.

F1 rearing Cohort1A animals

At the end of the administration period in females of the 1600 ppm test group, alanine aminotransferase (ALT) activities were higher compared to controls and slightly above historical control levels. In males of the 1600 ppm test group urea and calcium levels were higher compared to controls and in females of this test group cholesterol and potassium levels were increased. Potassium levels were already higher in females of the 800 ppm test group. The values of the mentioned parameters were within historical control ranges. In males of the 800 ppm test group sodium levels were increased, but the changes were not dose-dependent. Therefore, the effect on sodium levels was regarded as incidental and not treatment-related.

Table 54: Clinical chemistry parameters of F1 Cohort 1A animals

Parameter	sex	Dose level [ppm]							
		0		300		800		1600	
		mean	SD	mean	SD	mean	SD	mean	SD
ALT	♂	0.70	0.07	0.75	0.09	0.76	0.10	0.76	0.10
[µkat/L]	♀	0.62	0.09	0.63	0.10	0.70	0.14	**0.83****	0.17
	HC	0.54 - 0.81							
Urea	♂	5.27	0.28	5.24	0.27	5.50	0.65	**6.06****	0.72
[mmol/L]	HC	4.91 - 7.42							
	♀	5.81	0.61	5.53	0.69	5.96	0.79	5.91	0.62
Chol	♂	1.76	0.17	1.98	0.29	1.73	0.26	1.84	0.27
[mmol/L]	♀	1.56	0.27	1.48	0.30	1.77	0.32	**1.84***	0.26
	HC	1.06 - 2.27							
K	♂	4.50	0.21	4.40	0.42	4.58	0.21	4.56	0.24
[mmol/L]	♀	4.08	0.29	4.19	0.30	**4.36***	0.28	**4.40***	0.24
	HC	3.76 - 4.40							
Ca	♂	2.52	0.03	2.48	0.04	2.55	0.05	**2.57***	0.05
[mmol/L]	HC	2.49 - 2.67							
	♀	2.58	0.06	2.58	0.08	2.63	0.05	2.64	0.06
	HC	2.50 - 2.74							

* = p<0.05; * = p<0.01

HC = historical control data

Urinalyses (F1 rearing Cohort1A animals)

No treatment-related changes among urinalysis parameters were observed.

In females of the 300 ppm test group specific gravity of the urine was decreased, but the mean was not dose-dependently changed. In males of the 300 and 1600 ppm test groups the incidences of crystals in the urine sediment were statistically higher compared to controls, but there was no relevant change of the crystals

when regarding the individuals. Therefore, the mentioned alterations were regarded as incidental and not treatment-related.

Table 55*:* ***Urinalyses parameters of F1 rearing Cohort 1A animals***

Parameter	sex	Dose level [ppm]							
		0		300		800		1600	
		mean	SD	mean	SD	mean	SD	mean	SD
F_0 parental animals									
Cryst.	♂	2	0	**2***	1	2	0	**2***	1
	♀	2	0	2	0	2	0	2	0
Spec. gravity [g/L]	♂	1052	16	1051	21	1054	20	1058	16
	♀	1056	25	1040	10	1064	30	1047	13

* = p<0.05; * = p<0.01

Hormone analysis in F0 and F1 animals

F0 parental animals

At the end of the administration period no treatment-related changes of TSH and T4 hormone levels were observed [see Table 55].

F1 offspring (PND 4)

At PND4, in male and female F1 pups no treatment-related change of TSH hormones was observed. T4 levels in females of the 300 and 800 ppm test groups were lower compared to controls, but this change was not dose-dependent and it was not accompanied by an increase of TSH levels. Therefore, the T4 level changes in the mentioned test groups were regarded as incidental and not treatment-related. T4 levels in males were not altered [seeTable 56].

F1 offspring (PND 22)

At PND22, in male and female F1 pups no treatment-related changes of TSH and T4 hormones were observed [see Table 55].

F1 rearing Cohort1A animals

At the end of the administration period no treatment-related changes of TSH and T4 hormone levels were observed.

Table 56*:* ***Evaluation of hormone levels after Dimethomorph treatment***

Parameter	sex	Dose level [ppm]							
		0		300		800		1600	
		mean	SD	mean	SD	mean	SD	mean	SD

Parameter	sex	Dose level [ppm]							
		0		300		800		1600	
		mean	SD	mean	SD	mean	SD	mean	SD
F_0 parental animals									
T_4	♂	73.01	11.80	80.86	14.76	77.44	10.33	80.33	8.37
[nmol/L]	♀	43.26	11.13	44.25	9.26	48.56	10.89	45.37	13.64
TSH	♂	8.01	1.93	9.59	3.41	8.43	2.31	9.10	2.84
[µg/L]	♀	6.94	2.11	5.90	0.79	6.60	1.48	7.29	1.21
F_1 pups (PND 4)									
T_4	♂	43.45	8.76	40.53	8.44	41.73	9.92	37.24	5.52
[nmol/L]	♀	46.73	8.96	**35.05***	10.78	**36.41***	7.04	39.68	9.10
TSH	♂	7.12	1.19	6.30	0.83	6.85	1.13	7.27	0.89
[µg/L]	♀	6.84	0.61	6.68	0.90	6.74	0.71	7.35	1.04
F_1 pups (PND 22)									
T_4	♂	88.30	12.12	83.77	15.40	85.14	13.05	85.14	11.17
[nmol/L]	♀	85.02	16.32	86.59	11.86	79.81	8.04	87.11	13.96
TSH	♂	5.22	0.96	4.36	0.78	4.85	1.05	4.61	1.35
[µg/L]	♀	4.38	0.81	4.25	0.49	4.83	1.01	4.88	0.94
F_1 rearing Cohort1A animals									
T_4	♂	104.46	12.12	96.84	16.36	103.33	14.17	102.95	17.02
[nmol/L]	♀	65.01	13.25	57.56	9.93	58.97	9.99	68.49	15.29
TSH	♂	9.90	3.36	11.09	2.42	12.81	4.29	8.92	2.86
[µg/L]	♀	7.65	2.71	6.57	2.50	7.09	2.55	8.77	2.76
LH [µg/L]	♂	2.04	0.62	2.83	1.01	1.76	0.78	2.38	1.45
Testo [nmol/L[	♂	15.38	8.47	19.49	13.01	10.15	4.85	14.69	8.44

* = p<0.05; * = p<0.01

Three days prior to sacrifice of the males, no treatment-related changes of LH and testosterone levels were observed.

PATHOLOGY

PATHOLOGY IN F0 PARENTAL ANIMALS

Organ weights

Organ weight determination in parental animals revealed a number of significant changes of absolute and/or relative organ weights [seeTable 57]. All other mean absolute or relative weight parameters did not show significant differences when compared to the control group.

Table 57*:* ***Organ weights of F0 male and female parental animals***

Generation	F_0 Males	F_0 Females

		Dose [ppm]	Absolute weight	%&	Relative weight [% of b.w.]	%&	Absolute weight [mg]	%&	Relative weight [% of b.w.]	%&
Terminal weight	[g]	0	379				232			
		300	374	(99)			228	(98)		
		800	369	(97)			229	(99)		
		1600	362	(96)			222	(96)		
Adrenal gland	[mg]	0	55.96		0.015		75.21		0.032	
		300	56.88	(99)	0.015	(103)	74.16	(99)	0.033	(100)
		800	55.88	(97)	0.015	(102)	78.76	(105)	0.034	(106)
		1600	54.20	(96)	0.015	(102)	76.40	(102)	**0.035***	(106)
Heart	[g]	0	1.054		0.278		0.908		0.392	
		300	1.069	(101)	0.286	(103)	**0.845***	(93)	**0.372****	(95)
		800	1.046	(99)	0.284	(102)	0.911	(100)	0.398	(101)
		1600	1.034	(98)	0.286	(103)	0.870	(96)	0.392	(100)
Kidneys	[g]	0	2.324		0.615		1.733		0.749	
		300	2.349	(101)	0.628	(102)	1.712	(99)	0.753	(101)
		800	2.314	(100)	0.629	(102)	**1.838****	(106)	**0.802****	(107)
		1600	2.404	(103)	**0.665****	(108)	1.728	(100)	**0.778****	(104)
Liver	[g]	0	8.144		2.152		6.317		2.729	
		300	8.277	(102)	2.206	(103)	6.307	(100)	2.773	(102)
		800	8.358	(103)	**2.268***	(105)	**7.046****	(112)	**3.069****	(112)
		1600	8.728	(107)	**2.405****	(112)	**6.906****	(109)	**3.103****	(114)
Pituitary gland	[mg]	0	11.12		0.003		12.79		0.006	
		300	11.44	(103)	0.003	(105)	13.12	(103)	0.006	(104)
		800	11.36	(102)	0.003	(106)	12.84	(100)	0.006	(101)
		1600	11.96	(108)	0.003	(114)	12.20	(95)	0.006	(99)
Prostate	[g]	0	1.001		0.267					
		300	0.970	(97)	0.261	(98)				
		800	0.952	(95)	0.259	(97)				
		1600	**0.877****	(88)	0.243	(91)				
Seminal vesicle	[g]	0	1.356		0.362					
		300	1.273	(94)	0.343	(95)				
		800	**1.221****	(90)	0.334	(92)				
		1600	**1.190****	(88)	0.330	(91)				
Spleen	[g]	0	0.590		0.156		0.486		0.209	
		300	0.606	(103)	0.163	(104)	0.468	(96)	0.205	(98)
		800	0.589	(100)	0.160	(103)	0.501	(103)	0.218	(104)

Generation		Dose [ppm]	F_0 Males Absolute weight	%&	Relative weight [% of b.w.]	%&	F_0 Females Absolute weight [mg]	%&	Relative weight [% of b.w.]	%&
		1600	0.596	(101)	0.165	(105)	**0.428***	(88)	**0.193***	(92)
Testes	[g]	0	3.759		0.998					
		300	3.794	(101)	1.025	(103)				
		800	3.897	(104)	1.063	(107)				
		1600	3.948	(105)	1.100	(110)				
Ovaries	[g]	0					119.1		0.051	
		300					**110.2***	(92)	**0.048***	(94)
		800					123.8	(104)	0.054	(105)
		1600					122.8	(103)	0.055	(107)
Thyroid	[mg]	0	23.96		0.006		18.96		0.008	
		300	25.20	(105)	0.007	(106)	20.28	(107)	0.009	(109)
		800	24.56	(103)	0.007	(105)	21.64	(114)	**0.009****	(115)
		1600	24.92	(104)	0.007	(108)	20.92	(110)	**0.009****	(115)
Thymus	[mg]	0	258.4		0.068	(100)	334.17		0.144	
		300	267.04	(103)	0.072	(105)	324.68	(116)	0.142	(99)
		800	298.76	(116)	**0.08***	(118)	301.8	(112)	0.132	(91)
		1600	303.24	(117)	**0.085****	(124)	**278.12****	(109)	**0.125****	(87)

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

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

The increased absolute and relative liver weights in females of test groups 02 (800 ppm) and 03 (1600 ppm) were considered to be treatment-related.

The relative liver weights were significantly increased in males of test groups 02 (5.39%) and 03 (11.7%). Because there were no treatment-related histopathological findings, the increased relative liver weights in males of test groups 02 and 03 were not considerd to be adverse.

Because there was no dose-response relationship, the decreased absolute and relative weights of heart and ovaries in females of test group 01 (300 ppm) as well as the increased kidney weights in females of test groups 02 and 03 were regarded to be incidental. These organs did not show treatment-related findings, histopathologically.

The increased relative adrenal weight in females and the increased relative kidney weights in males of test group 03 were related to the slightly, but not significantly decreased terminal body weight (-4% in both sexes) in this test group. There were no histopathological correlates for the weight increase.

The absolute weight of the prostate (0.877 g) was significantly decreased in test group 03. The prostate weight was within the range of historical control data (0.796 g - 1.228 g) and there was no histopathological correlate. In addition, the relative prostate weight did not show a significant decrease. Therefore, the decrease of prostate weight was related to the slightly but not significantly reduced terminal body weight (-4%) in this test group.

The absolute weights of the seminal vesicles were significantly decreased in test groups 02 (1.221g) and 03 (1.190g). These weights were within the range of historical control data (0.905g - 1.426 g) and there were no histopathological correlates. In addition, the relative weights of the seminal vesicle did not show a significant decrease. The decreased weights of the seminal vesicles were considered to be due to the slight but not significantly reduced terminal body weight in these test groups (test group 02: -3%; test group 03: - 4%) in the study report. However, it is noted that although the effect on relative seminal vesicles weight is not statistically significant and within historical control it was also observed in the 1A and 1B cohort groups (seeTable 61 and Table 63).

The absolute and relative spleen weights were decreased in females of test group 03. Because there were no histopathological findings, and there was no effect in F1 cohort 1A females, the weight decrease was considered to be incidental.

The thymus weight was increased in males of test groups 02 (relative) and 03 (absolute and relative). Because there were no histopathological correlates, these weight changes were not regarded as adverse. In females the absolute and relative thymus weights were decreased in test group 03. Because there were no histopathological correlates and there was no effect in F1 cohort 1A females, these weight changes were regarded to be incidental.

The relative weights of the thyroid glands were increased in females of test groups 02 and 03. The absolute weights were not significantly changed and there were no histopathological correlates. Therefore, the weight increases were considered to be incidental.

Gross lesions

All gross lesions occurred singularly or were similar in distribution pattern. All of them were considered to be spontaneous lesions in origin and were not related to treatment.

The female animals, which were not pregnant, as well as the male mating partners did not show gross lesions.

Histopathology

In the liver, a minimal or slight centrilobular hepatocellular hypertrophy was noted in 13 females of test group 03 (1600 ppm). Five females in this test group showed a minimal increase of apoptotic hepatocytes that was observed mostly in centrilobular areas. In addition, the severity of lymphoid infiltration was slightly

increased in females of test group 03. All of these findings were considered to be treatment-related. The incidence and severity is given in the table below:

Table 58: Histopathology results in the liver of female animals

Liver	**Female animals**			
Test group [ppm]	**00**	**01 [300]**	**02 [800]**	**03 [1600]**
Organs examined	24	25	25	25
Hypertrophy, centrilobular	0	0	0	13
• Grade 1				11
• Grade 2				2
Apoptosis, increased	0	0	0	5
• Grade 1				5
Infiltration, lymphoid	24	25	25	25
• Grade 1	23	18	21	12
• Grade 2	1	7	4	7
• Grade 3				6

All other findings noted were single observations either, or were similarly in distribution pattern and severity in control rats compared to treatment groups. All of them were considered to be incidental and spontaneous in origin and without any relation to treatment.

The female animals, which were not pregnant, as well as their male mating partners did not show histopathological findings explaining the infertility.

Female No. 101 (test group 00) had unscheduled mated with male No. 40 (test group 01). It was sacrificed prematurely and no further investigations were performed.

PATHOLOGY IN F1 PUPS

Organ weights

The terminal body weight was significantly decreased in males of the 300 ppm test group as well as in males and females of the 1600 ppm test group, resulting in statistical significant secondary weight changes in various organs: decreased absolute spleen weight and increased relative weights of brain and thymus in males of the 300 ppm test group, as well as decreased absolute weights of brain, spleen and thymus and increased relative brain weight in males and females of the 1600 ppm test group. The increased relative weights of brain and thymus in females of the 300 ppm test group was related to the slightly but not significantly reduced terminal body weight (-2%) in this test group.

Table 59: Organ weights of F1 pups on PND 22

Generation: F_1 pups		Males				Females			
Organ	Dose [mg/kg]	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%	Absolute weight [g]	Δ%	Relative weight [% of bw]	Δ%
Terminal body weight	0	56.63				54.67			
	300	**54.95***	-3			53.25	-2		
	800	55.71	-2			53.46	-2		
	1600	**52.13****	-8			**48.41****	-11		
Brain	0	1.57		2.78		1.5		2.77	
	300	1.56	0	**2.85***	3	1.52	1	**2.86***	3
	800	1.55	-1	2.79	0	1.49	0	2.80	1
	1600	**1.52****	-3	**2.94****	6	**1.47****	-2	**3.05****	10
Thymus[#]	0	255.81		0.45		262.08		0.48	
	300	269.93	6	**0.49****	9	274.59	5	**0.52***	8
	800	253.22	-1	0.45	0	262.37	0	0.49	2
	1600	**230.83****	-9	0.44	-2	**225.84****	-14	0.47	-3
Spleen	0	0.30		0.53		0.29		0.52	
	300	**0.28***	-6	0.51	-3	0.28	-2	0.52	0
	800	0.31	4	0.56	5	0.29	2	0.54	4
	1600	**0.27****	-10	0.52	-1	**0.25****	-14	0.51	-3

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

[#] thymus weight in mg

Values may not calculate exactly due to rounding of figures

Gross lesions

A few F1 pups showed spontaneous findings at necropsy, such as discoloured liver lobe (pale), post mortem autolysis, discoloured left testis (red), malpositioned left testis (abdominal region) and abnormal liver lobation. These findings occurred without any relation to dosing and/or can be found in the historical control data at comparable or even higher incidences.

Thus, all these findings were not considered to be associated with the test substance.

Table 60: Incidence of gross necropsy observations in F1 pups

Dose [ppm]	0	300 / 150	800 / 400	1600 / 800
	F_1 pups (males/females days 0 - 21)			
Litters evaluated	24	23	25	22
Pups evaluated (m/f)	50 /31	37 / 42	45 / 50	32 / 41

Dose [ppm]		**0**	**300 / 150**	**800 / 400**	**1600 / 800**
		F_1 pups (males/females days 0 - 21)			
Litters evaluated		24	23	25	22
Pups evaluated (m/f)		50 /31	37 / 42	45 / 50	32 / 41
General (m/f)	[N]	3/1	0/1	2/2	2/4
	[%]	6.0/3.2	0.0/2.4	4.4/4.0	6.2/9.8
Post mortem autolysis (m/f)	[N]	3/1	0/0	1/0	0/0
	[%]	6.0/3.2	0.0/0.0	2.2/0.0	0.0/0.0
Historical control range(m+f)	[%]	0.0 - 2.7			
abnormal liver lobation (f)	[N]	0	0	0	1
	[%]	0.0	0.0	0.0	2.4
Historical control range(m+f)	[%]	0.0 - 0.4			
not assessed (m/f)	[N]	0/0	0/1	1/2	2/4
	[%]	0.0/0.0	0.0/2.4	2.2/4.0	6.2/9.8
liver lobe discoloured (m/f)	[N]	1/0	0/0	0/0	0/1
	[%]	2.0/0.0	0.0/0.0	0.0/0.0	0.0/2.4
Testes (m)	[N]	0	1	0	0
	[%]	0.0	2.7	0.0	0.0
discoloured	[N]	0	1	0	0
	[%]	0.0	2.7	0.0	0.0
malpositioned	[N]	0	1	0	0
	[%]	0.0	2.7	0.0	0.0
Total pup necropsy observations					
animals with signs	[N]	4	1	2	2
affected pups	[%]	8.0	2.7	4.4	6.2
Historical control range(m+f)	[%]	0.0 - 3.7			

PATHOLOGY IN F1 REARING ANIMALS (COHORT 1A)

Some weight parameters were significantly changed in one or more dose groups and were listed in the following table.

Table 61*: Organ weights of cohort 1A animals*

Generation	**F_1 Males (cohort 1A)**				**F_1 Females (cohort 1A)**			
Dose [ppm]	**Absolute weight**	**%&**	**Relative weight [% of b.w.]**	**%&**	**Absolute weight [mg]**	**%&**	**Relative weight [% of b.w.]**	**%&**

Generation			F_1 Males (cohort 1A)				F_1 Females (cohort 1A)			
		Dose [ppm]	Absolute weight	%&	Relative weight [% of b.w.]	%&	Absolute weight [mg]	%&	Relative weight [% of b.w.]	%&
Terminal weight	[g]	0	333				201			
		300	320	(96)			195	(97)		
		800	**304****	(91)			193	(96)		
		1600	**297****	(89)			192	(96)		
Brain	[g]	0	2.033		0.614		1.832		0.948	
		300	2.044	(101)	0.644	(105)	1.893	(100)	0.976	(103)
		800	**1.970****	(97)	**0.651***	(106)	1.851	(98)	0.960	(101)
		1600	1.994	(98)	**0.647****	(110)	1.866	(99)	0.978	(103)
Heart	[g]	0	0.989		0.297		0.677		0.338	
		300	0.956	(97)	0.299	(101)	0.668	(99)	0.344	(102)
		800	**0.906****	(92)	0.298	(100)	0.662	(98)	0.343	(101)
		1600	**0.916****	(93)	0.307	(103)	0.696	(103)	**0.363****	(107)
Kidneys	[g]	0	2.190		0.659		1.457		0.727	
		300	2.124	(97)	0.666	(101)	1.411	(97)	0.726	(100)
		800	2.070	(95)	0.681	(103)	1.460	(100)	0.755	(104)
		1600	**2.035****	(93)	0.686	(104)	1.499	(103)	**0.781****	(107)
Liver	[g]	0	8.252		2.482		5.203		2.599	
		300	7.956	(96)	2.486	(100)	5.111	(98)	2.627	(101)
		800	7.598	(92)	2.496	(101)	**5.215**	(100)	2.697	(104)
		1600	8.093	(98)	**2.725****	(110)	**5.798****	(111)	**3.026****	(116)
Cauda epididymis	[g]	0	0.388		0.117					
		300	0.382	(99)	0.121	(103)				
		800	0.373	(96)	0.123	(106)				
		1600	**0.344****	(89)	0.116	(100)				
Epididymides	[g]	0	1.036		0.312					
		300	1.023	(99)	0.322	(103)				
		800	1.008	(97)	0.333	(107)				
		1600	**0.946****	(91)	0.320	(102)				
Prostate	[g]	0	0.752		0.227					
		300	**0.669***	(89)	0.212	(93)				
		800	**0.643****	(86)	0.213	(94)				
		1600	**0.576****	(77)	0.194	(85)				
Seminal vesicle	[g]	0	1.004		0.304					
		300	0.958	(95)	0.302	(99)				

Generation			F_1 Males (cohort 1A)				F_1 Females (cohort 1A)			
		Dose [ppm]	Absolute weight	%&	Relative weight [% of b.w.]	%&	Absolute weight [mg]	%&	Relative weight [% of b.w.]	%&
		800	**0.831****	(83)	0.275	(91)				
		1600	**0.708****	(71)	**0.239****	(79)				
Testes	[g]	0	3.637		1.096					
		300	3.564	(98)	1.123	(102)				
		800	3.766	(104)	**1.244****	(113)				
		1600	3.746	(103)	**1.269****	(116)				
Ovaries	[g]	0					91.84		0.046	
		300					97.95	(107)	**0.050***	(110)
		800					94.95	(103)	0.049	(107)
		1600					103.20	(112)	**0.054****	(117)
Thyroid	[mg]	0	21.05		0.006		16.42		0.008	
		300	21.35	(101)	0.007	(106)	17.15	(104)	0.009	(108)
		800	22.00	(105)	**0.007***	(114)	17.35	(106)	0.009	(109)
		1600	23.15	(110)	**0.008****	(123)	17.65	(107)	0.009	(112)
Thymus	[mg]	0	383.9		0.115		345.9		0.173	
		300	372.6	(97)	0.116	(101)	355.1	(103)	0.181	(105)
		800	385.7	(100)	0.126	(110)	343.9	(99)	0.178	(103)
		1600	400.5	(104)	**0.134****	(117)	342.6	(99)	0.179	(104)

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

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

The increased absolute and relative liver weights in females of test group 13 (1600 ppm) were considered to be treatment-related. The the increased relative heart and kidney weight in female animals of test group 13 were related to the slightly, but not significantly decreased terminal body weights (-4%/ -4%) in these test groups. In all of these organs, there were no treatment-related histopathological findings. Because there was no dose-response relationship and due to the lack of a histopathological correlate, the increased relative weights of the ovaries in females of test groups 11 (300 ppm) and 13 (1600 ppm) were regarded to be incidental.

The terminal body weight was significantly decreased in males of test groups 12 and 13 resulting in statistical significant secondary weight changes in various organs: decreased absolute weights of the cauda epididymis, the epididymides and the kidneys in test group 13, in a decreased absolute brain weight in test group 12 (800 ppm), in decreased absolute weights of the heart and the prostate, increased relative weights of

the thymus in test group 13, as well as increased relative weights of the brain, testes and thyroid glands in test groups 12 and 13. The increased relative weights of the liver in test group 13 was only slight and without histopathological findings and therefore not considered to be adverse. A decrease in seminal vesicle weight was observed in test groups 12 and 13. The study author contributed this effect to the significantly decrease in terminal body weight in males. However, since the decrease in seminal vesicle weight was both on absolute and relative weight at the high dose level and since the effect was outside of the historical control range of the F0 generation (0.905 g – 1.426 g, no HCD for F1 generation) the RMS considers the effect to be treatment related.

Gross lesions

All gross lesions occurred singularly. All of them were considered to be spontaneous lesions in origin and were not related to treatment.

Histopathology

A minimal centrilobular hepatocellular hypertrophy was noted in 15 females of test group 13 (1600 ppm). In addition, the severity of lymphoid infiltration was slightly increased in females of this test group. All of these findings were considered to be treatment-related. The incidence and severity is shown inTable 62:

Table 62: Histopathology results in the liver of female animals

Liver	**Female animals**			
Test group [ppm]	**00**	**01 [300]**	**02 [800]**	**03 [1600]**
Organs examined	20	20	20	20
Hypertrophy, centrilobular	0	0	0	15
• Grade 1				15
Infiltration, lymphoid	19	19	20	20
• Grade 1	16	18	17	12
• Grade 2	3	1	3	5
• Grade 3				3

All other findings noted were single observations either, or were similarly in distribution pattern and severity in control rats compared to treatment groups. All of them were considered to be incidental and spontaneous in origin and without any relation to treatment. One control female died intercurrently but was without any histopathological findings.

Differential ovarian follicle count

The results of the differential ovarian follicle count (DOFC) – comprising the numbers of primordial and growing follicles, as well as the combined incidence of primordial plus growing follicles – did not reveal significant deviations between controls and animals of test group 13 (1600 ppm).

PATHOLOGY IN F1 REARING ANIMALS (COHORT 1B)

Some weight parameters were significantly changed in one or more dose groups and were listed in the following table.

Table 63: Organ weights of cohort 1B animals

Generation			**F_1 Males (cohort 1B)**				**F_1 Females (cohort 1B)**			
		Dose [ppm]	**Absolute weight**	**%&**	**Relative weight [% of b.w.]**	**%&**	**Absolute weight [mg]**	**%&**	**Relative weight [% of b.w.]**	**%&**
Terminal weight	[g]	0	318				200			
		300	311	(98)			198	(99)		
		800	**309***	(97)			199	(99)		
		1600	309	(97)			191	(95)		
Prostate	[g]	0	0.737		0.232					
		300	**0.665***	(90)	0.217	(93)				
		800	**0.625****	(85)	**0.203****	(87)				
		1600	**0.542****	(74)	**0.176****	(76)				
Seminal vesicle	[g]	0	0.949		0.300					
		300	0.899	(95)	0.293	(98)				
		800	**0.812****	(86)	**0.265***	(88)				
		1600	**0.721****	(76)	**0.234****	(78)				
Testes	[g]	0	3.554		1.124					
		300	3.569	(100)	1.159	(103)				
		800	3.709	(104)	**1.203***	(107)				
		1600	**3.847****	(108)	**1.248****	(111)				

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

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

For all other organs mean relative weight parameters in males and all mean relative weight parameters in females did not show significant differences when compared to the control group 10. The decreased absolute and relative weights of prostate and seminal vesicle in test groups 12 (800 ppm) and 13 (1600 ppm) were considered to be treatment-related.

Because the relative prostate weight in males of test group 11 (300 ppm) did not show a significant change, the decreased absolute prostate weight was related to the slightly, but not significantly decreased terminal body weights (-2%) in this test group.
The testes weights were significantly increased in test groups 12 (relative) and 13 (absolute and relative). There was no comparable effect in F0 generation parental males. In Cohort 1A males, the relative testes weights were increased in test groups 02 and 03 but there was no histopathological correlate and the increased testes weights could be related to reduced terminal body weights since no effect on absolute testes weight was observed. Therefore, a treatment-related effect was considered to be unlikely.

Gross lesions
All gross lesions occurred singularly. All of them were considered to be spontaneous lesions in origin and were not related to treatment.

CONCLUSIONS
Thus, under the conditions of the present extended one-generation reproduction toxicity study the NOAEL (no observed adverse effect level) for general, systemic toxicity is 300 ppm, based on decreased food consumption and body weight/body weight gain, as well as clinical-chemical changes and pathological evidence of liver toxicity at 800 and/or 1600 ppm, in the F0 parental animals and adult F1 offspring.
The NOAEL for fertility and reproductive performance for the parental rats is 800 ppm, based on a significant reduction in gestation length at 1600 ppm.
The NOAEL for developmental toxicity in the F1 progeny is 300 ppm, due to the decrease in the pre-weaning pup body weights/pup weight gains, as well as decreased anogenital distance/index (pup-based) at 800 ppm.

3.10.1.3 Preliminary developmental toxicity study, rat – study 1

Study reference:
B.6.6.2.1 - Anonymous 1986: ZHT 236Z50 – Preliminary Oral (Gavage) Embryotoxicity Study in Rat, unpublished BASF Document No. DK-432-001

Detailed study summary and results:
Dimethomorph was administered by intragastric intubation to groups of eight sexually mature and mated female Spragure-Dawley rats at dose levels of 50, 120 or 300 mg/kg bw/day from Days 6 to 15 of gestation. In this preliminary range-finding study, the administration of dimethomorph by oral gavage at dose level up to 300 mg/kg bw/day, did not elicit maternal toxicity or embryolethality.

Test type

Guideline: Not specified in the report

Deviations: Guideline not specified

GLP: Yes (This laboratory certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Acceptability: The study is considered to be acceptable as supplementary study.

Test substance

Dimethomorph; ZTH 236Z50; Batch No. T2/85; purity 98.7 %

Test animals

Female Spragure-Dawley rats, 8/dose group

Administration/exposure

Dimethomorph was administered by intragastric intubation to groups of eight sexually mature and mated female Spragure-Dawley rats at dose levels of 50, 120 or 300 mg/kg bw/day for ten consecutive days from Days 6 to 15 of gestation. A control group of eight rats received distilled water with admixture of 0.1 % Tween 80 for the same period of time. The test substance was prepared daily as an aqueous suspension; separate preparations were made for each dose level. The dose volume was 10 mL/kg, adjusted daily on the basis of the individual body weights. All animals were examined twice daily for signs of ill-health, toxicity and behavioral changes; daily mortality checks were also performed. Body weights were recorded on Days 0, 6, 10, 15 and 20 of gestation.

On Day 20 of pregnancy, the animals were sacrificed and examined macroscopically for pathological changes. The ovaries and uteri were removed and examined and the following data recorded: number of corpora lutea, number and position of implantation (live fetuses, early intra-uterine deaths, early-late intra-uterine deaths and late intra-uterine deaths), individual fetal weights and sex of the fetuses. All fetuses were examined for external malformations.

Results and discussion

No treatment-related clinical changes or necropsy findings were observed with dimethomorph. Also, there were no treatment-related effects on maternal body weight gain, on pregnancy incidence, pre-implantation loss or post-implantation loss. No treatment-related effects on number, weight or sex of the fetuses. No incidence of external malformations in the fetuses was recorded.

3.10.1.4 Preliminary developmental toxicity study, rat – study 2

Study reference:

B.6.6.2.2 - Anonymous 1987: CME 151 – Letter Report: Treatment of Pregnant Sprague Dawley rats with CME 151; unpublished BASF Document No. DK-432-006

Detailed study summary and results:

Pregnant Sprague Dawley rats were treated with dimethomorph by oral gavage, during the period of organogenesis (day 6 to 15 of gestation, 150 or 300 mg/kg dimethomorph/day).No conclusions were made in or this letter report. However, the findings from this study were used in combination with other acute studies to select doses used in the definitive oral teratogenicity study in the rat.

Test type

Guideline: Not specified in the report

Deviations: Guideline not specified

GLP: No

Acceptability: The report is considered to be acceptable as supplementary information.

Test substance

Dimethomorph; CME151, Batch No. not specified; purity not specified

Test animals

Female Spragure-Dawley rats, 4/dose

Administration/exposure

Pregnant Sprague Dawley rats were treated with dimethomorph by oral gavage, in a constant dosing volume of 10 mL/kg, during the period of organogenesis (day 6 to 15 of gestation). Four pregnant rats received 150 mg/kg dimethomorph and four other pregnant rats received 300 mg/kg dimethomorph.

Details regarding maternal or foetal gross and histopathological examinations were not provided in this letter report.

Results and discussion

One of four females treated with 150 mg/kg/day showed 100 percent intra-uterine deaths. In addition, one to three early resorptions in each of the further three females receiving 150 mg/kg/day was observed. Two out of three females receiving 300 mg/kg/day showed 100 percent intra-uterine deaths. Pups from the other female receiving 300 mg/kg/day had reduced mean fetal weight.

3.10.1.5 Developmental toxicity study, rat

Study reference:

B.6.6.2.3. – Anonymous 1989, SAG 151: Oral (gavage) teratogenicity study in the rat, unpublished, BASF Document No. DK-432-002

Detailed study summary and results:

Based on the reductions in food consumption, body weights and body weight gain in the 160 mg/kg bw/day group, the NOAEL for maternal toxicity was 60 mg/kg bw/day. Based on embryotoxicity (total litter loss in two animals) observed at 160 mg/kg bw/day, the NOAEL for developmental toxicity was 60 mg/kg bw/day. Thus, embryotoxicity was not observed at dose levels which were not maternally toxic. Dimethomorph is not teratogenic in the Sprague-Dawley rat at doses up to 160 mg/kg bw/day.

Test type

Guideline: Directive 96/54/EC B 31; OECD Guideline 414; (US EPA Guideline 83-3 claimed by the author)

Deviations: None from original guideline. Deviations from current OECD Guideline 414:
- Body weights were not determined every 3 days during the dosing period.
- Food consumption was not determined at three-day intervals.

GLP: Yes (This laboratory certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz.)

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (SAG 151); Batch No. DW 11/86; purity 96.6 %

Test animals

Female Sprague-Dawley rats (30 females/group)

Administration/exposure

Four groups of mated female Sprague-Dawley rats (30 females/group) were used in this study. Dimethomorph was administered by gavage as a suspension in distilled water containing 0.1 % Tween 80. Animals were treated on gestation days 6 through 15 at dose levels of 0, 20, 60 and 160 mg/kg bw/day. All animals were examined twice daily for signs of ill health, toxicity, and behavioural change. Daily mortality checks were also performed. Body weight was determined on day 0, 6, 15, and 20 of gestation.Food consumption was recorded for days 0-6, 6-10, 10-15 and 15-20. On Day 20 post coitum, all females were sacrificed and assessed by gross pathology (including weight determinations of the unopened uterus and the placentae). For each dam, corpora lutea were counted and the number and distribution of implantation sites (differentiated as resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus, sexed, weighed and further investigated for any external findings. Thereafter, nearly one half of the

fetuses of each litter was examined for soft tissue findings and the remaining fetuses for skeletal (incl. cartilage) findings.

Results and discussion

The stability and homogeneity distribution of the test substance in the water, as well as the correctness of the concentration, were confirmed by analysis.

Only one death, resulting from gavage error, was noted in the 160 mg/kg bw/day group. All other animals survived to scheduled sacrifice and there were no treatment-related clinical signs or macroscopic pathology observed in any dose group. Statistically significant reductions in food consumption (gestation days 6-15) and body weights (gestation days 10, 15 and 20) were observed for females in the 160 mg/kg bw/day group as compared to controls. Body weight gain during the treatment period (gestation days 6-15) was reduced 28 % for females in the 160 mg/kg bw/day group. This overall reduction, relative to controls, in body weight gain for the high-dose group, reflects a marked decrease (non-statistically significant) in body weight gain (7.0 grams versus 21.8 grams for controls) during the first half of the treatment period (gestation days 6-10). Body weight gains for the second half of treatment (gestation days 10-15), as well as during the post-treatment period (gestation days 15-20) for the 160 mg/kg bw/day group were comparable to controls.

Table 64: Oral (gavage) teratogenicity study in the rat - Group mean daily food consumption (g/day) during gestation calculated from animals with live fetuses at necropsy

Food consumption (g/day)				
Group/dose (mg/kg bw/day)	**0**	**20**	**60**	**160**
Days of gestation 6 - 15	24.9 ± 1.9	24.5 ± 2.1	24.0 ± 1.8	**19.2 ± 2.9****

** = p < 0.01, analysis of covariance followed by Dunnett`s t-test

Table 65: Oral (gavage) teratogenicity study in the rat - Group mean body weight (g) during gestation calculated from animals with live fetuses at necropsy

Group mean body weight (g)				
Group/dose (mg/kg bw/day)	**0**	**20**	**60**	**160**
Days of gestation 6	237.6 ± 15.1 (235.8)	233.1 ± 18.3 (233.8)	233.8 ± 15.2 (235.4)	233.5 ± 20.5
10	259.4 ± 14.7 (258.0)	256.5 ± 16.1 (255.5)	253.1 ± 14.4 (254.8)	**240.5 ± 22.0 (241.4)****

15	289.4 ± 16.0 288.2)	285.0 ± 17.0 (284.2)	283.1 ± 16.4 (284.6)	**270.8 ± 23.8** **(271.5)****
20	356.2 ± 22.1 (354.9)	352.3 ± 20.1 (351.3)	346.2 ± 21.9 (347.8)	**335.8 ± 29.1** **(336.6)****

** = $p < 0.01$, analysis of covariance with body weight on day 0 post-coitum taken as covariant, followed by Dunnett`s t-test;

( ) = number in parentheses show adjusted means for ANOVA (group mean body weight on day 0 post-coitum taken as covariant)

Table 66: Oral (gavage) teratogenicity study in the rat - Group mean body weight gain (g) during gestation calculated from animals with live fetuses at necropsy

Group mean body weight gain (g)				
Group/dose (mg/kg bw/day)	**0**	**20**	**60**	**160**
Days of gestation 6 - 10	21.8 ± 5.8	22.2 ± 6.7	19.3 ± 6.6	7.0 ± 9.2
6 - 15	51.8 ± 8.8	50.6 ± 10.6	49.3 ± 9.4	37.3 ± 13.8

Pregnancy rate for the 20 mg/kg bw/day group was 67 % as compared to the control pregnancy rate of 83 %; this finding was considered incidental as pregnancy rates in the 60 and 160 mg/kg bw/day groups (73 % and 77 %, respectively) were comparable to controls, and treatment was not initiated until after implantation had occurred.

Table 67: Oral (gavage) teratogenicity study in the rat - test animals

Group/dose (mg/kg bw/day)	**0**	**20**	**60**	**160**
Number of inseminated females	30	30	30	30
Number of pregnant females	25	20	22	23
Percentage	83.3	66.7	73.3	76.7
Number of females with 100 % intra-uterine deaths	0	0	1	2

An increased number (non-statistically significant) of early resorptions was observed in the 160 mg/kg bw/day group as compared to controls. However, this increase reflects data from two females (Animals Nos.

80 and 83) in this group. The implantation data from Animal Nos. 80 and 83 indicated no viable fetuses for either female with a total of 15 and 14 early resorptions, respectively. All other ovarian, uterine and fetal observations were unaffected by treatment at dose levels up to and including 160 mg/kg bw/day.The two high dose dams with total litter loss (Nos. 80 and 83) had markedly reduced food consumption of below 50% compared to controls as well as body weight loss of -15 and -20g, respectively. This is likely the cause of the early resortions observed in these dams. No treatment-related malformations or variations were evident from the fetal external, visceral or skeletal examination data.

Table 68: Oral (gavage) teratogenicity study in the rat

Group/dose (mg/kg bw/day)	**0**	**20**	**60**	**160**
Early resorptions				
Total number	13	18	13	48
Mean number	0.5 ± 0.8	0.9 ± 1.1	0.6 ± 0.7	2.2 ± 4.1
% Post-implantation loss	3.9 ± 6.1	6.3 ± 7.2	8.8 ± 21.2	15.6 ± 28.6
Litter size	12.6	12.6	12.1	12.6
Foetal weight	3.40	3.45	3.46	3.34
Proportion of male fetuses	50.2	59.5	46.8	50.4
Total malformations	1	1	0	2*
- Micrognathia	0	1	0	0
- Aplasia of the tail	0	0	0	1
- Shortened body	0	0	0	1
- Anorchia on the right side	1	0	0	0
- Scoliosis	0	0	0	1

* One fetus had both aplasia of the tail and a shortened body.

3.10.1.6 Preliminary developmental toxicity study, rabbit

Study reference:

B.6.6.2.4 - Anonymous 1987: CME 151 – Preliminary Oral (Gavage) Embryotoxicity Study in the Rabbit; unpublished. BASF Document No. DK-432-003

Detailed study summary and results:

Based on the results of this study, the administration of dimethomorph by oral route at a dose level of 1000 mg/kg bw/day caused maternal toxicity (reduced food consumption and body weight gain) and

embryotoxicity (high rate of abortions and increased number of intra-uterine deaths). Due to the low number of fetuses, teratogenic potential could not be evaluated.
At a dose level of 600 mg/kg bw/day, maternal toxicity (reduced body weight gain) and embryotoxicity (reduced fetal weight) were observed, but no teratogenicity. The administration of 300 mg/kg bw/day elicited slight maternal toxicity (slightly reduced body weight gain), but no embryotoxicity and teratogenicity.
Thus, dose levels not exceeding 600 mg/kg bw/day were recommended for a subsequent teratology study.

Test type

Guideline: The author claimed that the study was conducted according to guideline No. 83-3 of the "U.S. EPA Pesticide Assessment Guidelines, Subdivision F (November 1982)"

Deviations: Not specified

GLP: Yes (This laboratory certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Acceptability: The report is considered to be acceptable as supplementary study.

Test substance

Dimethomorph; CME 151; ZTH 236Z50; purity 98.7 %

Test animals

Female New Zealand White rabbits, 8 or 9/group

Administration/exposure

Dimethomorph was administered by oral gavage to groups of eight sexually mature and mated female New Zealand White rabbits at dose levels of 300 or 600 mg/kg bw/day and to a group of nine female New Zealand White rabbits at 1000 mg/kg bw/day for 13 consecutive days from Days 6 to 18 post-coitum. A control group of eight rabbits received distilled water with admixture of 0.1 % Tween 80 for the same period of time. The test substance was prepared daily as an aqueous suspension; separate preparations were made for each dose level. Formulations were prepared under darkroom condition immediately prior to dosing and used within two hours. The dose volume was 10 mL/kg, adjusted daily on the basis of the individual body weights.
All animals were examined twice daily for signs of ill-health, toxicity and behavioral changes and mortality. Body weights were recorded on Days 0, 6, 12, 18, 24 and 28 post-coitum.
On Day 28 post-coitum, the animals were sacrificed and examined macroscopically for pathological changes. The ovaries and uteri were removed and examined and the following data recorded: number of corpora lutea, number and position of implantation (live fetuses, early resorptions, late resorptions and dead fetuses), individual fetal weights and sex of the fetuses. All fetuses were examined for external malformations.

Results and discussion

In Group 4 (1000 mg/kg bw/day), one animal was killed on Day 7 post-coitum because of the left hindlimb injury and this injury was considered to be incidental. However, six animals aborted their litters between Days 16 – 23; in three of these animals, necropsy revealed findings in the liver and/or spleen. Also, some clinical signs (e.g., reduced food and/or water consumption) during or after the treatment period were observed in all nine animals. Two animals were severely emaciated when they aborted their litters, one further animal showed 100 per cent intra-uterine deaths at necropsy. In Group 2 (300 mg/kg bw/day), one animal aborted its litter on Day 20 post-coitum.

A slight reduction in maternal body weight gain was noted in the 300 and 600 mg/kg bw /day treatment groups. There were no treatment-related effects on pregnancy incidence or implantations. In Group 4 (1000 mg/kg bw/day), only two animals were available for implantation evaluation; these values were comparable with the control group. Post-implantation loss was increased only in Group 4 (1000 mg/kg bw/day).

No treatment-related effects were observed in the number and sex of fetuses, mean fetal weights and fetal defects in Groups 2 and 3 (300 and 600 mg/kg bw/day, respectively). As only seven fetuses of one litter were available for evaluation in Group 4 (1000 mg/kg bw/day), no statistics on fetal parameters were performed.

3.10.1.7 Developmental toxicity study, rabbit

Study reference:

B.6.6.2.5 - Anonymous 1989, SAG 151: Oral (gavage) teratogenicity study in the rabbit, unpublished, BASF Document No. DK-432-004.

Detailed study summary and results:

Based on a slightly increased abortion rate and reductions in food consumption and body weight gain in the 650 mg/kg bw/day group, the NOAEL for maternal toxicity was 300 mg/kg bw/day. The NOAEL for developmental toxicity was 300 mg/kg bw/day based on a slightly increased embryolethality presenting as abortion. Dimethomorph is neither a developmental toxicant nor a teratogenic agent in the New Zealand White rabbit.

Test type

Guideline: Directive 96/54/EC B31, OECD Guideline 414, (US EPA Guideline 83-3 claimed by the author)

Deviations: None from original guideline. Deviations from current OECD guideline 414:

- Body weights were not determined every 3 days during the dosing period.
- Food consumption was not determined at three-day intervals.

GLP: Yes (This laboratory certified by Ministerium für Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz.)

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (SAG 151); Batch No. DW 11/86; purity 96.6 %

Test animals

Female New Zealand White rabbits, 22/dose group

Administration/exposure

Four groups of inseminated female New Zealand White rabbits (22 per dose group) were used in this study. Dimethomorph was administered by gavage as a suspension in distilled water containing 0.1 % Tween 80. Animals were treated on gestation days 6 through 18 at dose levels of 0, 135, 300 or 650 mg/kg bw/day. All female rabbits were examined at least twice daily for sings of ill health, toxicity and behavioural change. Daily mortality checks were alos performed. Body weight was recorded on day 0, 6, 16, 24, and 28 post-coitum. Foord consumption was evaluated for days 0 to 6, 6 to 12, 12 to 18, 18 to 24 and 24 to 28 post-coitum. On Day 29 post insemination, all surviving females were sacrificed and assessed by gross pathology (including weight determination of the unopened uterus and the placentae). For each dam, corpora lutea were counted and the number and distribution of implantation sites (differentiated as resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus, sexed, weighed and further investigated for any external, soft tissue and skeletal findings.

Results and discussion

The stability and homogeneity distribution of the test substance in the water, as well as the correctness of the concentration, were confirmed by analysis. In the definitive study, a few accidental deaths occurred as a result of gavage errors, which were noted in all groups. There were no mortalities that were attributed to the test material. A slightly increased abortion rate in the 650 mg/kg bw/day group (3 of 22 does versus 1 of 22 does in the control group) was attributed to the test material. There were no treatment-related clinical signs or macroscopic pathology findings observed in any dose group.

Table 69: Oral (gavage) teratogenicity study in the rabbit - Test animals

Group/dose (mg/kg bw/day)	0	135	300	650
Number of inseminated females	22	22	22	22
Number of pregnant females	20	17	18	20

Number of pregnant females which were found dead	1	0	2	4
Number of females which aborted and were killed	1	1	0	3
Number of females with 100 % intra-uterine deaths	1	2	0	1
Number of females with live fetuses at necropsy	17	14	16	12

Because of several individual body weight losses early in the treatment period (gestation days 6-12), mean body weight gains for the entire treatment period (gestation days 6-18) were reduced in the 650 mg/kg bw/day group as compared to controls but the differences were not statistically significant. Body weight gains during the post-treatment period (days 18-24) were slightly increased for the 650 mg/kg bw/day group as compared to controls. These data indicate that most animals in the high-dose group were able to compensate for the earlier weight losses once treatment was terminated. Statistically significant reductions in absolute (g/day) food consumption values during the treatment period were observed in the 650 mg/kg bw/day group when compared to controls.

Table 70: Oral (gavage) teratogenicity study in the rabbit - Group mean body weight (kg)

Group mean body weight (kg)				
Group/dose (mg/kg bw/day)	**0**	**135**	**300**	**650**
Day of gestation 0	3.5 ± 0.3	3.3 ± 0.2	3.4 ± 0.2	3.5 ± 0.3
6	3.8 ± 0.3 (3.8)	3.6 ± 0.2 (3.7)	3.7 ± 0.3 (3.7)	3.8 ± 0.2 (3.7)
12	4.0 ± 0.3 (3.9)	3.7 ± 0.2 (3.8)	3.7 ± 0.3 (3.8)	**3.8 ± 0.2 (3.7)****
18	4.1 ± 0.3 (4.0)	3.8 ± 0.3 (3.9)	3.9 ± 0.3 (3.9)	**3.8 ± 0.2 (3.7)****
24	4.2 ± 0.3 4.2)	3.9 ± 0.3 (4.0)	4.0 ± 0.3 (4.1)	**4.0 ± 0.2 (3.9)***
28	4.3 ± 0.3 (4.2)	4.0 ± 0.3 (4.1)	4.1 ± 0.3 (4.2)	**4.1 ± 0.3 (4.0)****

** = result of Dunnett`s t-test versus control group: $p < 0.01$; * = result of Dunnett`s t-test versus control group: $p < 0.05$; ( ) = number in parentheses show adjusted means for ANOVA (group mean body weight on day 0 post-coitum taken as covariant)

Table 71: Oral (gavage) teratogenicity study in the rabbit - Group mean body weight gain

Group mean body weight gain (kg)				
Group/dose (mg/kg bw/day)	**0**	**135**	**300**	**650**
Days of gestation 6 - 18	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.2

Table 72: Oral (gavage) teratogenicity study in the rabbit - Group mean daily food consumption (g/day)

Food consumption (g/day)				
Group/dose (mg/kg bw/day)	**0**	**135**	**300**	**650**
Days of gestation 6 - 18	192.7 ± 40.8 (186.9)	170.5 ± 33.3 (177.3)	167.4 ± 30.2 (170.2)	**134.8 ± 28.7 (132.1)****

** = $p < 0.01$, analysis of covariance followed by Dunnett`s t-test

( ) = number in parentheses show adjusted means for ANOVA (group mean body weight of the first day of examined interval taken as covariant)

Pregnancy rates were comparable in all groups. Ovarian, uterine and fetal observations were unaffected at all dose levels. No treatment-related malformations or variations were evident from the fetal external, visceral or skeletal examination data.

Table 73: Oral (gavage) teratogenicity study in the rabbit

Group/dose (mg/kg bw/day)	0	135	300	650
Post-implantation loss (%)	10.0	4.6	5.3	11.3
Litter size (#)	6.1	6.3	6.9	7.2
Foetal weight (g)	40.9	38.1	37.4	37.5
Litter weight g)	232.4	226.6	238.1	260.9
Proportion of male fetuses	46.0	50.2	58.6	50.4
Total Malformations (#)	4	1	4	4
- Aplasia of the tail	0	1	2	0
- Arthrogryposis	0	0	0	1
- Open eye	0	0	0	1
- Acephaly	0	0	0	1
- Ventriculi cerebri enlarged	3	0	1	2
- Fused ribs	0	1	0	0
- Fissured ribs	0	1	0	0
- Bifurcated ribs	0	0	1	0
- Scoliosis	1	1	1	0

3.10.2 Human data

3.10.3 Other data (e.g. studies on mechanism of action)

3.10.3.1 Studies on endocrine disruption – YAS-assay

Study reference:

Woitkowiak C., 2011. BAS 550 F (Dimethomorph) - Testing for potential androgenic and antiandrogenic activity using the YAS-assay (AR) (yeast androgen screening). 2011/1140605

Detailed study summary and results:

BAS 550 F (Dimethomorph; Batch: COD-001244; purity 99.8%) was tested to assess an androgenic and/or antiandrogenic activity by using the Yeast Androgen Screening Assay (YAS-Assay) with the hAR yeast strain. Two independent experiments were carried out. Vehicle (DMSO) and positive controls for androgenic effects (5α-dihydrotestosterone: 10 pM - 1 µM) and antiandrogenic effects (5α-dihydrotestosterone, 5 nM; hydroxyflutamide, 10 µM) were included into the experiment. BAS 550 F was tested at concentrations from 100 pM up to 100 µM. No precipitation and no cytotoxicity were observed up to the highest concentration tested. An increase in the androgen receptor dependent enzyme expression (color development) was not observed. A reproducible inhibition of the androgen effect compared to 5 nM 5α-dihydrotestosterone was observed at a concentration of 100 µM onward.

Under the experimental conditions of the study, BAS 550 F (Dimethomorph) did not exert androgenic effects while anti-androgenic effects were observed in the Yeast Androgen Screening (YAS) assay using the hAR yeast strain.

Test type

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 550 F (Dimethomorph)

Description: Solid, beige

Lot/Batch #: COD-001244

Purity: 99.8% (tolerance ± 1.0%)

Stability of test compound: Expiry date: 01.03.2015

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control: DMSO 1% (v/v)

Positive control compounds: Androgenic control: 5α-dihydrotestosterone Antiandrogenic control: 5α-dihydrotestosterone combined with hydroxyflutamide

3. Test organisms:

Yeast cells (Saccharomyces cerevisiae) have been stably transformed with a gene encoding the human androgen receptor (hAR), which is constitutively expressed. Additionally, these cells are stably transformed with a reporter gene plasmid, containing an androgen response element and the LacZ gene, which encodes the reporter enzyme β-galactosidase. The hAR yeast strain was obtained from "Technische Universität Dresden", Prof. Dr. G. Vollmer on 11 Feb 2010.

B. TEST PERFORMANCE:

1. Dates of experimental work:

23-May-2011 to 27-May-2011

2. Test substance preparation:

The test substance was weighed and topped up with the chosen vehicle to achieve the required concentration of the stock solution. The test substance was dissolved in DMSO. To achieve a clear solution of the test substance in the vehicle, the test substance preparation was shaken thoroughly. The further concentrations

were diluted according to the planned concentrations. All test substance formulations were prepared immediately before administration. The stability of the test substance in the vehicle DMSO and in water was not determined analytically.
Final test substance concentrations: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/L

3. Test method:

A deep-frozen (-80°C) yeast stock culture was thawed at room temperature, inoculated in growth medium and incubated for preculture (24-72 h) and growth medium was exchanged after 72 h before use. Of the preculture, optical density (OD) was determined at 690 nm. For preparation of the test culture, 0.50 mL of the preculture with an OD of 1.0 was transferred into 50 mL fresh culture medium including 0.5 mL chromogenic substrate CPRG (chlorophenol red-β-D-galactopyranoside).
The study was carried out in 96-well microtiter plates in which 2 µL of different test substance solutions had been pipetted. 200 µL of the test culture was added to each well. The plates was sealed with breathable tape and incubated until measurement of the OD.

4. Controls:

Each experiment includes a negative control (vehicle control) and positive controls for the verification of the detection of androgenic and antiandrogenic activity in the yeast cells.

Negative controls / Vehicle controls

The vehicle control contains 2 µL of the vehicle used for the test substance. The final concentration of the vehicle in the culture medium will be 1% (v/v).

Positive controls

Androgenic control:

5α-dihydrotestosterone (dissolved in ethanol); 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} mol/L

Antiandrogenic Control:

5α-dihydrotestosterone combined with hydroxyflutamide (dissolved in DMSO); $5x10^{-9}$ mol/L (5α-dihydrotestosterone)/ $1x10^{-5}$ mol/L (hydroxyflutamide)

The stability of the selected positive controls is well-defined under the chosen culture conditions since they are well established reference endocrine disruptors.

5. Evaluation/Assessment

5.1 Endocrine activity

After 48 h (±4 h) incubation, absorbance of the plates is measured at 570 nm (color development, androgen receptor dependent enzyme expression) and 690 nm (turbidity due to growth of the yeast). Evaluation is performed by calculating the difference of the measured ODs at the two wavelengths (absorption at 570 nm - absorption at 690 nm). For the 4 replicates per concentration the median, minimum and maximum value are presented in a diagram. The findings of the 1st experiment were confirmed in an additional assay. The concentrations and test conditions were the same as in the first investigation.

5.2 Cytotoxicity

Cytotoxicity of the test substance is indicated by decrease of the yeast growth (measurement of the turbidity at 690 nm). For the evaluation of antiandrogenic activity only nontoxic test substance concentrations are taken into consideration.

5.3 Acceptance criteria

Generally, the experiment is considered valid, if the following criteria are met:

- The positive controls induced an agonistic / antagonistic effect within the range of the historical control data.
- The concentration $5x10^{-9}$ mol/L 5α-dihydrotestosterone achieved at least 40 percent of the maximum effected androgen receptor dependent enzyme expression of the positive control (color development) based on the experiment.
- The vehicle control did not show color development at 570 nm.

5.4 Assessment criteria

Generally, cytotoxicity (decrease of the yeast growth) is considered for data interpretation especially in the case of antiandrogenic activity.

A test substance is generally considered non-androgenic in this assay, if:

- Androgen receptor dependent enzyme expression was within the historical negative control range under all experimental conditions in two experiments carried out independently.

The test substance is considered as androgenic in this assay, if:

- A concentration-dependent and reproducible increase of the androgen receptor dependent enzyme expression (color development) by at least 20% compared to the vehicle control was observed.
- If a concentration-dependent and reproducible increase of the androgen receptor dependent enzyme expression (color development) by at least 10% and less than 20% compared to the vehicle control was observed, the test substance is considered to be slightly androgenic.

The test substance is considered as antiandrogenic in this assay, if:

- A concentration-dependent and reproducible inhibition of the androgenic effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected color development, without signs for cytotoxicity) by at least 20% was observed compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone alone.
- If a concentration-dependent and reproducible inhibition of the androgenic effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected color development, without signs for cytotoxicity) by at least 10% but less than 20% compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone alone was observed, the test substance is considered to be slightly antiandrogenic.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

No analytical determination of the test substance solutions was performed. No test substance precipitation was found.

B. ENDOCRINE ACTIVITY

Androgenicity:

An increase in the androgen receptor dependent enzyme expression (color development) was not observed.

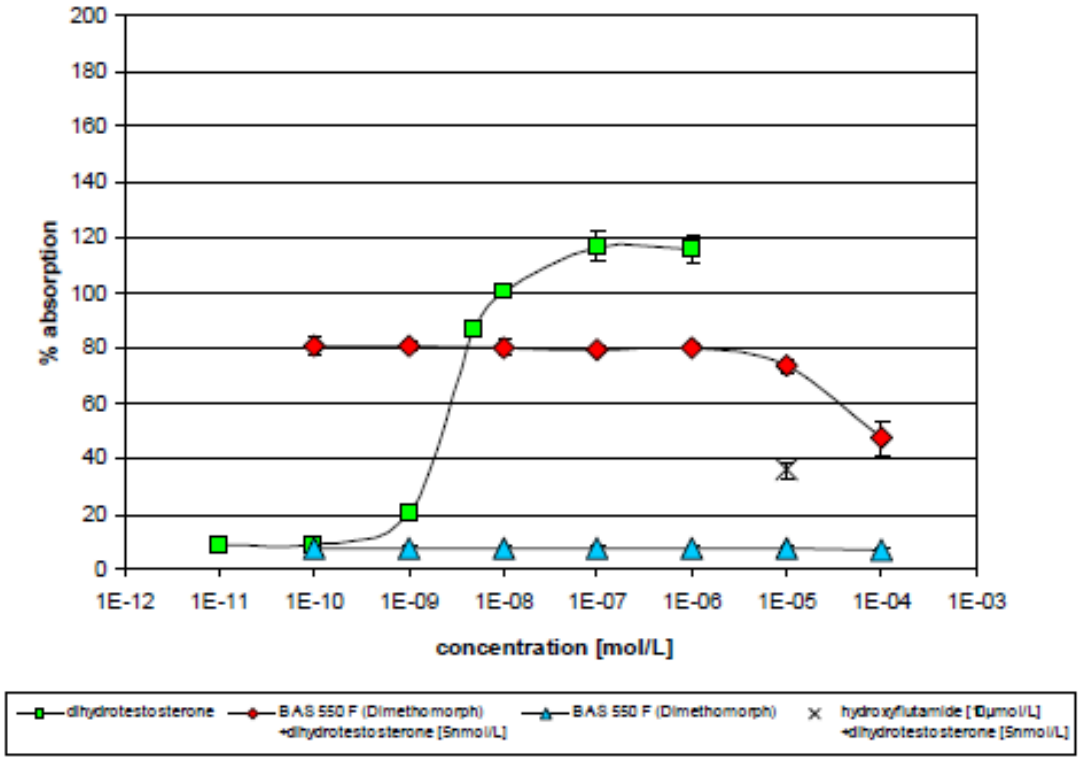
Antiandrogenicity:

A reproducible inhibition of the androgen effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected color development) was observed at a concentration of 10^{-4} mol/L. The inhibition was above 20% and therefore dimethomoprh is considered to antiandrogenic.

C. CYTOTOXICITY OF THE TEST SUBSTANCE

No cytotoxic effect (decrease of the yeast growth) was observed.

Figure 5: Androgen receptor-dependent enzyme expression; Normalized median (% absorption calculated relative to the absorbance values obtained for 10^{-8} M dihydrotestosterone).



III. CONCLUSIONS

Under the experimental conditions of the study, BAS 550 F (Dimethomorph) did not exert androgenic effects, while clear antiandrogenic effects were observed in the Yeast Androgen Screening (YAS) assay using the hAR yeast strain.

3.10.3.2 Studies on endocrine disruption – YES-assay

Study reference:

Woitkowiak C., 2011. BAS 550 F (Dimethomorph) - Testing for potential estrogenic and antiestrogenic activity using the YES-assay (ERalpha) (yeast estrogen screening). 2011/1140606

Detailed study summary and results:

BAS 550 F (Dimethomorph; Batch: COD-001244; purity 99.8%) was tested to assess an estrogenic and/or antiestrogenic activity by using the Yeast Estrogen Screening Assay (YES-Assay) with the hERα yeast strain. Two independent experiments were carried out. Vehicle (DMSO) and positive controls for estrogenic effects (17β-estradiol: 1 pM - 1 µM) and antiestrogenic effects (17β-estradiol, 1 nM; 4-hydroxytamoxifen, 1

µM) were included into the experiment. BAS 550 F was tested at concentrations from 100 pM up to 100 µM. No precipitation and no cytotoxicity were observed up to the highest concentration tested. An increase in the estrogen receptor dependent enzyme expression (color development) was not observed. Furthermore, no inhibition of the estrogen effect compared to 1 nM 17β-estradiol was observed.
Under the experimental conditions of the study, BAS 550 F (Dimethomorph) did not exert estrogenic or antiestrogenic effects in the Yeast Estrogen Screening (YES) assay using the hERα yeast strain.

Test type

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 550 F (Dimethomorph)

Description: Solid, beige
Lot/Batch #: COD-001244
Purity: 99.8% (tolerance ± 1.0%)
Stability of test compound: Expiry date: 01.03.2015
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control: DMSO 1% (v/v)
Positive control compounds: Estrogenic control: 17β-estradiol
Antiestrogenic control: 17β-estradiol combined with 4-hydroxytamoxifen

3.Test organisms:

Yeast cells (Saccharomyces cerevisiae) have been stably transformed with a gene encoding the human estrogen receptor α (hERα), which is constitutively expressed. Additionally, these cells are stably transformed with a reporter gene plasmid, containing an estrogen response element and the LacZ gene, which encodes the reporter enzyme β-galactosidase. The hERα yeast strain was obtained from "Technische Universität Dresden", Prof. Dr. G. Vollmer on 11 Feb 2010.

B. TEST PERFORMANCE:

1. Dates of experimental work:

23-May-2011 to 27-May-2011

2. Test substance preparation:

The test substance was weighed and topped up with the chosen vehicle to achieve the required concentration of the stock solution. The test substance was dissolved in DMSO. To achieve a clear solution of the test substance in the vehicle, the test substance preparation was shaken thoroughly. The further concentrations were diluted according to the planned concentrations. All test substance formulations were prepared immediately before administration. The stability of the test substance in the vehicle DMSO and in water was not determined analytically.

Final test substance concentrations: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/L

3. Test method:

A deep-frozen (-80°C) yeast stock culture was thawed at room temperature, inoculated in growth medium and incubated for preculture (24-72 h) and growth medium was exchanged after 72 h before use. Of the preculture, optical density (OD) was determined at 690 nm. For preparation of the test culture, 0.50 mL of the preculture with an OD of 1.0 was transferred into 50 mL fresh culture medium including 0.5 mL chromogenic substrate CPRG (chlorophenol red-β-D-galactopyranoside).

The study was carried out in 96-well microtiter plates in which 2 µL of different test substance solutions had been pipetted. 200 µL of the test culture was added to each well. The plates was sealed with breathable tape and incubated until measurement of the OD.

3. Controls:

Each experiment includes a negative control (vehicle control) and positive controls for the verification of the detection of estrogenic and antiestrogenic activity in the yeast cells.

Negative controls / Vehicle controls

The vehicle control contains 2 µL of the vehicle used for the test substance. The final concentration of the vehicle in the culture medium will be 1% (v/v).

Positive controls

Estrogenic control:

17β-estradiol (dissolved in ethanol)

Final concentrations: 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol/L

Antiestrogenic Control:

17β-estradiol combined with 4-hydroxytamoxifen (dissolved in DMSO)

Final concentrations:

$1x10^{-9}$ mol/L (17β-estradiol)/ $1x10^{-6}$ mol/L (4-hydroxytamoxifen)

The stability of the selected positive controls is well-defined under the chosen culture conditions since they are well established reference endocrine disruptors.

4. Evaluation/Assessment

4.1 Endocrine activity

After 48 h (±4 h) incubation, absorbance of the plates is measured at 570 nm (color development, estrogen receptor dependent enzyme expression) and 690 nm (turbidity due to growth of the yeast). Evaluation is performed by calculating the difference of the measured ODs at the two wavelengths (absorption at 570 nm - absorption at 690 nm). For the 4 replicates per concentration the median, minimum and maximum value are presented in a diagram. The findings of the 1st experiment were confirmed in an additional assay. The concentrations and test conditions were the same as in the first investigation.

4.2 Cytotoxicity

Cytotoxicity of the test substance is indicated by decrease of the yeast growth (measurement of the turbidity at 690 nm). For the evaluation of antiestrogenic activity only nontoxic test substance concentrations are taken into consideration.

4.2 Acceptance criteria

Generally, the experiment is considered valid, if the following criteria are met:

- The positive controls induced an agonistic / antagonistic effect within the range of the historical control data.
- The concentration $1x10^{-9}$ mol/L 17β-estradiol achieved at least 40 percent of the maximum effected androgen receptor dependent enzyme expression of the positive control (color development) based on the experiment.
- The vehicle control did not show color development at 570 nm.

4.3 Assessment criteria

Generally, cytotoxicity (decrease of the yeast growth) is considered for data interpretation especially in the case of antiestrogenic activity.

A test substance is generally considered non-estrogenic in this assay, if:

- Estrogen receptor dependent enzyme expression was within the historical negative control range under all experimental conditions in two experiments carried out independently.

The test substance is considered as estrogenic in this assay, if:

- A concentration-dependent and reproducible increase of the estrogen receptor dependent enzyme expression (color development) by at least 20% compared to the vehicle control was observed.
- If a concentration-dependent and reproducible increase of the estrogen receptor dependent enzyme expression (color development) by at least 10% and less than 20% compared to the vehicle control was observed, the test substance is considered to be slightly estrogenic.

The test substance is considered as antiestrogenic in this assay, if:

- A concentration-dependent and reproducible inhibition of the estrogenic effect compared to $1x10^{-9}$ mol/L 17β-estradiol (partly or total suppression of expected color development, without signs for cytotoxicity) by at least 20% was observed compared to $1x10^{-9}$ mol/L 17β-estradiol alone.
- If a concentration-dependent and reproducible inhibition of the estrogenic effect compared to $1x10^{-9}$ mol/L 17β-estradiol (partly or total suppression of expected color development, without signs for cytotoxicity) by at least 10% but less than 20% compared to $1x10^{-9}$ mol/L 17β-estradiol alone was observed, the test substance is considered to be slightly antiestrogenic.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

No analytical determination of the test substance solutions was performed. No test substance precipitation was found.

B. ENDOCRINE ACTIVITY

Estrogenicity:

An increase in the estrogen receptor dependent enzyme expression (color development) was not observed.

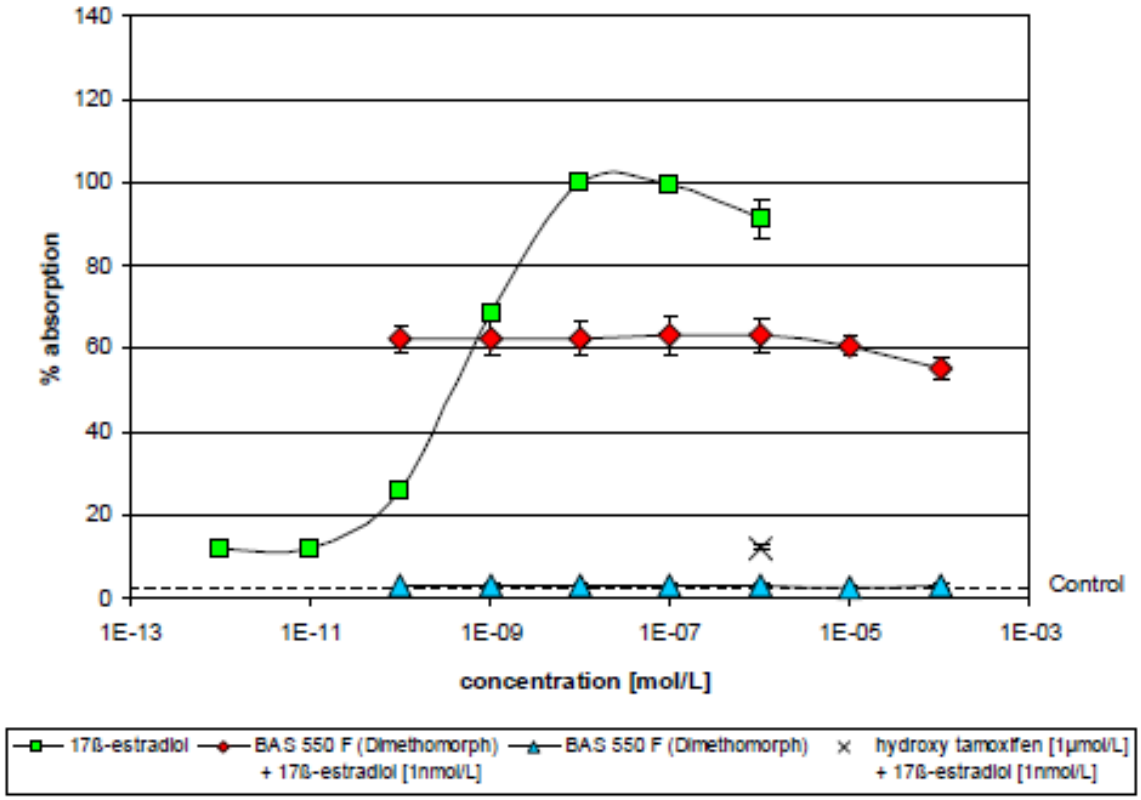
Antiestrogenicity:

A reproducible inhibition of the estrogen effect compared to $1x10^{-9}$ mol/L 17β-estradiol (partly or total suppression of expected color development) was not observed.

C. CYTOTOXICITY OF THE TEST SUBSTANCE

No cytotoxic effect (decrease of the yeast growth) was observed.

Figure 6: Estrogen receptor-dependent enzyme expression; Normalized median (% absorption calculated relative to the absorbance values obtained for 10-8 M 17β-estradiol)



III. CONCLUSIONS

Under the experimental conditions of the study, BAS 550 F (Dimethomorph) did not exert estrogenic or antiestrogenic effects in the Yeast Estrogen Screening (YES) assay using the hERα yeast strain.

3.10.3.3 Public literature information on endocrine disruption study 1

Study reference:

Bitsch N. et al, 2002. In vitro screening of the estrogenic activity of active components in pesticides (in German). Z. Umweltchem. Okotox. 14 (2) 76-84.

Study summary and results:

Abstract:

Over the last years, the obviously increasing, hormone-dependent impairments observed in human and animals, as well as the increased occurrence of hormone-dependent types of cancers, are sometimes associated with environmental chemicals which ae suspected to imitate or block the effects of natural hormones. For a variety of environmental chemicals an endocrine efficacy could already be demonstrated. Little is known, however, about a possible hormonal activity of plant protection agents which are at present certificated in the Federal Republic of Germany. The aim of the present study was the in vitro testing of at least 57 active ingredients of pesticides and growth regulators certificated in the Federal Republic of

Germany, for their possible estrogenic activity. The E-screen-Assay based on the human breast cancer cell line MCF-7 was used as the suitable test system. For at least 8 of the tested substances, an receptor-mediated estrogenic activity could be shown in vitro. With the exception of one substance, 7 active ingredients displayed a very weak affinity for the human estrogen receptor. Their ability to displace 17b-estradiol from the receptor was low. Only the herbicide pendimethalin is to be classified as a full estrogen receptor agonist, it is able to displace the 17b-estradiol almost completely from the receptor. However, the estrogenic potency of pendimethalin, in comparison to 17b-estradiol, was seen to be as small as the estrogenic potency of the other 7 substances tested positively in the E-Screen-Assay. Among these 7 substances, two could not be classified as estrogenically active anymore, because their affinity to the human estrogen receptor was too weak. The relevance of the available results for living organisms should be clarified in further in vivo investigations.

Analysis:

In this publication, dimethomorph was devoid of any estrogenic activity. The public literature study is in German with only the abstract translated to English. Therefore, no full reliability check could be carried out. Dimethomorph was not listed as one of the substances which resulted in a positive effect in the E-Screen Assay. Therefore, the public literature supports the results from the YES assay

3.10.3.4 Public literature information on endocrine disruption study 2

Study reference:

Orton F. et al, 2011. Widely used pesticides with previously unknown endocrine activity revealed as in vitro anti-androgens. Environ. Health Perspect., 109 (6), 794-800.

Study summary and results:

Abstract:

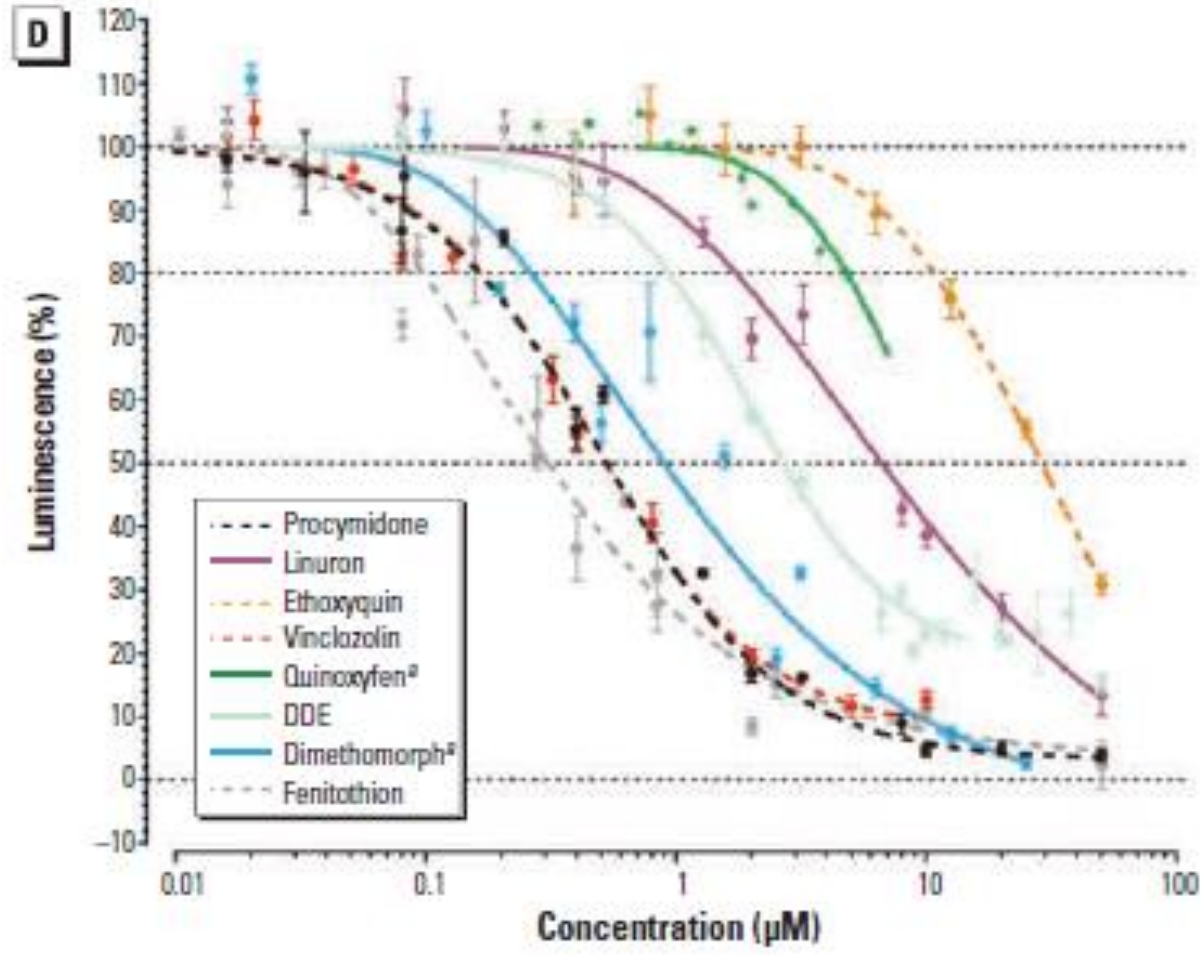
Evidence suggests that there is widespread decline in male reproductive health and anti-androgenic pollutants may play a significant role. There is also a clear disparity between pesticide exposure and endocrine disrupting data, with the majority of the published literature focused on pesticides that are no longer registered for use in developed countries. The aim of this study was to utilise estimated human exposure data to select pesticides to test for anti-androgenic activity, focusing on highest use pesticides. We used European databases to select 134 candidate pesticides based on highest exposure, followed by a filtering step according to known or predicted receptor mediated anti-androgenic potency, based on a previously published quantitative structure-activity relationship (QSAR) model. In total, 37 pesticides were tested for in vitro androgen receptor (AR) antagonism. Of these, 14 were previously reported to be AR antagonists (“active”), 4 were predicted AR antagonists using the QSAR, 6 were predicted to not be AR antagonists (“inactive”), and 13 with unknown activity, which were “out of domain” and therefore could not

be classified with the QSAR ("unknown"). All 14 pesticides with previous evidence of AR antagonism were confirmed as anti-androgenic in our assay and 9 previously untested pesticides were identified as anti-androgenic (dimethomorph, fenhexamid, quinoxyfen, cyprodinil, λ-cyhalothrin, pyrimethanil, fludioxonil, azinphos-methyl, pirimiphos-methyl). In addition, 7 compounds were classified as androgenic. Due to estimated anti-androgenic potency, current use, estimated exposure, and lack of previous data, we strongly recommend that dimethomorph, fludioxonil, fenhexamid, imazalil, ortho-phenylphenol and pirimiphos-methyl be tested for anti-androgenic effects in vivo. The lack of human biomonitoring data for environmentally relevant pesticides presents a barrier to current risk assessment of pesticides on humans.

Analysis:

The authors of this study performed an initial assessment of anti-androgenic properties using a QSAR model, the substances with positive predictions were tested in vitro in an assay in the stability transformed MDA-kb2 cell line. Substances demonstrating a positive response in the MDA-kb2 assay were additionally assessed using the Yeast Androgen Screen (YAS). The following results are presented for dimethomorph:

Figure 7: Results of the MDA-kb2 assay showing regression curves for antiandrogenic pesticides (A–D)



Values for luminescence were normalized to those of controls Regression lines end at the toxic threshold. Dashed lines indicate pesticides with lapsed registration, and solid lines indicate pesticides with current registration; data shown are mean ± SE.

a Newly described antiandrogens.

Table 74: Receptor mediated antiandrogenic activity and cytotoxicity in the MDA-kb2 and YAS assay

QSAR prediction	Anti-androgen IC20 (µM)		Cytotoxicty EC20 (µM)		Androgen IC20 (µM)
	MDA-kb2	YAS	MDA-kb2	YAS	MDA-kb2
Anti-androgen	0.263	38.5	> 25	> 50	Negative

The results of the YAS assay are confirmed by the study performed by BASF which also indicated that dimethomorph showed anti-androgenicity in the YAS.

3.10.3.5 Public literature information on endocrine disruption study 3

Study reference:

Reif D.M. et al, 2010. Endocrine profiling and prioritization of environmental chemicals using ToxCast data. Environ. Health Perspect., 118 (12), 1714-1720.

Study summary and results:

Abstract:

The prioritization of chemicals for toxicity testing is a primary goal of the U.S. Environmental Protection Agency (EPA) ToxCast program. Phase I of ToxCast used a battery of 467 in vitro, high-throughput screening assays to assess 309 environmental chemicals. One important mode of action leading to toxicity is endocrine disruption, and the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) has been charged with screening pesticide chemicals and environmental contaminants for their potential to affect the endocrine systems of humans and wildlife. The goal of this study was to develop a flexible method to facilitate the rational prioritization of chemicals for further evaluation and demonstrate its application as a candidate decision support tool for EDSP. Focusing on estrogen, androgen, and thyroid pathways, we defined putative endocrine profiles and derived a relative rank or score for the entire ToxCast library of 309 unique chemicals. Effects on other nuclear receptors and xenobiotic metabolizing enzymes were also considered, as were pertinent chemical descriptors and pathways relevant to endocrine-mediated signaling. Combining multiple data sources into an overall, weight-of-evidence Toxicological Priority Index (ToxPi) score for prioritizing further chemical testing resulted in more robust conclusions than any single data source taken alone. Incorporating data from in vitro assays, chemical descriptors, and biological pathways in this prioritization schema provided a flexible, comprehensive visualization and ranking of each chemical's potential endocrine activity. Importantly, ToxPi profiles provide a transparent visualization of the relative

contribution of all information sources to an overall priority ranking. The method developed here is readily adaptable to diverse chemical prioritization tasks.

Analysis:

In this publication used for screening of chemicals for prioritization for their potential endocrine effects, dimethomorph was cited in supplementary material. According to this data, no effects on estrogen or thyroid was identified and a potential effect on the androgen pathway reported. This publication is only considered as supplementary material as other studies have been performed to assess this issue.

3.10.3.6 Public literature information on endocrine disruption study 4

Study reference:

Orton F. et al, 2014. Mixture effects at very low doses with combination of anti-androgenic pesticides, antioxidants, industrial pollutant and chemicals used in personal care products. Tox. Appl. Pharmacol., 278, 201-208.

Study summary and results:

Abstract:

Many xenobiotics have been identified as in vitro androgen receptor (AR) antagonists, but information about their ability to produce combined effects at low concentration is missing. Such data can reveal whether joint effects at the receptor are induced at low levels and may support the prioritization of in vivo evaluations and provide orientations for the grouping of anti-androgens in cumulative risk assessment. Combinations of 30 AR antagonists from a wide range of sources and exposure routes (pesticides, antioxidants, parabens, UV-filters, synthetic musks, bisphenol-A, benzo(a)pyrene, perfluorooctane sulfonate and pentabromodiphenyl ether) were tested using a reporter gene assay (MDA-kb2). Chemicals were combined at three mixture ratios, equivalent to single components' effect concentrations that inhibit the action of dihydrotesterone by 1%, 10% or 20%. Concentration addition (CA) and independent action were used to calculate additivity expectations. We observed complete suppression of dihydrotestosterone effects when chemicals were combined at individual concentrations eliciting 1%, 10% or 20% AR antagonistic effect. Due to the large number of mixture components, the combined AR antagonistic effects occurred at very low concentrations of individual mixture components. CA slightly underestimated the combined effects at all mixture ratios. In conclusion, large numbers of AR antagonists from a wide variety of sources and exposure routes have the ability of acting together at the receptor to produce joint effects at very low concentrations. Significant mixture effects are observed when chemicals are combined at concentrations that individually do not induce observable AR antagonistic effects. Cumulative risk assessment for AR antagonists should apply grouping criteria based on effects where data are available, rather than on criteria of chem. similarity.

Analysis:

This publication is mainly focused on mixture effects. Dimethomorph is listed as a potential anti-androgen with an IC01 (produced 1% Androgen Receptor antagonistic effect) of 6.01 10^{-8} mole/L. These results are in line with assays already performed by the authors and also by BASF.

3.10.3.7 Public literature information on endocrine disruption study 5

Study reference:

Archer. E, et al, 2015. The potential anti-androgenic effect of agricultural pesticides used in the Western Cape: In vitro investigation of mixture effects. Water SA, 41 (1), 129-137.

Study summary and results:

Abstract:

Although it is known that environmental chemicals can affect the estrogenic system, far less attention has been paid to chemicals interacting with the androgen receptor (AR). Pesticides, particularly fungicides, have been shown to competitively bind or affect expression of the AR in an inhibiting manner. Few studies have addressed anti-androgenic effects of agrochemicals use in South Africa. The aim of this study was to screen for the ability of commonly-used pesticides (mostly fungicides) in Western Cape agricultural areas to alter the binding of an androgen (DHT) to the human AR (hAR) using a recombinant yeast androgen screen (YAS), and also to test the additivity mixture interaction hypothesis when commonly-used pesticides with similar modes of action (MOAs) are exposed in mixtures Fungicides vinclozolin, folpet, procymidone, dimethomorph, fenarimol, mancozeb, and the insecticide chlorpyrifos, all independently antagonized the binding of the androgen dihydrotestosterone (DHT) to the AR in a dose-dependent manner. The fungicide mancozeb was found to be the most potent anti-androgen in the assay. Binary, equimolar mixtures of the pesticides also antagonized the binding of DHT to the AR, but at lower IC50 concentrations potencies relative to their individual counterparts. The mixtures of the majority of the selected pesticides did not conform to the expected additive mixture interaction. Only the mixture between dimethomorph and mancozeb showed an additive mixture response at IC50 concentration, and, therefore, revealed a more severe AR antagonistic effect compared to their individual counterparts. This study confirmed that pesticides regularly used in agriculture inhibit the binding of androgens to the AR, but when in mixture do not always conform to the predictive addition mixture response model. Also, high relative potencies of individual chemicals in the assay were suppressed when combined with less potent chemicals, showing that the potent chemicals may not be granted access to bind with the AR when exposed in mixture.

Analysis:

In this publication, mixture effects of potential AR agonists were assessed. Individual values in the YAS assay were also reported. For dimethomorph, it was concluded that it was a weak AR agonist compared to

other substances with an IC50 of 0.38 mM (147.40 mg/L) and with a steep slope in the dose-dependent response indicating a small concentration range of AR agonistic activity. These results are in line with the YAS assay conducted by BASF where AR agonistic activity was seen at high doses with the same profile of the dose-response curve.

3.11 Specific target organ toxicity – single exposure

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Oral 28-day study in rats 1.

Study reference:

B.6.3.1.1 - Anonymous 1985, ZTH 236 Z50 Preliminary assessment of toxicity to rats by dietary admixture for four weeks, unpublished, BASF Document No. DK-420-005

Detailed study summary and results:

Test type

Guideline: Not specified in the report; however, the conduct of this study corresponds to EU Testing Method B7 and OECD 407.

Deviations: No microscopic examinations were performed. Deviations from current OECD 407 guideline (2008):
- reticulocytes and a measure of blood clotting time/potential not included.
- epididymides, prostate and thymus were not weighed.
- no histopathology was carried out.

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme).

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (ZTH 236 Z50); Batch No. L 5000; purity 99 %

Test animals

Male and female Sprague-Dawley rats (5/sex/group). At treatment initiation, animals were approximately 7-8 weeks of age, males weighed approximately 250 g and females weighed approximately 160 g.

Administration/exposure

Dimethomorph technical was fed to male and female Sprague-Dawley rats (5/sex/group) for 28 days at dietary concentrations of 0, 200, 1000 and 5000 ppm.

Food consumption and body weight were determined weekly. The state of health was checked each day. During the weekly weighing the animals were subjected to an additional comprehensive clinical examination. Clinical chemistry, haematological examinations and urinalysis were carried out towards the end of the administration period. The animals were subjected to gross-pathological assessment.

Results and discussion

The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The concentrations of the diets were analytically confirmed.

Table 75: Test substance intake

Dietary dose level	Test substance intake (mg/kg bw/day)	
(ppm)	Males	Females
200	15.8	17.5
1000	80.9	81.1
5000	305.9	283.3

Average test substance intake values for these concentrations were approximately 16, 80 and 300 mg/kg bw/day, respectively, for males, and 17, 80 and 280 mg/kg bw/day, respectively, for females based on food consumption data.

Clinical signs of toxicity were observed only in the 5000 ppm group and included soft feces, swollen abdomen, hunched posture, piloerection, emaciation, lethargy and unsteady gait. Due to the severity of these findings, two females and one male in the 5000 ppm group were sacrificed in a moribund condition and it was necessary to sacrifice the remaining animals in the 5000 ppm group following the completion of the clinical chemistry evaluations performed during week 4.

Statistically significant reductions in body weight gains were observed for males and females in the 5000 ppm group when compared to controls, with three males and three females exhibiting a net weight loss over the 28-day treatment period. These reductions in body weight gains correlated with reductions in food consumption noted for both sexes at 5000 ppm. No treatment-related effects on body weight or body weight gains were observed at 200 or 1000 ppm.

Table 76: 28-day oral toxicity study in rats - body weight gain

Male				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
Body weight gain (g)	176	157	157	**23****
Standard deviation	8.7	30.3	29.6	63.0
Female				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
Body weight gain (g)	51	60	57	**0.3****
Standard deviation	12.6	13.4	7.4	4.0

Level of significance (William`s test): * = 0.05 > p > 0.01 in comparison with control values

** = 0.01 > p values in comparison with control values

Table 77: 28-day oral toxicity study in rats – food consumption

Male				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
Body weight gain (g)	190	188	191	118
Standard deviation	14.4	16.6	21.0	25.2
Female				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
Body weight gain (g)	122	122	113	65
Standard deviation	11.9	8.7	9.9	8.1

Changes in hematology were observed for both sexes in the 5000 ppm group and included statistically significant increases in platelet and neutrophil counts. Total leukocyte counts were also increased for both sexes in the 5000 ppm group when compared to controls (reflecting the increases in neutrophil counts), but the differences were not statistically significant.

Table 78: 28-day oral toxicity study in rats – hematology

Male				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
platelet	1096	978	1044	**1422***
neutrophil	1.39	1.80	3.1	**4.56****
leukocyte	11.3	16.1	12.8	13.8
Female				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
platelet	996	1018	988	**1540****
neutrophil	1.41	1.80	1.07	**4.58****
leukocyte	8.5	10.5	8.6	12.0

Level of significance (William`s test): * = 0.05 > p > 0.01 in comparison with control values

** = 0.01 > p values in comparison with control values

Quantitative changes, relative to control values, in the concentration of plasma proteins were noted for males and females at the 5000 ppm level as indicated by a reduction in total albumin levels and a concomitant increase in globulin levels. Other clinical chemistry changes observed included a reduction in total plasma proteins for females at 5000 ppm, statistically significant increases in blood urea nitrogen (BUN) for both sexes at 5000 ppm and a slight but statistically significant increase in BUN for females at 1000 ppm. The increase in BUN observed for females at 1000 ppm was not considered adverse because no treatment-related renal effects, including BUN, were observed at dietary concentrations of 1000 ppm in the 13-week dietary toxicity study in rats or at dietary concentrations of 2000 ppm in two 24-month chronic feeding studies in rats.

Table 79: 28-day oral toxicity study in rats – clinical chemistry

Male				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
total plasma proteins	6.4	6.5	6.6	6.4
albumin	3.7	3.6	3.7	3.2
globulin	2.8	2.9	2.9	3.2
blood urea nitrogen	14	20	15	**28****
Female				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
total plasma proteins	7.1	7.1	7.0	**6.5***
albumin	4.3	4.2	4.1	**3.2****
globulin	2.8	2.8	2.9	**3.3****
blood urea nitrogen	16	15	**27***	**32****

Level of significance (William`s test): * = 0.05 > p > 0.01 in comparison with control values

** = 0.01 > p values in comparison with control values

Urinalyses data indicated that both males and females in the 5000 ppm group voided an increased volume of urine, when compared to control data, but the differences were only statistically significant for females.

Table 80: 28-day oral toxicity study in rats – urinalysis

Male				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
Volume	7.1	5.6	9.8	10.0
Female				
Group/dose (ppm)	**Control**	**200**	**1000**	**5000**
Volume	2.1	3.1	2.1	**10.0****

Level of significance (William`s test): * = 0.05 > p > 0.01 in comparison with control values

** = 0.01 > p values in comparison with control values

Absolute liver weights for both sexes in all treatment groups were comparable to controls, but increased liver to body weight ratios were observed for males (non-statistically significant) and females (statistically significant) in the 5000 ppm group when compared to controls. No other organ weight changes were observed. No microscopic examinations were performed in this study.

Table 81: 28-day oral toxicity study in rats - relative mean liver weights (g)

Males (5 animals/group)				
Group/dose (ppm)	**control**	**200**	**1000**	**5000**
Mean	15.6	16.8	17.6	19.5
Females (5 animals/group)				
Group/dose (ppm)	**control**	**200**	**1000**	**5000**
Mean	10.2	9.5	10.4	**14.6***

Level of significance (William`s test): * = 0.05 > p > 0.01 in comparison with control values
** = 0.01 > p values in comparison with control values; values adjusted using body weight of covariate

Conclusion:

The NOAEL for this study was 1000 ppm, based on increased moribundity and clinical signs of toxicity, reductions in body weight gains (or weight losses) and in food consumption, increased liver to body weight ratios and increased blood urea nitrogen for both sexes at 5000 ppm. Based on food consumption data, this dietary concentration representing the NOAEL is equal to an approximate daily intake of 80 mg/kg bw/day.

3.12.1.2 Oral 28-day toxicity study in rats 2.

Study reference:

B.6.3.1.2 - Anonymous 1986, CME 151: 4 week dietary dose range finding study in rats, unpublished, BASF Document No. DK-420-002

Detailed study summary and results:

Test type

Guideline: Not specified in the report; however, the conduct of this study corresponds to EU Testing Method B7 and OECD 407.

Deviations: Hematology, clinical chemistry and urinalysis studies were not performed in this study. Deviations from current OECD 407 guideline (2008):
- No haematological or clinical chemistry evaluation.
- Adrenals, testes, epididymides, prostate, thymus were not weighed.
- Histopapthology was only carried out on the liver.

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.)

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (CME 151); Batch No. DW 11/86; purity 96.6 ± 0.8 % reported in DK-380-002

Test animals

Male and female Sprague-Dawley rats (10/sex/group). At treatment initiation, animals were approximately 6-7 weeks of age, males weighed approximately 195 g and females weighed approximately 135 g.

Administration/exposure

Dimethomorph technical was fed to four groups of male and female Sprague-Dawley rats (10/sex/group) for 28 days at dietary concentrations of 0, 2000, 3000 and 4000 ppm.

Results and discussion

The concentrations of the diet were analytically confirmed. The stability and homogeneous distribution of the test substance in the diet have been confirmed in the above study.

Test substance intake values for the 2000, 3000 and 4000 ppm groups were approximately 175, 300 and 400 mg/kg bw/day, respectively, for males, and approximately 200, 300 and 400 mg/kg bw/day, respectively, for females based on food consumption data. The calculated test substance intake values for this study are higher than in the previous study because the rats in the second study were smaller in size and one week younger than in the first study. All animals in this study survived to study termination. Clinical signs of toxicity were observed only in the 4000 ppm group, which included piloerection, swollen abdomen and emaciation.

Dose-related reductions in body weights (statistically significant) and body weight gains (non-statistically significant) were noted for females at all dietary concentrations as compared to controls. Body weight gains for males at all dietary levels were reduced when compared to controls, but the differences were only statistically significant at 4000 ppm.

Table 82: 28-day oral toxicity study in rats - body weights and body weight gain

Male				
Body weight (g)				
Group/dose (ppm)	**Control**	**2000**	**3000**	**4000**
Treatment period (weeks)				
0	194	194	196	193
1	253	242	**241* (-4.7%)**	**224*** (-11.5%)**
2	303	285	286	**264*** (-12.9%)**
3	345	324	323	**292*** (-15.4%)**
4	380	357	353	**312*** (-17.9%)**
Body weight gain (g)	186	163	157	119
% of controls	-	88	84	64
Female				
Body weight (g)				
Group/dose (ppm)	**Control**	**2000**	**3000**	**4000**
Treatment period (weeks)				
0	135	133	137	130
1	160	150*	151	**140*** (-12.5%)**
2	183	**165** (-9.8%)**	**164** (-10.3%)**	**151*** (-17.5%)**
3	202	**179** (-11.4%)**	**170*** (-17.5%)**	**154*** (-23.8%)**
4	219	**190** (-13.2%)**	**172***(-21.5%)**	**155*** (-29.2%)**
Body weight gain (g)	84	57	35	25
% of controls	-	68	42	30

* = significantly different from controls, p < 0.05 (Student´s t-test, Kruskal-Wallis ANOVA)
** = significantly different from controls, p < 0.01 (Student´s t-test, Kruskal-Wallis ANOVA)
*** = significantly different from controls, p = < 0.001 (Student´s t-test, Kruskal-Wallis ANOVA)

Reductions in food consumption were noted for males and females at all dietary levels but the differences from the controls were not statistically significant at any level. Hematology, clinical chemistry and urinalysis studies were not performed in this study. Macroscopic findings noted at terminal necropsy included distended intestines, often fluid filled or with gelatinous contents, for both sexes in the 4000 ppm group and for females in the 3000 ppm group. Dose-related increases in liver-to-body weight ratios were observed for both sexes at 3000 and 4000 ppm and for females at 2000 ppm. Absolute organ weights for both sexes in all treatment groups were comparable to controls.

Table 83: 28-day oral toxicity study in rats - relative mean liver weights (g)

Males (10 animals/group)				
Group/dose (ppm)	**control**	**2000**	**3000**	**4000**
Mean	13.36	13.87	**14.75*** (+10%)**	**15.10*** (+13%)**
Females (10 animals/group)				
Group/dose (ppm)	**control**	**2000**	**3000**	**4000**
Mean	7.00	**7.98* (+14%)**	**8.73*** (+24%)**	**9.54*** (+36%)**

* = significantly different from controls, p < 0.05 (covariance analysis); ** = significantly different from controls, p < 0.01 (covariance analysis); *** = significantly different from controls, p = < 0.001 (covariance analysis); values adjusted using body weight of covariate

Histological examinations were only performed on livers from animals in all groups. Microscopic liver changes noted in this study included hepatocellular hypertrophy for both sexes at 3000 and 4000 ppm groups and for females at 2000 ppm.

Table 84: 28-day oral toxicity study in rats – histopathology liver

Males (5 animals/group)				
Group/dose (ppm)	**control**	**2000**	**3000**	**4000**
Hypertrophy				
-Mild	0	0	1	3
- Moderate	0	0	0	1
-Patchy	0	0	0	1
Vacuolation	0	0	0	0
Sunusoidal congestion	0	0	0	0
Females (5 animals/group)				
Group/dose (ppm)	**control**	**2000**	**3000**	**4000**
Hypertrophy				
-Mild	0	2	4	1
- Moderate	0	0	0	0
-Patchy	0	0	0	0
Vacuolation	0	0	0	3
Sunusoidal congestion	0	0	0	1

When comparing the food consumption data for both studies, animals in the 5000 ppm group (first study) ate substantially less food during the 4-week treatment period than animals in the 4000 ppm group (second study). The food consumption patterns for the control animals were comparable in both studies.

Conclusion:

The NOAEL is less than 2000 ppm, based on decreased body weight gains for both sexes at all dietary concentrations, and dose-related increases in liver-to-body weight ratios and hepatocellular hypertrophy for both sexes at 3000 and 4000 ppm and for females at 2000 ppm. Based on food consumption data, the 2000 ppm concentration is equal to an average daily intake of approximately 175 mg/kg bw/day.

3.12.1.3 Oral 28-day study in rats 3.

Study reference:

B.6.3.1.3 - Anonymous 1990, SAG 151 E isomer: A 28 day oral toxicity study in rats, unpublished, Shell Agrar Document No. 151AA-432-005

Detailed study summary and results:

Test type

Guideline: Not specified in the report; however, the conduct of this study corresponds to EU Testing Method B7 and OECD 407.

Deviations: None from the original guideline. Deviations from current OECD 407 guideline (2008):

- A measure of blood clotting time/potential was not included in the haematological evaluation.
- Epididymides, prostate and thymus were not weighed
- Histopathology was only carried out on gross macroscopic lesions, adrenals, heart, intestines, kidneys, liver, pituitary, spleen and stomach.

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.)

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (SAG 151 - E isomer); Batch No. L4785; purity: E isomer 99.5 - 99.7 %, Z-isomer 0.3 - < 0.5 %

Test animals

Male and female Fisher 344 rats (SPF) (7/sex/group). At treatment initiation, animals were approximately 6-8 weeks of age, males weighed approximately 105 g and females weighed approximately 93 g.

Administration/exposure

Dimethomorph technical E isomer was fed by oral gavage to four groups of male and female Fisher 344 rats (7/sex/group) for 28 days at dietary concentrations of 0, 10, 100 or 750 mg/kg bw/day.

Rats were checked for clinical signs twice daily. Bodyweight and food intake were measured weekly. At day 29 the rats were necropsied and blood samples were taken by cardiac puncture for hematology and clinical chemistry. All organs were subjected to macroscopic examination and brain, heart, liver, kidney, spleen, adrenals and testes were weighed. Histopathology was performed on the adrenals, heart, kidney, intestines, liver, lymph nodes, pituitary, spleen, caecum, stomach and gross lesions from the control and top dose

groups. Additionally livers were examined from the intermediate dose levels. Bone marrow smears were prepared from the femur of all rats.

Results and discussion

The concentrations of the diet were analytically confirmed. The stability and homogeneous distribution of the test substance in the diet have been confirmed in the above study.

There was no treatment-related effect on clinical signs. A statistical significant increase in food intake occurred in the top dose males during week 3 and 4. This coincided with a slight, but no statistical significant increase in body weigh, which was seen in 750 mg/kg bw/day dosed males during week 3 and 4.

Table 85: SAG 151 E isomer: 28-day oral toxicity study in rats - food intake

Male					
Food intake (g)					
Group/dose (mg/kg bw/day)	**Control**	**10**	**100**	**750**	**SD of a single obs.**
Treatment period (week)					
0	-	-	-	-	
1	86.1	89.1	87.9	91.3	6.10
2	84.6	83.4	87.4	90.1	8.80
3	84.8	84.2	85.5	**99.6****	8.04
4	72.3	73.7	72.8	**85.9****	8.60
Female					
Treatment period (week)					
0	-	-	-	**-**	
1	76.4	75.4	75.9	**70.1***	4.49
2	73.8	71.5	71.1	69.9	5.23
3	69.8	70.0	67.8	71.1	5.62
4	59.6	57.9	57.9	58.9	4.22

* = significantly different from controls, $p < 0.05$; ** = significantly different from controls, $p < 0.01$ (analysis of variance)

Table 86: SAG 151 E isomer: 28-day oral toxicity study in rats - body weights

Male					
Body weight (g)					
Group/dose (mg/kg bw/day)	**Control**	**10**	**100**	**750**	**SD of a single obs.**
Treatment period (week)					
0	104.2	103.8	106.7	107.5	7.02
1	129.6	130.7	132.1	132.6	7.92
2	153.9	155.6	156.7	160.7	10.40
3	175.8	177.1	176.5	185.6	12.96
4	189.7	193.4	190.3	203.7	16.00
Female					

Treatment period (week)					
0	92.8	93.9	94.4	94.5	5.29
1	107.8	109.5	109.2	108.1	5.63
2	122.2	123.9	124.3	121.2	6.84
3	131.1	134.3	134.7	133.0	7.94
4	137.8	139.7	139.3	138.5	8.20

Rats of both sexes treated with 750 mg/kg bw/day showed slight decreases in total blood hemoglobin indicating a mild normocytic normochromic anemia. Additionally, rats at the top dose group had increased platelet counts, males also had a small increase in platelet volume.

Table 87: SAG 151 E isomer: 28-day oral toxicity study in rats - hematology

Male					
Group/dose (mg/kg bw/day)	**Control**	**10**	**100**	**750**	**SD of a single obs.**
Hemoglobin (g/dL)	16.6	16.6	16.2	**15.8***	0.63
Platelet count (10^9/L)	774	798	792	**941****	74.9
Mean platelet volume (fl)	7.7	7.8	7.6	**7.9***	0.11
Female					
Hemoglobin (g/dL)	16.9	16.9	17.2	**16.2***	0.53
Platelet count (10^9/L)	779	775	833	**904****	72.8

* $p \leq 0.05$ significance of differences between control
** $p \leq 0.01$and treated means using Williams`test

A range of small but statistically significant differences were seen in the clinical chemistry parameters. The observed increases in protein, bilirubin, gamma glutamyl transpeptidase, cholesterol, calcium and triglyceride were attributed to treatment related hepatic changes at the 750 mg/kg bw/day treatment level. Increases in serum urea and creatinine concentrations suggested renal toxicity but no related histopathological observations were made.

Table 88: SAG 151 E isomer: 28-day oral toxicity study in rats – clinical chemistry

Male					
Group/dose (mg/kg bw/day)	**Control**	**10**	**100**	**750**	**SD of a single obs.**
protein	61.7	61.0	61.8	**65.0****	1.73
bilirubin	2.3	2.3	2.3	**2.7****	0.23
gamma glutamyl transpeptidase	0	0	0	**0.5**	
cholesterol	1.63	1.62	1.67	**1.82***	0.138
calcium	2.71	2.74	**2.77***	**2.85****	0.902
triglyceride	3.66	3.54	2.64	**2.41***	0.902

serum urea	4.9	5.2	**5.6****	**6.6****	0.35
creatinine	52	55*	**54***	**55***	2.3
Female					
protein	56.7	57.6	**60.0***	**64.8****	2.13
bilirubin	2.1	2.2	2.3	**3.0****	0.29
gamma glutamyl transpeptidase	0.0	0.3	0.5**	**6.6****	-
cholesterol	2.07	2.07	2.23	**2.40***	0.237
calcium	2.60	2.67	2.66	**2.80****	0.071
triglyceride	2.67	3.02	3.20	2.49	0.992
serum urea	4.8	5.2	5.6	**6.5****	0.64
creatinine	48	49	50	**52***	2.5

* $p \leq 0.05$ significance of differences between control
** $p \leq 0.01$ significance of differences between control

A slight dose increase in adjusted liver weight was seen in the males at 100 and 750 mg/kg bw/day and in females at the 750 mg/kg bw/day treatment level. The liver weight effect was dose related and supported by clinical chemical and histopathological changes indicating a treatment related response. Other organ weight differences included a decreased splenic weight in top dose males and an increase in unadjusted and adjusted adrenal weight in the 100 and 750 mg/kg bw/day dose group males. These differences were small and only seen in one sex and were considered to be chance effects.

Table 89: SAG 151 E isomer: 28-day oral toxicity study in rats - organ weight (adjusted for terminal bodyweight)

Male					
Group/dose (mg/kg bw/day)	**Control**	**10**	**100**	**750**	**SD of a single obs.**
Liver (g)	6.14	6.23	**6.46* (+5%)**	**8.14** (+33%)**	0.245
Spleen (g)	0.40	0.42	0.41	**0.36** (-10%)**	0.021
Adrenal glands	0.033	0.036	**0.038* (+15%)**	**0.044** (+33%)**	0.0039
Female					
Liver (g)	4.05	3.97	4.18	**5.81** (+43%)**	0.274
Spleen	0.33	0.31	0.30	0.31	0.023
Adrenal glands	0.045	0.043	0.045	0.049	0.0056

* $p \leq 0.05$ significance of differences between control
** $p \leq 0.01$and treated means using Williams`test

Slight to moderate hepatic enlargement was seen macroscopically in one male at 100 mg/kg bw/day and in all male and female rats at 750 mg/kg bw/day. Dark discoloration of the liver was observed in most males and one female at 750 mg/kg bw/day and occasionally in males at 100 mg/kg bw/day. Slight to moderate caecal enlargement and fluid caecal contents were seen at 750 mg/kg bw/day but no related changes were observed histologically. Patchy mid zonal hepatocellular cytoplasmic lipid vacuolation was observed at 750 mg/kg bw/day. The overall severity was slight and the frequency dose related, being apparent in all males and females at the top dose and some 100 mg/kg bw/day treated rats.

Table 90: SAG 151 E isomer: 28-day oral toxicity study in rats - histopathology liver findings

Male					
Findings	**Group/dose (mg/kg bw/day)**	**Control**	**10**	**100**	**750**
Liver: patchy midzonal cytoplasmic lipid vacuolation	Very slight	0	0	2	0
	Slight	0	0	0	**7*** **
	moderate	0	0	0	0
Caecal fluid content		0	0	0	7
Caecal enlargement	slight	0	0	1	0
	moderate	0	0	0	7
Female					
Liver: patchy midzonal cytoplasmic lipid vacuolation	Very slight	0	0	**4***	2
	Slight	0	0	0	**4***
	moderate	0	0	0	1
Caecal fluid content		0	0	0	7
Caecal enlargement	slight	0	0	0	0
	moderate	0	0	0	7

Significance of differences in a pairwise (Fisher`s) test between each treatment and control incidences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Conclusion:

Significant treatment-related changes were observed in the liver. The NOAEL is 10 mg/kg bw/day, based on a dark discoloration and enlargement of the liver, an increase in adjusted liver weight in the males and a mid zonal hepatocellular cytoplasmic lipid vacuolation in male and female rats at 100 mg/kg bw/day.

3.12.1.4 Oral 28-day study in rats 4.

Study reference:

B.6.3.1.4 - Anonymous 1991, SAG 151 Z isomer: A 28 day oral toxicity study in rats, unpublished, Report-No. SBGR.90.107; BASF document No. DK-470-016

Detailed study summary and results:

Test type

Guideline: Not specified in the report; however, the conduct of this study was consistent with Directive 92/69/EEC Method B7, the required guideline.

Deviations: None from the original guideline. Deviations from current OECD 407 guideline (2008):

- A measure of blood clotting time/potential was not included in the haematological evaluation.
- Epididymides, prostate and thymus were not weighed

- Histopathology was only carried out on gross macroscopic lesions, adrenals, heart, brain, intestines, kidneys, liver, pituitary, testes, spleen and stomach.

GLP: Yes, laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.

Acceptability: The study is considered to be acceptable.

Test substance

SAG 151 Z isomer; Batch No. Th H296, ST90/106; purity 95.6 %

Test animals

Male and female SPF Fischer 344 rats (7/sex/dose).

Administration/exposure

Dimethomorph was fed to groups of 7 male and 7 female rats for 4 weeks at dietary concentrations of 0, 10, 100 and 750 mg/kg bw/day. All animals were examined for clinical signs. Haematological and clinical chemistry examination was performed. Post-mortem examination included gross pathology, organ weights and histopathological evaluation.

Results and discussion

There was no compound related effect on body weight nor food consumption. No treatment related clinical findings were observed. No treatment-releated effect on the haematologic parameters was observed.

In both sexes increases in serum bilirubin and protein, as well as decreased albumin to globulin ration were seen at 750 mg/kg bw/day.

Table 91: SAG 151 Z isomer: 28-day oral toxicity study in rats – clinical chemistry

Male					
Group/dose (mg/kg bw/day)	**Control**	**10**	**100**	**750**	**SD of a single obs.**
Protein	60.4	61.6	62.6*	**63.7****	1.70
Albumin	37.7	38.2	38.0	38.9	1.20
Urea	5.2	4.9	5.6	**5.9****	0.37
Calcium	2.72	2.74	2.76	**2.80****	0.043
A/G	1.65	1.63	**1.54****	**1.57****	0.059
Female					
Protein	58.5	59.4	**60.7****	**39.4****	1.23
Albumin	36.9	37.7	**38.1****	**39.4****	0.74
Urea	5.2	5.4	5.1	5.6	0.51
Calcium	2.61	2.61	2.67	**2.70***	0.063
A/G	1.71	1.74	1.69	**1.64***	0.049

* $p \leq 0.05$ significance of differences between control

** $p \leq 0.01$and treated means using Williams`test

A dose related, statistical significant increase in relative liver weight was seen in rats treated with 100 or 750 mg/kg bw/day.

Table 92: SAG 151 Z isomer: 28-day oral toxicity study in rats - organ weight (adjusted for terminal bodyweight).

Male					
Group/dose (mg/kg bw/day)	**Control**	**10**	**100**	**750**	**SD of a single obs.**
liver	6.59	6.65	**7.20 (+9%)**	**7.97 (+21%)**	0.284
Female					
liver	4.27	4.21	**4.87****	**5.81****	0.189

* p ≤ 0.05 significance of differences between control
** p ≤ 0.01and treated means using Williams`test

Slight caecal enlargement was observed in 6 males and 2 females at 750 mg/kg bw/day. Slightly fluid caecal contents were recorded in 3 males at 750 mg/kg bw/day.

Table 93: SAG 151 Z isomer: 28-day oral toxicity study in rats – macroscopic findings

Male					
Findings	**Group/dose (mg/kg bw/day)**	**Control**	**10**	**100**	**750**
Liver: enlargement	Very slight	0	1	0	0
	Slight	0	0	2	7
Liver, dark discoloration		0	0	1	4
Caecal fluid content		0	0	0	3
Caecal enlargement	very slight				
	slight	0	0	0	6
Female					
Liver: enlargement	Very slight	0	0	3	0
	Slight	0	0	0	7
Liver, dark discoloration		0	1	3	7
Caecal fluid content		0	0	0	0
Caecal enlargement	very slight	0	0	0	1
	slight	0	0	0	1

Significance of differences in a pairwise (Fisher`s) test between each treatment and control incidences: * p < 0.05, ** p < 0.01, *** p < 0.001

Histopathological examination of the liver revealed a patchy midzonal cytoplasmic lipid vacuolation at 100 or 750 mg/kg bw/day in both sexes.

Table 94: SAG 151 Z isomer: 28-day oral toxicity study in rats – macroscopic findings

Male					
Findings	**Group/dose (mg/kg bw/day)**	**Control**	**10**	**100**	**750**
Liver: patchy midzonal cytoplasmic lipid vacuolation	Very slight	0	0	3	2
	Slight	0	0	0	**5***
Female					
Liver: patchy midzonal cytoplasmic lipid vacuolation	very slight	0	0	**4***	2
	slight	0	0	0	**5***

Significance of differences in a pairwise (Fisher`s) test between each treatment and control incidences: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Conclusion:

Some clinical chemistry variates showed changes at 750 mg/kg bw/day in both sexes, reflecting functional hepatic changes. Histopathological examination of the liver revealed a slight midzonal lipid vacuolation at 100 or 750 mg/kg bw/day in both sexes. No treatment related effects were seen at 10 mg/kg bw/day.

3.12.1.5 Oral 6-week study in mice.

Study reference:

B.6.3.1.5 - Anonymous 1986, CME 151 6 week dietary dose range finding study in mice, Celamerck Document Report No. 151AE-431-003, unpublished. BASF Document No. DK-420-003

Detailed study summary and results:

Test type

Guideline: Not specified in the report. The study did not specify a guideline and a limited number of endpoints were evaluated. No haematological or blood chemistry evaluation was carried out. Only the brain, heart kidney, liver, lungs and spleen were weighed and only the liver underwent histopathological evaluation. In addition, the dose levels were increased from day 23 of dosing. Therefore, the study is not suited to derive a NOAEL.

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.)

Acceptability: The study is considered to be supplementary information.

Test substance

Dimethomorph (CME 151); Batch No. DW 11/86; purity not specified in the report

Test animals

Male and female CD-1 mice (10/sex/group)

Administration/exposure

Dimethomorph was administered to mice (10/sex/group) at dietary concentrations of 0, 300, 800 and 2000 ppm. Due to the lack of any toxic effects the dose levels were increased from day 23 of dosing. The changes in dose level were as follows: 300 ppm was increased to 10000 ppm (males) and 8000 ppm (females); 2000 ppm was increased to 5000 ppm (males) and 4000 ppm (females). It was decided to incease the duration of dosing to a total of 6 weeks. All animals were cheked daily for death or moribundity. Each animal was given a weekly detailed physical examination for clinical signs. Body and food consumption was recorded weekly. After 6 weeks of dosing all animals were killed and autopsied with selected organs. Livers from selected animals were examined histopathologically.

Results and discussion

There were no premature deaths during the course of the study. There were no treatment-related clinical signs nor effects on body weight or food consumption. Males in the groups receiving 300/10000 ppm or 2000/5000 ppm and females in all dose groups showed significant increases in absolute liver weight and when adjusted for terminal body weight. There were not treatement related pathological phindings.

Table 95: 6-week oral toxicity study in mice - organ weight (adjusted for terminal bodyweight)

Male				
Group/dose (mg/kg bw/day)	**Control**	**300/10000**	**800**	**2000/5000**
liver	1.98	**2.63*** (+33%)**	2.18	**2.33** (+18%)**
Female				
Group/dose (mg/kg bw/day)	**Control**	**300/8000**	**800**	**2000/4000**
liver	1.44	**2.02*** (+40%)**	**1.66** (+15%)**	**1.87*** (+30%)**

** $p \leq 0.01$ significance of differences between control
*** $p \leq 0.001$ significance of differences between control

Conclusion:

There was a liver weight increase in males (5000 ppm and above) and females (4000 ppm and above) which could not be attributed to a specific histopathological lesion.

3.12.1.6 Oral 14 day study in dogs.

Study reference:

B.6.3.1.6 - Anonymous 1986, CME 151 Dietary maximum tolerated dose study in dogs, unpublished.BASF Document No. DK-420-001

Detailed study summary and results:

Test type

Guideline: Not specified in the report.

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.)

Acceptability: The study is considered to be supplementary information.

Test substance

Dimethomorph CME 151; Batch No. DW 11/86, purity not specified

Test animals

Male and female purebred Beagle dogs. (1/sex/dose)

Administration/exposure

The objective of the study was to determine the dietary maximum tolerated dose. The study was conducted in 2 parts. Dimethomorph was administered to groups of 1 male and 1 female dog at dietary concentrations of 1000, 750, 900 and 1200 ppm for 7 days in part A of the study. In part B of the study 1 male and 1 female dog were dosed with 1200 ppm dimethomorph for 14 consecutive days. The animals were observed daily for clinical signs and viability. Food consumption of the animals was determined daily and their body weight twice a week. Clinical chemistry and hematological examinations were carried out following overnight starvation pretrial and prior to necropsy. Urine samples were collected over the final period of water deprivation, pretrial and one day prior to necropsy. Faecal samples were collected at the time of urine collection. All animals were subjected to gross pathological evaluation and organ weight analysis.

Results and discussion

Emesis, subdued behaviour and increased micturition on very few occasions was observed for the male dog were absent in the last week of treatment in part A of the study. Body weight losses were demonstrated by the male dog when dosed with 900 ppm or above. There was a reduction in food intake for both animals when dosed with 1000 ppm. In case of the female dog, food intake was also noted to be below maximal pretrial. In part B of the study slightly body tremors were only observed on a few occasions for the male dog.

Conclusion:

It was concluded that the maximum tolerated dose of dimethomorph is > 1200 ppm.

3.12.1.7 Oral 90 day study in rats.

Study reference:

B.6.3.2.1 - Anonymous 1987, CME 151: Toxicity to rats by dietary admixture for 13 weeks with a 4 week withdrawal period, Report No. CMK 7/8624, unpublished, BASF Document No. DK-425-001

Detailed study summary and results:

Test type

Guideline: (US EPA Guideline 82-1 and OECD Guideline 408 claimed by the author); EU Testing Method B 26.

Deviations: None from original guideline. Deviations from current OECD guideline 408:
- Epididymides and thymus were not weighed.

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.)

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (CME 151); Batch No. T2/85; purity 98.7 ± 1.5 % reported in DK-380-003

Test animals

Male and female Sprague-Dawley rats (20/sex/dose)

Administration/exposure

Dimethomorph technical was fed to four groups of males and females Sprague-Dawley rats for 13 weeks at dietary concentrations of 0, 40, 200 and 1000 ppm. Twenty male and twenty female rats were assigned to the control and 1000 ppm groups while 10 animals/sex were assigned to the 40 and 200 ppm groups. Following 13 weeks of treatment, 10 animals/sex/group were sacrificed and 10 animals/sex in the control and 1000 ppm groups received control diet for a 4-week recovery period.

Prior to commencement of the study the proposed diet mixing procedures were checked by chemical analysis of trial diets to confirm that the proposed procedures product homogeneous diet, the accuracy of mixing and that the concentration of the test material remained unchanged between preparation and administration. A pre-mix was prepared each week. Samples of the diets prepared in the first week and week 13 were also analysed to check the accuracy of the preparation.

Mortality was checked twice daily. Clinical signs were recorded on a daily basis for the first four weeks. As no signs were recorded the frequency change to once weekly. Body weight and food consumption was recorded weekly. Ophthalmoscopic evaluation was carried out before treatment and during week 13 in the control and high dose group. During week 9 vaginal smear preparations were commenced to check the stage of oestrous cycles. As no treatment-related findings were noted this investigation was stopped at the end of week 12. Haematological, biochemistry and urinalysis evaluation were carried out at the end of the study. After termination the following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid and uterus. Full histopathological evaluation was carried out for the control animals

and the high dose. For the low and intermediate dose histopathology was carried out on the lungs, liver and kidneys only.

Results and discussion

The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The concentrations of the diets were analytically confirmed.

All animals survived to study termination and no overt signs of toxicity were observed that could be attributed to administration of the test material. Food consumption values for males and females in all treated groups were comparable to controls during the treatment and recovery periods. Average test substance intake values for the 40, 200 and 1000 ppm groups were 2.9, 14.2, and 73 mg/kg bw/day, respectively for males, and 3.2, 15.8 and 82 mg/kg bw/day, respectively for females, based on food consumption data.

Table 96: Test substance intake

Dietary Dose Level	Test Substance Intake (mg/kg bw/day)	
(ppm)	Males	Females
40	2.9	3.2
200	14.2	15.8
1000	73	82

No treatment-related changes in body weights or body weight gains were observed for either male or female rats at any concentration. No treatment-related ophthalmological findings were observed at study termination and microscopic examination of vaginal smears obtained during weeks 9 - 12 of the study did not indicate any treatment-related effects on the estrous cycle of female rats.

Hematological investigations performed in week 13 indicated that total white blood cell counts for males receiving 1000 ppm were lower than those of controls. This was associated with lower lymphocyte counts for these animals. Total white blood cell counts and lymphocyte counts for males of the high dose group were noted to be similar to those of controls at the investigation performed during week 4 of the withdrawal period. Marginal group differences noted among erythrocyte parameters in week 13 and week 4 of the withdrawal period were considered to be of no toxicological significance.

Table 97: 13 week dietary toxicity study in rats – hematalogy

Endpoint	Dose level (ppm)								HCD males age 18 weeks
	0		40		200		1000		
	M	F	M	F	M	F	M	F	
Week 13									
PCV%	51	48	51	47	51	48	**49***	49	44-59
Hb	15.3	15.2	15.0	15.0	15.6	15.3	15.4	15.3	14.0-18.1
RBC	8.6	7.7	8.2	7.7	8.4	7.7	**8.1***	7.7	6.5-9.8

MCHC	30.1	31.3	29.8	31.6	30.6	32.0	**31.2****	31.5	26.8-37.2
MCV	60	63	62	62	61	62	61	63	52-80
Total WBC	14.9	9.6	13.3	9.2	13.6	11.0	**10.9****	11.4	8.6-26.4
L	12.43	8.17	11.42	8.07	12.08	9.85	**9.15****	9.81	6.93-21.93
Week 4 of the withdrawal period									
PCV%	48	41	-	-	-	-	45	**45*****	
Hb	15.5	14.1	-	-	-	-	**14.7*****	14.3	
RBC	8.0	7.4	-	-	-	-	7.7	**6.9***	
MCHC	32.4	34.4	-	-	-	-	32.7	**32.1****	
Total WBC	12.7	7.0	-	-	-	-	11.6	7.0	
L	11.4	6.14	-	-	-	-	9.83	6.23	

*Kruskal-Wallis analysis performed, level of significans, Student`s test: * = p< 0.05 in comparison with controls; ** = p< 0.01 in comparison with controls; *** = p< 0.001 in comparison with controls;*
The background data ranges presented are the 1 % and 99 % percentiles taken directly from the observed distribution of control data for CD rats, obtained from studies performed at Anonymous Laboratory during the period 1982 - 1985.

No adverse effects of treatment were evident from the clinical chemistry data. A slight increase in the acidity of the urine of treated rats was noted in comparison with controls at the investigation performed in week 13, but this was not dose-related in degree. Urinary pH investigated during week 4 of the withdrawal period for rats which had received 1000 ppm showed regression of this effect for these animals. There were no other toxicological significant differences in urinalysis parameters.

Table 98: 13 week dietary toxicity study in rats - urinalysis

Group/dose (ppm)	Control		40		200		1000	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Week 13								
pH	6.8	6.4	**6.5****	**6.2****	**6.4****	**6.2****	**6.4****	**6.1****
Week 17 (withdrawal period)								
pH	6.7	6.2	-	-	-	-	6.7	6.2

*Level of significance, Williams`test: ** p < 0.01 in comparison with controls; no significans noted at withdrawal period using Student's t-test*

Relative liver weight was decreased in males at 200 and 1000 ppm. There were no other organ weight changes observed for males at termination of the 13-week treatment period. For females, absolute heart and liver weights, heart-to-body weight ratios and liver-to-body weight ratios were statistically significantly increased, relative to controls, at 1000 ppm following 13 weeks of treatment. Absolute and relative liver weight were also slightly increased (statistically significant) for females at 1000 ppm following the 4-week recovery period. Kidney weights for treated females were marginally higher than those of controls, after adjustment for final bodyweight as covariate, but not to a dose-related degree. After 4 weeks of withdrawal

there was no significant difference. There were no macroscopic or microscopic changes, which were attributable to treatment with dimethomorph technical in any of the tissues evaluated.

Table 99: 13 week dietary toxicity study in rats - organ weights

Group/dose (ppm)	Control		40		200		1000	
	M	F	M	F	M	F	M	F
Week 13								
Heart (g)	1.54 (1.55)	0.97 (1.02)	1.62 (1.59)	1.06 (1.01)	1.56 (1.59)	1.01 (1.00)	1.56 (1.57)	1.10** (1.10)
Liver (g)	23.3 (23.4)	10.5 (10.9)	21.9 (20.8)	11.0 (10.6)	**20.6*** (21.4)	11.3 (11.3)	**22.0*** (22.1)	**12.3**** (12.3)
Kidneys	4.05 (4.07)	2.24 (2.33)	4.16 (4.06)	**2.50*** (2.42)	4.10 (4.18)	**2.41*** (2.40)	4.14 (4.15)	**2.44*** (2.44)
Withdrawal								
Heart (g)	1.55 (1.59)	0.98 (0.98)	-	-	-	-	1.58 (1.54)	1.02 (1.01)
Liver (g)	23.0 (24.0)	10.7 (10.8)	-	-	-	-	23.6 (22.5)	**11.6*** (11.4))
Kidneys	4.01 (4.14)	2.38 (2.40)	-	-	-	-	4.35 (4.22)	2.51 (2.49)

*Values adjusted for final body weight as covariate, unadjusted values are shown in parantheses; level of significance, Williams` test: * p < 0.05 in comparison with control, ** p < 0.01 in comparison with control; withdrawal period: no significance noted using Student's t-test*

Conclusion:

Based on increased liver weights for females at 1000 ppm, the NOAEL for this study is 200 ppm. Based on food consumption data, this concentration is equal to an approximate average daily intake of 16 mg/kg bw/day.

3.12.1.8 Oral 90 day study in dogs.

Study reference:

B.6.3.2.2 - Anonymous 1986, CME 151: 13 week dietary toxicity study in dogs, Report No. 3722, unpublished.BASF Document No. DK-425-002

Detailed study summary and results:

Test type

Guideline: EU Testing Method B 27 (OECD Guideline 409 and US EPA Guideline 82-1 claimed by the author)

Deviations: None

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.)

Acceptability: The study is considered to be acceptable

Test substance

Dimethomorph (CME 151); Batch No. DW 11/86; purity 96.6 ± 0.8 % reported in DK-380-002

Test animals

Male and female purebred Beagle dogs (4/sex/group; 5-7.5 months old at the start of the study)

Administration/exposure

Dimethomorph technical was administered to purebred Beagle dogs (4/sex/group) at dietary concentrations of 0, 150, 450 and 1350 ppm. All animals were provided 400 grams of food per day for 90 days. Diets were prepared at the beginning of each study week. For analysis of the test material samples were taken at the start of treatment, during week 2, 7, 9 and 13 of the study.

The animals were observed daily for any signs of ill health or reaction to treatment. Food consumption was monitored daily and body weight was monitored weekly throughout the dosing period. Laboratory investigations of hematology, clinical chemistry and urinalysis were undertaken pretrial and during weeks 6 and 13 of treatment. Opthalmoscopic examination was undertaken pretrial and during week 13 of treatment. All animals were subjected to detailed gross and histopathological evaluation and organ weight analysis.

Results and discussion

The concentrations of the diets were analytically confirmed.

Table 100: Test substance intake

Dietary Dose Level	Test Substance Intake (mg/kg bw/day)	
ppm	Males	Females
150	5	6
450	15	15
1350	43	44

All animals survived to study termination. Treatment-related clinical signs were noted at 1350 ppm and consisted of lip-licking occasional subdued behaviour and a few incidences of body tremors. Lip-licking was frequently observed over the dosing period and was considered to be due to the fact that 1350 ppm was approximately the emetic threshold demonstrated in a previous maximum tolerated dose study in dogs. Salivation was observed in all groups, including controls, during week 1 of the study and was observed intermittently in all treatment groups at various times during treatment. Because no dose-related increase in the incidence of salivation was evident, this finding was not considered treatment-related. No treatment-related ophthalmoscopic abnormalities were observed at study termination.

Table 101: 13 week dietary toxicity study in dogs - incidence of clinical signs (total number of observations per week)

Week of study	Clinical signs	Group/dose (ppm)							
		Males				Females			
		0	150	450	1350	0	150	450	1350
1	Salivation Body tremors	3	1	4	1 1		2	1	3
2	Salivation Subdued		1		 1				
3	Salivation Lip licking Body tremors			1	 6				 2 1
4	Salivation Lip licking				 9	1			 5
5	Lip licking Subdued				10 2				7
6	Salivation Subdued Lip licking Body tremors				 2 2 1		1		 1 4
7	Salivation Lip licking Subdued				 6 4				1 1 4
8	Lip licking Subdued Body tremors				5				2 1 1
9	Salivation Lip licking Subdued	1 1	 1 2		 7 2				 2 1
10	Lip licking Subdued		 5		5 4				
11	Lip licking Subdued		 7		7				1
12	Lip licking Subdued		 7		3				1
13	Lip licking Subdued Salivation		 7 1	 2	3 2				2 1

Food consumption patterns for males and females in all treated groups were comparable to controls during the 13-week treatment period. Average test substance intake values during the 13-week treatment period for the 150, 450 and 1350 ppm groups were 5, 15 and 43 mg/kg bw/day, respectively for males, and 6, 15 and 44 mg/kg bw/day, respectively for females, based on food consumption data.

No treatment-related changes in body weights or body weight gains were observed at any dietary concentration.

No changes in hematology parameters were observed for any treatment group during the study.

Statistically significant increases in serum alkaline phosphatase activity were observed at weeks 6 and 13 for males at 1350 ppm when compared to controls. Although increases in serum alkaline phosphatase activity were not observed for females at any dietary concentration at weeks 6 and 13 during the 13-week study,

statistically significant increases for this enzyme were observed at weeks 13, 26, and 51 for male and female dogs at 1350 ppm in the 1-year dietary toxicity study. No other clinical chemistry changes were observed during the study.

Table 102: 13 week dietary toxicity study in dogs – clinical chemistry

Endpoint	Group/dose (ppm)							
	Males				Females			
	0	150	450	1350	0	150	450	1350
Alkaline phosphatase								
- Pretrial	144	124	139	166	196	142	**144*** (-27%)	**146*** (-26%)
- Week 6	164	176	291	**546*** (333%)	206	233	304	325
- Week 13	137	149	223	**348**** (254%)	180	166	212	278

No adverse effects of treatment were evident from the urinalysis data.

Anatomic pathology findings showed no treatment-related macroscopic changes during necropsy. Absolute thymus weights and thymus-to-body weight ratios were statistically significantly increased in males at 1350 ppm when compared to controls, but these organ weight changes were not correlated with any microscopic lesions, and therefore, were not considered to be treatment-related. Increases in mean absolute liver weight (non-statistically significant) and mean liver-to-body weight ratios (+27%, statistically significant) were observed for females at 1350 ppm when compared to controls. Absolute and relative liver weights for males at 1350 ppm were comparable to controls. Absolute prostate weights and prostate-to-body weight ratios were significantly reduced, relative to controls, for males at 1350 ppm. The changes in prostate weights noted for males in the 1350 ppm group were consistent with an apparent increase in the incidence of prostatic interstitial fibrosis for this group, as compared to the control group. No other treatment-related organ weight changes or microscopic findings were observed.

Table 103: 13 week dietary toxicity study in male dogs - organ weights

Group/dose (ppm)	Control	150	450	1350
Number of animals	**4**	**4**	**4**	**4**
Absolute organ weights, group mean value (g) ± SD				
Thymus	8.34 ± 1.35	5.63 ± 3.10	9.86 ± 3.42	**14.95 ± 6.84*** (+79%)
Prostate	8.56 ± 3.67	6.63 ± 1.18	6.49 ±2.08	**3.27 ± 1.31**** (-62%%)
Relative organ weights (as % body weight), group mean value ± SD				
Thymus	0.069 ± 0.013	0.051 ± 0.029	0.083 ± 0.022	**0.13 ± 0.049*** (+76%)
Prostate	0.070 ± 0.026	0.060 ± 0.008	0.057 ± 0.023	**0.027 ±**

				0.010** (-61%)

*S.D. = standard deviation; ** = significantly different from control, p < 0.01 ("F-max" test; parametric ANOVA; Student`s t-test)*

Table 104: 13 week dietary toxicity study in female dogs - organ weights

Group/dose (ppm)	Control	150	450	1350
Number of animals	**4**	**4**	**4**	**4**
Absolute organ weights, group mean value (g) ± SD				
Liver	279.20 ± 10.42	297.90 ± 49.56	295.39 ±28.37	319.93 ± 51.26
Relative organ weights (as % body weight), group mean value ± SD				
Liver	2.82 ± 0.21	**3.30 ± 0.09*** (+17%)	3.21 ± 0.25 (+14%)	**3.59 ± 0.51**** (+27%)

*S.D. = standard deviation; ** = significantly different from control, p < 0.01 ("F-max" test; parametric ANOVA; Student`s t-test)*

Table 105: 13 week dietary toxicity study in dogs - histopathology findings of the prostate

Group/dose (ppm)	Control	150	450	1350
Number of animals	**4**	**4**	**4**	**4**
Incidence of lesions (numeric)				
Prostatitis		1		2
Increased fibrosis				**4***

* = significance of differences in a pairwise (Fisher`s) test between each treatment and control incidence: p < 0.05

Conclusion:

Based on increases in alkaline phosphatase activity (males and females), increases in mean relative liver weight in females, reductions in absolute and relative prostate weights, and an increased incidence of prostatic interstitial fibrosis in males at 1350 ppm, the NOAEL for this study is 450 ppm. Based on food consumption data, this concentration is equal to an approximately average daily intake of 15 mg/kg bw/day.

3.12.1.9 Oral 52 week study in dogs.

Study reference:

B.6.3.2.3 - Anonymous 1990, SAG 151: 52 week dietary toxicity study in dogs, unpublished, BASF Document No. DK-427-003

Detailed study summary and results:

Test type

Guideline: Directive 96/54/EC B 30 (OECD Guideline 409 and US EPA Guideline 83-1 claimed by the author)

Deviations: None

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.)

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (SAG 151; CME 151); Batch No. DW 11/86; purity 96.6 %

Test animals

Male and female purebred Beagle dogs (4/sex/dose)

Administration/exposure

Dimethomorph was administered to groups of 4 male and 4 female purebred Beagle dogs at dietary concentrations of 0, 150, 450 and 1350 ppm for about 12 months. All dogs were offered limited diet (400 g/dog/day) for 52 weeks. The test diet was prepared at the beginning of each week. For analysis of the test material samples were taken from all treatment groups at approximately 2-3 month intervals. The animals were observed daily for any signs of ill health or reaction to treatment. Food consumption of the animals was determined daily and their body weight once a week. Clinical chemistry and hematological examinations as well as urinalyses were carried out once pretrial and during weeks 13, 26, and 51 of the administration period. Ophthalmological examinations were carried out pretrial and during weeks 26 and 51 of treatment. All animals were subjected to detailed gross and histopathological evaluation and organ weight analysis.

Results and discussion

The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis.

Table 106: Test substance intake

Dietary Dose Level	Test Substance Intake (mg/kg bw/day)	
(ppm)	Males	Females
150	4.9	5.0
450	14.7	15.7
1350	44.6	47.0

All dogs survived to study termination and no treatment-related clinical signs of toxicity or ophthalmological abnormalities were detected during the 52-week study period. Sporadic convulsive seizures (lasting for 30-60 seconds) were observed in one male at the 1350 ppm treatment level on two days during week 44 and two days during week 47. Since the incidence of spontaneous convulsive episodes may occur up to 12 % in a closed colony of Beagle dogs, the sporadic convulsions observed in the male dog in the 1350 ppm group were not considered treatment-related. In addition, a slight convulsive seizure was observed for one male in the 150 ppm group prior to weighing during weeks 32 and 34, and was attributed to stress.

Food consumption patterns for males and females in all treated groups were comparable to controls during the 52-week treatment period. Average test substance intake values during the 52-week treatment period for the 150, 450 and 1350 ppm groups were 5, 15 and 45 mg/kg bw/day, respectively, for males and 5, 16 and 47 mg/kg bw/day, respectively, for females based on food consumption data. Slightly reduced body weight gains were observed for dimethomorph treated groups with the exception of the 150 mg/kg bw/d dose group females which showed an increase of body weight gain.

Table 107: 52 week dietary toxicity study in dogs – body weight gain (kg)

	0		150		450		1350	
	M	F	M	F	M	F	M	F
Mean body weight gain (week 0–52)	3.1	2.9	2.2	4.1	2.1	2.5	2.5	2.6
% of controls			71	141	68	85	81	90

Hematology and urinalysis parameters were unaffected by treatment with dimethomorph technical. Statistically significant increases in serum alkaline phosphatase were observed at weeks 13, 26 and 51 for males and females at 1350 ppm when compared to controls. No other treatment-related clinical chemistry changes were observed for either sex at any dietary level.

Table 108: 52 week dietary toxicity study in dogs – clinical chemistry

	0		150		450		1350	
	M	F	M	F	M	F	M	F
Alkaline phosphatase								
- Pretrial	324	296	333	341	333	288	324	345
- Week 13	226	207	217 (-4%)	241 (+16%)	279 (+23%)	246 (+19%)	**379*** (+68%)	**411***** (+99%)
- Week 26	153	173	160	180	220 (+44%)	207 (+20%)	**375***** (+145%)	**399*** (+131%)
- Week 52	127	150	133	159	196 (+54%)	193 (+29%)	**310**** (+144%)	**495***** (+230%)

** Significantly different from control, p<0.05, *** Significantly different from control, p<0.001*

There were no treatment-related macroscopic changes observed at necropsy. Absolute liver weights and liver-to-body weight ratios were significantly increased for males at 1350 ppm when compared to controls. Liver-to-body weight ratios were increased, relative to controls, for females at 450 ppm (not statisticaly significant) and at 1350 ppm (statisticaly significant). At the 450 ppm group there was a high varation in absolute organ weights. The individual liver weights in females were 451.03 g, 376.35, 215.85 and 272.65 and therefore clearly above the control range (264.96-303.28 g) for two out of four animals.

Statistically significant increases in testes-to-body weight ratios were observed for males at 450 and 1350 ppm. Decreases in absolute prostate weights (statistically significant) and prostate-to-body weight ratios (non-statistically significant) were observed for males at 1350 ppm when compared to controls.

Table 109: 52 week dietary toxicity study in male dogs - liver, testes and prostate organ weight (g)

Group/dose (ppm)	Control	150	450	1350
Number of animals	**4**	**4**	**4**	**4**
Liver – absolute	371.65	327.71	346.35	**433.44*** (+17%)
Liver – adjusted[a]	368.14	328.01	349.20	**433.82*** (+18%)
Testes – absolute	27.25	27.41 (+0.5%)	30.04 (+10%)	32.53 (+19%)
Testes – adjusted[a]	25.44	27.56 (+8%)	**31.49*** (+24%)	**32.73*** (+29%)
Prostate – absolute	8.25	8.97 (+9%)	5.55 (-33%)	**4.27*** (-48%)
Prostate – adjusted[a]	7.40	9.04 (+22%)	6.23 (-16%)	4.36 (-41%)

* = significantly different from control, $p < 0.05$ (analysis of covariance); [a]values adjusted using body weight of covariate

Table 110: 52 week dietary toxicity study in female dogs - liver relative organ weight (g)

Group/dose (ppm)	Control	150	450	1350
Number of animals	4	4	4	4
Liver – absolute	281.83	297.15	328.97	399.36
Liver – adjusted[a]	280.25	264.79 (-5.5%)	350.02 (+24.9%)	**412.26**** (+47%)

** = significantly different from control, $p < 0.01$ (analysis of covariance); [a]values adjusted using body weight of covariate

The changes in prostate weights noted for males in the 1350 ppm group were consistent with a slight increase in the incidence and severity of prostatic interstitial fibrosis, as compared to controls. Thus, the tissue shrinkage resulting from interstitial fibrosis was reflected by a reduction in prostate weights only in the 1350 ppm group. The study author indicated that some of this effect may be due to a slightly different level of section and indicated that in none of the animals was the degree of interstitial tissue higher than in the control. However, it is noted that this finding was also observed in the 13-week study. The only other microscopic finding was increased hepatic lipid content which was observed in 3 of 4 males at 1350 ppm as compared to none in control males. This small increase in hepatic lipid was more equivocal in females where the incidences for the control, 150, 450 and 1350 ppm groups were 1 of 4, 0 of 4, 2 of 4 and 2 of 4, respectively (see Table 111).

Table 111: 52 week dietary toxicity study in female dogs – histopathological findings of the liver

Group/dose (ppm)	Control	150	450	1350
Number of animals	4	4	4	4
Hepatocyte Lipid-minimal	3	4	2	2
Hepatocyte Lipid-mild	1	0	2	2

** = significantly different from control, $p < 0.01$ (analysis of covariance); values adjusted using body weight of covariate

Conclusion:

Based on increased relative liver weights in females (+24.9%) and increased adjusted testes weights (+23.8%) in males at 450 ppm, the NOAEL for this study is 150 ppm. Based on food consumption data, this concentration is equal to an approximate daily intake of 4.9 mg/kg bw/day.

The study author claimed a NOAEL of 450 ppm.

3.12.1.10 Dermal 28 day study in rats.

Study reference:

B.6.3.3.1 - Anonymous 2010. BAS 550 F (Dimethomorph) - Repeated-dose 28-day dermal toxicity study in Wistar rats. 2010/1151903

Detailed study summary and results:

The dermal administration of dimethomorph (BAS 550 F) to male and female Wistar rats over a period of 28 days did not reveal test substance related adverse signs of systemic toxicity at dose levels of 100, 300, or 1000 mg/kg bw/d. Moreover, during functional observational battery as well as measurement of motor activity, no signs of neurotoxicity were obtained. Regarding clinical pathology no treatment-related, adverse effects were observed during dermal application of the compound corresponding to a dose of 1000 mg/kg bw/d. Regarding pathology significant weight changes noted in adrenal glands and liver of male and females occurred without dose-relationship and, therefore, were regarded as incidental and not related to treatment. Histopathology did not reveal treatment-related findings.

The no observed adverse effect level (NOAEL) for systemic toxicity and local effects under the conditions of the present study was 1000 mg/kg bw/day in male and female Wistar rats.

Test type

Guidelines: OECD 410, EPA 870.3200

Deviations: None

GLP: yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Test substance

BAS 550 F (Dimethomorph). Lot/Batch #: COD-001244. Purity: 99.8%. Stability of test compound: The test substance was stable over the study period (until 01 Mar 2015)

Test animals

Crl:WI(Han) rats. (10/sex/dose). Age: 61-63 days. Weight at dosing: 235-279 g (males), 158-178 g (females).

Administration/exposure

STUDY DESIGN

Animal assignment and treatment:

BAS 550 F was administered dermally to groups of 10 male and 10 female Wistar rats at doses of 0, 100, 300, and 1000 mg/kg bw/day for 28 days. The animals were randomized and assigned to the treatment groups. The test substance was administered uniformly to the clipped dorsal skin (dorsal and dorsolateral parts of the trunk; at least 10% of the body surface) using 3 mL syringes (Becton Dickinson & Co. USA) for about 28 days (5 days per week). The administration volume was 4 mL/kg bw, based upon the latest individual body weight determination. The skin was covered for 6 hours after administration using a semiocclusive dressing, consisting of 4 layers of porous gauze dressing ("Verbandmull Ph. Eur.", Lohmann GmbH & Co KG, Neuwied, Germany) and an elastic dressing (Fixomull Stretch, Beiersdorf AG, Hamburg, Germany). After removal of the dressing, the treated skin was washed with lukewarm water. Control animals received the vehicle, only. At the end of the administration period all surviving animals were sacrificed after a fasting period (withdrawal of food) for about 16-20 hours.

Test substance preparation and analysis:

The test substance was applied as a suspension in 1% aqueous carboxymethylcellulose (CMC). To prepare the suspension, the appropriate amount of test substance was weighed out depending on the desired concentration. Then the vehicle was filled up to the desired volume, subsequently mixed using a magnetic stirrer. During application the test substance preparations were kept homogeneous using a magnetic stirrer. The test substance preparations were prepared twice a week and kept cold in a refrigerator during this time.

The stability of the test substance in the vehicle over a period of 7 days was proven prior to the study (study code PCP06240). For homogeneity and concentration control analyses, each 6 samples of all concentrations were drawn before the start of the administration period (will be used as a concentration control at the same time). The sampling was done under administration conditions out of the beaker (each 2 samples from bottom, mid, and top of the beaker). Each one sample was analyzed in the analytical laboratory; the other one was frozen until finalization of this report.

Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Statistics applied

Parameter	Statistical test
Body weight, feed consumption, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
feces, rearing, grip strength forelimbs, grip strength hindlimbs, footsplay test, motor activity	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians
Clinical pathology parameters, urine volume, urine specific gravity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians
Urinalysis, except color, turbidity, volume and specific gravity	Pairwise comparison of each dose group with the control group using FISHER's exact test for the hypothesis of equal proportions
Weight parameters (pathology)	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians

METHODS

Observations:

A check for moribund and dead animals was made twice daily on working days and once daily on Saturdays, Sundays and public holidays. If animals were in a moribund state, they were sacrificed and necropsied.

All animals were checked daily for any clinically abnormal signs. Abnormalities and changes were documented for each animal. Moreover, the findings on the treated skin were obtained once each workday (as a rule, immediately before application).

Detailed clinical observations (DCO) were performed in all animals prior to the administration period and thereafter at weekly intervals. The findings were ranked according to the degree of severity, if applicable. The animals were transferred to a standard arena (50×37.5 cm with sides of 25 cm high). The following parameters were examined:

1. abnormal behavior during “handling”	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. posture	13. palpebral closure

5. salivation	14. exophthalmus
6. respiration	15. feces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

Body weight:

During the administration period the body weight was determined on study day 0 (start of the administration period) and twice weekly thereafter. The difference between the body weight on the respective day of weighing and the body weight on study day 0 was calculated as body weight change.

Food and water consumption:

Food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and per day. Drinking water consumption was observed by daily visual inspection of the water bottles for any overt changes in volume.

Functional observational battery:

A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 h. The FOB started with passive observations without disturbing the animals, followed by removal from the home cage, open field observations in a standard arena and sensorimotor tests as well as reflex tests. The findings were ranked according to the degree of severity, if applicable. The observations were performed at random.

Home cage observations:

The animals were observed in their closed home cages; any disturbing activities (touching the cage or rack, noise) were avoided during these examinations in order not to influence the behavior of the animals. Attention was paid to:

1. posture	4. abnormal movements
2. tremor	3. convulsions
5. impairment of gait	6. other findings

Open field observations:

The animals were transferred to a standard arena (50×50 cm with sides of 25 cm high) and observed for at least 2 minutes. Following parameters were examined:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions

4. salivation	13. abnormal movements
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

Sensorimotor Tests/Reflexes:

The animals were removed from the open field and subjected to following sensorimotor or reflex tests:

1. approach response	8. behavior during "handling"
2. touch response	9. vocalization
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hindlimbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

Motor activity assessment:

Motor activity assessment (MA) was carried out in all animals at the end of the administration period. Motor activity was measured on the same day as FOB was performed. The examinations were performed using the TSE Labmaster System supplied by TSE Systems GmbH, Bad Homburg, Germany. For this purpose, the animals were placed in new clean polycarbonate cages for the time of measurement. Eighteen beams were allocated per cage. The numbers of beam interrupts were counted over 12 intervals of 5 minutes. The sequence at which the animals were placed in the polycarbonate cages was selected at random.

Ophthalmoscopy:

The eyes of all animals were examined prior to the start of the administration period. At the end of the administration period, i.e. study day 28, the eyes of animals in test groups 0 (control) and 3 (1000 mg/kg bw/d) were examined for any changes using an ophthalmoscope (HEINE OPTOTECHNIK, Herrsching, Germany) after administration of a mydriatic (Mydrum, Chauvin ankerpharm GmbH, Rudolstadt, Germany).

Hematology and clinical chemistry:

In the morning, blood was taken from the retroorbital venous plexus from fasted animals. The animals were anaesthetized using isoflurane (Isoba®, Essex GmbH, Munich, Germany). The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence. For urinalysis the individual animals were transferred to metabolism cages (withdrawal of food and water) and

urine was collected overnight. The following examinations were carried out in 10 animals per test group and sex.

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Platelet count
✓	Hemoglobin (Hb)	✓	Differential blood count	✓	Prothrombin time
✓	Hematocrit (Hct)				
✓	Mean corpuscular volume (MCV)				
✓	Mean corpuscular hemoglobin (MCH)				
✓	Mean corpuscular hemoglobin concentration (MCHC)				
✓	Reticulocytes				
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Potassium	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Sodium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Chloride	✓	Globulin (by calculation)	✓	Lactic dehydrogenase
✓	Phosphate (inorganic)	✓	Glucose (fasted)	✓	γ-GT
✓	Magnesium	✓	Protein (total)		
		✓	Urea		
		✓	Creatinine		
		✓	Triglycerides		
		✓	Bile acids		
	Urinalysis				
			Semiquantitative parameters		
✓	Specific gravity	✓	Bilirubin	✓	Protein
✓	Colour	✓	Glucose	✓	pH-value
✓	Turbidity	✓	Ketones	✓	Urobilinogen
✓	Volume		Blood	✓	Sediment (microscopical exam.)

Sacrifice and pathology:

The animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Weight assessment was carried out on all animals sacrificed at scheduled dates. The organs were sampled, weighed, and examined histopathologically as indicated in the table below.

Pathology:											
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).											
C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓		#	lymph nodes[#]	✓	✓	#	testes
✓		#	aorta	✓		#	mammary gland (♀)	✓	✓	#	thymus
✓		#	bone marrow[§]	✓			muscle, skeletal	✓	✓	#	thyroid glands
✓	✓	#	brain	✓		#	nerve, peripheral (sciatic n.)	✓		#	trachea
✓		#	caecum	✓		#	nose	✓		#	urinary bladder
✓		#	colon	✓	✓	#	ovaries	✓	✓	#	uterus, oviducts, vagina

✓		#	duodenum	✓		#	pancreas	✓	✓		body (anesthetized animals)
✓		#	esophagus	✓	✓	#	parathyroid glands				
✓	✓	#	epididymides	✓		#	pharynx				
✓		#	eyes (with optic nerve)	✓		#	pituitary				
✓		✓	gross lesions	✓		#	prostate				
			Harderian gland	✓		#	rectum				
✓	✓	#	heart	✓		#	salivary glands				
✓		#	ileum	✓		#	seminal vesicle				
✓		#	jejunum (w. Payer's plaque)	✓		#	skin (treated and untreated)				
✓	✓	#	kidneys	✓		#	spinal cord (3 levels)				
✓	✓	✓	liver	✓	✓	#	spleen				
✓		#	larynx	✓			sternum w. marrow				
✓		#	lung	✓		#	stomach (cardia, fundus and pylorus)				
§ from femur; # axillary and mesenteric											

The organs or tissues were fixed in 4% formalin solution. The hematoxylin-eosin (HE) stained slides were examined and assessed by light microscopy.

Results and discussion

A. ANALYSIS

The stability of the test substance BAS 550 F (Dimethomorph) in M4-, OECD- and tap water over a period of 7 days at room temperature was verified analytically before the start of the study. Considering the low standard deviation in the homogeneity analysis, it can be concluded that BAS 550 F (Dimethomorph) was distributed homogeneously in drinking water containing 1% carboxymethylcellulose. The concentration control analyses of all concentrations revealed that the values were in the expected range of the target concentrations, i.e. were always in a range of 97.9-102.5% of the nominal concentration.

B. OBSERVATIONS

Clinical signs of toxicity

No test substance-related effects were observed.

Mortality

No animal died prematurely in the present study.

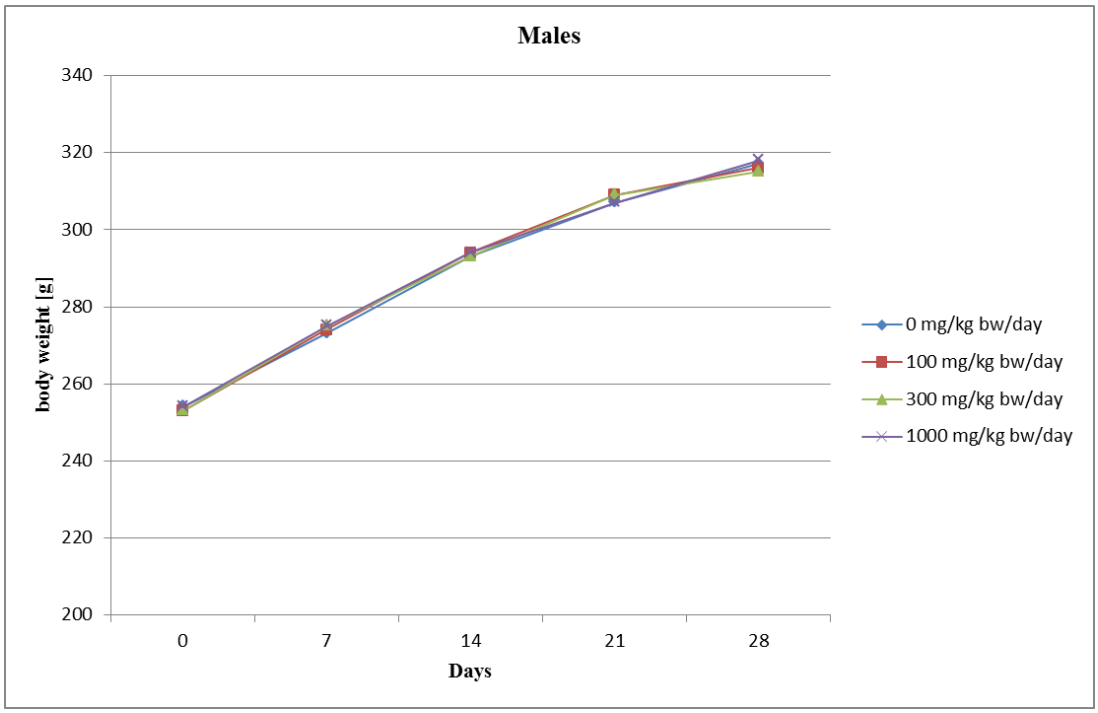
Ophthalmoscopy

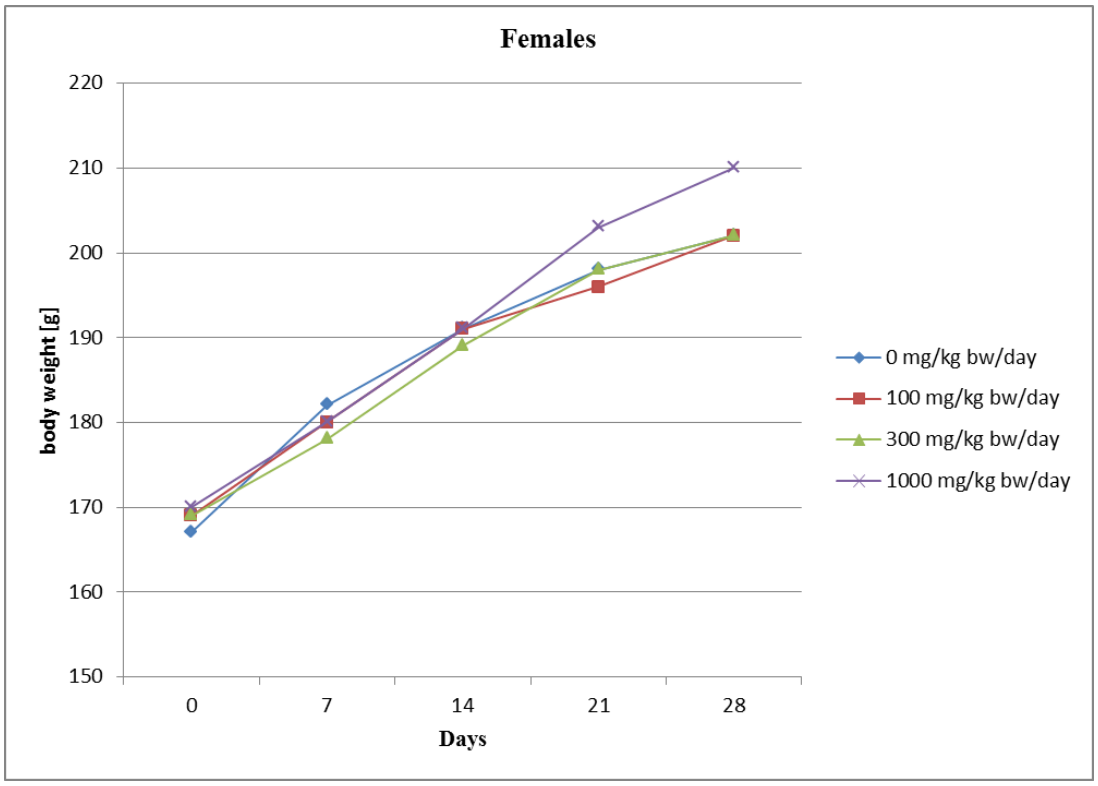
Because findings occurred in single animals only and a dose-response relationship was not observed all findings were assessed as being incidental in nature.

C. BODY WEIGHT AND BODY WEIGHT GAIN

No test substance-related effects on body weights in both sexes were observed. Body weight change in females was decreased in all dosed animals, with the exception of animals of test group 3 (1000 mg/kg bw/day) on days 21 and 28 (+15.7%) and a statistically significant maximum of -35.4% in females of test group 2 on day 7 of the application period. Due to the fact that no correlation to food consumption and body weight was observed this variability could be assessed as not related to treatment.

Figure 8: Body weight development of rats administered dimethomorph for 28 days





D. FOOD AND WATER CONSUMPTION

No test substance-related changes on food and water consumption were observed.

E. FUNCTIONAL OBSERVATIONAL BATTERY

Deviations from "zero values" were obtained in several animals. However, as most findings were equally distributed between test substance-treated groups and controls, were without a dose-response relationship or occurred in single animals only, these observations were considered to have been incidental. Furthermore no test substance-related findings were observed at “Home cage observations”, “Open field observations”, “Sensorimotor tests/reflexes”, and “Quantitative parameters”.

F. MOTOR ACTIVITY MEASUREMENT

Regarding the overall motor activity, a significantly increased value was detected only in female animals of test group 2 (300 mg/kg bw/d). As there was no comparable effect at higher doses and, thus, no dose-response relationship observable this finding was assessed as being not related to treatment. Comparing the single intervals of test substance-treated groups with the control groups, the isolated significant increase of interrupts in females of test group 2 (300 mg/kg bw/d) at interval 6 and of test group 3 (1000 mg/kg bw/d) at interval 3 and was considered to be spontaneous in nature and not test substance-related.

F. CLINICAL PATHOLOGY

1. Hematological findings

No treatment-related changes among hematological parameters were detected. In males of test group 1 (100 mg/kg bw/d) red blood cell (RBC) counts and hematocrit values were lower compared to controls, but this parameter was not dose-dependently changed. Therefore, this alteration was regarded as incidental and not treatment-related. In females of test group 3 (1000 mg/kg bw/d) relative reticulocyte counts were higher compared to controls, but this increase was still within the historical control range (1.0-4.9%, HCD:study years 2008-2010, 31 studies in female Wistar rats age 10 weeks) and, this effect has not been observed in other studies therefore, it was regarded as incidental and not treatment-related.

Table 112: Selected hematology findings in rats administered dimethomorph for 28 days (group means)

	RBC [tera/L]	HGB [mmol/L]	HCT [L/L]	RETI [%]
Males				
Control	8.65±0.28	9.7±0.2	0.434±0.009	1.5±0.3

	RBC	HGB	HCT	RETI
100 mg/kg bw/day	**8.18±0.30****	9.4±0.2	**0.419±0.008****	1.6±0.2
3000 mg/kg bw/day	8.49±0.24	9.6±0.2	0.426±0.008	1.4±0.3
1000 mg/kg bw/day	8.57±0.44	9.5±0.3	0.430±0.012	1.6±0.3
Females				
Control	8.07±0.22	9.3±0.3	0.411±0.016	2.3±1.1
100 mg/kg bw/day	8.04±0.34	9.2±0.3	0.407±0.013	1.9±0.5
300 mg/kg bw/day	8.22±0.40	9.5±0.5	0.421±0.017	1.9±0.6
1000 mg/kg bw/day	7.69±0.61	9.2±0.5	0.397±0.033	**2.8±0.7***

* = p≤0.05; ** = p≤0.01

2. Clinical chemistry findings

No treatment-related, adverse changes among clinical chemistry parameters were measured. In males of test group 3 (1000 mg/kg bw/d) inorganic phosphate levels were higher compared to controls but these values were within the historical control range (1.90-2.63 mmol/L, HCD: study years 2008-2010, 31 studies in male Wistar rats age 10 weeks) and, therefore, were regarded as incidental and not treatment-related. In females of test group 2 (300 mg/kg bw/d) the potassium levels were higher and the total bilirubin levels were lower compared to controls and, additionally, in females of test group 1 (100 mg/kg bw/d) the total bilirubin levels were decreased. These values were not changed dose-dependently and, therefore, the alterations were considered as incidental and not treatment-related. In females of test group 3 (1000 mg/kg bw/d) the cholesterol levels were increased, but the values were still within the historical control range (0.95-1.96 mmol/L, HCD: study years 2008-2010, 31 studies in female Wistar rats age 10 weeks) and was not observed in males. Therefore, this effect was regarded as incidental and not treatment-related.

Table 113: Selected clinical chemistry findings in rats administered dimethomorph for 28 days (group means)

Dose	Males				Females			
[mg/kg bw/d]	0	100	300	1000	0	100	300	1000
Phosphate (inorganic) [mmol/L]	2.21±0.11	2.31±0.18	2.22±0.13	**2.38±0.11****	2.08±0.23	2.12±0.23	2.14±0.24	2.04±0.24
Potassium [mmol/L]	4.75±0.20	4.70±0.29	4.62±0.29	4.71±0.27	4.23±0.23	4.35±0.18	**4.66±0.21****	4.22±0.44
Cholesterol [mmol/L]	1.92±0.21	1.87±0.26	1.94±0.33	1.89±0.33	1.37±0.44	1.20±0.23	1.22±0.31	**1.71±0.40***
Bili, total [µmol/L]	2.29±0.42	2.31±0.51	2.36±0.23	2.60±0.33	3.15±0.70	**2.32±0.37****	**2.48±0.48***	2.60±0.59

* = p≤0.05; ** = p≤0.01

3. Urinalysis

No treatment-related changes among urinalyses parameters were detected.

E. NECROPSY

1. Organ weight

Absolute weights

The significant weight increase of the adrenal glands in males of test groups 1 (100 mg/kg bw/d,) and 3 (1000 mg/kg bw/d) occurred without dose-relationship and was therefore regarded as incidental and not related to treatment. All other mean absolute weight parameters did not show relevant differences when compared to the control group and were considered to be within the normal range.

Table 114: Mean absolute organ weights in rats administered dimethomorph for 28 days (group means)

	Terminal bw [g]		Adrenals [mg]	
	Males	Females	Males	Females
Control	288±18	183±7	58.7±7.6	77.9±9.3
100 mg/kg bw/day	288±19	181±11	**66.7±4.5****	74.5±6.6
300 mg/kg bw/day	285±22	182±10	63.4±8.0	77.8±10.3
1000 mg/kg bw/day	288±22	188±11	**68.0±8.8***	79.5±5.7

Relative weights

The significant weight increase observed in adrenal glands in males of test groups 1 (100 mg/kg bw/d) and 3 (1000 mg/kg bw/d), as well as the significant liver weight decrease in male animals of test groups 1-3 (100, 300 and 1000 mg/kg bw/d) occurred without a dose-relationship. In females, a weak significant liver weight increase without dose-relationship was observed at all dose levels. All these changes in males and females were slight and without a histopathological correlate and considered to be incidental and not treatment-related. All other mean relative weight parameters of treated animals did not show relevant differences when compared to the control groups.

Table 115: Mean relative organ weights in rats administered dimethomorph for 28 days (group means)

	Liver [%]		Adrenals [%]	
	Males	Females	Males	Females
Control	2.63±0.1	2.51±0.12	0.02±0.003	0.043±0.006
100 mg/kg bw/day	**2.50±0.05***	**2.62±0.09***	**0.023±0.002***	0.041±0.004
300 mg/kg bw/day	**2.44±0.18****	**2.71±0.24***	0.022±0.003	0.043±0.005
1000 mg/kg bw/day	**2.51±0.13***	**2.70±0.18***	**0.024±0.003****	0.042±0.005

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

2. Gross and histopathology

Gross lesions:

All macroscopic findings occurred either individually and were considered to be incidental or spontaneous in origin and without any relation to treatment.

Histopathology
All findings were either single observations, or were biologically equally distributed between control and treated rats. All of them were considered to be incidental and/or spontaneous in origin.

III. CONCLUSIONS
The dermal administration of dimethomorph (BAS 550 F) to male and female Wistar rats over a period of 28 days did not reveal test substance related adverse signs of systemic toxicity at dose levels of 100, 300, or 1000 mg/kg bw/d. Increased liver and adrenal weights were observed, but were considered incidental, lacking a dose-response relationship. Histopathology revealed no findings. Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) for systemic and local effects was 1000 mg/kg bw/d in male and female animals. The study author concluded the same NOAEL.

3.12.1.11 Long term toxicity and carcinogenicity in rats 1.

Study reference:

B.6.5.1.1-study 1 - Anonymous 1990, SAG 151: 104 week dietary toxicity study in rats, unpublished, BASF Document No, DK-427-006 (BASF Document No. DK-427-004 [Addendum BASF Document No. DK-427-005/1991 (revised figures)])

Detailed study summary and results:

Test type

Guideline: EU Testing Method B 30 (US EPA Guideline 83-5; Guidelines of OECD and Japanese MAFF, claimed by the author)

Deviations: None from the original guideline. Deviations from current OECD guideline:
- Cholesterol not measured.
- Thyroid and uterus were not weighed.

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme).

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (SAG 151; CME 151); Batch No. DW 11/86; purity 96.6 %

Test animals

Male and female Sprague-Dawley rats, 20/sex/dose (age: 4 weeks on arrival, weight: 80-103 g males, 51-63 g females).

Administration/exposure

Dimethomorph was administered to groups of 20 male and 20 female Sprague-Dawley rats at dietary concentrations of 0, 200, 750, and 2000 ppm for 104 weeks.

Food consumption and body weight were determined once a week during the first 13 weeks, and thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Ophthalmological examinations were carried pretrial and during weeks 51 and 102 on 10 males and 10 females from each dose group. Urinalysis, clinical chemistry and hematological examinations were carried out during weeks 25, 51, 77 and 102 of the study. All animals were subjected to detailed gross and histopathological evaluation and organ weight analysis.

Results and discussion

The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis. Based on food consumption data, the average test substance intake values during the 2-year treatment period for the 200, 750, and 2000 ppm groups are given in Table 116.

Table 116: Test substance intake

Dietary Dose Level (ppm)	Test Substance Intake (mg/kg bw/day)	
	Males	Females
200	9.4	11.9
750	36.2	57.7
2000	99.9	157.8

Survival rates at study termination for the control, 200, 750 and 2000 ppm groups were 45 %, 65 %, 70 % and 75 %, respectively, for males, and 50%, 55 %, 70 % and 50 %, respectively, for females. There were no clinical signs of toxicity related to dimethomorph treatment.

There was a marked reduction in overall body weight gain for the 2000 ppm males (33 %) which in terms of absolute body weight, attained statistical significance from weeks 65 – 104. Males treated at 200 and 750 ppm exhibited decreases in body weight gain during the 18-month to 2-year period of the study, as compared to controls, which corresponded to non-statistically significant decreased mean body weights at study termination. However, these reductions in body weight gain/mean body weights for males at 200 and 750 ppm were not considered treatment related because they occurred only at the end of the study, were not evident at 90 days, 1 year or 18 months, and no clear dose-response was evident. In addition, males at 200

and 750 ppm did not show a decreased weight gain as compared to control in the corresponding oncogenicity study, using a higher number of animals per dose group. For females at 2000 ppm, a moderate reduction in overall body weight gain (17%) was observed, which in terms of absolute body weight, attained statistical significance from week 1 – 40 and 45 – 96. Females at 750 ppm showed reduced body weight gains compared to controls over the first 88 weeks of study. However, this effect was mainly due to a strong increase in body weight between week 72 and week 88 in the control group. Considering that the overall body weight gain was only reduced by 2% the effect on body weight was not considered to be adverse.
No effect on food consumption was observed.

Table 117: 104-week dietary toxicity study in rats – body weight (g) group mean values

Treatment Period (weeks)	Males				Females			
	Dose group/Dose Level (ppm)							
	0	**200**	**750**	**2000**	**0**	**200**	**750**	**2000**
0	181	187	181	186	124	121	124	120
13	459	465	480	456	284	270	**267***	**239*****
52	596	618	**633***	578	360	364	340	**301*****
72	630	637	661	**560*****	398	412	376	**330*****
88	662	666	667	**545*****	422	441	**369***	**338*****
104	692	626	633	**530*****	396	463	390	346
Weight gain (g) Weeks 0 – 104	511	439	452	344	272	342	266	226
% of Controls	-	86	88	67	-	126	98	83

(F-max test, parametric ANOVA, Student`s t-test, Kruskal-Wallis ANOVA)
* = Significant different from control, p <0.05
** = Significant different from control, p <0.01
*** = Significant different from control, p <0.001

Changes in red blood cell parameters indicative of a mild anemia occurred at 2000 ppm, including decreases in red blood cell count (males and females) and decreases in hemoglobin level and hematocrit (females). Females receiving 2000 ppm exhibited increased bone marrow cellularity, consistent with erythropoiesis, indicative of a compensatory effect related to the anemia.
No treatment-related effects on clinical chemistry or urinalysis were observed.

Table 118: 104-week dietary toxicity study in rats – red blood cell parameters

Treatment Period (weeks)	Males				Females			
	Dose group/Dose level (ppm)							
	0	200	750	2000	0	200	750	2000
Week 11								
Hb	15.6 ± 0.8	15.6 ± 0.4	15.04 ± 0.6	15.2 ± 0.5	15.1 ± 0.6	15.11 ± 0.5	15.0 ± 0.8	**14.5** ± 0.5**
RBC	7.63 ± 0.47	7.64 ± 0.27	7.50 ± 0.29	**7.32** ± 0.31**	7.43 ± 0.26	7.41 ± 0.25	7.33 ± 0.41	**7.00*** ± 0.27**
HCT	0.433 ± 0.021	0.439 ± 0.012	0.429 ± 0.017	0.425 ± 0.013	0.428 ± 0.016	0.429 ± 0.011	0.425 ± 0.021	**0.414* ± 0.018**
Week 25/26								
Hb	15.2 ± 0.5	15.2 ± 0.6	15.2 ± 0.4	**14.8* ± 0.6**	14.6 ± 0.8	14.6 ± 0.9	14.5 ± 0.6	**13.5*** ± 0.7**
RBC	8.05 ± 0.32	8.07 ± 0.36	7.72 ± 1.76	**7.76* ± 0.38**	7.44 ± 0.51	7.46 ± 0.51	7.44 ± 0.30	**6.83*** ± 0.30**
HCT	0.440 ± 0.029	0.445 ± 0.023	0.439 ± 0.021	0.431 ± 0.022	0.413 ± 0.019	0.415 ± 0.026	0.412 ± 0.015	**0.391*** ± 0.019**
Week 51/52								
Hb	14.8 ± 0.7	14.8 ± 0.5	**14.1** ± 0.8**	**14.3* ± 0.7**	13.8 ± 0.9	13.9 ± 1.0	13.5 ± 1.3	**12.7 ± 0.9****
RBC	7.87 ± 0.51	7.71* ± 0.22	**7.49*** ± 0.49**	**7.44*** ± 0.42**	7.02 ± 0.49	7.05 ± 0.52	6.87 ± 0.76	**6.35*** 0.48**
HCT	0.399 ± 0.019	0.402 ± 0.014	**0.382** ± 0.020**	0.390 ± 0.018	0.380 ± 0.024	0.384 ± 0.028	0.375 ± 0.032	**0.354** ± 0.025**
Week 77/78								
Hb	14.5 ± 0.6	14.1 ± 1.4	13.9 ± 0.7	13.8 ± 1.0	14.7 ± 0.9	14.1 ± 1.7	14.0 ± 1.0	**12.5*** ± 1.2**
RBC	7.49 ± 0.23	7.17 ± 0.73	7.26 ± 0.36	**7.00** ± 0.51**	7.03 ± 0.51	6.63 ± 1.06	6.65 ± 0.53	**5.84*** ± 0.61**
HCT	0.397 ± 0.025	0.400 ± 0.038	0.383 ± 0.021	0.387 ± 0.033	0.393 ± 0.029	0.376 ± 0.043	0.375 ± 0.025	**0.339*** 0.029**
Week 102/103								
Hb	14.7 ± 1.0	13.3 ± 2.8	13.8 ± 2.0	13.9 ± 1.3	13.6 ± 1.6	13.5 ± 1.4	13.3 ± 1.4	12.6 ± 1.9
RBC	7.54 ± 0.38	6.71 ± 1.44	7.08 ± 1.05	7.15 ± 0.58	6.63 ± 0.74	6.37 ± 0.75	6.28 ± 0.85	5.86 ± 1.20
HCT	0.419 ± 0.029	0.378 ± 0.074	0.393 ± 0.051	0.396 ± 0.033	0.391 ± 0.043	0.389 ± 0.036	0.380 ± 0.036	0.366 ± 0.049

Hb = hemoglobin g dL^{-1} RBC = total red blood cell count 10^{12} L^{-1} HCT = hematocrit l L^{-1}
* = significantly different from control, $p < 0.05$; ** = significantly different from control, $p < 0.01$; *** = significantly different from control, $p < 0.001$ (F-max test, Student`s t-test, log or square root transformation, Kruskal-Wallis ANOVA))

No treatment related effects on organ weights was observed. Histopathological findings included a statistically significant increased incidence of hepatocellular hypertrophy and/or increased amount of pigment in hepatocytes for females at 2000 ppm. In addition, an increased incidence of ”ground-glass” foci of cellular alteration in the liver for both sexes was observed at 2000 ppm and for females at 750 ppm.

However, in neither sex did this change attain statistical significance. No historical control data is available. The altered hepatocytes contained pale, granular, acidophilic cytoplasm and a variable content of glycogen.

Table 119: 104-week dietary toxicity study in male rats – incidence of histopathological findings in the liver

Dose (ppm)	0	200	750	2000
Liver - ground glass focus of cellular alteration				
Number of animals	20	20	20	20
1 - 5 foci	7	6	9	12
5 - 10 foci	0	0	1	2
15 - 20 foci	0	1	0	0
total	7	7	10	14

Fisher`s exact test

Table 120: 104-week dietary toxicity study in female rats – incidence of histopathological findings in the liver

Dose (ppm)	0	200	750	2000
Liver - ground glass focus of cellular alteration				
Number of animals	20	20	20	20
1 - 5 foci	1	4	6	5
5 - 10 foci	0	0	0	1
total	1	4	6	6
Accumulation of pigment in periacinar hepatocytes				
Number of animals	20	20	20	20
minimal	0	0	3	7
slight	0	0	0	4
moderate	0	0	0	5
total	0	0	3	**16***
Hypertrophy of periacinar hepatocytes				
Number of animals	20	20	20	20
minimal	0	0	1	4
slight	0	0	1	4
total	0	0	2	**8***

Fisher`s exact test

There were increased incidences of dilated mesenteric blood vessels, arteritis and testicular interstitial cell proliferation in males at 2000 ppm. No treatment-related organ weight changes were observed for either males or females at study termination.

Table 121: 104-week dietary toxicity study in male rats – incidence of histopathological findings in the testes

Testes - focal interstitial cell hyperplasia				
Dose (ppm)	**0**	**200**	**750**	**2000**
Number of animals	19	20	20	20
Focal hyperplasia	2	5	6	7
Testicular tumors				
One interstitial cell adenoma	1	4	3	5
Two interstitial cell adenoma	1	1	1	1
Total animals bearing one or more tumors	2	5	4	6

* There was no significant difference in the incidence of interstitial cell hyperplasia or adenoma when these findings were analysed independently (Fisher`s test or age-adjusted analysis).

Conclusion:

Based on the liver findings in females at 750 ppm, the NOAEL for chronic toxicity in this study was 200 ppm. This concentration of 200 ppm is equal to approximately 9 mg/kg bw/day, based on food consumption data.

3.12.1.12 Long term toxicity and carcinogenicity in rats 2.

Study reference:

B.6.5.1.1- study 2 - Anonymous 1991, SAG 151: 104 week dietary carcinogenicity study in rats, unpublished, BASF Document No. DK-428-005

Detailed study summary and results:

Test type

Guideline: EU Testing Method B 32 (US EPA Guideline 83-5; Guidelines of OECD and Japanese MAFF claimed by the author)

Deviations: None

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.)

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (SAG 151; CME 151); Batch No. DW 11/86; purity 96.6 %

Test animals

Male and female Sprague-Dawley rats, 50/sex/dose

Administration/exposure

Dimethomorph technical was administered to groups of 50 male and 50 female Sprague-Dawley rats at dietary concentrations of 0, 200, 750, and 2000 ppm for 104 weeks.

Food consumption and body weight were determined once a week during the first 13 weeks, and thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Differential blood counts were determined for all surviving animals during weeks 51/52, 78/79 and 103/104 of the study. After 24 months, the animals were subjected to detailed gross and histopathological examinations.

Results and discussion

The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis. Based on food consumption data, the average test substance intake values during the 2-year treatment period for the 200, 750, and 2000 ppm groups are given in Table 122.

Table 122: Test substance intake

Dietary Dose Level (ppm)	Test Substance Intake (mg/kg bw/day)	
	Males	Females
200	8.8	11.3
750	33.9	46.3
2000	94.6	132.5

There were no treatment-related effects on mortality or clinical signs of toxicity throughout the 104-week study period. Survival rates were 44%, 60%, 68% and 54% (males) and 60%, 62%, 60% and 52% in females, respectively. A slight decrease (7 %) in absolute food consumption was noted for females fed 2000 ppm. Treatment related reductions in overall body weight gain were observed for males and females at 2000 ppm (14 and 38 %, respectively) and for females at 750 ppm (23 %) over a 2-year period, as compared to controls.

Table 123: 104-week dietary carcinogenicity study in rats – body weight (g) group mean values

Treatment Period (weeks)	Males				Females			
	Dose group/Dose Level (ppm)							
	0	200	750	2000	0	200	750	2000

0	192	192	195	193	120	123	121	120
13	489	484	501	**456****	282	279	**267*****	**245*****
52	642	668	670	617	363	370	**340***	**301*****
72	663	689	688	640	403	396	**361*****	**317*****
104	680	716	716	**611***	478	450	**395*****	**341*****
Weight Gain (g) Weeks 0 – 104	488	524	521	418	358	327	274	221
% of Controls	-	107	107	86	-	91	77	62

(F-max test, parametric ANOVA, Student`s t-test, Kruskal-Wallis ANOVA)
* = Significant different from control, $p < 0.05$
** = Significant different from control, $p < 0.01$
*** = Significant different from control, $p < 0.001$

There were no treatment-related effects in differential blood counts at any timepoint. Non-neoplastic findings, similar to those observed in the 2-year dietary toxicity study in rats included a statistically significant increased incidence of dilated mesenteric blood vessels and arteritis in the abdominal vessels, predominantly in the pancreas, for males at 2000 ppm. There was a statistically significant increase in "ground-glass" foci of cellular alteration in the liver for males and females at 2000 ppm. The altered hepatocytes contained pale, granular, acidophilic cytoplasm and a variable content of glycogen. Furthermore there was a statistically significant increased incidence in hepatocellular pigmentation and hypertrophy and increased severity of bone marrow cellularity for females at 2000 ppm.

Table 124: 104-week dietary carcinogenicity study in rats – Incidence of histopathological findings in the liver

Liver - ground glass focus of cellular alteration				
Dose (ppm)	**0**	**200**	**750**	**2000**
Males				
Number of animals	50	50	50	50
1 - 5 foci	17	16	18	21
5 - 10 foci	0	1	2	2
10 -15 foci	0	0	0	2
15 - 20 foci	0	1	1	0
25 - 30 foci	0	0	0	1
30 - 35 foci	0	0	0	1
total	17	18	21	**27***
Females				
Dose (ppm)	0	200	750	2000
Number of animals	50	50	50	50

1 - 5 foci	12	9	16	**18***

* = Fisher`s Exact Probability test: significant different from control, p < 0.01 for males; p < 0.05 females

Table 125: 104-week dietary carcinogenicity study in rats – Incidence of histopathological findings in the liver

Liver - accumulation of pigment in periacinar hepatocytes				
Dose (ppm)	**0**	**200**	**750**	**2000**
Females				
Number of animals	50	50	50	50
Minimal	1	0	2	10
Slight	0	0	2	9
Moderate	0	0	0	6
Marked	0	0	0	1
Total	1	0	4	**26***

* = Fisher`s Exact Probability test: significant different from control, p < 0.05 females

Table 126: 104-week dietary carcinogenicity study in rats – Incidence of histopathological findings in the liver

Liver - hypertrophy of periacinar hepatocytes				
Dose (ppm)	**0**	**200**	**750**	**2000**
Females				
Number of animals	50	50	50	50
Slight	0	0	1	**15***

* = Fisher`s Exact Probability test: significant different from control, p < 0.05 females

Table 127: 104-week dietary carcinogenicity study in male rats – Incidence of testicular hyperplasia and adenoma

Dose /Group (ppm)	**0**	**200**	**750**	**2000**
Number of animals	50	49	50	50
Focal Interstitial Cell Hyperplasia	6	6	10	10
Adenoma	5	7	8	10
Adenoma and Focal Hyperplasia	8	12	12	15

Statistical analysed using methods described by Peto et al.

In both 104-week dietary toxicity studies in rats the incidences of testicular tumors in treatment groups were higher than incidences in control groups. However, for the following reasons dimethomorph is not considered to be oncogenic:

(1) Increased longevity in the chronic toxicity study

For the 2-year chronic toxicity study, increased longevity observed for males at 2000 ppm likely predisposed these animals to increased incidences of benign interstitial cell tumors given that more animals in this group lived longer than animals in the other treatment groups or control group. Specifically, the survival rate at 104 weeks for males at 2000 ppm was 75 %, as compared to 45, 65, and 70 % for males at 0, 200, and 750 ppm, respectively. For premature male decedents, only one rat per each dietary concentration had a benign interstitial cell tumor. All other testicular tumors of this cell type were observed in males that survived until terminal sacrifice. In the 104 week carcinogenicity study the survival rate of male animals of the control group was also lower than the survival rates of male animals in the treatment groups. However, the differences are lower than in the chronic toxicity study.

(2) No statistical significant oncogenic effects

There was no statistical significance in the incidence of testicular interstitial cell adenomas between the control group and any of the dimethomorph treated groups for either study ($p > 0.05$). Moreover, in a trend analysis, considered to be a more sensitive test to detect dose-related trends than a pair-wise comparison, no statistically significant positive trends were observed ($p > 0.05$).

(3) Incidence values for benign testicular interstitial cell tumors are within or just slightly higher the historical ranges for the testing laboratory.

The range of the historical spontaneous incidence of testicular interstitial cell adenomas for carcinogenicity studies performed at Anonymous Laboratory the laboratory which conducted the rat carcinogenicity study on dimethomorph, is from 4 % (minimal control incidence) to 20 % (maximal control incidence). Therefore, the 20 % incidence of interstitial cell adenoma of the testes in the high-dose group from the rat carcinogenicity study with dimethomorph is within the range of the historical control incidence of this laboratory. In addition, the incidence from the combined two studies for interstitial cell adenoma of the testes in the high-dose group is 16/70 (22.9 %), just slightly higher the maximum historical incidence. For the first study (DK-427-006) the incidence is outside the historical control range. However, this is the study with a much higher survival rate in the high dose group compared to the control.

Table 128: Historical incidence of neoplastic and focal hyperplastic lesions in testicular interstitial cells of control Sprague-Dawley rats in 104 week studies at Anonymous Laboratory

Study I.D.	Date	No examined	Incidence *		
			Adenoma	Hyperplasia	Combined
198	1986-1988	50	10 (20)[a]	0	10 (20)
507	1987-1989	50	9 (18)	1 (2)	10 (20)
827	1986-1988	48	4 (8.3)	9 (18.8)	13 (27.1)
261	1985-1987	49	2 (4.1)	7 (14.3)	9 (18.4)
717	1985-1987	48	5 (10.4)	3 (6.2)	8 (16.7)
671	1984-1986	50	6 (12)	1 (2)	7 (14)
729	1984-1986	50	4 (8)	0	4 (8)
802	1984-1986	50	6 (12)	0	6 (12)
Range			4.1 – 20 %	0 – 18.8 %	8 – 27.1 %

*There was no single case of malignant tumours recorded in these studies
a Numbers in bracket represents incidence in %

(4) No progression of benign testicular adenomas to malignant carcinomas

There was no evidence of progression of benign interstitial cell adenomas to malignant interstitial cell carcinomas.

(5) No decreased latency for the benign testicular interstitial cell tumors from the high-dose group versus the controls.

There was no indication that testicular interstitial cell adenomas developed earlier in treated rats, as compared to controls.

(6) Lack of oncogenic effects for the testes in the 2-year oncogenicity study in the mouse

(7) Absence of mutagenic or genotoxic activity of dimethomorph

(8) Very conservative size criterion was used on microscopic examination to diagnose benign testicular interstitial cell adenomas. Thus, some of these benign tumors may be downgraded to interstitial cell hyperplasia. The major criterion used for the differential diagnosis of benign testicular interstitial cell adenoma versus hyperplasia was based on size. The pathologists considered that any focal, expansile aggregates of interstitial cells of greater diameter than an average arteriole but smaller than a cross section of a seminiferous tubule were recorded as focal hyperplasia. Those greater in diameter than an average cross section of seminiferous tubule were classified as an adenoma. However, less conservative, present day diagnostic criteria for standardization purposes recommend that the diameter (or cross sections) of 3 or more seminiferous tubules be set as the arbitrary separation of interstitial cell neoplasia, as distinguished from focal hyperplasia. Thus, some of the benign interstitial cell adenomas may possibly downgraded to focal interstitial hyperplasia, using the present-day, less conservative size criterion.

Conclusion:

Based on a decrease in overall body weight gain for females at 750 ppm, the NOAEL for chronic toxicity for this study is 200 ppm, equivalent to approximately 9 mg/kg bw/day. Dimethomorph is not considered to be oncogenic. The NOAEL for carcinogenicity is 750 ppm (equivalent to 94.6 mg/kg bw in males and 132.5 mg/kg bw in females).

3.12.1.13 Long term toxicity and carcinogenicity study in mice.

Study reference:

B.6.5.2 - Anonymous 1991, SAG 151: 104 week dietary carcinogenicity study in mice, unpublished, BASF Document No. DK-428-004

Detailed study summary and results:

Test type

Guideline: EU Testing Method B.32 (US EPA Guideline 83-2; Guidelines of OECD and Japanese MAFF, claimed by the author)

Deviations: None

GLP: Yes (This laboratory certified by the Department of Health of the Government of the United Kingdom as part of the UK GLP Compliance Programme.)

Acceptability: The study is considered to be acceptable.

Test substance

Dimethomorph (SAG 151; CME 151); Batch No. DW 11/86; purity 96.6 %

Test animals

CD-1 mice, 50/sex/dose (~4 weeks old, males 20-22 g, females 16-19 g)

Administration/exposure

Dimethomorph technical was administered to groups of 50 male and 50 female CD-1 mice at adjusted nominal dietary dose levels of 0, 10, 100, and 1000 mg/kg bw/day for 104 weeks. The concentrations of the test material in the diet were adjusted according to body weights and dietary intake at weekly intervals for the first 13 weeks of the study and every 4 weeks thereafter, in order to maintain a constant test material intake. Food consumption and body weight were determined once a week during the first 13 weeks, and thereafter at 4-week intervals. A check of the general state of health of the animals was made at least daily. Additionally, the animals were examined in detail and palpated once a week. Differential blood counts were evaluated after 52, 78 and 104 weeks of treatment for all surviving animals in the control and 1000 mg/kg bw/day groups. A satellite group of 15 mice/sex received the test material at a dose level of 1000 mg/kg bw/day and a control satellite group of 4 males and 6 females were designed to provide differential blood

counts, clinical chemistry, organ weight and liver histology data following 13 and 52 weeks of treatment. All satellite control animals and 8 satellite animals/sex in the 1000 mg/kg bw/day group were sacrificed following 13 weeks of treatment. All other satellite animals in the 1000 mg/kg bw/day group were sacrificed after 52 weeks of treatment (without concurrent controls).

Results and discussion

The stability and homogeneity distribution of the test substance in the diet, as well as the correctness of the concentration, were confirmed by analysis. Based on food consumption data, the average test substance intake values during the 2-year treatment period for the nominal dose levels of 10, 100 and 1000 mg/kg bw/day are given in Table 129.

Table 129: Test substance intake

Nominal Dose Level (mg/kg b.w./day)	Actual Test Substance Intake (mg/kg b.w./day)	
	Males	Females
10	9.8	9.8
100	98.0	96.8
1000	978.0	977.0

Survival for males and females was unaffected by administration of the test material as adjusted survival rates for the treated groups were comparable to or greater than the controls. Specifically, adjusted survival rates at study termination for the control, 10, 100 and 1000 mg/kg bw/day groups were 54 %, 78 %, 50 % and 74 %, respectively for males, and 52 %, 44 %, 50 % and 54 %, respectively for females. No overt clinical signs were attributed to administration of the test material during the 24-month study period.

Food consumption values for males and females at all treatment levels were comparable to controls for most measurement intervals during the 24-month treatment period. Statistically significant reductions in body weights were observed during weeks 17 through 84 and from weeks 92-104 for males at 1000 mg/kg bw/day when compared to controls. Overall body weight gains was reduced 17 % for males at 1000 mg/kg bw/day when compared to controls. Although no significant differences in body weights were observed during the 24-month treatment period for females at any treatment level when compared to controls, overall body weight gains for females at 100 and 1000 mg/kg bw/day were reduced 10% and 21.8 %, respectively, when compared to controls. The reductions in overall body weight gain for females at 100 and 1000 mg/kg bw/day were considered treatment-related.

Table 130: 104-week dietary carcinogenicity study in mice – Body Weight Group Mean Values (g)

Treatment period (weeks)	Males				Females			
	Group Dose level (mg/kg bw/day)							
	0	10	100	1000	0	10	100	1000
0	30.7	30.6	30.4	30.1	23.2	23.7	23.3	**24.2***
13	37.2	36.5	36.5	35.6	30.2	30.8	30.1	30.8

17	38.8	**37.3***	37.7	**36.7****	30.9	31.9	31.4	31.7
52	43.8	**41.4***	42.4	**40.5****	34.9	36.0	34.7	34.6
84	44.4	42.4	42.3	**41.1****	37.5	36.9	36.9	36.5
92	45.0	42.9	**42.5***	**41.4****	37.9	37.5	37.0	37.2
104	43.0	43.4	42.0	**40.3***	38.8	38.1	37.3	36.4
Weight Gain (g) Weeks 0 – 104	12.3	12.8	11.6	10.2	15.6	14.4	14.0	12.2
% of Controls	-	104	94	83	-	92	90	78

(F-max test, parametric ANOVA, Student's t-test, Kruskal-Wallis ANOVA)
* = Significant different from control, p < 0.05
** = Significant different from control, p < 0.01
*** = Significant different from control, p < 0.001

Differential blood counts evaluated at approximately 52, 78 and 104 weeks of treatment in the control and 1000 mg/kg bw/day groups were unaffected by treatment with dimethomorph technical.

After 13 weeks of treatment, alkaline phosphatase activity was significantly increased in males at 1000 mg/kg bw/day and aspartate aminotransferase activity was significantly increased for females in the 1000 mg/kg bw/day group (satellite animals) when compared to controls. In addition, slight but non-statistically significant increases in alanine aminotransferase activity (both sexes) and aspartate aminotransferase activity (males) were observed at 13 weeks for the 1000 mg/kg bw/day satellite group when compared to controls.

Table 131: 104-week dietary carcinogenicity study in mice – clinical chemistry

Endpoint	**Males**		**Females**	
	Group Dose level (mg/kg bw/day)			
	0	**1000**	**0**	**1000**
ALP - Week 13	98	**129***	191	190
AST - Week 13	69	105	82	**103***

*Significantly different from Control, p<0.05

Increases in absolute liver weights and liver-to-body weight ratios were observed for satellite male and female mice (1000 mg/kg bw/day) sacrificed after 13 weeks, when compared to concurrent controls. Absolute and adjusted liver weights remained elevated after 52 weeks of treatment when compared to historical controls; no concurrent control organ weight data were available for comparison. No dose-related microscopic liver findings were observed in the satellite animals at either the 13 or 52-week sacrifice.

Table 132: 104-week dietary carcinogenicity study in mice – organ weight

Endpoint	**Males**	**Females**
	Group Dose level (mg/kg bw/day)	

	0	1000	0	1000
Absolute liver weight - Week 13 - Week 52	 1.98 2.07[1]	 **2.20*** 2.42	 1.45 1.65	 **2.06***** 2.17
Adjusted liver weight	1.93	2.22	1.55	**1.99****

*Significantly different from Control, p<0.05; **Significantly different from Control, p<0.01; ***Significantly different from Control, p<0.001
[1]Based on historical control data, no concurrent control was available.

No treatment-related macroscopic or microscopic changes (neoplastic or non-neoplastic) were observed in any of the tissues evaluated from animals sacrificed following 13, 52 or 104 weeks of treatment nor from any of the animals found dead or sacrificed moribund during the study. An increase in pulmonary tumors was observed at terminal sacrifice for males when compared to controls. However, this increase was not considered treatment-related for the following reasons:

(1) The incidence of pulmonary adenomas for males in the high-dose (1000 mg/kg bw/day) group (17 of 50) was not statistically significantly different from controls (12 of 50) by the Fisher Exact Test ($p < 0.05$). Thus, there was no dose-response relationship for the increased incidence of benign lung tumors for males because the high-dose level (1000 mg/kg bw/day), a dose level that is 10 times greater than the mid-dose level, showed no increase in this lesion.

(2) Males in the 1000 mg/kg bw/day group (highest dose tested) demonstrated a higher survival rate at termination as compared to the survival rate of males in the control and 100 mg/kg bw/day groups. The survival rate at termination (Weeks 104-105) for males at 1000 mg/kg bw/day was 74 % versus survival rates of 52 % and 50 % for males in the control and 100 mg/kg bw/day groups, respectively.

(3) There was no decreased time in appearance of benign pulmonary adenomas for either the mid-dose or high-dose males, when compared to controls.

(4) There was no evidence of any treatment-related increase in the incidence of pulmonary pre-neoplastic lesions (i.e., hyperplastic foci) or neoplastic lesions (adenomas or adenocarcinomas) in female CD-1 mice in the 24-month dietary oncogenicity study with dimethomorph technical.

Table 133: 104-week dietary carcinogenicity study in mice – Incidence of histopathology findings – males – pulmonary tumors – premature deaths and terminal kill combined

Dose /Group (mg/kg b.w./day)	0	10	100	1000
Number of animals	50	50	50	50
Benign tumor – total - One bronchiolar/alveolar hyperplastic focus - One pulmonary adenoma - Two pulmonary adenomas - Three pulmonary adenomas	**12*** 7 5 0 2	14 10 2 2 0	**22*** 20 2 0 0	17 15 2 0 0
Malignant tumor (one pulmonary adenomcarcinoma)	2	0	2	0

Fisher`s Exact Probability test: significant different from control, p < 0.01 for males
*= Incidences in the study report were changed to omit redundancy. One male in the control and the 100 mg/kg bw/day group also exhibited an adenocarcinoma which, therefore, was tabulated as an adenocarcinoma only.

Conclusion:

Based on reduced body weight in males and reduced body weigth gains in males and females at 1000 mg/kg bw/day (approximately 977 mg/kg bw/day, based on food consumption data), the data for this chronic oncogenicity study in the mouse support a systemic toxicity NOAEL of approximately 97 mg/kg bw/day, based on food consumption data. Dimethomorph is not considered to be oncogenic. The NOAEL for carcinogenicity is 977 mg/kg bw/day.

3.12.2 Human data

3.12.3 Other data

3.12.3.1 Subchronic neurotoxicity study - rat

Report:	CA 5.7.1/2
	B.6.7.1.2 - Anonymous, 2004 a
	BAS 550 F - Subchronic neurotoxicity study in Wistar rats - Administration in the diet for 3 months
	2004/1013810
Guidelines:	EPA 870.6200, OECD 424
Deviations:	None
GLP:	yes
	(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The aim of this study was to determine the effects of BAS 550 F (Dimethomorph) on neurotoxicity in rats after administration of 300, 800, and 2400 ppm test substance via the diet over 90 days. The mean daily intake was 21.7, 58.7, and 177.9 mg/kg bw/day for males and 25.7, 69.6, and 204.0 mg/kg bw/day for females in the 300, 800, and 2400 ppm dose group, respectively. In the high dose groups reduced food consumption, impaired body weight gain and food efficiency was observed. FOB and MA measurement revealed no test substance-related effects at any dose level. Furthermore, neither necropsy nor the light microscopy examinations of the central and peripheral nervous system did reveal any test substance-dependent neuropathological lesions in the organ samples examined. Based on these findings the no

observed adverse effect level (NOAEL) was 800 ppm for general systemic toxicity and 2400 ppm for neurotoxicity under the conditions of this study, respectively.

(DocID 2004/1013810)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 550 F (Dimethomorph)
Description:	solid, powder / white
Lot/Batch #:	AC9978-131
Purity:	98.3%
Stability of test compound:	proven by reanalysis
2. Vehicle and/or positive control:	None
3. Test animals:	
Species:	Rats
Strain:	CrlGlxBrlHan:WI
Sex:	Male and female
Age:	49±1 days (start of administration)
Weight at dosing:	Males: 165-211 g
	Females: 121-164 g
Acclimation period:	approx. 2 weeks
Diet:	ground Kliba maintenance diet (rat/mouse "GLP"), Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	single housing in type DK III (Becker&Co., Castrop-Rauxel, Germany)
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	not specified (fully air-conditioned room)
Photo period:	12 h light / 12 h dark
	(06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 08-July-2003 - 24-Mar-2004

(In life dates: 21-July-2003 (start of administration) to 23-Oct-2003 (necropsy)

2. Animal assignment and treatment:

BAS 550 F was administered to groups of 10 male and 10 female rats at concentrations of 300, 800, and 2400 ppm in the diet. On the day of arrival an acclimatization period started, in which the animals were accustomed to housing and diet. The animals were randomly assigned to the groups based upon body weight and separated by sex prior to the first functional observational battery. At the start of the administration period (day 0) the rats were 49±1 days old. The weight variation of the animals did not exceed 20 percent of the mean weight of each sex.

3. Test substance preparation and analysis:

For each concentration, the test substance was weighed out and mixed with a small amount of food. Then corresponding amounts of food, depending on dose group, were added to this premix in order to obtain the desired concentrations. Mixing was carried out for about 10 minutes in a laboratory mixer. The test substance preparations were mixed monthly.

The stability of the test substance in the diet over a period of 49 days at room temperature was verified analytically before the start of the study.

Homogeneity analyses of the test substance preparations were performed in samples of all concentrations on the first week of the administration period. These samples also served for concentration control analyses. Additional concentration control analyses were performed with samples of all concentrations at the end of the administration period.

4. Statistics:

Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further parameters and statistical tests are listed below.

Parameters	**Statistical test**	**Markers in the tables**	**References**

body weight, body weight change, food consumption, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096-1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482-491
feces, rearing, grip strength forelimbs, grip strength hindlimbs, footsplay test, motor activity	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York
Weight parameters (neuropathology)	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON-test for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	HETTMANNSPERGER, T.P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3 MILLER, R.G. (1981): Simultaneous Statistical Inference, Springer-Verlag New York Inc., 165-167 NIJENHUIS, A. and S.W. WILF (1978): Combinatorial Algorithms, Academic Press, New York, 32-33

C. METHODS

1. Observations:

The animals were examined for evident signs of toxicity or mortality twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays.

2. Body weight:

The body weight was determined before the first neurofunctional test in order to randomize the animals. During the conduct of the study, the body weight was determined on day 0 and thereafter in weekly intervals. In addition the body weight was determined on the days when functional observational batteries were carried out. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

3. Food and water consumption, food efficiency and compound intake:

Individual food consumption was determined weekly over a period of 7 days and calculated as mean food consumption in grams per animal and day. Individual water consumption was determined daily by visual inspection.

Food efficiency was calculated based upon individual values for body weight and food consumption. The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

4. Ophthalmoscopy:

Prior to the start of the administration period the eyes of all animals, and to the end the eyes of the control and high dose animals were examined for any changes using an ophthalmoscope (HEINE OPTOTECHNIK, Herrsching, Germany) after administration of a mydriatic (Chauvin, ankerpharm GmbH, Rudolfstadt, Germany).

5. Clinical assessment of neurotoxicity:

Functional observational battery

FOBs were performed in all animals before the administration (day -7) and on days 1, 22, 50 and 85. The FOBs started with passive observations without disturbing the animals, followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. For *home cage observations* attention was paid to: posture, tremor, convulsions, abnormal movements, and impairment of gait.

Open field observations:

The animals were transferred to a standard arena (50×50 cm with sides of 25 cm high) and observed for at least 2 minutes. Following parameters were examined:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

Sensorimotor tests/reflexes:

The animals were removed from the open field and subjected to following sensorimotor and reflex tests:

1. approach response	8. behavior during "handling"
2. touch response	9. vocalization
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hindlimbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

Motor activity assessment

Motor activity was measured on the same day as FOB was performed. The measurement was performed in the dark using the Multi-Varimex-System (Columbus Instruments Int. Corp., Ohio, USA) with 4 infrared beams per cage. Motor activity measurements were from 2.00 a.m. onwards and the number of beam interrupts was counted over 12 intervals, each lasting 5 minutes. Measurement did not commence at the same instant for all cages; the period of assessment for each animal started when the first beam was interrupted by pushing the cage into the rack (staggered start). Measurements ended exactly 60 minutes thereafter. During the measurements the animals received no food and no water.

6. Neuropathology:

The five surviving animals per sex and test group that are selected for neuropathology, were deeply anesthetised (Narcoren®, about 4 mL/kg body weight) at the end of the study and sacrificed by perfusion fixation.SOERENSEN's phosphate buffer served as rinsing solution and the fixation solution according to KARNOVSKY served as fixative.

The sacrificed animals were necropsied and the visible organs assessed by gross pathology as thoroughly as possible for perfused animals. The organ/tissue samples listed in paragraphs below were carefully removed.

Weight determinations

Weight assessment of the brain (without olfactory bulb) was carried out on all perfused animals after removal of the brain but before any further preparation.

Organ/tissue preservation list

Paraffin embedding, sectioning, staining and preservation:

Organ samples from:	Test groups (dose)			
	0 control	1 300 ppm	2 800 ppm	3 2,400 ppm
Brain (cross sections):		F5*	F5*	
– Frontal lobe	A5			A5
– Parietal lobe with *diencephalon*	A5			A5
– Midbrain with occipital and temporal Lobe	A5			A5
– Pons	A5			A5
– Cerebellum	A5			A5
– Medulla oblongata	A5			A5
Brain-associated organs/tissues				
– Eyes with retina and optical nerve	A5	F5	F5	A5
Spinal cord (cross and longitudinal sections):				
– Cervical swelling (C3-C6)	A5	F5	F5	A5
– Lumbar swelling (L1-L4)	A5	F5	F5	A5
Peripheral nervous system:				
– Gasserian ganglia with nerve	A5	F5	F5	A5
– Gastrocnemius muscle	A5	F5	F5	A5

METHODS/SCOPE OF EXAMINATIONS:

A = Paraffin embedding (paraplast), sectioning and staining with hematoxylin-eosin (H & E)
F = Preservation in 4% formaldehyde
5 = all perfused animals per group and sex
* = in toto

Plastic embedding, sectioning, staining and storage:

Organ samples from:	Test groups (dose)			
	0 control	1 300 ppm	2 800 ppm	3 2,400 ppm
Peripheral nervous system:				
– Dorsal root ganglion, 3 of (C3-C6)	T5	P5	P5	T5
– Dorsal root fiber (C3-C6)	T5	P5	P5	T5
– Ventral root fiber (C3-C6)	T5	P5	P5	T5
– Dorsal root ganglion, 3 of (L1-L4)	T5	P5	P5	T5
– Dorsal root fiber (L1-L4)	T5	P5	P5	T5
– Ventral root fiber (L1-L4)	T5	P5	P5	T5
– Proximal sciatic nerve	T5	P5	P5	T5
– Proximal tibial nerve (at knee)	T5	P5	P5	T5
– Distal tibial nerve (at lower leg)	T5	P5	P5	T5

METHODS/SCOPE OF EXAMINATIONS:

T = Plastic embedding (epoxy resin), semithin sectioning and staining with Azure II-Methylene blue-basic Fuchsin (AMbf)
P = Storage of fixed specimen in buffer solution
5 = all perfused animals per group and sex

In addition the following organs/tissues (after trimming) were preserved in neutrally buffered 4% formaldehyde solution:

1. Brain (remaining material after trimming)
2. Spinal cord (sections from cervical and lumbar cords)
3. All gross lesions

The remaining animal body was stored in neutrally buffered 4% formaldehyde solution.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

Test substance preparations were found to be homogenous and of correct concentrations (97.6-104.1% of nominal concentrations).

B. OBSERVATIONS

1. Clinical signs of toxicity

One male animal of group 0 showed unpalpable left testis in scrotum from day 49 to the end of the study. One male animal of group 3 showed sparse hair on the ventral region and on both forelimbs from day 78 to the end of the study. These isolated findings were spontaneous in nature and therefore not substance-related.

2. Mortality

No animal died during the study.

C. BODY WEIGHT AND BODY WEIGHT GAIN

In male animals of group 3, body weight was reduced over the whole study period, statistically significantly on most days, up to -14.4 % on day 84. In male animals of group 3, body weight change was reduced statistically significantly over the whole study period up to -33.2 % on day 7.

In female animals of group 3, body weight change was reduced over the whole study period, statistically significantly on day 21 and from day 42 until the end of the study period, up to -27.5 % on day 42. These findings are considered to be treatement related.

Table 134: **Mean body weight and body weight change of rats administered BAS 550 F for 90 days**

	Males				**Females**			
Dose level [ppm]	**0**	**300**	**800**	**2400**	**0**	**300**	**800**	**2400**
Body weight [g]								
day 0	190	187	186	183	137	139	137	140
day 56	356	341	335	**313****	204	202	201	194
day 91	390	377	366	**340****	219	214	213	206
Body weight change [g] (% control)								
day 0-91	199	190 (-5)	179 (-10)	**157**** **(-21)**	81	76 (-6)	75 (-6)	**66*** **(-18)**

* = p≤0.05; ** = p≤0.01

D. FOOD CONSUMPTION AND FOOD EFFICIENCY

In male animals of group 3, food consumption was reduced over the whole study period, statistically significantly on days 7, 42, 70 and 84 up to -12.7 % on day 7. This finding was assessed as substance-related.

In female animals of group 1, food consumption was statistically significantly reduced on day 42 (-8.7 %). Since it was an isolated finding it is considered to be incidental. In female animals of group 3 food consumption was substance-related reduced over the whole study period, statistically significantly on days 7, 35, 42, 56, 63 and 70 up to -20.9 % on day 7.

In male animals of group 3, food efficiency was statistically significantly impaired on days 7 and 35. This finding was assessed as related to the test compound. Food efficiency was substance-related impaired in female animals of group 3 (statistically significant on days 28 and 42).

E. TEST SUBSTANCE INTAKE

The approximate mean daily test substance intake in mg/kg body weight/day over the entire study period is shown in the following table:

Table 135: **Test substance intake over the study period**

Test group	**Concentration in the diet (ppm)**	**Mean daily substance intake (mg/kg bw/day)**	
		males	**females**
1	300	21.7	25.7
2	800	58.7	69.6
3	2400	177.9	204.0

F. OPHTHALMOSCOPY

No substance-related effects were obtained.

G. CLINICAL ASSESSMENT OF NEUROTOXICITY

Functional observational battery

Deviations from "zero values" were obtained in several animals. However, as most findings were equally distributed between treated groups and controls, were without a dose-response relationship or occurred in single animals, these observations were considered to have been incidental.

Home cage observations:

No significant deviations from controls were observed.

Open field observations:

One male animal of group 0 showed unpalpable left testis in scrotum on days 22, 50 and 85. One male animal of group 3 showed sparse hair on the ventral region and on both forelimbs on day 85. These findings were spontaneous in nature and therefore not substance-related.

Quantitative parameters:

No significant deviations from controls were observed.

Sensorimotor tests/reflexes

No significant deviations from controls were observed.

Motor activity assessment

Regarding the overall motor activity (summation of intervals 1-12), a statistically significant reduced value was only obtained in female animals of dose group 1 on day 85. This single occurrence in the low dose group was not considered to be treatement-related. The comparison of the single intervals resulted in the following deviations:

Group 1: In female animals at interval 11 on day 85.

Group 2: In female animals at intervals 10 and 11 on day 50.

Group 3: In female animals at interval 2 on day - 7 as well as at interval 11 on day 85.

However, due to the isolated occurrences and alternating results in the sense of increased as well as decreased motor activity and moreover, as all findings were without any dose-response relationship, the above-mentioned deviations were assessed as being incidental and not related to treatment.

H. NEUROPATHOLOGY

No significant changes were noted for absolute or relative organ weights, and no gross lesions were recorded. The only lesion found was a single "axonal degeneration" in the peripheral nerves in 2 control and 1 top dose test animal. This lesion is regarded to be spontaneous in nature and not related to treatment.

III. CONCLUSIONS

The administration of BAS 550 F at concentrations of 300, 800, and 2400 ppm in the diet revealed signs of toxicity, such as reduced food consumption and impaired body weight gain in the high dose group. No test substance-related effects were observed concerning clinical observations, FOB, motor activity or in the neurohistopathology investigation at any dose level. The no observed adverse effect level (NOAEL) under the conditions of this study was 800 ppm (58.7 mg/kg w/day) for general systemic toxicity and 2400 ppm (177.9 mg/kg bw/day) for neurotoxicity, respectively. The same conclusion was drawn by the study author.

3.12.3.2 4 week immunotoxicity study - rat

Report: CA 5.8.2/1

B.6.8.2.15 - Anonymous, 2010 b

BAS 550 F (Dimethomorph) - Immunotoxicity study in male Wistar rats - Administration via the diet for 4 weeks

2010/1043718

Guidelines: EPA 870.7800

GLP: yes

(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The immunotoxic potential of BAS 550 F (Batch: AC9978-131; Purity: 97.5%) in male Wistar rats was analyzed using dietary dose levels of 0, 300, 800 and 2400 ppm (corresponding to mean intake levels of 23, 61 and 184 mg/kg bw/day, respectively) for 28 days. The parameters used for detection of potential test substance related alterations in the morphology of the immune system included a) the determination of lymphoid organ weights (spleen and thymus) and b) the analysis of the primary humoral (IgM response) immune response to sheep red blood cells (SRBC).

Treatment with BAS 550 F did not result in clinical signs or mortality in any test group. However, in the high dose group (2400 ppm) a significantly lower body weight gain (about 12-19% less as compared to the control) was observed from day 7 until day 14 of treatment. None of the immunotoxicologically relevant parameters mentioned above were affected by treatment with BAS 550 F up to the highest dose level tested.

Concurrent treatment with positive control substance, cyclophosphamide monohydrate (CPA, 4.5 mg/kg bw/day) induced clear signs of immunotoxicity, demonstrating the reliability of the test system under the study conditions employed.

Based on the obtained results it can be concluded that BAS 550 F does not bear an immunomodulatory/immunotoxic potential under the conditions of this study. The NOAEL for the immunotoxicologically relevant endpoints was determined to be 2400 ppm corresponding to 184 mg/kg bw/day. The NOAEL for systemic toxicity was 800 ppm corresponding to 61 mg/kg bw/day in male Wistar rats.

(DocID 2010/1043718)

I. MATERIAL AND METHODS

1. Test Material: BAS 550 F (Dimethomorph)

Description:	solid/ white
Lot/Batch #:	AC9978-131
Purity:	97.5%
Stability of test compound:	The test substance was stable over the study period (Expiry date Mar 01, 2015).

2. Vehicle control: Rodent diet

3. Positive control: Cyclophosphamide monohydrate (CPA)

Description:	Solid / white
Lot/Batch #:	1362353
Purity:	100% (according to supplier)
Stability of test compound:	According to the supplier the positive control substance was stable over the study period (Expiry date Oct. 2010).
Vehicle for CPA:	Drinking water

4. Test animals:

Species:	Rat
Strain:	Crl:WI(HAN)
Sex:	Male (more sensitive gender based on previous studies)
Age:	34 ± 1 days at delivery; approx. 42 ± 1 days at start of administration
Reason for the selection:	The rat is a frequently used laboratory animal, and there is comprehensive experience with this animal species. Moreover, the rat has been proposed as a suitable animal species by the OECD and the EPA for this type of study.
Weight at dosing:	155-183 g
Acclimation period:	9 days
Diet:	Kliba maintenance diet for mouse/rats "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water in bottles, ad libitum
Housing:	4 animals per cage in H-Temp (PSU, floor area about 2065 cm^2) cages (TECNIPLAST, Hohenpeißenberg, Germany)
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	10/hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 01-Sep-2009 - 24-Mar-2010

(In life dates: 09-Sep-2009 (start of administration) to 08-Oct-2009 (necropsy))

2. Animal assignment and treatment:

BAS 550 F was administered to groups of 8 male rats at dietary concentrations of 0, 300, 800 and 2400 ppm for 28 days. Additionally, 8 male rats were treated orally (gavage) with 4.5 mg/kg bw/day Cyclophosphamide monohydrate (CPA; positive control substance). CPA was administered as a solution in drinking water at a volume of 10 mL/kg. The administered volume was determined based on the most recently determined body weights. The animals were assigned to the treatment groups by means of computer generated randomization list based on body weights.

On day 23 of the study all animals received a single intraperitoneal injection (0.5 mL) of a sheep red blood cell (SRBC)-suspension containing 4 x 10^8 cells/mL for immunization.

3. Test substance preparation and analysis:

The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Test substance preparations were mixed once before the start of administration.

The stability of the test substance dimethomorph in the diet over a period of up to 49 days was proven before the start of the study. Homogeneity and concentration analyses of the diet preparations were performed at the beginning of the administration period for all concentrations.

Table 136: **Results of homogeneity and concentration control analysis of dimethomorph in rodent diet**

Nominal Dose level [ppm]	Sampling	Mean of nominal concentration [%]	Relative standard deviation [%]
300	Sep. 08, 2009	94.7	1.1
800	"	99.1	2.9
2400	"	100.8	1.3

Relative standard deviations of the homogeneity of the dimethomorph samples were quite low, which indicates the homogenous distribution of dimethomorph in the diet preparations. The actual (mean)

average test-substance concentrations were in the range of 94.7 to 100.8% of the nominal concentrations confirming the correctness of the concentrations.

The positive control substance preparation (CPA in drinking water) was prepared once at the beginning of the study, split in daily aliquots and deep frozen at -18°C. The mixtures were applied when reaching room temperature. The concentration control of the CPA solution was performed at the beginning of the study. Since the CPA formulation in drinking water was a solution a homogeneity analysis was redundant. The stability analysis conducted revealed the stability of the CPA solution for 66 days when stored frozen.

The actual CPA concentrations were 108.2 and 108.7% of the nominal concentration confirming the correctness of the concentration.

Table 137: **Results of concentration control analysis of CPA in drinking water**

Nominal Concentration [g/100 mL]	Sampling / Analysis	Analytical concentration [g/100 mL]	Mean of nominal concentration [%]
0.045	Sep. 08, 2009 / Nov. 09, 2009	0.0432	96.0
0.045	Sep. 08, 2009 / Nov. 09, 2009	0.0430	95.6

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Table 138: **Statistics of clinical examinations**

Parameter	Statistical test	Markers in the tables	References
body weight and body weight change	For the test substance and the control groups: A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means For the vehicle and positive control groups: A comparison of the dose group with the control group was performed using the t-	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096-1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482-491 WINER, B.J. (1971): Statistical principles in experimental design. McGraw-Hill, New York, 2nd

Parameter	Statistical test	Markers in the tables	References
	test (two-sided) for the hypothesis of equal means		edition
Clinical pathology parameters	For the test substance and the control groups: Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each test group with the control group was performed using WILCOXON test for the equal medians For the vehicle and positive control groups: Pair-wise comparison of the dose group with the control group was performed using Wilcoxon test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Nonparametric statistics for the behavioural sciences. McGraw-Hill New York SIEGEL, S. (1956): Non-parametric statistics for the behavioral sciences. McGraw-Hill, New York
Pathology weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each test group with the control group was performed using the WILCOXON test for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	HETTMANNSPERGER, T.P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3 MILLER, R.G. (1981): Simultaneous Statistical Inference, Springer-Verlag New York Inc., 165-167 NIJENHUIS, A. and S.W. WILF (1978): Combinatorial Algorithms, Academic Press, New York, 32-33

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Observation for overt clinical signs of toxicity was performed at least once daily. Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. The animals were transferred to a standard arena (50 x 37.5

cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable.

1. abnormal behavior during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. feces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

2. Food consumption and compound intake:

Food consumption was determined weekly for each cage. The average food consumption per cage was used to estimate the mean food consumption in grams per animal per day.

3. Water consumption:

Drinking water consumption was observed by daily visual inspection of the water bottles for any overt changes in volume.

4. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined on day 0 (start of the administration period) and thereafter at weekly intervals. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

5. Analysis of the primary immune response:

In the morning blood was taken from the retro-orbital venous plexus from not-fasted animals. The animals were anaesthetized using isoflurane (Isoba®, Essex GmbH Munich, Germany). The blood sampling procedure and the subsequent analysis of the blood and plasma samples were carried out in a randomized sequence. The following examinations were carried out in 8 male animals per test group.

Primary T-cell dependent antibody response (anti-SRBC IgM ELISA)

Plasma samples from all SRBC immunized animals were analyzed for their specific anti SRBC-IgM titer in an ELISA. Each serum sample was diluted to 1:64 and 1:128. SRBC-IgM concentrations outside the standard curve range were measured in a second test run with an appropriate dilution. Generally, two in-house controls were used for a standard curve. The ELISA was measured with a Sunrise MTP-reader (Tecan AG, Maennedorf, Switzerland), and evaluated with the Magellan-Software of the instrument producer.

6. Necropsy and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following weights were determined for all animals sacrificed at scheduled dates:

1. Anesthetized animals
2. Spleen
3. Thymus

No further histopathological examinations were performed.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of the test substance BAS 550 F (Dimethomorph) in the diet was demonstrated over a period of up to 49 days at room temperature. As the mixtures were stored no longer than this time period, the stability was guaranteed. The stability of Cyclophosphamide monohydrate (positive control substance) in drinking water was demonstrated for 66 days under study conditions.

Considering the low standard deviation in the homogeneity analysis, it can be concluded that BAS 550 F (Dimethomorph) was distributed homogeneously in feed. Due to the fact that Cyclophosphamide monohydrate (positive control substance) was a solution in drinking water no homogeneity analysis was performed.

The concentration control analyses revealed that the values of the test substance BAS 550 F (Dimethomorph) in feed and the positive control substance (Cyclophosphamide monohydrate) in drinking water were in the expected range of the target concentrations, i.e. were always in a range of 93.8 - 102.4% for the test-substance preparations and 95.6% and 96.0% for the positive control substance, of the nominal concentrations.

B. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related clinical signs were observed in all animals treated with BAS 550 F (Dimethomorph) and in animals which received Cyclophosphamide monohydrate as positive control.

2. Mortality

No mortality was observed in this study.

B. FOOD CONSUMPTION, DRINKING WATER CONSUMPTION, AND COMPOUND INTAKE

Food consumption was slightly reduced in all animals treated with BAS 550 F (Dimethomorph) during the study. However, since the effect was only marginal it was not considered to be adverse. In contrast, a more pronounced effect with regard to food consumption was observed for the positive control animals (Cyclophosphamide monohydrate) with a maximum of -12.9% on days 7 and 14. This change was assessed as treatment-related.

No test substance-related findings were observed regarding drinking water consumption.

Table 139: **Effect on food consumption**

Treatment	**BAS 550 F**				**CPA**
Dose level	**0**	**300**	**800**	**2400**	**4.5 mg/kg**
	ppm	**ppm**	**ppm**	**ppm**	
Food consumption per cage [g]					
- Day 6 to 7	18.56	17.35	17.15	17.08	16.16
- Day 13 to 14	20.95	20.05	20.71	19.68	18.25
- Day 20 to 21	20.00	18.38	19.09	18.99	17.70
- Day 27 to 28	22.06	21.04	22.52	21.96	20.06

The mean daily test substance intake in mg/kg body weight/day over the entire study period was calculated and is shown in the following table:

Table 140: **Calculated intake of BAS 550 F**

Test group	**Concentration in the diet**	**Mean daily test-substance**

	(ppm)	intake (mg/kg bw/d)
		Males
1	300	23
2	800	61
3	2400	184

C. BODY WEIGHT AND BODY WEIGHT GAIN

Body weights in animals of test group 3 (2400 ppm; BAS 550 F) and 1 (300 ppm; BAS 550 F) were slightly lower from day 7 until day 28 but not significantly different when compared to the control animals. Body weights of rats treated with Cyclophosphamide monohydrate were significantly impaired on days 21 and 28, with a maximum of -7.6% on day 28. This finding was considered as being related to treatment with the positive control substance. Body weight changes in animals of test group 3 (2400 ppm; BAS 550 F) were significantly lower from day 7 until day 28 with a maximum of 19.3% less on day 7 when compared to the controls. As the body weight change was nearly 12% less on study day 28 a relation to treatment was indicated. Body weight change of male rats treated with Cyclophosphamide monohydrate was impaired throughout the whole administration period, with a maximum of 21.7% less on day 7. This finding was assessed as treatment-related.

Table 141: **Mean body weights (g) of rats administered BAS 550 F or Cyclophosphamide (CPA) for 28 days**

Treatment	**BAS 550 F**				**CPA**
Dose level	**0 ppm**	**300 ppm**	**800 ppm**	**2400 ppm**	**4.5 mg/kg**
- Day 0	163	168	169	168	170
- Day 7	210	208	211	206	206
- Day 14	249	246	250	242	240
- Day 21	285	276	284	273	**268***
- Day 28	308	300	308	296	**285***
- Day 28 % compared to control	-	-2.77	-0.01	-3.98	-7.64

Table 142: **Mean body weight changes (g) of rats administered BAS 550 F or Cyclophosphamide (CPA) for 28 days**

Treatment	BAS 550 F				CPA
Dose level	**0 ppm**	**300 ppm**	**800 ppm**	**2400 ppm**	**4.5 mg/kg**
- Day 0-7	46.88	40.58	41.95	**37.81****	**36.70****
- Day 0-14	85.53	77.74	81.55	**74.20***	**70.96****
- Day 0-21	121.29	107.86	115.15	**105.34***	**98.44****
- Day 0-28	144.60	131.75	139.21	**127.81***	**114.99****

*: p≤0.05, **: p≤0.01

D. IMMUNOLOGICAL ANALYSES

1. Analysis of the primary T-cell dependent immune response

Six days after immunization, no changes in the SRBC IgM titres were found in male rats dosed with the test substance, whereas the SRBC titres were significantly lower in rats of test group 4 (Cyclophosphamide Monohydrate, positive control group) [see Table below].

Table 143: **Analysis of the specific primary (IgM) immune response to SRBC in rats treated with BAS 550 F or Cyclophosphamide (CPA) for 28 days**

Treatment	BAS 550 F				CPA
Dose [ppm]	**0**	**300**	**800**	**2400**	**4.5 mg/kg**
Specific IgM Titer (U/mL)					
- Mean ± SD	2653 ± 2147	5946 ± 7542	2018 ± 777	3728 ± 3253	**740** ± **266**
- Median	1477	2711	1873	2303	**820**

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

G. NECROPSY

1. Terminal body weight and organ weights

The absolute mean weights of animals in test groups 1-3 (300, 800 and 2400 ppm, BAS 550 F) did not show relevant differences compared to the control group. The positive control group (test group 4, Cyclophosphamide monohydrate) revealed significant decreases of spleen and thymus weights, which corresponded to the expected result. In addition, the terminal body weights were significantly reduced.

The relative mean weights of animals in test groups 1-3 (300, 800 and 2400 ppm, BAS 550 F) did not show relevant differences compared to the control group. The positive control group (test group 4, Cyclophosphamide monohydrate) revealed significant decreases of spleen and thymus weights, which corresponded to the expected result.

Table 144: **Mean absolute and relative organ weights of male rats treated with BAS 550 F or Cyclophosphamide for at 28 days**

Sex		**Metconazole**				**CPA**
Dose [ppm]		**0**	**300**	**800**	**2400**	**4.5 mg/kg**
Terminal bodyweight	[g]	289	278	289	279	**265***
	[% of control]	-	96	100	97	**92**
Spleen, absolute	[g]	0.609	0.55	0.586	0.584	**0.366****
	[% of control]	-	90	96	96	**60**
Spleen, relative	[%]	0.21	0.198	0.202	0.207	**0.138****
	[% of control	-	94	96	99	**66**
Thymus, absolute	[mg]	535	496	554	553	**220****
	[% of control]	-	93	103	103	**41**
Thymus, relative	[%]	0.185	0.178	0.192	0.201	**0.083****
	[% of control]	-	96	103	109	**45**

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

2. Gross pathology

No gross lesions were observed in any test animal. In the absence of any gross lesions, no histopathological investigation was carried out.

III. CONCLUSIONS

Under the conditions of the study BAS 550 F did not reveal any signs of immunotoxicity when administered via the diet over a period of 4 weeks to male Wistar rats. The NOAEL for the immunotoxicologically relevant endpoints was determined to be 2400 ppm (184 mg/kg bw/d; highest dose tested). The NOAEL for systemic toxicity was set to 800 ppm (61 mg/kg bw/d) based on reduced body weight change at 2400 ppm. The oral administration of the positive control substance Cyclophosphamide Monohydrate (4.5 mg/kg bw/d) led to clear findings indicative of immunotoxicity. This was represented by significantly lower SRBC IgM

antibody titres as well as reduced spleen and thymus weights. Thus, assay sensitivity was verified in the present immunotoxicity study performed in male Wistar rats.

3.13 Aspiration hazard

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.2 Bioaccumulation

4.3 Acute toxicity

4.4 Chronic toxicity

4.5 Acute and/or chronic toxicity to other aquatic organisms