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

Substance Name: Dimoxystrobin

EC Number: **-**

CAS Number: **149961-52-4**

Index Number: **616-164-00-7**

Contact details for dossier submitter: National Public Health Center
Department of Chemical Safety and Competent Authority
Albert Flórián út 2-6.
1097 Budapest
Hungary

Version number: 1 **Date: May 2019**

CONTENTS

1 PHYSICAL HAZARDS

1.1 Explosives

1.1.1 [Study 1]

Study reference:
Achhammer G (2013): Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008), 2013/1065793, BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep.

Detailed study summary and results:
please refer also to
RAR (2017) – Volume 3. Annex B-2, chapter B 2.11

Guideline and method	Test material purity and specification	Findings	GLP Y/N
EEC A.14	TGAI, batch No. FF18850, 99.9 %	Pre-test via DSC: 1st reaction: onset 170°C, peak 296°C, energy release 470 J/g (exothermal) 2nd reaction: onset 390°C, energy release > 70 J/g (exothermal) Testing on explosive properties (thermal and mechanical sensitivity and friction): negative Conclusion: The test substance is not considered to exhibit a danger of explosion in the sense of the directive.	Y

1.2 Flammable gases (including chemically unstable gases)

Not applicable as dimoxystrobin is a solid

1.3 Oxidising gases

Not applicable as dimoxystrobin is a solid

1.4 Gases under pressure

Not applicable as dimoxystrobin is a solid

1.5 Flammable liquid

Not applicable as dimoxystrobin is a solid

1.6 Flammable solids

1.6.1 [Study 1]

Study reference:
Loeffler U. (1998): Safety characteristics of the crop protection product PS 285 028 (BAS 505 F), 1998/11588, BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.

Detailed study summary and results:
please refer also to
RAR (2017) – Volume 3. Annex B-2, chapter B 2.9
EFSA Scientific Report (2005) 46, 1-82
DAR (2005) – Volume 3, Annex B-2, chapter B-2.1.20

Guideline and method	Test material purity and specification	Findings	GLP Y/N
EEC A.10	N6, 97.6 %	Information previously reported and peer-reviewed: Not considered highly flammable	Y

1.7 Self-reactive substances

1.7.1 [Study 1]

Study reference:
Loeffler U. (1998): Safety characteristics of the crop protection product PS 285 028 (BAS 505 F), 1998/11588, BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.

Detailed study summary and results:
please refer also to
RAR (2017) – Volume 3. Annex B-2, chapter B 2.9
EFSA Scientific Report (2005) 46, 1-82
DAR (2005) – Volume 3, Annex B-2, chapter B-2.1.20

Guideline and method	Test material purity and specification	Findings	GLP Y/N
EEC A.16	N6, 97.6 %	Information previously reported and peer-reviewed: At temperatures up to the melting point no self heating was registered.	Y

1.8 Pyrophoric liquids

Not applicable as dimoxystrobin is a solid

1.9 Pyrophoric solid

1.9.1 [Study 1]

Study reference:
Achhammer G (2013): Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008), 2013/1065793, BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep.

Detailed study summary and results:
please refer also to
RAR (2017) – Volume 3. Annex B-2, chapter B 2.11

Guideline and method	Test material purity and specification	Findings	GLP Y/N
EEC A.13 UN RTDG, Annex 6	TGAI, batch No. FF18850, 99.9 %	The test for Pyrophoric Properties (A.13) has not been carried out because the substance is known to be stable at room temperature for prolonged periods of time (days).	Y

1.10 Self-heating substances

1.10.1 [Study 1]

Study reference:
Loeffler U. (1998): Safety characteristics of the crop protection product PS 285 028 (BAS 505 F), 1998/11588, BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.

Detailed study summary and results:
please refer also to
RAR (2017) – Volume 3. Annex B-2, chapter B 2.9
EFSA Scientific Report (2005) 46, 1-82
DAR (2005) – Volume 3, Annex B-2, chapter B-2.1.20

Guideline and method	Test material purity and specification	Findings	GLP Y/N
EEC A.16	N6, 97.6 %	Information previously reported and peer-reviewed: At temperatures up to the melting point no self heating was registered.	Y

1.11 Substances which in contact with water emit flammable gases

1.11.1 [Study 1]

Study reference:
Achhammer G (2013): Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008), 2013/1065793, BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep.

Detailed study summary and results:
please refer also to
RAR (2017) – Volume 3. Annex B-2, chapter B 2.11

Guideline and method	Test material purity and specification	Findings	GLP Y/N
EEC A.12, UN N.5 UN RTDG, Annex 6	TGAI, batch No. FF18850, 99.9 %	The test for substances which in contact with water emit flammable gases (A.12, N.5) has not been carried out because the substance is known to be stable in water to form a stable mixture.	Y

1.12 Oxidising liquids

Not applicable as dimoxystrobin is a solid

1.13 Oxidising solids

1.13.1 [Study 1]

Study 1 reference:

Study reference:
Loeffler U. (1998): Safety characteristics of the crop protection product PS 285 028 (BAS 505 F), 1998/11588, BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep.

Detailed study summary and results:
please refer also to
RAR (2017) – Volume 3. Annex B-2, chapter B 2.13
EFSA Scientific Report (2005) 46, 1-82
DAR (2005) – Volume 3, Annex B-2, chapter B-2.1.23

Guideline and method	Test material purity and specification	Findings	GLP Y/N
EEC A.17	N6, 97.6 %	Information previously reported and peer-reviewed: Not oxidizing.	Y

1.14 Organic peroxides

Not applicable as dimoxystrobin is not an organic peroxide

1.15 Corrosive to metals

Not applicable as dimoxystrobin is a solid with a melting point of approx. 138-139°C

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1 Studies on Absorption, Distribution, Metabolism and Excretion in Mammals

Two new studies have been submitted for the renewal of approval of Dimoxystrobin. The objective of one of the new studies was to quantify the metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) in rat excreta as both metabolites became of interest. For a detailed assessment of the metabolic behavior of BAS 505 F in rats, urine and faeces samples from a study previously assessed in the DAR 2003 were reanalyzed. In an other new in vitro study rat and human metabolites were compared.

Information regarding the absorption, distribution, excretion and metabolism of Dimoxystrobin in the DAR 2003 was based on two acceptable studies. These studies were not re-evaluated; the original evaluation of the studies is copied here from the DAR 2003.
An extensive literature search was carried out by the notifier (references can be provided upon request) and the search method was accepted by the RMS. No new information relevant to the absorption, distribution, metabolism and excretion of Dimoxystrobin was found.

2.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Report:	KIIA 5.1.1/1 Anonymous, 1999: 14C-BAS 505 F - Study of the biokinetics in rats BASF DocID 1999/11642
Guidelines:	OECD guideline 417 (1984)
GLP:	yes
Previously evaluated:	in the DAR 2003
Acceptability:	yes

The biokinetics of ^{14}C-dimoxystrobin in male and female Wistar rats (Strain Chbb-THOM) were investigated at dose levels of 10 mg/kg bw and 100 mg/kg bw ("low" and "high" dose). These dose levels were selected to represent levels at which no toxic effects and slight toxic effects, respectively, would be expected (based on subacute and subchronic toxicity data). In a pre-test for this study, clinical signs were not seen for 24 h following a single dose of 100 mg/kg bw.

The experiments were performed with ^{14}C-dimoxystrobin labelled in the benzyl ring (radiochemical purity > 98%) and ^{14}C-dimoxystrobin labelled in the phenyl ring (radiochemical purity >98%), see Figure 2.1-1. The radiolabelled test compound was diluted with the non-labelled compound to achieve the desired specific activity.

[^{14}C-benzyl]dimoxystrobin [^{14}C-phenyl]dimoxystrobin

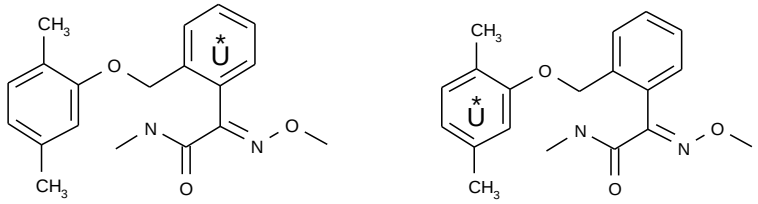


Figure 2.1-1 Structure and position of the ^{14}C-label for benzyl and phenyl-labelled dimoxystrobin

Dimoxystrobin was administered by oral gavage after being suspended in 0.5 % Tylose CB 30.000 in water containing 1% Cremophor EL. The stability, homogeneity and correctness of the test substance preparation were reported to be satisfactory (data not presented).

The design of the biokinetics experiments in this study are outlined in Table 2.1-1, together with the metabolic investigations which are reported later in this chapter.

Table 2.1-1 Summary of dose groups and analysed samples in the biokinetics and metabolism studies with dimoxystrobin

Experiment no.* Dose Groups**	**1 D**	**2 B**	**3 D**	**4 C**	**9 S**	**10 R**	**- DX**
Purpose	Excretion balance, Metabolite paterns	excretion balance, metabolite paterns	excretion balance, metabolite paterns	excretion balance, metabolite paterns	biliary excretion, metabolite identification	biliary excretion, metabolite identification	metabolite identification
Dosing	oral high	oral low	oral high	oral high/low***	oral high	oral low	oral high
Nominal dose level (mg/kg b.w.)	100	10	100	14 x 100 1 x 10	100	100	150
No. of animals per label (male/female)	4/4	4/4	4/4	4/-	4/4	4/4	10/10
^{14}C-label	Benzyl	benzyl	phenyl	benzyl	benzyl	benzyl	benzyl
Duration [h]	120	120	120	120	48	48	96
Samples	Urine, faeces, Tissues, Exhaled air	urine, faeces, tissues	urine, faeces, tissues	urine, faeces, tissues	bile	bile	urine faeces
Methods of analysis	Total ^{14}C, HPLC	total ^{14}C, HPLC	total ^{14}C, HPLC	total ^{14}C, HPLC	total ^{14}C, HPLC, MS	total ^{14}C, HPLC, MS	total ^{14}C, HPLC, MS

Experiment no.* Dose Groups**	**5 -**	**6 -**	**7 -**	**8 -**	**- V**	**- W**	**- DX**
Purpose	blood/plasma level	blood/plasma level	tissue distribution	tissue distribution	metabolite patterns	metabolite patterns	metabolite identification
Dosing	oral high	oral low	oral high	oral low	oral low	oral high	oral high
Nominal dose level (mg/kg b.w.)	100	10	100	10	15	150	150

Experiment no.* **Dose Groups****	**5** -	**6** -	**7** -	**8** -	**-** **V**	**-** **W**	**-** **DX**
No. of animals per label (male/female)	4/4	4/4	12/12 (3 per time point)	12/12 (3 per time point)	4/4	4/4	10/10
^{14}C-label	Benzyl	benzyl	Benzyl	benzyl	benzyl	benzyl	phenyl
Duration [h])	120	120	8, 24, 48, 68	0.5, 8, 20, 32	8	8	96
Samples	Blood/ plasma	blood/ plasma	Tissues	tissues	plasma, liver, kidney	plasma, liver, kidney	urine faeces
Methods of analysis	Total ^{14}C	total ^{14}C	total ^{14}C	total ^{14}C	total ^{14}C, HPLC	total ^{14}C, HPLC	total ^{14}C, HPLC, MS

* Designation used in the biokinetics study report
** Designation used in the metabolism study report
*** 14 non-radiolabelled doses at the high dose level followed by one ^{14}C-labelled dose at the low dose level

For the determination of the excretion balance, urine and faeces were collected separately 6, 12, 24h after application of the radiolabelled test substance and then in 24h intervals till the end of the experiment (120h). During Experiment 1 (high dose), exhaled air was also collected. At the end of the excretion balance experiments the following organs and tissues were collected: heart, liver, kidney, spleen, bone and bone marrow, skin, lung, ovaries and uterus, or testes, brain, pancreas, thyroid, adrenal glands, fat tissue, muscle, blood/plasma, stomach and contents, intestinal tract and contents, and carcass.

For the determination of blood/plasma levels, blood was taken retro-orbitally 0.5, 1, 2, 4, 8, 24, 48, 72, 96 and 120 hours post dose.

For the determination of the time-dependent tissue distribution, animals were sacrificed at time points close to maximum plasma concentration (MPC) and at 1/2MPC, 1/4MPC and 1/8MPC. The same organs and tissues were removed as described above.

For the determination of the biliary excretion, the bile duct of the animals was cannulated and bile was collected in 3 hour intervals up to a maximum of 48h.

Total radioactivity in urine, faeces, bile, blood/plasma and tissues was determined by liquid scintillation counting; in the case of solid samples, after solubilisation. Biokinetic data were analysed using the TOPFIT V 2.0 Program Package.

Findings

Excretion balance

In all dose groups and for both labelled forms of the test substance, the total amount of radioactivity was almost completely excreted, predominantly via the faecal route (Table 2.1-2). At 120h after administration, 57 - 82% of the dose was recovered from faeces and 14 - 39 % from urine. Renal excretion was more pronounced in female than in male animals (22 - 39% vs. 14 - 22% of dose after 120 hours). No radioactivity was detectable in the exhaled air. Within the first 24h after dosing, 56-83% of the administered radioactivity was excreted in faeces and urine. Radioactivity remaining in the body 120h post dose was below 0.6% of the dose administered, and the level in any tissue/organ sampled was < 2µg eq./g (except for the GI tract of males at the high dose). The overall recovery of radioactivity was in the range of 93-100 %.

Within 48 hours after administration of 10 and 100 mg/kg bw ^{14}C-dimoxystrobin (benzyl label), 34 - 55% of the administered radioactivity was excreted in bile. The lower percentage of the dose excreted in the bile at the higher dose indicates saturation of bilary excretion and/or formation of bilary metabolites and/or dose dependent oral absorption.

The amount of radioactivity excreted via bile and urine essentially reflects the absorbed proportion of the dose. Based on the amount of radioactivity excreted from 0 to 48 hours, the extrapolated total excretion and, hence, the bioavailability was assumed by the applicant to be in the range of 85 - 90% at the low dose level and 58 - 71% at the high dose level.

The RMS prefers to calculate oral absorption (bioavailablity) based on measured data for urine and bile rather than on extrapolated data. Oral absorption is therefore considered by the RMS to be:

75.3% (males) to 85.5% (females) at the low dose (mean for both sexes of 80%)
52.5% (males) to 62.8% (females) at the high dose (mean for both sexes of 58%).

Table 2.1-2 Excretion balance (in percent of dose) after administration of [benzyl-^{14}C]-dimoxystrobin and [phenyl-^{14}C]-dimoxystrobin to male and female rats (including biliary excretion)

Dose		**10 mg/kg bw p.o.**		**100/10 mg/kg bw multiple p.o.***	**100 mg/kg bw p.o.**		**100 mg/kg bw p.o.**	
Label		Benzyl		benzyl	benzyl		phenyl	
Sex		male	female	male	male	Female	male	female
Urine	0 – 6 h	3.53	11.84	4.33	0.82	2.88	0.98	2.37
	6 - 12 h	4.45	13.02	2.44	2.85	4.83	1.80	3.83
	12 - 24 h	6.49	10.09	7.83	5.78	9.99	4.70	8.71
	24 - 48 h	4.12	3.06	5.52	4.85	8.06	4.20	5.04
	48 – 120 h	1.88	1.15	2.18	2.41	2.94	2.52	2.06
	Subtotal urine	20.46	39.16	22.28	16.70	28.68	14.20	22.00
Faeces	0 – 12 h	8.66	21.00	1.92	7.36	3.04	3.99	2.64
	12 - 24 h	41.55	27.04	40.48	39.04	38.27	48.45	59.42
	24 - 48 h	17.65	7.21	25.93	25.12	16.77	22.92	10.15
	48 – 120 h	6.48	2.01	7.09	7.02	5.04	6.86	5.37
	Subtotal faeces	74.33	57.23	75.41	78.52	63.11	82.20	76.92
Excreted in urine and faeces 0- 24h		*65*	*83*	*57*	*56*	*59*	*60*	*77*
Cagewash		0.60	0.95	0.97	0.60	1.11	1.42	0.85
Tissues/carcass		0.42	0.24	0.55	0.43	0.16	0.22	0.14
Total	0 – 120 h	95.81	97.59	99.18	96.23	93.07	98.05	99.91
Bile	0 - 48 h	54.84	46.33	--	35.83	34.16	--	--

* 14 non-radiolabelled doses at the high dose level followed by 1 ^{14}C-labelled dose at the low dose level

Kinetic parameters

Key biokinetic parameters are summarised in Table 2.1-3. After oral administration at both dose levels a first peak plasma level was reached 0.5 hours post dose and second peak occurred after ca. 8 hours at the low dose level and between 8 and 24 hours at the high dose level. The study investigators considered that the first peak was probably due to absorption of dimoxystrobin dissolved in the preparation and the second peak to the slow dissolution of initially undissolved (suspended) test material in the gut. Up to 24 hours after dosing, the concentrations in whole blood were lower than plasma concentrations indicating that radioactivity preferably stayed in the plasma and was not bound to erythrocytes or other blood cells (the situation was reversed after 24h). After reaching the second peak plasma level, radioactivity concentrations declined monophasically. The plasma AUC-values increased less than proportionally with the dose indicating a lower absorption at the high dose level.

Table 2.1-3 Biokinetic parameters derived from plasma level vs. time curves after oral ad-ministration of [benzyl-^{14}C]-dimoxystrobin to male and female rats

Dose level	**10 mg/kg p.o.**		**100 mg/kg p.o.**	
Sex	**Male**	**Female**	**male**	**female**
C_{max} [µg/g]	0.77	1.00	1.67	1.72
T_{MAX} [H]	0.5	0.5	24.0	0.5
$t_{1/2}$ [h]	11.4	11.9	18.6	15.2
AUC [µG*H/G]	11.17	7.97	72.82	59.66
Total clearance [g/min]	14.9	20.9	22.9	27.9

Tissue distribution

There was wide distribution of radiolabel.

At the low dose, animals that were sacrificed 0.5 hours after dosing (at the first and, at this dose level, more pronounced plasma peak) showed highest radioactivity concentrations in the gastro-intestinal tract/contents. Tissues and organs which showed radioactivity concentrations higher than or close to the plasma concentration were liver, kidney, lung, fat tissue, thyroid, pancreas, adrenals, ovaries, uterus. Tissues with lowest concentrations included bone marrow and brain. At later time points the concentrations mostly declined continuously in all tissues and dropped to values below 1 µg equiv./g at 32 hours post dose, except for the gastro-intestinal tract/contents.

At the high dose, animals that were sacrificed 8 hours after dosing (close to the second and at this dose level more pronounced plasma peak) showed highest radioactivity concentrations in the gastro-intestinal tract/contents. Tissues and organs, which showed radioactivity concentrations higher than or close to the plasma concentration, were the same as at the low dose. Again, tissues with lowest concentrations included bone marrow and brain. At 24 hours after dosing, corresponding to the long lasting peak plasma level, the concentration dropped in the gastro-intestinal tract but remained more or less unchanged in the tissues except for fat, pancreas, ovaries and uterus where the concentration had increased. From 48 hours onwards, the concentrations declined continuously in most tissues and dropped to values below 2.5 µg equiv./g at 68 hours post dose, except for the gastro-intestinal tract/contents.

RMS comments and conclusion 2017:
The RMS agrees with the conclusion drawn in the DAR 2003.

Report:	KIIA 5.1.1/2 Anonymous, 1999: Metabolism of 14C-BAS 505 F (14C-285028) in rats 1999/12013
Guidelines:	not stated
GLP:	yes
Previously evaluated:	in the DAR 2003
Acceptability:	yes

Information on metabolite patterns/identification was obtained from the 10 and 100 mg/kg bw dose groups described in the biokinetics study (see Table 2.1-1).

Additional information on metabolite patterns/identification was obtained by oral dosing male and female Wistar rats with [^{14}C]-dimoxystrobin suspended in 1% carboxymethylcellulose in water containing ca. 10% Cremophor EL at a nominal dose level of 15 mg/kg and 150 mg/kg body weight (Dose Groups V, W, and DX, see Table B.6.1-1). Both benzyl and phenyl ^{14}C-labels were used.

Patterns of radioactive metabolites in urine, faeces (methanol extract), bile, plasma (acetonitrile/methanol/water extraction), liver (methanol extract) and kidney (methanol extract) were analysed chromatographically by HPLC. Where extraction was used the following percentages of radiolabel present were subject to HPLC analysis.

Faeces: 75-100%
Plasma: 59-74%
Liver: 70-104%
Kidney: 83-102%

Relevant metabolites were identified by MS analysis and in some cases by ^{1}H-NMR analysis of isolated fractions. The latter allowed in some cases the decision between isomeric structures. Intact conjugates were identified by API-LC-MS.

Prior to analysis, samples were pooled according to dose group and sex (and sometimes collection period).

Findings

A large number of metabolites were detected; a total of 45 (including conjugates) were identified mainly in urine and faeces.

Comparison of metabolite patterns in urine and faeces from animals especially generated for the isolation of metabolites (dose group DX) with those obtained from the biokinetics study showed that metabolite patterns were essentially the same (see Tables 2.1-4, 2.1-5, 2.1-7, 2.1-8). About 69-89% of the administered dose was identified in urine and faeces.

Because of the complexity of the metabolite patterns, most peaks in the chromatograms corresponded to several components that were quantified together in the study report (see Tables 2.1-4 – 2.1-8). The applicant however often considers one component in such a multicomponent peak to be predominant

but provides no supporting justification. Such statements by the applicant that are not apparently supported by data presented in the study report are indicated below by use of the phrase, " the applicant states that….".

These tables are as presented by the applicant (but with added footnotes). In certain cases HPLC peaks containing components common to 2 or more peaks have been simply amalgamated by the applicant (as compared with the summary tables in the study report). However, for a few metabolites present in urine some other regroupings of components have been presented by the applicant (see footnotes to tables).

The a.s. is an E-isomer. Only two rat metabolites were stated to be Z-isomers:
505M23 (the Z-isomer of 505M42) and 505M46 (the Z-isomer of 505M09). Both were identified as Z-isomers from a weaker m/z 116^+ identified by mass spectroscopy (as also seen with the Z-isomer of dimoxystrobin, which is an impurity in dimoxystrobin).

Benzyl-labelled metabolites

Urine (see Table 2.1-4): No unchanged parent was found. The applicant states that the isomeric acid metabolites 505M08/505M09 were the most abundant metabolites (together ca. 4-6% of dose in males and ca. 14-19% of dose in females). A series of further metabolites (e.g. 505M23, 505M37, 505M39, 505M34, 505M40, 505M42) are derived from 505M08 and 505M09 by oxidation and subsequent loss of the N-methyl group and/or cleavage of the methoxyimino group. The applicant states that, as far as these metabolites could be quantified separately, they were found in amounts of less than 4 % of the applied dose. Two groups of metabolites formed by the cleavage of the benzyl ether bond (505M01/505M22 and 505M12/505M17/505M18/505M19) together comprise 1-5 % of dose. Another metabolite derived from 505M09, produced by oxidation of the second aromatic methyl group, is 505M67 which was found in amounts of 1-5 % dose. Numerous other metabolites were found, but the applicant states that none exceeded 1 % of the applied dose.

Faeces (see Table 2.1-5): contained significant amounts of unchanged parent compound 505M00. A metabolite group, which the applicant states consisted mainly of parent, comprised 33-53% of the applied dose. The next most abundant faecal metabolites were 505M42 (2-8% dose), 505M67 (1-8% dose) and 505M37/505M39/505M41 (3-8% dose), all of which had also been found in urine.

Bile (see Table 2.1-6): contained 6 metabolites but no unchanged parent. The applicant states that two glucuronides formed the major portion of the biliary metabolites (505M50: 19-30% dose; 505M81: 6-10% dose).

Plasma (sampled at 8h = approx. time of second peak plasma level of a.s./metabolites): No unchanged parent compound was detected. At the low dose level, the only metabolite found was the acid 505M09 (50-61% plasma radioactivity). At the high dose level, 505M09 was still the major component in plasma (22-35% of plasma radioactivity) with smaller percentages of unknown metabolites.

Liver and kidney (sampled at 8h = approx. time of second peak plasma level of a.i/metabolites): contained 505M09 and 505M67 as major metabolites (505M67: 25- 58% tissue radioactivity; 505M09: 17-48% tissue radioactivity) at the low and the high dose level. In addition, smaller percentages of unchanged parent compound were found in liver (4-6% tissue radioactivity). Some individual unidentified metabolites were present in significant amounts (up to 6% of radioactivity in liver, up to 13-31% in kidney, although the 31% value appears to be spurious).

Phenyl-labelled metabolites

Urine (see Table 2.1-7): numerous metabolites were found but no unchanged parent. Metabolites containing the intact molecule backbone (*i.e.* with the benzyl ether bond uncleaved) were found in

comparable quantities as after dosing of the benzyl label. Metabolites specific for the phenyl label were the highly polar cleaved metabolites 505M15, 505M53, 505M55, 505M57, 505M59, and 505M60. However, they were only present in amounts of ca. 2% of the administered dose or less.

Faeces (see Table 2.1-8): a similar pattern was seen as after dosing with the other label. The applicant states that unchanged parent compound was the dominating component (48-61% of dose). No phenyl-label specific metabolites were found.

Overall metabolic picture

The overall picture is that dimoxystrobin was mainly metabolised by hydroxylation of the phenyl ring and by oxidation of the aromatic methyl groups to the corresponding benzyl alcohols and subsequently to the carboxylic acids. The cleavage of the benzyl ether bond was observed as well as the N-demethylation and O-demethylation. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid led to the large number of observed metabolites. The fact that faeces contained several radioactive components that cannot be derived from biliary metabolites indicates a considerable contribution of microbial intestinal metabolism. As no unchanged parent compound was found in plasma and dimoxystrobin is stable in aqueous solutions at pHs 4-9, a pronounced first pass metabolism has to be concluded.
The study investigators considered that unchanged parent was the main component of the faeces at the high dose level which was probably due to saturation of absorption.

The proposed metabolic pathway is depicted in Figure 2.1-2. Between sexes and generally between dose levels, metabolism was qualitatively similar and there were no substantial quantitative differences (notable differences in metabolic profile between dose levels were however seen in plasma sampled at 8 h post dose). Pre-dosing with parent had no marked effect on the metabolic profile.(Anonymous, 1999)

Table 2.1-4: Summary of identified metabolites in urine after single and multiple low and single high dose administration of [benzyl-^{14}C]-dimoxystrobin. Total excretion in % of dose

	Dose Group B (10 mg/kg single oral)		**Dose Group C (100/10 mg/kg multiple oral*)**	**Dose Group D (100 mg/kg single oral)**		**Dose Group DX (150 mg/kg single oral)**	
Metabolite identity	**male**	**female**	**male**	**Male**	**female**	**male**	**female**
	0 - 48 h					0 - 72 h	
505M10** 505M11**	0.17	0.12	0.36	0.16	-	0.08	-
505M12**	0.23	0.11	0.52	0.13	-	0.22	0.09
505M13**	0.36	0.22	0.47	0.24	-	0.40	0.12
505M14**	0.70	0.69	0.68	0.73	0.68	0.38	0.19
505M12** 505M17** 505M18** 505M19**	2.57	2.53	3.50	2.06	1.24	1.23	0.98
505M01** 505M22**	0.76	0.69	0.98	0.49	-	0.52	0.61
505M24 505M66	0.61	0.44	0.82	0.34	-	0.33	0.21
505M26 505M28 505M29 505M30 505M31 505M32	1.21	1.61	1.04	0.71	0.65	0.63	0.68
505M67	1.53	5.12	1.51	1.08	3.74	0.98	2.62
505M34	0.40	0.80	0.57	0.22	0.37	0.28	0.50
505M37 505M38 505M39 505M33	0.28	0.34	0.31	0.14	-	0.17	0.25
505M39 505M40 505M41	2.33	3.81	2.50	1.00	1.43	1.74	2.82
505M09 505M23 505M42 505M08	6.20	18.50	5.83	5.38	17.66	4.47	13.54
505M09 505M46	0.57	1.95	0.89	0.94	-	0.19	0.98
Total (urine)	17.92%	36.93%	19.98%	13.62%	25.77%	11.62%	23.59%
Total (urine+ faeces)* **	**75.90%**	**87.29%**	**81.92%**	**72.47%**	**68.86%**	**76.80%**	**78.66%**

* 14 non-radiolabelled doses at the high dose level followed by 1 ^{14}C-labelled dose at the low dose level
** Metabolites formed after cleavage of benzyl ether bond
Underlined metabolites: these groupings (as provided in the applicant's Tier II summary) show some differences to the groupings in the study report summary table
*** faeces data taken from Table 2.1-5

Table 2.1-5 Summary of identified metabolites in faeces after single and multiple low and single high dose administration of [benzyl-^{14}C]-dimoxystrobin. Total excretion in % of dose

	Dose Group B (10 mg/kg single oral)		Dose Group C (100/10 mg/kg multiple oral*)	Dose Group D (100 mg/kg single oral)		Dose Group DX (150 mg/kg single oral)	
Metabolite identity	**male**	**female**	**male**	**male**	**female**	**male**	**female**
	6 - 48 h		**0 - 48 h**				
505M17** 505M18**	-	-	-	-	-	-	0.31
505M01**	0.05	-	-	-	-	-	0.72
505M26	1.90	0.50	3.45	-	-	-	0.62
505M30	0.34	0.54	1.33	-	-	1.39	0.76
505M67	4.11	3.57	8.14	2.67	1.22	0.74	3.03
505M29	-	-	-	-	-	2.68	0.42
505M37 505M39 505M41	6.71	5.63	7.70	4.32	2.59	3.66	2.97
505M42	8.10	5.04	8.13	4.91	2.42	3.89	3.94
505M00 (parent)*** 505M02 505M08 505M09 505M29 505M44 505M63	36.77	35.08	33.19	47.95	36.86	52.82	42.30
Total (faeces)	57.98%	50.36%	61.94%	58.85%	43.09%	65.18%	55.07%

* 14 non-radiolabelled doses at the high dose level followed by 1 ^{14}C-labelled dose at the low dose level
** Metabolites formed after cleavage of benzyl ether bond
*** applicant states that parent was the major component but study report concludes that parent became major component at the high dose

Table 2.1-6 Summary of identified metabolites in bile after single low and high dose administration of [benzyl-^{14}C]-dimoxystrobin. Total excretion in % of dose

	Dose Group R (10 mg/kg single oral)		Dose Group S (100 mg/kg single oral)	
Metabolite identity	**male**	**female**	**male**	**female**
	0 - 48 h	**0 - 36 h**	**0 - 33 h**	**0 - 39 h**
505M33 505M50	29.38	23.60	20.54	18.47
505M81	8.67	9.87	6.68	6.08
505M33 505M39	0.82	1.00	0.60	0.55
505M42	1.77	1.23	0.78	0.93
505M09	8.10	5.89	3.89	4.79
Total (bile)	48.74%	41.59%	32.49%	30.82%

Table 2.1-7 Summary of identified metabolites in urine after single high dose administration of [phenyl-^{14}C]-dimoxystrobin. Total excretion in % of dose

Metabolite	Dose Group D (100 mg/kg single oral)		Dose Group DX (150 mg/kg single oral)	
	male	female	male	female
	0 - 48 h	0 - 48 h	0 - 72 h	0 - 72 h
505M60**	0.28	0.13	0.10	-
505M57**	-	-	0.16	-
505M55**	1.00	0.37	0.73	0.48
505M53** 505M59**	0.64	0.31	0.79	0.39
505M15**	0.19	-	0.17	-
505M24	0.22	0.18	0.25	0.15
505M66	0.38	0.19	0.39	0.14
505M26	0.44	0.26	0.35	0.30
505M28 505M29 505M30 505M33	0.90	0.52	0.53	0.34
505M67 505M64	1.04	1.91	0.91	2.54
505M34	0.19	0.34	0.27	0.32
505M08 505M37 505M40	0.24	0.24	0.18	0.19
505M39 505M42	2.14	3.80	2.18	3.52
505M08 505M09 505M44 505M46	3.57	11.53	3.65	12.15
Total (urine)	11.23%	19.78%	10.66%	20.52%
Total (urine + faeces)* **	**86.44%**	**86.13%**	**88.95%**	**75.69%**

** Metabolites formed after cleavage of benzyl ether bond

Underlined metabolites: these groupings (as provided in the applicant's Tier II summary) show some differences to the groupings in the study report summary table

*** faeces data taken from Table 2.1-8

Table 2.1-8 Summary of identified metabolites in faeces after single high dose administration of [phenyl-^{14}C]-dimoxystrobin. Total excretion in % of dose

	Dose Group D (100 mg/kg single oral)		Dose Group DX (150 mg/kg single oral)	
Metabolite identity	**male**	**female**	**male**	**Female**
	6 - 48 h		0 - 48 h	
505M26	1.58	-	2.36	0.42
505M30	-	-	0.98	-
505M67	3.82	1.92	4.51	2.39
505M29	-	-	-	0.3
505M39 505M41	4.37	2.33	4.88	1.87
505M42	4.51	2.86	5.96	2.66
505M00 (parent)* 505M02 505M08 505M09 505M29 505M44 505M63	60.93	59.24	59.60	47.53
Total (faeces)	75.21%	66.35%	78.29%	55.17%

* applicant states that parent was the major component but study report concludes that parent became major component at the high dose

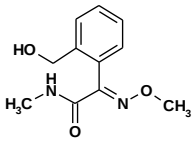
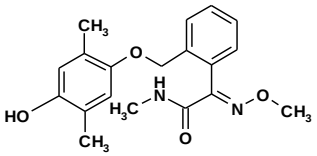
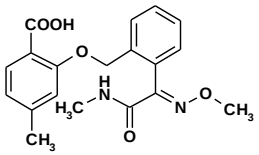
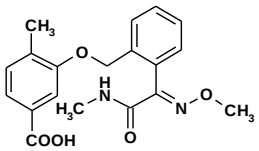
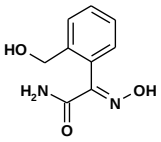
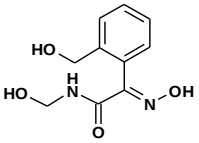
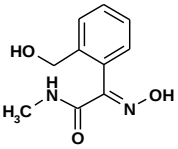
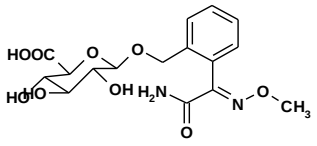
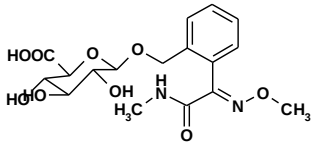
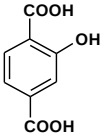
For reasons of convenience, a short summary of the main conclusions and an overview about the integration of rat metabolism data into the TTC concept is given in table 2.1-9.

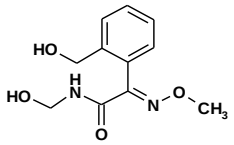
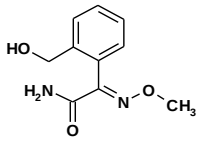
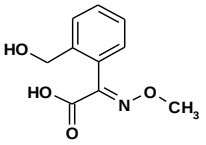
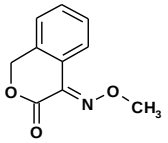
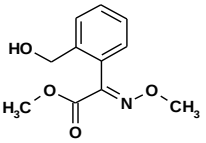
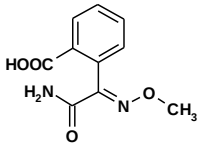
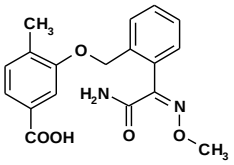
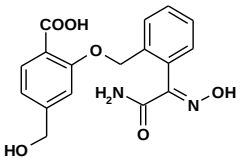
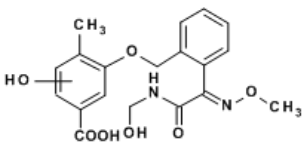
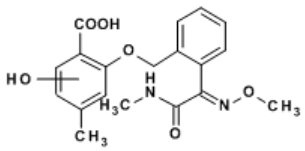
Table 2.1-9: The integration of rat metabolism data into the TTC concept

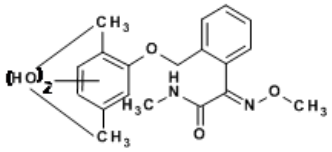
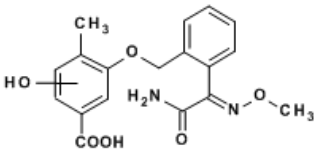
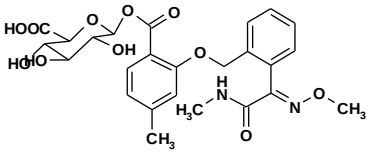
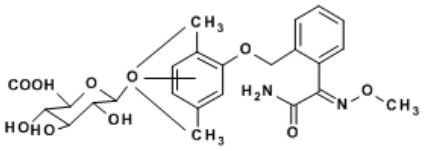
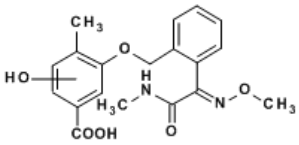
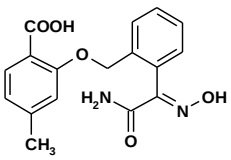
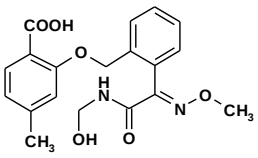
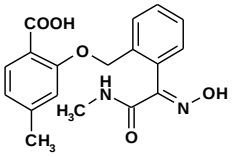
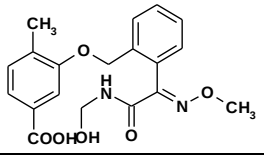
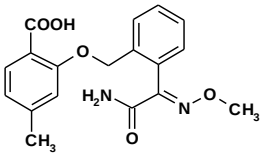
Category of test	**Dose ranges**	**Results**	**Reference (BASF DocID)**
Metabolism of ^{14}C-BAS 505 F (^{14}C-285028) in rats	– 10/100/150 mg/kg bw/d (single ^{14}C-exposure) – 10 mg/kg bw ^{14}C-spike after 14 d pre-treatment (unlabelled) – 15 and 150 mg/kg bw/d (single ^{14}C-exposure sacrifice after 8h, plasma, liver, kidney analysis) – 10 and 100 mg/kg bw/d (single ^{14}C-exposure, bile fluid analysis)	BAS 505 F was rapidly and intensively metabolised to a large number of biotransformation products. Combinations of Phase I biotransformation and conjugation reactions led to the large number of observed metabolites. No major differences were observed with regard to sex and dose level.	KIIA 5.1.1/2 (BASF DocID 1999/12013)
^{14}C-BAS 505 F - Study of the Biokinetics in rats	10 and 100 mg/kg bw	Radioactivity excretion half-life 11.4 - 18.6 h. Faecal excretion 57 - 82% of applied dose Biliary excretion 34 - 55% of applied dose. Bioavailability was 58 - 71% (high dose level) and 85 - 90% (low dose level).	KIIA 5.1.1./1 (BASF DocID 1999/11642)

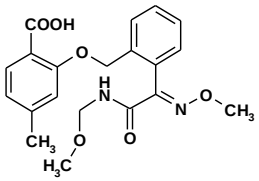
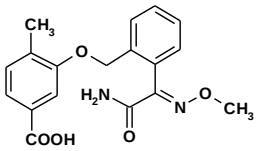
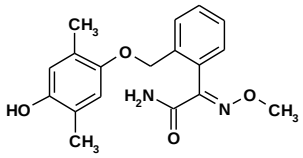
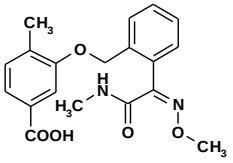
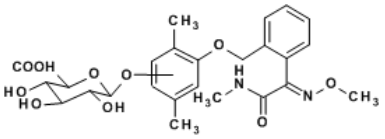
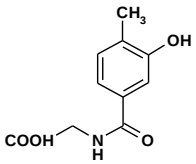
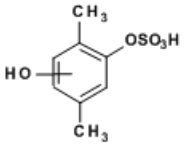
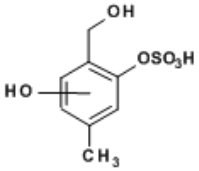
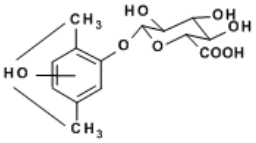
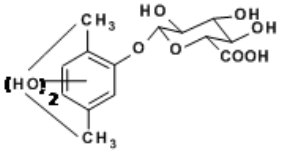
An overview of all metabolites identified in rat metabolism studies is presented in table 2.1-10.

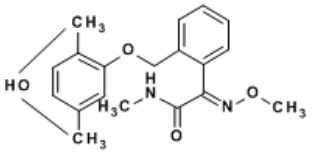
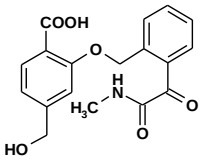
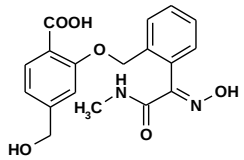
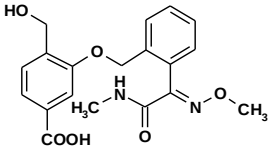
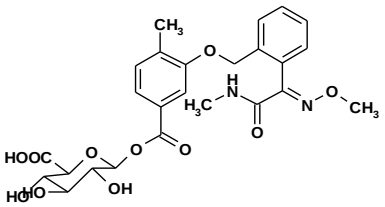
Table 2.1-10: All metabolites identified in rat

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M01	rat	urine, feces	
505M02	rat	feces	
505M08	rat	urine, feces	
505M09	rat	urine, feces, bile, plasma, liver, kidney	
505M10	rat	urine	
505M11	rat	urine	
505M12	rat	urine	
505M13	rat	urine	
505M14	rat	urine	
505M15	rat	urine	

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M17	rat	urine, feces	
505M18	rat	urine, feces	
505M19	rat	urine	
505M20	rat	urine	
505M21	rat	urine	
505M22	rat	urine	
505M23	rat	urine	
505M24	rat	urine	
505M26	rat	urine, feces	
505M28	rat	urine	

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M29	rat	urine, feces	
505M30	rat	urine, feces	
505M31	rat	urine	
505M32	rat	urine	
505M33	rat	urine, bile	
505M34	rat	urine	
505M37	rat	urine, feces	
505M38	rat	urine	
505M39	rat	urine, feces, bile	
505M40	rat	urine	

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M41	rat	urine, feces	
505M42	rat	urine, feces, bile	
505M44	rat	urine, feces	
505M46	rat	urine	
505M50	rat	bile	
505M53	rat	urine	
505M55	rat	urine	
505M57	rat	urine	
505M59	rat	urine	
505M60	rat	urine	

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M63	rat	feces	
505M64	rat	urine	
505M66	rat	urine	
505M67	rat	urine, feces, liver, kidney	
505M81	rat	bile	

Dosing and dose groups

The biokinetics of ^{14}C-BAS 505 F (BASF DocID ID 1999/11642) in male and female Wistar rats (Strain Chbb-THOM) were investigated at dose levels of 10 mg/kg bw and 100 mg/kg bw ("low" and "high" dose, respectively). For the isolation and identification of metabolites (BASF DocID 1999/12013), male and female Wistar rats were orally dosed in a separate study with [^{14}C]-BAS 505 F at a nominal dose level of 10 mg/kg, 100 mg/kg and 150 mg/kg body weight.

Excretion balance

In all dose groups and for both labelled forms of the test substance, the total amount of radioactivity was almost completely excreted, predominantly via the faecal route: 120 hours after administration 57 - 82% of the dose were recovered from faeces and 14 - 39% from urine. The renal excretion was more pronounced in female than in male animals (22 - 39% vs. 14 - 22% of dose after 120 hours). No radioactivity was detectable in the exhaled air. Already within the first 24 hours after dosing, 41 - 62% of the administered radioactivity was found in faeces and 8 - 35% in urine. Radioactivity remaining in tissues and organs 120 hours post dosing was below 0.6% of the dose (< 2µg eq./g). The overall recovery of radioactivity was in the range of 93-100 %. Within 48 hours after administration of 10 and 100 mg/kg bw of both ^{14}C-BAS 505 F, 34 - 55% of the administered radioactivity were excreted with the bile.

The amount of radioactivity excreted via bile and urine essentially reflects the absorbed proportion of the dose. Based on the amount of radioactivity excreted from 0 to 48 hours, the extrapolated total excretion and hence the bioavailability is assumed to be in the range of 85 - 90% at the low dose level and 58 - 71% at the high dose level with indications of saturation with increasing dose.

Pharmacokinetics

After oral administration at both dose levels a first peak plasma level was reached 0.5 hours post dose and second peak occurred after ca. 8 hours at the low dose level and between 8 and 24 hours at the high dose level. The second peak plasma level can be explained with a slow dissolution of initially undissolved (suspended) test material in the gut. The plasma level maxima were 0.77 – 1.00 µg eq/ after dosing of 10 mg/kg bw and 1.67 - 1.72 µg eq/g after dosing of 100 mg/kg. Up to 24 hours after dosing, the concentrations in whole blood were lower than plasma concentrations and thus indicate that the radioactivity preferably stayed in the plasma and was not bound to erythrocytes or other blood cells. After reaching the second peak plasma level, radioactivity concentrations declined monophasically with a half-life of 11.4 to 18.6 hours. The plasma AUC-values increased less than proportionally with the dose indicating a lower absorption at the high dose level.

Tissue distribution

At the low dose level, animals that were sacrificed 0.5 hours after dosing (at the first and, at this dose level, more pronounced plasma peak) showed highest radioactivity concentrations in the gastro-intestinal tract. Tissues and organs which showed radioactivity concentrations higher than or close to the plasma concentration were kidney, liver, lung, fat tissue, thyroid, pancreas, adrenals, ovaries, uterus. At later time points the concentrations declined continuously in all tissues and dropped to values below 1 µg equiv./g at 32 hours post dose, except for the gastro-intestinal tract.

At the high dose level, animals that were sacrificed 8 hours after dosing (close to the second and at this dose level more pronounced plasma peak) showed highest radioactivity concentrations in the gastro-intestinal tract. Tissues and organs which showed radioactivity concentrations higher than or close to the plasma concentration were kidney, liver, fat tissue, thyroid, pancreas, adrenals, and ovaries. At 24 hours after dosing, according to the long lasting peak plasma level, the concentration dropped in the gastro-intestinal tract but remained more or less unchanged in the tissues except for fat, where the concentration had increased. From 48 hours onwards, the concentrations declined continuously in all tissues and dropped to values below 2.5 µg equiv./g at 68 hours post dose, except for the gastro-intestinal tract.

Proposed metabolic pathway

The overall picture is that BAS 505 F was mainly metabolised by hydroxylation of the phenyl ring and by oxidation of the aromatic methyl groups to the corresponding benzyl alcohols and subsequently to the carboxylic acids. The cleavage of the benzyl ether bond was observed as well as the N-demethylation and O-demethylation. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid led to the large number of observed metabolites. The fact that faeces contained several radioactive components that cannot be derived from biliary metabolites indicates a considerable contribution of microbial intestinal metabolism. As no unchanged parent compound was found in plasma, a pronounced first pass metabolism has to be concluded. Comparison of metabolite patterns in samples from animals especially generated for the isolation of metabolites with those obtained from the biokinetic study showed that metabolite patterns were essentially the same. The metabolic pathway is presented below (Figur 2.1-2).

2.1.2 Comparative in-vitro metabolism

Report:	KCA 5.1.2/1 Funk D. et al., 2015 Comparative in-vitro metabolism with 14C-BAS 505 F 2015/1017699
Guidelines:	none
GLP :	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability	yes

EXECUTIVE SUMMARY

The radiolabeled test item was incubated with hepatocytes from humans and rats (both mixed genders) at a final concentration of 5 µM. Human hepatocytes were incubated with 5 µM Dimoxystrobin (^{14}C-phenyl and ^{14}C-benzyl label). As no label-specific metabolites were detected, the rat hepatocytes were only incubated with 5 µM phenyl labeled Dimoxystrobin. The viability of the hepatocytes was determined after 180 min incubation time using a luminescent cell viability assay. Negative and positive controls were conducted in parallel to prove the absence of non-metabolic degradation and the metabolic activity of the hepatocytes (phase I and phase II metabolic reactions). The control experiments yielded the expected results. After incubation for 10, 30, 60 or 180 min, the reaction was terminated by addition of cold ethanol and the resulting supernatant after concentration was analyzed by LSC and HPLC. Selected samples were additionally investigated by HPLC-MS. If the supernatant contained less than 90 % of the applied radioactivity (% AR), the corresponding cell pellet was extracted with acetone and in selected samples additionally with methanol, acetonitrile or treated with protease. The radioactive residues in the resulting extracts and the final pellet were determined by LSC. The extracts were also analyzed by HPLC.

The HPLC analyses of human and rat cell samples were compared to determine whether a unique human metabolite occurred. Selected human and rat supernatant samples were also analysed by HPLC-MS to assign *m/z* values to prominent peaks representing more than 5 % AR in human samples. After incubation of Dimoxystrobin with human hepatocytes, seven peaks were detected in the radio-chromatograms that represented more than 5 % AR for at least one time point. One of these signals represented the unchanged active substance Dimoxystrobin. The other peaks corresponded to metabolites of Dimoxystrobin (peaks at 2.2 min, 12.8 min, 19.0 min and 23.4 min and the metabolites M505F063 (28.5 min) and M505F108 (29.8 min)). The peaks at 2.2 min, 12.8 min, 19.0 min and 23.4 min and the active substance were also detected in rat hepatocyte samples.
The metabolites M505F063 and M505F108 were only detected in human hepatocyte samples. Nevertheless, they are described in the metabolic pathway of Dimoxystrobin in rats as shown in a previous rat metabolism study. As metabolic degradation of Dimoxystrobin was significantly faster in rat hepatocytes than in human hepatocytes it is likely that these phase I metabolites were not detected due to their fast conversion into other compounds.

In summary it can be concluded, that no human-specific metabolites were found.

MATERIALS

1. **Test Material:** BAS 505 F (Dimoxystrobin, Reg. No. 285028)
   Batch # / purity: see table below

Label	Batch No	Radiochemical purity [%]	Chemical purity [%]	Specific radioactivity a.s. [1] (MBq/mg)
benzyl-U-C14	596-3201	99.5	99.0	7.27
phenyl-U-C14	597-1501	99.9	98.7	4.67
acetamide-2-C13 (only for viability test)	596-2010	-	>97	-
unlabeled Dimoxystrobin (only for viability test)	01171-55	-	99.7	-

[1]active substance

Stability of test compound:
Stable over testing period. Stability control experiments (w/o cells) showed no degradation

2. **Positive controls:**
   Hepatocytes were incubated with 7-ethoxycoumarin and testosterone instead of the active substance to validate the metabolic activity of the hepatocytes.

3. **Test animals:**
   Species/Strain: Rat (Wistar, hepatocytes from male and female animals as mixture, ratio 1/1, source: Xenotech, Germany)
   Human (hepatocytes from males and females, source: Xenotech, Germany)

STUDY DESIGN AND METHODS

The study was carried out at the Agricultural Research Centre of BASF SE in Limburgerhof, Germany.
Dates of work: July 31, 2014 – November 20, 2014

2. Test system preparation

Test items
The radiolabeled and non-radiolabeled test materials were prepared according to the following procedures: for the benzyl and phenyl label, the radiolabeled test item (solution in toluene) was concentrated to dryness and taken up in an appropriate volume of acetonitrile. For the stock solutions of the benzyl and phenyl label, a concentration of approximately 2.3 mg/mL was determined. For preparation of the stock solutions of the acetamide label and unlabeled Dimoxystrobin, a defined weight was dissolved in acetonitrile with final concentrations of 0.7 mg/mL and 1.1 mg/mL.
The stock solution for testosterone contained about 0.5 mg/mL ^{14}C-labeled test material dissolved in ethanol. For the ^{14}C-7-ethoxycoumarin stock solution, a concentration of 0.3 mg/mL of the radio-labeled substance in dimethylsulphoxide (DMSO) was prepared.

For the preparation of application solutions for experiments with 1 µM Dimoxystrobin (benzyl label) and 5 µM Dimoxystrobin (benzyl and phenyl label), specific amounts of the ^{14}C-labeled test item were concentrated to dryness in a stream of nitrogen and redissolved in DMSO. For the preparation of the application solution for experiments with 10 µM Dimoxystrobin, calculated amounts of unlabeled, ^{13}C-labeled and ^{14}C-labeled test item were combined, concentrated to dryness in a stream of nitrogen and redissolved in DMSO. The actual concentrations in the application solutions were determined by LSC measurements. The purity of each application solution was confirmed by HPLC analysis and the identity as well as the isotope ratio was confirmed by MS analysis.
For the preparation of the application solution for experiments with 10 µM radiolabeled testosterone, a specific amount of the stock solution was concentrated to dryness in a stream of nitrogen and redissolved in DMSO.
For the preparation of the application solution for experiments with 10 µM radiolabeled 7-ethoxycoumarine, a specific amount of the stock solution was used as the application solution.

Hepatocytes
Cell suspensions (2×10^6 viable cells/mL) were prepared from cryopreserved rat and human hepatocytes. In the case of rat hepatocytes, male and female cells were combined at a ratio of 1:1. The human hepatocytes were purchased as a mixture of male and female cells.

Viability tests
The viability of human hepatocytes after incubation with 1 µM, 5 µM and 10 µM Dimoxystrobin (benzyl) was tested to select an appropriate concentration of the test item. Therefore, 250 µL of the respective application medium were incubated in a 24-well plate with 250 µL hepatocyte cell suspension at 37°C and 5% CO_2 for 180 min. The cell viability was determined using a luminescent cell viability assay. The viability of the human hepatocytes after the incubation with Dimoxystrobin at a concentration of 5 µM accounted for 101% in comparison to untreated cells. At a concentration of 10 µM the viability of the cells accounted only for 79%. Therefore, a final concentration of 5 µM Dimoxystrobin for both labels was chosen.
For the experiments with 5 µM Dimoxystrobin (each label), the viability of the hepatocytes was determined for each incubation date by a luminescent cell viability assay and is discussed in section II.

3. *In-vitro* assays

Hepatocytes were incubated with Dimoxystrobin at a final concentration of 5 µM for both labels. On each incubation day, the application solutions in DMSO were diluted with incubation media by a factor of 100 to prepare the respective application media. Each sample was prepared by adding equal amounts of the application medium and of the hepatocyte cell suspension into a well of a 24-well cell culture plate (maximum concentration of DMSO: 0.5%).

For human hepatocytes, both labels (benzyl and phenyl) were used in incubation experiments, while for rat hepatocytes, only the phenyl label was used. The incubations were performed for 10 min, 30 min, 60 min and 180 min at 37°C. Incubations were terminated by pipetting the incubation mixture into a weighed tube containing cold ethanol (final ethanol concentration: 70%) and cell lysis was assisted by ultrasonication. At this stage, the samples were stored frozen after concentration.

In addition, two negative controls (“stability control” and “zero incubation control”) and a blank control (application medium with DMSO instead of test item), in which no metabolism occurs, and two positive controls were performed for each species. For the “stability control”, the application medium was mixed only with incubation medium instead of cell suspension. For the “zero incubation control” (t = 0 min), the reaction was stopped immediately after addition of the cell suspension.
For the positive controls, 10 µM testosterone or 7-ethoxycoumarin instead of the active substance were incubated with hepatocytes from the different species to prove the metabolic activity of the different hepatocytes.

4. Work-up of samples

The stopped incubation mixture was centrifuged and concentrated by a centrifugal evaporator. The supernatant and the pellet were frozen separately. All samples were stored at -18°C or below.

Analysis of the supernatants
All supernatants were analyzed by RP- HPLC. The metabolites detected in the supernatants of human hepatocytes at relative concentrations above 5% of the applied radioactivity were further investigated by HPLC-MS analysis of selected samples. For all other samples, the radio-HPLC peaks were assigned to the identified metabolites by comparison of retention times and m/z values. The metabolite patterns obtained by incubation of rat hepatocytes were compared to the metabolite pattern obtained with human hepatocytes.

Work-up of the residual pellet
If the radioactive residues in the supernatants of the terminated incubation mixtures yielded less than 90% AR, the pellets after centrifugal evaporation were resuspended in 50 µL water and mixed with 250 µL acetone followed by ultrasonication for 5 min. After centrifugation, the radioactive residues in the acetone extract were determined by LSC analysis of aliquots. In some cases the remaining pellets were additionally extracted with methanol and/or acetonitrile. The methanol and acetonitrile extractions were performed as described for the acetone extraction and the radioactive residues in the supernatants were determined by LSC analysis. The final pellets were resuspended in 320 µL water, ultrasonicated for 10 min and the radioactive residues in the suspended samples were measured by LSC.

In some samples low recoveries of the applied radioactivity were observed. Therefore, enzyme solubilization was performed for affected samples to examine a potential incorporation into amino acids. The pellet after centrifugal evaporation was resuspended in 750 µL TRIS buffer and solubilized with protease at 37°C . After 210 min, an equal amount of protease was added and the sample was incubated overnight. After ultrasonication for 5 min and subsequent centrifugation the supernatants were analyzed by LSC.

A polar peak at 2.2. min was detected at relevant levels after incubation of hepatocytes with Dimoxystrobin. In order to investigate the polar peak in more detail, selected samples were subjected to fractionation. The fraction containing the polar peak was concentrated and analyzed by HPLC.

RESULTS AND DISCUSSION

1. Control Experiments

The blank controls (w/o test item) performed for human and rat hepatocytes showed no significant amounts of radioactive residues (LSC measurements), and no radioactive peaks were detected by HPLC analysis.

The negative control samples (stability control without cells and zero incubation control showed nearly identical HPLC profiles, which contained only one peak corresponding to the unchanged active substance Dimoxystrobin. Some acetone extracts of the pellet showed two closely adjacent peaks both most likely representing Dimoxystrobin. No significant metabolism or degradation of Dimoxystrobin was observed.

The positive controls showed that the metabolic activity of the hepatocytes in respect to phase I and phase II metabolic reactions was sufficiently high. Testosterone was metabolised completely after incubation with human and rat hepatocytes. The degree of metabolization for 7-ethoxycoumarin reached values above 69% and 78% for human and rat hepatocytes, respectively.

2. Cell viability

The hepatocyte suspensions were adjusted to a cell density of approximately 10^6 cells/mL in the incubation assays. After incubation for 180 min, the viability of the cells was determined using a

luminescent cell viability assay. The viability of human hepatocytes incubated with 5 µM Dimoxystrobin was 74% (phenyl label) and 84% (benzyl label) in comparison to untreated cells. The viability of the rat hepatocytes incubated with 5 µM Dimoxystrobin (phenyl label) was 106% compared to untreated cells. Therefore, it can be stated that the viability in the *in-vitro* assays was sufficient to generate valid results.

3. Determination of radioactivity in supernatants and pellets

In some samples, the radioactive residues recovered in the concentrated supernatants after terminating the incubation were below 90% AR. The corresponding residual pellets were extracted with acetone and if necessary with methanol and acetonitrile. The radioactive residues in the acetone extracts were below or equal to 15.01% AR in all experiments, while the methanol and acetonitrile extracts accounted for less than 1% AR. Likewise, the radioactive residues in the final pellets of all experiments were below 1% AR. In some cases the sum of recovery of radioactive residues accounted for less than 90% AR. Therefore, some acetone extraction pellets were subjected to an enzyme solubilisation step with protease. The radioactive residues in the enzyme solubilizates were below or equal to 0.53% AR. Thereby, it can be stated that no significant amounts of radioactive residues were incorporated into amino acids. As the sum of recovery of radioactive residues in general accounted for more than 90% AR and losses were mostly observed in stability control samples, no significant influence on the objective of this study is expected.

4. Kinetics of biodegradation of Dimoxystrobin after incubation of hepatocytes

The peak representing the unchanged active substance is present in most of the samples after incubation of 5 µM Dimoxystrobin with human hepatocytes. The mean % AR of the active substance decreased in samples of the benzyl and the phenyl label from 91.15% and 90.83% AR at 0 min to 21.86 % (benzyl label) and 21.63 % AR (phenyl label) after 60 min. After 180 min, 10.17 % AR was recovered for the benzyl label, while no Dimoxystrobin was detected for the phenyl label.

After incubation of rat hepatocytes with Dimoxystrobin, the active substance (phenyl label) showed a faster decrease compared to human hepatocytes from 89.55 % AR at 0 min to 11.92 % AR after 10 min and after 30 min, no Dimoxystrobin was detectable. The transformation of Dimoxystrobin thus proceeded faster in rat hepatocytes than in human hepatocytes.

5. Peaks detected after incubation of hepatocytes with Dimoxystrobin

Radio-HPLC and HPLC-MS analysis of samples after incubation with human hepatocytes with Dimoxystrobin resulted in the assignment of seven relevant peaks. The amounts of detected metabolites in different test species after designated incubation times are summarized in Table 2.1-11.

Apart from the active substance Dimoxystrobin the following peaks were detected in human hepatocytes samples incubated with 5 µM Dimoxystrobin:

Metabolites M505F063 and M505F108 were detected after 10 min in samples of both labels. Both metabolites reached their maximum concentration after 60 min and subsequently decreased with M505F063 not being detectable after 180 min.

Four additional peaks were assigned for both labels, whereby for the benzyl label one component was detected after 10 min (P23.4) and two (P2.2 and P12.8) after 30 min, which all constantly increased within the observation period. Additionally for the benzyl label, a peak (P19.0) was detected after 10 min reaching its maximum after 30 min followed by a subsequent decrease below the detection limit (180 min). Similar observations were made for the phenyl label, except for the component P23.4, which was initially detected after 30 min.

The results obtained from incubation of human hepatocytes with Dimoxystrobin were comparable for both labels. Therefore, no label-specific evaluation was required for the data from rat hepatocyte samples.

In samples obtained from rat hepatocytes, metabolites M505F063 and M505F108 were not detectable after incubation with Dimoxystrobin.

The peaks P2.2, P12.8, P19.0, P23.4 and the parent compound Dimoxystrobin, also found after incubation of human hepatocytes, were detected. The formation of the metabolites corresponding to P12.8 and P23.4 occurred faster than in human hepatocytes. Both metabolites were already detected after 10 min and constantly increasing until reaching plateau or maximum, respectively, after 60 min.

The peak P19.0 was detected after 10 min and its levels subsequently decreased below the detection limit after 60 min.

In summary, the comparative *in-vitro* experiments demonstrated that the metabolites, which were identified in human hepatocyte samples, are also observed in rat hepatocyte samples and/or rat metabolism studies.

Table 2.1-11: Comparison of Relevant Metabolites of Dimoxystrobin after Incubation of Human or Rat Hepatocytes

Relevant Peak		**P2.2**	**P12.8**	**P19.0**	**P23.4**	**M505F063**	**M505F108**	**Dimoxy-strobin**
		[% AR]						
0 min	Human (Benzyl)	-	-	-	-	-	-	91.15
	Human (Phenyl)	-	-	-	-	-	-	90.83
	Rat (Phenyl)	-	-	-	-	-	-	89.55
10 min	Human (Benzyl)	-	-	7.50	1.50	2.57	1.92	81.55
	Human (Phenyl)	-	-	6.68	-	2.32	1.47	75.45
	Rat (Phenyl)	-	4.59	12.15	32.30	-	-	11.92
30 min	Human (Benzyl)	0.57	6.32	17.15	7.38	5.91	3.72	48.01
	Human (Phenyl)	0.67	5.85	14.06	6.85	5.44	3.91	53.08
	Rat (Phenyl)	-	16.76	4.27	53.34	-	-	-
60 min	Human (Benzyl)	3.45	20.82	16.89	13.72	7.13	5.49	21.86
	Human (Phenyl)	4.51	20.72	13.55	13.00	6.91	5.55	21.63
	Rat (Phenyl)	-	22.20	-	53.46	-	-	-
180 min	Human (Benzyl)	4.48	46.32	-	14.93	-	2.65	10.17
	Human (Phenyl)	8.05	51.46	-	15.36	-	1.88	-
	Rat (Phenyl)	4.49	21.29	-	26.33	-	-	-

In the present comparative *in-vitro* metabolism study, no significant differences between the two different radiolabels were evident, while some species differences were observed.
After the incubation of human hepatocytes with the active substance, seven ^{14}C-peaks were detected by HPLC analysis that represented more than 5% AR on at least one time point in one label (sum of supernatant and pellet extract). One of these signals represented the unchanged active substance Dimoxystrobin.
The metabolites M505F063 and M505F108 were only detected in human hepatocyte samples. These metabolites were identified in the metabolic pathway in rats as shown in a previous rat metabolism study. The metabolic degradation of Dimoxystrobin occurred considerably faster in rat hepatocytes than in human hepatocytes. Therefore, these two phase I metabolites were likely not detected due to their fast conversion to other compounds.
The other peaks of Dimoxystrobin (peaks at 2.2 min, 12.8 min, 19.0 min and 23.4 min) were also detected in rat hepatocyte samples.

CONCLUSION:
The metabolic pattern of ^{14}C-Dimoxystrobin was similar in the tested species and no human-specific metabolites were found.

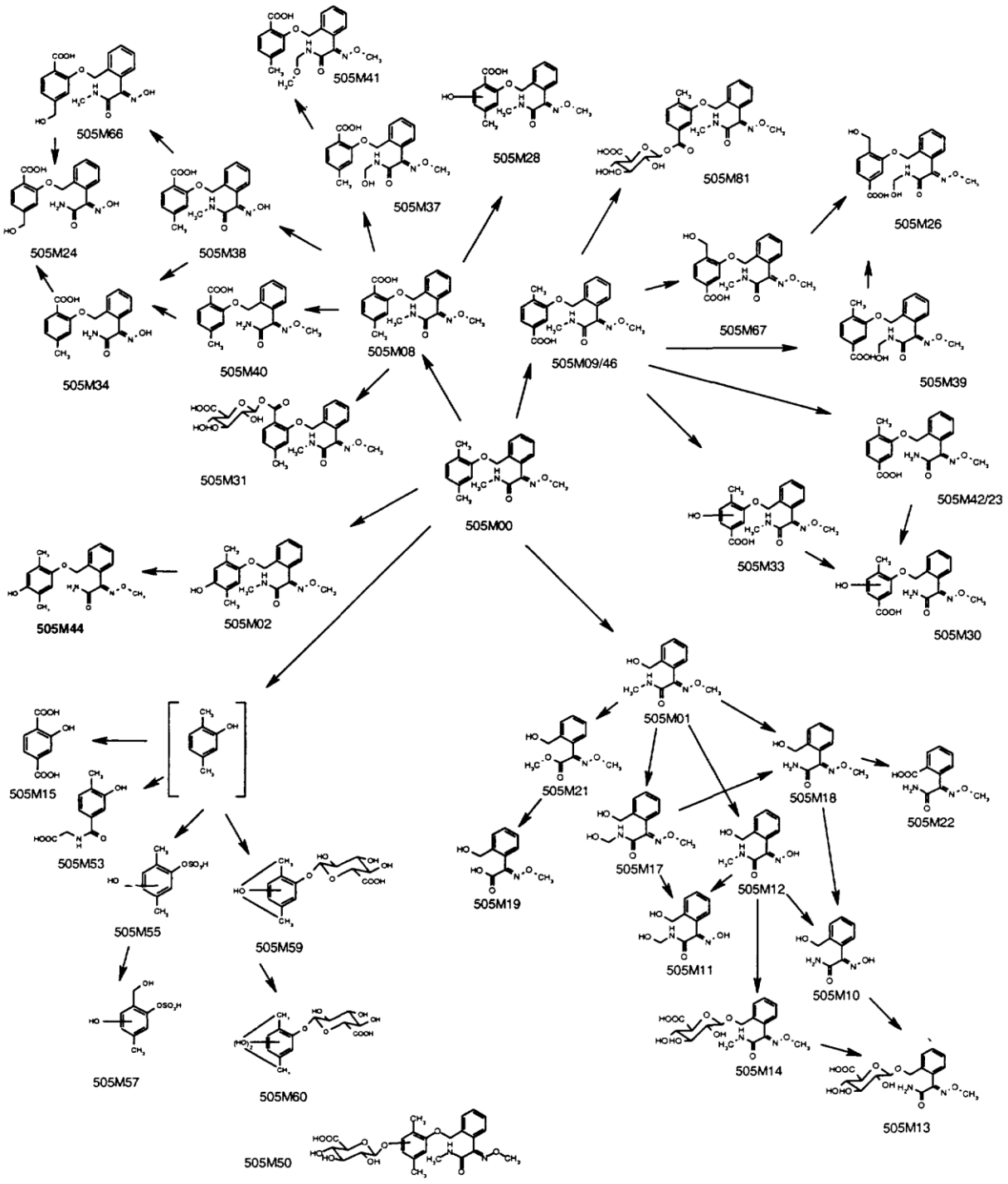


Figure 2.1-2: Proposed metabolic pathway of dimoxystrobin in rats

2.1.3 Quantification of metabolites 505M08 and 505M09 in rat excreta

Report:	KCA 5.1.1/1 Fabian E., 2006a Quantification of BF 505-7 and BF 505-8 in rat excreta 2004/1006526
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	yes

A rat metabolism study with ^{14}C-BAS 505 F was performed from August 1996 to March 1999 (Anonymous, 1999). In this study the applied HPLC systems did not allow separate quantification of metabolites 505M08 and 505M09. As both metabolites became of interest for a detailed assessment of the metabolic behavior of BAS 505 F in rats, urine and faeces samples of study were reanalyzed.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Material:**	urine and faeces samples of study Velic I.. 1999:
	Description:	a) Reg.No. 354562 (radiolabeled) b) Reg.No. 354562 (unlabeled) c) Reg.No. 354563 (radiolabeled) d) Reg.No. 354563 (unlabeled)
	Lot/Batch #:	a) 695-1018 b) 01196-241 c) 664-1013 d) 01196-245
	Purity:	a) >96% (chemical/radiochemical; benzyl-U-^{14}C) b) 97.8% c) >99% (radiochemical; phenyl-U-^{14}C), >85% (chemical) d) 99.6%
	Metabolite code:	a) BF 505-7 (505M08) b) BF 505-7 (505M08) c) BF 505-8 (505M09) d) BF 505-8 (505M09)
	Stability of test compound:	NA

B. STUDY DESIGN AND METHODS

1. Dates of work: August 06, 2003 – February 6, 2004

The urine and faeces samples reanalyzed in this study were sampled from 10 male and 10 female Wistar rats treated with single oral dose of 150 mg/kg benzyl- or phenyl-labeled BAS 505 F (dose group DX, see B.6.1.1). The test substance was suspended in 1% carboxymethylcellulose in water containing 10% Cremophor EL. Urine and faeces from dose group DX were collected after 24, 48, 72, and 96 h. In this re-analysis the metabolites 505M08 and 505M09 were quantified in pooled faeces (0-48 h) and pooled urine (0-72 h) samples.

Urine samples were investigated by the application of several HPLC methods without any work up / purification steps. For the re-analysis of faeces samples, methanol extracts of pool samples were also measured by means of HPLC. For radioactivity measurement aliquots of liquid samples were mixed with scintillator and measured in a liquid scintillation counter. For the detection of radioactivity in extraction residues of faeces, five aliquots of the extraction residue were dried and combusted, followed by determination of the radioactivity.

II. RESULTS AND DISCUSSION

Storage stability
Before quantification, all pool samples were analyzed applying the chromatographic conditions of the study of Leibold *et al*., 1999. and the received metabolic profiles were compared to the original profiles. Since the metabolic profiles of the study of Leibold *et al*., 1999 and this study were qualitatively and quantitatively comparable, the storage stability of samples was proven.

Extractability
The extracts of faeces homogenates from dose group DX (label B) amounted to 100.12% (71.56% dose) and 80.58% (54.44% dose) of the faeces activity for male and female rats, respectively. The extracts of faeces homogenates from dose group DX (label P) amounted to 93.51% (76.73% dose) and 89.77% (52.75% dose) of the faeces activity for male and female rats, respectively. Therefore, the measured extractabilities of the generated pool samples were in good accordance with the values described in the main study report.

Table B.6.1-12: Extractability of faeces samples with methanol after dosing of rats with [benzyl-U-^{14}C]- or [phenyl-U-^{14}C]-BAS 505 F

Matrix	Faeces	Methanol extract		Residue	
	% dose	% dose	% faeces activity	% dose	% faeces activity
Benzyl-U-^{14}C label					
Male rats (0-48 h)	71.47	71.56	100.12	9.87	13.81
Female rats (0-48 h)	67.56	54.44	80.58	9.98	14.77
Phenyl-^{14}C label					
Male rats (0-48 h)	82.06	76.73	93.51	13.38	16.30
Female rats (0-48 h)	58.76	52.75	89.77	7.61	12.95

Metabolism
Since the goal of this study was the quantification of 505M08 and 505M09, the evaluation of the chromatograms concentrated on these metabolites and the peaks of additional metabolites were not assigned.

In the urine samples from DX (label B) dose group 505M08 amounts to 11.64% and 11.08% of the total peak area corresponding to 1.36% and 2.61% dose for male and female rats, respectively. 505M09 amounts to 31.57% and 53.68% of the total peak area corresponding to 3.69% and 12.66% dose for male and female rats, respectively.
505M08 and 505M09 are also the main metabolites in the pool samples of faeces extracts of dose group DX (label B). 505M08 amounts to 7.82% and 6.12% of the total peak area corresponding to 5.60% and 3.33% dose for male and female rats, respectively. 505M09 amounts to 25.61% and 21.48% of the total peak area corresponding to 18.33% and 11.69% dose for male and female rats, respectively.
The sum of the amounts of 505M08 in pooled urine and faeces samples accounts for 6.96% and 5.94% dose in male and female rats of dose group DX (label B), respectively. The sum of the amounts of 505M09 in the same dose groups is 22.02% and 24.35%
dose in male and female rats, respectively.

Similarly, 505M08 and 505M09 are the main metabolites in urine samples from dose group DX (label P). 505M08 amounts to 13.78% and 11.36% of the total peak area corresponding to 1.49% and 2.33% dose for male and female rats, respectively.
505M09 amounts to 32.95% and 59.23% of the total peak area corresponding to 3.57% and 12.15% dose for male and female rats, respectively. 505M08 and 505M09 are also the main metabolites in the pool samples of faeces extracts of dose group DX (label P). 505M08 amounts to 9.35% and 6.09% of the total peak area corresponding to 7.17% and 3.21% dose for male and female rats, respectively. 505M09 amounts to 22.33% and 25.34% of the total peak area corresponding to 17.13% and 13.36% dose for male and female rats, respectively.
The sum of the amounts of 505M08 in pooled urine and faeces samples accounts for 8.66% and 5.54% dose in male and female rats of dose group DX (label P), respectively. The sum of the amounts of 505M09 in the same dose groups is 20.70% and 25.51% dose in male and female rats, respectively.

It could be demonstrated that 505M08 and 505M09 can be found in significant amounts in urine and faeces samples of rats.

Table 2.1-13: Summary of metabolites identified in urine and faeces from dose group DX (label B) and DX (label P)

Designation	Females			Males		
	Urine (0-72 h) [% dose]	**Faeces (0-48 h) [% dose]**	**Sum [% dose]**	**Urine (0-72 h) [% dose]**	**Faeces (0-48 h) [% dose]**	**Sum [% dose]**
Benzyl-U-^{14}C label						
505M08	2.61	3.33	5.94	1.36	5.60	6.96
505M09	12.66	11.69	24.35	3.69	18.33	22.02
Phenyl-^{14}C label						
505M08	2.33	3.21	5.54	1.49	7.17	8.66
505M09	12.15	13.36	25.51	3.57	17.13	20.70

III. CONCLUSION

It could be demonstrated by re-analysing urine and faeces samples from study Velic I.. 1999: that 505M08 and 505M09 are found in significant amounts in urine and faeces samples of rats treated with BAS 505 F by the oral route.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

3.1.1 Animal data

[Study 1]

Report:	Anonymous 1998a BAS 505 F - Acute oral toxicity in rats BASF DocID 1998/11002
Guidelines:	OECD guideline 401 (1987)
GLP:	yes

Dimoxystrobin (batch/purity: N 6 Lot 3004: 98.8%) as a suspension in 0.5% aqueous Tylose was administered as a single gavage dose to five male and five female fasted Wistar rats at dose levels of 2,000 and 5,000 mg/kg bw. The application volume was 10 ml/kg bw. The observation period lasted for up to 14 days.
The study investigators report that the stability of the test substance in the vehicle, the correctness of the concentration and its homogeneity were analytically confirmed.

Findings
There was no mortality. Signs of toxicity noted at 2,000 and 5,000 mg/kg bw included impaired or poor general state, dyspnoea, apathy, staggering, and diarrhoea in males and females. All animals appeared normal within six days after application.

Body weight gain generally appeared to be normal. There were no macroscopic pathological findings in animals sacrificed at the end of the observation period.

Conclusion
The oral LD_{50} of dimoxystrobin was > 5,000 mg/kg bw for male and female rats.

3.1.2 Human data
No human data are available.

3.1.3 Other data
No other data are available.

3.2 Acute toxicity - dermal route

3.2.1 Animal data
[Study 1]

Report:	Anonymous.1998b BAS 505 F-Acute dermal toxicity in rats BASF DocID 1998/11001
Guidelines:	OECD guideline 402 (1987)
GLP:	yes

Dimoxystrobin (batch/purity: N 6 Lot 3004: 98.8%) as a suspension in 0.5% aqueous Tylose was applied dermally to five male and five female Wistar rats for 24 hours under semi-occlusive dressing at a dose level of 2,000 mg/kg bw. The application area was about 50 cm² (at least 10% of body surface area). The observation period lasted for up to 14 days.

Findings
The study investigators report that the stability of the test substance in the vehicle was analytically confirmed and its homogeneity was guaranteed by stirring.
No mortality occurred and no clinical signs of toxicity were observed. No effects on the skin were observed (recorded 30-60 minutes after removal of dressing and weekly thereafter). Body weight development appeared to be normal with exception of one female animal, which showed weight reduction and another female animal, which showed no gain in body weight, in the first week of observation.
No pathological findings were detected in the animals.

Conclusion
The dermal LD_{50} of dimoxystrobin was > 2,000 mg/kg bw for male and female animals.

3.2.2 Human data

No human data are available.

3.2.3 Other data

No other data are available.

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

[Study 1]

Report:	Anonymous 1997; BAS 505 F-Acute inhalation toxicity in Wistar rats BASF DocID 1997/10971 Anonymous 1998.: Amendment to the report "Anonymous 1997; BAS 505 F-Acute inhalation toxicity in Wistar rats" BASF DocID 1998/10626
Guidelines:	OECD guideline 403 (1981)
GLP:	yes

Five male and five female Wistar rats per dose level were exposed (head and nose) to a dust aerosol of dimoxystrobin (batch/purity: N 6 Lot 3004: 98.8%) for four hours in a head/nose inhalation system at mean analytical concentrations of 0.51, 1.28 and 5.9 mg/l. The observation time was 14 days.

The study investigators report that the homogenous distribution of atmospheres in this inhalation system has been proven with model aerosols and vapours.

The particle size distribution revealed mass median aerodynamic diameters (MMADs) of 2.5 µm for the low and mid concentration, which are within the respirable range for humans. The MMAD of the high concentration was of 5.1 µm in spite of several technical measures; this was probably due to the high concentration.

Findings

All animals exposed to 5.9 mg/l died during exposure. In the intermediate concentration of 1.28 mg/l one male and two female animals died during or apparently immediately after exposure (see Table 3.3.1-1).

Table 3.3.1-1 Lethality

Cumulated lethality on day	Test group (concentration)					
	1 (0.51 mg/L)		2 (1.28 mg/L)		3 (5.9 mg/L)	
	M	F	M	F	M	F
0	0/5	0/5	1/5	2/5	5/5	5/5
1	-	-	-	-	-	-
2	-	-	-	-	-	-
7	-	-	-	-	-	-
14	-	-	-	-	-	-
End of study	0/5	0/5	1/5	2/5	5/5	5/5

In the low and mid dose concentration group clinical examination revealed attempts to escape, irregular accelerated and intermittent respiration, as well as squatting posture and piloerection. No clinical signs could be detected from post exposure day 5 onward (see Table 3.3.1-2).

Table 3.3.1-2 Maximum incidence of clinial findings

Clinical signs Duration	Test group (concentration)					
	1 (0.51 mg/L)		2 (1.28 mg/L)		3 (5.9 mg/L)	
	M	F	M	F	M	F
All animals without findings	D5 – d14		D8 – d14		n.d.	
Attemps to escape	0/5	5/5	5/5	5/5	5/5	5/5
Irregular respiration	5/5	5/5	1/5	n.d.	5/5	3/5
Acceleration respiration	5/5	5/5	5/5	5/5	3/5	5/5
Intermittent respiration	1/5	2/5	n.d.	n.d.	4/5	5/5
Squatting posture	5/5	5/5	4/5	3/5	n.d.	n.d.
Piloerection	5/5	5/5	4/5	3/5	n.d.	n.d.
Smeared fur	n.d.	n.d.	4/5	3/5	n.d.	n.d.

The mid concentration resulted additionally in smeared fur. In the surviving animals no clinical signs were detected from day 8 onward.
The study investigators considered that there was no effect on body weight gain in the low concentration animals. Body weight gain in the surviving mid concentration males was slightly depressed in the first post exposure week but recovered in the second.
Necropsy of the decedent mid (1.28 mg/l) concentration animals showed agonal congestive hyperaemia. No macroscopic pathologic findings were noted in all other animals that died or were examined at the end of the study.

Conclusion
The inhalation LC_{50} of dimoxystrobin was 1.3 mg/l (males 1.9 mg/l, females 1.3 mg/l). The LC_{50} of both sexes combined is about 1.7 mg/L.

3.3.2 Human data
No human data are available

3.3.3 Other data
No other data are available.

3.4 Skin corrosion/irritation

3.4.1 Animal data

[Study 1]

Report:	Anonymous, 1998c BAS 505 F - Acute dermal irritation/corrosion in rabbits BASF DocID 1998/10999
Guidelines:	OECD guideline 404 (1992)
GLP:	yes

The study complied with OECD guideline 404 (1992), although more animals were used than required by the OECD guideline.

The 0.5 g dimoxystrobin (batch/purity: N 6 Lot 3004: 98.8%) moistened with distilled water was applied dermally to the intact skin of three male and three female White New Zealand rabbits. The application was for 4 hours on a 2.5 cm x 2.5 cm test patch under a semi-occlusive dressing. After the patches were removed the treated area was rinsed with Lutrol (= polyethyleneglycol) and Lutrol/water (1 : 1). The animals were observed for skin irritation for 8 days after test material application. Skin readings were performed at 1 h, 24 h, 48 h, and 8 days after removal of the patch.

Findings
The average score (24 to 72 h) for dermal irritation was calculated to be 0.6 for erythema (mean individual scores at 24, 48 and 72 h: 0.0, 0.0, 0.0, 0.7, 1.0 and 2.0) and 0.0 for oedema. No evidence of skin irritation was seen at 8 days.

Conclusion
Dimoxystrobin caused slight irritation with mean individual scores (24, 48, 72 h timepoints) for erythema of 0.0, 0.0, 0.0, 0.7, 1.0, and 2.0. The mean score for oedema was 0,0. The skin reactions were fully reversible within 8 days.

3.4.2 Human data
No human data are available.

3.4.3 Other data
No other data are available.

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

[Study 1]

Report:	Anonymous 1998d BAS 505 F - Acute eye irritation in rabbits BASF DocID 1998/11000
Guidelines:	OECD guideline 405 (1987)
GLP:	yes

The study complied with OECD guideline 405 (1987), although more animals were used than required by the OECD guideline.

Undiluted dimoxystrobin (batch/purity: N 6 Lot 3004: 98.8%) was applied once to the conjunctival sac of six male White New Zealand rabbits. The application volume was about 0.1 ml bulk volume (about 46 mg). The test substance was washed out with tap water 24 hours after the application. Readings of the eyes were carried out at 1 h, 24 h, 48 h and 72 h after the application of the test material.

Findings

No signs of cornea opacity were seen at any of the observation time points. Slight conjunctival responses were seen at 1h, including grade 1-2 conjunctival discharge. The average score (24 to 72 h) for eye irritation was calculated to be 0.1 for iritis (mean individual scores at 24, 48 and 72 h: 0.0, 0.0, 0.0, 0.3, 0.0, 0.0), 0.6 for conjunctival redness (mean individual scores at 24, 48 and 72 h: 0.0, 0.3, 0.7, 1.7, 0.7, 0.3) and 0.1 for chemosis (mean individual scores at 24, 48 and 72 h: 0.0, 0.0, 0.0, 0.7, 0.0, 0.0). One animal at 24 h only showed slight conjunctival discharge and a contracted pupil. No evidence of eye irritation was seen at 72h.

Conclusion
Dimoxystrobin caused only slight, reversible eye irritation with mean individual scores (24, 48 and 72 h timepoints of 0.0, 0.0, 0.0, 0.3, 0.0, 0.0 for iritis, 0.0, 0.3, 0.7, 1.7, 0.7, 0.3 for conjunctival redness and 0.0, 0.0, 0.0, 0.7, 0.0, 0.0 for chemosis. The eye effects were fully reversible within 72 h.

3.5.2 Human data
No human data are available.

3.5.3 Other data
No other data are available.

3.6 Respiratory sensitisation

3.6.1 Animal data

No data are available. No evidence for respiratory tract irritation was seen from the necropsy findings of the acute inhalation toxicity study.

3.6.2 Human data

No human data are available

3.6.3 Other data

No other data are available.

3.7 Skin sensitisation

3.7.1 Animal data

[Study 1]

Report:	Anonymous 1998e: BAS 505 F - Maximisation Test based on the method of Magnusson and Kligman in guinea pigs BASF DocID 1998/10998
Guidelines:	OECD guideline 406 (1992)
GLP:	yes

The study mostly complied with OECD guideline 406 (1992). However the guideline recommends for non-irritant substances pre-treatment with sodium lauryl sulfate (SLS) before dermal induction. Although there was no SLS pre-treatment in this study, this is considered acceptable (see conclusion).

Dimoxystrobin (batch/purity: N 6 Lot 3004: 98.8%) was tested for its skin sensitising effect in Pirlbright White (Dunkin-Hartley) guinea pigs using the Maximisation Test based on the method of Magnusson and Kligman. Twenty female animals were used for the test group and ten female animals in each of two control groups.

In a pre-test, two 24-hour percutaneous occlusive applications within 96 hours were performed with test substance concentrations up to 50% in a 1% aqueous preparation of Tylose CB 30.000 (animals had been pre-treated with Freund's adjuvant 4 weeks previously). No skin reactions were observed.

It was possible to inject a 5% test substance preparation in 1% Tylose CB 30.000 in aqua bidest. or in Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1) with a syringe. This concentration caused local findings and was well-tolerated systemically.

The following concentrations for induction and challenge were selected on the basis of the pretests:

Table 3.7.1-1 Preparations used for test animals in the maximisation test

Intradermal induction	Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1) test substance 5% in 1% Tylose CB 30.000 in aqua bidest test substance in Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)
Percutaneous induction	test substance 50% in 1% Tylose CB 30.000 in aqua bidest.
Challenge	test substance 50% in 1% Tylose CB 30.000 in aqua bidest. 1% Tylose CB 30.000 in aqua bidest

Six intradermal induction injections in groups of two were given to each test animal (see Table 3.7.1.1). One week later a percutaneous induction exposure was conducted. 2 x 4 cm gauze patches coated with a c.0.5 mm thick layer of the test substance formulation were applied to the skin of the shoulder under an occlusive dressing for 48 h. Control animals received the same induction treatment but without test material. The challenge was conducted 14 days after the percutaneous induction with a test substance preparation and vehicle control as indicated in the above table. 2 x 2 cm gauze patches coated with a c.0.5 mm thick layer of the test substance formulation were applied to the skin of the flank under an occlusive dressing for 24 h.

Skin irritation readings were made at 24 and 48 hours after removal of the patch.

Separate tests using alpha-hexylcinnamaldehyde as a positive control are conducted twice a year in the laboratory to determine the ability of the test procedures to detect sensitising compounds. Satisfactory results were presented.

Findings
The study investigators report that the stability of the test substance in the vehicle was confirmed by analysis and that homogeneity of the preparation was ensured by stirring.

2 control and 2 test animals died 10-13 days after the start of the study. Macroscopic examination revealed that the animals had pneumonia. The cause of death was not related to treatment.

After intradermal induction, well defined skin responses were seen where the test substance and or Freunds had been injected. After dermal induction, incrustation (caused by intradermal injection), well defined erythema and slight oedema were seen in test and control animals.

After challenge no skin responses were seen in test or control animals.

Conclusion
It is concluded, that dimoxystrobin has no sensitizing potential to the skin of the guinea pig in the maximisation test.

The concentrations of dimoxystrobin used in this study are considered to be acceptable. Although the concentration of dimoxystrobin used for topical induction was non-irritant in the pre-test, the absence of the use of pre-treatment with SLS prior to dermal induction in the main study is considered to be acceptable. This is because ECETOC (2000)[4] have recommended that SLS is no longer used as a pre-treatment in the guinea pig maximisation test.

[4] ECETOC, 2000. Skin sensitisation testing for the purpose of hazard identification and risk assessment. Monograph No.29, ECETOC, Brussels, Belgium.

[Study 2]
The more recently conducted Local Lymph Node Assay of the representative dimoxystrobin formulation, which is also included in the Air 3 RAR of dimoxystrobin is added to this Annex I, in order to confirm the absence of a sensitising potential of dimoxystrobin. Dimoxystrobin is contained in this formulation at a concentration of 200 g/L.

Report:	Anonymous, 2015 a BAS 540 01 F - skin sensitisation: Local Lymph Node Assay 2015/1229507
Guidelines:	OECD 429 (2010), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.42
GLP:	Yes

Executive Summary
For the determination of potential sensitizing properties of the test item BAS 540 01 F the Murine Local Lymph Node Assay (LLNA) was conducted. Groups of 5 female mice were treated with three different concentrations of the test substance (25, 50, and 100% (w/w) in Pluronic® Water) or with the vehicle alone for three consecutive days.
A skin sensitizing effect for BAS 540 01 F is not considered since Stimulation Indices (S.I.) of 1.05, 1.01 and 1.93 were determined with BAS 540 01 F at concentrations of 25, 50, and 100% in Pluronic® Water, respectively, which stay below the cut-off value of ≥ 3 for ^{3}H-thymidine incorporation. Likewise, no biologically relevant increase in lymph node cell count (S.I. of 1.44, 1.12, and 1.34) was observed in any dose group in comparison to the vehicle control group. The cut-off value for a positive response regarding the lymph node cell count index (1.55) was not exceeded. A statistically significant but biologically not relevant increase in lymph node weights was observed in the high dose group in comparison to the vehicle control group. A biologically relevant or statistically significant increase in ear weights was not observed. The cut-off value (1.1) of the ear weight index for a positive response regarding ear skin irritation (as reported for BALB/c mice) was exceeded in the high dose group (index of 1.15). This increased index was caused by the single heightened ear weight of animal No. 20, probably induced by the scratch wound, as the other four animals in this dose group had lower ear weights. No signs of systemic toxicity and no mortalities were observed. No erythema was observed in any of the animals in the main experiment. Substance residues were reported in the animals treated with the test item. Hair loss and scratch wound was observed in one animal of the high dose group at day 6. Positive control studies performed twice a year with the sensitizer alpha-hexylcinnamaldehyde proved the sensitivity of the method used.
In conclusion, based on the results of this study BAS 540 01 F does not display skin sensitizing properties under the conditions of the test.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 540 01 F
Description:	liquid / beige, milky
Lot/Batch #:	FRE-001178
Purity:	Boscalid, BAS 510 F: 200.2 g/L
	Dimoxystrobin, BAS 505 F: 199.8 g/L
Stability of test compound:	Stable until 31 March 2016. Stability in solvent was not indicated.
2. Vehicle and/or positive control:	Pluronic® Water (1%, v/v), batch: 120815
3. Test animals:	
Species:	Mouse
Strain:	CBA/CaOlaHsd
Sex:	females
Age:	9 to 10 weeks (pre-test), 8 to 9 weeks (main study)
Weight at dosing:	18.9 ± 0.9 g
Source:	Envigo RMS B.V., Inc., 5960 AD Horst / The Netherlands
Acclimation period:	at least 5 days
Diet:	2018C Teklad Global 18% protein rodent diet (certified), ad libitum
Water:	tap water ad libitum
Housing:	group housing in Makrolon Type II (pre-test) / III (main test) cages with wire mesh top

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 22-July-2015 to 18-Aug-2015

2. Animal assignment and treatment:

Pre-test:

Two mice were treated by (epidermal) topical application to the dorsal surface of each ear with test item concentrations of 50 and 100% once daily each on three consecutive days. Prior to the first application of the test item and before sacrifice, the body weight was determined. Clinical signs were recorded at least once daily. Any signs of local irritation were documented and a score was used to grade a possible erythema of the ear skin. Furthermore, prior to the first application of the test item (day 1), on day 3 and before sacrifice (day 6) the ear thickness was determined using a micrometer. Additionally, for both animals, the ears were punched after sacrifice (day 6) at the apical area using a biopsy punch (Ø 8 mm corresponding to 0.5 cm^2) and were immediately pooled per animal and weighed using an analytical balance.

Main test:

The skin sensitizing potential of BAS 540 01 F was assessed using the radioactive Murine Local Lymph Node Assay. For the main experiment, female mice were allocated to groups of 5 animals at random.

The groups were treated either with

- the control group with Pluronic® (1%, v/v),
- a 25% (w/w) dilution of the test article in Pluronic® (1%, v/v),
- a 50% (w/w) dilution of the test article Pluronic® (1%, v/v), or
- a 100% (w/w) dilution of the test article in Pluronic® (1%, v/v).

3. Analysis of treatment solutions:

The highest test item concentration, which could be technically used was a 100% of the undiluted test item. For the dilutions, the test item was placed into an appropriate container on a tared balance and 1% aqueous Pluronic® (v/v) was added. The different test item concentrations were prepared individually. Homogeneity of the test item in vehicle was maintained during treatment using a magnetic stirrer.

The preparations were made freshly and used within two hours before each dosing occasion. Concentrations were in terms of material as supplied.

A concentration control analysis of all three doses was performed as a separate study under the responsibility of the sponsor. For this, samples of all dose formulations (1 mL, each) and of the vehicle control (5 mL) were taken on the first treatment day immediately after the last application of the test item. Also, corresponding reserve samples were taken. These aliquots were stored at -18°C until shipment. Furthermore, a small amount of test item was taken as a sample and stored at room temperature until shipment.

4. Statistics:
The mean values and standard deviations were calculated in the body weight tables, for the ear weights, the lymph node weights and lymph node cell count, and for the DPM values (group mean DPM ± standard deviation).
A statistical analysis was conducted on the DPM values, the ear weights, the lymph node weights and the lymph node cell count to assess whether the difference was statistically significant between the test item groups and negative control group. For all statistical calculations validated statistical program R Script DecisionTree_2.Rnw was used. Statistical significance was set at the five per cent level ($p < 0.05$).
The Dean-Dixon-Test and Grubb's Test were used for identification of possible outliers (performed with validated program R Script Outlier.Rnw).
However, both biological and statistical significance were considered together.

5. Clinical observation:
Mortality was checked at least once daily from experimental start to necropsy. Clinical signs (local irritation at the application site or systemic toxicity) were recorded at least once daily. Especially the treatment sites were observed carefully.

6. Body weights:
In the pre-test: prior to the first application and prior to sacrifice. In the main experiment: prior to the first application and prior to treatment with ^{3}HTdR.

7. Treatment of animals:
The dosing solutions were applied daily to the dorsal part of the ears at a volume of 25 µL per ear per day for 3 consecutive days. A further group of mice (control animals) was treated with an equivalent volume of the relevant vehicle alone. Five days after the first topical application (day 6), 19.5 µCi of ^{3}H-methyl thymidine (equivalent to 78 µCi/mL ^{3}HTdR) in 250 µL phosphate-buffered saline was injected into the tail vein of each mouse.

8. Terminal procedures:
Approximately 5 hours after ^{3}H-thymidine injection the animals were euthanized by using CO_2.
After the death of each animal a circular piece of tissue (diameter 0.8 cm) was punched out of the apical part of each ear of all animals. The weight of the pooled punches was determined for each test group. These measurements served for the detection of a potential inflammatory ear swelling.
Just before removal of the ear punches the left and right auricular lymph nodes were dissected. The weight of the pooled lymph nodes from both sides was determined for each animal.
After weight determination, a single cell suspension was prepared per test group from the pooled lymph nodes by carefully passing all lymph nodes through an iron mesh (mesh size 200 µm) into phosphate-buffered physiological saline. Subsequently the cell counts were determined with an aliquot of each suspension using a Casy®- Counter.
The remaining cell suspensions were washed twice with phosphate buffered saline (PBS) and precipitated with 5% trichloro-acetic acid. Each precipitate was transferred to scintillation fluid and incorporation of ^{3}H-thymidine into the cells was measured in a β-scintillation counter.

9. Data evaluation and interpretation

The stimulation indices (SI) of cell count, ^{3}H-TdR incorporation, lymph node weight and ear weight were calculated as the ratio of the test group values for these parameters divided by those of the vehicle control group.

The lymph node cell count and the ^{3}H-TdR incorporation into the lymph node cells as well as to a certain extent lymph node weight are used to determine the potential sensitizing properties of a test article. Because not only sensitization induction but also irritation of the ear skin by the test substance may induce lymph node responses, the weight of ear punches taken from the area of test-substance application is determined as a parameter for inflammatory ear swelling as an indicator for the irritant action of the test substance.

Stimulation indices of > 1.55 for cell count and/or of ≥ 3 for ^{3}H-TdR incorporation are generally considered as indicative for a sensitizing potential of a test substance. If applicable, the EC (estimated concentration) leading to the respective SI values were calculated by linear or semi-logarithmical regression. The cut-off value for the ear weight index regarding a positive response (ear irritation) was reported to be 1.1.

If the increase in cell count, ^{3}H-thymidine incorporation and/or lymph node weight is accompanied by a biologically relevant increase in ear weights it cannot be ruled out that the lymph node response was caused by irritation and not by skin sensitization. Depending on the magnitude of lymph node response, based on expert judgment, the evaluation of the sensitizing potential may be modified or additional studies might be necessary.

If a test article – despite of concentration related increase - does not elicit a biological relevant increase in cell count and/or ^{3}H-thymidine incorporation, further investigation of the sensitization potential at higher concentrations may be considered.

10. Positive controls

A concurrent positive control (reliability check) with a known sensitizer was not included into this study. Studies using the positive control substance alpha-hexylcinnamaldehyde are performed twice a year in the laboratory in order to show that the test system is able to detect sensitizing compounds under the test conditions chosen.

II. RESULTS AND DISCUSSION

A. PRE-TEST

At the tested concentrations the animals showed neither signs of local skin irritation nor systemic toxicity. Due to the colour of the test item and substance residuals, redness of the ear skin could not easily be determined. Furthermore, a possible erythema could not be assessed in both animals after the first application on day 1. Thus, the test item in the main study was assayed at 25, 50, and 100%. The highest concentration tested was the highest level that could be achieved whilst avoiding systemic toxicity and excessive local skin irritation as confirmed in the pre-experiment.

B. OBSERVATIONS

No deaths occurred during the study period. No signs of systemic toxicity were observed during the study period. On day 6, during the lymph node preparation, a scratch wound was observed in one animal (animal No. 20), which was not observed during the measurement of the body weight in the morning. Animal No. 20 also showed loss of hair on day 6.

C. BODY WEIGHTS

There were no effects on body weight development. The increase of body weights during the study was within the expected range.

D. STIMULATION, CELL COUNT AND WEIGHT INDICES

The stimulation indices (SI) for ^{3}HTdR incorporation and indices for lymph node cell counts, lymph node weights and ear weights are given in Table 3.7.1-2, Table 3.7.1-3 and Table 3.7.1-4.

Table 3.7.1-2: Stimulation indices for cell counts, ^{3}H-thymidine incorporation, lymph node and ear weight in mice after treatment with BAS 540 01 F

Test Group	Treatment	Parameter evaluated	Stimulation index[1]
		Cell count	
		[counts/lymph node pair]	
1	vehicle	7.5 ± 1.4	1.00
2	25%	**10.9* ± 3.2**	1.44
3	50%	8.5 ± 0.5	1.12
4	100%	10.1 ± 1.2	1.34
		^{3}HTdR incorporation	
		[DPM/lymph node pair]	
1	vehicle	753.9 ± 172.8	1.00
2	25%	789.3 ± 241.9	1.05
3	50%	762.3 ± 101.2	1.01
4	100%	**1458.3* ± 495.4**	1.93
		Lymph node weight	
		[mg/lymph node pair]	
1	vehicle	4.4 ± 0.6	1.00
2	25%	4.8 ± 0.7	1.11
3	50%	4.8 ± 0.4	1.09
4	100%	**5.9* ± 0.8**	1.34
		Ear weight	
		[mg/animal]	
1	vehicle	22.6 ± 0.9	1.00
2	25%	22.9 ± 0.9	1.01
3	50%	23.5 ± 1.0	1.04
4	100%	25.9 ± 4.4	**1.15**

[1] test group x / test group 1 (vehicle control)

* statistically significant increase vs. control group ($p < 0.05$)

Vehicle: Pluronic® Water (1%, v/v)

Lymph node weights and cell counts

The measured lymph node weights and –cell counts of all animals treated were recorded after sacrifice. A statistically significant but biologically not relevant increase in lymph node weights was observed in the high dose group and in lymph node cell counts of the low dose group in comparison to the vehicle control group (see Table B.6.1-13). For BALB/c mice, a cut-off value for the lymph node cell count index of 1.55 was reported to be a positive response. The indices determined for the lymph node cell count did not exceed this threshold in any of the dose groups.

Table 3.7.1-3: Lymph node weight (abs. and rel.) in mice after treatment with BAS 540 01 F (single animal data)

Test Group	Treatment	Parameter evaluated	Index[1]
		Mean ear weight [mg]	
1	vehicle	3.56, 4.18, 4.23, 4.76, 5.13	1.00
2	25%	4.11, 5.24, 4.47, 4.59, 5.81	1.11
3	50%	4.89, 4.37, 4.45, 4.77, 5.41	1.09
4	100%	5.31, 5.86, 5.64, 5.30, 7.15#	1.34

[1] test group x / test group 1 (vehicle control); #: animal no. 20
* statistically significant increase vs. control group ($p < 0.05$)
Vehicle: Pluronic® Water (1%, v/v)

Table 3.7.1-4: Lymph node cell count (abs. and rel.) in mice after treatment with BAS 540 01 F (single animal data)

Test Group	Treatment	Parameter evaluated	Index[1]
		Mean ear weight [mg]	
1	vehicle	5.74, 7.29, 7.00, 9.35, 8.30	1.00
2	25%	7.05, 12.14, 9.19, 10.52, 15.54	1.44
3	50%	8.98, 7.81, 8.72, 8.67, 8.15	1.12
4	100%	8.70, 9.14, 10.16, 11.07, 11.44	1.34

[1] test group x / test group 1 (vehicle control); #: animal no. 20
* statistically significant increase vs. control group ($p < 0.05$)
Vehicle: Pluronic® Water (1%, v/v)

Ear weights

The measured ear weight of all animals treated was recorded on test day 6 (after necropsy). A biologically relevant or statistically significant increase in ear weights was not observed. The cut-off value (1.1) of the ear weight index for a positive response regarding ear skin irritation reported for BALB/c mice was exceeded in the high dose group (index of 1.15). This increased index was caused by the single heightened ear weight observed in animal No. 20, probably induced by the scratch wound, compared to the lower ear weights of the other four animals in this dose group (see Table 3.7.1.5).

Table 3.7.1-5: Ear weight (abs. and rel.) in mice after treatment with BAS 540 01 F (single animal data)

Test Group	Treatment	Parameter evaluated	Index[1]
		Mean ear weight [mg]	
1	vehicle	23.44, 22.27, 21.15, 23.19, 23.06	1.00
2	25%	21.94, 23.99, 23.39, 21.97, 23.19	1.01
3	50%	22.93, 23.39, 25.15, 23.05, 22.80	1.04
4	100%	23.65, 24.64, 24.55, 23.13, 33.63#	1.15

[1] test group x / test group 1 (vehicle control); #: animal no. 20
* statistically significant increase vs. control group ($p < 0.05$)
Vehicle: Pluronic® Water (1%, v/v)

^{3}HTdR incorporation

Stimulation Indices (S.I.) of 1.05, 1.01, and 1.93 were determined with the test item at concentrations of 25 and 50% in 1% aqueous Pluronic® (v/v) as well as 100%, respectively (see Table 1). Although a statistically significant increase in DPM value and also in lymph node weight was observed in the high dose group in comparison to the vehicle control group, this was not considered to be biologically relevant as the S.I. determined for these concentrations did not exceed the threshold values of 3 for ^{3}H-thymidine incorporation.

The EC3 value could not be calculated, since none of the tested concentrations induced a S.I. greater than the threshold value of 3. The statistically significant increased DPM value and lymph node weight of the high dose group was probably caused by the heightened single value for animal No. 20 due to the scratch wound, which might have led to an inflammatory response in this animal (see Table 3.7.1-6).

Table 3.7.1-6: DPM and stimulation indices in mice after treatment with BAS 540 01 F (single animal data)

Test Group	Treatment	Parameter evaluated	Stimulation index[1]
		^{3}HTdR incorporation [DPM/lymph node pair]	
1	vehicle	804, 646, 1027, 586, 704	1.00
2	25%	507, 1122, 650, 934, 731	1.05
3	50%	653, 774, 813, 897, 672	1.01
4	100%	955, 1153, 1422, 1506, 2253#	**1.93***

[1] test group x / test group 1 (vehicle control); #: animal no. 20
* statistically significant increase vs. control group ($p < 0.05$)
Vehicle: Pluronic® Water (1%, v/v)

E. POSITIVE CONTROL

The sensitivity of mice (CBA) and the reliability of experimental techniques are assessed regularly using a known sensitizer. Positive results were consistently obtained over the years using several variations of the methods and different vehicles. The results of 6 control studies are presented in table 3.7.1-7.

Table 3.7.1-7: Positive control LLNA studies performed

Date of performance	Apr 2015	Oct 2014	Apr 2014	Oct 2013	Apr 2013	Oct 2012
Name of test substance	alpha-hexylcinnam-aldehyde	alpha-hexylcinnam-aldehyde	alpha-hexylcinnam-aldehyde	alpha-hexylcinnam-aldehyde	alpha-hexylcinnam-aldehyde	alpha-hexylcinnam-aldehyde
Concentrations tested	25%	25%	25%	25%	25%	25%
Vehicle	acetone/olive oil (4:1, v/v)	acetone/olive oil (4:1, v/v)	acetone/olive oil (4:1, v/v)	acetone/olive oil (4:1, v/v)	acetone/olive oil (4:1, v/v)	acetone/olive oil (4:1, v/v)
Stimulation index ^{3}H-thymidine incorporation[b]	9.5	13.8	6.8	5.8	5.9	5.7
Evaluation of study results	Positive	Positive	Positive	Positive	Positive	Positive

[b] = Ratio of test group values to control group values (Stimulation index) greater than 3.0 indicates a positive result

III. CONCLUSION

Based on the results of this study it is concluded that BAS 540 01 F has no sensitizing properties under the test conditions chosen.

3.7.2 Human data

No human data are available.

3.7.3 Other data

No other data are available.

3.8 Germ cell mutagenicity

Not assessed in this CLH dossier..

3.9 Carcinogenicity

Not assessed in this CLH dossier..

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1

Multigenerational studies, rat

Study reference:

Anonymous, (2000): BAS 505 F Two-generation reproduction study with dimoxystrobin in Wistar rats, continuous dietary administration. BASF AG, Ludwigshafen, Germany; BASF DocID 2000/1016869; August 2001

Detailed study summary and results (see also RAR (2017) Vol. 3. Annex B-6, pp 124 – 148):

Report:	KIIA 5.6.1/1 Anonymous (2000) BAS 505 F Two-generation reproduction study with dimoxystrobin in Wistar rats, continuous dietary administration 2000/1016869
Guidelines:	OPPTS 870.3800, OECD 416
GLP:	yes
Previous evaluation:	in the DAR (2003)
Acceptability:	Yes

This detailed study broadly complied with OECD guideline 416 (2001) but did show some deviations. Notably, although an extensive histopathological examination on parental animals was conducted, the duodenum (a known target organ) was only investigated histopathologically when changes were seen grossly. Also there was no histopathological examination of weanlings. No functional investigation (eg motor activity) of F1 offspring was conducted but this is not of concern as there are no indications that dimoxystrobin is neurotoxic. The absence of food consumption measurement for day 21 of lactation is acceptable as by this time pups would also be eating the food.

Executive summary:

In a 2-generation reproduction toxicity study, dimoxystrobin (Batch: N 15; Purity 98.4%) was administered in the diet to groups of 25 male and 25 female Wistar rats (Chbb = THOM (SPF)) at dietary concentrations of 0, 50, 150, 500 and 1200 ppm (0, 5, 17, 55 and 136 mg/kg bw) throughout two generations.

No treatment-related mortality was observed in any of the male and female parental animals throughout the study. Food consumption and body weight development were impaired in the 500 and 1200 ppm dose F0 and F1 female parental animals and F1 male parental animals. In F0 male parental animals, food consumption and body weight development were impaired in the 1200 ppm dose group. There were no treatment-related clinical, gross, or histopathological observations indicating general, systemic toxicity in both parental generations at 150 ppm.

Treatment with dimoxystrobin up to the concentration of 1200 ppm had no effects on the estrous cycle, the number, morphology and motility of sperm as well as on male or female fertility. Male and female fertility indices ranged between 80 and 100% without relation to dose. Dimoxystrobin treatment did not affect the reproductive performance as was evident from the absence of effects on the pre-coital interval or gestation lengths as well as gestation (96 to 100%) or live birth indices (95 to 99%). Some high dose effects were not consistent between the first and the second generation in the 2-generation study with dimoxystrobin and were well covered by data compiled from historical control data. Overall, all the effects on reproductive parameters can be regarded as incidental and not treatment-related.
Gross- and histopathological examination of the reproductive organs of apparently infertile males and females did not reveal any common cause for the lack of reproductive success and thus were considered to be unrelated to treatment. Finally, ovarian follicle counts did not reveal any treatment-related differences between control and high dose groups.

Survival of pups was not affected by treatment as viability and lactation indices were in the range of 92 to 100% and 97 to 100%, respectively. Body weight development of the 1200 and 500 ppm dose F1 and additionally the 150 ppm F2 pups was significantly impaired. Pup body weight effects were absent at birth and developed over time during lactation being most prominent at PND 14 and 21 after start of self-feeding of the pups. Calculated dimoxystrobin doses of the pups are considerably higher compared to the dams at the same dietary dose levels.

Other pup parameters like sex ratio, clinical observations and organ weights findings did not reveal any treatment-related effects. The observed effects on absolute and relative thymus and spleen weights were secondary to the lower terminal pup body weights. Gross necropsy findings comprised pale yellowish discoloration of the liver, cardiomegaly, milky fluid in the abdomen and/or thorax after organ evisceration, and hypoplasia of thymus, which was related to the reduced pup body weights. The findings on hearts, and liver were considered to be secondary to a microcytic hypochromic anemia. The milky fluid reported in the abdomen and the breast cavity is considered to be secondary to the heart-insufficiency induced by the chronic microcytic anemia. A decompensated right heart insufficiency is known to lead to fluid retention in the body as observed by edema in the legs as well as ascites in the big body cavities (abdomen and breast cavity).

Cardiomegaly reported in the PND21 pups is regarded as a secondary and reversible effect via lactation. No cardiomegaly was seen in PND4 pups macroscopically. There is information in the literature that young animals undergo cardiac remodeling secondary to anemia. Cardiomegaly is a transient effect and not an irreversible structural malformation. Parental F1 animals did not show cardiomegaly.

The delay in vaginal opening and preputial separation is considered to be the secondary consequence of reduced body weight.

The NOAEL for effects on reproduction is 1200 ppm (136 mg/kg bw/day), the highest dose tested.
The parental NOAEL for general, systemic toxicity is 150 ppm (17 mg/kg bw/day), based on decreased body weight.
The NOAEL for developmental toxicity is 50 ppm (5 mg/kg bw/day in adults; about 12 mg/kg bw/day estimated value in pups) in the F2 progeny based on impairments in pup body weight.
When doing benchmark dose calculations the no effect levels (= PODs) for body weight effects between dams (25.5-45.3 mg/kg bw during lactation) and pups (about 39.8 mg/kg bw; PND 21) are essentially comparable.

Materials and Methods:
Dimoxystrobin (batch/purity: N 15: 98.4%) was administered to groups of 25 male and 25 female young Wistar rats (F0 parental generation) as a constant homogeneous addition to the food in different concentrations (0, 50, 150, 500 and 1200 ppm). At least 74 days after the beginning of treatment, F0 animals were mated (1:1) to produce a litter (F1). Litters were standardised to 8 pups on day 4. Mating pairs were from the same dose group and F1 animals selected for breeding were continued in the same dosing group as their parents. Groups of 25 males and 25 females selected from F1 pups as F1 parental generation were offered diets containing 0, 50, 150, 500 and 1200 ppm of the test substance post weaning, and the breeding program was repeated to produce F2 litter. The study was terminated with the terminal sacrifice of the F2 weanlings and F1 adult animals.

Results:
1. Clinical signs of toxicity
No treatment-related clinical observations were detected in the male and female F0 and F1 parental animals in any group during the administration period.
The F1 dams revealed no treatment-related clinical finding during the lactation period.

2. Mortality
No treatment-related mortality was observed in any of the male and female F0 parental animals throughout the study.
No mortality was observed in F1 parental animals.

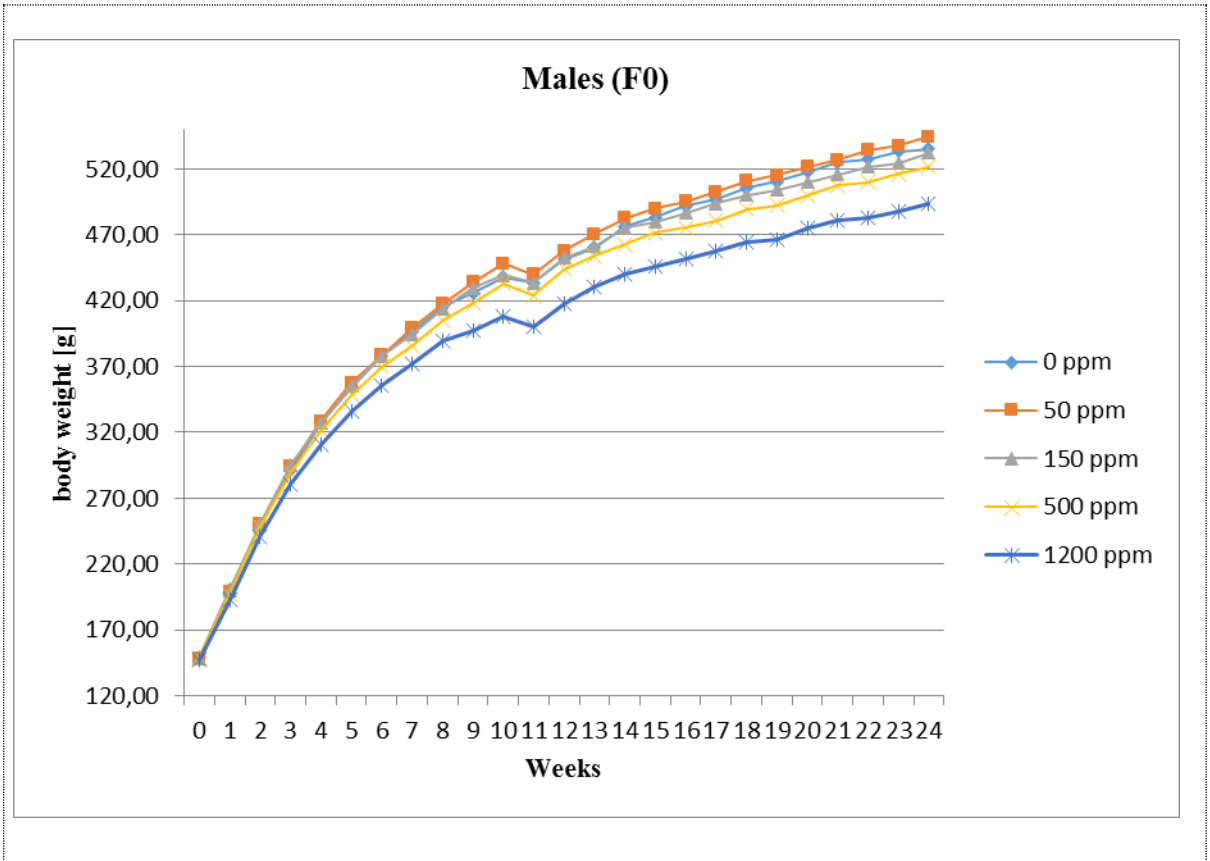
3. Parental body weight

Body weight development was impaired in 500 ppm and 1200 ppm female F0 and F1 animals as well as in 1200 ppm male F0 animals and 500 ppm and 1200 ppm F1 males.

F0 parental animals:

Mean body weights of F0 parental males were impaired at 1200 ppm by test substance administration during the entire study period [see Figure 3.10.1.1-1].
Statistically significantly reduced body weight in the high dose (1200 ppm) males was observed from study week 4 until the end of the study, about 8% below controls at termination of the study. This was in-line with the reduced food consumption in this dose group. Mean body weight gains of this dose group were also clearly impaired (about 10% below control).

Mean body weights and mean body weight gains of high dose (1200 ppm) F0 parental females were comparable to controls during premating. During gestation and lactation, as well as after weaning, however, mean body weights were below controls, reaching statistically significance during some intervals [see Figure 3.10.1.1-1]. Throughout gestation and lactation, as well as after weaning, mean body weights of high dose females were decreased (-5%, -6%, -7%). Moreover, the mean body weights of the 500 ppm dose group females were slightly below controls during lactation and post-weaning phase. The impairments in the body weight of the 500 and 1200 ppm dams were regarded as treatment-related. As concurrent impairments in food consumption also occurred in these rats, the impairments in body weight were considered to be treatment-related.



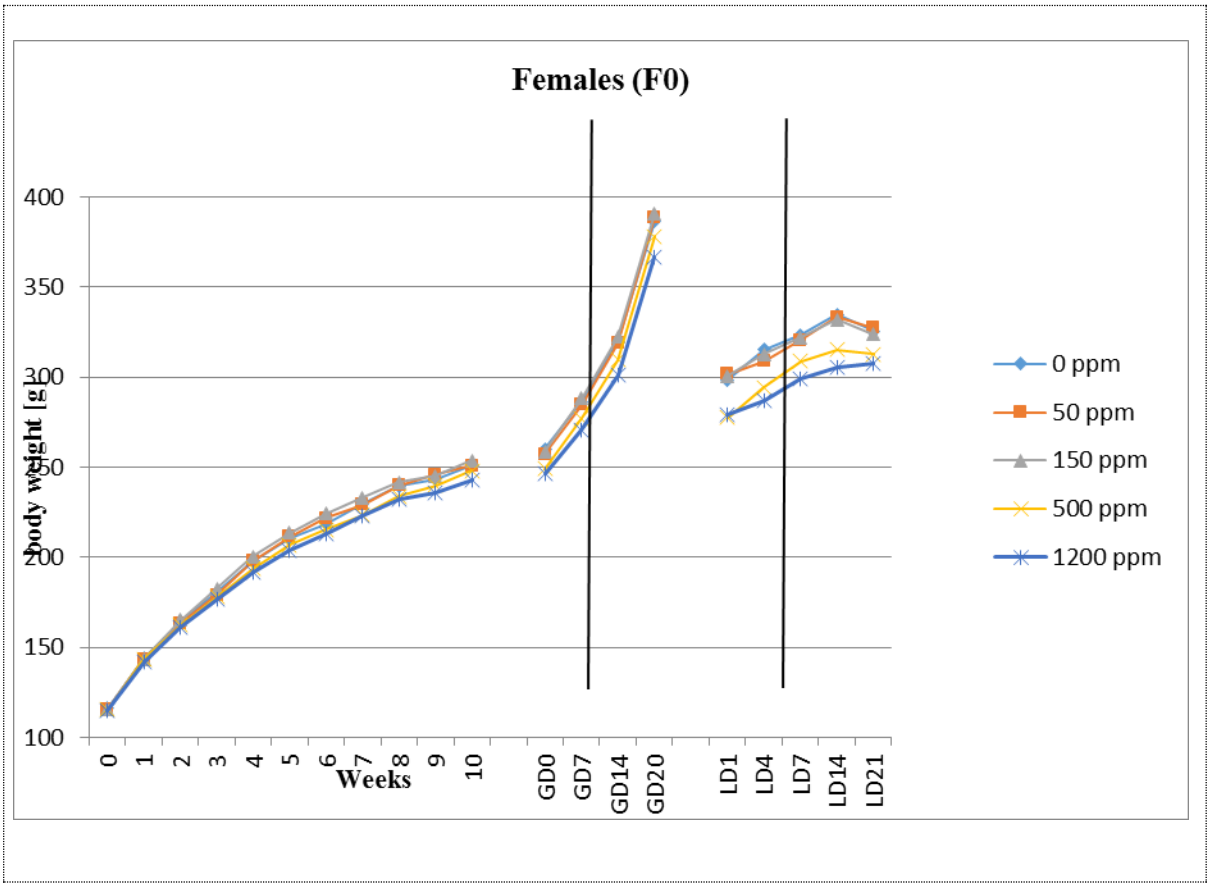
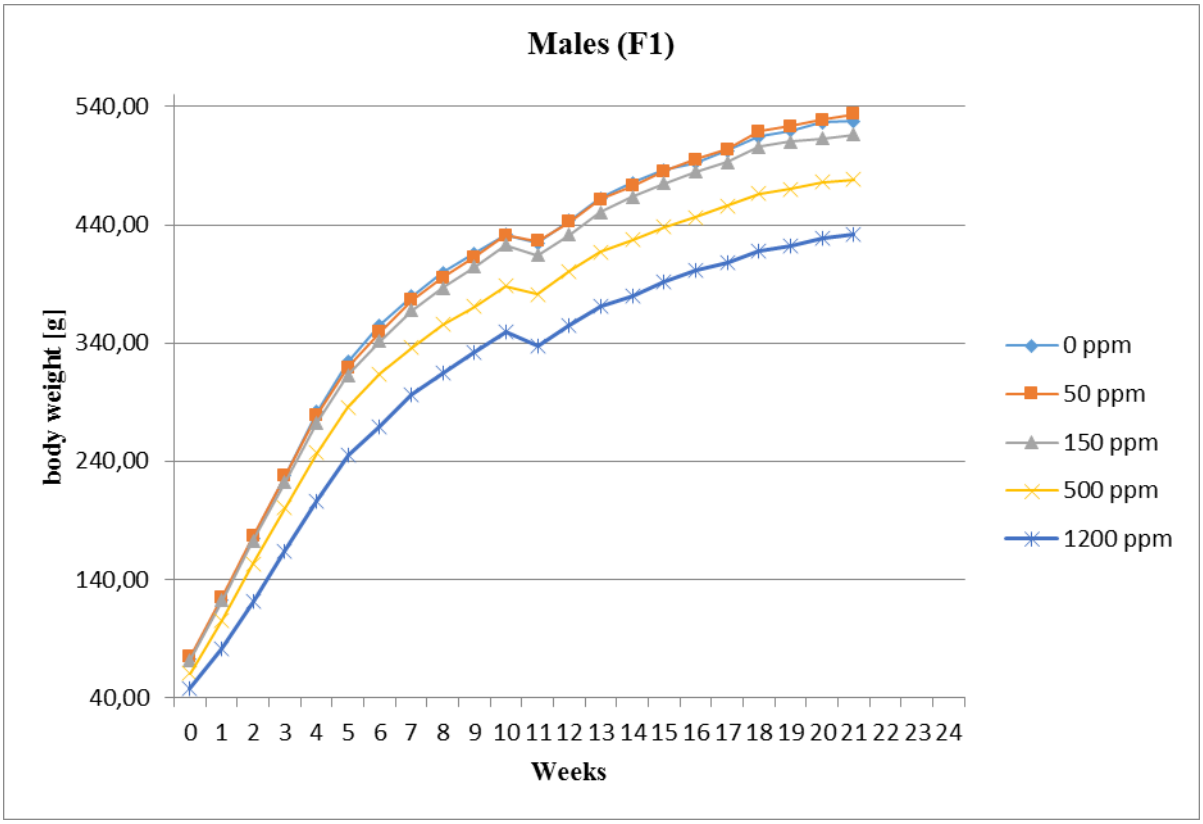


Figure 3.10.1.1-1: Body weight development of parental F0 rats

F1 parental animals:

Mean body weights of the 500 ppm and 1200 ppm parental F1 males were statistically significantly impaired during the entire pre-mating period [see Figure 3.10.1.1-2]. Before sacrifice mean body weighs were about 9% and 18% below controls. Mean body weight gains were similarily affected, but attaining only sporadically statistical significance. This was in-line with the reduced food consumption in this dose group.

Mean body weights of the 500 and 1200 ppm F1 parental females were reduced during various phases of the entire study [see Figure 3.10.1.1-2]. Throughout pre-mating, gestation and lactation mean body weights of high dose females were decreased (-16%, -19%, -15%). Body weights of the 500 ppm females were also reduced during pre-mating, gestation and lactation (about -8%, -9% and -5% below controls). The impairments in the body weight of the 500 and 1200 ppm dams were regarded as treatment-related. As concurrent impairments in food consumption also occurred in these rats, the impairments in body weight were considered to be treatment-related.



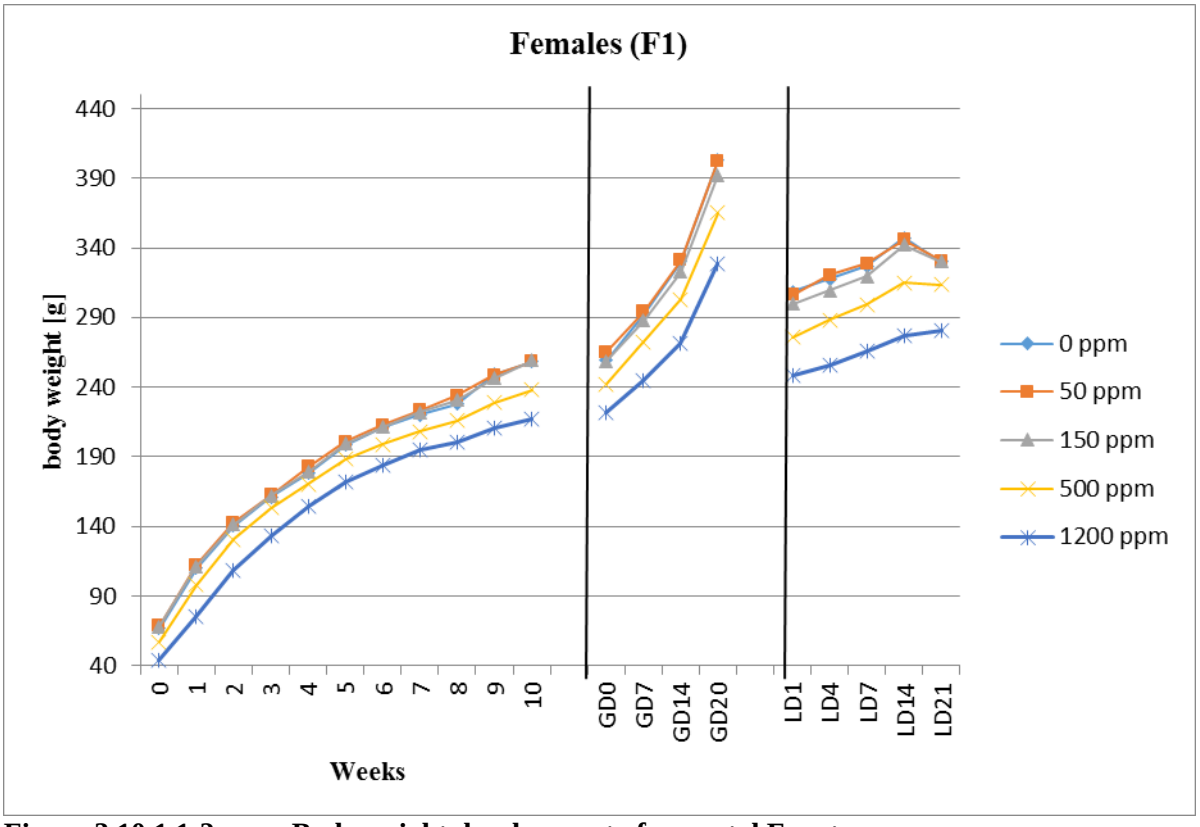


Figure 3.10.1.1-2: Body weight development of parental F_1 rats

Body weight development in the 50 and 150 ppm parental male and females were not affected by treatment.

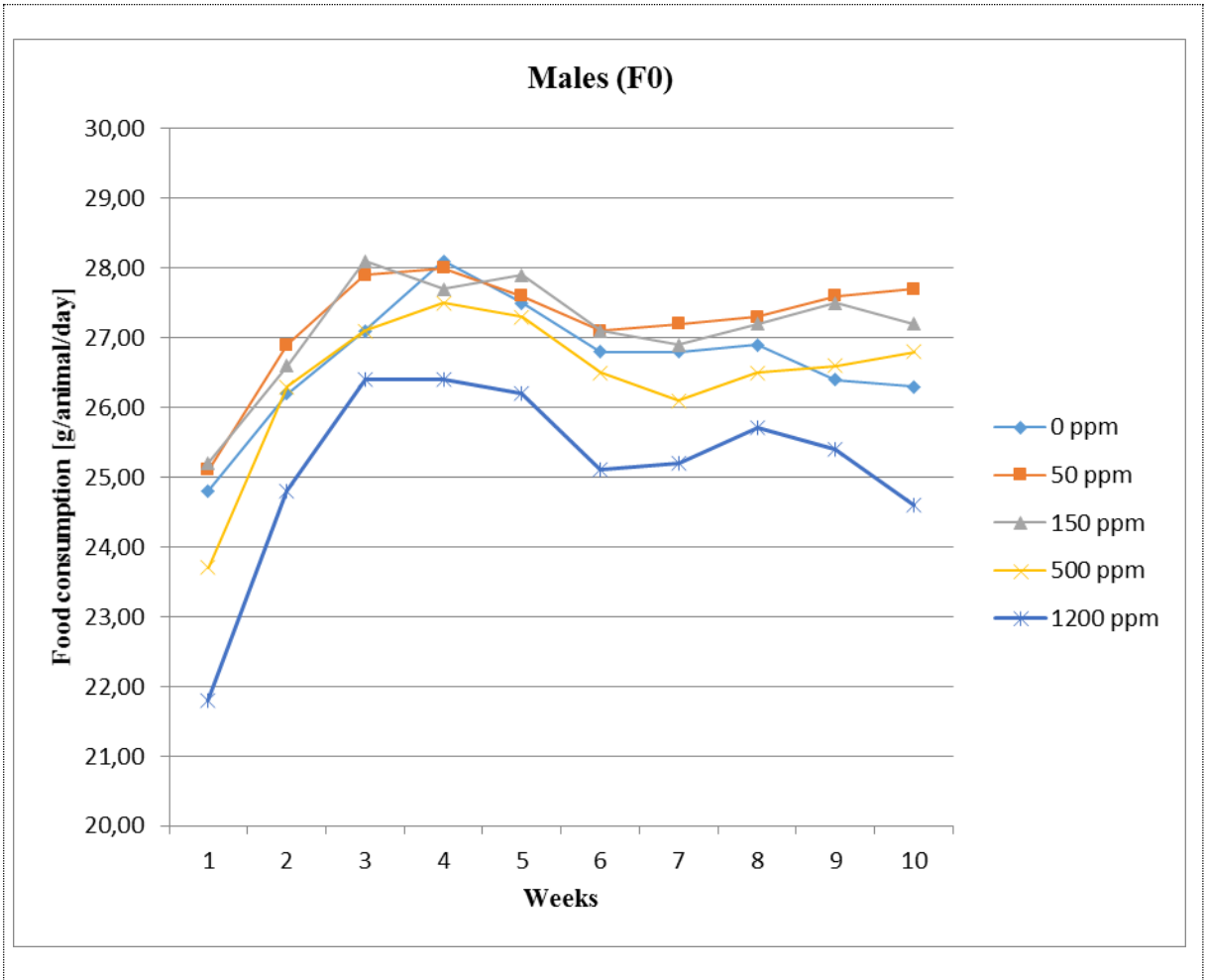
4. Parental food consumption and compound intake

Treatment-related effects on food consumption were restricted to high dose (1200 ppm) F0 males and females and 500 and 1200 ppm F1 males and females.

F0 parental animals:

Food consumption was reduced to about 4% in high dose (1200 ppm) F0 parental male animals during the entire premating period (statistically significant at weeks 7-8 and 9-10) [see Figure 3.10.1.1-3].

During pre-mating food consumption of the high dose (1200 ppm) F0 parental females was only slightly below the corresponding controls (about 4%) [3.10.1.1-3]. During gestation and lactation however food consumption in this group was about 8-13% lower compared to the control group, which also had an effect on the body weight of dams and was thus considered substance-related.



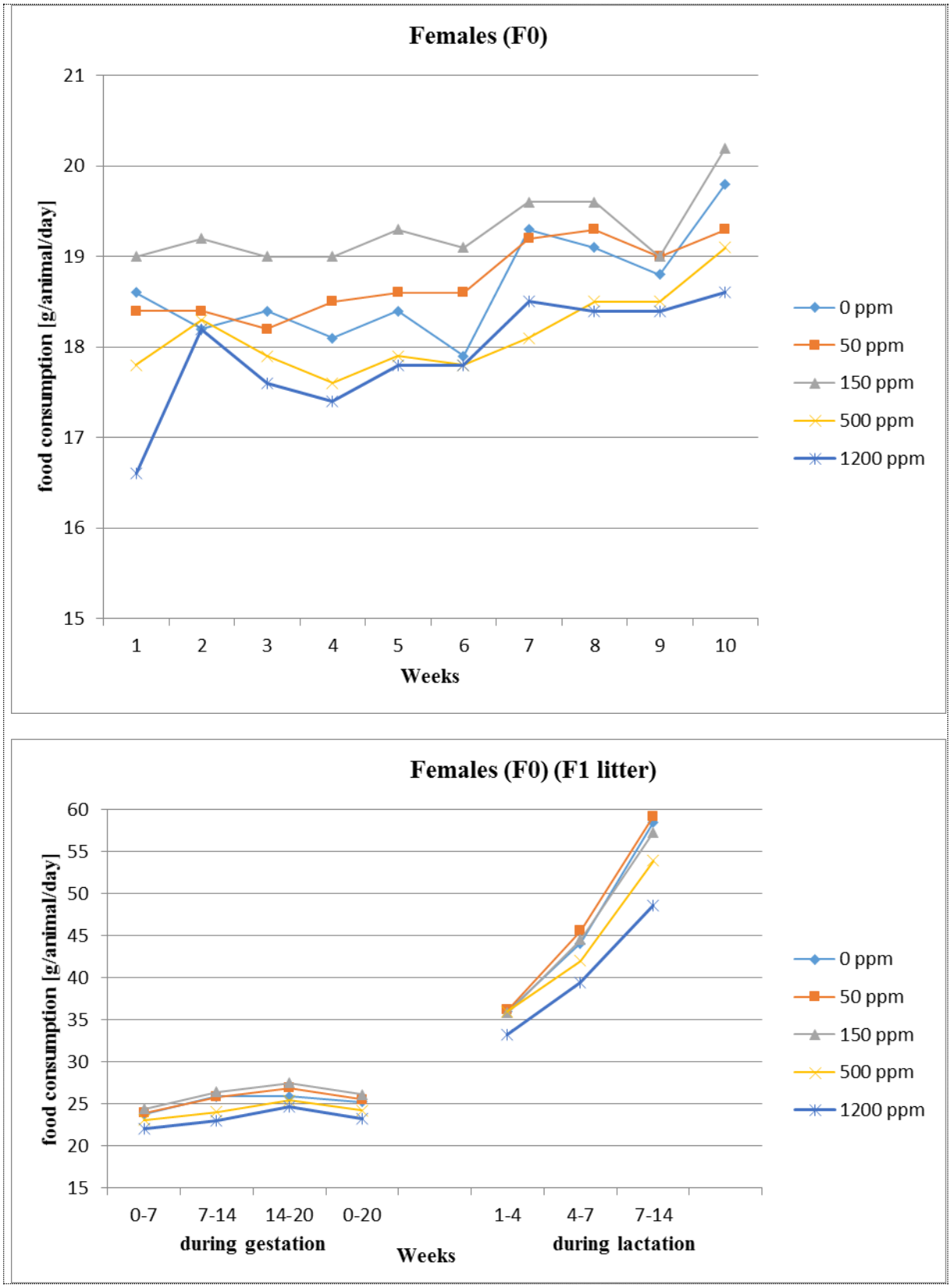


Figure 3.10.1.1-3: Food consumption of parental F0 males and females

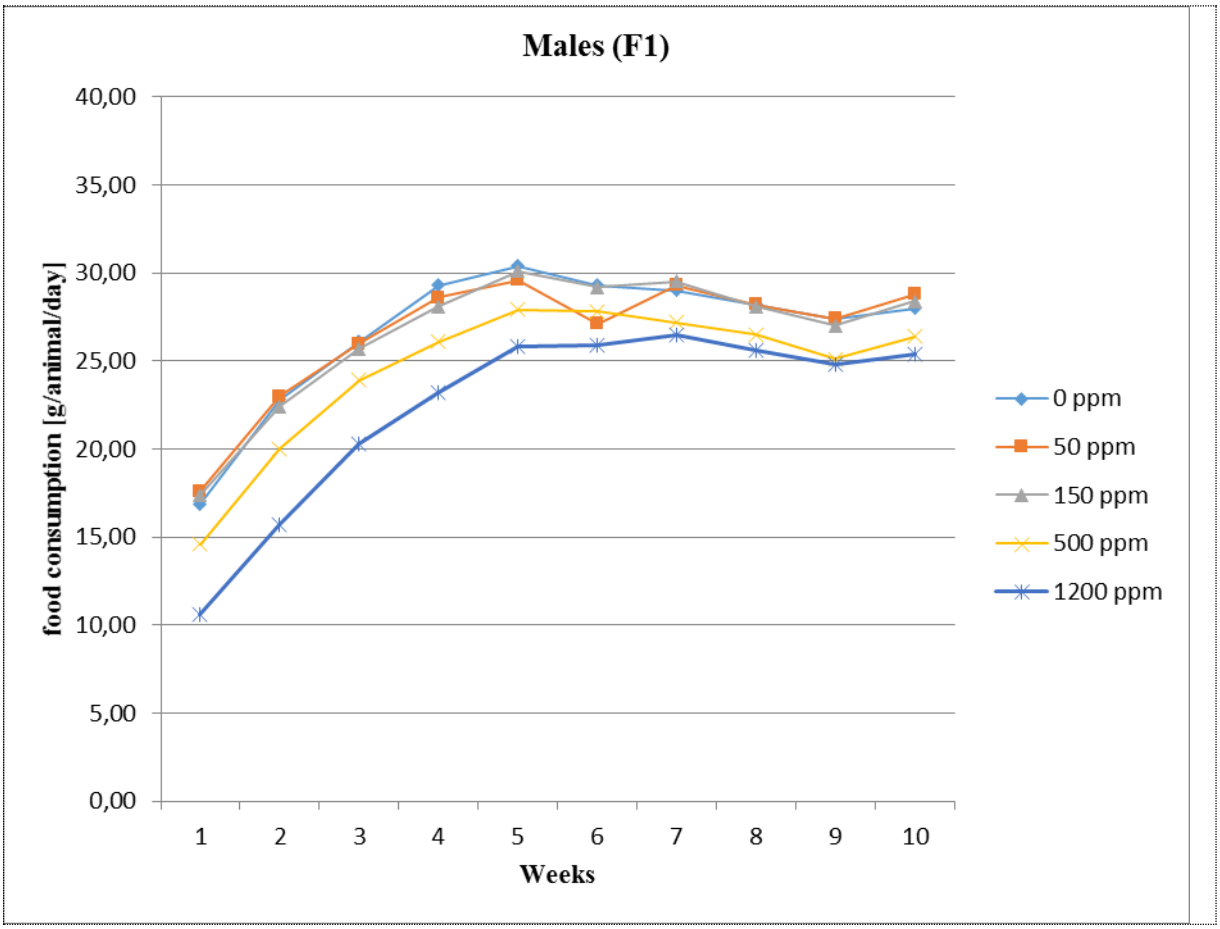
Food consumption of all other treated F0 males and females was not affected by treatment.
The impairments of food consumption of the high dose F0 parental animals were considered to be treatment-related, due to concurrent impairments in body weight/body weight gain in these rats.

F_1 parental animals:

Food consumption was statistically significantly reduced in the 500 (during most weeks) and 1200 ppm parental F1 male animals during pre-mating [see Figure 3.10.1.1-4]. Food consumption over the entire pre-mating period was impaired by about 8 or 16% compared to the concurrent control. This is in-line with the impaired body weights at 500 and 1200 ppm.

Food consumption in 500 and 1200 ppm F1 parental females was reduced during the whole premating period, but less pronounced compared to males [see Figure 3.10.1.1-4]. Food consumption in these dose groups was further impaired during gestation and lactation attaining statistical significance during several intervals. Compared to the corresponding controls food consumption at 500 ppm was about 4, 6, and 10%, that of the 1200 ppm dose group about 12, 18, and 26% lower over the entire pre-mating, gestation and lactation phase.

Food consumption of all treated 50 ppm and 150 ppm F1 males and females was not affected by treatment.



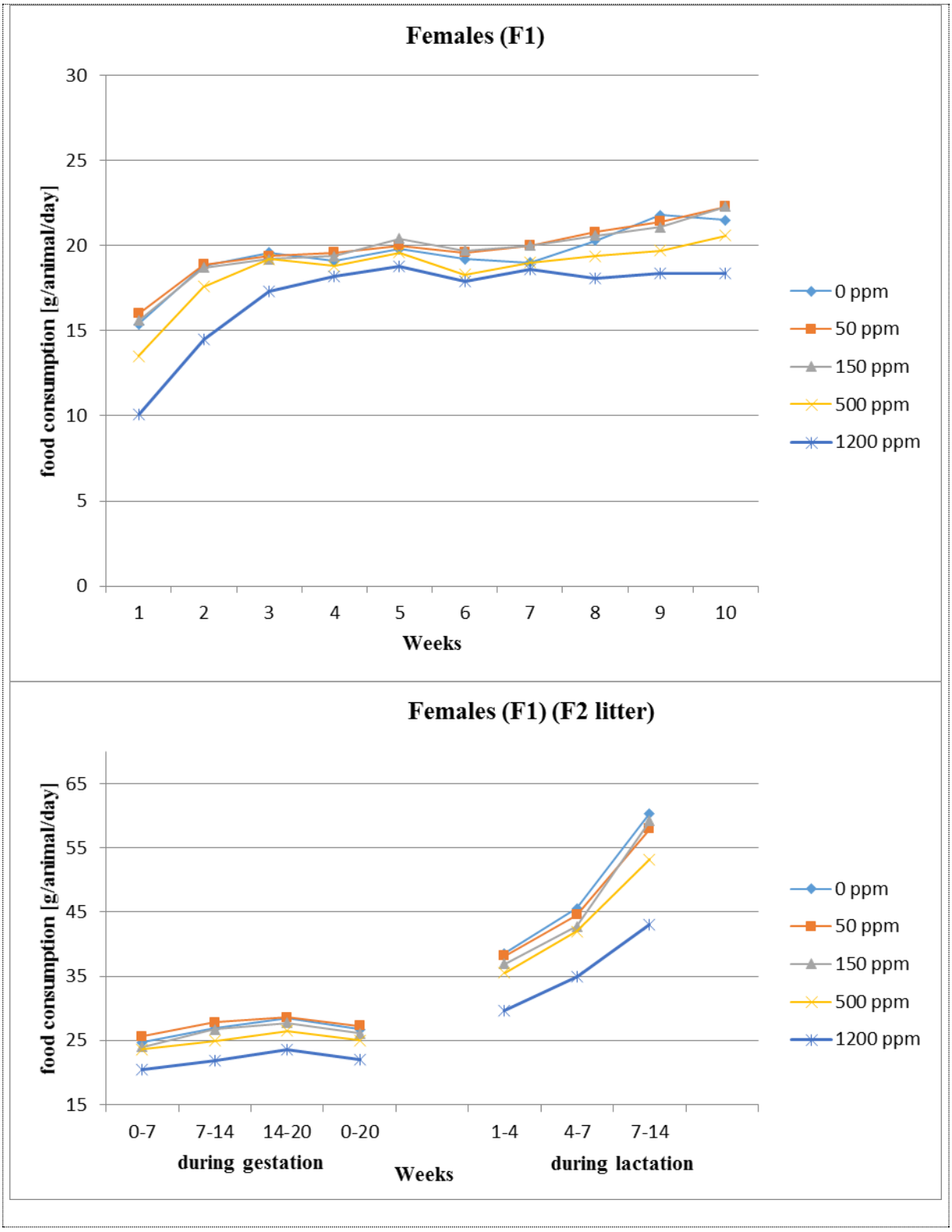


Figure 3.10.1.1-4: Food consumption of parental F_1 males and females

The impairments of food consumption of the F1 parental animals of the 500 and 1200 ppm dose groups were considered to be treatment-related, due to concurrent impairments in body weight in these rats.

5. Effects on oestrous cycle

No effects were observed.
Estrous cycles as determined during 3 weeks prior to mating were very regular in all dose groups in F0 and F1 females. The mean cycle length was 3.9, 3.8, 3.9, 3.9, and 3.8 days in F0 and 4.9, 5.0, 5.0, 4.4, and 4.7 days in F1 females at 0, 50, 150, 500, and 1200 ppm, respectively.

6. Effects on mating and gestation

Male reproductive performance

Male reproductive performance was not affected by treatment. The effects on mating and fertility displayed no dose-response relationship and were within the normal variation of this strain of rats used [see Table 3.10.1.1-1].

Table 3.10.1.1-1: Reproduction parameters of male rats treated with dimoxystrobin

Parental generation	F0					F1				
Dose [mg/kg]	0	50	150	500	1200	0	50	150	500	1200
Animals per dose	25	25	25	25	25	25	25	25	25	25
Male fertility										
- placed with females	24	25	25	25	25	25	25	25	25	25
- mated [n]	23	25	25	25	25	24	24	24	25	24
- mating index [%]	96	100	100	100	100	96	96	96	100	96
- pregnant [n]	23	23	25	22	24	23	20	23	25	22
- Fertility index [%]	96	92	100	88	96	92	80	92	100	88

For only one of the control male rats (#1) corroborative histopathological findings occurred in testes and epididymides, which might explain the observed infertility.
The apparently infertile F1 males did not show histopathological findings that could explain infertility, with the exception of males #444 (50 ppm dose group, F1 adults) and #514 (1200 ppm dose group, F1 adults) showing an altered size of testes and epididymides.

Sperm analysis

Sperm analysis (number of homogenization resistant testicular spermatids or caudal sperm, % abnormal and normal sperm and motility) did not indicate any effects of treatment in F0 and F1 males [see Table 3.10.1.1-2]. All values were within the range of historical data.

Table 3.10.1.1-2: Sperm parameters of males administered dimoxystrobin

Parental generation	F0					F1				
Dose [ppm]	0	50	150	500	1200	0	50	150	500	1200
Sperm count [10^6/g]										
Testis	86	107	103	104	89	96	110	113	121	95
HCD*	Mean: 92 (range of study means: 82 – 205); Range of individual values: 59 - 128									
Cauda epididymis	514	551	545	543	462	668	573	598	612	635
HCD*	Mean: 531 (range of study means: 451 – 660); Range of individual values: 234 - 844									
Normal sperm [%]	98.3	97.2	96.9	98.4	93.0	96.6	94.7	97.7	98.0	96.3
HCD*	Mean: 97.3 (range of study means: 95.4 – 98.5%%); Range of individual values: 81.5 – 100%									
Abnormal sperm [%]	1.7	2.8	3.1	1.6	7.0	3.4	5.3	2.3	2.0	3.7
HCD*	Mean: 2.7 (range of study means: 1.5 – 4.6%); Range of individual values: 0.0 – 18.5%									
Sperm motility [%]	90	90	92	88	83	92	88	89	92	89
HCD*	Mean: 90% (range of study means: 86 – 93%); Range of individual values: 65 – 99%									

*mean of 5 studies, n=125)

Female reproductive performance

Female reproductive performance was not affected by treatment [see Table 3.10.1.1-3].

The pre-coital interval was in the range of 2.6 to 3.1 days in F0 females and of 2.2 to 2.7 days in F1 females and displayed no relation to treatment level. Likewise, duration of gestation was similar in F0 (21.8 to 21.9 days) and F1 females (21.6 to 21.9 days) [see Table 3.10.1.1-3 and Table 3.10.1.1-4].

The number of F0 females with stillborn pups and thus the total number of stillborn F1 pups were slightly increased in the parental F0 generation (F1 litters) at 1200 ppm [see Table 3.10.1.1-3], but within the historical control data range. Consequently, the live birth index was slightly, but statistically significantly reduced at 1200 ppm (95%), which was within the historical control data (HCD 90-99%).

In the second generation of this study, the mean number of implantations was statistically significantly decreased compared to controls at 1200 ppm in the F1 dams (F2 litters) [see Table 3.10.1.1-4]. The evaluations of implantation sites was not required at that time, therefore, no historical control data are available from this lab. The decreased number of implantations in the high dose group however was in the range of that published in literature (HCD 11.5–18.3; Hood, 2006). The mean number of delivered F2 pups (11.8; see Table 3.10.1.1-4) was statistically significantly reduced in the high dose, but was covered by historical control data (HCD 11.1-16.4). The mean number of pups per litter alive on PND 0 was not statistically significantly lower than controls and there was no increase in stillbirths in this generation.
The number of stillborn F2 pups was comparable between control and treated groups. There was no effect on the live birth index. It ranged from 97 to 99% in F2 pups. The values were well within the range of biological variation (90-99%).

The gestation index in F0 females was 100% in all groups, indicating that all pregnant females delivered live F1 pups [see Table 3.10.1.1-3]. The gestation index in F1 females ranged between 96 and 100% [see Table 3.10.1.1-4]. This is considered to be incidental due to the isolated occurrence and lack of a dose-response relationship.

Table 3.10.1.1-3: Reproduction and gestational parameters of female F0 rats treated with dimoxystrobin

Parental generation		F0				
Dose	[ppm]	0	50	150	500	1200
Animals per dose		25	25	25	25	25
Female fertility						
- placed with males						
- mated	[n]	24	25	25	25	25
- mating index	[%]	96	100	100	100	100
- pregnant	[n]	24	23	25	22	24
- **Fertility index**	[%]	100	92	100	88	96
Pre coital interval	[days]	3.1	2.6	2.8	2.6	2.7
Duration of gestation	[days]	21.9	21.9	21.8	21.9	21.9
Implantation sites, total	[n]	361	338	395	338	363
- dto per dam	[n]	15.0	15.4	15.8	15.4	15.1
Post implantation loss	[n]	44	39	54	21	28
- dto per dam	[n]	1.8	1.8	2.2	1.0	1.2
- dto per litter	[mean %]	11.7	11.4	14.4	5.5	7.3
Females with liveborn		24	23	25	22	24
- **Gestation index**	[%]	100	100	100	100	100
- with stillborn pups	[n]	2	6	4	3	11**
(HCD 3-13)						
- with all stillborn	[n]	0	0	0	0	0
Pups delivered	[n]	317	303	341	317	335
- per dam	[mean n]	13.2	13.2	13.6	14.4	14.0
- liveborn	[n]	315	296	336	314	318**
- stillborn	[n]	2	7	5	3	17**
(HCD 4-35)						
- **Live birth index**	[%]	99	98	99	99	95
(HCD 90-100%)						

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test, two sided or Fisher's exact test, one sided)
Values may not calculate exactly due to rounding of values

Table 3.10.1.1-4: Reproduction and gestational parameters of female F1 rats treated with dimoxystrobin

Parental generation		F1				
Dose	[ppm]	0	50	150	500	1200
Animals per dose		25	25	25	25	25
Female fertility						
- placed with males						
- mated	[n]	24	24	24	25	24
- mating index	[%]	96	96	96	100	96
- pregnant	[n]	23	20	23	25	22
- **Fertility index**	[%]	96	83	96	100	92
Pre coital interval	[days]	2.2	2.5	2.3	2.6	2.7
Duration of gestation	[days]	21.9	21.7	21.6	21.6	21.7
Implantation sites, total	[n]	378	308	364	349	383
- dto per dam	[n]	16.4	15.4	16.5	14.0	12.9**
(HCD 11.5–18.3)[a)]						
Post implantation loss	[n]	36	33	36	35	23
- dto per dam	[n]	1.6	1.6	1.6	1.4	1.0
- dto per litter	[mean %]	9.2	11.1	13.4	10.7	8.4
Females with liveborn		23	20	22	25	22
- **Gestation index**	[%]	100	100	96	100	100
- with stillborn pups	[n]	4	7	4	4	5
- with all stillborn	[n]	0	0	0	0	0
Pups delivered	[n]	342	275	330	314	260
- per dam	[mean n]	14.9	13.8	15.0	12.6	11.8*
(HCD 11.1-15.0)						
- liveborn	[n]	338	266	322	310	253
- stillborn	[n]	4	9	8	4	7
- **Live birth index**	[%]	99	97	98	99	97

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test, two sided or Fisher's exact test, one sided)
Values may not calculate exactly due to rounding of values
[a)]HCD derived from publlished literature (Hood, 2006), since evaluations of implantation sites was not a standard parameter in studies at that time.

The high dose effects described above were not consistent between the first and the second generation in the 2-generation study with dimoxystrobin and can be conclusively explained by data compiled from historical control data. Overall, all the effects on reproductive parameters can be regarded as incidental and not treatment-related.

7. Effects on pups

Survival

Survival of pups was not affected by the treatment in either generation.

The viability index (survival days 0 to 4 pre cull) ranged between 93% and 96% in F1 pups [see Table 3.10.1.1-5] and between 92 and 97% in F2 pups [see Table 3.10.1.1-6] and was covered by historical control data (83-99%).

The total number of F2 pups that died, was statistically significantly increased at 150 and 1200 ppm [see Table 3.10.1.1-6], but within the historical control data range. In the high dose group this was predominantly caused by just one litter (eight pups of this dam (#707) died/were found dead on day 1 after delivery, which can also happen sporadically in control animals of this rat strain). This increased number of died pups in the high dose did not affect the viability index, which was with 92% still within the historical control range (83 - 99%). Therefore, the effect was considered to be spontaneous.
This is supported by another 2-generation toxicity study (Study No. 96172) performed with the same rat strain at the same lab. In this study the number of pups that died was actually the highest in the control (31) and low dose animals (40) in the F1 generation and not affected in the second generation, showing the biological variability of this parameter.

The statistically significantly increased number of cannibalized pups in the 500 ppm dose group is considered to be spontaneous in nature as this was predominantly caused by just one dam and was not dose-related.

Table 3.10.1.1-5: Pup survival, sex-ratio and body weights (F1 pups)

Pup generation		F1				
Dose	ppm	0	50	150	500	1200
Number of litters		24	23	25	22	24
- with liveborn pups		24	23	25	22	24
- with stillborn pups		2	6	4	3	11**
Pups liveborn	[n]	315	296	336	314	318**
Pups died *(HCD 4-31)*	[n]	9	8	11	78	17
Pups cannibalized	[n]	4	2	5	12*	7
Pups culled day 4	[n]	117	111	133	126	110
Pups day 4 - pre-cull	[n]	302	285	321	293	296
- **Viability index** *(HCD 83-99%)*	[%]	96	96	96	93	93
Pups day 4 - post cull	[n]	185	171	187	164	183
Pups day 21	[n]	185	171	187	164	183
- **Lactation index**	[%	100	98	99	98	98
Sex ratio	[% live males]					
- Day 0		47.6	50.3	48.2	50.0	50.6
- Day 21		49.7	50.3	50.3	49.4	50.3
Male pup weight	[g]					
- day 1	[g]	6.6	6.6	6.5	6.3	6.3
- day 4 - pre cull	[g]	9.5	9.4	9.8	8.4	8.3*
- day 4 - post cull	[g]	9.5	9.4	9.5	8.4	8.3*
- day 7	[g]	15.2	15.1	15.3	13.3*	12.2**
- day 14	[g]	32.6	32.2	31.9	28.0**	23.8**
- day 21	[g]	54.0	54.4	52.6	44.4**	34.7**
Male body weight gain						
- day 4 to 21	[g]	44.5	45.0	43.1	36.0**	26.4**
	[□%]		1.1	-3.1	-19.1	-40.7
Female pup weight	[g]					
- day 1	[g]	6.3	6.2	6.3	6.0	6.0
- day 4 - pre cull	[g]	9.1	8.9	9.1	8.0*	7.9*
- day 4 - post cull	[g]	9.1	9.0	9.1	8.0*	7.9*
- day 7	[g]	14.7	14.6	14.9	12.9*	11.7**
- day 14	[g]	31.4	31.5	31.6	27.4**	22.9**
- day 21	[g]	51.4	51.6	51.1	43.2**	33.2**
Female body weight gain						
- day 4 to 21	[g]	42.3	42.7	42.0	35.2**	25.3**
	[Δ%]		0.9	-0.7	-16.8	-40.2

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test, two sided or Fisher's exact test, one sided)
Values may not calculate exactly due to rounding of values

Table 3.10.1.1-6: Pup survival, sex-ratio and body weights (F2 pups)

Pup generation		F2				
Dose	ppm	0	50	150	500	1200
Number of litters		23	20	22	25	22
- with liveborn pups		23	20	22	25	22
- with stillborn pups		4	7	4	4	5
Pups liveborn	[n]	338	266	330	314	260
Pups died	[n]	5	10	14*	10	19**
(HCD 4-31)						
Pups cannibalized	[n]	4	5	4	7	4
Pups culled day 4	[n]	145	104	132	110	65
Pups day 4 - pre-cull	[n]	329	253	308	295	232**
- **Viability index**	[%]	97	95	96	95	92
Pups day 4 - post cull	[n]	182	147	171	183	165
Pups day 21	[n]	182	147	171	183	165
- **Lactation index**	[%	99	99	97	99	99
Sex ratio	[% live males]					
- Day 0		53.3	50.4	50.6	53.3	48.6
- Day 21		51.6	48.3	53.2	51.4	48.5
Male pup weight	[g]					
- day 1	[g]	6.5	6.5	6.3	6.5	6.5
- day 4 - pre cull	[g]	9.4	9.6	8.5	9.3	8.7
- day 4 - post cull	[g]	9.3	9.7	8.6	9.4	8.7
- day 7	[g]	15.0	15.5	13.3*	14.4	12.1**
- day 14	[g]	32.3	33.0	29.5*	29.2*	22.8**
- day 21	[g]	52.3	53.2	47.8*	45.0**	33.5**
Male body weight gain						
- day 4 to 21	[g]	43.0	43.6	39.2*	35.6**	24.8**
	[Δ%]		1.4	-8.8	-17.2	-42.3
Female pup weight	[g]					
- day 1	[g]	6.1	6.3	6.0	6.2	6.2
- day 4 - pre cull	[g]	9.0	9.4	8.3	8.9	8.3
- day 4 - post cull	[g]	9.0	9.4	8.3	8.9	8.3
- day 7	[g]	14.6	15.1	13.1	13.8	11.5**
- day 14	[g]	31.4	32.2	29.0	28.2**	21.8**
- day 21	[g]	50.0	51.1	46.4*	43.2**	31.9**
Female body weight gain						
- day 4 to 21	[g]	41.0	41.7	38.1*	34.3**	23.5**
	[Δ%]		1.7	-7.1	-16.3	-42.7

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test, two sided or Fisher's exact test, one sided)
Values may not calculate exactly due to rounding of values

The lactation index (survival day 4 post cull to 21) was not affected by treatment. The lactation indices ranged for F1 pups between 98 and 100% [see Table 3.10.1.1-5], for F2 pups between 97 and 99% [see Table 3.10.1.1-6].

Sex ratio

The sex ratios at day 0 and 21 were not affected by treatment in both generations. All differences were within the historical control range and not indicative of a treatment-related effect [see Table 3.10.1.1-5 and Table 3.10.1.1-6].

Pup clinical observations

The F1 pups did not show any clinical signs up to weaning which could be attributed to treatment.

Body weight

Significant effects on body weight were essentially absent at birth in the offspring animals. Therefore, an in utero effect can be excluded. In line with maternal body weight development, mean pup body weights of F1 pups in the 500 and 1200 ppm dose groups were statistically significantly reduced compared to controls from postnatal day (PND) 4 onward.

Body weight effects in the offspring became more pronounced in the later phase of lactation, especially in the last week of lactation (time points PND 14 and 21), when the pups start self-feeding (around PND12; Hood, 2011; Tyl et al., 2008 (BASF DocID 2008/1102837)). Table 3.10.1.1-7 shows the comparison of maternal body weights (F0 generation) with pup body weights (F1 generation).

Table 3.10.1.1-7: Maternal (F0) and pup (F1) body weights during lactation

F0 maternal	**ppm**				
day	**0**	**50**	**150**	**500**	**1200**
1	298.5	301.7	300.9	277.6* (-7.0)	278.9* (-6.6)
4	315.3	309	312.8	294.4** (-6.6)	287.2** (-8.9)
7	323.6	320.6	322.1	308.9 (-4.5)	299.2** (-7.5)
14	334.9	333.6	332	315.2* (-5.9)	305.2** (-8.9)
21	325.7	327.3	324	312.7 (-4.0)	307.7* (-5.5)
F1 litters	**ppm**				
day	**0**	**50**	**150**	**500**	**1200**
1	6.4	6.4	6.4	6.2 (-3.1)	6.1 (-4.7)
4 preculling	9.2	9.1	9.3	8.2 (-10.9)	8.1* (-12.0)
4 postculling	9.3	9.2	9.3	8.2* (-11.8)	8.1* (-12.9)
7	14.9	14.8	15.1	13.1* (-12.1)	11.9** (-20.1)
14	32	31.8	31.7	27.7** (-13.4)	23.4** (-26.9)
21	52.6	52.9	51.8	43.8** (-16.7)	34.0** (-35.4)

*$p \leq 0.05$; ** $p \leq 0.01$
In brackets (% vs. control)

The pups show more severe effects on body weights since they receive higher doses of dimoxystrobin compared to the dams at the same dietary doses. This is due to the fact that dietary exposure was continuous throughout the 2-generation study (and the supplementary one-generation study, see below), and dietary concentrations were not adjusted during gestation or lactation. Therefore, actual maternal dose levels during the lactation phase are increased due to a physiologically higher need for food. This leads in turn to a considerably higher dosing of the pups due to an assumed higher concentration in milk and additionally at the later lactational phase via self-feeding starting at around PND 12. A comparison of the actually measured maternal (F0 and F1) dimoxystrobin doses is shown in Table 3.10.1.1-8, including the estimated mg/kg bw/day exposures for the F1 and F2 pups during the last week of lactation only, which consides estimated direct exposure of pups via treated feed. Assuming the presence of dimoxystrobin in milk even increases the doses of offspring animals.

Table 3.10.1.1-8: Approximate mg/kg bw/day compound exposure to parental (F0 and F1) animals and estimated mg/kg bw/day exposure to F1 and F2 pups during the last week of lactation (excluding amount transferred in milk) in the 2-generation study

	Compound exposure (mg/kg bw/day)				
ppm in diet	**0**	**50**	**150**	**500**	**1200**
F0 male (premating)	0	4.7	14.1	46.4	108.8
F0 female (premating)	0	5.1	15.6	49.9	118.9
F0 Female (gestation)	0	4.5	13.6	43.6	102.5
F0 Female (lactation)[a)]	0	7.6	22.1	74.5	168.2
F1 pups[b)]	0	9.8	29.7	96.3	227.7
F1 pups (not corrected)[c)]	0	6.1	17.9	59.1	135.4
F1 Male (premating)	0	5.9	18.2	61.8	156.4
F1 Female (premating)	0	6.2	18.6	63.7	159
F1 Female (gestation)	0	4.6	13.6	46.1	107.8
F1 Female (lactation)[a)]	0	7.4	22.4	75.4	168
F2 pups[b)]	0	12.1	36.8	125.5	315.4
F2 pups (not corrected)[c)]	0	6	18	60.8	138

a) Excludes final week of lactation because of pup self-feeding

b) 2.0 fold factor for estimated pup dietary consumption on a mg/kg bw/day basis as adults through self feeding behavior in the last week of lactation based on pre-weaning pup consumption of radiolabelled microsphere recorded by Hanley and Watanabe (1985; BASF DocID 1985/1002252) (weaning at PND 28), plus estimated compound consumption during late lactation supported by the dietary 2,4-D rangefinding TK study (Saghir et al., 2013; BASF DocID 2013/1419940). This factor was applied to the compound intake based on mean premating adult male and female feed consumption.

c) Not corrected values for pup dietary test substance intake based on approximate compound exposure to females during gestation and lactation (mean values)

The estimated values for pups substance intake (in the last week of lactation) is considerably higher in pups compared to female adults (a dose of 227.7 mg/kg bw is estimated for F1 pups, while females consume only 168.2 mg/kg bw over the lactation period in the 1200 ppm dose group). This difference is even more pronounced in the F2 pups of the 1200 ppm dose group with an estimated test substance intake of 315.4 mg/kg bw/day compared to 168 mg/kg bw/day in the respective high dose females (Table 3.10.1.1-8).
A similar picture with regard to pup body weights is seen in the second generation of this 2-generation toxicity study [see Table 3.10.1.1-9].

Table 3.10.1.1-9: Maternal (F1) and pup body weights (F2 litters) during lactation

F1 maternal	**Body weight [g]**				
day	**0 ppm**	**50 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	308.7	306.0	299.9	275.8** (-10.7)	248.7** (-19.4)
4	317.9	320.3	309.6	288.4** (-9.3)	256.2** (-19.4)
7	327	328.9	319.4	299.5** (-8.4)	265.7** (-18.7)
14	347.2	345.9	342	314.9** (-9.3)	276.6** (-20.3)
21	330	330.6	330.2	313.4 (-5.0)	280.7** (-14.9)
F2 litters	**Body weight [g]**				
day	**0 ppm**	**50 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	6.4	6.4	6 (-6.3)	6.2 (-3.1)	6.2 (-3.1)
4 preculling	9.2	9.5	8.4 (-8.7)	9.2 (0)	8.5 (-7.6)
4 postculling	9.2	9.6	8.5 (-7.6)	9.2 (0)	8.5 (-7.6)
7	14.8	15.3	13.2* (-10.8)	14.2 (-4.1)	11.8** (-20.3)
14	31.9	32.7	29.2* (-8.5)	28.8** (-9.7)	22.3** (-30.1)
21	51.2	52.3	47.1* (-8.0)	44.3** (-13.5)	32.7** (-36.1)

*$p \leq 0.05$; ** $p \leq 0.01$
In brackets (% vs. control)

A clearly increased trend in pup body weight effects especially after PND 14 can be observed with no effects at birth. The NOAEL for pup body weight effects was 50 ppm, while the NOAEL for maternal body weight effects was 150 ppm. Although this picture seems to indicate a higher susceptibility of the pups compared to the adults, it needs to be considered, that again the actual doses between pups and adults are considerably different at the same dietary concentrations (see Table 3.10.1.1-8). Even, when comparing the measured substance intakes between the first and the second generation of this study, it is evident, that F1 parents during premating and F2 pups had considerably higher substance intakes compared to the respective values of the F0/F1 generation: top dose F0 females had a compound intake of 118.9 mg/kg bw during premating, while the respective compound intake of F1 females was 159 mg/kg bw.

Organ weights

There were several increased and/or decreased mean absolute and relative F1 and F2 pup organ weights in the 500 and 1200 ppm pups [see Table 3.10.1.1-10]. A treatment relationship is considered likely, if the same effect (increase or decrease) was noted for the absolute or for the relative organ weight and a dose-relationship was evident. The distinct decrease in the thymus, spleen and brain weights in the 500 and 1200 ppm pups were assessed as being a direct consequence of the significant delays in mean pup body weight gains at these dose levels.
All other differences in absolute and/or relative F1 and F2 pup organ weights were assessed as being incidental.

Table 3.10.1.1-10: Organ weights of F_1 and F_2 pups

Generation		F_1 (Males & Females combined)				F_2 (Males & Females combined)			
	Dose [mg/kg]	Absolute weight [g]	Δ%	Relative weight [% of b.w.]	Δ%	Absolute weight [mg]	Δ%	Relative weight [% of b.w.]	Δ%
Terminal weight	0	52.6				51.2			
	50	52.9	(0.6)			52.3	2.1		
	150	51.8	(-1.5)			47.1*	-8.0		
	500	43.8**	(-16.7)			44.3**	-13.5		
	1200	34.0**	(-35.4)			32.7**	-36.1		
Brain	0	1.439		2.747		1.458		2.873	
	50	1.451	1.0	2.734	-0.5	1.473	1.0	2.869	-0.1
	150	1.458	1.3	2.800	1.9	1.434	-1.6	3.101**	7.9
	500	1.420	-1.3	3.228**	17.5	1.448	-0.7	3.278**	14.1
	1200	1.351**	-6.1	3.970**	44.5	1.340**	-8.1	4.188**	45.8
Thymus	0	0.197		0.375		0.181		0.354	
	50	0.191	-3.0	0.356	-5.1	0.182	0.6	0.349	-1.4
	150	0.182	-7.6	0.347	-7.5	0.161*	-11.0	0.343	-3.1
	500	0.140**	-28.9	0.316**	-15.7	0.149**	-17.7	0.336	-5.1
	1200	0.083**	-57.9	0.238**	-36.5	0.087**	-51.9	0.264**	-25.4
Spleen	0	0.235		0.445		0.223		0.435	
	50	0.234	-0.4	0.435	-2.2	0.220	-1.3	0.422	-3.0
	150	0.224	-4.7	0.427	-4.0	0.198*	-11.2	0.420	-3.4
	500	0.174**	-26.0	0.393**	-11.7	0.180**	-19.3	0.400*	-8.0
	1200	0.112**	-52.3	0.322**	-27.6	0.101**	-54.7	0.313**	-28.0

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))
Values may not calculate exactly due to rounding of figures

Pup necropsy findings

The most obvious necropsy observations in F1 pups, which were considered to be treatment-related were

- Pale yellowish discoloration of the liver at 500 and 1200 ppm
- Cardiomegaly at 500 and 1200 ppm
- Milky fluid in the abdomen and/or thorax after organ evisceration at the high dose

Hypoplasia of thymus at the high dose related to decreased body weights and reduced thymus weights. An overview over the findings is given in Table 3.10.1.1-11.

The above mentioned findings on liver and heart are considered to be a consequence of an iron-deficiency microcytic hypochromic anemia (Cluzeaud et al., 1981; BASF DocID 1981/1001522; Tanne et al., 1994; BASF DocID 1994/1005569; Crowe et al., 1995; BASF DocID 1995/1008574; Rothenbacher and Sherman, 1980; BASF DocID 1980/1001747; Roth and Smith, 1988; BASF DocID 1988/1003436), which was seen in several other repeated-dose toxicity studies with dimoxystrobin and furthermore in the modified one-generation reproduction toxicity study in Wistar rats. The milky fluid reported in the abdomen and the breast cavity is considered to be secondary to the heart-insufficiency (cardiomegaly) induced by the chronic microcytic anemia. The same effects are also seen in the F2 pups.

Cardiomegaly was reported in the PND21 pups only and was not seen in PND4 pups macroscopically, but no effect on heart and arteries was found at histopathological examination. Furthermore, parental F1 animals did not show cardiomegaly or other effects on the heart [see Table 3.10.1.1-12]. There is information in the literature indicating that young animals undergo cardiac remodelling secondary to anemia (Cluzeaud et al., 1981; BASF DocID 1981/1001522; Tanne et al., 1994; BASF DocID 1994/1005569; Crowe et al., 1995; BASF DocID 1995/1008574).

The cardiomegaly of PND 21 pups observed is considered to be an adaptive, secondary and reversible consequence of microcytic hypochromic anemia.

Furthermore, a few of the large number of examined F1 and F2 pups showed some spontaneous findings at necropsy.

Table 3.10.1.1-11: Incidence of gross necropsy observations in F_1 and F_2 pups

Dose [mg/kg]	0	50	150	500	1200
	F_1 pups				
Litters evaluated	23	23	25	21	24
Pups evaluated	263	250	286	255	278
- Live	261	243	281	252	261
- Stillborn	2	7	5	3	17
Milky fluid in abdomen	0	0	0	0	3 (2)
Hypoplasia of thymus	0	0	0	0	30 (13**)
Cardiomegaly	0	0	2 (1)	5 (3)	56 (19**)
Liver: pale-yellowish	0	0	2 (1)	6 (3)	42 (15**)
Total pup necropsy observations - % affected pups/litter	9 (8) 4.3	12 (8) 4.4	11 (8) 3.5	19 (9) 7.5	67 (20**) 23.9**
	F_2 pups				
Litters evaluated	23	20	22	25	22
Pups evaluated	338	270	326	307	256
- Live	334	261	318	303	249
- Stillborn	4	9	8	4	7
Milky fluid in abdomen	0	0	0	0	8 (1)
Hypoplasia of thymus	0	0	0	0	6 (3)
Cardiomegaly	0	0	0	7 (5*)	52 (12**)
Liver: pale-yellowish	0	0	1 (1)	1 (1)	41 (9**)
Total pup necropsy observations - % affected pups/litter	10 (9) 2.9	6 (5) 2.8	19 (9) 5.7	15 (10) 4.9	70 (16*) 25.5**

* $p \leq 0.05$, ** $p \leq 0.01$ (Wilcoxon-test, one-sided)
( ) values in brackets give litter incidence

Table 3.10.1.1-12: Overview on cardiac effects in the 2-generation toxicity study

Dose [ppm]	**50**	**150**	**500**	**1200**
F0 parental	No effect	No effect	No effect	No effect
F1 pups	No effect	No effect	**Cardiomegaly** (only in PND21 pups, not PND4)	**Cardiomegaly** (only in PND21 pups, not PND4)
F1 parental	No effect	No effect	Heart dilation in 1 male animal*	No effect
F2 pups	No effect	No effect	**Cardiomegaly** (only in PND21 pups, not PND4)	**Cardiomegaly** (only in PND21 pups, not PND4)

*incidental finding due to single occurrence and no dose-relationship

Sexual maturation

Male and female F1 pups selected to become F1 parental animals were examined for sexual maturation. No treatment-related effects on sexual maturation were observed [see Table 3.10.1.1-13 and Table 3.10.1.1-14.]

Table 3.10.1.1-13: Sexual maturation of F1 female pups

Sex & parameter	Vaginal opening				
Dose [ppm]	0	50	150	500	1200
Animals per dose	25	25	25	25	25
- Days to criterion	34.9	36.0	35.8	36.4*	40.6**

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

Table 3.10.1.1-14: Sexual maturation of F1 male pups

Sex & parameter	Preputial Separation				
Dose [ppm]	0	50	150	500	1200
Animals per dose	25	25	25	25	21
- Days to criterion	43.4	43.7	43.6	45.1	47.8**

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

In females vaginal opening was not affected by treatment in the 50 and 150 ppm dose groups [see Table 3.10.1.1-13]. In the 500 and 1200 ppm dose females the mean age for vaginal opening was slightly but statistically significantly delayed. This effect was related to the impaired body weights/body weight gains at the respective dose groups.
In males preputial separation was not affected by treatment in the 50 and 150 ppm dose groups [see Table 3.10.1.1-14]. In the 500 and 1200 ppm dose males the mean age for preputial separation was slightly, -for the 1200 ppm dose group statistically significantly- delayed. This effect is considered to rather reflect the general retardation of the young animals due to impaired body weights/body weight gains at the respective dose groups in males and females. Lower male body weights at PND 21 strongly correlate to a delayed onset of puberty (Anonymous, 2014; BASF DocID 2014/1326033). There is broad evidence in the literature that lower body weights of the offspring, as well as the decreased food consumption or lower body weights of dams during pregnancy, correlate with the age at preputial separation of its male offspring and may cause delayed onset of puberty in male and female rats (Carney et al., 2004; BASF DocID 2004/1041034; Chernoff et al., 2009; BASF DocID 2009/1132002).
The fact that both parameters preputial separation in males and vaginal opening in females are delayed further supports the assumption that the delayed onset of puberty is a secondary effect of lower offspring body weights and not a direct endocrine mediated effect.

8. Effects on parent at terminal investigation

Organ weights

Organ weight determination in parental animals revealed a number of significant changes of absolute and/or relative organ weights [see Table 3.10.1.1-15].

Alterations of absolute and/or relative organ weights in F0 and F1 parental rats were attributed to decreased body weights. None of the organs with weight changes in the adults showed correlating histopathology.

Table 3.10.1.1-15: Major findings in parental F0 and F1 males and females[a)]

Dose, ppm [mg/kg]	0	50	150	500	1200
	F0 males				
Organs weights	No effect	No effect	↑testes (relative)	↑kidney, brain, testes, epididymides, cauda epididymides (relative)	↓ liver, spleen, thymus (absolute); ↑ kidney, brain, testes, epididymides, cauda epididymides (relative)
Necropsy findings	No effect	No effect	No effect	No effect	No effect
Histopathology	No effect	No effect	No effect	No effect	No effect
	F0 females				
Organs weights	No effect	No effect	No effect	↓ adrenal (absolute); ↑kidney, brain (relative)	↓ pituitary (absolute); ↑liver, kidney, brain (relative)
Necropsy findings	No effect	No effect	No effect	No effect	No effect
Histopathology	No effect	No effect	No effect	No effect	No effect
	F1 males				
Organs weights	No effect	No effect		↓ liver, kidney, testes, spleen; ↑thymus (absolute); ↑ epididymides, cauda epididymis, thymus and brain (relative)	↓ liver, kidney, spleen, testes, prostate, brain, adrenal (absolute); ↑ kidneys, testes, epididymides, cauda epididymis, seminal vesicle, thymus, brain (relative)
Necropsy findings	No effect	No effect	No effect	No effect	No effect
Histopathology	No effect	No effect	No effect	No effect	No effect
	F1 females				
Organs weights	No effect	No effect	↑brain (relative)	↓ kidney, adrenal (absolute); ↑ liver, kidney, ovary, spleen, brain (relative)	↓ kidney, ovary, brain, adrenal, pituitary (absolute); ↑ liver, kidney, spleen, brain (relative)
Necropsy findings	No effect	No effect	No effect	No effect	No effect
Histopathology	No effect	No effect	No effect	No effect	No effect

a) Increases (↑) and decreases (↓) relative to control.

b) Changes in organ weights attributed to body weight decreases; there were no histopathological correlates for organ weight findings. Organ weights listed are statistically significantly different from control.

c) Gross lesions few and sporadic across exposure groups; regarded as of spontaneous origin and not exposure related.

d) Histopathological lesions few and spread across exposure groups; regarded as of spontaneous origin and not exposure-related.

Differential ovarian follicle count

The results of the differential ovarian follicle count (DOFC) – comprising the numbers of primordial, growing and antral follicles, as well as the combined incidence of primordial plus growing follicles and corpora lutea – did not reveal significant deviations between controls and the high dose groups of F0 and F1 generation animals [see Table 3.10.1.1-16 and Table 3.10.1.1-17].

The increased mean numbers of primordial, growing and antral follicles, as well as the combined incidence of primordial plus growing follicles and corpora lutea were higher in the F0 high dose group than in the control. However, the deviations from the control were regarded to be within the biological variability and they were not indicative for a treatment related adverse alteration.
In the F1 high dose group the results of the differential ovarian follicle count did result in a slight although significant decrease in the number of growing follicles and of corpora lutea. Overall, the mean numbers of primordial, as well as the combined incidence of primordial plus growing follicles was higher in the high dose group than in the control, whereas the mean number of growing and antral follicles and corpora lutea was lower. However, although the decrease in the number of growing follicles and of corpora lutea was significant, both the significant and the numerical deviations from the control were regarded to be within the biological variability.

Table 3.10.1.1-16: Ovarian follicle count in F_0 maternal females

Group	Absolute number Primordial	Growing	Primordial + growing	Antral	Corpora lutea
Control	2355	1021	3376	159	705
1200 ppm	2874	1284	4158	208	878
	Mean number				
Control	94	41	135	6.4	28
1200 ppm	115	51	166	8.3	35

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

Table 3.10.1.1-17: Ovarian follicle count in F_1 maternal females

Group	Absolute number Primordial	Growing	Primordial + growing	Antral	Corpora lutea
Control	2923	1212	4135	165	650
500 ppm	3237	1345	4582	216	660
1200 ppm	3178	1063	4241	146	570
	Mean number				
Control	117	48	165	6.6	26
500 ppm	129	54	183	8.6	26
1200 ppm	127	43*	170	5.8	23*

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

CONCLUSIONS

In conclusion, dimoxystrobin causes decreased body weight developments in pups and dams. There are no effects on pup body weights seen at birth, the effects only develop over time during lactation, being most pronounced at PND 14 and 21, when pups consume solid feed.
Under the conditions of the present 2-generation reproduction toxicity study the NOAEL for general, systemic toxicity is 150 ppm (about 17 mg/kg bw/day) for the F0 and F1 parental rats, based on impairments in food consumption and body weight/body weight gain observed at the LOAEL of 500 ppm in the F1 parental animals.
The NOAEL for fertility and reproductive performance for the F0 and F1 parental rats is 1200 ppm (about 136 mg/kg bw/day). At necropsy, the F1 and the F2 pups show increased incidences of liver discoloration and cardiomegalies at the top dose of 1200 ppm. These findings are correlated to the microcytic hypochromic anemia caused by dimoxystrobin. The cardiomegalies occur only at PND 21, not at PND 4 and not in the F1 adults, indicating an adaptive, transient effect. The NOAEL for developmental toxicity in the F1 progeny is 150 ppm (17 mg/kg bw/day in adult animals; about 30 mg/kg bw/day estimated value for pups) and in F2 progeny 50 ppm (about 5 mg/kg bw/d in adults; about 12 mg/kg bw/day estimated value for pups) based on impairments in pup body weight. The apparently more severe effects in F2 pups compared to F1 adults is related to a considerably higher compound intake of the pups compared to the dams at the same dietary dose levels.

Please note: For the convenience of the reviewer, relevant historical control data (HCD; live birth index, pups delivered per dam, viability index), as included in the study report (pp. 1173 - 1186) are depicted below explicitely:

Selected reproduction an litter data obtained for the 1st generation with multi-generational studies on Wistar rats supplied by Thomae /Boehringer from 1989 – 1998 by the laboratory of the Experimental Toxicology and Ecology, BASF AG

Study No.	Generation	Study Start Date	Live Birth Index [%]	Pups delivered per dam [mean]	Viability Index [%]
83078	F1a	27-Aug-1989	96	14.5	98
	F1b	29-Oct-1989	97	15.8	99
85121	F1a	10-Sep-1987	96	14.4	96
	F1b	04-Nov-1987	97	16.2	96
87099	F1a	22-May-1988	99	14.3	91
	F1b	24-Jul-1988	94	15.6	95
88032	F1a	02-Oct-1988	99	13.6	91
	F1b	11-Dec-1988	96	15.7	97
88053	F1a	12-Feb-1989	98	14.0	92
	F1b	23-Apr-1989	97	15.2	97
88076	F1a	31-Jul-1989	99	13.7	96
88098	F1a	06-May-1990	95	14.0	**90**
	F1b	29-Jul-1990	95	14.4	95
88119	F1a	05-Apr-1992	94	14.2	92
	F1b	07-Jun-1992	96	16.1	93
89046	F1a	18-Feb-1990	97	15.0	95
	F1b	22-Apr-1990	97	14.8	98
89090	F1a	24-Mar-1991	99	14.0	96
	F1b	26-May-1991	97	15.3	95
90102	F1a	04-Aug-1991	97	14.0	91
	F1b	06-Oct-1991	96	**16.4**	94
90134	F1	09-Aug-1993	97	14.3	95
90141	F1	09-Jul-1994	92	**12.6**	97
91093	F1a	14-Dec-1992	98	14.0	97
	F1b	14-Feb-1993	98	15.7	95
91100	F1a	24-Jan-1993	99	13.9	98

Study No.	**Generation**	**Study Start Date**	**Live Birth Index [%]**	**Pups delivered per dam [mean]**	**Viability Index [%]**
	F1b	28-Mar-1993	96	15.2	95
92011	F1	02-Aug-1992	98	14.1	96
92072	F1a	30-Aug-1993	99	13.6	97
	F1b	01-Nov-1993	97	15.0	97
92095	F1a	21-Aug-1994	94	14.5	96
	F1b	23-Oct-1994	96	15.0	97
94041	F1a	10-Apr-1995	94	13.2	96
	F1b	12-Jun-1995	**90**	14.6	94
95119	F1	14-Feb-1999	98	13.1	**90**
96172	F1	16-Aug-1998	98	14.5	93
97008	F1a	17-Aug-1997	98	13.1	96
	F1b	19-Oct-1997	**100**	14.0	92
Range Min.	**F1(a/b)**		**90**	**12.6**	**90**
Max.	**F1(a/b)**		**100**	**16.4**	**99**

Selected reproduction an litter data obtained for the 2nd generation with multi-generational studies on Wistar rats supplied by Thomae /Boehringer from 1989 – 1998 by the laboratory of the Experimental Toxicology and Ecology, BASF AG

Study No.	Generation	Study Start Date	Live Birth Index [%]	Pups delivered per dam [mean]	Viability Index [%]
83078	F2	28-Jan-1990	99	13.0	95
85121	F2	27-Jan-1988	94	**11.1**	89
87099	F2	23-Oct-1988	98	12.3	95
88032	F2	12-Mar-1989	96	12.4	95
88053	F2a	23-Jul-1989	94	12.7	93
	F2b	01-Oct-1989	97	**15.2**	93
88098	F2	21-Oct-1990	98	14.0	90
88119	F2a	06-Sep-1992	96	12.1	93
	F2b	08-Nov-1992	**93**	12.6	**97**
89046	F2a	22-Jul-1990	97	12.6	94
	F2b	23-Sep-1990	95	13.9	95
89090	F2	25-Aug-1991	97	12.8	96
90102	F2	05-Jan-1992	97	12.9	95
90134	F2	17-Jan-1994	96	12.6	**83**
91093	F2	23-May-1993	94	12.5	96
91100	F2	27-Jun-1993	97	13.7	96
92011	F2	17-Jan-1993	97	12.9	94
92072	F2	31-Jan-1994	98	12.4	92
92095	F2	22-Jan-1995	**99**	12.4	95
94041	F2	11-Sep-1995	**99**	14.1	93
96172	F2	20-Dec-1998	94	**11.1**	**97**
97008	F2a	22-Feb-1998	98	13.6	96
	F2b	26-Apr-1998	97	13.9	96
Range Min.	**F2(a/b)**		**93**	**11.1**	**83**
Max.	**F2(a/b)**		**99**	**15.2**	**97**

3.10.1.2 Study 2

Multigenerational studies, rat

Study reference:

Anonymous (2015): BMD report, 2-generation reproduction toxicity study (BMD calculations for dam body weight on lactation day 0, 4, 7, 14, 21 and pup body weight on day 21). BASF SE, Ludwigshafen, Germany; BASF DocID 2015/1172904, July 2015

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp 148 – 150):

Report:	KCA 5.6.1/2 Anonymous BMD report, 2-generation reproduction toxicity study (BMD calculations for dam body weight on lactation day 0, 4, 7, 14, 21 and pup body weight on day 21) 2015/1172904
Guidelines:	N/A
GLP:	N/A
Acceptability:	Yes

Summary

Benchmark dose modeling was conducted using EPA's BMD software (www.epa.gov) with measured body weights and calculated doses for F1 dams and estimated doses of F2 pups in the 2-generation study (BASF docID: 2000/1016869). Estimated doses of F2 pups were derived from doses of adults (males, females, not pregnant, not lactating) using a factor of two.

A dose with 5% decrease of the body weight in comparison to the control group mean (BMD) and 95% lower confidence limits (BMDL) were calculated. All models of BMDS software were used. The model with the best fit and least complexity was used. Therefore the BMD calculation was taken from the model with the lowest measure of fit (Akaike Information Criteria, AIC).

Input data are summarized in table **3.10.1.2-1.**

Table 3.10.1.2-1: Input data

	dose mg/kg bw /day	number of animals	Mean response mg bw	Standard Deviation.
dams lactation day 1	0	23	308.7	25.84
	6.3	20	306.0	22.60
	18.6	22	299.9	26.94
	64.5	25	275.8	18.98
	144.1	22	248.7	24.03
dams lactation day 4	0	23	317.9	28.24
	6.3	20	320.3	23.46
	18.6	22	309.6	25.03
	64.5	25	288.4	20.00
	144.1	22	256.2	23.89
dams lactation day 7	0	23	327.0	27.58
	7	20	328.9	25.27
	20.8	22	319.4	25.42
	72.9	25	299.5	20.22
	164.7	22	265.7	25.16
dams lactation day 14	0	23	347.2	25.63
	8.9	20	345.9	22.34
	27.9	22	342.0	27.14
	88.9	25	314.9	22.24
	195.3	22	276.6	25.84
dams lactation day 21	0	23	330.0	25.95
	7.4	20	330.6	24.14
	22.4	22	330.2	32.22
	75.4	25	313.4	24.01
	168.0	22	280.7	28.33
pups lactation day 21	0	23	51.2	3.1
	12.1	20	52.3	3.4
	36.8	22	47.1	5.6
	125.5	25	44.3	5,0
	315.4	22	32.7	5.4

Results

The $BMDL_{05}$ is comparable between F1 dams and F2 pups (see table 3.10.1.2-2).

Table 3.10.1.2-2: Results of the calculations

Endpoint	model	BMD	$BMDL_{05}$
dam bw on day 1	Exponental (M2)	33.4	28.6
dam bw on day 4	Exponental (M2)	33.1	28.4
dam bw on day 7	Exponental (M4)	39.7	25.5
dam bw on day 14	Linear	46.9	41.5
dam bw on day 21	Linear	54.7	45.3
pup bw on day 21	Linear	43.5	39.8

Conclusion

$BMDL_{05}$ values of F1 dams and F2 pups indicate similar dose–response relationships. Dimoxystrobin is as toxic for pups as it is for dams.

3.10.1.3 Study 3

Multigenerational studies, rat

Study reference:

BASF SE (2017): Historical control data from one- and two-generation reproduction toxicity studies using the Wistar rats supplied by Charles River, Germany performed at BASF SE between May 2000 and February 2003. BASF AG, Ludwigshafen, Germany; BASF DocID 2017/1201528; November 2017

Detailed study summary and results:

Please note: The Wistar rats supplied by the breeder Thomae, which have been used in the Dimoxystrobin reproduction toxicity study (BASF DocID 2000/1016869) have significant biological differences to the Wistar rats supplied by Charles River. e.g. the litter sizes of the Thomae Wistar rats are considerably larger, than the litters from the Charles River Wistar rats, which also leads to a higher biological pup mortality of the Thomae Wistar rats compared to the Charles River rats. In order to demonstrate these biological differences, historical control data from Charles River Wistar rats, as included in a reproduction toxicity study conducted in the year 2000, are summarized below. Only those parameter that are relevant for the dimoxystrobin assessment (live birth index, pups delivered per dam, and viability index), i.e. the comparison of the Wistar rats from different suppliers, are listed here:

Selected reproduction an litter data obtained with multi-generational studies on Wistar rats supplied by Charles River from 2000 – 2003 by the laboratory of the Experimental Toxicology and Ecology, BASF AG

Study No.	Generation	Study Start Date	Live Birth Index [%]	Pups delivered per dam [mean]	Viability Index [%]
00037	F1	16-Oct-2000	**97**	10.6	97
	F2	19-Feb-2001	99	10.1	99
00120	F1	29-Oct-2001	99	11.5	97
01021	F1a	05-Nov-2001	97	11.0	98
	F1b	07-Jan-2002	98	10.7	100
	F2	11-Mar-2002	99	10.3	100
01057	F1	13-May-2002	99	11.0	98
	F2	16-Sep-2002	99	11.4	97
01058	F1a	27-May-2002	100	10.7	99
	F1b	30-Sep-2002	98	**9.3**	100
	F2	03-Feb-2003	98	11.0	**94**
02017	F1	04-Nov-2002	98	10.0	98
97170	F1	29-May-2000	99	10.4	98
	F2	02-Oct-2000	98	10.2	97
98132	F1	16-Oct-2000	99	10.4	98
	F2	19-Feb-2001	100	10.1	98
98136	F1	18-Dec-2000	99	10.8	100
	F2	23-Apr-2001	99	10.1	98
99080	F1	17-Apr-2000	96	9.8	98
99099	F1	-	98	10.5	97
99118	F1a	29-Jan-2001	99	9.9	100
	F1b	02-Apr-2001	100	11.0	99
	F2a	04-Jun-2001	100	11.2	97
	F2b	06-Aug-2001	100	**11.7**	96
99140	F1	28-Jan-2002	98	11.0	98
	F2	03-Jun-2002	98	10.6	96
Range Min.			**97**	**9.3**	**94**
Max.			**100**	**11.7**	**100**

3.10.1.4 Study 4

Multigenerational studies, rat

Study reference:

Anonymous (1998): Reg.No. 270 351 - Two-Generation Reproduction Toxicity Study in Wistar rats – Continuous Dietary Administration. BASF AG, Ludwigshafen, Germany; BASF DocID 1998/10130; Two-Generation Reproductive Toxicity **Study No. 94041**; January 1998

Detailed study summary and results:

Please note: This study (Two-Generation Reproductive Toxicity Study No. **94041**) is one of studies included in the HCD set used for the dimoxystrobin assessment (see 3.10.1.1, BASF DocID 2000/1016869, above). Therefore, only those parameter that are relevant for the dimoxystrobin evaluation are listed here:

Test type

Guideline:	OECD 416; 87/302/EEC; EPA 83-4; JMAFF
Deviations:	NA
GLP:	yes

Test substance

Test substance used is the equivalent to substance in CLH dossier: no (profoxydim), only control animal data relevant

Analytical purity:	94.4%
Impurities:	not specified
Batch number:	N 86
Storage conditions:	Refrigerator at -20°C, in the dark

Test animals

Species:	rat
Strain:	Wistar rats (Chbb = THOM (SPF))
Sex:	male/female
Source:	Karl THOMAE, Biberach/Riss, Germany
No. of animals/sex/dose:	25 (P, F1)
Age at study initiation:	24 ± 1 days old
Weight at study initiation:	125 - 151 g (males), 105 - 126 g (females)

Housing:	During the study period, the rats were housed individually in type DK III stainless steel wire mesh cages supplied by BECKER & CO., Castrop-Rauxel, Germany (floor area of about 800 cm²), with the following exceptions: from day 18 of gestation until day 14 after birth, the pregnant animals and their litters were also housed in Makrolon type M III cages. The M III cages were again supplied by BECKER & CO. Pregnant females were provided with nesting material (cellulose wadding) toward the end of gestation. The bedding used throughout the study was SSNIFF (type 3/4) supplied by SSNIFF SPEZIALDIÄTEN GmbH, Soest, Germany
Diet:	ground Kliba maintenance diet rat/ mouse/hamster, 343 meal, supplied by KLINGENTALMÜHLE AG, Kaiseraugst, Switzerland,*ad libitum*
Water:	tap water, *ad libitum*
Acclimation period:	at least 7 days
Environmental conditions:	Temperature: 20- 24°C Humidity: 30 - 70% Air changes: not specified Photoperiod (h dark/h light): 12/12

Administration/exposure

Route of administration:	oral; dietary
Vehicle:	basal diet
Preparation of dosing solutions:	A solution of the test substance in acetone was prepared for each concentration. These solutions were sprayed each on about 2.5 kg diet in a rotation vaporizer (Heidolph VV20) under partial vacuum. Acetone was removed by heating up to about 40°C for about 40 minutes. Thereafter, these premixes were adjusted to the desired concentrations with appropriate amounts of food and mixed for about 10 minutes in a laboratory mixer supplied by GEBR. LÖDIGE. The mixtures were prepared weekly. Due to the limited stability of the test substance in the diet (for 14 days at 4°C and for 4 days at room temperature) the food in the food hoppers was changed twice a week: first the food was filled into the food hoppers and offered to the animals no longer than 4 days; the residual amounts of the mixtures were stored in parallel at 4°C for this time period, then filled into the hoppers and offered to the animals for another 3 or 4 days, respectively.

Analytical verification of doses or concentrations: yes; homogeneity, stability, actual concentration

Duration of treatment:	P: females: at least 70 days prior to 1. mating, up to 3 weeks 1. mating period, continuously exposed during gestation and weaning (PND 21) of F1a litter; 10 days prior to 2. mating, up to 3 weeks 2. mating period, continuously exposed during gestation and and weaning (PND 21) of F1b litter male: at least 70 days prior to 1. mating, mating, up to 3 weeks mating period up to 3 weeks 1. mating period, continuously exposed during gestation and weaning (PND 21) of F1a litter; 10 days prior to 2. mating, up to 3 weeks 2. mating period F1a: females: at least 98 days prior to mating, up to 3 weeks mating period, continuously exposed during gestation and weaning (PND 21) of F2 litter; male: at least 98 days prior to mating, mating, up to 3 weeks mating period
Frequency of treatment:	continuously in diet
Dose levels:	0, 100, 500 and 2000 ppm
Control group and treatment:	yes, basal diet
Statistical analysis:	data were, where appropriate, expressed as group mean values and standard deviation. Dunnett-test (two-sided), Fischer's exact test,Wilcoxon-test (one-sided) or Kruskal-Wallis test (two-sided) were applied

Details on mating procedure

M/F ratio per cage:	1:1
Length of cohabitation:	up to 21 days
Proof of pregnancy:	vaginal plug and sperm in vaginal smear referred to as day 0 of pregnancy

After successful mating each pregnant female was caged: individually

Observations and examinations performed and frequency:

Clinical signs and mortality:
The animals were observed at least once daily for cases of mortality and animals *in extremis* were killed. Animals were observed daily for to record clinical signs. No detailed weekly examination was performed.

Body weight and food consumption:
Males were weighed weekly. Females were weighed weekly until mating was confirmed; on Day 0, 7, 14, and 20 *post-coitum*; and on Day 1, 4, 7, 14 and 21 *post-partum*.
The food consumption was recorded analogous to body weight determination, except for females between Days 14 and 21 p.p.

Test substance intake:
Not relevant

Hematology:
Blood was taken from the retroorbital venous plexus in the morning from non-fasted, unanesthetized animals.
The following parameters were determined in blood with EDTA-K_3 as anticoagulant using a particle counter: leukocytes, erythrocytes, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular hemoglobin concentration and platelets. Additionally, the clotting analyses (prothrombin time: Hepato Quick's test) were carried out using a ball coagulometer.

Clinical chemistry:
The following parameters were determined: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, y-glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol and magnesium.

Urinalysis:
The following examinations were carried out: volume, color, turbidity, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity and sediment.

Gross pathology:
All animals were subjected to detailed gross pathology and organ weight analysis. The following organs were weighed: liver, kidney, epididymis and testes. Animals that died during the study or were sacrificed non-scheduled were subjected to gross pathology and specimens of tissue considered abnormal were retained for further histological examination.

Histopathology:
Histopathology was performed on adult P- and F1a-animals in the control and high-dose group. Additionally, animals of suspected impaired fertility were also analysed histopathologically. The following tissues/organs were examined: liver, kidney, epididymis, ovaries, oviduct, pituitary, prostate, seminal vesicles, coagulating gland, testes, uterus, cervix uteri, vagina, abnormal tissues/organs.

Reproductive parameters:
Oestrus cycle: not evaluted
Sperm parameters: not evaluated

Other: the pre-coital interval, gestation time, parturition time, and number of implantation sites were recorded

Reproductive indices:
mating index, fertility index, gestation index.

Offspring
Parameters examined in F1-, F2-offspring:
On Day of birth all the offspring was examined as soon as possible and the number born alive/dead was recorded. Mortality was recorded daily. The body weight was recorded on Day 1, 4 (before culling), 7, 14 and 21 post-*partum*. The offspring were sexed on Day 0 and 21 *post-partum*; sex ratios were calculated for Day 0 and 21. Developmental landmarks like pinna unfolding (on PND 4, before standardization), opening of the auditory canal (on PND 13) and opening of the eyes (on PND 15) were recorded. Reflexes like grip reflex (on PND 13), pupillary reflex (on PND 20) were monitored and haring test (on PND 21) was performed.

Gross examination of dead pups:
Dead pups were examined for external and internal macroscopic abnormalities.

Standardisation of litters:
Yes, on Day 4 *post-partum,* to reduce the litter size to eight (4 males and 4 females, where possible)

Sacrifice:
After scheduled sacrifice (i.e. pups, which were culled on day 4 p.p., and pups, which were sacrificed on day 21 after birth or subsequent days) by CO_2.

Gross necropsy:
Offspring were examined extemally and eviscerated, their organs were assessed macroscopically.

Histopathology:
The pups with macroscopic findings were further processed and examined additionally using appropriate methods (e.g., skeletal staining according to modified DAWSON's method and/or further processing of head according to WILSON's method All pups without any notable findings or abnormalities were discarded after their macroscopic evaluation.

Offspring indices:
post-implantation survival index, live birth index, viability index, lactation index.

Results and discussion

Please note: This study (Two-Generation Reproductive Toxicity Study No. **94041**) is one of studies included in the HCD set used for the dimoxystrobin assessment (see 3.10.1.1, BASF DocID 2000/1016869, above). Therefore, only those parameter that are relevant for the dimoxystrobin evaluation are listed here:

Selected reproduction an litter data from 1st generation of the Two-Generation Reproductive Toxicity Study No. 94041

PPM in Diet	**0**		**100**		**500**		**2000**	
Generation	**F1a**	**F1b**	**F1a**	**F1b**	**F1a**	**F1b**	**F1a**	**F1b**
Females with liveborn pups (Gestation Index - %)	25 (100)	25 (100)	22 (96)	24 (100)	23 (96)	24 (100)	24 (96)	25 (100)
Females with stillborn pups (% of litters delivered)	9 (36)	**13** (52)	7 (32)	8 (33)	5 (22)	7 (29)	5 (21)	6 (24)
Number of liveborn pups (Live Birth index - %)	310 (94)	331 (**90**)	314 (96)	311 (95)	329 (98)	328 (94)	340 (98)	361 (97)
Mean number of pups delivered	13.2	14.6	14.8	13.7	14.7	14.5	14.4	14.8
Number of pups stillborn (% of number delivered)	20 (6.1)	**35** (9.6)	12 (3.7)	17 (5.2)	8 (2.4)	20 (5.7)	6 (1.7)	10 (2.7)
Pups died	11	15	28	23	20	19	39	27
Pups (%) surviving Days 0-4 (Viability Index)	96	94	91	93	94	93	86	92

Selected reproduction an litter data from 2nd generation of the Two-Generation Reproductive Toxicity Study No. 94041

PPM in Diet	**0**	**100**	**500**	**2000**
Generation	**F2**	**F2**	**F2**	**F2**
Females with liveborn pups (Gestation Index - %)	23 (100)	23 (100)	23 (96)	24 (96)
Females with stillborn pups (% of litters delivered)	4 (17)	5 (22)	6 (26)	4 (14)
Number of liveborn pups (Live Birth index - %)	320 (99)	290 (97)	296 (97)	326 (98)
Mean number of pups delivered	14.1	13.0	13.3	13.3
Number of pups stillborn (% of number delivered)	4 (1.2)	8 (2.7)	9 (3.0)	7 (2.1)
Pups died	21	25	10	30
Pups (%) surviving Days 0-4 (Viability Index)	93	89	96	90

Conclusion:

The selected reproduction and litter parameter observed in the 2-generational reproduction toxicity study on Wistar rats with dimoxystrobin [see Study 1 with DocID 2000/1016869] were within the range of the historical control data, since comparable values were obtained in the control animals of comparable studies.

3.10.1.5 Study 5

Multigenerational studies, rat

Study reference:

Anonymous (1999): BAS 500 F Two-generation reproduction study in Wistar rats, continuous dietary administration. BASF AG, Ludwigshafen, Germany; BASF DocID 1999/11869; Two-Generation Reproductive Toxicity **Study No. 96172**; November 1999

Detailed study summary and results:

Please note: This study (Two-Generation Reproductive Toxicity Study No. 96172) is one of studies included in the HCD set used for the dimoxystrobin assessment (see 3.10.1.1, BASF DocID 2000/1016869, above). Therefore, only those parameter that are relevant for the dimoxystrobin evaluation are listed here:

Test type

Guideline:	OECD 416; 87/302/EEC; EPA OPPTS 870.3800; JMAFF
Deviations:	NA
GLP:	yes

Test substance

Test substance used is the equivalent to substance in CLH dossier: no (pyraclostrobin), only control animal data relevant

Analytical purity:	98.7%
Impurities:	not specified
Batch number:	J.-No. 27882/199/b (Toxlll/part 2)
Storage conditions:	Refrigerator, after grinding at -20°C

Test animals

Species:	rat
Strain:	Wistar rats (Chbb = THOM (SPF))
Sex:	male/female
Source:	Boehringer Ingelheim, Pharma KG, Biberach/Riss, Germany
No. of animals/sex/dose:	25 (P, F1)
Age at study initiation:	28 ± 1 days old
Weight at study initiation:	108 - 162 g (males), 107 - 145 g (females)
Housing:	During the study period, the rats were housed individually in type DK III stainless steel wire mesh cages supplied by BECKER & CO., Castrop-Rauxel, Germany (floor area of about 800 cm^2), with the following exceptions: from day 18 of gestation until day 14 after birth, the pregnant animals and their litters were also housed in Makrolon type M III cages. The M III cages were again supplied by BECKER & CO. Pregnant females were provided with nesting material (cellulose wadding) toward the end of gestation. The bedding used throughout the study was SSNIFF (type 3/4) supplied by SSNIFF SPEZIALDIÄTEN GmbH, Soest, Germany

Diet:	ground Kliba maintenance diet rat/ mouse/hamster, 343 meal, supplied by KLINGENTALMÜHLE AG, Kaiseraugst, Switzerland,*ad libitum*
Water:	tap water, *ad libitum*

Acclimation period:	at least 7 days
Environmental conditions:	Temperature: 20- 24°C Humidity: 30 - 70% Air changes: not specified Photoperiod (h dark/h light): 12/12

Administration/exposure

Route of administration:	oral; dietary
Vehicle:	basal diet
Preparation of dosing solutions:	The test substance was frozen and mechanically crushed. Then an acetonic solution of the respective concentration was made. These solutions were sprayed on about 3 kg diet in a rotation vaporizer (Büchi, Rotavapor R 153) under partial vacuum. Acetone was removed by heating up to about 40°C for about 30 minutes. Thereafter these premixes were adjusted to the desired concentrations with appropriate amounts of food and mixed for about 10 minutes in a Ruberg (EM 100) laboratory mixer.

Analytical verification of doses or concentrations: yes; homogeneity, stability, actual concentration

Duration of treatment:	P/F1: females: at least 74 days prior to mating, up to 3 weeks mating period, continuously exposed during gestation and weaning; male: at least 74 days prior to mating, mating, up to 3 weeks mating period
Frequency of treatment:	continuously in diet
Dose levels:	0, 25, 75 and 300 ppm
Control group and treatment:	yes, basal diet
Statistical analysis:	data were, where appropriate, expressed as group mean values and standard deviation. Dunnett-test (two-sided), Fischer's exact test,Wilcoxon-test (one-sided) or Kruskal-Wallis test (two-sided) were applied

Details on mating procedure

M/F ratio per cage:	1:1
Length of cohabitation:	up to 21 days
Proof of pregnancy:	vaginal plug and sperm in vaginal smear referred to as day 0 of pregnancy

After successful mating each pregnant female was caged: individually

Observations and examinations performed and frequency:

Clinical signs and mortality:
The animals were observed at least once daily for cases of mortality and animals *in extremis* were killed. Animals were observed daily for to record clinical signs. No detailed weekly examination was performed.

Body weight and food consumption:
Males were weighed weekly. Females were weighed weekly until mating was confirmed; on Day 0, 7, 14, and 20 *post-coitum*; and on Day 1, 4, 7, 14 and 21 *post-partum*.
The food consumption was recorded analogous to body weight determination, except for females between Days 14 and 21 p.p.

Test substance intake:
Not relevant

Gross pathology:
All animals were subjected to detailed gross pathology and organ weight analysis. The following organs were weighed: liver, kidney, epididymis (total and cauda), ovaries, prostate, seminal vesicles (with coagulating glands and their fluids), testes, uterus with cervix and oviduct, thymus, brain, pituitary gland, adrenal gland and spleen. Animals that died during the study or were sacrificed non-scheduled were subjected to gross pathology and specimens of tissue considered abnormal were retained for further histological examination.

Histopathology:
Histopathology was performed on adult P- and F1-animals in the control and high-dose group, except for kidneys where animals of all dose groups were assessed. Additionally, animals of suspected impaired fertility were also analysed histopathologically. The following tissues/organs were examined: liver, kidney, left epididymis, thymus, adernal glands, ovaries, oviduct, pituitary, prostate, seminal vesicles, coagulating gland, left testes, uterus, cervix uteri, vagina, abnormal tissues/organs.

Reproductive parameters:
Oestrus cycle:
Oestrous cycle length and normality were evaluated daily for all P and F1 female parental rats for a minimum of 3 weeks prior to mating and were continued throughout the mating period until the female exhibited evidence of mating. Moreover, at necropsy a vaginal smear was examined to determine the stage of the estrous cycle for each P and F1 female with scheduled sacrifice.

Sperm parameters:
Immediately after necropsy and organ weight determination the right testis and cauda epididymis were taken from the males of all dose groups.
The following parameters were determined:
• sperm motility
• sperm morphology
• sperm head count (cauda epididymis)
• sperm head count (testis)
Sperm morphology and sperm head count (cauda epididymis and testis) were evaluated for the control and highest dose group, only

Other: the pre-coital interval, gestation time, parturition time, and number of implantation sites were recorded and post-implantation loss calculated, Differential ovarian follicle count

Reproductive indices:
mating index, fertility index, gestation index.

Offspring

Parameters examined in F1-, F2-offspring:
On Day of birth all the offspring was examined as soon as possible and the number born alive/dead was recorded. Mortality was recorded daily. The body weight was recorded on Day 1, 4 (before culling), 7, 14 and 21 post-*partum*. The offspring were sexed on Day 0 and 21 *post-partum*; sex ratios were calculated for Day 0 and 21. The day of onset of sexual maturation (vaginal opening and preputial separation) with the respective body weight were recorded.

Gross examination of dead pups:
Dead pups were examined for external and internal macroscopic abnormalities.

Standardisation of litters:
Yes, on Day 4 *post-partum,* to reduce the litter size to eight (4 males and 4 females, where possible)

Sacrifice:
After scheduled sacrifice (i.e. pups, which were culled on day 4 p.p., and pups, which were sacrificed on day 21 after birth or subsequent days) by CO_2, brain, spieen and thymus of 1 pup/sex and litter from the F1 and F2 pups were weighed.

Gross necropsy:
Offspring were examined extemally and eviscerated, their organs were assessed macroscopically.

Histopathology:
The pups with macroscopic findings (macrophthalmia, anophthalmia) were further processed and examined according to WILSON's method. All pups without any notable findings or abnormalities were discarded after their
macroscopic evaluation.

Offspring indices:
post-implantation survival index, live birth index, viability index, lactation index.

Results and discussion

Please note: This study (Two-Generation Reproductive Toxicity Study No. **96172**) is one of the studies included in the HCD set used for the dimoxystrobin assessment (see 3.10.1.1, BASF DocID 2000/1016869, above). Therefore, only those parameter that are relevant for the dimoxystrobin evaluation are listed here:

Selected reproduction an litter data from 1st generation of the Two-Generation Reproductive Toxicity Study No. 96172

PPM in Diet	0	25	75	300
Generation	F1	F1	F1	F1
Females with liveborn pups (Gestation Index - %)	25 (100)	25 (100)	24 (100)	25 (100)
Females with stillborn pups (% of litters delivered)	5 (20)	10 (40)	4 (17)	5 (20)
Number of liveborn pups (Live Birth index - %)	356 (98)	336 (96)	295 (98)	322 (98)
Mean number of pups delivered	14.5	14.0	12.5	13.1
Number of pups stillborn (% of number delivered)	7 (1.9)	15 (4.3)	6 (2.0)	6 (1.8)
Pups died	31	40	1	8
Pups (%) surviving Days 0-4 (Viability Index)	93	87	100	93

Selected reproduction an litter data from 2nd generation of the Two-Generation Reproductive Toxicity Study No. 96172

PPM in Diet	0	25	75	300
Generation	F2	F2	F2	F2
Females with liveborn pups (Gestation Index - %)	25 (100)	23 (96)	23 (96)	25 (100)
Females with stillborn pups (% of litters delivered)	5 (20)	3 (13)	3 (13)	1 (4.0)
Number of liveborn pups (Live Birth index - %)	320 (98)	295 (99)	283 (99)	346 (99)
Mean number of pups delivered	13.1	13.0	12.5	14.0
Number of pups stillborn (% of number delivered)	8 (2.4)	4 (1.3)	4 (1.4)	4 (1.1)
Pups died	7	4	11	8
Pups (%) surviving Days 0-4 (Viability Index)	97	98	95	96

Conclusion

The selected reproduction and litter parameter observed in the 2-generational reproduction toxicity study on Wistar rats with dimoxystrobin [see Study 1 with DocID 2000/1016869] were within the range of the historical control data, since comparable values were obtained in the control animals of comparable studies.

3.10.1.6 Study 6

Multigenerational studies, rat

Study reference:

Anonymous (2001): Modified one-generation reproduction toxicity study in Wistar rats, continuous dietary administration. BASF AG, Ludwigshafen, Germany; BASF DocID 2000/1016870; August 2001

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp. 150 – 165):

Report:	KIIA 5.6.1/2 Anonymous Modified one-generation reproduction toxicity study in Wistar rats, continuous dietary administration 2000/1016870
Guidelines:	OECD 415
GLP:	yes
Previous evaluation:	in the DAR (2003)
Acceptability:	yes

This one-generation study was designed to investigate whether indications of anaemia could be detected in F0 parental animals and F1 litters following administration of dimoxystrobin at and above the overall NOAEL determined in the previous multigeneration study. The design of this special study was based partly on OECD guideline 415 (1983). However, as the study used smaller numbers of parental animals than a typical reproductive toxicity study (which is acceptable given the purpose of the study) reproductive performance and fertility findings need to be interpreted with care.

Executive summary:

Dimoxystrobin was administered to groups of 10 male and 10 female healthy young Wistar rats (F0 parental generation) as a constant homogeneous addition to the food in different concentrations (0, 150, 500 and 1200 ppm). This study was conducted in order to further elucidate the mode of action for the observed cardiomegalies and liver discolorations in offspring, and therefore only administered expected "effect doses". At least 47 days after the beginning of treatment, F0 animals were mated to produce a litter (F1). Mating pairs were from the same dose group. The study was terminated with the terminal sacrifice of the F1 weanlings and F0 adult animals. Test diets containing dimoxystrobin were offered continuously throughout the study.
The administration of 150, 500 and 1200 ppm of the test compound to male and female rats in this modified one-generation reproduction toxicity study caused a dose-dependent regenerative microcytic hypochromic anemia in the parental animals and the F1 pups at all doses. The anemia was characterized by reduced haemoglobin concentrations and mean corpuscular indices as well as increased microcytosis and reticulocytes.
Furthermore, 500 and 1200 ppm induced other substance-induced adverse effects, primarily on parental food consumption, parental and pup body weight data and pup necropsy findings, which are in-line with the observed anemia.

The slight effects on hematological parameters observed in the 150 ppm parental animals indicate a beginning anemia, which is treatment-related, but not considered to represent an adverse outcome, thus the lowest NOAEL for parental toxicity is considered to be 150 ppm (about 17 mg/kg bw). The apparently more severe effects on hematology in pups compared to dams can be related to a considerably higher compound intake at the same dietary dose levels. When more accurate calculated doses between pups and dams are used for estimation, the no effect levels are essentially comparable.

Materials and Methods:

Dimoxystrobin (batch/purity: N 15: 98.4%) was administered to groups of 10 male and 10 female young Wistar rats (F0 parental generation) as a constant homogeneous addition to the food in different concentrations (0, 150, 500 and 1200 ppm). At least 47 days after the beginning of treatment, F0 animals were mated (1:1) to produce a litter (F1). Litters were standardised to 8 pups on day 4. Mating pairs were from the same dose group. The study was terminated with the terminal sacrifice of the F1 weanlings and F0 adult animals. Test diets containing dimoxystrobin were offered continuously throughout the study.

Results:

Table 3.10.1.6-1 shows the approximate mg/kg bw/day compound exposure to parental animals and estimated mg/kg bw/day exposure to pups during last week of lactation (excluding amount, if any, transferred in milk) in the modified one-generation study.

Table 3.10.1.6-1: Approximate mg/kg bw/day compound exposure to parental animals and estimated mg/kg bw/day exposure to pups during last week of lactation (excluding amount, if any, transferred in milk) in the modified one-generation study

ppm in diet	**0**	**150**	**500**	**1200**
Male (pre-mating)	0	17.5	56.6	128.4
Female (pre-mating)	0	18.3	58.2	131.9
Female (gestation)	0	14.5	47.4	109.3
Female (lactation)[a)]	0	23.3	82.8	170.5
Pups [b)]	0	36	114	260
Pups (not corrected)[c)]	0	18	57	130

a) Excludes final week of lactation because of pup self-feeding

b) 2.0 fold factor for estimated pup dietary consumption on a mg/kg bw/day basis as adults through self-feeding behaviour in the last week of lactation based on pre-weaning pup consumption of radiolabelled microsphere recorded by Hanley and Watanabe (1985; BASF DocID 1985/1002252) (weaning at PND 28), plus estimated compound consumption during late lactation supported by the dietary 2,4-D range-finding TK study (Saghir et al., 2013; BASF DocID 2013/1419940). This factor was applied to the compound intake based on mean premating adult male and female feed consumption.

c) Not corrected values for pup dietary test substance intake based on approximate compound exposure to females during gestation and lactation (mean values)

1. Clinical signs of toxicity

No abnormal clinical signs of toxicity were observed.

2. Mortality

There were no substance-related or spontaneous mortalities in any of the male and female F0 parental animals in any groups.

3. Effects on body weight

Mean body weights of the mid (500 ppm) and high dose (1200 ppm) males were influenced by the test substance administration during the entire study period [see Figure 3.10.1.6-1]. From week one until the end of the study the differences attained statistical significance in comparison to that of the control males. At termination of the study (week 13), the mean body weight of the 500 ppm males was about 11 %, that of the 1200 ppm males about 17% below the corresponding control value. This is in-line with the concurrent reductions in food consumption in these groups. Mean body weight gain of the mid and high dose F0 males were also clearly impaired (with or without attaining statistical significance); if calculated for study weeks 0 - 13, the mean weight gain in the mid dose group was about 14 %, that of the high dose group about 24% below the respective control value. The mean body weights and mean body weight gains of the 150 ppm F0 males were substantially similar to control values.

Mean body weights of the substance-treated F0 females were substantially similar to controls during the premating period, but the weight gain of the high dose females (1200 ppm) was about 11% below the corresponding control value if calculated for weeks 0 – 6 (without attaining statistical significance) [see Figure 3.10.1.6-1].
During gestation the mean body weights and body weight gains of the high dose F0 parental females were also impaired. The mean body weight of the 1200 ppm dams on day 20 p.c. was about 9%, the body weight gain from days 0 - 20 p.c. was about 18% below the corresponding control value.
During the lactation period the mean body weights of the 1200 ppm females were also below the corresponding control values (up to about 12% on day 14 p.p.), the differences, however, did not always reach statistical significance. The mean body weight gains of the substance-treated females did not show a consistent trend throughout the lactation period; particularly at the high dose, the weight gains were sometimes distinctly above, sometime distinctly below the corresponding control values. Mean body weights and mean body weight gains of the 150 and 500 ppm females were not affected by the test substance administration during premating, gestation or lactation, particularly if the normal range of biological variation is taken into consideration.

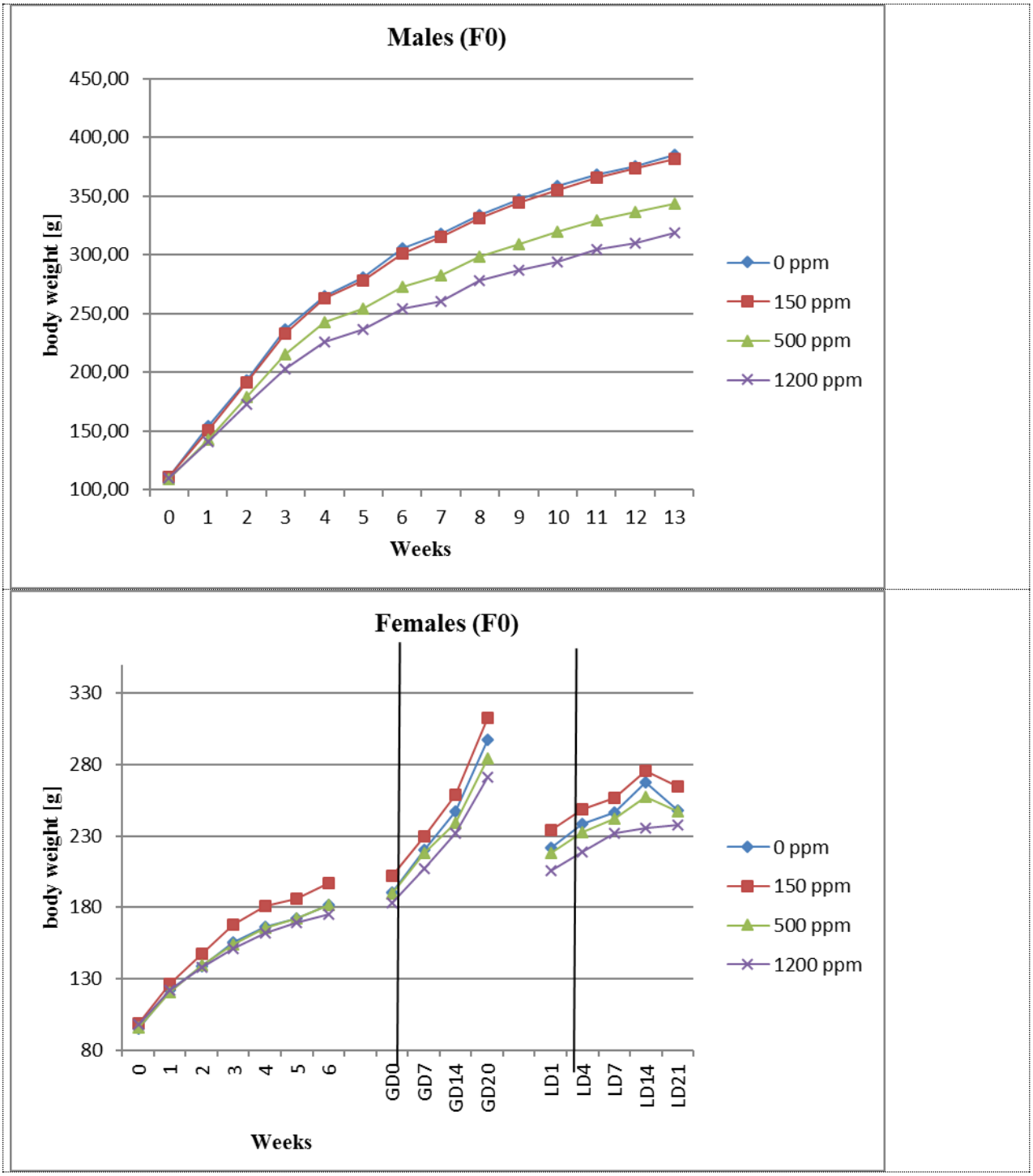


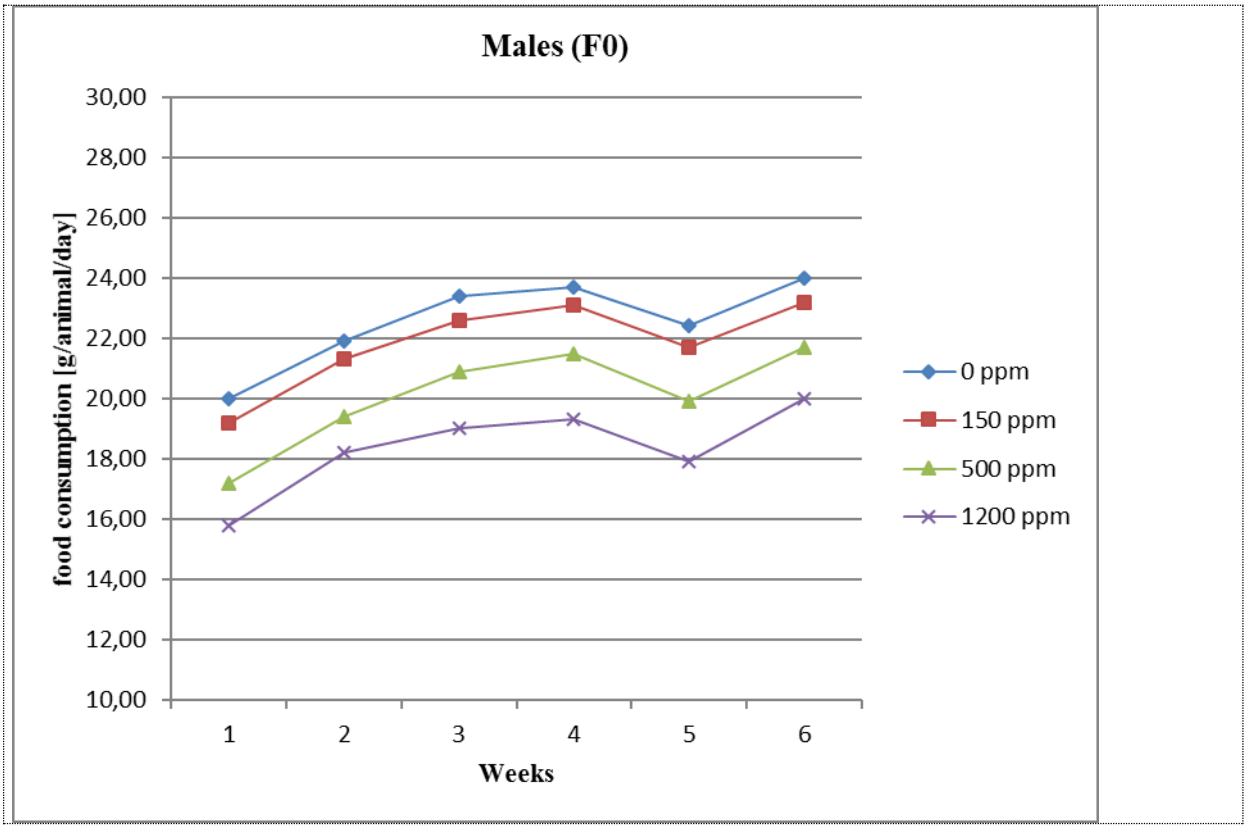
Figure 3.10.1.6-1: Body weight development of parental F_0 animals

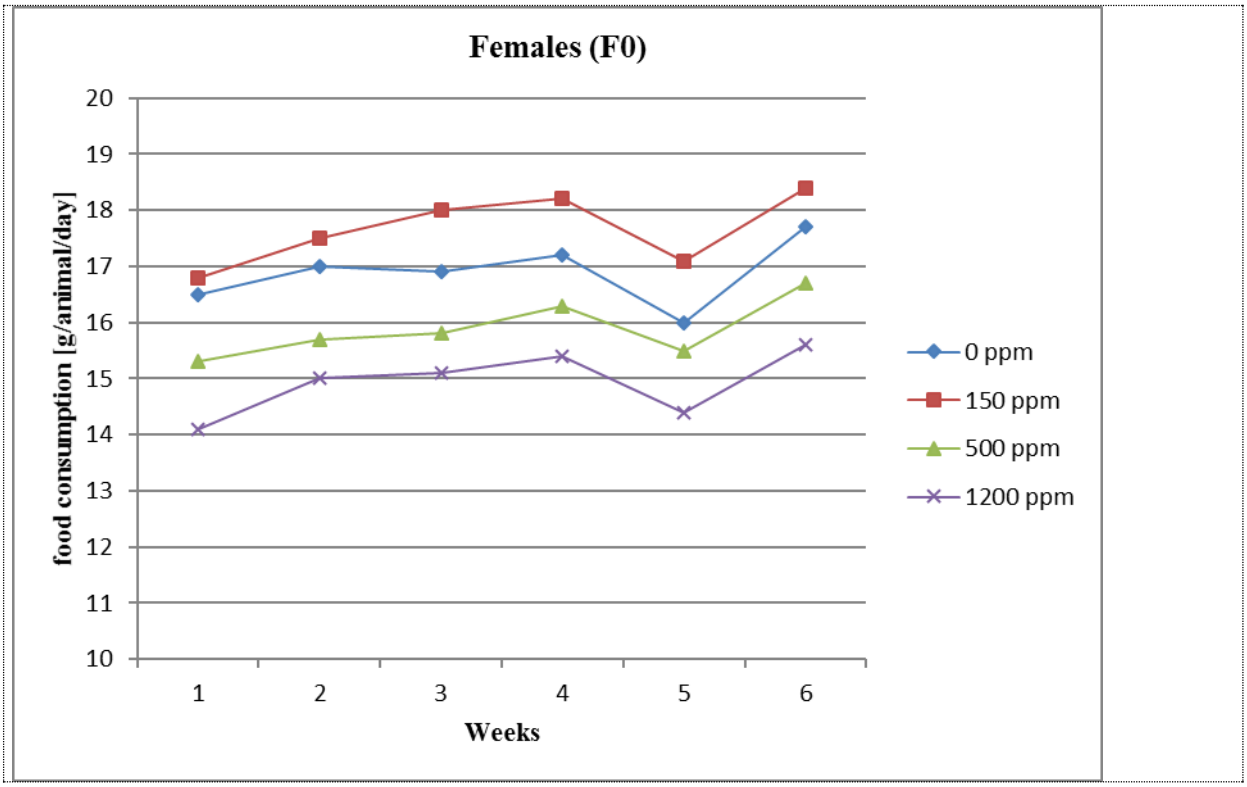
4. Effects on feed consumption

In the F0 parental male animals of the mid and high dose group (500 or 1200 ppm) the feed consumption was statistically significantly reduced during the premating period (study weeks 0 - 6); if calculated for the entire premating period the food intake was about 11% (500 ppm) or 19% (1200 ppm), respectively, below the corresponding control value [see Figure 3.10.1.6-2]. This is considered to reflect a substance-related effect as body weight data of the mid and high dose males were concomitantly affected. The food intake of the low dose males, however, was similar to control values.

The feed consumption of the high dose F0 parental females was statistically significantly reduced during the premating period (study weeks 0 - 6); if calculated for the entire premating period, the feed intake was about 12% below the corresponding control value [see Figure 3.10.1.6-2].

During gestation and lactation of the F1 litter, food consumption of the 1200 ppm F0 dams was also statistically significantly impaired and on average about 14 - 20% lower than in the control group. As the impairments in the dams' feed intake at the high dose had also some concurrent effects on the dams' body weight data, these effects are considered as substance - related. The food consumption of the 150 and 500 ppm female F0 parental rats during premating, gestation and lactation did not show any statistically significant differences and was generally comparable to that of the controls taking the normal range of biological variation into consideration.





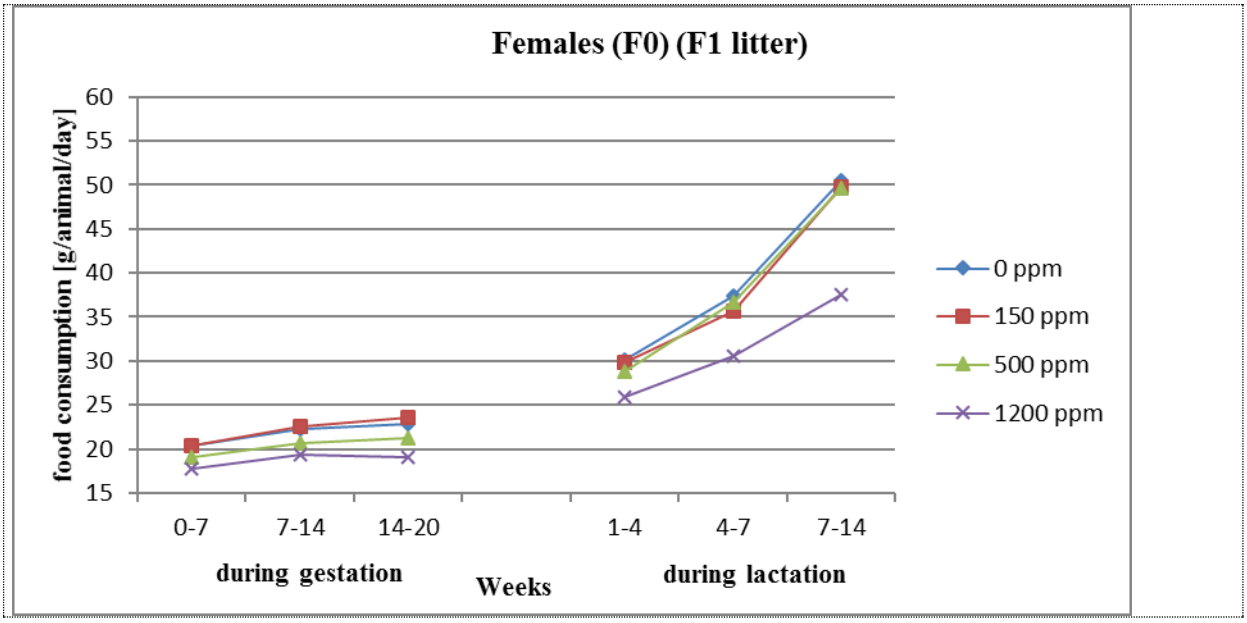


Figure 3.10.1.6-2: Food consumption of parental F_0 animals during premating

5. Effects on reproduction

The following table summarizes the reproduction and gestational parameters of female F0 rats treated with dimoxystrobin in the modified one-generation toxicity study

Table: Female reproduction and delivery data (Wistar rats bred by Charles River)

Parental generation (F0)					
Dose	[ppm]	0	150	500	1200
Animals per dose		10	10	10	10
Female fertility					
- placed with males					
- mated	[n]	10	9	10	10
- mating index	[%]	100	90	100	100
- pregnant	[n]	6	8	10	10
- **Fertility index**	[%]	60	89	100	100
Pre coital interval	[days]	2.8	3.2	2.7	3.7
Duration of gestation	[days]	21.8	22.0	22.0	21.7
Implantation sites, total	[n]	72	98	99	103
- dto per dam	[n]	12.0	12.3	11.0	10.3
Post implantation loss	[n]	2	6	11	1
- dto per dam	[n]	0.3	0.8	1.1	0.1
- dto per litter	[mean %]	2.8	5.3	17.5	0.8
Females with liveborn		6	8	9	10
- **Gestation index**	[%]	100	100	90	100
- with stillborn pups	[n]	1	1	0	0
- with all stillborn	[n]	0	0	0	0
Pups delivered	[n]	70	92	90	102
- per dam	[mean n]	11.7	11.5	10.0	10.2
HCD 9.3 – 11.7 [mean]					
- liveborn	[n]	65	91	90	102
- stillborn	[n]	5	1	0	0
- **Live birth index**	[%]	100	85*	99	97
(HCD 97-100%)					

*p<0.05

a. Male reproduction data

Male mating index

The male mating index reached 100% in the control group and test groups 02 and 03 (500 and 1200 ppm), while it was 90% at 150 ppm.

Male fertility index

Fertility could be proven for most F0 parental males within the scheduled mating interval for F1 litter. The male fertility index varied between 60% and 100% without showing any relation to dosing [see Table 3.10.1.6-2]. Actually, four control males (males Nos. 3, 4, 9 and 10 mated with female Nos. 103, 104, 109 and 110) and two males of test group 01 (males Nos. 12 and 15 mated with female Nos. 112 and 115 - 150 ppm) did not generate F1 pups. Moreover, one male of test group 02 (male No. 24 mated with female No. 124 - 500 ppm) did generate F1 progeny, but all implants of the corresponding dam died already in utero. The sporadic occurrence of infertility without relation to dosing in several male rats of the different test groups including the controls is assessed as being incidental and spontaneous in nature.

Table 3.10.1.6-2: Male fertility index for F0 males (%)

	Test group 00 (0 ppm)	Test group 01 (150 ppm)	Test group 02 (500 ppm)	Test group 03 (1200 ppm)
Concerning F1 litters	60	80	100*	100

*= The female mating partner of mid dose male No. 24 had only dead implants in utero at necropsy and did not deliver pups.

b. Female reproduction data

Female mating index

The female mating index calculated after the mating period for F1 litter was 90% for the low dose group and 100% for all remaining groups. In one low dose F0 female rat (No. 112) no sperm was detected within the 14-day mating period; this female rat did not give birth to a litter/had no implants in utero.

The mean duration until sperm was detected (day 0 p.c.) varied between 2.7 and 3.7 days without relation to dosing. These values reflect the normal range of biological variation inherent in the strain of rats used for this study.

Female fertility index

During the mating interval, all sperm positive rats delivered pups except four females of test group 00 and one female of test group 01, which did not become pregnant. One female of test group 02 did not deliver pups, but showed two implants in utero. As all females of test group 03 were pregnant, the fertility index varied between 60% (test group 00), 90% (test group 01) and 100% (test group 02 and 03) [see Table 3.10.1.6-3].

The occurrence of infertility in several female rats of test groups 0 and 1 (0 and 150 ppm) is assessed as being incidental and spontaneous in nature, also because no substance-induced impairments on the fertility occurred in the previous extensive two generation reproduction toxicity study.

The mean duration of gestation was very similar in all groups and the variation was negligible (between 21.7 and 22.0 days).

The gestation index was 100% for test group 00, test group 01 and test group 03, indicating that all pregnant females delivered live F1 pups.

In the test group 02 the gestation index reached 90% due to the fact, that one pregnant female delivered no pups, but had only dead implants in utero. Implantation was not affected by the treatment since the mean number of implantation sites was comparable between all test groups if the normal range of biological variation is taken into consideration. Furthermore, there were no indications for a substance-induced increase in intrauterine embryo-/feto-lethality since the postimplantation loss values were unaffected by treatment and did not show dose-response relationship.

Table 3.10.1.6-3: Female fertility index for F0 females (%)

	Test group 00 (0 ppm)	Test group 01 (150 ppm)	Test group 02 (500 ppm)	Test group 03 (1200 ppm)
Concerning F1 litters	60	90	100	100

F1 generation litter/pups

Litter data

The mean number of delivered pups/dam and the rate of liveborn and stillborn pups were not affected by the administration of the test substance considering the overall low litter number, the lack of a dose response and the normal biological variation.

Pup viability/mortality

There were no substance-related differences between the control and the substance-treated groups concerning mortality and viability of the F1 pup generation. The viability indices, as indicators for perinatal mortality varied between 93% and 100%, are in the range of biological variation and thus reflect the normal range of biological variation inherent in the strain of rats used for this study. This includes the statistically significantly lower viability index at 150 ppm.

The lactation indices as indicators for pup mortality between days 4 - 21 p.p. ranged between 100% (control group, test groups 01 and 03), and 99% (test group 02) and therefore did not show differences of biological relevance between the substance-treated groups and the control group.

Sex ratio

The sex distribution and sex ratios of live F1 pups on the day of birth and on day 21 p.p. did not show any substantial differences between controls and treated groups; all differences observed are regarded to be spontaneous in nature.

Pup body weight data

Effects on body weights of pups were essentially absent at birth in the offspring animals. Mean body weights of F1 pups in test groups 03 (1200 ppm) were statistically significantly reduced from day 7 p.p. onwards until scheduled sacrifice on day 21 p.p. [see Table 3.10.1.6-4]. Body weight effects became more pronounced in the later phase of lactation, especially in the last week of lactation (time points PND 14 and 21), when the pups start self-feeding (around PND12; Tyl et al., 2008 (BASF DocID 2008/1102837)). On day 21 p.p. mean body weights of the F1 pups of test group 03 (1200 ppm) were about 38% below the concurrent control value if both sexes were combined. Mean body weights of F1 pups in test group 02 (500 ppm) were slightly reduced on day 21 p.p. (about 12% below the concurrent control group). Table 3.10.1.6-4 shows the comparison of maternal body weights (F0 generation) with pup body weights (F1 generation). The impairments in pup body weights at 500 and 1200 ppm are considered to be a consequence of test substance administration.

The F1 pups of the 150 ppm group did not show any statistically significant or biologically relevant differences for body weights/body weight gains.

Table 3.10.1.6-4: Maternal and pup body weights during lactation

Maternal	**Body weight [g]**			
day	**0 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	221.5	233.8	218.2 (-1.5)	206 (-7.0)
4	238.7	248.6	232.4 (-2.6)	219.1 (-8.2)
7	246.6	256.3	242 (-1.9)	231.7 (-6.0)
14	267.2	275.7	257.2 (-3.7)	235.4** (-11.9)
21	248.2	264.7	247.2 (-0.4)	237.4 (-4.4)
Litters	**Body weight [g]**			
day	**0 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	5.8	5.6	5.1 (-12.1)	5.3 (-8.6)
4 preculling	8.6	8.4	9.1 (5.8)	7.4 (-14)
4 postculling	8.7	8.4	9.1 (4.6)	7.5 (-13.8)
7	14	13.9	13.7 (-2.7)	10.8** (-22.9)
14	29.1	29.6	27.1 (-6.9)	20.2** (-30.6)
21	47.1	46.3	41.4 (-12.1)	29** (-38.4)

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

In brackets (% vs. control)

Pup clinical observations
The F1 generation pups did not show any clinical signs up to weaning which could be attributed to the treatment.
Only one spontaneous clinical finding occurred in one low dose F1 pup (filiformed tail).
Pup organ weights
The mean absolute heart weights of the F1 pups of test groups 01 - 03 (150, 500 or 1200 ppm) did not show any substance-induced differences in comparison to the control values.

The mean relative heart weights of the pups of test groups 02 and 03 (500 and 1200 ppm) were statistically significantly increased (if both sexes are combined), whereas the relative mean heart weights at 150 ppm were similar to control values. If calculated for both sexes, the mean relative heart weight was increased above control values to about 20% (mid dose) or 57% (high dose) [see Table 3.10.1.6-5].
The increased mean relative heart weights of the PND21 F1 pups in test groups 02 - 03 (500 and 1200 ppm) are considered to be substance-related and directly in-line with the cardiomegaly observed macroscopically at the high dose (1200 ppm).

Table 3.10.1.6-5: Overview on cardiac effects in the modified one-generation study

Dose [ppm]	**150**	**500**	**1200**
Parental	No effect	Absolute heart weight slightly decreased (m);	Absolute heart weight slightly decreased (m) (related to decreased bw); ↑Relative heart weights due to decreased bw (not treatment-related) Gross lesions: no effects on hearts
Pups PND 21	No effect	↑Relative heart weight (20%) (males statistically significantly increased)	↑Relative heart weight (57%) (males and females statistically significantly increased)
Pups Necropsy findings PND21	No effect	-	Cardiomegaly (only in PND21 pups, not PND4)

Pup necropsy observations

The most obvious necropsy observations in the F1 pups, which are considered to be substance-induced, were increased occurrences of:

- pale yellowish discoloration of the liver at 500 and 1200 ppm
- cardiomegaly at 1200 ppm
- milky fluid in abdomen and/or thorax after organ evisceration at 500 and 1200 ppm
- pale discoloration of kidney(s) at 500 and 1200 ppm

The liver, kidney and heart findings are considered to be a consequence of a microcytic hypochromic anemia, which occurs in this study. The milky fluids reported in the abdomen and the breast cavity are considered to be secondary to the heart-insufficiency (cardiomegaly) induced by the chronic microcytic anemia.

In-line with the 2-generation study cardiomegaly was evident in the PND21 pups only and not seen in PND4 pups. Cardiomegaly occurs secondary to the microcytic hypochromic iron-deficiency anemia (see below) after the start of direct food intake of the pups.

A few of examined F1 pups showed some spontaneous findings at necropsy or in the additional examinations carried out later (e.g. incisors sloped, post mortem autolysis, dilated renal pelvis, hydroureter, filiformed tail) scattered throughout the test groups. These findings occurred without a relation to dosing and/or do also occur sporadically in the strain of rats used.

6. Clinical pathological findings

Hematology

After 4 weeks of test substance administration (before mating period) decreased haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were found in the peripheral blood of the high dose males (1200 ppm). Increased red blood cell counts, anisocytosis, microcytosis and hypochromasia were also detected in these males. Moreover, in the mid dose males (500 ppm) MCV and MCH were reduced and microcytosis was increased. In the females, decreased MCH and MCHC values and slightly increased microcytosis and hypochromasia were found in the high dose animals at this time interval [see Table 3.10.1.6-6].
After 3 months of test substance administration (shortly before sacrifice) slightly increased microcytosis was seen in the high dose males (1200 ppm), only. In the peripheral blood of the high dose dams, decreased haemoglobin, MCV, MCH and MCHC values and increased red blood cells, platelets, reticulocytes, microcytosis and hypochromasia were found. In the dams of the mid dose group (500 ppm) decreases in MCH and MCHC as well as increases in microcytosis were detected. Slight effects were detected in the low dose group of 150 ppm.

On day 21 after birth the following hematology changes were observed in the high dose male and female F1 pups (1200 ppm): decreases in red blood cells, haemoglobin, haematocrit, MCV and MCH; increases in platelets, reticulocytes, microcytosis and anisochromasia. Moreover, in the high dose male pups MCHC was increased. In the male and female pups of the mid dose group (500 ppm) haemoglobin, haematocrit, MCV and MCH were decreased and reticulocytes, microcytosis, anisochromasia and normoblasts were increased. Significantly reduced erythrocytes were also measured in the blood of the mid dose male pups. In the low dose male pups (150 ppm) MCV was reduced and microcytosis and anisochromasia were increased. In the low dose female pups reticulocytes and microcytosis were elevated.

An overview on the hematological changes for parental animals and PND21 pups can be found in Table 3.10.1.6-7.

Table 3.10.1.6-6: Summary of hematology parameters in the modified one-generation study

Dose [ppm]	Hematological parameters						
Males, day 29	**RBC (TERA/L)**	**HGB (MMOL/L)**	**HCT (L/L)**	**MCV (FL)**	**MCH (FMOL)**	**MCHC (MMOL/L)**	**PLT (GIGA/L)**
0	7.71	9.3	0.411	53.3	1.21	22.69	819
150	7.49	9.1	0.399	53.4	1.22	22.79	800
500	7.87	9.1	0.404	51.4**	1.16**	22.47	856
1200	8.2**	8.5***	0.387	47.2***	1.04***	22.04**	896
Males, day 98							
0	8.84	9.5	0.484	54.7	1.08	19.68	709
150	8.6	9.5	0.474	55.2	1.11	20.09**	700
500	9	9.6	0.489	54.3	1.06	19.55	706
1200	8.97	9.4	0.479	53.4	1.05	19.63	723
Females, day 29							
0	7.68	9.4	0.409	53.3	1.22	22.94	740
150	7.58	9.3	0.403	53.1	1.22	22.59	768
500	7.72	9.2	0.403	52.2	1.19	22.78	728
1200	7.96	9	0.405	51	1.14**	22.28**	841
Females, day 100							
0	8.5	10.4	0.498	58.8	1.22	20.76	779
150	8.31	9.5**	0.471	56.7	1.14*	20.07*	763
500	9.09	10	0.505	55.7	1.11*	19.90***	793
1200	9.54*	9.4***	0.491	52.0*	1.00**	19.21***	981**
Male pups, PND 21							
0	4.64	5.4	0.305	65.9	1.17	17.67	825
150	4.67	5.1	0.286	61.4**	1.08	17.62	821
500	4.28*	3.9***	0.223***	52.0***	0.91***	17.48	1065
1200	3.10***	3.0***	0.152***	47.1***	0.97**	20.59*	1227**
Female pups, PND 21							
0	4.47	5.1	0.281	63	1.14	18.08	759
150	4.78	5.1	0.291	61	1.07	17.6	690
500	4.37	4.0***	0.230**	52.3***	0.91***	17.39	1084
1200	3.02**	2.7***	0.139**	44.5***	0.91***	20.65	1471*

*$p \leq 0.05$; ** $p \leq 0.02$; *** $p \leq 0.002$

Table 3.10.1.6-7: Overview of hematological changes in the modified one-generation study

Dose [ppm]	150	500	1200
Adult males	No effect	**Premating:** ↓ MCH (-4%), MCV (-4%); ↑ microcytosis	**Premating:** ↓ Hgb (-9%), MCV (-11%), MCH (-14%), MCHC (-3%); ↑ RBC (+6%), anisocytosis, microcytosis and hypochromasia, reticulocutes (+52%) **Study day 100:** slight↑ microcytosis
Adult females	**Study day 100:** ↓ Hgb (-9%), MCH (-7%), MCHC (-3%); ↑ microcytosis	**Study day 100:** ↓ MCH (-9%), MCHC (-4%); ↑ microcytosis, reticulocytes (+44%)	**Premating:** ↓ MCV (-3%), MCH (-7%), ↑ RBC (+4%), microcytosis, hypochromasia, reticulocytes (+20%) **Study day 100:** ↓ Hgb (-10%), MCV (-12%), MCH (-18%), MCHC(-7%); ↑ RBC (+12%), microcytosis, hypochromasia, platelets and reticulocytes (+333%)
Pups PND 21	↓MCV (M:-7%) ↑microcytosis and reticulocytes (M:+6%; F:+56%); ↑anisochromasia (males)	↓ Hgb (M:-28%; F:-22%), Hct (M:-27%; F:-18%), MCV (M:-21%; F:-17%), MCH (M:-22%,F: -20%); ↓ RBC (M:-8%); ↑ anoisochromasia, microcytosis, reticulocytes (M:+57%; F:+58%), normoblasts (both sexes)	↓ RBC (M: -33%; F:-32%), Hgb (M:-45%; F:-47%), Hct (M:-50%; F:-51%), MCV (M:-29%; F:-29%), MCH (M:-17%; F:-20%), MCHC (M: -19%); ↑ anisochromasia, microcytosis, platelets and reticulocytes (M:+66%; F:+216%)

The changes in hematological parameters observed are indicative for an iron-deficiency microcytic hypochromic anemia. Dimoxystrobin reduces iron uptake in the duodenum and thus causes lower serum iron levels in rats and a microcytic hypochromic anemia (also seen in subchronic and chronic studies), which is characterized by reduced blood haemoglobin (HGB), reduced mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). The correlation of anemia and reduced iron levels was clearly shown in a mechanistic study, where a reduction in serum iron levels was accompanied by changes in hematological parameters indicative for anemia (see KCA 5.8.2/5; BASF DocID 2002/1005354).

Anemia was seen at the same dietary concentrations in pups and parental animals. However, the individual hematology parameters were more pronounced in the PND 21 pups.
This is not considered to be indicative for a higher susceptibility in the pups, because

- the milk of anemic dams contains less iron compared to control dams,
- pups are lacking body iron stores, and
- pups were exposed to higher dimoxystrobin doses (see above; estimated pup intake is 260 mg/kg bw, while parental females consume 170.5 mg/kg bw during lactation; see Table 3.10.1.6-1).

Milk is generally a poor source of iron, milk of dams suffering from iron deficiency anemia contains less iron than usual (e.g. 34 µg/g dry wt in controls vs. 22 µg/g dry wt in treated dams; Roth and Smith, 1988; BASF DocID 1988/1003436), so that these dams are even less able to transfer sufficient iron to the young via the milk in the early postpartum period (Anaokar and Garry, 1981; BASF DocID 1981/1001521).
Compared to adult animals, that can store excessive amounts of iron in tissues in either two forms, ferritin or hemosiderin, which can be mobilized in an iron deficient state, pups have physiologically only very small iron stores. Therefore, the body iron stores of nursing pups can easily be depleted since their blood volume expands to accommodate the increasing body size (Roth and Smith, 1988; BASF DocID 1988/1003436).

The iron-deficiency anemia is occurring in dams and offspring of the reproduction toxicity studies at the same dose levels (≥ 150 ppm). The susceptibility of the pups to develop an anemia is not higher compared to the adults, as the iron deficiency is the first event to occur after dimoxystrobin treatment and calculations show, that the maternal and offspring BMDL values are comparable.

7. Pathological findings

Organ weights

The mean terminal body weight was significantly decreased in males of mid and high dose groups showing dose response relationship. This was regarded as treatment-related. In females of the high dose group, the mean terminal body weight was slightly decreased (-4.7%), however, this was not significant. In contrast, in females of the low dose group, the mean terminal body weight was slightly although significantly increased. This was, however, regarded as unrelated to treatment.

In males of the mid and high dose groups, the mean weight of heart was slightly although significantly decreased. This was regarded to be related to the decreased mean terminal body weight [see Table 3.10.1.6-8].

In females of the high dose group, the mean liver weight was slightly although significantly increased. In females of the low dose group, the mean weights of liver and spleen were significantly increased. This was regarded to be related to the increased mean terminal body weight rather than to treatment [see Table 3.10.1.6-9].

The other mean absolute weight parameters did not show significant differences when compared with the control group.

Due to the significantly decreased mean terminal body weight, the mean relative weights of liver and heart (males, high dose group) were significantly increased. This was not considered treatment-related.

In females of the high dose group, the mean relative weights of liver and heart were also significantly increased. Although the decrease of the mean terminal body weight in this group was not significant, the increased mean weights of both organs are interpreted to be related to the decreased mean terminal body weight rather than to a treatment-related effect. The other mean relative weight parameters did not show significant differences when compared with the control group.

A comparison of the cardiac effects in parental animals and pups can be found in Table 3.10.1.6-5.

Table 3.10.1.6-8: Absolute and relative organ weights of F1 males (Mean ± SD[#])

Dose group		0	150	500	1200
Terminal bw [g]	absolute	366 ± 27	362 ± 30	**325 ± 26****	299 ± 19
	relative	-	-	-	-
Liver [g]	absolute	9.03 ± 1.02	8.63 ± 0.85	8.25 ± 0.73	8.45 ± 0.39
	relative	2.47 ± 0.18	2.38 ± 0.10	2.54 ± 0.11	**2.83 ± 0.18****
Heart [g]	absolute	1.07 ± 0.10	1.04 ± 0.08	**0.96 ± 0.08***	**0.96 ± 0.08***
	relative	0.292 ± 0.017	0.288 ± 0.019	0.295 ± 0.010	**0.323 ± 0.028****

#: numbers were rounded and thus may not exactly reflect the numbers given in the study report

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

Table 3.10.1.6-9: Absolute and relative organ weights of F1 females (Mean ± SD[#])

Dose group		0	150	500	1200
Terminal bw [g]	absolute	202.8 ± 17.4	224.5 ± 21.0	205.0 ± 17.4	193.2 ± 13.4
	relative	-	-	-	-
Liver [g]	absolute	5.98 ± 1.36	**6.98 ± 0.74***	6.76 ± 1.48	**7.16 ± 1.30***
	relative	2.94 ± 0.56	3.13 ± 0.40	3.29 ± 0.59	**3.69 ± 0.49****
Heart [g]	absolute	0.816 ± 0.120	0.868 ± 0.066	0.874 ± 0.094	0.872 ± 0.043
	relative	0.402 ± 0.043	0.389 ± 0.037	0.427 ± 0.042	**0.452 ± 0.022***

#: numbers were rounded and thus may not exactly reflect the numbers given in the study report
* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))

Gross lesions

Thickening of the wall of the duodenum was noted in one male of the high dose group. This adaptive observation was regarded to be treatment-related. A few other gross lesions were noted in the glandular stomach (erosion ulcer in each one female of all groups), mammary gland (mass in a low dose female), and axillary and iliac lymph nodes (enlarged in a low dose group female). They were all regarded incidental and unrelated to treatment.

Histopathology

Histopathology was not performed.

Conclusion

The administration of 150, 500 and 1200 ppm of the test compound to male and female rats in this modified one-generation reproduction toxicity study caused a dose-dependent regenerative microcytic hypochromic anemia in the parental animals and the pups. The anemia was characterized by reduced haemoglobin concentrations and mean corpuscular indices as well as increased microcytosis and reticulocytes.

The **NOAEL for reproduction** is **1200 ppm (170.5 mg/kg bw/day),** the highest dose tested.

The **NOAEL for parental toxicity is 150 ppm (about 17 mg/kg bw/day**), as the slight anemia, which was observed at 150 ppm in maternal animals, is regarded treatment-related but not adverse.

The **NOAEL for offspring** toxicity

The apparently more severe effects on hematological parameter in pups compared to dams can be related to a considerably higher compound intake (260 mg/kg bw in pups vs 170.5 mg/kg bw in parental females) at the same dietary dose levels.

3.10.1.7 Study 7

Multigenerational studies, rat

Study reference:

Anonymous (2011): Enhanced one-generation reproduction toxicity study in Wistar rats. Administration via the diet. BASF SE, Ludwigshafen, Germany; BASF DocID 2011/1211676; August 2011

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp 166 - 180:

Report:	KCA 5.6.1/1 Anonymous Enhanced one-generation reproduction toxicity study in Wistar rats. Administration via the diet 2011/1211676
Guidelines:	OPPTS 870.3800 OECD 416
GLP:	yes
Acceptability:	yes

Executive Summary

In an enhanced one generation toxicity study, dimoxystrobin (BAS 505 F; Batch: OP-No. 13; Purity 98.5%) was administered in the diet to groups of 25 male and 25 female Crl:WI(Han) Wistar rats at nominal dose levels of 0, 10, 20 and 50 ppm. The dietary concentrations of dimoxystrobin were adjusted to 0, 5, 10, 25 ppm during lactation, thereby maintaining constant dose-levels during this period of increased food intake. The overall mean dose of dimoxystrobin administered to the male Wistar rats during the entire study period was approx. 0.8 mg/kg bw/day in the 10 ppm group, approx. 1.6 mg/kg bw/day in the 20 ppm group and approx. 4.0 mg/kg bw/day in the 50 ppm group. For the females mean doses of 0.9, 1.8, and 4.5 mg/kg bw/day were administered to groups 1, 2, and 3, respectively, during the premating period. During gestation and lactation mean doses of 0.8/0.7, 1.6/1.5, and 3.8/3.8 were administered to the test groups 1, 2, and 3, respectively.

No treatment-related mortality was observed throughout the study. No clinical signs or changes of general behavior, which may be attributed to the test substance, were detected in any of the male and female parental animals. Body weight development and food consumption was sporadically different to the control group, but without biological relevance.

Overall, male and female fertility indices ranged between 96 and 100% without relation to dose. Dimoxystrobin treatment did not affect the reproductive performance as was evident from the absence of effects on the pre-coital interval or gestation lengths as well as gestation (100%) or live birth indices (98 to 100%). The observed numerical differences displayed no dose-response relationship and were thus not indicative of a relation to treatment.

Survival of pups was not affected by treatment as viability indices in the range of 97 to 99% without dose relation were observed. Lactation indices indicating pup mortality between PND 4-21 varied between 49% and 55%. The lowered lactation index was caused by the fact that selected pups were sacrificed for blood sampling on PND 7, 14, and 21.
No treatment-related, adverse changes among hematological parameters as well as transferrin and iron levels were detected in the F_0 generation or in the F_1 generation on day 7, 14, and 21.
Under the conditions of the present enhanced one-generation reproduction toxicity study the NOAEL for the F_0 parental rats for general, systemic toxicity is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.
The NOEL for fertility and reproductive performance for the F_0 parental rats is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.
The NOEL for developmental toxicity in the F_1 progeny is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 505 F (dimoxystrobin)

Description:	solid / beige
Lot/Batch #:	OP-No. 13
Purity:	98.5% (tolerance +- 1.0%)
Stability of test compound:	The test substance was stable over the study period; (Expiry date: 01.11.2015)

2. Vehicle and/or positive control: rodent diet

3. Test animals:

Species:	Rat, Wistar
Strain:	Crl:WI(Han)
Sex:	Male and female
Age:	F_0 parental animals: 28 ± 1 days at delivery; 36 ± 1 days at beginning of treatment
Weight at dosing:	♂: 127.6 g- 159.8 g, ♀: 95.6 g – 124.0 g
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH

B. STUDY DESIGN AND METHODS

2. Animal assignment and treatment:

Dimoxystrobin was administered in the diet to groups of 25 male and 25 female rats at nominal dose levels of 0, 10 (low dose), 20 (mid dose), and 50 ppm (high dose). The dietary concentrations of dimoxystrobin were adjusted to 0, 5, 10, 25 ppm during lactation, thereby maintaining constant dose-levels during this period of increased food intake.

Females were allowed to deliver and rear their pups (F_1 generation pups) until day PND 4 (standardization; see below) or day 21 after parturition. After weaning of F_1 pups the F_0 generation parental animals were sacrificed.

Standardization of litters: 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.

4. Statistics:

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

Statistics of clinical examinations

Parameter	Statistical test
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, postimplantation loss and % postimplantation 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 with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, number of litters with affected pups at necropsy	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test for the hypothesis of equal proportions
Proportions of affected pups per litter with necropsy observations	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-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

1. 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 behavior 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 behavior 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.

2. Body weight:

Body weight of **parental animals** was determined on the first day of the premating period and weekly thereafter at the same time of the day. The following exceptions are notable for female parental animals:

a. The F_0 generation parental females were weighed on the day of positive evidence of sperm (GD 0) and on GD 7, 14, and 20.
b. Females were not weighed during mating until there was a positive evidence of sperm in vaginal smears
c. Females with litter were weighed on the day after parturition (PND 1) and on PND 4, 7, 14, and 21.
d. Females without litter were not weighed during the lactation phase.

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

3. Food consumption, food efficiency and compound intake:

Food consumption was determined once a week (for a period of 6 days) for parental animals and calculated as mean food consumption in grams per animal and day. The following exceptions are notable for female parental animals:

a. Food consumption of females during pregnancy was determined weekly for GD 0-7, 7-14, and 14-20
b. During the lactation period food consumption of the females was determined for PND 1 - 4, 4 - 7, 7 - 14, and 14 - 21.
c. 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/d) was calculated based upon individual values for body weight and food consumption:

4. Ophtalmoscopy:
Not performed in this study

5. Hematology and clinical chemistry:

Blood was withdrawn from the adult animals from the retro-orbital venous plexus, and from pups after decapitation, following isoflurane anesthesia.
Blood samples were withdrawn from 12 adult F_0 animals at sacrifice and from 1 pup per sex from each litter on PND 7, 14 and 21. The following hematological and clinical chemistry parameters were determined:

Clinical chemistry:					
	Electrolytes				
✓	Iron				
✓	Transferrin				
Hematology:					
✓	Leukocytes	✓	Reticulocytes	✓	Blood smears
✓	Erythrocytes	✓	Differential blood count		
✓	Hemoglobin	✓	Mean corpuscular volume		
✓	Hematocrit (calculation)	✓	Mean corpuscular hemoglobin (calculation)		
✓	Platelets	✓	Mean corp. hemoglobin conc. (calculation		

6. Male reproduction data

For the males, mating and fertility indices were calculated for F_1 litters.

7. Female reproduction and delivery data

For the females, mating, fertility and gestation indices were calculated for F_1 litters.

The total number of pups delivered and the number of liveborn and stillborn pups were noted, and the live birth index was calculated for F_1 litters:

The implantations were counted and the postimplantation loss (in %) was calculated.

8. Litter data

All F_1 pups were examined on the day of birth to determine the total number of pups and the number of liveborn and stillborn members of each litter. Pups, which died before the first examination on the day of birth, were designated as stillborn pups.

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.

On the day of birth (PND 0) the sex of F_1 pups was determined by determination of the anogenital distance. Subsequently the sex of the pups was assessed by the external appearance of the anogenital region and/or the mammary line. The sex of the animals was finally confirmed at necropsy. The sex ratio was calculated at PND 0 and PND 21 after birth.

9. Sacrifice and pathology:

All F_0 parental animals were sacrificed, necropsied and assessed by gross pathology with special attention given to the reproductive organs.

On PND 4, as a result of standardization, the surplus pups were sacrificed, examined externally, eviscerated and their organs were assessed macroscopically.

On PND 7, 14 and 21, all F1 generation pups used for blood sampling were sacrificed. The surplus pups on PND 21 were sacrificed, examined externally, eviscerated and their organs were assessed macroscopically.

Animals with notable findings or abnormalities were further evaluated on a case-by-case basis, depending on the findings noted.

II. RESULTS AND DISCUSSION

1. Clinical signs of toxicity

No treatment related clinical observations, which may be attributed to the test substance, were detected in any of the male **F_0** parental animals. Clinical observation of **F_0** male animals revealed a skin lesion at the throat in one high dose male (#83). This finding is considered to be spontaneous in nature.
There were no test substance-related clinical findings in all **F_0** females of all dose groups during premating, gestation and lactation periods for F1 litter. One low-dose **F_0** dam (#127) did not nurse its pups properly. Therefore it had no more pups alive on PND 1 (most of its pups were stillborn or died on PND 0). This observation is not considered to be treatment-related.

2. Mortality

No treatment-related or spontaneous mortality was observed throughout the study.

C. PARENTAL BODY WEIGHT AND BODY WEIGHT GAIN

Mean body weights and body weight gain of the mid- and high-dose F_0 males (20 and 50 ppm) were comparable to the concurrent control group throughout the study (see Figure 3.10.1.7-1). However, high-dose body weight was slightly, but statistically significantly, reduced in week 6. Furthermore, F_0 males gained either less body weight during study weeks 0-1 and weeks 3-5 (test group 2 and 3) or more body weight during weeks 13-14 (test group 2).
However, low-dose parental males had statistically significantly lower body weights from study week 5 onwards until the end of the study in week 15. Their body weight gain was statistically significantly decreased during weeks 0-5. During the entire study, the low-dose males gained about 8% less weight than in controls.
All statistical significant differences noted during isolated periods between the substance treated groups and the concurrent control are without biological relevance and considered not treatment-related due to the lack of dose-response relationship.
Neither mean body weights nor mean body weight gain of the F_0 parental females in all dose groups were influenced by the test substance during premating, gestation and lactation periods. The statistically significantly increased body weight gain during GD 0-7 (test group 1 and 2) and during PND 7-14 (test group 2) was considered as spontaneou.

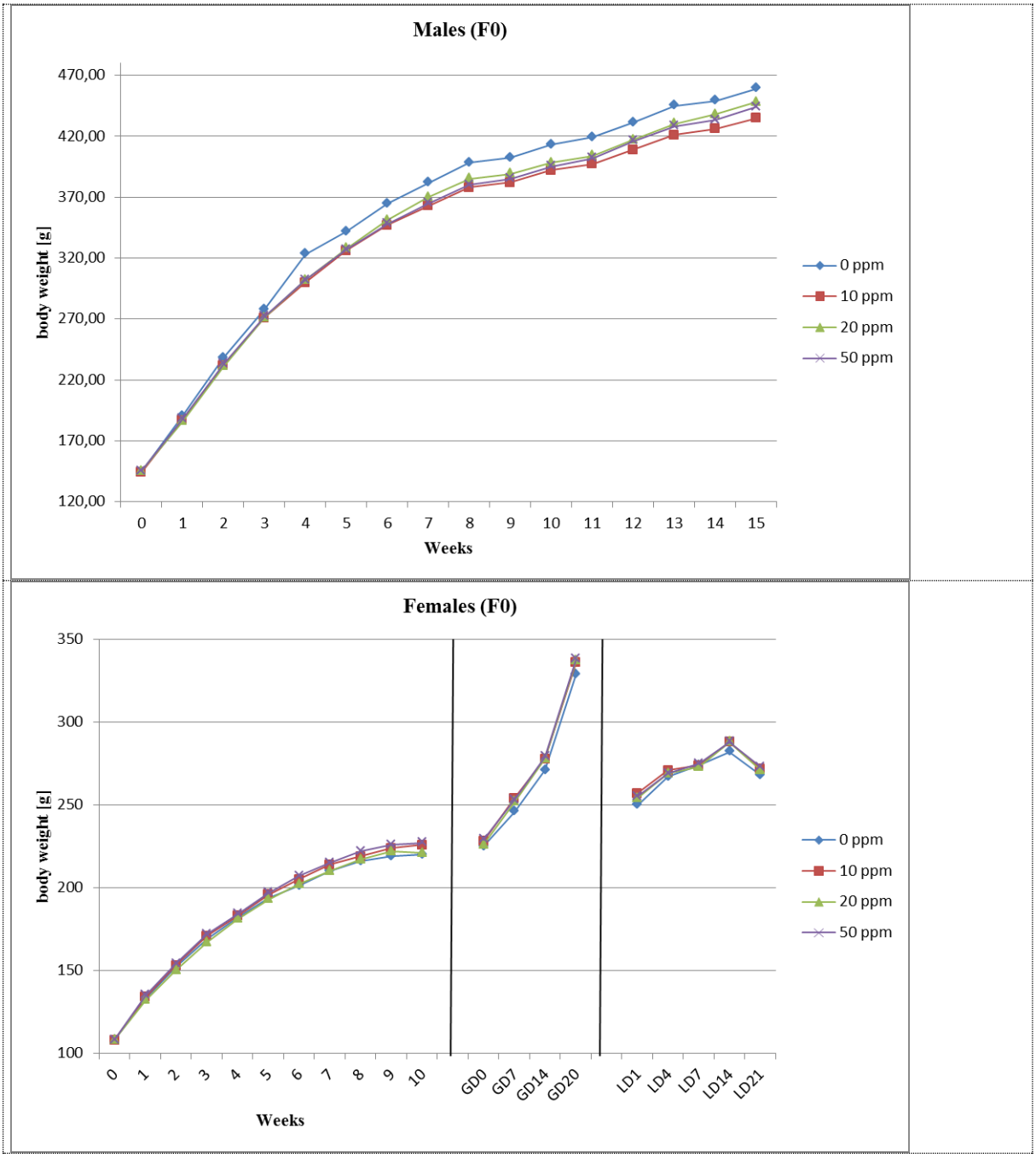


Figure 3.10.1.7-1: Body weight development of parental F_0 animals

D. PARENTAL FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption of the F_0 male and female animals in all test groups (10, 20, and 50 ppm) was generally comparable to the concurrent control group during the entire study, covering premating, gestation and lactation periods (see Figure 3.10.1.7-2 and Figure 3.10.1.7-3).

This includes the sporadically significant food consumption values during the different study periods:

Food consumption of the low-dose F_0 males was decreased during premating weeks 4-6. Food consumption of the F_0 females was increased during premating weeks 5-7 and 8-10 in test group 1 (10 ppm) and during week 8-9 in test group 2 (20 ppm). Furthermore, it was increased in test group 1 and 3 calculated for GD 0-7 and in test group 2 for GD 0-14. During lactation the food consumption of the mid-dose F_0 females was increased if calculated for PND 7-14.

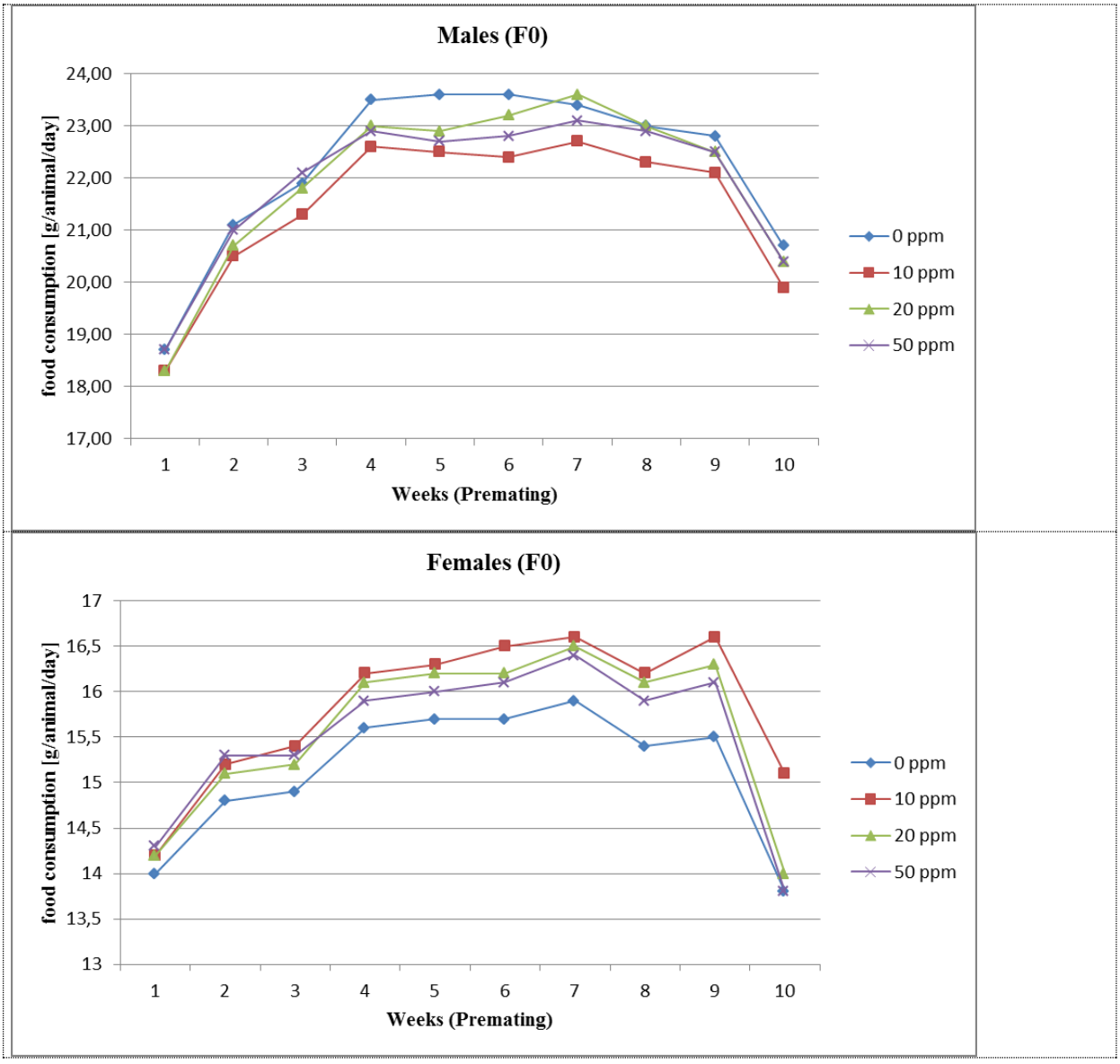


Figure 3.10.1.7-2: Food consumption of parental F_0 animals during premating

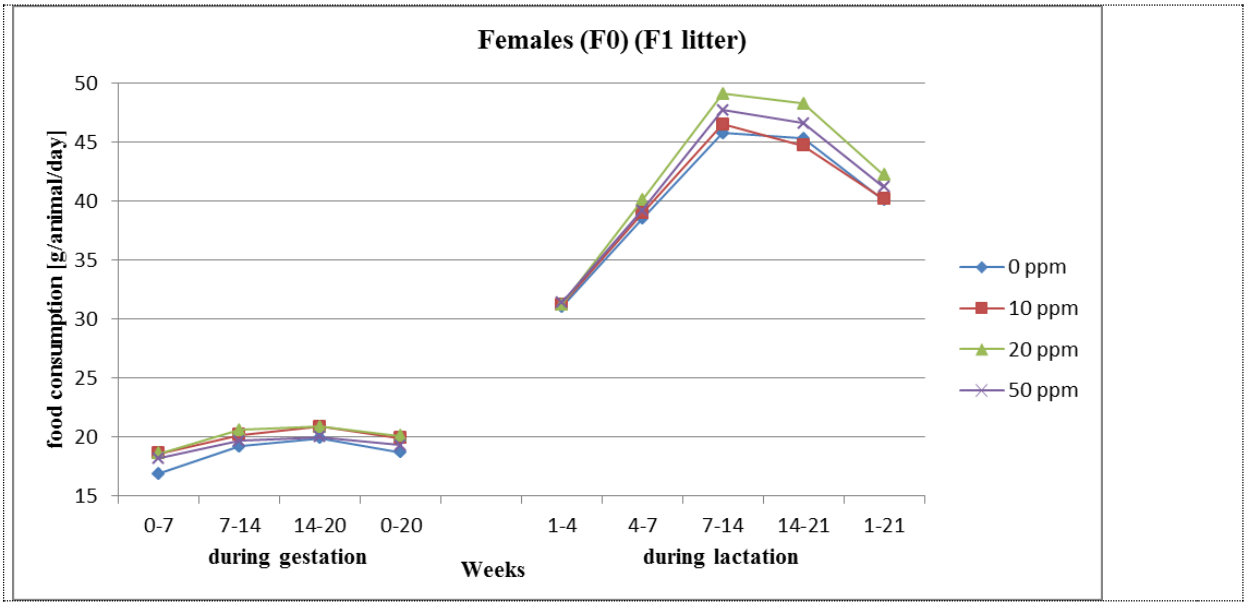


Figure 3.10.1.7-3: Food consumption of parental F_0 animals during gestation and lactation

For all test groups the intake of dimoxystrobin 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 3.10.1.7-1.

Table 3.10.1.7-1: Average dimoxystrobin intake (mg/kg bw/d) in parental animals

Dose	10 ppm		20 ppm		50 ppm	
Group & sex	average	min/max	average	min/max	average	min/max
F_0 males	0.8	0.5 / 1.3	1.6	1.0 / 2.5	4.0	2.6 / 6.5
F_0 females (premating)	0.9	0.7 / 1.3	1.8	1.3 / 2.6	4.5	3.1 / 6.6
F_0 females (F1 litter)						
- gestation period	0.8	0.8 / 0.8	1.6	1.5 / 1.7	3.8	3.6 / 4.0
- lactation period	0.7	0.6 / 0.8	1.5	1.2 / 1.8	3.8	3.1 / 4.3

E. MATING AND GESTATION DATA

The following table summarizes the reproduction and gestational parameters of female F0 rats treated with dimoxystrobin in the enhanced one-generation toxicity study

Table: Female reproduction and delivery data

Parental generation					
Dose	[ppm]	0	10/5[a)]	20/10 [a)]	50/25 [a)]
Animals per dose		25	25	25	25
Female fertility					
- placed with males					
- mated	[n]	25	25	25	25
- mating index	[%]	100	100	100	100
- pregnant	[n]	25	24	25	25
- **Fertility index**	[%]	100	96	100	100
Pre coital interval	[days]	2.9	2.4	3.0	2.5
Duration of gestation	[days]	22.0	22.0	22.0	22.0
Implantation sites, total	[n]	323	323	331	331
- dto per dam	[n]	12.9	13.5	13.2	13.2
Post implantation loss	[n]	17	23	13	16
- dto per dam	[n]	0.7	1.0	0.5	0.6
- dto per litter	[mean %]	5.0	7.3	4.2	5.3
Females with liveborn		25	24	25	25
- **Gestation index**	[%]	100	100	100	100
- with stillborn pups	[n]	1	2	0	2
- with all stillborn	[n]	0	0	0	0
Pups delivered	[n]	306	300	318	315
- per dam	[mean n]	12.2	12.5	12.7	12.6
- liveborn	[n]	305	294	318	313
- stillborn	[n]	1	6	0	2
- **Live birth index**	[%]	100	98	100	99

[a)]The test concentration in the diet was reduced during lactation to account for the higher feeding rate of the dams

1. Male reproductive performance

For all F_0 parental males, which were placed with the females to generate F_1 pups, copulation was confirmed. Thus, the male mating index was 100% in all groups including the controls.
Fertility was proven for nearly all F_0 parental males within the scheduled mating interval for F_1 litter. One low-dose male (#39) did not generate F_1 pups.

Thus, the male fertility index was 96% in test group 1 and 100% in all remaining groups including the control. 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.

Parental generation				
Dose [ppm]	**0**	**10/5**	**20/10**	**50/25**
Males placed with females	25	25	25	25
Mated [n]	25	25	25	25
Male mating index [%]	100	100	100	100
did not mate [n]	0	0	0	0
with females pregnant [n]	25	24	25	25
Male fertility index [%]	100	96	100	100
without females pregnant [n]	0	1	0	0
without females pregnant [%]	0	4	0	0

2. Female reproductive performance

The female mating index calculated after the mating period for F_1 litter was 100% in all test groups. The mean duration until sperm was detected (GD 0) varied between 2.4 and 3.0 days

without any relation to administered doses. All sperm positive rats delivered pups or had implants in utero with the exception of low-dose female #139 (mated with male #39), that did not become pregnant.
The female fertility index varied between 96% and 100%. All respective values are within the range of historical control data of the test facility and do not show any relation to dosing.
The mean duration of gestation was identical in all test groups: 22 days for control, low-, mid- and high-dose groups. The gestation index was 100% in all test groups. Implantation was not affected by the treatment since the mean number of implantation sites was comparable between all test substance-related groups and the control, taking normal biological variation into account (12.9/13.5/13.2 and 13.2 implants/dam in test groups 0-3.
Furthermore, there were no indications for test substance-induced intrauterine embryo-/fetolethality since the postimplantation loss did not show any statistically significant differences between the groups, and the mean number of F_1 pups delivered per dam remained unaffected (12.2/12.5/12.7 and 12.6 pups/dam at 0, 10, 20 and 50 ppm).
The rate of liveborn pups was also not affected by the test substance, as indicated by live birth indices of 100, 98%, 100, and 99% in the control, 10, 20, and 50 ppm dose groups, respectively. Moreover, the number of stillborn pups was comparable between the groups.

Table 3.10.1.7-2: Summary of female reproduction and delivery data

Parental generation		**F_0**			
Dose	**[ppm]**	**0**	**10/5**	**20/10**	**50/25**
Animals per dose		25	25	25	25
Female fertility					
- placed with males		25	25	25	25
- mated	[n]	25	25	25	25
- mating index	[%]	100	100	100	100
- pregnant	[n]	25	24	25	25
- **Fertility index**	[%]	100	96	100	100
Pre coital interval	[days]	2.9	2.4	3.0	2.5
Duration of gestation	[days]	22.0	22.0	22.0	22.0
Implantation sites, total	[n]	323	323	331	331
- dto per dam	[n]	12.9	13.5	13.2	13.2
Post implantation loss	[n]	17	23	13	16
- dto per dam	[n]	0.7	1.0	0.5	0.6
- dto per litter	[mean %]	5.0	7.3	4.2	5.3
Females with liveborn		25	24	25	25
- **Gestation index**	[%]	100	100	100	100
- with stillborn pups	[n]	1	2	0	2
- with all stillborn	[n]	0	0	0	0
Pups delivered	[n]	306	300	318	315
- per dam	[mean n]	12.2	12.5	12.7	12.6
- liveborn	[n]	305	294	318	313
- stillborn	[n]	1	6	0	2
- **Live birth index**	[%]	100	98	100	99

F. PUP DATA

1. Survival

The mean number of delivered F_1 pups per dam and the rates of liveborn and stillborn F_1 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% (control), 98% (group 1), and 99% (groups 2 and 3).
The lactation index indicating pup mortality on PND 4-21 varied between 49% (group 1), 50% (group 3), 54% (control) and 55% (group 2). The lowered lactation index in all test groups is caused by the fact, that selected pups were sacrificed for blood sampling on PND 7, 14 and 21.
Thus, the test substance did not influence pre-weaning pup survival in any of the treated groups.

Table 3.10.1.7-3: Summary of litter data

Pup generation		F_1			
Dose	[ppm]	**0**	**10/5**	**20/10**	**50/25**
Number of litters		25	24	25	25
- with liveborn pups		25	24	25	25
- with stillborn pups		1	2	0	2
Pups liveborn	[n]	305	294	318	313
Pups stillborn [a]	[n]	1	6	0	2
Pups died	[n]	5	6	3	1
Pups cannibalized	[n]	4	1	1	4
Pups culled day 4	[n]	98	105	114	110
Pups day 4 - pre-cull	[n]	296	287	314	309
- Viability index	[%]	97	98	99	99
Pups day 4 - post cull	[n]	198	182	200	199
Pups day 21	[n]	106	90	109	99
- Lactation index	[%	54	49	55	50
Sex ratio	[% live males]				
- Day 0		48.9	54.8	45.9	48.6
- Day 21		48.1	56.7	45.0	44.4
Male pup weight	[g]				
- Day 1	[g]	6.4	6.3	6.5	6.5
- Day 4 - pre cull	[g]	9.6	9.8	10.0	10.0
- Day 4 - post cull	[g]	9.6	9.8	10.0	10.1
- Day 7	[g]	15.7	16.0	16.3	16.5
- Day 14	[g]	35.1	36.1	36.2	36.7
- Day 21	[g]	58.3	59.5	58.9	60.1
Male body weight gain					
- Day 4 to 21	[g]	48.6	49.8	48.9	50.3
Female pup weight	[g]				
- Day 1	[g]	6.1	6.1	6.1	6.2
- Day 4 - pre cull	[g]	9.3	9.6	9.5	9.6
- Day 4 - post cull	[g]	9.3	9.6	9.6	9.6
- Day 7	[g]	15.2	15.7	15.7	15.7
- Day 14	[g]	34.2	35.4	35.2	35.6
- Day 21	[g]	55.7	56.8	56.3	57.0
Female body weight gain					
- Day 4 to 21	[g]	46.4	47.3	46.7	47.4

2. Sex ratio

The sex ratios at day 0 and 21 were not affected by treatment. All differences were within the historical control range and not indicative of a treatment-related effect [see Table 3.10.1.7-3]

3. Pup clinical observations /

The F_1 generation pups did not display any clinical signs until weaning.

4. Body weight

No test compound-related influence on F_1 pup body weights and pup body weight changes was noted for all test groups.

5. Pup necropsy findings

A few F_1 pups showed spontaneous findings at necropsy, such as post mortem autolysis, hemorrhagic thymus, dilated renal pelvis, hydroureter, hemorrhagic testis and small testis. These findings occurred without relation to dosing and/or can be found in the historical control data at comparable or even higher incidences. None of the findings was considered to be related to the treatment.

Table 3.10.1.7-4: Incidence of gross necropsy observations in F_1 pups

Dose [ppm]	0	10/5	20/10	50/25
	F_1 pups			
Litters evaluated	25	24	25	25
Pups evaluated	302	299	317	311
- Live	301	293	317	309
- Stillborn	1	3	0	2
Post mortem autolysis	0	1	0	1
Hemorrhagic thymus	0	1	0	0
Dilated renal pelvis	6	5	4	13
Hydrouereter	1	0	0	0
Hemorrhagic testis	1	0	0	0
Small testis	1	0	1	0
Total pup necropsy observations - % affected pups/litter	2.7	2.6	1.5	4.5

G. CLINICAL CHEMISTRY AND HEMATOLOGY

F_0 generation (adults)
No treatment-related, adverse changes among hematological parameters as well as transferrin and iron levels were measured.
In males of test group 50 ppm absolute lymphocyte counts and also total white blood cell counts were higher compared to controls (lymphocytes represent the main fraction of white blood cells). This finding was isolated among the differential blood cell counts. No other hematological parameter was changed in these rats. Therefore, high lymphocyte counts in rats of this test groups were regarded as incidental.

In female rats of test groups 10 ppm and 50 ppm absolute neutrophil counts were increased, and female high dose group additionally had high absolute eosinophil counts, without any significant alteration of total white blood cell counts in these animals. The neutrophil counts were not dose-dependently changed and the means were within the historical control range and the absolute eosinophil counts were only marginally above the historical range. Therefore, these alterations were regarded as incidental and not treatment-related.

F_1 pups 7 days pp
No treatment-related changes among hematological parameters as well as transferrin and iron levels were measured.

F_1 pups 14 days pp
No treatment-related changes among hematological parameters as well as transferrin and iron levels were measured.
In female pups of test groups 1 and 3 (10 and 50 ppm) relative lymphocyte counts were decreased and relative neutrophil counts were increased. Additionally, in pups of test group 3 absolute neutrophil counts were higher compared to controls. The mentioned differential blood cell counts were not changed dose-dependently. Total white blood cell (WBC) counts in these pups were not affected. No change in the hematology parameters in pups 7 days pp as well as 21 days pp was measured. Therefore, the described alterations were regarded as incidental and not treatment-related.
At day 14 pp, in all dosed male pups serum transferrin values were higher compared to controls. The values were not dose-dependently altered and they were not changed in pups at day 7 pp and 21 pp. Therefore, this change was regarded as incidental and not treatment-related.

F_1 pups 21 days pp
No treatment-related changes among hematological parameters as well as transferrin and iron levels were measured.

III. CONCLUSIONS

Under the conditions of the present enhanced one-generation reproduction toxicity study the NOAEL for the F0 parental rats for general, systemic toxicity is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.
The NOEL for fertility and reproductive performance for the F0 parental rats is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.
The NOEL for developmental toxicity in the F_1 progeny is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.

3.10.1.8 Study 8

Developmental toxicity studies, rat

Study reference:

Anonymous (1999): Prenatal developmental toxicity study in Wistar rats. Oral administration (gavage). BASF AG, Ludwigshafen, Germany; BASF DocID 1999/11680; November 1999

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp 181 - 191:

Report:	KIIA 5.6.2.1/1 Anonymous 1999 Prenatal developmental toxicity study in Wistar rats. Oral administration (gavage) 1999/11680
Guidelines:	OPPTS 870.3700, OECD 414 (2001) Japan MAFF guidelines
GLP:	yes
Previous evaluation:	in the DAR (2003)
Acceptability:	yes

Executive summary:

Dimoxystrobin was administered by oral gavage to groups of 25 presumably pregnant rats at dose levels of 0, 60, 120 and 300 mg/kg bw/day during days 6 to 19 of gestation. Maternal toxicity was observed at dose levels ≥ 120 mg/kg as evident by reduced food consumption, body weights and body weight gain during certain intervals of the treatment period. Additionally, reduced corrected body weight gains were noted.

There were no treatment-related effects on cesarean section parameters. There were no external malformations/variations or visceral malformations. A low number of skeletal malformations was observed in the low and high dose groups. Neither the incidence nor the type or inter-group distribution of malformations indicated a relation to treatment. Examination of fetuses for visceral and skeletal variations revealed no statistically significant differences between treated groups and the control group. Furthermore, the incidences either displayed no dose-response relationship or were within the historical control range.

Based on the findings of this study the maternal NOAEL was determined at 60 mg/kg bw/day. The NOAEL for developmental toxicity was the highest dose tested (300 mg/kg bw/day).

Materials and Methods:

Dimoxystrobin (batch/purity: N 6 Lot 3004: 98.8%) was tested for its prenatal developmental toxicity in Wistar rats. The test substance was administered as an aqueous suspension to 25 mated female rats/group by stomach tube at doses of 0, 60, 120 and 300 mg/kg bw/day on day 6 through day 19 post coitum (day 0 = detection of sperm). A standard dose volume of 10 ml/kg body weight was used for each group. The control group was dosed with the vehicle only (0.5 % Tylose CB 30.000 in doubly distilled water).

Results:

1. Clinical signs of toxicity

No treatment-related clinical signs nor any disturbance of the general behavior were observed throughout the study.

One mid dose dam and a high dose dam showed apathy and lateral position prior to death respectively sacrifice in moribund state.

2. Mortality

No substance-related mortalities were observed in this study.

2. Food consumption

The mean food consumption of the high dose (300 mg/kg) dams was statistically significantly reduced on several days of treatment (Day 6-8 and 10-19 p.c.); if calculated for the entire treatment period (Day 6-19 p.c.) these dams consumed about 8% less food than the concurrent control.

Food consumption in the mid dose group (120 mg/kg) was statistically significantly reduced at initiation of dosing (Day 6-8 p.c.); no relevant deviation if calculated for the entire treatment period.

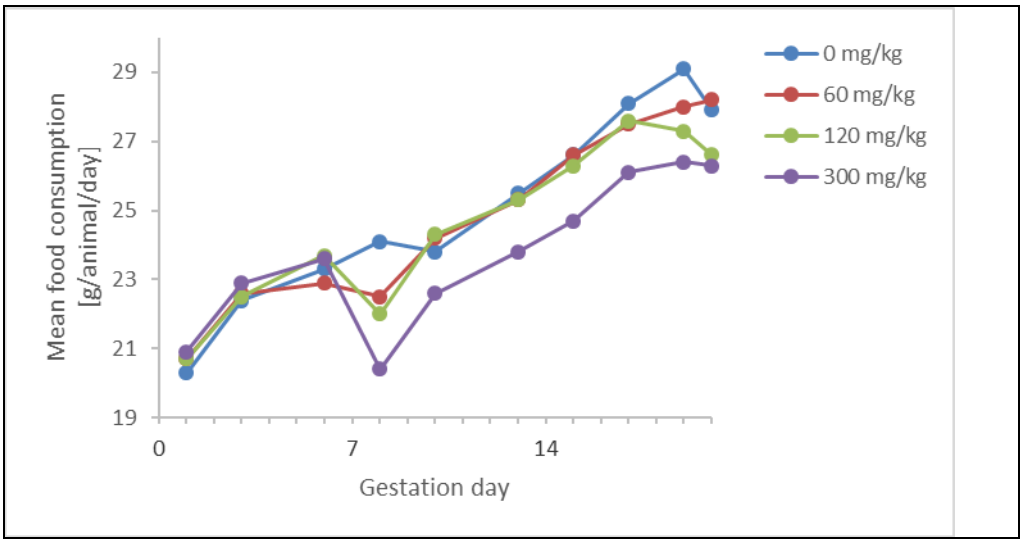
These changes were considered treatment-related as they were corroborated by a treatment-related impairment of body weight gain [see Table 3.10.1.8-1 and Table 3.10.1.8-2, and Figure 3.10.1.8-1].

Food consumption of low dose dams did not show any difference in food consumption compared to concurrent controls [see Table 3.10.1.8-1].

3. Body weight and body weight gain

No statistically significant differences of absolute body weights were noted for the substance-treated groups during the administration period [see Table 3.10.1.8-1]. A statistically significant decrease of body weight gain was observed at the high dose group immediately following commencement of treatment (Day 6-8). If calculated for the entire treatment period body weight gains were about 10% lower compared to concurrent control. A statistically significant decrease of body weight gain of about 12% was observed at the mid dose over the whole treatment period (Day 6-19). These changes in the mid and high dose group were considered to be treatment-related as they were consistent with the effects on food consumption and the lower corrected body weight gain at this dose level.

Body weight gains of the females of the low dose groups were similar to those of the concurrent control.



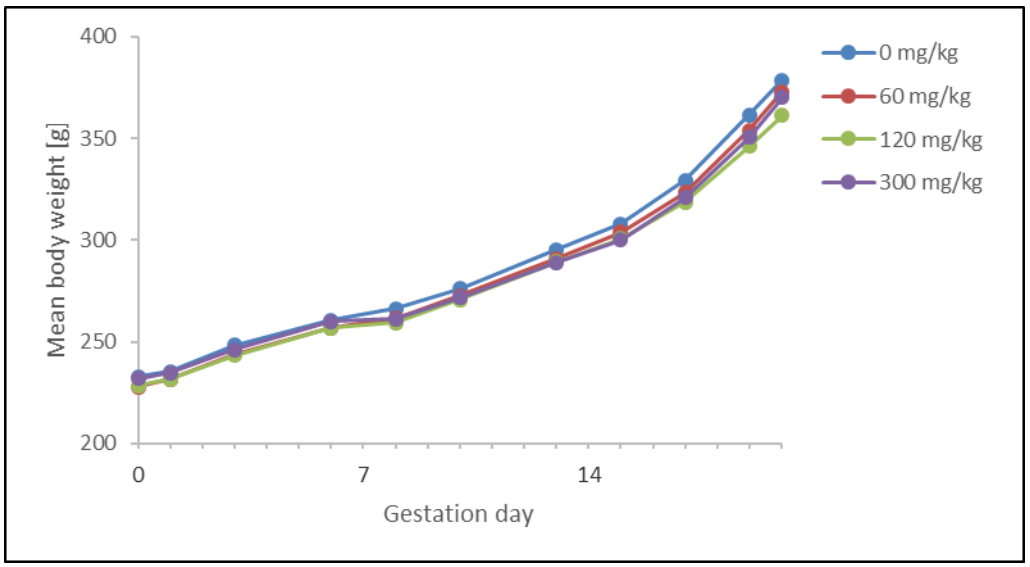


Figure 3.10.1.8-1: Food consumption and body weight development in rats administered dimoxystrobin during days 6 to 19 of gestation

Table 3.10.1.8-1: Food consumption and body weight development in rat administered dimoxystrobin during days 6 to 19 of gestation

Dose level [mg/kg bw/day]	0	60	120	300
Food consumption [g/animal/day]				
Day 0 to 6 □%	22.0 ± 1.6	22.1 ± 1.2 0.5	22.3 ± 1.5 1.4	22.5 ± 1.4 2.3
Day 6 to 19 □%	26.2 ± 2.1	25.7 ± 2.1 -1.9	25.5 ± 2.1 -2.7	24.0 ± 2.3 -8.4
Day 0 to 20 □%	25.1 ± 2.8	24.9 ± 2.6 -0.8	24.6 ± 2.4 -2.0	23.8 ± 2.1 -5.2
Body weight [g]				
Day 0 □%	233.0 ± 8.1	227.8 ± 9.1 -2.2	228.4 ± 7.8 -2.0	231.9 ± 8.1 -0.5
Day 6 □%	260.6 ± 10.2	257.0 ± 9.6 -1.4	256.9 ± 12.2 -1.4	260.1 ± 9.7 -0.2
Day 19 □%	361.7 ± 20.1	354.2 ± 18.4 -2.1	346.1 ± 27.9 -4.3	350.7 ± 22.1 -3.0
Day 20 □%	378.6 ± 22.9	373.0 ± 21.7 -1.5	361.2 ± 32.9 -4.6	370.0 ± 23.1 -2.3
Body weight gain [g]				
Day 0 to 6 □%	27.6 ± 6.2	29.2 ± 6.6 5.8	28.5 ± 7.3 3.3	28.2 ± 5.1 2.2
Day 6 to 19 □%	101.1 ± 13.5	97.2 ± 12.8 -3.9	**89.2** ± **21.4*** -11.8	90.7 ± 16.2 -10.3
Day 0 to 20 □%	145.6 ± 21.4	145.2 ± 18.8 -0.3	132.8 ± 29.6 -8.8	138.1 ± 19.8 -5.2

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

4 Necropsy observations

Gravid uterus weight, carcass weight and corrected (net) body weight gain
Gravid uterus weights were comparable between control and treated groups. The differences between the groups did not reveal dose-dependency. A treatment-related statistically significant decrease of net body weight gain was observed at 300 mg/kg. Furthermore, the corrected body weight gain at 120 mg/kg dose group was slightly decreased without statistical significance. The effects were considered to be treatment-related direct signs of maternal toxicity as they were in line with the decrease in food consumption and body weight gain. The carcass weight of the high dose group was slightly but not statistically significantly reduced [see Table 3.10.1.8-2].

No treatment related effects were noted at the low dose of 60 mg/kg.

Table 3.10.1.8-2: Mean gravid uterus weights and net body weight change of pregnant rats administered dimoxystrobin during Days 6 to 19 of gestation

Dose level [mg/kg bw/d]	**0**	**60**	**120**	**300**
Gravid uterus (g)	81.1 ± 14.5	78.9 ± 12.4	70.0 ± 24.6	83.0 ± 9.8
Carcass (g)	297.4 ± 15.0	294.0 ± 15.7	291.2 ± 17 8	286.9 ± 15.7
Net weight change from Day 6 (g)	36.8 ± 7.8	37.0 ± 9.3	34.3 ± 9.0	**26.9 ± 10.8****

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

4.2. Gross necropsy observations
No treatment-related findings were observed. All findings were observed in single animals only without any relation to treatment. Edema and/or marginal emphysema of the lung were associated with the method of the females sacrifice. Findings like perforation of upper oesophagus and/or acute fibrinous pleuritic, pericarditis and exsudate in the thoracic cavity were typical for misgavaging [see Table 3.10.1.8-3].

Table 3.10.1.8-3: Gross necropsy findings in rats administered dimoxystrobin during days 6 to 19 of gestation

Dose group	Animal #	Observation
Control	-	-
60 mg/kg bw/day	-	-
120 mg/kg bw/day	67	Lung: edema/marginal emphysema
	54	Bilateral hydrometra
	75	Findings after gavage error
300 mg/kg bw/day	82	Lung: Edema
	89	Findings after gavage error

4.3 Cesaren section data
25, 24, 24 and 25 females were pregnant at 0, 60, 120 and 300 mg/kg [see Table 3.10.1.8-4]. None of the pregnant dams aborted, or gave premature birth. One mid dose dam had all fetuses resorbed. This was considered incidental.

There were no treatment-related differences in the number of corpora lutea, implantation sites, pre- and post-implantation losses, resorptions, number of live fetuses between controls and treated groups [see Table 3.10.1.8-4]. The statistically significant higher post-implantation loss at the mid dose level (120 mg/kg) was due to the higher rate of early resorptions. However, this was mainly caused by one female #72, which had only six early resorptions but no viable fetuses. Due to the isolated occurrence and the lack of dose-dependency this finding was considered incidental. Placental and fetal weights were comparable between all groups and not affected by treatment [see Table B.6.6.2-4]

Table 3.10.1.8-4: Caesarean section data

Dose level [mg/kg bw/d]		**0**	**60**	**120**	**300**
Pregnancy status					
- mated	[n]	25	25	25	25
- pregnant	[n]	25	24	24	24
conception rate	[%]	100	96	96	96
- aborted	[n]	0	0	0	0
- premature birth	[n]	0	0	0	0
- dams with viable fetuses	[n]	25	24	22	24
- dams with all resorptions	[n]	0	0	1	0
- mortality		0	0	0	0
- pregnant terminal sacrifice	[n]	25	24	23	24
Cesarean section data[a]					
- Corpora lutea	[n]	15.8	15.5	15.0	15.3
total number	[n]	394	372	346	367
- Implantation sites	[n]	14.8	14.7	13.3	14.5
total number	[n]	370	353	307	348
- Pre-implantation loss	[%]	6.4	5.2	12.2	5.0
- Post-implantation loss	[%]	5.2	8.6	14.7*	3.7
- Resorptions	[n]	0.8	1.3	1.5	0.5
total number	[n]	21	30	35	13
- Early resorptions		0.8	1.2	1.3	0.5
total number	[n]	20	28	30	13
- Late resorptions		0.0	0.1	0.2	0.0
total number	[n]	1	2	5	0
- Dead fetuses	[n]	0	0	0	0
- Dams with viable fetuses	[n]	25	24	22	24
- Live fetuses		14.0	13.5	12.4	14.0
total number	[n]	349	323	272	335
- Total live female fetuses	[n]	6.1	6.5	5.9	6.8
total number	[n]	153	156	129	164
Mean	[%]	41.7	43.4	43.3	47.0
- Total live male fetuses	[n]	7.8	7.0	6.5	7.1
total number	[n]	196	167	143	171
Mean	[%]	53.2	48.0	45.9	49.4
- Percent live females		43.8	48.3	47.4	49.0
- Percent live males		56.2	51.7	52.6	51.0
Placental weights	[g]	0.44	0.44	0.45	0.45
- male fetuses	[g]	0.44	0.45	0.45	0.46
- female fetuses	[g]	0.43	0.43	0.45	0.44
Mean fetal weight	[g]	3.9	3.9	3.9	4.0
- males	[g]	3.9	4.0	4.0	4.1
- females	[g]	3.8	3.9	3.8	3.9

[a] Mean ± SD on litter basis; Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett-test, two-sided)

4.4 External, visceral and skeletal examination of fetuses

External examination

No external malformations or variations were found in any fetuses of any group [see Table 3.10.1.8-5]. One unclassified observation, fused placentae with one of the neighbored littermates or resorptions, appeared in one control (#4 of dam #16), one mid dose (#2 of dam #55) and three high dose fetuses (#2 of dam #77, #11 and 13 of dam #86). The scattered occurrence of this one finding dams not suggest any treatment-relationship.

Table 3.10.1.8-5: Incidence of external malformations and variations

Dose level [mg/kg]	**0**	**60**	**120**	**300**
Litters Evaluated	25	24	22	24
Fetuses Evaluated	349	323	272	335
Live	349	323	272	335
Dead	0	0	0	0
Total external malformations				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Litter incidence [a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total external variations				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Total external unclassified observations				
- Fetal incidence [No. (%)]	1 (0.3)	0 (0.0)	1 (0.4)	3 (0.9)
- Litter incidence [No. (%)]	0 (4.0)	0 (0.0)	1 (4.5)	2 (8.3)
- Affected fetuses/litter (Mean ± SD) [%]	0.2 ± 1.18	0.0 ± 0.00	0.3 ± 1.52	0.9 ± 3.15
Individual external unclassified observations				
- fused placenta				
- Fetal incidence [No. (%)]	1 (0.3)	0 (0.0)	1 (0.4)	3 (0.9)
- Litter incidence [No. (%)]	0 (4.0)	0 (0.0)	1 (4.5)	2 (8.3)
- Affected fetuses/litter (Mean ± SD) [%]	0.2 ± 1.18	0.0 ± 0.00	0.3 ± 1.52	0.9 ± 3.15

Visceral examination

No treatment-related visceral (soft tissue) malformations were noted in this study in any of the fetuses [see Table 3.10.1.8-6].

Soft tissue variations, namely dilated renal pelvises and dilated ureter, were found in all dose groups including the control. Since these findings were not statistically significant, not dose-dependent and represent very frequent findings in the rat strain a treatment-related origin was excluded. The mean percentage of affected fetuses/litter with total visceral variations (21.0, 17.4, 31.3, 28.9% for the control, 60, 120, and 300 mg/kg bw/day dose groups respectively) was fully within the historical control range (5.0-33.3%) for all dose groups [see Table 3.10.1.8-6].

Table 3.10.1.8-6: Incidence of visceral (soft tissue) malformations and variations

Dose level [mg/kg]	**0**	**60**	**120**	**300**
Litters Evaluated	25	24	22	24
Fetuses Evaluated	166	156	132	160
Live	166	156	132	160
Dead	0	0	0	0
Total visceral malformations				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Litter incidence [a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Total visceral variations				
- Fetal incidence [No. (%)]	34 (20)	26 (17)	36 (27)	46 (29)
- Litter incidence [No. (%)]	20 (80)	15 (63)	20 (91)	20 (83)
- Affected fetuses/litter (Mean ± SD) [%]	21.0 ± 15.35	17.4 ± 18.42	31.3 ± 24.43	28.9 ±23.75
Individual visceral variations				
- **Dilated renal pelvis**				
- Fetal incidence [No. (%)]	34 (20)	26 (17)	36 (27)	46 (29)
- Litter incidence [No. (%)]	20 (80)	15 (63)	20 (91)	20 (83)
- Affected fetuses/litter (Mean ± SD) [%]	21.0 ± 15.35	17.4 ± 18.42	31.3 ± 24.43	28.9 ±23.75
- **Dilated ureter**				
- Fetal incidence [No. (%)]	2 (1.2)	1 (0.6)	4 (3.0)	6 (3.8)
- Litter incidence [No. (%)]	2 (8.0)	1 (4.2)	4 (18)	3 (13)
- Affected fetuses/litter (Mean ± SD) [%]	1.2 ± 4.30	0.7 ± 3.40	2.6 ± 5.73	4.0 ± 13.95

No unclassified visceral findings were noted.

Skeletal examination

No treatment-related skeletal malformations were noted in this study. Low incidences of skeletal malformations occurred in the low and high dose group [see Table 3.10.1.8-7].

Absent lumbar vertebra, misshapen scapula, malpositioned and bipartite sternebra, absent and/or fused ribs occurred in 3 low and 3 high dose fetuses. The mean percentage of affected fetuses/litter with skeletal malformations amounted to 0.0, 1.6, 0.0, 1.7% for the control, low, mid and high dose group respectively. All of the skeletal malformations occurred without a dose-relationship, can be found at a comparable frequency in the historical control data range for skeletal malformations (0.0-3.1%), and were therefore assessed as incidental.

Table 3.10.1.8-7: Incidence of skeletal malformations

Dose level [mg/kg]	**0**	**60**	**120**	**300**
Litters Evaluated	25	24	22	24
Fetuses Evaluated	183	167	140	175
Live	183	167	140	175
Dead	0	0	0	0
Total skeletal malformations				
- Fetal incidence [No. (%)]	0 (0.0)	3 (1.8)	0 (0.0)	3 (1.7)
- Litter incidence [a]	0 (0.0)	3 (13)	0 (0.0)	2 (8.3)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	1.6 ± 4.48*	0.0 ± 0.00	1.7 ± 6.26
Individual skeletal malformations				
- **Absent lumbar vertebra**				
- Fetal incidence [No. (%)]	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.5 ± 2.27	0.0 ± 0.00	0.0 ± 0.00
- **Misshapen scapula**				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.5 ± 2.55
- **Malpositioned and bipartite sternebra**				
- Fetal incidence [No. (%)]	0 (0.0)	3 (1.8)	0 (0.0)	1 (0.6)
- Litter incidence [No. (%)]	0 (0.0)	3 (13)	0 (0.0)	1 (4.2)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	1.6 ± 4.48*	0.0 ± 0.00	0.6 ± 2.92
- **Fused rib**				
- Fetal incidence [No. (%)]	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.5 ± 2.27	0.0 ± 0.00	0.0 ± 0.00
- **Absent rib**				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.6 ± 2.92

* $p \leq 0.05$, ** $p \leq 0.01$ (Fisher's exact test, one-sided; Wilcoxon-test, one-sided)

Table 3.10.1.8-8: Incidence of skeletal variations

Dose level [mg/kg]	0	60	120	300
Litters Evaluated	25	24	22	24
Fetuses Evaluated	183	167	140	175
Live	183	167	140	175
Dead	0	0	0	0
Total skeletal variations				
- Fetal incidence [No. (%)]	143 (78)	132 (79)	113 (81)	132 (75)
- Litter incidence [No. (%)]	25 (100)	24 (100)	22 (100)	24 (100)
- Affected fetuses/litter (Mean ± SD) [%]	78.0 ±18.92	78.6 ±15.18	79.7 ±18.73	75.6 ±17.25
Selected individual skeletal variations				
- Unossified sternebra – unchanged cartilage				
- Fetal incidence [No. (%)]	8 (4.4)	11 (6.6)	10 (7.1)	15 (8.6)
- Litter incidence [No. (%)]	4 (16)	8 (33)	8 (36)	9 (38)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 4.6-11.6%)*	4.2 ± 10.65	6.5 ± 10.38	6.9 ± 11.91	8.3 ± 13.22
- Short rib (13th) – cartilage not present				
- Fetal incidence [No. (%)]	19 (10)	26 (16)	29 (21)	35 (20)
- Litter incidence [No. (%)]	12 (48)	14 (58)	12 (55)	15 (63)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 7.7-26.4%)*	9.9 ± 13.17	15.2 ± 15.80	19.7 ± 23.96	19.3 ± 22.62
- Cervical Rib – cartilage not present				
- Fetal incidence [No. (%)]	2 (1.1)	3 (1.8)	0 (0.0)	5 (2.9)
- Litter incidence [No. (%)]	2 (8.0)	3 (13)	0 (0.0)	3 (13)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0.0-4.1%)*	0.9 ± 3.08	1.8 ± 4.81	0.0 ± 0.00	3.0 ± 8.55

Skeletal variations were observed with a high incidence of > 75% of the fetuses and in all litters [see Table 3.10.1.8-8].

The observed variations were related to skull (bipartite ossification of supraoccipital bone, supraoccipital holes, extra ossification site between parietal and interparietal bones, incomplete ossification of parietal, interpartietal, supraoccupital, and/or the total skull bones), the ventral column (incomplete or missing ossification of thoracic and/or lumbar vertebra), the ribs (short 13th, supernumary 14th or cervical ribs), and the sternum (misshapen, fused or bipartite sternebra, extra sternebral ossification site, incomplete or missing ossification of sternebra).

The mean percentage of affected fetuses/litter with skeletal variations amounted to 78.0, 78.6, 79.7, and 75.6% at 0, 60, 120, or 300 mg/kg be/day.

No statistically significant differences were observed between control and treated groups. The variations were observed without dose response-relationship or at incidences comparable to the historical control range. Therefore, these skeletal variations were not considered to be related to treatment.

Some isolated unclassified observations were observed in all groups including the control in this study. They were related to the vertebral column, the ribs and the sternum. As they were found in similar incidences in all dose groups, and did not show any relation to dosing (mean percentage of affected fetuses/litter 15.6, 10.6, 16.0, 10.9% at 0, 60, 120, 300 mg/kg) they were regarded incidental.

CONCLUSION

Based on the effects on maternal food consumption and body weight development the maternal NOAEL was 60 mg/kg bw/day. In absence of any treatment-related developmental effects the developmental NOAEL was 300 mg/kg bw/day. Dimoxystrobin is not teratogenic in rats.

The NOAELs did not change compared to the evaluation in the DAR 2003.

3.10.1.9 Study 9

Developmental toxicity studies, rabbit

Study reference:

Anonymous (2001): BAS 505 F - Prenatal developmental toxicity study in Himalayan rabbits. Oral administration. BASF AG, Ludwigshafen, Germany; BASF DocID 2000/1016867; March 2001

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp 191 - 200:

Report:	KIIA 5.6.2.2/1 Anonymous 2001Prenatal developmental toxicity study in Himalayan rabbits. Oral administration 2000/1016867
Guidelines:	OPPTS 870.3700, OECD 414 (2001) Japan MAFF guidelines
GLP:	yes
Previous evaluation:	in the DAR (2003)
Acceptability:	yes

Executive summary:

Dimoxystrobin was administered daily to presumably pregnant Himalayan rabbits by stomach tube during gestation days 7-28 post insemination (p.i.) at dose levels of 0, 25, 50 and 100 mg/kg bw/day. A dose related maternal toxicity was noted. One high dose doe was found dead, while another high dose female was sacrificed after abortion. Both effects were considered to be treatment-related. Furthermore, a moderate to excessive reduction of food consumption and impaired body weight gain was seen in high dose females. Diarrhea was present in almost all high dose, 12 mid dose and two low dose does along with no defecation in several high dose animals. Most probably secondary to the massive maternal toxicity immediately after implantation an increased post implantation loss (mainly early resorptions) was noted at 100 mg/kg. This high dose was clearly too toxic to does.

No treatment-related effects on external or visceral (soft tissue) malformations or variations or skeletal variations were observed. There was a statistically significant increase in the skeletal variations fused and misshapen sternebra without a dose-response. The statistical significance in the incidence of fused sternebra was due to the low incidence of this finding in the concurrent control. At the highest dose level - where severe maternal toxicity was evident - the mean value % affected fetuses per litter was substantially influenced by the litter of one doe. The only fetus of this doe displayed fused sternebrae, which resulted in a 100% value of % affected fetuses per litter. Excluding this animal from calculation results in a value of 11.1% well covered within the extended historical control range. Therefore, the increased number of skeletal variations can be considered to be incidental.

Based on the findings in this study no maternal NOAEL can not be set (< 25 mg/kg bw/day), whereas the developmental NOAEL was 50 mg/kg bw/day. No maternel and no developmental NOAEL was set in the previous evaluation, in the DAR 2003.

Materials and Methods:
Dimoxystrobin (batch/purity: N 15: 98.4%) was tested for its prenatal developmental toxicity in Himalayan rabbits. The test substance was administered as an aqueous suspension to 25 inseminated female Himalayan rabbits/group by stomach tube at doses of 0, 25, 50 and 100 mg/kg bw/day on day 7 through day 28 post insemination (insemination = day 0). A standard dose volume of 10 ml/kg bw was used. The control group was dosed with the vehicle only (0.5% Tylose CB 30.000 in doubly distilled water).

Results:

1. Clinical signs of toxicity

Treatment-related clinical signs in all dose groups consisted of diarrhea (24/25 high dose, 12/25 mid dose, 2/25 low dose), and no defecation (8/25 high dose showing previously diarrhea, 1 mid dose female without diarrhea) [see Table 3.10.1.9-1].
Blood in bedding was observed in 1 high dose rabbit.

Table 3.10.1.9-1: Clinical observations in rabbits administered dimoxystrobin during days 6 to 28 of gestation

Dose level [mg/kg]	Animal #	Gestation Day	Observation
Control	-	-	-
25 mg/kg	28	8	diarrhea
	37	8	diarrhea
50 mg/kg	51, 52, 54, 55, 58, 59, 60, 64, 68, 71, 74	7-8	diarrhea
	69	7-8	No defecation
100 mg/kg	All (except 99) 84, 85, 86, 87, 93, 96, 98, 100	7-10 7-8	diarrhea No defecation
	90	25-26 27	Blood in bedding; aborted

2. Mortality

One high dose doe (#94) was found dead on day 8 p.i., while another high dose female (#90) was sacrificed after abortion on day 27 p.i.. Both effects were considered to be treatment-related.
There was no mortality in the control, low and mid dose groups (0, 25, and 50 mg/kg bw)

3. Food consumption

Instantly with the start of treatment at GD 7, a marked, statistically significant and treatment related decrease of food consumption was noted in all treated groups [see Table 3.10.1.9-2]. Mean food consumption fell to 62, 39 and 17% of the control for the day 7 to 8 interval at 25, 50, and 100 mg/kg, respectively. Thereafter and in a dose-dependent matter food consumption improved and reached control levels latest on GD 15. Calculated for the entire period high dose rabbits consumed about 15% less food, mid dose rabbits about 6% less food than concurrent controls.

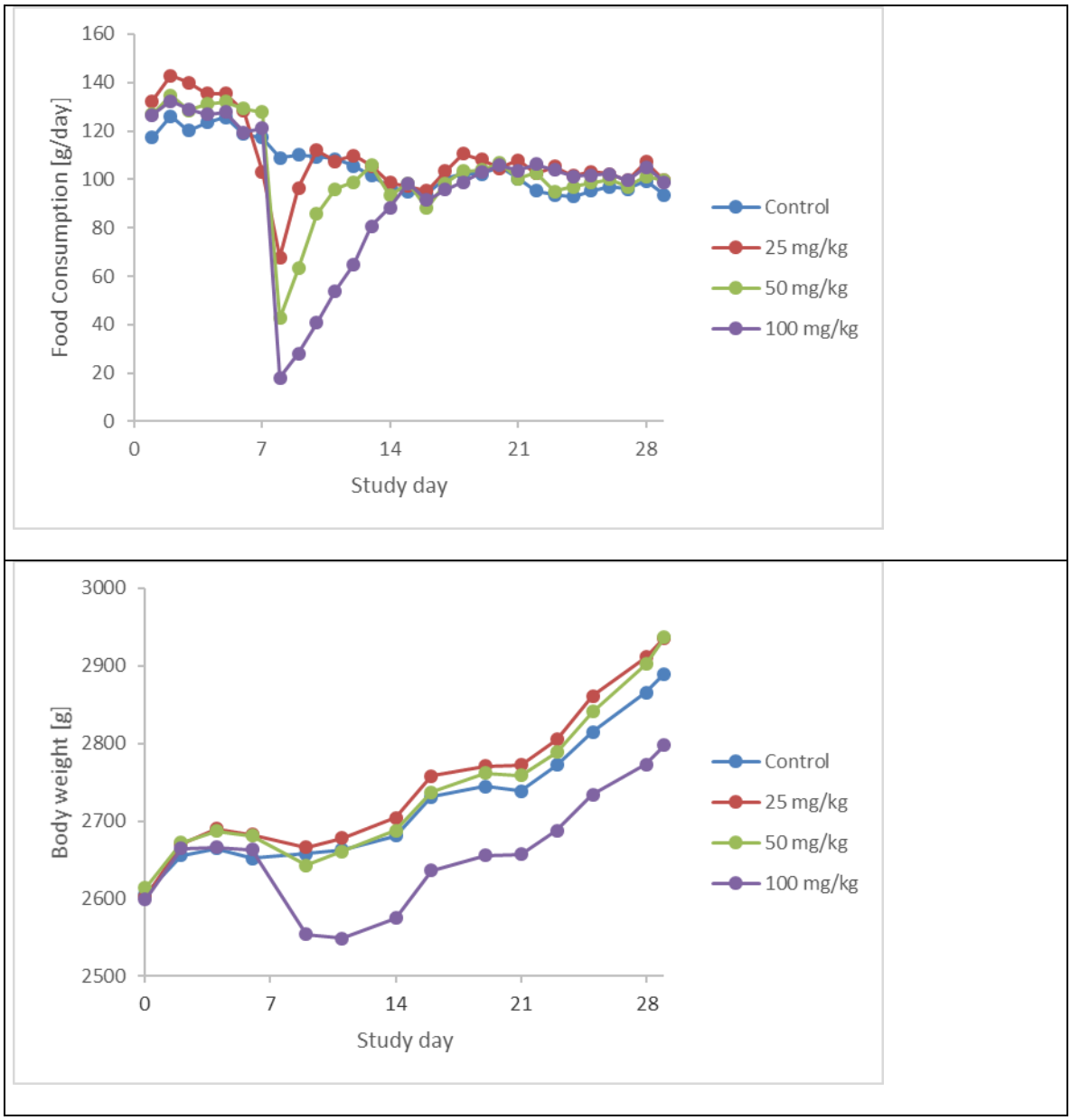


Figure 3.10.1.9-1: Food consumption and body weight development in rabbits administered dimoxystrobin during days 7 to 28 of gestation

4. Body weight and body weight gain

Secondary to the temporary decrease of food consumption at initiation of treatment (days 7-9) a dose-dependent body weight loss was noted in all treated groups [Figure 3.10.1.9-1 and Table 3.10.1.9-2]. Absolut body weights did not show any statistically significant differences. For the gestation day interval 7-9 a statistically significant decrease of body weight gain was noted in the mid and high dose groups. Mean body weight gain during treatment was significantly decreased at the high dose of 100 mg/kg (about 40%) [see Table 3.10.1.9-2]. The impaired mean body weight gains were assessed as substance-related.

Table 3.10.1.9-2: Food consumption and body weight development in rabbit administered dimoxystrobin during days 7 to 28 of gestation

Dose level [mg/kg]	0	25	50	100
Food consumption [g/animal/day][§]				
Day 0 to 7 □%	121.3	135.0 11.3	130.2 7.3	126.1 4.0
Day 7 to 28 □%	100.4	102.2 1.8	94.1 -6.3	85.3 -15.0
Day 0 to 29 □%	105.4	110.0 4.4	103.0 -2.3	95.6 -9.3
Body weight gain [g]				
Day 0 to 7 □%	46.3	81.3 75.6	67.2 45.1	64.2 38.7
Day 7 to 28 □%	213.3	227.6 6.7	221 3.6	**127*** -40.5
Day 0 to 29 □%	283.4	334.2 17.9	322.6 13.8	214.1 -24.5

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

5. Necropsy observations

Corrected (net) body weight gain, carcass and gravid uterus weights

No treatment-related effects on carcass and corrected (net) body weight gain were observed at any dose level [see Table 3.10.1.9-3]. The mean gravid uterus weight of the high dose animals was decreased (about 81% of that of the control) without attaining statistical significance. The finding was in line with the increased resorption rate.

Table 3.10.1.9-3: Mean gravid uterus weights and net body weight change of pregnant rabbits administered dimoxystrobin during Days 7 to 28 of gestation

Dose level [mg/kg bw/d]	**0**	**25**	**50**	**100**
Gravid uterus (g)	310.5	336.8	353.1	252.6
Carcass (g)	2579.1	2599.1	2583.6	2545
Net weight change from Day 6 (g)	-73.4	-83.9	-97.7	-100.7

Gross necropsy observations

There occurred no substance-related necropsy findings in any of the rabbits. A number of gross necropsy findings were noted in all groups including control at Cesarian section [see Table 3.10.1.9-4]. Findings were either single observations or associated with the method of sacrifice and/or equally distributed over the treatment groups including the control. The most frequent findings were congestions, edema and marginal emphysema of/in lungs and congested livers. Neither the incidence nor the type of observations indicated a relation to treatment.

Table 3.10.1.9-4: Gross necropsy findings in rabbits administered dimoxystrobin during days 7 to 28 of gestation

Dose group	Animal #	Observation
Control	10	Lungs: edema, marginal emphysema
	14	Lungs: edema
	19	Lungs: edema
	20	Lungs: edema, marginal emphysema Liver: congested
	23	Lungs: edema, marginal emphysema
25 mg/kg bw/day	42	Lungs: edema, marginal emphysema
	44	Lungs: congested
	48	Lungs: edema, marginal emphysema
	49	Lungs: edema
	50	Lungs: edema, marginal emphysema
50 mg/kg bw/day	60	Liver: congested
	61	Lungs: edema, marginal emphysema
	64	Lungs: edema, marginal emphysema
	66	Lungs: edema
	68	Lungs: petechia
	69	Lungs: edema, marginal emphysema
	72	Lungs: edema, marginal emphysema
	74	Lungs: marginal emphysema
	75	Lungs: edema, marginal emphysema
100 mg/kg bw/day	76	Liver: necrosis
	82	Lungs: edema, marginal emphysema
	88	Lungs: edema
	90	Particular findings on implants in females which aborted
	92	Liver: congested
	94	Particular findings on implants in females died
	97	Liver: congested Lungs: congested, edema

6. Cesaren section data

25, 25, 23 and 24 does were pregnant at 0, 25, 50 and 100 mg/kg [see Table 3.10.1.9-5]. As already indicated one high dose female died and one was sacrificed after abortion.

The number of corpora lutea, implantations and the pre-implantation loss was comparable between all groups.

Three high dose females (#84, 92, 97) had total resorptions. In addition, a non-statistically significant increase of the post-implantation loss was noted at the high dose. This was due to an increased number of early resorptions. The increase of early resorptions is considered to be secondary to the marked maternal toxicity observed at the high dose.

Table 3.10.1.9-5: Pregnancy status and caesarean section data of does treated with dimoxystrobin during days 7 to 28 of pregnancy

Dose level [mg/kg bw/d]		0	25	50	100
Pregnancy status					
Females					
- mated	[n]	25	25	25	25
- pregnant	[n]	25	25	23	24
conception rate	[%]	100	100	92	96
- aborted	[n]	0	0	0	1
- premature birth	[n]	0	0	0	0
- females with viable fetuses	[n]	25	25	23	19
- females with all resorptions	[n]	0	0	0	3
- mortality		0	0	0	2
- pregnant terminal sacrifice	[n]	25	25	23	22
Cesarean section data[a]					
- Corpora lutea	[n]	8.0	7.9	8.5	8.0
total number	[n]	200	198	196	177
- Implantation sites	[n]	7.0	6.9	7.5	7.0
total number	[n]	175	173	172	153
- Pre-implantation loss	[%]	13.1	14.1	13.1	14.3
- Post-implantation loss	[%]	14.2	5.0	6.6	27.5
- Resorptions	[%]	14.2	5.0	6.6	27.5
number	[n]	1.0	0.4	0.5	**2.2***
total number	[n]	24	10	11	48
- Early resorptions	[%]	11.6	2.7	5.2	25.7
number	[n]	0.8	0.2	0.3	**2.0***
total number	[n]	19	5	8	45
- Late resorptions	[%]	2.6	2.3	1.5	1.8
number	[n]	0.2	0.2	0.1	0.1
total number	[n]	5	5	3	3
- Dead fetuses	[n]	0	0	0	0
- Females with viable fetuses	[n]	25	25	23	19
- Live fetuses		6.0	6.5	7.0	5.5
total number	[n]	151	163	161	105
Mean	[%]	85.8	95.0	93.4	84.0
- Total live female fetuses	[n]	3.0	3.3	3.8	2.8
total number	[n]	74	83	87	53
Mean	[%]	40.7	47.7	50.8	41.6
- Total live male fetuses	[n]	3.1	3.2	3.2	2.7
total number	[n]	77	80	74	52
Mean	[%]	45.1	47.3	42.6	42.4
- Percent live females		49.0	50.9	54.0	50.5
- Percent live males		51.0	49.1	46.0	49.5
Placental weights	[g]	4.3	4.2	4.2	4.4
- male fetuses	[g]	4.4	4.2	4.3	4.4
- female fetuses	[g]	4.3	4.2	4.0	4.2
Mean fetal weight	[g]	38.0	38.5	37.8	38.1
- males	[g]	38.2	38.6	38.1	38.4
- females	[g]	37.9	38.3	37.2	37.6

[a] Mean ± SD on litter basis; Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett-test, two-sided)

The sex ratio as well as placental and fetal weights were not affected by treatment. No substance-related differences occurred between control, low and mid dose group animals.

7. External, visceral and skeletal examination of fetuses

External examination

External malformations were recorded in each one low, mid and high dose fetus [see Table 3.10.1.9-6]. Low dose fetus 1 of female #33 displayed microphthalmia. Mid dose fetus 4 of female #67 had meningoencephalocele. High dose fetus 3 of female #83 had a short tail. Findings were regarded as incidental and not treatment-related.
External variations observed consisted of paw hyperflexion in single control, mid and high dose fetuses. Neither the incidence nor the type of external variations indicated a relation to treatment.
There appeared two unclassified external observations. Placenta necrobiotic combined with blood coagulum around placenta was observed in one control fetus 6 of female #4.

Table 3.10.1.9-6: Incidence of external malformations and variations

Dose level [mg/kg bw/day]	**0**	**25**	**50**	**100**
Litters Evaluated	25	25	23	19
Fetuses Evaluated	151	163	161	105
Live	151	163	161	105
Dead	0	0	0	0
Total external malformations				
- Fetal incidence [N (%)]	0 (0.0)	1 (0.6)	1 (0.6)	1 (1.0)
- Litter incidence [a]	0 (0.0)	1 (4.0)	1 (4.3)	1 (5.3)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	2.0 ± 10.00	0.7 ± 3.48	1.8 ± 7.65
Total external variations				
- Fetal incidence [N (%)]	1 (0.7)	0 (0.0)	1 (0.6)	3 (2.9)
- Litter incidence [N (%)]	1 (4.0)	0 (0.0)	1 (4.3)	2 (11)
- Affected fetuses/litter (Mean ± SD) [%]	0.6 ± 2.86	0.0 ± 0.00	0.6 ± 2.98	3.9 ± 11.61
Individual external variations				
- Paw hyperflexion				
- Fetal incidence [N (%)]	1 (0.7)	0 (0.0)	1 (0.6)	3 (2.9)
- Litter incidence [N (%)]	1 (4.0)	0 (0.0)	1 (4.3)	2 (11)
- Affected fetuses/litter (Mean ± SD) [%]	0.6 ± 2.86	0.0 ± 0.00	0.6 ± 2.98	3.9 ± 11.61

( ) Values in brackets give % fetal respectively litter incidence

Visceral examination

A variety of visceral (soft tissue) malformations were observed in all groups including control [see Table 3.10.1.9-7]. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range. The only statistically significant difference was observed for muscular ventricular septal defects in low dose fetuses. The incidence of this malformation was within the historical control range. All other findings were observed in single cases only. None of the findings was considered to be treatment-related.

Table 3.10.1.9-7: Incidence of visceral (soft tissue) malformations

Dose level [mg/kg]	0	25	50	100
Litters Evaluated	25	25	23	19
Fetuses Evaluated	151	163	161	105
Live	151	163	161	105
Dead	0	0	0	0
Total visceral malformations				
- Fetal incidence [No. (%)]	3 (1.3)	3 (1.8)	3 (1.9)	2 (1.9)
- Litter incidence	2 (8.0)	3 (12)	3 (13)	2 (11)
- Affected fetuses/litter (Mean ± SD) [%]	1.4 ± 4.82	1.6 ± 4.55	2.3± 6.15	2.8 ± 8.70
Selected Individual visceral malformations				
- Muscular ventricular septum defect				
- Fetal incidence [N (%)] *(HCD 0-2.6%)*	0 (0.0)	3 (1.8)	2 (1.2)	0 (0.0)
- Litter incidence [N (%)] *(HCD 0-17.6%)*	0 (0.0)	3 (12)	2 (8.7)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-2.6%)*	0.0 ± 0.00	**1.6***± 4.55	1.4 ± 4.80	0.0 ± 0.00

( ) Values in brackets give % fetal respectively litter incidence
* $p \leq 0.05$, ** $p \leq 0.01$ (Fisher's exact test, one-sided; Wilcoxon-test, one-sided)

Two different visceral variations was observed in all treated groups [see Table 3.10.1.9-8]. Malpositioned carotid branch was the most common finding with the highest incidence in mid dose fetuses, without attaining statistically significance. The other finding (dilated renal pelvis) was observed in a single case only. None of the findings was considered to be treatment-related.

Table 3.10.1.9-8: Incidence of visceral (soft tissue) variations

Dose level [mg/kg]	0	25	50	100
Litters Evaluated	25	25	23	19
Fetuses Evaluated	151	163	161	105
Live	151	163	161	105
Dead	0	0	0	0
Total visceral variations				
- Fetal incidence [N (%)]	11 (7.3)	11 (6.7)	17 (11)	11 (10)
- Litter incidence [N (%)]	10 (40)	9 (36)	11 (48)	8 (42)
- Affected fetuses/litter (Mean ± SD) [%]	8.1 ± 13.09	6.7 ± 10.42	9.2 ± 11.09	10.2 ± 15.12
Individual visceral variations				
- Malpositioned carotid branch				
- Fetal incidence [N (%)]	11 (7.3)	11 (6.7)	16 (9.9)	11 (10)
- Litter incidence [N (%)]	10 (40)	9 (36)	11 (48)	8 (42)
- Affected fetuses/litter (Mean ± SD) [%]	8.1 ± 13.09	6.7 ± 10.42	8.7 ± 10.74	10.2 ± 15.12
- Dilated renal pelvis				
- Fetal incidence [N (%)]	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
- Litter incidence [N (%)]	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.0 ± 0.00	0.5 ± 2.32	0.0 ± 0.00

( ) Values in brackets give % fetal respectively litter incidence

Skeletal examination

A number of skeletal malformations were observed in all groups including controls [see Table 3.10.1.9-9]. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range.

There were no statistically significant differences for the total or individual skeletal malformations. The highest incidence of severely fused sternebra (bony plate) occurred with 3 affected fetuses in the control group.

Table 3.10.1.9-9: Incidence of skeletal malformations and variations

Dose level [mg/kg]	**0**	**25**	**50**	**100**
Litters Evaluated	25	25	23	19
Fetuses Evaluated	151	163	161	105
Live	151	163	161	105
Dead	0	0	0	0
Total skeletal malformations				
- Fetal incidence [N (%)]	4 (2.6)	2 (1.2)	3 (1.9)	5 (4.8)
- Litter incidence	3 (12)	2 (8.0)	3 (13)	4 (21)
- Affected fetuses/litter (Mean ± SD) [%]	3.4 ± 9.87	2.5 ± 10.21	2.4 ± 7.46	6.9 ± 16.66
Individual skeletal malformations				
- **Sternebra severely fused (Bony plate)**				
- Fetal incidence [N (%)]	3 (2.0)	0 (0.0)	0 (0.0)	1 (1.0)
- Litter incidence [N (%)]	3 (12)	0 (0.0)	0 (0.0)	1 (5.3)
- Affected fetuses/litter (Mean ± SD) [%]	2.6 ± 7.23	0.0 ± 0.00	0.0 ± 0.00	1.8 ± 7.65
- **Absent lumbar vertebra**				
- Fetal incidence [N (%)]	2 (1.3)	0 (0.0)	1 (0.6)	1 (1.0)
- Litter incidence [N (%)]	1 (4.0)	0 (0.0)	1 (4.3)	1 (5.3)
- Affected fetuses/litter (Mean ± SD) [%]	1.6 ± 8.00	0.0 ± 0.00	0.5 ± 2.32	0.8 ± 3.28
Total skeletal variations				
- Fetal incidence [N (%)]	111 (74)	114 (70)	100 (62)	77 (73)
- Litter incidence [N (%)]	25 (100)	25 (100)	23 (100)	19 (100)
- Affected fetuses/litter (Mean ± SD) [%]	73.7 ± 18.73	73.0 ± 22.87	62.3 ± 22.53	76.7 ± 18.06
Individual skeletal variations				
- **Fused sternebra**				
- Fetal incidence [N (%)] *(HCD 0-10.7%)*[1)]	2 (1.3)	9 (5.5)	10 (6.2)	11 (10)
- Litter incidence [N (%)] *(HCD 0-50.0%)*[1)]	2 (8.0)	9* (36)	8 (35)	9** (47)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-13.5%)*[1)]	0.9 ± 3.08	5.1*± 6.97	6.5**±10.07	15.2** ±24.50 11.1[2)] (excluding female #87)
- **Misshapen sternebra**				
- Fetal incidence [N (%)] *(HCD 0-11.6%)*[1)]	5 (3.3)	16 (9.8)	9 (5.6)	7 (6.7)
- Litter incidence [N (%)] *(HCD 0-55.0%)*[1)]	4 (16)	11* (44)	8 (35)	6 (32)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-15.8%)*[1)]	2.6 ± 6.37	10.5*± 14.47	5.5 ±8.85	7.6 ±12.39
- **Supernumary Rib (13th)**				
- Fetal incidence [N (%)] *(HCD 2.5-11.7%)*[1)]	7 (4.6)	7 (4.3)	7 (4.3)	12 (11)
- Litter incidence [N (%) *(HCD 16.0-52.5%)*[1)]	6 (24)	4 (16)	7 (30)	7 (37)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 2.1-11.1%)*[1)]	5.6 ± 11.06	4.3 ± 13.76	4.8 ±8.59	10.0 ±15.62

* $p \leq 0.05$, ** $p \leq 0.01$ (Fisher's exact test, one-sided; Wilcoxon-test, one-sided)

[1)] extended historical control data covering a time span of roughly ± 5 years around the experimental date

[2)] when excluding dam #87 (which had 100% affected fetuses (=1 affected pup)/litter) from the evaluation the incidence decreases to 11.1%

Skeletal variations were observed in about 62 to 74% of the fetuses in all litters [see Table 3.10.1.9-9]. No statistically significant differences (exception: fused or misshapen sternebra) were observed between control and treated groups. The variations were observed without dose response-relationship or at incidences comparable to the historical control range. This includes the statistically significantly increased incidence of fetuses with misshapen sternebra in the low dose group only. Furthermore, the incidences are covered within an extended historical control data covering a time span of roughly ± 5 years around the experimental date (The historical control data considered during the last evaluation only comprised studies conducted before the respective study summarized.). Therefore, these skeletal variations were not considered to be related to treatment.

There was an apparent increase in the fetal, litter and affected fetuses/litter incidence of fused sternebrae at all dose levels without a clear dose-response relationship. The statistical significance was due to the low incidence of this finding in the concurrent control. At the highest dose level - where severe maternal toxicity was evident - the % affected fetuses per litter was substantially influenced by the reduced litter size. Especially, the litter of female #87 influenced the group mean value substantially. The only fetus of this female displayed fused sternebrae, which resulted in a 100% value of % affected fetuses per litter. Excluding this female from calculation results in a group mean of 11.1% , which is within the extended historical control range.

The incidence of supernumerary rib (13^{th}) was not statistically significantly increased in the high dose group. No clear dose-relationship could be observed and the incidence is covered by the extended historical control data range.

No skeletal unclassified observations were observed in this study.

CONCLUSION

Severe maternal toxicity was seen at 100 mg/kg bw, indicated by maternal deaths (2 females died). At ≥ 50 mg/kg bw increased incidences of no defecation was observed and the animals showed diarrhea at all dose levels. Marked to excessive, but transient drop of food consumption and body weight loss was observed at all dose levels.

At 100 mg/kg bw/day this resulted in increased resorptions (mainly early resorptions) and post implantation loss. As a consequence gravid uterus weights were lower at this dose level without attaining statistical significance. No treatment-related malformations or variations were recorded.

Based on these effects the maternal NOAEL was < 25 mg/kg and developmental NOAEL was 50 mg/kg bw/day.

3.10.1.10 Study 10

Developmental toxicity studies, rabbit

Study reference:

Anonymous (2001): BAS 505 F - (Second) Prenatal developmental toxicity study in Himalayan rabbits. Oral administration (Gavage). BASF AG, Ludwigshafen, Germany; BASF DocID 2001/1016351; October 2001

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp 200 - 211:

Report:	KIIA 5.6.2.2/2 Anonymous, 2001 (Second)Prenatal developmental toxicity study in Himalayan rabbits. Oral administration (Gavage) 2001/1016351
Guidelines:	OPPTS 870.3700, OECD 414 (2001) Japan MAFF guidelines
GLP:	yes
Previous evaluation:	in the DAR (2003)
Acceptability:	yes

Executive summary:

Dimoxystrobin was administered daily to presumably pregnant Himalayan rabbits by stomach tube during gestation days 7-28 post insemination (p.i.) at dose levels of 0, 5, 20 and 75 mg/kg bw/day.

Two high dose does were found dead. This effect was considered to be treatment-related. Moreover, dose-related maternal toxicity was noted as indicated by a moderate to excessive reduction of food consumption and impaired body weight gain. Diarrhea was seen in 16 high dose, and 6 mid dose females and no defecation in 10 high dose does, most of them showing previously diarrhea and was assessed as treatment-related. A slightly increase in early resorptions and post implantation loss was noted secondary to the massive maternal toxicity at the high dose of 75 mg/kg. This high dose was clearly too toxic to does.

No treatment-related effects on external or visceral (soft tissue) malformations or variations or skeletal variations was observed. There was an increase in the skeletal variation fused sternebra without attaining statistically significance, which was well covered within the extended historical control range. Further statistically significant increases in skeletal variations did not show any dose-relationship and were not found in the first rabbit prenatal developmental toxicity study. Therefore, the increased number of skeletal variations can be considered to be incidental.

Based on the findings in this study the maternal NOAEL was 5 mg/kg bw/day. NOAEL for developmental effects is 20 mg/kg bw/day.

Materials and Methods:
Dimoxystrobin (batch/purity: N 15: 98.4%) was administered as an aqueous suspension to 25 inseminated female Himalayan rabbits/group by stomach tube at doses of 0, 5, 20 and 75 mg/kg bw on day 7 through day 28 post insemination (insemination = day 0). A standard dose volume of 10 ml/kg bw was used. The control group was dosed with the vehicle only (0.5% Tylose CB 30.000 in doubly distilled water).
Results:

1. Clinical signs of toxicity

Treatment-related clinical signs in the mid and high dose groups consisted of diarrhea (16/25 high dose, 6/25 mid dose), and no defecation (10/25 high dose, most of them showing previously diarrhea) [see Table 3.10.1.10-1]. Blood in bedding was observed in 1 high dose rabbit. This rabbit delivered no viable fetuses but had only early resorptions. These effects were considered treatment-related. One low dose doe showed ne defecation towards the end of treatment, which was assessed as incidental due to the late occurrence.

Table 3.10.1.10-1: Clinical observations in rabbits administered dimoxystrobin during days 6 to 28 of gestation

Dose level [mg/kg]	Animal #	Gestation Day	Observation
Control	17	29	Abortion, sacrificed
5 mg/kg	33	27-29	No defecation
20 mg/kg	51, 54, 63, 64, 70, 74	8	Diarrhea
75 mg/kg	76, 79, 81, 87, 89, 91-100	7-8	Diarrhea
	81, 84, 85, 87, 93, 96-100	8-14	No defecation
	98	19-24	Blood in bedding

2. Mortality

Two high dose does (#77 and 78) were found dead on day 8 p.i.. This effect was considered to be treatment-related.
One low dose doe (#30) was found dead on day 16 p.i.. This was an incidental death since necropsy showed findings indicative for misgavage. One control doe (#17) was sacrifice after abortion on day 29 p.i..
There were no further substance-related or spontaneous mortalities in any of the groups.

3. Food consumption

Instantly with the start of treatment at GD 7, a marked, statistically significant and treatment related decrease of food consumption was noted in all treated groups [see Figure 3.10.1.10-1]. Mean food consumption fell to 75, 33 and 11% of the control for the day 7 to 8 interval at 5, 20, and 75 mg/kg, respectively. Thereafter and in a dose-dependent matter food consumption improved and reached control levels latest on GD 15. Calculated for the entire period high dose rabbits consumed about 18% less food than concurrent controls.

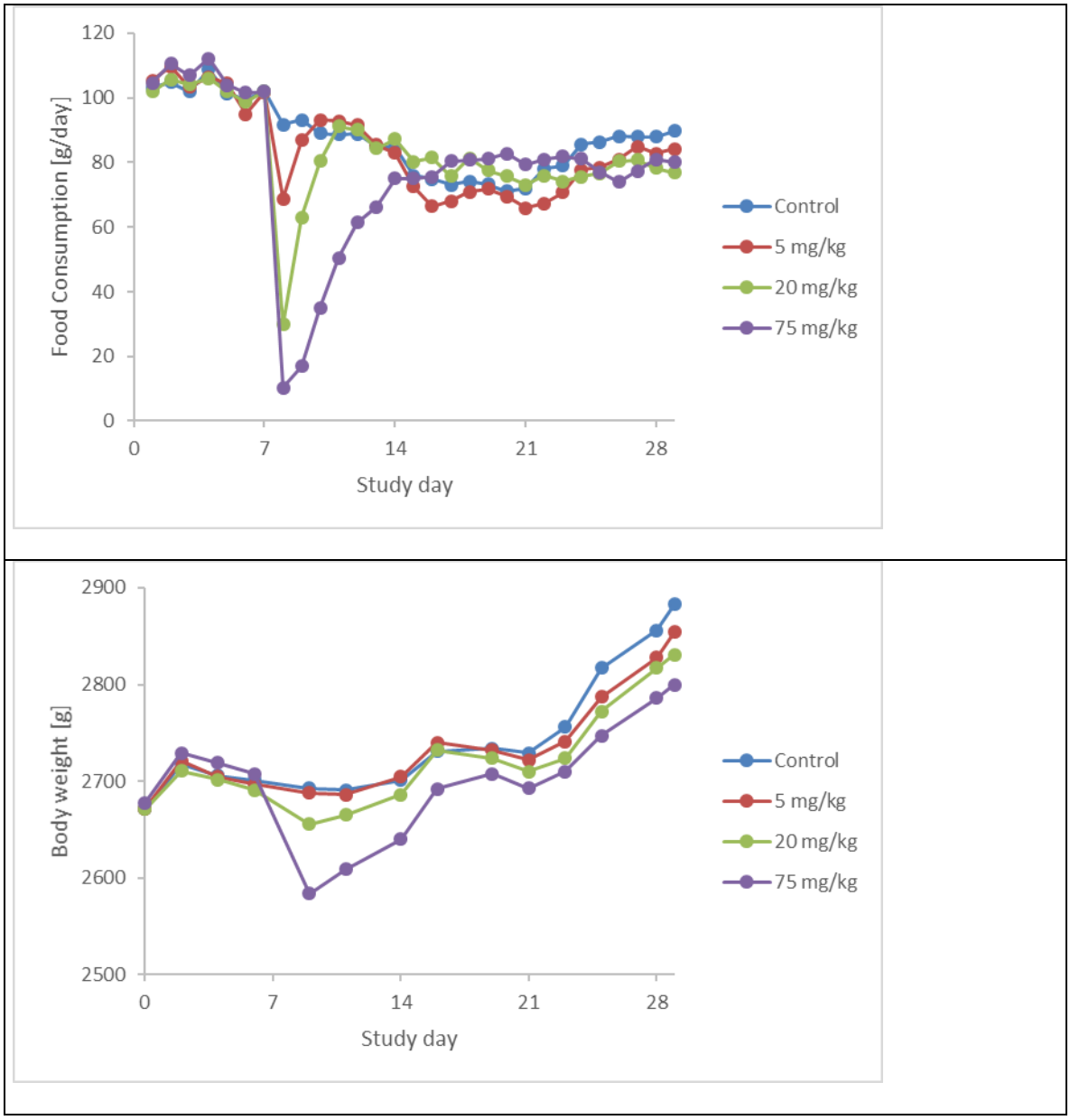


Figure 3.10.1.10-1: Food consumption and body weight development in rabbits administered dimoxystrobin during days 7 to 28 of gestation

4. Body weight and body weight gain

Secondary to the temporary decrease of food consumption at initiation of treatment (days 7-9) a dose-dependent body weight loss was noted in mid and high dose groups [Figure B.6.6.2-3 and Table 3.10.1.10-2]. Absolut body weights did not show any statistically significant differences. For the gestation day interval 7-9 a statistically significant decrease of body weight gain was noted in the mid and high dose groups. Mean body weight gain during treatment was significantly decreased at the high dose of 75 mg/kg (about 55%) and non-statistically significantly at the mid dose of 20 mg/kg (about 19%) [see Table B.6.6.2-19]. The impaired mean body weight gains in the mid and high dose group were assessed as substance-related.

Table 3.10.1.10-2: Food consumption and body weight development in rabbit administered dimoxystrobin during days 7 to 28 of gestation

Dose level [mg/kg]	0	5	20	75
Food consumption [g/animal/day][§]				
Day 0 to 7	103.1	103.7	102.9	106.0
□%		0.6	-0.2	2.8
Day 7 to 28	82.3	77.6	76.8	67.8
□%		-5.7	-6.7	-17.6
Day 0 to 29	87.6	84.1	83.1	77.4
□%		-4.0	-5.1	-11.6
Body weight gain [g]				
Day 0 to 7	29.7	25.2	20.5	29.2
□%		-15.2	-31.0	-1.7
Day 7 to 28	155.2	136.4	125.3	**69.2***
□%		-12.1	-19.3	-55.4
Day 0 to 29	212.1	188.4	160.2	**107.6***
□%		-11.2	-24.5	-49.3

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

5. Necropsy observations

Corrected (net) body weight gain, carcass and gravid uterus weights

No treatment-related effects on carcass and corrected (net) body weight gain were observed at any dose level [see Table 3.10.1.10-3]. The mean gravid uterus weight of the high dose animals was decreased (about 85% of that of the control) without attaining statistically significance. The finding was in line with the slightly increased resorption rate.

Table 3.10.1.10-3: Mean gravid uterus weights and net body weight change of pregnant rabbits administered dimoxystrobin during Days 7 to 28 of gestation

Dose level [mg/kg bw/d]	**0**	**5**	**20**	**75**
Gravid uterus (g)	328.9	323.6	292.1	279.4
Carcass (g)	2561.1	2530.5	2539.1	2521.1
Net weight change from Day 6 (g)	-131.1	-161.1	-152.4	-195.5

Gross necropsy observations

There occurred no substance-related necropsy findings in any of the rabbits. A number of gross necropsy findings were noted in all groups including control at Cesarian section [see Table 3.10.1.10-4]. Findings were either single observations or associated with the method of sacrifice and/or equally distributed over the treatment groups including the control. The most frequent findings were congestions, edema of/in lungs and congested livers. Neither the incidence nor the type of observations indicated a relation to treatment.

Table 3.10.1.10-4: Gross necropsy findings in rabbits administered dimoxystrobin during days 7 to 28 of gestation

Dose group	Animal #	Observation
Control	11	Lungs: congested
	17	Large intestine: tympanic distension Thoracic cavity: filled with serous fluid Lungs: congested Stomach: filled with fluid bloody Particular findings on implants in females which aborted
	19	Lungs: congested
	20	Lungs: congested
	25	Lungs: edema Thoracic cavity: filled with serous fluid
5 mg/kg bw/day	28	Lungs: congested
	30	Thoracic cavity: filled with bloody fluid Lungs: congested Particular findings on implants in females died intercurrently
	41	Lungs: congested
20 mg/kg bw/day	56	absence of uterine horns
	57	Lungs: congested Liver: congested
	65	Lungs: edema Liver: pale Thoracic cavity: filled with serous fluid
	66	Lungs: congested
	75	Lungs: edema Liver: pale
75 mg/kg bw/day	77	Stomach: tympanic distension Watery feces Particular findings on implants in females died intercurrently
	78	Stomach: ulceration(s) Watery feces Particular findings on implants in females died intercurrently
	87	Lungs: congested
	91	Blind ending uterine horn(s)
	98	Lungs: congested Liver: congested
	100	Blind ending uterine horn(s)

6 Cesaren section data

25 does were pregnant at 0, 5, 20 and 75 mg/kg each [see Table 3.10.1.10-5]. As already indicated two high dose and one low dose female died.

The number of corpora lutea, implantations and the pre-implantation loss was comparable between all groups.

The total resorption rate was slightly but not statistically significantly increased in the high dose group due to an increase in early resorptions. This was mainly caused by one doe (#98), which had no viable fetuses but inly early resorptions. Consequently, the post-implantation loss value was slightly increased without attaining statistical significance. The increase of early resorptions is considered to be secondary to the marked maternal toxicity observed at the high dose as evident by maternal deaths, the marked reduction of food consumption, body weight loss and diarrhea during the early phase of treatment.

Table 3.10.1.10-5: Pregnancy status and caesarean section data of females treated with dimoxystrobin during days 7 to 28 of pregnancy

Dose level [mg/kg bw/d]		0	5	20	75
Pregnancy status					
Females					
- mated	[n]	25	25	25	25
- pregnant	[n]	25	25	25	25
conception rate	[%]	100	100	100	100
- aborted	[n]	1	0	0	0
- premature birth	[n]	0	0	0	0
- females with viable fetuses	[n]	24	24	25	22
- females with all resorptions	[n]	0	0	0	1
- mortality		1	1	0	2
- pregnant terminal sacrifice	[n]	24	24	25	23
Cesarean section data[a]					
- Corpora lutea	[n]	8.6	8.9	8.5	8.5
total number	[n]	206	214	213	195
- Implantation sites	[n]	7.6	7.5	6.8	6.7
total number	[n]	183	181	213	155
- Pre-implantation loss	[%]	12.4	14.9	19.7	21.7
- Post-implantation loss	[%]	10.1	8.8	12.5	16.0
- Resorptions	[%]	10.1	8.8	12.5	16.0
number	[n]	0.8	0.6	0.8	0.9
total number	[n]	19	15	21	20
- Early resorptions	[%]	8.4	8.4	9.4	15.5
number	[n]	0.7	0.6	0.6	0.8
total number	[n]	16	14	16	19
- Late resorptions	[%]	1.6	0.4	3.1	0.5
number	[n]	0.1	0.0	0.2	0.0
total number	[n]	3	1	5	1
- Dead fetuses	[n]	0	0	0	0
- Females with viable fetuses	[n]	24	24	25	22
- Live fetuses		6.8	6.9	6.0	6.1
total number	[n]	164	166	150	135
Mean	[%]	89.9	91.2	87.5	87.8
- Total live female fetuses	[n]	3.6	3.1	3.1	2.8
total number	[n]	86	75	78	62
Mean	[%]	48.1	41.9	43.8	37.6
- Total live male fetuses	[n]	3.3	3.8	2.9	3.3
total number	[n]	78	91	72	73
Mean	[%]	41.8	49.3	43.7	50.3
- Percent live females		52.4	45.2	52.0	45.9
- Percent live males		47.6	54.8	48.0	54.1
Placental weights	[g]	4.3	4.1	4.4	4.3
- male fetuses	[g]	4.3	4.2	4.6	4.3
- female fetuses	[g]	4.3	4.0	4.3	4.2
Mean fetal weight	[g]	36.1	34.7	36.0	35.3
- males	[g]	36.2	34.8	36.1	35.3
- females	[g]	35.8	34.2	35.9	34.4

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

The sex ratio as well as placental and fetal weights were not affected by treatment. No substance-related differences occurred between control, low and mid dose group animals.

7 External, visceral and skeletal examination of fetuses

External examination

No treatment related external malformations were recorded in treated groups. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range [see Table 3.10.1.10-6].
Microglossia was observed in two low, one mid and two high dose fetuses. There was no dose-response relationship and the mean percentage of affected fetuses/litter did not attain statistical significance. The same was true for the finding malroted limb.
External variations observed consisted of paw hyperflexion in all treated dose groups including control to a comparable number. Neither the incidence nor the type of external variations indicated a relation to treatment.
Moreover, none of the observed external malformations or variations was found in the preceding prenatal developmental toxicity study up to and including the high dose level of 100 mg/kg bw/day.
No unclassified external observations were found in either the control or the treated dose groups.

Table 3.10.1.10-6: Incidence of external malformations and variations

Dose level [mg/kg bw/day]	**0**	**5**	**20**	**75**
Litters Evaluated	24	24	25	22
Fetuses Evaluated	164	166	150	135
Live	164	166	150	135
Dead	0	0	0	0
Total external malformations				
- Fetal incidence [N (%)]	0 (0.0)	8 (1.8)	1 (0.7)	3 (2.2)
- Litter incidence [a]	0 (0.0)	2 (8.3)	1 (4.0)	1 (4.5)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	1.7 ± 6.26	0.6 ± 2.86	1.9 ± 9.14
Individual external malformations				
- Microglossia				
- Fetal incidence [N (%)]	0 (0.0)	2 (1.2)	1 (0.7)	2 (1.5)
- Litter incidence [N (%)]	0 (0.0)	1 (4.2)	1 (4.0)	1 (4.5)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	1.2 ± 5.83	0.6 ± 2.86	1.3 ± 6.09
- Malrotated limb				
- Fetal incidence [N (%)]	0 (0.0)	2 (1.2)	1 (0.7)	2 (1.5)
- Litter incidence [N (%)]	0 (0.0)	1 (4.2)	1 (4.0)	1 (4.5)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	1.2 ± 5.83	0.6 ± 2.86	1.3 ± 6.09
Total external variations				
- Fetal incidence [N (%)]	4 (2.4)	7 (4.2)	5 (3.3)	5 (3.7)
- Litter incidence [N (%)]	4 (17)	5 (21)	4 (16)	4 (18)
- Affected fetuses/litter (Mean ± SD) [%]	1.8 ± 4.24	5.3 ± 11.26	2.6 ± 6.42	2.8 ± 6.97
Individual external variations				
- Paw hyperflexion				
- Fetal incidence [N (%)]	4 (2.4)	7 (4.2)	5 (3.3)	5 (3.7)
- Litter incidence [N (%)]	4 (17)	5 (21)	4 (16)	4 (18)
- Affected fetuses/litter (Mean ± SD) [%]	1.8 ± 4.24	5.3 ± 11.26	2.6 ± 6.42	2.8 ± 6.97

( ) Values in brackets give % fetal respectively litter incidence

Visceral examination

A variety of visceral (soft tissue) malformations were observed in all groups including control [see Table 3.10.1.10-7]. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range. There were no statistically significant differences in treated compared to control fetuses. A slight increase in membranous ventricular septum defect was seen in the control, mid and high dose with 1, 1, and 3 affected fetuses without attaining statistical significance. The incidence of this malformation was within the historical control range. Furthermore, ventricular septum defects were seen only in the low and mid dose of the first prenatal developmental toxicity study in rabbits, but not in the highest dose tested (100 mg/kg bw), showing the variability of this effect. It was therefore considered to be incidental. All other findings were observed in single cases only. None of the findings was considered to be treatment-related.

Table 3.10.1.10-7: Incidence of visceral (soft tissue) malformations

Dose level [mg/kg]	**0**	**5**	**20**	**75**
Litters Evaluated	24	24	25	22
Fetuses Evaluated	164	166	150	135
Live	164	166	150	135
Dead	0	0	0	0
Total visceral malformations				
- Fetal incidence [No. (%)]	1 (0.6)	3 (1.8)	2 (1.2)	3 (2.2)
- Litter incidence	1 (4.2)	3 (13)	2 (8.0)	3 (14)
- Affected fetuses/litter (Mean ± SD) [%]	0.5 ± 2.55	1.7 ± 4.64	1.8± 7.02	2.4 ± 6.56
Selected Individual visceral malformations				
- Membranous ventricular septum defect				
- Fetal incidence [N (%)] *(HCD 0-2.6%)*	1 (0.6)	0 (0.0)	1 (0.7)	3 (2.2)
- Litter incidence [N (%)] *(HCD 0-17.6%)*	1 (4.2)	0 (0.0)	1 (4.0)	3 (14)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-2.6%)*	0.5 ± 2.55	0.0 ± 0.00	0.5 ± 2.50	2.4 ± 6.56

( ) Values in brackets give % fetal respectively litter incidence

Visceral variations were observed in all treated groups [see Table 3.10.1.10-8]. Malpositioned carotid branch was the most common finding with the highest incidence in mid dose fetuses, without attaining statistically significance. The other finding (dilated cerebral ventricle) was observed in a single case only. None of the findings was considered to be treatment-related.

Table 3.10.1.10-8: Incidence of visceral (soft tissue) variations

Dose level [mg/kg]	0	5	20	75
Litters Evaluated	24	24	25	22
Fetuses Evaluated	164	166	150	135
Live	164	166	150	135
Dead	0	0	0	0
Total visceral variations				
- Fetal incidence [N (%)]	31 (19)	29 (17)	34 (23)	27 (20)
- Litter incidence [N (%)]	18 (75)	11 (71)	18 (72)	14 (64)
- Affected fetuses/litter (Mean ± SD) [%]	17.8 ± 15.69	17.4 ± 14.85	24.6 ± 20.86	20.5 ± 18.61
Individual visceral variations				
- Malpositioned carotid branch				
- Fetal incidence [N (%)]	30 (18)	25 (15)	34 (23)	24 (18)
- Litter incidence [N (%)]	17 (71)	16 (67)	18 (72)	12 (55)
- Affected fetuses/litter (Mean ± SD) [%]	17.2 ± 16.09	15.2 ± 13.23	24.6 ± 20.86	18.0 ± 18.99
- Dilated cerebral vertricle				
- Fetal incidence [N (%)]	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
- Litter incidence [N (%)]	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.6 ± 2.92	0.0 ± 0.00	0.0 ± 0.00

( ) Values in brackets give % fetal respectively litter incidence

Low incidences infarct of liver (2 fetuses of the mid and 1 fetus of the high dose) and fluid-filled abdomen (1 fetus of the mid dose only) were the only unclassified findings. The incidence was not indicative for a relation to treatment.

Skeletal examination

A number of skeletal malformations were observed in all groups including controls [see Table 3.10.1.10-9]. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range. The total skeletal malformations were statistically significantly increased in the high dose group on a litter and affected fetuses/litter basis without a dose-relationship. Individual skeletal malformations did not shown significant changes. Severely fused sternebra were slightly but statistically significantly increased in the high dose group (fetal incidence of 3). The incidence was observed to the same incidence in the control of the preceding prenatal developmental study in rabbits performed with dimoxystrobin and was therefore regarded as being incidental. No other individual skeletal malformation was significantly increased.

Table 3.10.1.10-9: Incidence of skeletal malformations and variations

Dose level [mg/kg]	**0**	**5**	**20**	**75**
Litters Evaluated	24	24	25	22
Fetuses Evaluated	164	166	150	135
Live	164	166	150	135
Dead	0	0	0	0
Total skeletal malformations				
- Fetal incidence [N (%)]	3 (1.8)	6 (3.6)	3 (2.0)	11 (8.1)
- Litter incidence	3 (13)	6 (25)	2 (8.0)	9* (41)
- Affected fetuses/litter (Mean ± SD) [%]	2.2 ± 6.16	3.4 ± 6.07	1.6 ± 5.65	9.5*± 14.07
Individual skeletal malformations				
- **Sternebra severely fused (Bony plate)**				
- Fetal incidence [N (%)]	0 (0.0)	0 (0.0)	1 (0.7)	3 (2.2)
- Litter incidence [N (%)]	0 (0.0)	0 (0.0)	1 (4.0)	3 (14)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.0 ± 0.00	0.5 ± 2.50	2.2*± 6.16
Total skeletal variations				
- Fetal incidence [N (%)]	100 (61)	107 (64)	92 (61)	94 (70)
- Litter incidence [N (%)]	24 (100)	24 (100)	25 (100)	22 (100)
- Affected fetuses/litter (Mean ± SD) [%]	62.7 ± 24.39	61.7 ± 20.88	59.4 ± 25.72	71.5 ± 17.00
Individual skeletal variations				
- **Fused sternebra; unchanged cartilage**				
- Fetal incidence [N (%)] *(HCD 0-10.7%)[1)]*	5 (3.0)	8 (4.8)	3 (2.0)	16 (12)
- Litter incidence [N (%)] *(HCD 0-50.0%)[1)]*	4 (17)	5 (21)	2 (8.0)	8 (36)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-13.5%)[1)]*	2.7 ± 6.29	4.6 ± 9.92	2.5 ±10.21	11.2 ±18.17
- **Incomplete Ossification of cervical centrum; unchanged cartilage**				
- Fetal incidence [N (%)]	16 (9.8)	17 (10)	28 (19)	22 (16)
- Litter incidence [N (%)]	8 (33)	9 (38)	**17*** (68)	12 (55)
- Affected fetuses/litter (Mean ± SD) [%]	9.5 ± 16.62	9.3 ± 15.01	**18.3***±17.68	14.7 ±16.84
- **Hemicentric cervical centrum; unchanged cartilage**				
- Fetal incidence [N (%)]	0 (0.0)	4 (2.4)	1 (0.7)	0 (0.0)
- Litter incidence [N (%)]	0 (0.0)	3 (13)	1 (4.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	**2.1***± 5.88	0.6 ±2.86	0.0 ± 0.00
- **incomplete ossification of thoracic centrum; unchanged cartilage**				
- Fetal incidence [N (%)]	0 (0.0)	1 (0.6)	3 (2.0)	0 (0.0)
- Litter incidence [N (%)]	0 (0.0)	1 (4.2)	3 (12)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ± 0.00	0.5 ±2.55	**1.4***±3.85	0.0 ± 0.00

* $p \leq 0.05$, ** $p \leq 0.01$ (Fisher's exact test, one-sided; Wilcoxon-test, one-sided)

[1)] extended historical control data covering a time span of roughly ± 5 years around the experimental date

Skeletal variations were observed in about 61 to 70% of the fetuses in all litters [see Table 3.10.1.10-9].

The variations were observed either singly, without dose response-relationship or at incidences comparable to the historical control range.

This includes the statistically significantly increased incidence of incomplete ossification of cervical centrum, which was statistically significantly increased only in the mid dose group. Furthermore, incomplete ossification of cervical centrum was not increased in the first prenatal developmental rabbit study up to the high dose of 100 mg/kg bw.

Incomplete ossification of thoracic centrum was statistically significantly increased in the mid dose only, but did not occur in the high dose group. Therefore, the effect is not considered treatment-related. Again, this finding was not evident in the first prenatal developmental rabbit study up to the high dose of 100 mg/kg bw, but was actually highest in the control animals with fetal incidence 6, litter incidence 5 and affected fetuses/litter 3.2.
Slight incidence of hemicentric cervical centrum was statistically significantly increased in the low dose only and did not show any dose-relationship.

An increased incidence in fused sternebrae was observed in the high dose group (11.2% affected fetuses/litter), which was not statistically significantly different from control. The incidence is well covered by the extended historical control range described above.

Discussion

Severe maternal toxicity was seen at 75 mg/kg bw, indicated by maternal deaths (2 females died) and no defecation. At ≥ 25 mg/kg bw the incidence of diarrhea was increased. Marked to excessive, but transient drop of food consumption and lower mean body weights / body weight gains were seen at ≥ 25 mg/kg bw.
At 75 mg/kg bw/day this resulted in increased non-statistically significant resorptions (mainly early resorptions) and post implantation loss. As a consequence gravid uterus weights were lower at this dose level without attaining statistical significance. No treatment-related malformations or variations were recorded.

Based on these effects the maternal NOAEL was 5 mg/kg. Developmental toxicity was observed at 75 mg/kg bw/day.

Based on the results of both prenatal toxicity studies in rabbits the overall **maternal NOAEL was 5 mg/kg bw/day**, the same value as concluded in the DAR 2003.
For the **developmental NOAEL 50 mg/kg bw/day** (from the first rabbit study) is proposed. In the previous evaluation (DAR 2003) "the NOAEL for prenatal developmental toxicity = 20 mg/kg bw/day based an increased incidence of a skeletal variation, fused sternebrae, at 25 mg/kg bw/day. Although the incidences of fused sternebrae at 25 and 50 mg/kg bw/day were within the historical control range there is some evidence of a dose response in the incidence of affected fetuses/litter at 25 mg/kg bw/day and above."

However, only a significant change outside the HCD is considered adverse. The results for fused sternebrae (variation) in the two rabbit studies are summarized in the table 3.10.1.10-10 and the table 3.10.1.10-11. Dose dependency can be seen in rabbit study 1, but not in rabbit study 2.
In rabbit study 1, adverse effect is observed at 100 mg/kg bw/day: fused sternebra, increased % of affected fetuses /litter, outside the HCD range.
In rabbit study 2, adverse effect is observed at 75 mg/kg bw/day: fused sternebra, increased fetal incidence, outside the HCD range.

Table 3.10.1.10-10: Fused sternebrae (variation) in the two rabbit studies

fused sternebrae (variation)	rabbit study 1 0, 25, 50, 100 mg/kg bw/day	rabbit study 2 0, 5, 20, 75 mg/kg bw/day	HCD, %
fetal incidence, %	1.3, 5.5, 6.2, 10	3.0, 4.8, 2.0, **12**	**0-10.7**
litter incidence, %	8, 36, 35, 47	17, 21, 8, 36	**0-50.0**
% affected fetuses/litter	0.9, 5.1, 6.5, **15.2**	2.7, 4.6, 2.5, 11.2	**0-13.5**

Table 3.10.1.10-11: Fused sternebrae (variation) in the two rabbit studies (pooled results)

fused sternebrae (variation)	dose, mg/kg bw/day							HCD, %
	0	**5**	**20**	**25**	**50**	**75**	**100**	
fetal incidence, %	1.3, 3.0	4.8	2.0	5.5	6.2	**12**	10	**0-10.7**
litter incidence, %	8, 17	21	8	36	35	36	47	**0-50.0**
% affected fetuses/litter	0.9, 2.7	4.6	2.5	5.1	6.5	11.2	**15.2**	**0-13.5**

Conclusion on the rabbit developmental toxicity studies

Maternal NOAEL was 5 mg/kg bw/day, the same value as concluded in the DAR 2003.
For the **developmental NOAEL 50 mg/kg bw/day** (from the first rabbit study) is proposed.
Dimoxystrobin is **not teratogenic** in rabbits.

3.10.1.11 Study 11

Developmental toxicity study in rabbits – collection of historical control data (BASF DocID 2013/1421980)

Control data of rabbit studies (Himalayan strain) conducted in the laboratories of BASF SE between April 1999 and November 2003 were collected to better understand the treatment relationship of the variation: "Fused sternebra" and the malformation "Severely fused sternebrae – bony plate". It is generally to be noted, that for some of the skeletal observations (variations or malformations), the historical incidences are presented with the addition of the comment "unchanged cartilage" or without this addition. This is because in the older studies the skeletons of the animals were assessed with single staining (alizarine red) only. With this staining technique, the cartilage structures can´t be assessed. In the later studies, a double staining technique was used to assess the cartilage (with alcian blue) and the bone (with alizarin red) structure. This is the reason, why there are two entries for the background incidences of e.g. "fused sternebra" with or without the addition of the comment "unchanged cartilage". These two entries could be added together and can be used synonymously – if the timeframe, in which the findings were noted is appropriate - to assess the treatment relationship of the observed findings in the studies.

The first rabbit developmental toxicity study (DocID 2000/1016867) conducted with dimoxystrobin (start date was October 1997) was using alizarin red staining technique only, the second rabbit developmental toxicity study (DocID 2001/1016351; start date was April 1999) used the double staining technique.

For the fetal skeletal variations "fused sternebra" litter incidences ranged from 0 – 41.7% with a mean value of 13.6%, the affected foetuses per litter varied from 0 – 9.0% (mean: 3.0%)

For the observation "fused sternebra unchanged cartilage" the litter incidences ranged from 0 – 50% with a mean value of 9.9%, the affected foetuses per litter varied from 0 – 13.5% (mean: 3.1%).

The fetal skeletal malformation "Sternebrae severely fused (bony plate)" litter incidences ranged from 0 – 8.3% with a mean value of 1.9%, the affected foetuses per litter varied from 0 – 0.8% (mean: 0.2%)

For the observation "Sternebrae severely fused (bony plate) unchanged cartilage" litter incidences ranged from 0 – 4.5% with a mean value of 0.6%, the affected foetuses per litter varied from 0 – 0.9% (mean: 0.1%)

25-OCT-13

TABLE :

SPECIES RABBIT
STRAIN HIMALAYAN RABBIT
SUPPLIER ALL

HISTORICAL CONTROL DATA
FETAL SKELETAL VARIATIONS

DATES: 01-JAN-98 - 31-DEC-03

	FETUSES N	FETUSES %	%RANGE (per study) %	%RANGE (per study) %	LITTERS N	LITTERS %	%RANGE (per study) %	AFFECTED FETUSES /LITTER %MEAN %	AFFECTED FETUSES /LITTER RANGE (per study) %	AFFECTED FETUSES /LITTER RANGE (per study) %
NUMBER EVALUATED	1081				162					
Live	1081									
Dead	0									
V SUPERNUMERARY THORACIC VERTEBRA	5	0.5	0.0	1.7	5	3.1	0.0	10.0	0.5	0.0	1.4
V SUPERNUMERARY LUMBAR VERTEBRA	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.1	0.0	0.7
V INCOMPLETE OSSIFICATION OF LUMBAR ARCH	2	0.2	0.0	0.7	2	1.2	0.0	4.2	0.2	0.0	0.7
V INCOMPLETE OSSIFICATION OF SACRAL ARCH	6	0.6	0.0	2.7	6	3.7	0.0	16.7	0.5	0.0	2.2
V UNOSSIFIED STERNEBRA	159	14.7	0.0	30.1	66	40.7	0.0	79.2	13.4	0.0	27.8
V UNOSSIFIED STERNEBRA Unchanged cartilage	91	8.4	0.0	30.8	35	21.6	0.0	68.2	8.3	0.0	29.6
V INCOMPLETE OSSIFICATION OF STERNEBRA	154	14.2	0.0	29.2	76	46.9	0.0	87.5	13.9	0.0	31.6
V INCOMPLETE OSSIFICATION OF STERNEBRA Unchanged cartilage	154	14.2	0.0	44.5	52	32.1	0.0	87.5	13.8	0.0	43.4
V MISSHAPEN STERNEBRA	27	2.5	0.0	6.2	26	16.0	0.0	37.5	2.6	0.0	6.5
V MISSHAPEN STERNEBRA Unchanged cartilage	36	3.3	0.0	11.6	27	16.7	0.0	55.0	4.5	0.0	15.8
V FUSED STERNEBRA	35	3.2	0.0	8.5	22	13.6	0.0	41.7	3.0	0.0	9.0
V FUSED STERNEBRA Unchanged cartilage	24	2.2	0.0	10.7	16	9.9	0.0	50.0	3.1	0.0	13.5
V EXTRA STERNEBRAL OSSIFICATION SITE	4	0.4	0.0	1.2	4	2.5	0.0	8.3	0.3	0.0	1.1
V EXTRA STERNEBRAL OSSIFICATION SITE Unchanged cartilage	3	0.3	0.0	1.4	3	1.9	0.0	9.1	0.8	0.0	4.2
V SHORT RIB (12TH)	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.1	0.0	0.5

OBSERVATION CODE: V=Variation

Figure 3.10.1.11-1: Summary of historical control data for the fetal skeletal variations (among others) in Himalayan rabbit studies

25-OCT-13

TABLE :

SPECIES RABBIT
STRAIN HIMALAYAN RABBIT
SUPPLIER ALL

HISTORICAL CONTROL DATA
FETAL SKELETAL MALFORMATIONS

DATES: 01-JAN-98 - 31-DEC-03

	FETUSES N	FETUSES %	%RANGE (per study) %	%RANGE (per study) %	LITTERS N	LITTERS %	%RANGE (per study) %	AFFECTED FETUSES /LITTER %MEAN %	AFFECTED FETUSES /LITTER RANGE (per study) %	AFFECTED FETUSES /LITTER RANGE (per study) %
NUMBER EVALUATED	1081				162					
Live	1081									
Dead	0									
M FETUS WITH MULTIPLE MALFORMATIONS	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.1	0.0	0.5
M FUSED SKULL BONES	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.1	0.0	0.8
M THORACIC CENTER, ARCH AND CORRESPONDING RIB ABSENT	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.2	0.0	1.0
M FUSED CERVICAL VERTEBRA	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.2	0.0	1.4
M ABSENT LUMBAR VERTEBRA	5	0.5	0.0	1.4	5	3.1	0.0	9.1	0.5	0.0	1.6
M MISSHAPEN LUMBAR VERTEBRA	1	0.09	0.0	0.7	1	0.6	0.0	4.2	0.1	0.0	0.5
M LUMBAR HEMIVERTEBRA	1	0.09	0.0	0.7	1	0.6	0.0	4.2	0.1	0.0	0.5
M MISSHAPEN SACRAL VERTEBRA	1	0.09	0.0	0.7	1	0.6	0.0	4.5	0.1	0.0	0.8
M STERNEBRAE SEVERELY FUSED (BONY PLATE)	3	0.3	0.0	1.1	3	1.9	0.0	8.3	0.2	0.0	0.8
M STERNEBRAE SEVERELY FUSED (BONY PLATE) Unchanged cartilage	1	0.09	0.0	0.7	1	0.6	0.0	4.5	0.1	0.0	0.9
M SEVERELY MALFORMED STERNUM	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.1	0.0	0.7
M BRANCHED RIB	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.1	0.0	0.7
M SHORT RIB Cartilage not present	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.6	0.0	4.2
M SEVERELY MALFORMED FORELIMB	1	0.09	0.0	0.6	1	0.6	0.0	4.2	0.1	0.0	0.7
TOTAL FETAL SKELETAL MALFORMATIONS	16	1.5	0.0	2.1	16	9.9	0.0	13.6	2.2	0.0	5.0

OBSERVATION CODE: M=Malformation

Figure 3.10.1.11-2: Summary of historical control data for the fetal skeletal malformations (among others) in Himalayan rabbit studies

3.10.1.12 Study 12

Mechanistical studies, rat

Study reference:

Anonymous (2002): Determination of early changes in parameters of Clinical Pathology in male Wistar rats after 36 days of oral administration in the diet. BASF AG, Ludwigshafen, Germany; BASF DocID 2002/1005354; April 2002

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp 336 – 339):

Title:	Determination of early changes in parameters of Clinical Pathology in male Wistar rats after 36 days of oral administration in the diet (KIIA 5.8.2/5, BASF DocID 2002/1005354)
Guidelines:	No test guideline exists for this type of study.
Deviations:	NA
GLP:	Yes
Acceptance:	The study was considered acceptable in the EU registration process 2003.

Assessment from the DAR 2003:

This study was designed to investigate short-term effects of the test compound on biochemical parameters in the serum of Wistar rats in order to provide clues as to the possible cause of duodenal thickening observed in previous studies. For this purpose several enzymes and chemical substrates were examined after administration of 4,500 ppm (the highest dose level used in the subchronic rat study, at which slight to moderate thickening of the duodenal mucosa was seen).
No test guideline exists for this type of study. The study was not conducted in full compliance of GLP because the study protocol, experimental phase and the report were not checked by QAU. However the study is considered to be scientifically acceptable.

Dimoxystrobin (batch/purity: N 15: 98.4%) was administered to groups of 10 male Wistar rats (CrlGlxBrlHan:Wi), aged 11 weeks at the start of administration, at dietary concentrations of 0 ppm and 4,500 ppm (232 mg/kg bw/day) for 3 weeks. The study then continued for a further 2 weeks with all rats dosed as before (mean intake of test substance over this period was 264 mg/kg bw/day) except that 5 rats from the test group were fed control food to determine the reversibility of effects.

Test diets were prepared once and used over a period within the known stability of the test material in diet. Homogeneity of the dietary test substance preparation and correctness of the concentrations were analytically demonstrated.

The animals were examined for evident signs of toxicity or mortality at least once a day. Body weight was determined before the start of the administration period, and then weekly. Food consumption was determined weekly. Blood sampling was performed on study days –5, 1, 2, 5, 15, 30 and 36. Blood was taken from all rats (non fasted) except on day 30 when blood was taken from 5 fasted control rats.

Haematology was investigated on day 30 only. The following clinical-chemical parameters were determined in serum from day –5 to day 30:

alanine aminotransferase
alkaline phosphatase
serum-γ-glutamyltransferase
glutamate dehydrogenase
lipase
α-amylase
aldolase
calcium
urea
glucose
total bilirubin
albumin
triglycerides
iron (also on day 36)*
bile acids
transferrin (on day 30 only)
unsaturated iron binding capacity (on day 36, only)
[* iron was measured by the guanidine/ferrozine method without deproteinisation.] (Eisenwiener *et al* 1979)[17].

The applicant has confirmed (Kieczka, 2003) that this method measures the concentration of total iron in serum. Total serum iron includes transferrin-bound iron, any free iron (if present) and the marginal portion of iron bound to other proteins, in particular ferritin (but not haemoglobin). The measured serum iron is therefore predominantly transferrin-associated Fe^{3+} iron. The RMS also considers it probable that any iron bound by chelation to dimoxystrobin and/or its metabolites would also be measured by this technique. The method seems to involve use of guanidine to liberate iron from transferrin, the use of ascorbic acid to reduce ferric to ferrous iron and then reaction of free ferrous iron with ferrozine to form a chromogen.

Findings
GENERAL OBSERVATIONS

THERE WERE NO DEATHS OR CLINICAL SIGNS OF TOXICITY.

There was a clear impairment of food consumption at 4,500 ppm (by about 22- 50% below control) but food consumption returned to normal by day 33 in the recovery group.
Body weight was depressed (by 19% at day 33, which equated to slight body weight loss) in the group feed 4,500 ppm continuously but had returned to normal by day 33 in the recovery group.

[17] Eisenwiener *et al.*, 1979. Die bestimmung des eisens mit der guanidiniumchlorid/ferrozin-methode. J. Clin.Chem.Clin. Biochem. 17, 149.

Clinical chemistry
Probably as a consequence of the reduced food consumption, parameters like alanine aminotransferase, alkaline phosphatase, glucose, and triglycerides were reduced for most of the exposure period in the study (starting at days 1-2). Serum iron and glutamate dehydrogenase were also reduced for most of exposure period in the study (from day1).

The most marked effects were on iron (reduced by up to 74%), alkaline phosphatase (reduced by 40-54% on days 1-15) and triglycerides (reduced by 30-60% on days 1-15).

Total bilirubin was increased from days 1-15 (by 32-70%).
All these changes returned to normal levels during the recovery periods.

The effects on serum iron are considered the most notable findings (see Table 3.10.1.12-1).

Table 3.10.1.12-1 Serum iron concentrations in rats administered dimoxystrobin for 3-5 weeks

Dose ppm	Serum iron concentration (µmol/l)								
	day -0	**Day 1**	**Day 2**	**Day 5**	**Day 15**	**Day 30a**	**Day 36a**	**Day 30b 19 + 11†**	**Day 36b 19 + 17†**
0	42.2	52.8	46.8	57.3	45.9	37.2	43.0	37.2	43.0
4,500	48.5	25.6**	25.4**	14.7**	16.2**	24.4*	13.4**	55.9	54.9
% change from control	+15%	-51%	-46%	-74%	-65%	-34%	-69%	+50%	+28%

* $p \leq 0.05$, ** $p \leq 0.01$ Wilcoxon test (2-sided)
† 19+11 and 19+17 = 19 days on test diet then a recovery period of 11 or 17 days
group sizes = 10/group, except a) =5/group and b) = 10 controls + 5 test

Already after 1 day of test substance administration the iron level in the serum of the treated animals was decreased by 51% compared to the corresponding control. On day 5, serum iron concentration of the treatment group was decreased by about 74% of the respective control. There was no effect on the serum level of transferrin (the carrier of iron in plasma) when measured on day 30. However after 36 days there was a significant increase in unsaturated iron binding capacity (indicating a depletion of iron reserves in the body). However no increase was seen in unsaturated binding capacity following 19 days of exposure and a 17-day recovery period.

The applicant considers that due to "excessive" content of iron in the diet, the decrease in serum iron concentration in this study was mainly related to an interaction of dimoxystrobin with iron kinetics rather than to the decreased food consumption.

[**Note:** The iron content of the diet in all studies with dimoxystrobin was 250 mg/kg for rats and mice, and 200 mg/kg for dogs. The applicant considers that these iron contents are sufficiently high ("excessive") to satisfy the demands of the animal even if a reduction in food intake occurs or a (hypothetical) chemical interaction of food iron with the test compound is taken into account.]

Since it is known that iron deficiency causes thickening of the duodenum in rats (Smith *et al*. 2000)[12], it is assumed that the thickening of the duodenum induced by dimoxystrobin is also correlated to the decrease in serum iron concentration.

Haematology

After exposure for 30 days, there were statistically significant decreases in haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. There were also significant increases in white blood cells, platelets and reticulocytes.

In animals exposed for 19 days followed by a 11 day recovery period, there were still some statistically significant reductions in some parameters (haemoglobin, haematocrit, MCV and MCH) but the magnitude of the reductions was clearly less than in animals exposed for 30 days, i.e. there was some but not complete recovery in haematological effects.

Conclusion

Dietary administration of dimoxystrobin at a dose level of 4,500 ppm caused reduced food consumption and body weight gain, a number of clinical chemistry changes (fully reversible) and haematological changes indicative of microcytic hypochromic anaemia (partly reversible within a 11 day recovery period).

Many of the clinical chemistry changes were probably caused by reduced food consumption. However it is proposed that the distinct decrease in serum iron was caused by dimoxystrobin affecting iron kinetics. It is also proposed that the decrease in serum iron resulted in an iron deficiency anaemia (iron deficiency is a known cause of microcytic hypochromic anaemia, see Howard and Hamilton 1997)[7] and an adaptive thickening of the duodenal mucosa (also a known response to iron deficiency) in order to increase the absorption of iron.

The marked decrease in serum iron after dosing for only one day is particularly notable. The RMS (UK) notes that this raises the mechanistic question as to whether dimoxystrobin simply affected the absorption rate of iron and/or whether some other mechanism was involved e.g. possible chelation of iron by dimoxystrobin/metabolites in food and/or serum.

12 Smith *et al*., 2000. Structural and cellular adaptation of duodenal iron uptake in rats maintained on an iron-deficient diet. Pfluegers Arch - Eur. J. Physiol. 439, 449-454.

7 Howard & Hamilton, 1997. Haematology, an illustrated colour text. Churchill Livingstone, New York, USA.

3.10.1.13 Study 13

Mechanistical studies, rat

Study reference:

Anonymous (2002): Screening study BAS 505F: Administration I the diet and determination of serum iron after 2 and 6 days. BASF AG, Ludwigshafen, Germany; BASF DocID 2002/1014245; December 2002

and

Anonymous (2003): Amendment to the study: Screening study BAS 505F: Administration I the diet and determination of serum iron after 2 and 6 days. BASF AG, Ludwigshafen, Germany; BASF DocID 2003/1009198; March 2003

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp 339 – 340):

Title:	Screening study, BAS 505F- Administration of serum iron after 2 and 6 days (Anonymous, KIIA 5.8.2/7, BASFDoc ID: **2002/1014245)**
Guidelines:	No test guideline exists for this type of study.
Deviations:	NA
GLP:	Yes
Acceptance:	The study was considered acceptable in the EU registration process 2003.

Assessment from the DAR 2003:

This screening study was to determine the NOEL for the decrease in serum iron after oral administration of dimoxystrobin.

Dimoxystrobin (batch/purity:N15 : 98.4%) was administered to groups of 6 male Wistar rats (CrlGlxBrlHan:Wi), aged 10 weeks, at dietary concentrations of 0, 10, 50 250 and 500 ppm for 7 days. Body weights and food intake were not investigated. Therefore intake of dimoxystrobin has been calculated approximately based on intakes for rats of similar age (but female and therefore probably of lower body weight) administered 500 ppm for 7 days in the following study. On this basis intakes of dimoxystrobin in the present study are calculated to be approximately:

0, 1, 4, 20 and 40 mg/kg bw/day

Test diets were prepared once and used over a period within the known stability of the test material in diet. No analysis of the test diets for homogeneity, stability or test substance concentrations were performed. The test diet was basic maintenance diet, the same as used in other rat studies, and therefore is expected to have contained 250 mg iron/kg.

Animals were observed for clinical signs of toxicity. Blood was taken from non-fasted rats on the morning of days 2 and 6. Serum iron was measured by the guanidine/ferrozine method without deproteinisation. Animals were killed on day 7. No further investigations were perfomed.

Findings

Table 3.10.1.13-1 Serum iron concentrations for rats administered dimoxystrobin in the diet for up to 6 days

		Serum iron concentration (micromol/l)				
		0 ppm	**10 ppm**	**50 ppm**	**250 ppm**	**500 ppm**
Day 2	Mean (SD)	52.7 (11.4)	52.6 (6.9)	47.6 (8.5)	37.35* (4.9)	34.25** (7.5)
	Range	38.5-72.1	43.4-61.0	32.5-57.2	31.4-43.0	24.5-43.8
	% change in mean from control		-0.1%	-9.7%	-29%	-35%
Day 6	Mean (SD)	58.2 (12.8)	61.73 (13.5)	55.78 (8.0)	39.62* (6.6)	37.92** (5.7)
	Range	45.4-77.8	43.5-85.0	44.4-67.7	34.0-51.4	31.2-46.8
	% change in mean from control		+6.2%	-4.1%	-32%	-35%

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

There were no deaths or clinical signs of toxicity.
There was a clear statistically significant decrease in serum iron, by about 30%, after administration of 250 or 500 ppm for 2 and 6 days (Table 3.10.1.13-1). There was also a marginal decrease, by 4-10%, at 50 ppm which was not statistically significant. The study investigators considered that the biological and toxicological relevance of this finding to be questionable.

The RMS notes that consideration of the individual serum levels shows that the values at 50 ppm were broadly similar to controls, with only 1 rat having a serum iron level marginally outside the concurrent control range. Hence, even in the absence of historical control data or predose serum iron levels for the rats in this study, it would appear that there was no toxicologically important effect at 50 ppm. This is also supported by the fact that the mean serum iron level at 50 ppm on day 6 was greater than the mean control value on day 2.

Conclusion (from the DAR 2003)

A NOAEL of 50 ppm (equivalent to about 4 mg/kg bw/day) is proposed by the RMS for decreased serum iron in this study based on effects at 250 ppm (equivalent to about 20 mg/kg bw/day). The extent of serum iron depression at 250 and 500 ppm was similar after 2 and 6 days.

3.10.1.14 Study 14

Mechanistical studies, rat
Study reference:

Anonymous (2005): BAS 505 F - Determination of serum iron concentration in young and adult male Wistar rats after oral administration in the diet over 7 days. BASF AG, Ludwigshafen, Germany; BASF DocID 2005/1004845; January 2005

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp 356 – 364):

Report:	KCA 5.8.2/5 Anonymous , 2005b BAS 505 F - Determination of serum iron concentration in young and adult male Wistar rats after oral administration in the diet over 7 days 2005/1004845
Guidelines:	none
GLP:	yes
peer-reviewed:	not (the former RMS evaluated the study in an Addendum to DAR in June 2005)
Acceptability:	considered acceptable by RMS (2005), considered supplementary by the RMS (2017)

Executive Summary

The aim of this study was to determine the level of iron in serum after dietary administration of dimoxystrobin. Dimoxystrobin was administered to groups of 10 young (3 weeks old) and adult (10 weeks old) male Wistar rats at dietary concentrations of 0, and 500 ppm over a period of 7 days. Due to the low body weights and the higher basal metabolic rate of young rats as compared to adult animals, the animals had a higher test substance intake (65.3 mg/kg bw/day) during the treatment period as compared to adults (33.4 mg/kg bw/day). Thus, an additional group of young rats was included in the study receiving a test substance concentration of 250 ppm in the diet (33.8 mg/kg bw/day) in order to achieve a similar test substance intake at the end of the 7 days of administration.
Food consumption was impaired in all treatment groups. Body weight was slightly reduced in young animals treated with 250 ppm of test substance.
Serum iron concentrations were decreased in all treated animals. The reduction was -42% and -37.9% in young animals and -20.8% and -18.3% in adult animals at study day 2 and 7, respectively. Since iron levels in young animals were distinctly higher than in adult animals at the start of the study, a comparison between serum iron reductions between young and adult animals may be misleading. Therefore, young animals are not more sensitive to the test substance compared to adult animals. During macroscopic examination a thickening of the duodenum was observed only in adult animals after treatment with the test substance. No duodenum findings were observed in young animals treated with 250 or 500 ppm of dimoxystrobin.

In conclusion, dimoxystrobin reduced serum iron concentration in young and adult Wistar rats with no higher susceptibility in young rats. The RMS did not agree with this conclusion by the notifier.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Dimoxystrobin (BAS 505 F)

Description:	solid / powder / white
Lot/Batch #:	N15
Purity:	98.4%
Stability of test compound:	Stable - Expiry date January 2007

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Wistar (CrlGlxBrlHan:WI)
Sex:	Male
Age:	3 - 10 weeks
Weight at dosing:	35.6 – 47.2 (young), 230.5 – 252.8 (adult)
Source:	Charles River, Sulzfeld, Germany
Acclimation period:	6 days
Diet:	basic maintenance diet for rat/mouse "GLP", meal from Provimi KLIBA SA, Kaiseraugust, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	single housing in type DK III stainless steel wire mesh cages, floor area about 800 cm²
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	not reported
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: 21-Sep-2004 - 28-Oct-2004

2. Animal assignment and treatment:

Dimoxystrobin was administered to groups of 10 male rats (3 weeks old) at dietary concentrations of 0, 250, and 500 for 7 days. In a second experiment, Dimoxystrobin was administered to groups of 10 male rats (10 weeks old) at dietary concentrations of 0 and 500 for 7 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

3. Test substance preparation and analysis:

For each preparation, the test substance was weighed out and thoroughly mixed with a smaller amount of diet. Then food was added in order to obtain the desired concentrations, and mixing was carried out for about 10 minutes in a laboratory mixer. The mixtures were prepared once before the start of the study.

No analyses of the test substance preparations were carried out for this study.

4. Statistics:

Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Statistics of clinical examinations, clinical pathology and pathology

Parameter	Statistical test
Food consumption, body weight	A comparison of the dose group with the control group was performed using Welch t-test (two-sided) for the hypothesis of equal means.
Blood chemistry	Pair-wise comparison of each substance with a control group was performed using Wilcoxon-test (two-sided) for the equal means.

C. METHODS

1. Observations:

The animals were examined for evident signs of toxicity or mortality twice daily on working days and once daily on weekends and public holidays. Observations for general clinical signs of toxicity were performed once daily.

2. Body weight:

The body weight of the animals was determined on the day of randomization and at the end of the study.

3. Food consumption and compound intake:

Individual food consumption was determined at the end of the study and calculated as mean food consumption in grams per animal and day.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$Substance\ intake\ for\ day\ x = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in mg/kg, and BW_x as body weight on day x of the study (in g).

4. Clinical pathology:

Non-fasted animals were anesthetized with Isoflurane anesthesia. The blood sampling procedure and the subsequent analysis of the serum samples were carried out in a randomized sequence. For all animals iron levels were determined.

5. Urinalysis:

Not determined in the study.

6. Sacrifice and pathology:

The animals were sacrificed by decapitation under carbon dioxide anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of dimoxystrobin in the diet over a period of 49 days at room temperature was determined in a previous study (08B0264/966003; BASF DocID 1997/1008341). No further analyses of the test substance were carried out for this study.

B. OBSERVATIONS

1. Clinical signs of toxicity

There was nothing abnormal detected in any animal.

2. Mortality

Two control animals (group 4) died during the blood sampling.

C. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight of animals treated with 250 ppm of dimoxystrobin was found slightly reduced (-6%) but statistically significant at the end of the study (see Table 3.10.1.14-1).

Table 3.10.1.14-1: Body weight (g) of male rats (3 and 10 weeks old) at day 0 and day 7 of the experiment

	Males (3 weeks)		Males (10 weeks)	
Day	0	7	0	7
Dose level [ppm]				
Experiment 1				
0	39.3 ± 2.7	73.9 ± 4.0	240.6 ± 7.1	277.2 ± 16.1
500	39.2 ± 2.7	70.5 ± 4.3	240.1 ± 6.2	276.7 ± 9.9
	-0.4% vs control	-4.6% vs control	-0.2% vs control	-0.2% vs control
Experiment 2				
0	42.1 ± 3.3	78.3 ± 4.1	-	-
250	41.2 ± 2.9	73.6 ± 4.8*	-	-
	-2.2% vs control	-6.0% vs control	-	-

*: $p \leq 0.05$

D. FOOD CONSUMPTION AND COMPOUND INTAKE

An impairment of food consumption (group 1: -8.8%; group 3: -9.4%; group 5: -8.5%) was observed in all animal groups treated with dimoxystrobin (see Table 3.10.1.14-2).

The approximate mean daily test substance intake was calculated to be 33.8 and 65.3 mg/kg bw/day in young males (3 weeks old) in the 250 and 500 ppm test group. The mean daily test substance intake for the older males (10 week old) in the 500 ppm test group was 33.4 mg/kg bw/day.

Table 3.10.1.14-2: Daily food consumption (g/animal per day) of male rats (3 and 10 weeks old) after administration of dimoxystrobin for 7 days

	Males (3 weeks)	Males (10 weeks)
day	7	7
Dose level [ppm]		
Experiment 1		
0	10.1 ± 0.7	20.4 ± 1.9
500	9.2 ± 6**	18.5 ± 1.0*
	-8.8% vs control	-9.4% vs control
Experiment 2		
0	10.9 ± 0.6	-
250	10.0 ± 0.8*	-
	-8.5% vs control	-

*: $p \leq 0.05$, **: $p \leq 0.01$

E. BLOOD ANALYSIS

1. Clinical chemistry

All animals showed significant reduction of iron concentration in serum after 2 and 7 days of dimoxystrobin treatment compared to control animals [see Table 3.10.1.14-3]. The serum iron concentrations determined in the young control animals (92.6 and 95.3 µmol/L at days 2 and 7) were roughly factor 2 higher than the respective control values in 10 week old rats (43.1 and 44.0 µmol/L at days 2 and 7). The determined serum iron concentrations were decreased by 42.0 and 37.9 % at 33.8 mg/kg bw in young animals, compared to reductions of only -20.8 and -18.0% in the 10 week old animals, however the measured serum iron concentrations were higher in young 56.0 and 59.7 µmol/L) compared to the adult animals (34.2 and 36.0 µmol/L) at equivalent dose levels. Thus the apparent higher susceptibility of young animals to show decreased serum iron levels after dimoxystrobin treatment is not plausible.

Table 3.10.1.14-3: Serum iron concentration (µmol/L) of male rats (3 and 10 weeks old) after administration of dimoxystrobin for 2 and 7 days

Animal	Blood sampling		0 ppm	250 ppm (33.8 mg/kg bw)	500 ppm (65.3 mg/kg bw
Males (3 weeks)	Day 2	Mean % Dev.	92.6 ± 7.5	56.0 ± 27.8** -42.0	35.8 ± 14.0** -61.3
	Day 7	Mean % Dev.	95.3 ±6.0	59.7 ± 23.3** -37.9	33.6 ± 13.1** -64.8
			0 ppm		500 ppm (33.4 mg/kg bw)
Males (10 weeks)	Day 2	Mean % Dev.	43.1 ±5.9	-	34.2 ± 3.1** -20.8
	Day 7	Mean % Dev.	44.0 ±3.5	-	36.0 ± 3.5** -18.0

** $p \leq 0.01$

F. NECROPSY

A thickening of the duodenum was observed during macroscopic examination in seven rats from group 3 (10 weeks old animals receiving 500 ppm of dimoxystrobin). This finding is considered as being related to treatment. No duodenum findings were observed in the other animal groups.

Discussion (notifier):
In order to assess, whether the ≈ 40% decrease seen in young animals dosed with 33.8 mg dimoxystrobin/kg bw for one week compared to the ≈ 20% decrease seen in 10 week old rats dosed with 33.4 mg dimoxystrobin/kg bw is an indication for a higher sensitivity of young animals, a small literature review had been conducted. In Naylor and Harrison, 1995 (BASF DocID 1995/1003711), the gastrointestinal iron absorption and the iron status of young rats at different ages has been compared. A difference in iron status and in iron absorption has been determined depending of the age of rats. Rats were either fed or intraperitoneally injected ^{59}Fe solutions and the % retentions in the body were measured. The fractional absorption (absorption which is specifically measured after oral ingestion compared to parenteral administration) values for iron from rats at different ages are displayed in the following table:

Table 3.10.1.14-4: Fractional absorption values for Fe from rats and total quantity of Fe in the rat livers at different ages

Age (days)	Fractional Fe absorption*	Rat liver	
		µg Fe/g	Total Fe [µg]
1	0.97 ± 0.02	524 ± 80	75 ± 18
10	1.02 ± 0.01	158 ± 18	66 ± 5
20	0.69 ± 0.02	105 ± 16	165 ± 35
30	0.24 ± 0.02	233 ± 6	735 ± 29
60	0.23 ± 0.02	242 ± 25	1670 ± 97
200	0.08 ± 0.01	327 ± 23	4530 ± 480

*Mean ± SD for the ratio of retention after oral administration (n = 5-8) and intraperitoneal injection (n=3-6)

Comparing the fractional iron absorption of the 20-day old rats with the mean value determined for 60-day old rats, it becomes obvious, that younger rats absorb 3 times higher percentages of iron after oral administration compared to parenteral absorption. At the same time the total quantity of iron in the livers of rats indicates significantly higher values of µg Fe/g liver and in total Fe in the 60-day old rats compared to the 20-day old rats. A comparison of middle-aged (8-10 months) and old (20 – 22 months) Lewis rats also gave evidence, that the plasma iron levels are lower in older rats (by roughly a factor of 2) (Ahluwalia N et al., 2000; BASF DocID 2000/1023158)

In the present study the effect of dimoxystrobin on plasma iron levels in 3 week old rats compared to 10 week old rats was studied. An age-related difference in plasma iron levels had been confirmed as well. The levels were 92.6 and 95.3 µmol/L in untreated young rats (age of 22 and 28 days) and 43.1 and 44.0 µmol/L in 72 and 77 day old rats. These values are considered to be indicative for a higher physiological iron absorption in untreated rats at 3 week of age compared to the 10-week old rats, as shown by Naylor and Harrison, 1995. As the physiological iron absorption had not been measured in the present study, but only the absolute iron plasma levels, an assessment of different sensitivities between 3-week old and 10-week old rats based on the present study is not possible. It is assumed, that the physiological iron absorption in the 3-week old rats is considerably higher than in 10 week old rats (compared to Naylor and Harrison) by roughly a factor of 3 (0.69 vs 0.23) and thus the observed factor of 2 (percent decrease in serum iron concentrations from treated compared to untreated 3 week and 10 week old rats) is **not** an indicator of a higher sensitivity of weanling rats compared to young adult rats. This is further confirmed by the more recently conducted studies, where a clear NOAEL for changes in serum iron level had been confirmed in 3-week old rats (DocID 2010/1026748) and also clear NOAELs of 4 mg/kg bw for serum iron changes and hematology had been determined in an enhanced One-Generation toxicity study in offspring and adult rats.

III. CONCLUSION (notifier)

Dietary administration of dimoxystrobin to male rats at dose levels of 250 and 500 ppm (young, 3 weeks old corresponding to 33.8 and 65.3 mg/kg bw) or 500 ppm (adult, 10 weeks old, corresponding to 33.4 mg/kg bw) over a period of 7 days resulted in a decrease of serum iron concentrations in all treated animals. The percent decrease of serum iron levels was about -40% in young animals and about -20% in adult animals, however the iron levels in young control animals were factor 2 higher compared to the respective control values in the adults (95.3 vs 44.0 µmol/L at day 7 after administration). Thus, regarding the decrease of serum iron concentration in all treated animals, the no observed effect level was < 250 ppm.

Based on the existing age-related differences in hematologic and iron status it cannot be assumed, that young animals are more sensitive to the test substance than adult animals.
This assessment is further supported by the NOAEL of roughly 4 mg/kg bw determined for decreased serum iron levels in young (3 week old) rats in the new study (see BASF DocID 2010/1026748). 4 mg/kg bw was also the NOAEL determined for adult rats (see BASF DocID 2002/1014245).

Conclusion of the RMS (2005)

Exposure to dimoxystrobin in the diet reduced serum iron concentration in young and adult Wistar rats. The evidence indicates that young rats are more sensitive (by 2-8 times, depending how the comparison is made) than adult rats to the effect of dimoxystrobin on serum iron levels. However for the purposes of risk assessment a difference in sensitivity of up to c.5 times would seem to be more relevant.
The RMS does not agree with the study authors who concluded that:
Dimoxystrobin reduces serum iron concentration in young and adult Wistar rats. Since iron levels in young animals were distinctly higher than in adult animals at the start of the study, a comparison of serum iron reductions between young and adult animals may be misleading. Therefore it cannot be assumed that young animals are more sensitive to the test substance than adult animals.

[The RMS notes that a smaller difference in sensitivity between adults and young is apparent if the serum iron findings for young from this study are compared with those for 10 week-old males exposed to 500 ppm dimoxystrobin for up to 6 days in a previous study, see Table B.6.8.2-8 (Table B.6.51 in the DAR 2003). However less weight should be placed on comparing results from separate studies (eg methods of measuring serum iron were slightly different) than on data derived from within the same study.]

Conclusion of RMS (2017):
It is known that there are age related differences in hematological parameters and iron status in rats. Untreated control animals in this study (22-28 days of age) however did not show age related differences in serum iron level. (In study BASF DocID 2010/1026748 the serum iron concentration of untreated animals/22-28 days of age/ decreased by about 30% between day 2 and day 7 of the experiment.) There are some deviations from GLP requirements: no analytical determinations of the test substance preparations were carried out, no acclimatization period was kept between the arrival of the animals and the beginning of the treatment and the GLP inspection certificate was almost 4 years old. In addition the standard deviation of iron concentration values in the 250 ppm group are very high, with a range of 13.8-94.7 µmol/L on day2 and 36.0-112 µmol/L on day7.
On the basis of the study results sensitivity of 3 week and 10 week old rats to Dimoxystrobin cannot be compared and the study can not to be used for risk assessment or classification purposes. It can be concluded that feed consumption relative to body weight of 3 week old male rats is 2 times higher than that of 10 week old male rats.

In order to derive a NOAEL for effects of dimoxystrobin on serum iron in young rats, a further mechanistic study was performed to determine serum iron levels in young (3-week old) rats after oral administration of dimoxystrobin.

3.10.1.15 Study 15

Mechanistical studies, rat

Study reference:

Anonymous (2010): BAS 505 F (Dimoxystrobin) - Determination of serum iron concentration of young male Wistar rats after oral administration via the diet over 7 days. BASF SE, Ludwigshafen, Germany; BASF DocID 2010/1026748; February 2010

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-6, pp 365 - 370:

Report:	KCA 5.8.2/6 Anonymous, 2010a BAS 505 F (Dimoxystrobin) - Determination of serum iron concentration of young male Wistar rats after oral administration via the diet over 7 days 2010/1026748
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Acceptability:	Yes

Executive Summary

The aim of this study was to determine the level of iron and transferrin in serum after oral administration of dimoxystrobin. Dimoxystrobin was administered to groups of 10 young, male Wistar rats at dietary concentrations of 6, 11 and 22 ppm over a period of 7 days. A target dose of 22 ppm (4 mg/kg body weight/day) has been chosen, as this dose was found to be the NOAEL for adult rats with respect to iron concentrations in blood serum.

Based on clinical findings the oral administration of dimoxystrobin caused no signs of general systemic toxicity. With regard to clinical pathology findings, no treatment-related findings or differences were observed after gross examination. In summary, oral administration of Dimoxystrobin via dietary incorporation at levels of 6, 11 and 22 ppm, over a period of 7 days caused no test substance-related adverse effects. The study did not reveal any toxicologically relevant difference.

(BASF DocID 2010/1026748)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Dimoxystrobin **(**BAS 505 F, 06/0629-2)

Description:	solid / white
Lot/Batch #:	01171-55
Purity:	99.7% (±1.0%)
Stability of test compound:	The test substance was stable at room temperature over the study period.

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Crl:WI (Han)
Sex:	Male
Age:	21 days (start of administration)
Weight at dosing:	♂: 48.17 – 48.69 g
Source:	Charles River Laboratories, Sulzfeld, Germany
Acclimation period:	8 days
Diet:	basic maintenance diet for rat/mouse, meal from Provimi KLIBA SA, Kaiseraugust, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	individually during administration period in Makrolon, type M III cages, floor area about 800 cm^2

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 AND METHODS

1. Dates of experimental work: 06-Oct-2009 - 04-Feb-2010
(In life dates: 06-Oct-2009 (start of administration) to 21-Oct-2009 (necropsy)

2. Animal assignment and treatment:
Dimoxystrobin was administered to groups of 10 male rats at dietary concentrations of 0, 6 (low dose), 11 (mid dose) and 22 ppm (high dose) for up to 7 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

3. Test substance preparation and analysis:
For each preparation, the test substance was weighed out and mixed with a small amount of food. Then, corresponding amounts of food, depending on test 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. Details of the mixers used are retained with the raw data. The mixtures were prepared once before the start of the study.
The animals received dietary concentrations of dimoxystrobin based on control data for food consumption and body weight of a previous study (48C0360/96072; BASF DocID 2005/1004845).
Mean daily food consumption males, age 21 to 28 days: 10 g.
Mean body weight males, age 21/28 days: 56 g.
The dietary concentrations of dimoxystrobin were calculated using the following formula:

$$ppm = \frac{BW_x \times D}{FC_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, D as the dose in mg/kg bw, and BW_x as mean body weight on day x of the study (in g).

The stability of the test substance in the diet over a period of up to 49 days at room temperature was proven earlier. Homogeneity was verified from the low and high concentration at the beginning of the study (was used as a concentration control at the same time). Concentration control analysis was carried out from the mid concentration.

4. Statistics:
Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Statistics of clinical examinations, clinical pathology and pathology

Parameter	Statistical test
Body weight, body weight change	A comparison of the dose group with the control group was performed using DUNNETT´s test (two-sided) for the hypothesis of equal means.
Clinical phathology	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.

C. METHODS

1. Observations:
The animals were examined for evident signs of toxicity or mortality twice daily on working days and once daily on weekends and public holidays. Observations for general clinical signs of toxicity were performed once daily.

2. Body weight:
The body weight of the animals was determined on day 0 (start of administration period), 2 and 7. The difference between the consecutive days of weighing was calculated as body weight change.

3. Food consumption and compound intake:
Individual food consumption was determined on study days 2 and 7 and calculated as mean food consumption in grams per animal and day.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$Substance\ intake\ for\ day\ x = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm, and BW_x as body weight on day x of the study (in g).

4. Water consumption:

Water consumption was observed daily by visual inspection of the water bottles for any changes in volume.

5. Hematology and clinical chemistry:

Blood samples were taken from the retroorbital venous plexus in the morning (day 7) from fasted animals. For all animals iron and transferrin was determined.

6. Sacrifice and pathology:

The animals were sacrificed using carbon dioxide, necropsied and assessed by gross pathology. No further examinations were carried out.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

There was nothing abnormal detected in dams and test animals.

2. Mortality

No animal died during the study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

No test group showed significant deviations in comparison to the control animals [see Table 3.10.1.15-1].

Table 3.10.1.15-1: Body weight and body weight gain data after administration of dimoxystrobin

Sex	Blood sampling		0 ppm	6 ppm	11 ppm	22 ppm
Males	Day 0	Mean±SD [µmol/L]	48.62 ± 2.89	48.63 ± 3.03	48.69 ± 2.63	48.17 ± 2.82
	Day 2	Mean±SD [µmol/L]	53.86 ± 5.00	52.57 ± 4.46	53.50 ± 3.42	51.51 ± 3.83
		deviation vs control		0.02	0.14	-0.93
	Day 7	Mean±SD [µmol/L]	79.52 ± 5.70	77.11 ± 5.85	79.04 ± 4.39	77.68 ± 3.87
		deviation vs control		-3.03	-0.60	-2.31

C. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption was not influenced by dosing with the test substance during the study (see Table 3.10.1.15-2).

Table 3.10.1.15-2: Food consumption after administration of dimoxystrobin

Sex	Blood sampling		0 ppm	6 ppm	11 ppm	22 ppm
Males	Day 2	Mean ± SD [µmol/L]	6.96 ± 1.32	7.14 ± 1.36	7.38 ± 1.03	6.92 ± 0.91
		deviation vs control		2.66	6.11	-0.43
	Day 7	Mean ± SD [µmol/L]	10.52 ± 0.64	10.72 ± 0.91	10.61 ± 0.77	10.46 ± 0.73
		deviation vs control		1.90	0.89	-0.53

The approximate mean daily test substance intake was calculated to be 0.95, 1.71 and 3.42 mg/kg bw/day at dietary dose levels of 6, 11 and 22 ppm, respectively.

D. BLOOD ANALYSIS

1. Clinical chemistry findings

No treatment-related changes of the iron and transferrin values were measured (see Table 3.10.1.15-3 and Table 3.10.1.15-4)

Table 3.10.1.15-3: Iron concentration (µmol/L) in the serum of male rats after administration of dimoxystrobin for 2 and 7 days

Sex	Blood sampling		0 ppm	6 ppm	11 ppm	22 ppm
Males	Day 2	Mean ± SD [µmol/L]	107.8 ± 14.4	102.8 ± 12.7	102.2 ± 16.2	102.5 ± 19.6
	Day 7	Mean ± SD [µmol/L]	77.3 ± 12.7	77.7 ± 10.3	73.1 ± 18.0	82.2 ± 9.7

Table3.10.1.15-4: Transferrin concentration (g/L) in the serum of male rats after administration of dimoxystrobin for 2 and 7 days

Sex	Blood sampling		0 ppm	6 ppm	11 ppm	22 ppm
Males	Day 2	Mean ± SD [µmol/L]	6.52 ± 0.38	6.30 ± 0.41	6.34 ± 0.39	6.67 ± 0.50
	Day 7	Mean ± SD [µmol/L]	5.02 ± 0.39	5.21 ± 0.48	5.05 ± 0.37	4.96 ± 0.35

E. NECROPSY

No test substance-related findings were observed during macroscopically assessment.

III. CONCLUSIONS

The administration of dimoxystrobin in a dose up to 22 ppm (about 4 mg/kg bw/day) via the diet did not lead to dose-dependent changes of serum iron and transferrin values in young (21 to 28 days old) male Wistar rats when fed for two and seven days.

The NOAEL concerning both clinical pathology parameters was at least 22 ppm (about 4 mg/kg bw/day) in young male Wistar rats.

Conclusion on effects on decreased serum iron concentrations and relevance for reference dose setting

Dimoxystrobin causes decreased serum iron concentrations in rats. Clear NOAELs of 4 mg/kg bw for the induction of this effect were derived for adult (7-day study in 10 week old rats, BASF DocID 2002/1014245), for young (7-day study in 3-week old rats, BASF DocID 2010/1026748), for dams and offspring (enhanced one generation toxicity study, BASF DocID 2011/1211676). Only a slight tendency of decreased serum iron in blood was detected in the new mechanistic study in rats at high doses (BASF DocID 2011/1001622,). Thus the 4 mg/kg bw is considered to represent the lowest relevant NOAEL identified in the most sensitive species after administration of dimoxystrobin and should be used for reference dose setting.

For humans average nutrient requirements (ANR) for iron are known. When recommended iron intakes are compared, differences in the iron demands in humans depending on sex, and age are described. Comparing the recommended intakes for males, females premenopausal, and females postmenopausal a factor of about 2 can be derived for differences in dietary iron requirement over the different human population groups (Doets et al., 2011, BASF DocID 2011/1297651).

3.10.1.16 Study 16

Published, mechanistical studies, rat
Study reference:

Rothenbacher and Sherman (1980): Target organ pathology in iron-deficient suckling rats. The Journal Of Nutrition 110 (8), 1648-1654. (BASF DocID 1980/1001747)

Detailed study summary and results:
Test type
Target organ pathology in iron-deficient suckling rats. Mechanistical study, no guideline available.

Test substance
Test substance used is the equivalent to substance in CLH dossier: no; iron
Analytical purity: not specified

Test animals
Species: rat
Strain: Sprague-Dawley CD
Sex: female
Source: Charles River Breeding Labs, Wilmington, MA, US
No. of animals/group: dams: 8 bred females; pups: 3 males and 3 females / litter
Weight at study initiation: ~ 220 g
Housing: individually, in stainless steel screen-bottom cages during gestation and in solid bottom "maternity" cages with iron-free nesting material during lactation
Diet: for the diet composition, see table below

Composition	**307 ppm iron[1] (control group)**	**5 ppm iron (iron-deficient group)**
	[%]	**[%]**
Casein	22.0	22.0
Sucrose	29.70	29.76
Cornstarch	29.70	29.76
Iron-free salt mix	5.48	5.48
Vitamin mix	1.00	1.00
Corn oil	10.00	10.00
Cellulose	2.00	2.00

[1]: Iron levels were determined by atomic absorption

Water: tap water, *ad libitum*
Environmental conditions: Photoperiod (h dark/h light): 12/12

Administration/exposure
Route of administration: oral; dietary
Vehicle: basal diet
Duration of treatment: from GD0 until LD18
Frequency of treatment: continuously

Dose levels: 5 ppm iron-deficient diet
Control group and treatment: yes, 307 ppm iron-containing diet
Statistical analysis: data were analysed using the Student's t-test

Observations and examinations performed and frequency:
Groups of bred female rats received either iron-deficient (5 ppm) or iron-containing (307 ppm) diet. On the second day of lactation, litters were standardized to contain three female and three male pups. The litters were suckled by their dams until day 18 of lactation when tail blood samples were collected for hemoglobin and hematocrit determinations. Dams and pups were fasted for 4 hours and sacrificed following chloroform anesthesia. Thymuses, hearts, spleens, kidneys and livers were removed from pups for histopathological analyses. Tissues for pathological examination were fixed in 10% buffered formalin, dehydrated and embedded in paraffin and cut at 6 µm. Histological and histochemical stains included hematoxylin and eosin, oil-red-O, PAS, Giemsa and toluidine blue.

Results and discussion
Pups and dams fed the 5 ppm iron-deficient diet weighed significantly less than the controls (receiving 307 ppm iron-containing diet) and appeared less developed with sparse coats.
Eighteen-day-old pups of rats fed the iron-deficient diet developed an anemia characterized by lower blood haemoglobin and lower PCV (packed cell volume) levels than pups of rats fed the diet containing 307 ppm iron. The gross pathology of the spleen of iron-deficient pups revealed decreased weights and size. Microscopically, hemopoiesis as well as lymphopoiesis were markedly depressed in spleen and thymus.

Body weight, hematological values and organ weights of 18-day old iron-deficient (5 ppm) and control (307 ppm) pups

Parameter		**5 ppm iron iron-deficient group**		**307 ppm iron control group**	
		mean	**SE**	**mean**	**SE**
Body weight	[g]	**24*** **	2	40	5
Hemoglobin	[g/dL]	**4.2*** **	0.2	11.2	1.2
Thymus weight					
- absolute	[g]	**0.041** **	0.006	0.154	0.023
- relative	[% of bw]	**0.176*** **	0.024	0.355	0.028
Heart weight					
- absolute	[g]	**0.297** **	0.021	0.198	0.020
- relative	[% of bw]	**1.275** **	0.129	0.504	0.036
Spleen weight					
- absolute	[g]	**0.061** **	0.008	0.154	0.021
- relative	[% of bw]	0.262	0.036	0.364	0.038
Kidney weight					
- absolute	[g]	0.187	0.014	0.219	0.020
- relative	[% of bw]	**0.789*** **	0.042	0.554	0.035
Liver weight					
- absolute	[g]	1.174	0.093	1.242	0.129
- relative	[% of bw]	**4.677*** **	0.235	3.090	0.116

** $p \leq 0.01$; *** $p \leq 0.001$ (Student's t-test)

The most pronounced gross changes were observed in the fatty livers of iron-deficient rat pups with concurrently statistically significantly increased relative liver weights. The livers from deficient rats had tan-yellow discoloration and increased mottling. Hemopoietic foci were present in control livers and absent in the iron-deficient livers. Similar fatty changes and cellular degeneration were found in the cortical tubular epithelium of the kidneys of iron-deficient rat pups. Renal glomeruli appeared shrunken and/or hypercellular when compared to control kidneys. Additionally, relative kidney weight was statistically significantly increased in iron-deficient pups. Hearts from iron-deficient pups were pale in color and were statistically significantly increased in size and weight than hearts from control pups. Heart sections from iron-deficient rats had up to 10% fatty-hydropic and/or hyalingranular degeneration. Correspondingly, there were more pyknotic and degenerateing myocardial nuclei. Increased perivascular accumulations of mononuclear cells and mast cells were seen in the iron-deficient hearts when compared to control hearts.

Conclusion

Iron deficiency during gestation and lactation result in morphological changes of immunocompetent tissues (spleen, thymus, liver) and impair the function of the liver, kidney and heart by hyperlipidemia in rat offspring.

3.10.1.17 Study 17

Published, mechanistical studies, rat

Study reference:

Tanne et al. (1994): Ultrastructural and cytochemical changes in the heart of iron-deficient rats. Biochemical Pharmacology 47 (10), 1759-1766. (BASF DocID 1994/1005569)

Detailed study summary and results:

Test type

Changes in the heart of iron-deficient rats. Mechanistical study, no guideline available.

Test substance

Test substance used is the equivalent to substance in CLH dossier: no; iron

Analytical purity:	not specified

Test animals

Species:	rat
Strain:	Sprague-Dawley
Sex:	male
Source:	not specified
No. of animals/dose:	treatment/control groups: 15 males; recovery group: 20 males
Weight at study initiation:	not specified
Age:	21 days
Housing:	not specified
Diet:	For food composition of the iron-deficient (5 ppm) diet see the table below, *ad libitum*

Ingredient	Amount (g)
Main mix	
Household sugar (sucrose)	1530
Choline dihydrogen citrate	100
Non-supplemented milk powder	2600
Salt mixture (iron free)	120
Water-soluble vitamin mix	40
Fat-soluble vitamin mix	4
Fat mix	640
Salt mixture (iron-free)	
NaCl	100
$NaIO_3 \cdot H_2O$	0.001
$MnSO_4 \cdot 4H_2O$	5.406
$CuSO_4 \cdot 5H_2O$	1.361
D-glucose*	393.25
Water-soluble vitamin mix	
Pyridoxine hydrochloride	0.25
Pantothenic acid calcium salt	0.60
Aneurine hydrochloride	0.50
Nicotinic acid	0.50
Menadione (Vitamin K_3)	0.05
Folic acid	0.05
4-amino-benzoic acid	0.50
Biotin	0.01
Inositol	10.00
Cyanocobalamin (B_{12})	0.00075
Riboflavin	0.025
D-glucose	487.25
Fat-soluble vitamin mix	
Retinol palmitate (A)	0.40
Ergocalciferol (D_2)	0.05
DL-α-tocopherol	17.50
Soya bean oil	32.50 mL
Fat mixture	
Pig lard	240
Soya bean oil	80 mL

* For the iron-sufficient (Fe^+) control diet 27 g of D-glucose was replaced by 27 g $(NH_4)_2SO_4.FeSO_4.6H_2O$.

Water: distilled water, *ad libitum*

Environmental conditions: Photoperiod (h dark/h light): 12/12

Administration/exposure

Route of administration: oral; dietary

Vehicle: basal diet

Duration of treatment: 5 weeks

Frequency of treatment: continuous

Recovery period: 2 weeks, receiving iron-containing (427 ppm) diet

Dose levels: 5 ppm iron-deficient diet

Control group and treatment: yes, 427 ppm iron-containing diet

Statistical analysis: data were analysed using the Student’s t-test

Observations and examinations performed and frequency:
Groups of male rats received iron-deficient (5 ppm) or iron-containing (427 ppm) diet for 5 weeks. At weekly intervals, the animals were weighed and blood samples removed from the tail for haemoglobin and non-heme determinations. A recovery group of iron-deficient rats of 5 week duration were fed an iron-sufficient (427 ppm) diet for a further 2 weeks in order to determine the degree of restoration of hemoglobin levels and cardiac ultastructure. At the end of the treatment / recovery periods, rats were anesthetised with ether and following neck dislocation, the hearts were removed and prepared for light and electron microscopy. Additonally, succinate dehydrogenase activity was determined in heat sections.

Results and discussion
After 5-week of iron deficient diet, those rats differed markedly from the controls in both their physical appearance and behavior. The iron-deficient rats were much smaller and weighed considerably less than the iron-sufficient controls. The iron-deficient rats showed a generalized lethargy. The body fur in these animals was in very poor condition with substantial focal hair loss. Gross signs of anemia were clearly detectable. In particular, this was seen in the pallid color of the ears and eyes compared with the normal ruddy color of the control rats and in pallid color of the liver, kidneys, spleen and heart. Moreover, the amount of subcutaneous fat and fat surrounding the internal organs was markedly reduced.

Nutritional and physiological parameters

Parameter		**427 ppm iron control group (N = 15)**		**5 ppm iron iron-deficient group (N = 15)**		**recovery group (N = 20)**	
		mean	**SEM**	**mean**	**SEM**	**mean**	**SEM**
Body weight	[g]	208.0	1.79	**167.4***	1.45	205.0	2.50
Hemoglobin	[g/dL]	12.9	0.11	**3.94***	0.14	15.5	0.20
Heart weight							
- absolute	[g]	0.86	0.02	**1.26***	0.05	**1.16***	0.50
- relative	[g/100 g bw]	0.42	0.06	**0.75***	0.09	**0.57***	0.06
Iron concentration							
- in heart	[µg Fe/g heart]	47.37	3.13	**17.38***	2.69	**31.50***	2.50
- in serum	[µg/mL]	6.29	0.38	**1.55***	0.07	4.70	0.32
Liver weight	[g]	6.20	0.30	**10.45***	0.05	8.47	0.40

* $p \leq 0.05$ (Student's t-test)

The heart size and weights were statistically significantly increased in rats receiving the iron-deficient diet for 5 weeks. The hypertrophy had occurred in the left ventricle and in the left papillary muscles. The degree of hypertrophy was so extreme in most cases as to occlude almost completely the lumen of the left ventricle. At ultrastructural level, the myocytes of the left ventricle of the iron-deficient rats showed extensive interfibrillar edema. Sarcomeres were typically thrown out of register and showed myofilament loss, discontinuities and diorganization. The interfibrillar mitochondria were grossly enlarged and more pleomorphic than those of the controls. Succinate dehydrogenase activity, which is found exclusively in mitochondria and is used as a measure of oxidative capacity of myocytes, was markedly reduced in the iron-deficient heart.

SDH activity in myocytes of the left ventricle of control and iron-deficient rats

Parameter		**427 ppm iron control group (N = 6)**		**5 ppm iron iron-deficient group (N = 8)**	
		mean	**SEM**	**mean**	**SEM**
SDH activity	[integrated extinction / fiber / min x 10^3]	35.0	2.1	**20.4*** **	1.3

*** $p \leq 0.001$ (Student's t-test)

Iron-deficient rats restored to an iron-containing diet for 2 weeks revealed body and liver weights, haemoglobin and serum iron levels that were not significantly different to the control rats. However, the hearts of those rats remained grossly hypertrophic with no indication of any amelioration of the degenerative changes caused by the former iron-deficiency. The myocytes of the left ventricles and left papillary muscles continued to show enlarged mitochondria with electronlucent matrices, occasional dense amorphous inclusions, myofilament loss and edema.

Conclusion

Iron deficiency in young rats induced anemia and degenerative cardiac changes in rats, the later still persistent after 2-weeks recovery.

3.10.1.18 Study 18

Published, mechanistical studies, rat
Study reference:

Crowe et al. (1995): The effects of anaemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. Journal of Physiology, 488 (2): 515-519. (BASF DocID 1995/1008574)

Detailed study summary and results (see RAR (2017) Volume 3, Annex B-6, pp 216 – 218):

Report:	KCA 5.6.1/5 Crowe C & Dandekar P & Fox M & Dhingra K & Bennet L & Hanson MA (1995) (IDD0055, BASF DocID 1995/1008574)
Title:	The effects of anaemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. Journal of Physiology, 488 (2): 515-519
Guideline:	Not stated
Test substance:	none
Previous evaluation:	none
GLP:	Non-GLP
Relevance check:	Relevant
Reliability check:	Reliable (Klimish score: 2, the number of animals, the period of treatment before mating is lower than recommended in the relevant guideline)

Executive summary:

The effects of nutritional anaemia on placenta and body weight, blood pressure and heart of fetal and neonatal rats were studied. Anaemia was induced by feeding low-iron diet (< 6 p.p.m.) prior to mating and throughout gestation and postnatally. Rats were divided into three study groups and their controls to study (1) the effects of iron-deficient diet in fetal development, (2) the effects on pups until postnatal day (PND) 20, and (3) the effects of returning to normal iron-rich diet on PND20 and its effects up until PND40. Maternal anaemia was associated with increased postnatal systolic blood pressure. Increased heart weights in anaemic pups indicate an effect on cardiovascular development. This effect appears to be related to rate of postnatal growth and blood pressure development. Placental and birth weight, as well as placental: birth weight ratio was lower in anaemic animals.

Aim: The aim of the study was to investigate the effects of anaemia during pregnancy on placental, fetal, and birth weight and the development of blood pressure in postnatal period.

Method: Female Sprague Dawley rats were treated with low iron diet (<6 ppm) from weeks prior to mating, and treatment continued throughout gestation.

Group 1 (E20) animals were sacrificed on gestation day 20 and in postnatal period (in relevant study groups). Blood pressure of mothers, maternal and foetal haemoglobin content, maternal and foetal body weight and placental weight were recorded.

Group 2 (PND20) animals received low iron diet (<6 ppm) until, postnatal day (PND) 20. On PND 20 blood pressure of dams and pups was measured and the animals were sacrificed. Maternal and pup haemoglobin content, body weight and organ weight of pups were recorded.

Group 3 (PND40) animals received normal, balanced diet from PND 20 to PND 40. Then the pups were sacrificed, their haemoglobin content, body weights and organ weights were recorded.

The number of animal in the three goups:

Group	Number of dams		Number of fetuses / pups	
	Anaemic	Control	Anaemic	Control
1. E20	4	4	47	59
2. PND20	4	6	18	39
3. PND40	8	7	80	64

Control group: Yes

Species: Sprague-Dawley rats

Parameters: Haemoglobin, body weight, placental weight, Placental:body weight ratio

Statistical method Student's unpaired T test.

Findings:

- reduced haemoglobin level of pups by PND20 (through lactation and self-feeding). After weaning on PND 21 and receiving normal diet the haemoglobin level of the pups increased (see table 3.10.11.18-1below).

Table -3.10.11.18-1: Haemoglobin content of the pups

Postnatal day	Hemoglobin (g/dl)	
	control pups	anaemic pups
Group 2, PND20 (N=39, 18)	10.1 ± 0.4	4.4 ± 0.9***
Group 3, PND40 (N=64, 80)	14.6 ± 0.5	13.3 ± 0.4*

***P<0.001
* P<0.05

- reduced body weight in pups (see table 3.10.11.18-2 below).

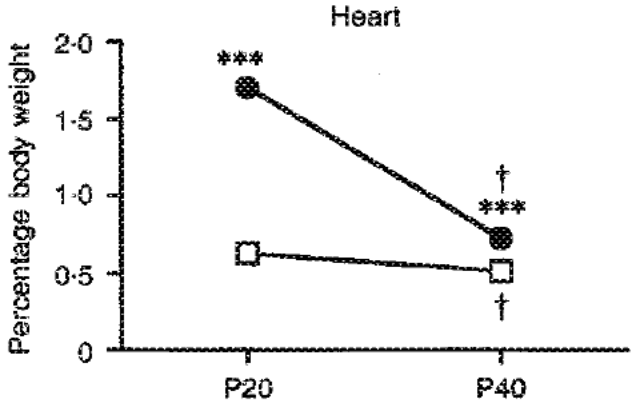
Table -3.10.11.18-2: **Effect on body weight:**

Postnatal day	body weight, g (mean ± S.D.)	
	control pups	anaemic pups
Group 2, PND20 (N=39, 18)	48.0 ± 1.0	28.0 ± 0.7***
Group 3, PND40 (N=64, 80)	157.3 ± 2.9	103.0 ± 3.5***

***P<0.001

- increased liver, kidney and heart weights and a low systolic blood pressure before weaning that raised thereafter. Effects on hearth weight of the pups is shown in figure 1. below.

Figure 1: Effect on hearth weight of the pups



anaemic (●) and control (□) animals

***P<0.001

Conclusion (relevant to the assessment of dimoxystrobin):
Anaemia in pups due to iron deficient diet fed to their mothers is associated with decreased body weight and increased liver, kidney and heart weight and a low systolic blood pressure before weaning that raised thereafter. The effects on organ weights tend to be reversible, as the pups were fed balanced diet from PND 21 until PND 40: Differences in body weight and relative heart weight between treated and control pups diminished considerably during the recovery period.

3.10.1.19 Study 19

Lactating animal studies, goat
Study reference:

Anonymous (2001): Nature of the residue of ^{14}C BAS 505F in lactating goats, amended report. BASF Corporation Agricultural Products Center; Research Triangle Park, NC, US; BASF DocID 2001/5002332; September 2001

Detailed study summary and results (see also RAR (2017) Volume 3 Annex B-7, pp 35 - 37:
The nature of dimoxystrobin residues in commodities of animal origin was investigated in the context of the Annex I inclusion of dimoxystrobin. Reported metabolism studies include two studies in lactating goats using [^{14}C]-phenyl labeled dimoxystrobin; one nature of the residue study and one study on the extractability of the analytical methods used. The studies were found adequate, however, the goat metabolism study was conducted with only a ^{14}C-label in the phenyl moiety. As metabolism studies in hens and rats indicate cleavage of the molecule, an additional metabolism study in goat was conducted with a ^{14}C-label in the benzyl moiety (**KCA 6.2.3/1**) to provide a comprehensive view on the metabolism in ruminants. Furthermore, the extraction efficiency of representative analytical methods used for animal matrices was investigated during this study.

Report Nr.	**IIA 6.2.1/1 (included in dossier submitted to the Annex I inclusion process)**
Title	Nature of the residue of 14C BAS 505F in lactating goats, amended report BASF Doc ID: 2001/5002332
Authors	Anonymous 2001
GLP	Yes

Report Nr.	**IIA 6.2.1/2 (included in dossier submitted to the Annex I inclusion process)**
Title	14C-Validation of the extractability of methods 478/0 and D0006 for the determination of BAS 505 F (Reg. No. 354563) and its metabolites in matrices of animal origin BASF Doc ID: 2001/5002312
Authors	Anonymous, 2001
GLP	Yes

For the sake of completeness a brief summary of the former metabolism study is given below.

Table 3.10.1.19-1 Summary of available and evaluated metabolism studies in goat

Group	Species	Label position	Application details		Sample details	
			Rate (mg/kg bw per day	**Duration** (days)	**Commodity**	**Time**
Lactating ruminants	Goat	[^{14}C]-phenyl	0.5 and 10.3	5	Milk	twice daily
					Urine and faeces	daily
					Tissues	after sacrifice (23 h after final dose)

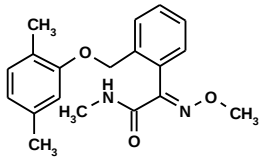
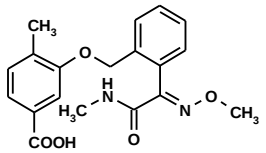
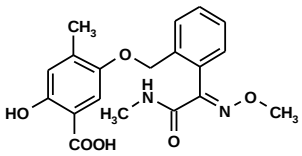
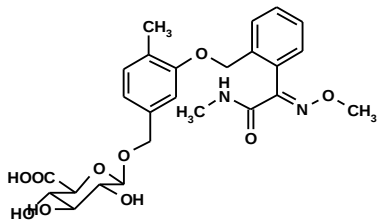
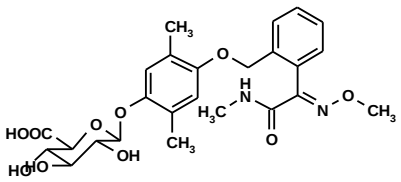
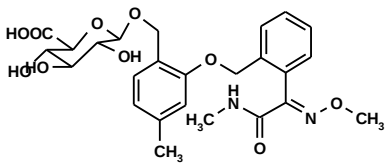
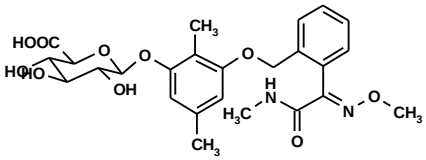
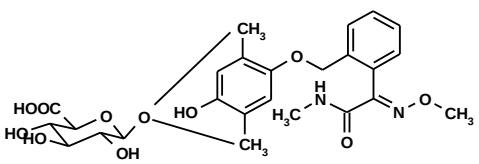
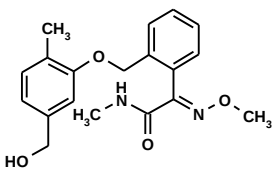
After 5 consecutive daily oral administrations ^{14}C-BAS 505 F (phenyl label) was rapidly absorbed and almost completely (86-98.1%) excreted mainly in the faeces. There was no indication of accumulation of ^{14}C-BAS 505 F in goat tissues and milk. Milk and tissues of goats that had received a dose of 12.6 mg/kg feed (equivalent to 3.3 N) contained residues of 8 identified metabolites at levels of 0.05 mg/kg or less. The parent compound was detected in milk, liver and kidney at levels of 0.02 mg/kg or less. Identity of the radioactivity in muscle and fat was not investigated due to the low levels of radioactivity present. Carbon atoms on the phenyl ring and the methyl side chains of the phenyl ring were oxidised to form hydroxy groups (505M04 and intermediates). Further oxidation of the benzyl groups formed the corresponding carboxylic acids (505M09, 505M76) which are the major constituents in all matrices. Conjugation of the OH-groups with glucuronic acid (505M86, 505M49, 505M78, and 505M79) resulted in the formation of numerous polar metabolites.

In liver, the major components were the carboxylic acid metabolite 505M0918 (21.8% TRR; 0.05 mg/kg) and the hydroxyl acid metabolite 505M7619 (19.2% TRR; 0.05 mg/kg). Glucorunide conjugates of the metabolites 505M02 and 505M03 also accounted for more than 10% TRR (12.1%TRR; 0.03 mg/kg and 10%TRR; 0.02 mg/kg, respectively) whereas dimoxystrobin accounted for 7.5% TRR (0.018 mg/kg). All other components were below 10% of the TRR and below 0.01 mg/kg.

In kidney, glucuronide conjugate of 505M03 (22.8%; 0.017 mg/kg), 505M09 (17.1%, 0.013 mg/kg), glucuronide conjugates of 505M04 (13.6%TRR; 0.009 mg/kg) and of 505M02 (12%TRR; 0.008 mg/kg) constituted the most important components of the residue (13.6% and 12.0% TRR respectively). All other components were below 10% TRR.

In milk, no compound was detected at levels above 0.001 mg/kg. The major components were 505M09 (14.4% TRR) and 505M76 (10.3% TRR). Dimoxystrobin accounted for 9.2% TRR. All other components were below 10% of the TRR.

Table 3.10.1.19-2 Summary of identified metabolites and quantities in edible matrices of lactating goats after dosing with ^{14}C-BAS 505 F (phenyl label)

Metabolite Code	Structure	Liver* mg/kg (% TRR)	Kidney* mg/kg (% TRR)	Milk mg/kg (% TRR)
BAS 505 F		0.018 (7.5)	0.001 (1.7)	<0.001 (9.2)
505M09 (BF 505-8)		0.052 (21.8)	0.013 (17.1)	<0.001 (14.4)
505M76 (BF 505-11)		0.046 (19.2)	0.005 (7.5)	<0.001 (10.3)
505M49		0.008 (3.8)	0.009 (13.6)	----- -----
505M78		0.029 (12.1)	0.008 (12.0)	----- -----
505M79		0.024 (10.1)	0.017 (22.8)	----- -----
505M84		0.008 (3.4)	0.005 (6.9)	----- -----
505M86		----- -----	----- -----	<0.001 (4.9)
505M04		----- -----	----- -----	<0.001 (3.9)

* includes protease digest

From these findings it is rather unlikely that the parent compound or any of the identified metabolites would reach quantifiable concentrations in milk or tissues from ruminants under normal agricultural practice. However, for the analysis of samples generated in the course of a cow feeding study which was performed to confirm this extrapolation, unchanged BAS 505 F and the metabolites 505M09 and 505M76 were considered suitable marker analytes.

In the frame of the renewal process a new goat metabolism study using [^{14}C]-benzyl labeled dimoxystrobin was submitted. The assessment of this study is presented below.

3.10.1.20 Study 20

Lactating animal studies, goat
Study reference:

Anonymous (2015): The metabolism of ^{14}C-Reg. No. 285028 (BAS 505 F) in the lactating goat. Charles River, Edinburgh, UK; BASF DocID 2015/1001730; May 2015 and Anonymous (2015): Report amendment 1 - The metabolism of ^{14}C-Reg. No. 285028 (BAS 505 F) in the lactating goat. Charles River, Edinburgh, UK; BASF DocID 2015/1125782; May 2015

Detailed study summary and results (see also RAR (2017) Volume 3 Annex B-7, pp 37 – 45):

Report Nr.	**KCA 6.2.3/1**
Title	The metabolism of ^{14}C-Reg. No. 285028 (BAS 505 F) in the lactating goat
Authors	Anonymous, 2015a
Guidelines	EPA 860.1300: Nature of the Residue in Plants Livestock, EEC 91/414 (7030(VI/95 Rev. 3), OECD Test Guideline 503 - Metabolism in livestock
GLP	Yes
Acceptability	Yes

Report Nr.	**KCA 6.2.3/2**
Title	Report amendment 1 - The metabolism of ^{14}C-Reg. No. 285028 (BAS 505 F) in the lactating goat
Authors	Anonymous, 2015b
Guidelines	EPA 860.1300: Nature of the Residue in Plants Livestock, EEC 91/414 (7030(VI/95 Rev. 3), OECD Test Guideline 503 - Metabolism in livestock
GLP	Yes
Acceptability	Yes

The purpose of the study was to investigate the extent to which dimoxystrobin residues may be transferred to food products destined for human consumption and establish the nature of any transferred residues. The study was designed to investigate the disposition of [U-^{14}C Benzyl]-BAS 505 F in the lactating goat following multiple oral administration. The levels of radioactivity in milk, urine, faeces and edible tissues was determined following 7 consecutive daily doses of [U-^{14}C Benzyl]-BAS 505 F.

Materials and Methods

1. **Test Material:**

Description: ^{14}C-dimoxystrobin (benzyl-U-^{14}C label) 98.2%, specific activity: 7.6 MBq/mg, Batch nr: 596-3013, chemical purity: 96.1%
^{13}C-dimoxystrobin (acetamide-2-^{13}C), Batch nr: 596-2010, chemical purity: >97%
^{12}C-dimoxystrobin (unlabeled), Batch nr: 00956-117, chemical purity: 99.9%

2. **Test Animal:**

Species:	Goat
Variety:	"Saanen cross Toggenburg"
Gender:	Female
Age:	approx. 2 years
Weight at dosing:	63 kg
Number of animals:	1
Acclimation period:	14 days
Diet:	2 x 0.5 kg non-medicated commercially available concentrate (Dodson and Horrell Goat Mix supplied by Dodson and Horrell Limited, Kettering Road) + hay *ad libitum*

Table 3.10.1.20-1 Study design

Group	Species	Label position	Application details			Sample details	
			dose (mg/kg feed dry matter/day	**dose** (mg/kg bw)*	**Duration** (days)	**Commodity**	**Time**
Lactating ruminants	Goat	[^{14}C]-benzyl	11.8	0.15-0.27** (mean 0.19)	7	Milk	twice daily
						Urine and faeces	daily
						Tissues	after sacrifice (6 h after final dose)

* Body weight mean calculated based on weight on 1 day before dosing and day 7 of dosing = 60.75 kg

** Since the goat was sacrificed 6 h after the seventh dose administration (not a full day) day seven was not taken into account

Sampling and storage

Blood samples were taken prior and at 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post first dose. Urine and faeces samples were collected for the 24 h period prior to first dose and for each 24 h period until sacrifice. The goat was milked twice daily and immediately prior to sacrifice. At approximately 6 h post final dose the goat was humanely killed and edible tissues (liver, kidney, muscle and fat), bile, blood and the GI tract were removed *post mortem*. All samples were stored at ca.–20°C.

Description of analytical methods

The radioactivity of homogenized extracts of the liquid samples (milk, bile, urine, blood) was determined by LSC (liquid scintillation counting) analysis. Solid debris (faeces, tissues) were analysed for radioactivity content by combustion analysis followed by LSC.

Faeces, liver and kidney were generally homogenized and extracted using 3 extraction solutions with different water:methanol ratios. Further investigation of non-solvent extractable residues in liver and kidney debris (RRR) was conducted using protease enzyme. In addition larger subsamples of liver and kidney were extracted to aid metabolite elucidation and these extracts were also used for the enzyme deconjugation experiments and chiral analysis of BAS 505 F. Extracts containing significant amounts of radioactivity were proportionately combined and concentrated. Additionally, subsamples of liver and kidney were extracted using 2 different extraction methods [BASF method D0006 (methanol based) and BASF method L0232/01 (acetone and ethyl acetate:cyclohexane based] in order to measure the components of the proposed residue definition (BAS 505 F and 505M09).

Samples were analysed using two HPLC methods; on-line radiodetection for quantification and fraction collection and TopCount analysis to confirm the assignment and quantification. A mix of the reference standards was prepared and each sample/extract analysed by HPLC was fortified with the standard mix. The identity of radiolabeled components was then based on co-chromatography with the authentic reference items. Two HPLC methods were used to quantify individual residues and to confirm the assignment and quantification. To identify components which did not correspond to a reference standard and to confirm the assignments made using co-chromatography HPLC-MS/MS was used.

Results and Discussion

The total radioactive residues (TRR) in all matrices are summarized in Table 3.10.1.20-2. Rapid excretion was observed. Until sacrifice, the radioactive residues excreted via urine and faeces amounted to 17.6% and 62.6% of the total radioactivity administered, respectively. Of approximately 94% of the total dose that was recovered 11.3% was found in the GI tract and contents (bile). Radioactivity associated with edible portions (milk and tissues) accounted for <1% of the administered dose. Radioactive residues in milk were low and accounted for a plateau concentration of approximately 0.002 mg eq/kg within 2 days for the test goat. The radioactive residues in muscle and fat were low and ranged from 0.005-0.009 mg/kg and from 0.005-0.006 mg/kg, respectively. The residues in the liver were 0.365 mg/kg and in kidney 0.110 mg/kg.

Table 3.10.1.20-2 Total radioactive residues in edible matrices and excreta after dosing of lactating goat with benzyl-U-^{14}C-BAS 505 F

Matrix	**TRR** (mg/kg)	
	measured	**calculated**
Benzyl-U-^{14}C label		
Milk[1]	0.002	-
Bile	9.180	-
Liver	0.365	0.385
Kidney	0.110	0.113
Fat (omental/renal/subcutaneous)	0.005/0.006/0.005	-
Muscle (loin/flank)	0.005/0.009	-

TRR: Total radioactive residue (sum of ERR + RRR)
[1] Mean of six pooled milk samples (PM and AM milk collection)
[2] Pool samples day 4-6

The extractability of the edible tissues was high, ranging from 78.0% (liver) to 85.0% (kidney) of the TRR (see Table 3.10.1.20-3). The residue after solvent extraction of liver and kidney was 21.9% TRR and 15.1% TRR, respectively and was further investigated with protease enzyme. An additional 23% and 16.1% of the TRR was released for liver and kidney, respectively.

Table 3.10.1.20-3 Residues after [^{14}C]-BAS 505 F treatment in goat matrices extracted with methanol and methanol/water (4:1 and 3:7, v/v)

Matrix	**TRR**		**ERR[1]**		**RRR[2]**		**Total** (ERR+RRR)
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	%
Liver	0.385	100.0	0.300	78.0	0.084	21.9	99.9
Kidney	0.113	100.0	0.096	85.0	0.017	15.1	100.1

[1] ERR = Extractable radioactive residue
[2] RRR = Residual radioactive residues

Summaries of identified and characterized residues are shown in Table 3.10.1.20-4 and Table 3.10.1.20-5. A summary of all identified metabolites and their distribution in excreta, bile, milk, muscle, fat, liver, and kidney is given in Table 3.10.1.20-6. Identification was accomplished by HPLC co-chromatography with reference standards. To confirm the thus identified metabolic pattern and to identify additional components HPLC-MS/MS was utilized.

For quantification of labeled components, liver concentrated extract was analyzed directly by HPLC pre- and post treatment with glucuronidase enzyme (see Table 3.10.1.20-4 and Table 3.10.1.20-6). Analysis of pre-enzyme hydrolysis liver extract showed 17 peaks; two of which were identified as unchanged BAS 505 F (0.014 mg/kg; 3.8% TRR) and 505M09 (0.051 mg/kg; 13.4 % TRR). Of the other peaks five were identified as glucuronide conjugates and two of them were identified as isomers of 505M50 and individually accounted for 0.037 mg/kg (9.7% TRR) and 0.022 mg/kg (5.6% TRR). Three other conjugates were assigned as 505M105 (0.033 mg/kg; 8.6% TRR), 505M51 (0.022 mg/kg; 5.8% TRR) and 505M81 (0.044 mg/kg; 11.5% TRR). The other peaks were not identified and individually accounted for less than 0.006 mg/kg (1.6% TRR). Deconjugation experiments with glucuronidase enzyme confirmed the formation of aglycons form the glycon identified glucuronide conjugates.

Table 3.10.1.20-4 Summary of identified and characterized residues in liver of lactating goat dosed with [benzyl-U-^{14}C]-BAS 505 F

Components	Liver	
	(mg/kg)	(% TRR)
BAS 505 F (Reg No.285028) [1]	0.014	3.8
505M105 (M505F105)[2]	0.033	8.6
505M50 (M505F050)[2,3]	0.037	9.7
505M51 (M505F051)[2]	0.022	5.8
505M81 (M505F081)[2]	0.044	11.5
505M50 (M505F050)[2,3]	0.022	5.6
505M09 (Reg. No. 354563)[1]	0.051	13.4
Total identified in extractable radioactivity (ERR)	**0.223**	**58.4**
Total characterized in extractable radioactivity (ERR)	**0.033**	**8.6**
Total identified/characterized radioactivity (RRR[4])	**0.089**	**23.0**
Sum identified/characterized (from ERR+RRR)	**0.345**	**90.0**
Final Residue	0.008	2.2
Grand Total	**0.353**	**92.2**

[1] Identified metabolites were resolved and quantified by co-chromatography using HPLC. Qualitative confirmation was achieved by co-chromatography using HPLC-MS/MS

[2] Identified metabolites were resolved and quantified using HPLC and identification was performed by mass spectral analysis

[3] Two isomers of 505M50 have been detected

[4] Further investigated using protease enzyme

For quantification of labeled components, kidney concentrated extract was analyzed directly by HPLC pre- and post treatment with glucuronidase enzyme (see Table 3.10.1.20-5 and Table 3.10.1.20-6), without further purification. Analysis of pre-enzyme hydrolysis kidney extract showed 19 peaks; two of which were identified as unchanged BAS 505 F (0.005 mg/kg; 4.6% TRR) and 505M09 (0.009 mg/kg; 7.5% TRR). Of the other peaks four were identified as glucuronide conjugates and two of them were assigned as isomers 505M50 and individually accounted for 0.009 mg/kg (7.8% TRR) and 0.008 mg/kg (6.8%TRR). Two other conjugates were assigned as 505M51 (0.014 mg/kg; 12.3% TRR) and 505M81 (0.022 mg/kg; 19.5% TRR). The other peaks were not identified and individually accounted for less than 0.003 mg/kg (2.7% TRR).

Table 3.10.1.20-5: Summary of identified and characterized residues in kidney of lactating goat dosed with [benzyl-U-^{14}C]-BAS 505 F

Components	Kidney	
	(mg/kg)	(% TRR)
BAS 505 F (Reg No.285028)[1]	0.005	4.6
505M50 (M505F050)[2,3]	0.009	7.8
505M51 (M505F051)[2]	0.014	12.3
505M81 (M505F081)[2]	0.022	19.5
505M50 (M505F050)[2,3]	0.008	6.8
505M09 (M505F009, Reg No.354563)[1]	0.009	7.5
Total identified in extractable radioactivity (ERR)	**0.067**	**58.5**
Total characterized in extractable radioactivity (ERR)	**0.020**	**17.4**
Total identified/characterized radioactivity (RRR[4])	**0.018**	**16.1**
Sum identified/characterized (from ERR+RRR)	**0.105**	**92.0**
Final Residue	0.001	1.1
Grand Total	**0.106**	**93.1**

1 Identified metabolites were resolved and quantified by co-chromatography using HPLC. Qualitative confirmation was achieved by co-chromatography using HPLC-MS/MS

2 Identified metabolites were resolved and quantified using HPLC and identification was performed by mass spectral analysis

3 Two isomers of 505M50 have been detected

4 Further investigated using protease enzyme

Deconjugation experiments with liver and kindney samples with glucuronidase enzyme confirmed the formation of aglycons from the identified glucuronide conjugates.

Table 3.10.1.20-6 Summary of identified residues in goat after dosing with [benzyl-U-^{14}C]-BAS 505 F

Metabolite	Urine		Faeces		Bile		Liver		Kidney	
	(mg/kg)	(% TRR)	(mg/kg)	(% TRR)	(mg/kg)	(% TRR)	(mg/kg)	(% TRR)	(mg/kg)	(% TRR)
BAS 505 F	ND	ND	3.181	62.5	ND	ND	0.014	3.8	0.005	4.6
505M105 (M505F105)	ND	ND	ND	ND	ND	ND	0.033	8.6	ND	ND
505M109 (M505F109)	0.246	7.5	ND	ND	ND	ND	ND	ND	ND	ND
505M50[1] (M505F050)	0.823	24.9	ND	ND	2.087	22.7	0.037	9.7	0.009	7.8
505M51 (M505F051)	0.698	21.2	ND	ND	1.050	11.4	0.022	5.8	0.014	12.3
505M81 (M505F081)	ND	ND	ND	ND	3.858	42.0	0.044	11.5	0.022	19.5
505M50[1] (M505F050)	0.234	7.1	ND	ND	1.272	13.9	0.022	5.6	0.008	6.8
505M02 (Reg. No 356310)	ND	ND	0.509	10.0	ND	ND	ND	ND	ND	ND
505M04 (Reg. No 035807)	ND	ND	0.259	5.1	ND	ND	ND	ND	ND	ND
505M09 (Reg. No 354563)	0.882	26.7	1.586	31.2	0.329	3.6	0.051	13.4	0.009	7.5

[1] Two isomers of 505M50 have been detected
ND Not detected

Comparability of residue extraction methods used for liver and kidney samples

A comparison summary table detailing the residues observed for BAS 505 F and 505M09 between the profiling and two residue extraction methods is presented in Table 3.10.1.20-7, below.

Table 3.10.1.20-7 Comparison of residues in liver and kidney (BAS 505 F and 505M09) following extraction using three different extraction methods

Extraction	ERR			BAS 505 F			505M09		
	(mg/kg)	(% TRR)	(% Met)[1]	(mg/kg)	(% TRR)	(% Met)[1]	(mg/kg)	(% TRR)	(% Met)[1]
Liver									
Residue Method D0006 (Methanol)	0.243	64.7	88.1	0.017	4.5	118.4	0.076	20.1	150.0
Residue Method L0232/01 (Acetone and ethyl acetate:cyclohexane)	0.146	39.9	54.4	0.005	1.3	34.2	0.058	15.8	117.9
Goat Metabolism Study (Methanol and Water)	0.283	73.4	N/A	0.014	3.8	N/A	0.051	13.4	N/A
Kidney									
Residue Method D0006 (Methanol)	0.087	78.0	96.2	0.004	4.0	89.0	0.009	8.5	88.2
Residue Method L0232/01 (Acetone and ethyl acetate:cyclohexane)	0.013	11.6	14.3	0.005	4.4	95.7	0.009	8.5	88.2
Goat Metabolism Study (Methanol and Water)	0.092	81.1	N/A	0.005	4.6	N/A	0.009	7.5	N/A

ERR Extractable radioactive residue (solvents: acetonitrile, isohexane, water)
[1] % Met = Extraction efficiency compared to the extraction method used in the metabolism study
N/A not applicable

Subsamples of liver and kidney were extracted by residue methods BASF Method D0006 and BASF Method L0232/01 in order to measure the components of the residue definition (BAS 505 F and 505M09) and to identify these components by HPLC using co-chromatography with authentic reference standards. The levels of 505M09 and BAS 505 F observed in liver and kidney extracts generated by the D0006 and L0232/01 residue methods are generally comparable with the levels following analysis of the extracts obtained by the metabolite profiling extraction method (used in the already evaluated goat metabolism study with phenyl label). Slightly less 505M09 (0.051 mg/kg; 13.4% TRR) and BAS 505 F (0.014 mg/kg; 3.8% TRR) was observed in the liver extract following the metabolite profiling extraction method when compared to the D0006 and L0232/01 residue methods, respectively.

Storage stability
Initial analyses of the tissue and excreta extracts were carried out within 6 months of sacrifice and storage at -20°C for liver, kidney, urine, faeces and bile. Liver and kidney samples were extracted and profiled by HPLC-MS/MS twelve months after the original extraction and analysis was carried out. The original concentrated and reconstituted extracts from liver and kidney were analysed fifteen months after their initial analysis. The profiles were comparable showing stability over the course of the study in both the tissues and extracts.

Metabolic pathway
The proposed metabolic pathway of BAS 505 F in the lactating goat is provided in *Figure 3.10.2.20-1*. The unchanged parent BAS 505 F was extensively metabolized in the lactating goat. Metabolic transformations occurred mainly *via* two routes *i)* hydroxylation followed by glucuronide conjugation and *ii)* carboxylation followed by glucuronide conjugation. Main components of the former category in extracts of liver and kidney were 505M50 (as two isomers) and 505M51. The main component of the latter category in extracts of liver and kidney was 505M81, a glucuronide conjugate of 505M09. A glucuronide conjugate of the hydroxy carboxylic acid of BAS 505 F was observed in liver and designated as 505M105. 505M01, a cleavage product also observed in rat and laying hens was identified indirectly in the new goat study. The aglycon 505M01 was detected upon glucuronidase treatment of urine, bile and liver.

Stereoisomer analysis
The E/Z ratio of BAS 505 F was investigated in the dose solution and in extracts of liver and kidney. The ratio of the E/Z isomers of BAS 505 F was *ca* 9:1 in the dose solution and in the liver extract. Due to the low overall amount of radioactivity in the sample only the E-isomer (BAS 505 F) was detected in the extract of kidney extract.

Conclusion
Parent BAS 505 F was extensively metabolized in the lactating goat. Among all the metabolites identified from the tissues and urine, all structural modifications occurred at the phenyl ring and the basic structural backbone of the parent compound remained intact. To a small extent the parent compound was cleaved at the connecting ether bridge eliminating the substituted phenyl ring to form a benzyl alcohol 505M01 of the entirely unchanged benzyl part of the molecule. This could only be shown indirectly by treating urine, bile and liver with glucuronidase enzyme and is in alignment with the results of rat and hen metabolism.

Metabolic transformations occurred mainly *via* two routes *i)* hydroxylation followed by glucuronide conjugation and *ii)* carboxylation followed by glucuronide conjugation. Main components of the former category in extracts of liver and kidney were two isomers of 505M50 and 505M51. The main component of category ii) in extracts of liver and kidney of the study was 505M81, a glucuronide conjugate of 505M09. A glucuronide conjugate of the hydroxy carboxylic acid of BAS 505 F was observed in liver and designated as 505M105. Deconjugation experiments with glucuronidase enzyme confirmed the formation of aglycons from the identified glucuronide conjugates. By treatment with glucoronidase 505M01, a cleavage product observed in rat and laying hens was also identified indirectly.

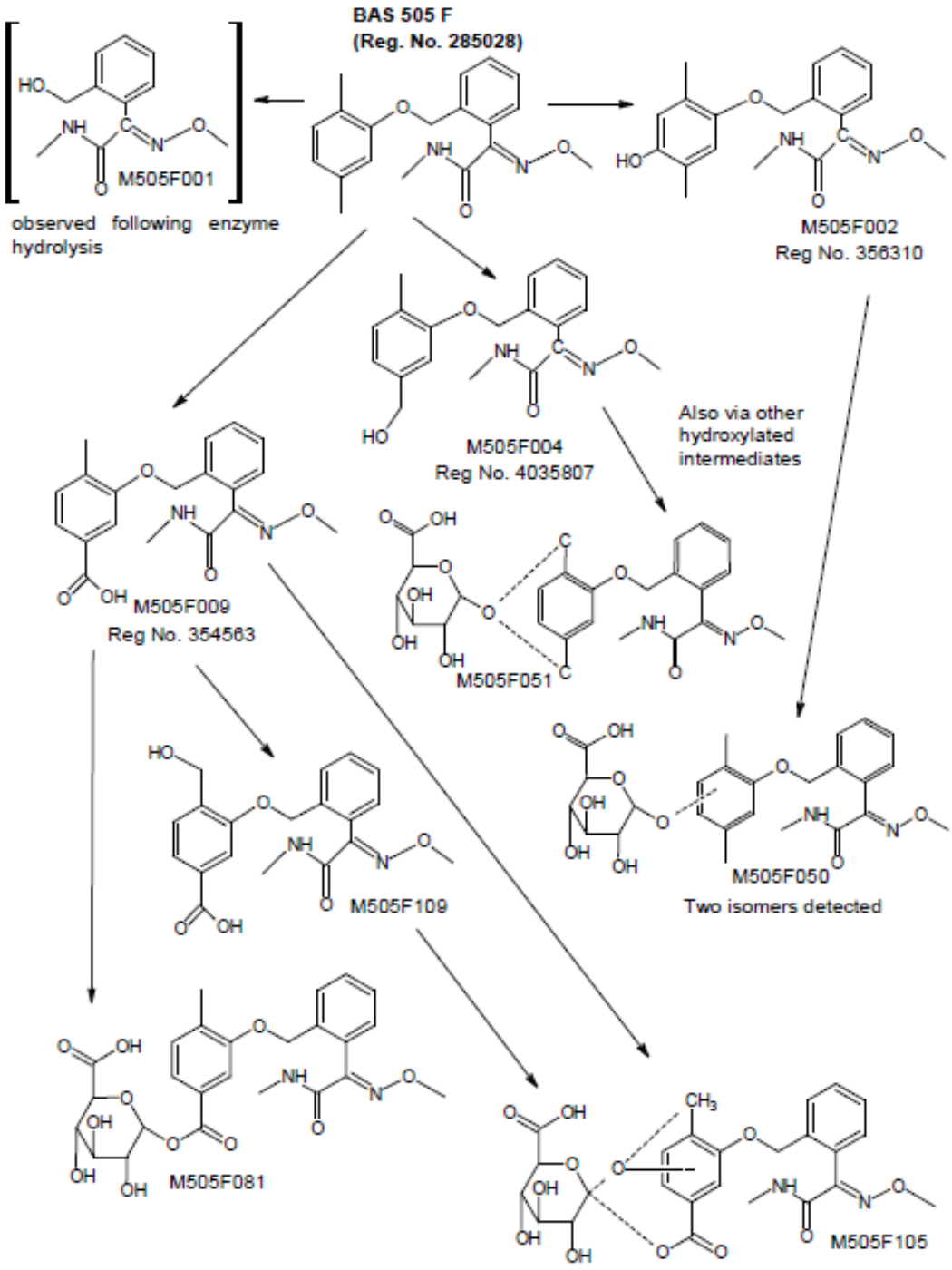


Figure 3.10.1.20-1 Biotransformation pathway for Dimoxystrobin in lactating goat

Overall conclusion on goat metabolism studies

BAS 505 F is extensively metabolized in the lactating goat. In terms of encountered metabolization pathways the two goat studies are largely comparable. The following general conclusions can be drawn:

- In all identified metabolites, structural modification occurred at the phenyl ring while the basic structural backbone of the parent compound remained unchanged.
- 505M01, a cleavage product observed in rat and laying hens was also identified indirectly in the new goat study. The aglycon 505M01 was detected upon glucuronidase enzyme treatment of goat urine, bile and kidney. This underlines the similarity of dimoxystrobin metabolism in different animal species.
- As observed in the older goat study metabolic transformations occurred mainly *via* two routes *i)* hydroxylation followed by glucuronide conjugation and *ii)* carboxylation followed by glucuronide conjugation. Main components of category *i)* were 505M50 and 505M51 (new study) and 505M49, 505M78 and 505M79 (old study). The main components of category *ii)* were 505M09 and its glucuronide conjugate 505M81 (new study) and 505M09 and 505M76 in the old study.
- The observed metabolic pathways are similar to the ones observed in rat and laying hens. Main routes are based on hydroxylation and carboxylation of the aromatic ring system, cleavage of the two ring system was observed to a lesser extent.
- The ratio of the E and Z stereoisomers of BAS 505 F was investigated in the new study in the dose solution and extracts of liver and kidney. The ratio of the E/Z-isomers of BAS 505 F was approximately 9:1 in the dose solution and in the liver extract. Due to the low overall amount of radioactivity only the E-isomer (BAS 505 F) was detected in the kidney extract. The isomer ratio of parent to its Z-isomer (505M98) remained constant throughout the study and no change of the ratio is to be expected.

Summarizing the above, it can be noted that the metabolic processing in the two lactating goat studies aligns very well and provides a comprehensive picture of dimoxystrobin metabolism in lactating ruminants. Additionally, it can be concluded that the metabolic pathways in the different animal species (rat, hen, goat) are quite similar. Main routes are based on hydroxylation and carboxylation of the aromatic ring system, cleavage of the two ring system was observed to a lesser extent.

3.10.1.21 Study 21

Lactating animal studies, cow
Study reference:

Anonymous (2001): A meat and milk magnitude of the residue study with BAS 505 F in lactating dairy cows. BASF Corporation Agricultural Products Center; Research Triangle Park, NC, US; BASF DocID 2000/5259; February 2001

Detailed study summary and results (see also RAR (2017) Volume 3, Annex B-7, pp 82 – 83):
Based on the dietary burden calculation (Table 3.10.1.21-1), the potential intake for dairy and beef cattle is above the trigger value of 0.004 mg/kg bw/day.
Data/information on a lactating ruminant feeding study for dimoxystrobin were reviewed during the Annex I inclusion process and were considered to be acceptable (DAR, 2003).
The summary of this study is presented below.

Report Nr.	**IIA 6.4.1/1 (included in dossier submitted to the Annex I inclusion process)**
Title	A meat and milk magnitude of the residue study with BAS 505 F in lactating dairy cows (BASF Doc ID 1998/11235)
Authors	Anonymous, 2001
GLP	Yes

Three groups of lactating cows, each consisting of three animals were dosed for 28 consecutive days with dimoxystrobin at levels of 2.5, 7.5 and 25 mg/kg in the diet (equivalent to 0.07, 0.18 and 0.64 mg/kg bw). The samples of milk and tissues were analysed for parent dimoxystrobin, 505M09 and 505M76 using the LC/MS/MS method D0006. The description and the validation of the analytical method are presented in Volume 3, section B.5.1.2 of this report.
All samples were analysed within 26 days of freezer storage, therefore no storage stability study was required. Residues in all milk samples from the animals dosed at 7.5 and 25 mg/kg feed DM were individually all below the LOQ of 0.01 mg/kg. Residues in samples of muscle, liver, kidney and fat (composite of renal, mesenteric and peripheral) from the dose group of 2.5 and 7.5 mg/kg feed DM were also all individually below the LOQ of 0.025 mg/kg.

The suitability of the study was recently confirmed by EFSA during the re-evaluation of the established MRLs according to Regulation 396/2005, Art. 12 (EFSA Journal 2013;11(11):3464). Results of ruminant livestock feeding studies are summarised in Table B.7.4-3.

Table 3.10.1.21-1 Overview of the values derived from the livestock feeding studies

Commodity	Dietary burden		Results of the livestock feeding study						Median residue (mg/kg)[b]	Highest residue (mg/kg)[c]	MRL proposal (mg/kg)	CF for RA [d]
	Median (mg/kg bw/day)	Maximum (mg/kg bw/day)	Dose level (mg/kg bw/day)[a]	No	Results for enforcement		Results fo RA					
					Mean (mg/kg)	Max. (mg/kg)	Mean (mg/kg)	Max. (mg/kg)				
Enforcement residue definition: 505M09, expressed as dimoxystrobin **Risk assessment residue definition**: sum of 505M09 and 505M76, expressed as dimoxystrobin												
Ruminant liver	0.011	0.037	0.07	3	<0.025	<0.025	<0.050	<0.050	0.025*	0.025*	0.03*	2
			0.18	3	<0.025	<0.025	<0.050	<0.050				
			0.64	3	<0.025	<0.025	<0.050	<0.050				
Enforcement and risk assessment residue definition: 505M09, expressed as dimoxystrobin												
Ruminant kidney	0.011	0.037	0.07	3	<0.025	<0.025	<0.025	<0.025	0.025*	0.025*	0.03*	1
			0.18	3	<0.025	<0.025	<0.025	<0.025				
			0.64	3	<0.025	<0.025	<0.025	<0.025				
Ruminant muscle	0.011	0.037	0.07	3	<0.025	<0.025	<0.025	<0.025	0.025*	0.025*	0.03*	1
			0.18	3	<0.025	<0.025	<0.025	<0.025				
			0.64	3	<0.025	<0.025	<0.025	<0.025				
Ruminant fat	0.011	0.037	0.07	3	<0.025	<0.025	<0.025	<0.025	0.025*	0.025*	0.03*	1
			0.18	3	<0.025	<0.025	<0.025	<0.025				
			0.64	3	<0.025	<0.025	<0.025	<0.025				
Milk	0.018	0.060	0.07	3	#	#	#	#	0.01*	0.01*	0.01*	1
			0.18	3	<0.01	n.a.	<0.01	n.a.				
			0.64	3	<0.01	n.a.	<0.01	n.a.				

n.a.: Not applicable – only the mean values are considered for calculating MRLs in milk
(a): Based on a 654-688 kg animal consuming 15-18 kg feed DM/day.
(b): Median residue value according to the enforcement residue definition
(c): Highest residue value (tissues) or mean residue value (milk) according to the enforcement residue definition
(d): The median conversion factor for enforcement to risk assessment.
(*): Indicates that the MRL is set at the limit of analytical quantification.
(#) – not analyzed – no residues are expected since no residues were detected in the milk samples from the higher dose group

3.10.1.22 Study 22

Published lactating animal studies, rat
Study reference:

Roth AC, Herkert GE, Bercz JP, Smith K (1987) Evaluation of the developmental toxicity of sodium nitritie in Long-Evans rats. Fundamental and Applied Toxicology 9, 668 – 677 (BASF DocID 1987/1003494)

Detailed study summary and results:

Test type
Maternally mediated, nitrite-associated iron-deficiency in dams and offspring rats. Mechanistic perinatal study design including a cross-fostering experminet, no guideline available.

Test substance
Test substance used is the equivalent to substance in CLH dossier: no, $NaNO_2$ (sodium nitrile)
Analytical purity: no data

Test animals

Species:	rat
Strain:	Long-Evans hooded
Sex:	male and female (females were bred, only pregnant females were dosed)
Source:	Charles River Laboratories
No. of animals/sex/dose:	5 - 10 females (depending on the experiment)
Weight at study initiation:	180 - 200 g
Housing:	before mating: Male rats were single-housed and virgin females were maintained three to a cage until; at GD0: females were single-housed in plastic shoebox cages
Diet:	Purina starndard lab chow, *ad libitum*
Water:	tap water, *ad libitum*
Environmental conditions:	Photoperiod (h dark/h light): 12/12

Administration/exposure

Route of administration:	oral; by drinking water
Vehicle:	drinking water
Duration of treatment:	Experiment I: GD0 – LD16 (Group I, II and III) Experiment II: GD0 – LD20 (Selected pup sacrifice days for blood sampling on LD 7, 9, 13, 16 and 20) (Groups IV, V, VI, VII) Experiment III (Cross-fostering): GD0 – LD21 (4 groups of pups)
Frequency of treatment:	continously
Dose levels:	Experiment I: Control, (Group I, 8 animals), 2 g $NaNO_2$/L (Group II, 10 animals); 3 g $NaNO_2$/L (Group III, 8 animals) Experiment II: Control (Group IC, 5 animals), 0.5 g $NaNO_2$/L (Group V, 8 animals), 1.0 g $NaNO_2$/L (Group VI, 8 animals), 2.0 g $NaNO_2$/L (Group VII, 6 animals Experiment III: 2 group of dams (tap water and 2 g $NaNO_2$/L); 4 group of pups (5 – 6 litters)
Control group and treatment:	yes, tap water
Statistical analysis:	Maternal body weights and fluid consumption: repeated measures analysis of variance (WINER); followed by Tukey´s multiple comparison procedure. Hematological parameters: One-way analysis of variance followed by Duncan´s post hoc evaluation; mean body weights per litter: analysis of variance (m & f separately); litter size and sex ratio: Kruskal-Wallis

Observations and examinations performed and frequency:

Experiment I – pilot dose response: Three groups of females were maintained throughout gestation and lactation on tap water control, 2 g $NaNO_2$/L and 3 g $NaNO_2$/L throughout gestation and lactation. On day 1 postpartum, litters were culled to eight. On days 9 and 16 postpartum (LD 9 and 16), selected pups from each litter were killed, blood was taken and hematological parameters were determined

Experiment II – dose response: Four groups of females were kept on tap water, 0.5 g $NaNO_2$/L, 1 g $NaNO_2$/L, or 2 g $NaNO_2$/L throughout gestation and lactation. Litters were culled to 10 on day 2 postpartum. Pups selected across litters were killed for blood samples on days 7, 9, 13, 16, 20 postpartum.

Experiment III – cross-fostering: Pregnant females were given either tap water of 2 g $NaNO_2$/L from GD=. At birth, all pups were removed from the natural dams and replaced to foster dams, such that four groups of pups resulted: **A**: pups born to and weaned on dams drinking tap water (5 litters); **B**: pups born to dams dams drinking 2 g $NaNO_2$/L and weaned on dams drinking tap water (6 litters); **C** pups born to dams drinking tap water and weaned on dams drinking 2 g $NaNO_2$/L (5 litters); **D** pups born to and suckled on dams drinking 2 g $NaNO_2$/L (5 litters). Each dam received 10 pups. Pups selected across litters were killed on days 7, 14, 21 postpartum, blood was taken and tissues were preserved for histopathological examination

Histopathology: Tissues preserved in 10% phosphate-buffered formalin were trimmed and embedded in paraffin. Sections 4 µm thick were stained with hematoxyxlin and eosin and evaluated for pathological changes. Tissues routinely sectioned were kidney, liver, femur, ribs, spleen, thymus, salivary glands, mandibular lymph nodes, heart, and lungs.

Clinical chemistry and hematology: Red blood cell (RBC) counts and mean corpuscular volumes (MCV) were determined. Hemoglobin levels were measured. Blood smears were stained with either Wright´s stain or with cresyl blue vital stain and counterstained with Wright´s. Methemoglobin was determined. A microspectrophotometric assay based on the Greiss reaction was employed for analysis of nitrite in blood plasma.

Results and discussion

Experiment I:

Maternal weights and fluid consumption are shown in Table 1.

TABLE 1

MATERNAL BODY WEIGHTS AND FLUID CONSUMPTION[a]

	Gestation		Postpartum	
	Day 18 body weight (% Day 0 body weight)	Fluid intake[b] to Day 18 (ml/g body weight)	Day 20 body weight (% Day 0 gestation body weight)	Fluid intake[c] Days 1–20 postpartum (ml/g body weight)
Experiment I				
Control, Group I	140.1 ± 9.2	2.41 ± 0.36	136.8 ± 13.3[d]	5.07 ± 0.56[d]
2 g $NaNO_2$/liter, Group II	133.9 ± 9.4	1.97 ± 0.32[e]	123.3 ± 9.5[e]	4.19 ± 0.43[e]
3 g $NaNO_2$/liter, Group III	132.1 ± 6.9	1.80 ± 0.21[e]	114.9 ± 8.5[e]	3.43 ± 0.40[f]
Experiment II				
Control, Group IV	129.7 ± 0.2	2.45 ± 0.18[d]	123.4 ± 11.4	3.86 ± 0.35[e,f]
0.5 g $NaNO_2$/liter, Group V	132.2 ± 7.9	2.61 ± 0.32[d]	122.7 ± 10.0	4.71 ± 0.51[d]
1.0 g $NaNO_2$/liter, Group VI	131.7 ± 6.0	2.14 ± 0.46[d,e]	120.2 ± 5.8	4.44 ± 0.61[d,e]
2.0 g $NaNO_2$/liter, Group VII	129.1 ± 3.6	1.80 ± 0.29[e]	117.8 ± 6.2	3.04 ± 0.48[f]

[a] Values are $\bar{X}$ ± SD of 5 to 12 animals.
[b] Normalized to Day 18 body weight.
[c] Normalized to Day 20 postpartum body weight.
[d,e,f] From each experiment values within a column with different superscripts are significantly different, $p < 0.05$.

There were no significant differences in maternal body weights at GD18 between treated and untreated dams. Fluid consumption was lower in the treated groups compared to controls. At LD20, mean body weight of dams in both grops II and III was less than that of controls. At parturition, there were no significant differences in litter size, sex ratio, and mean pup weights. Thereafter pups of treated dams manifested poor weight gain compared to controls (see Fig. 1A).

≥

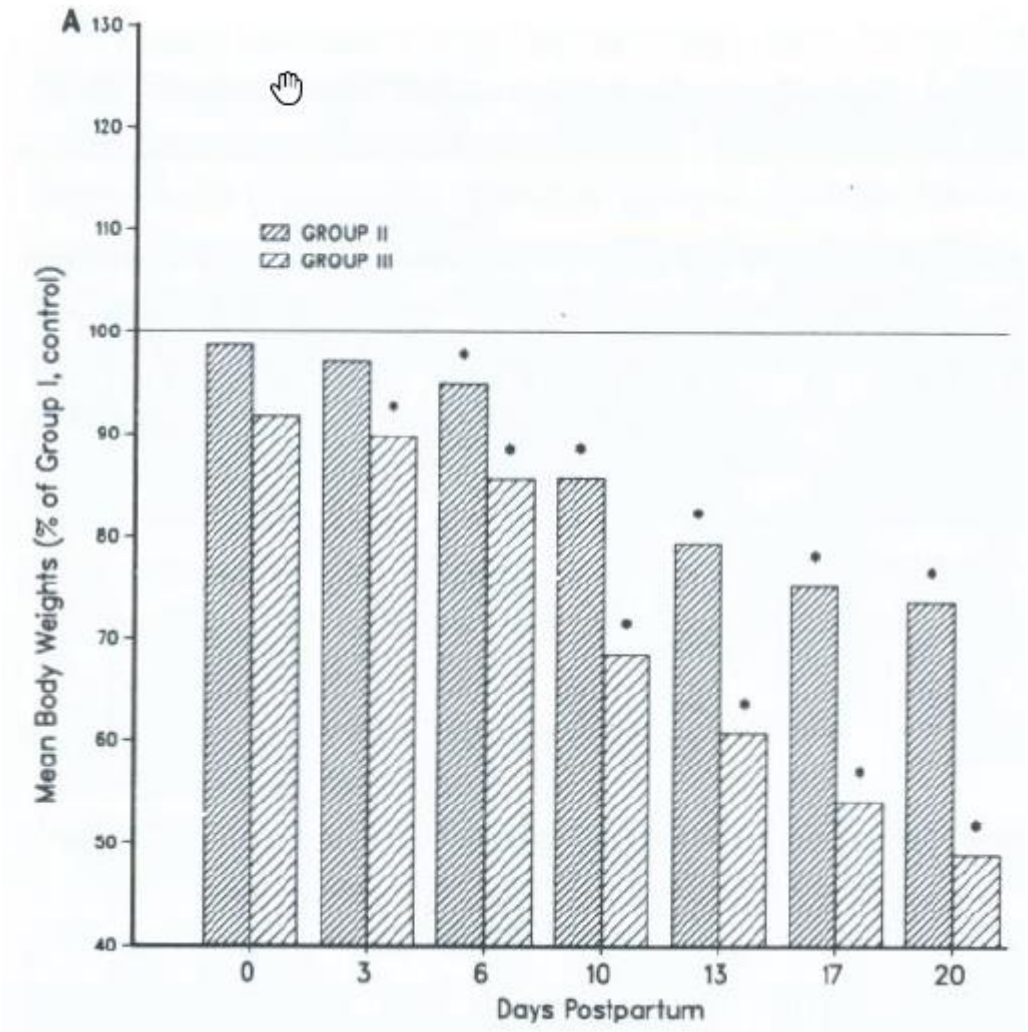


Fig. 1A: Effect of maternal consumption of sodium nitrite on pup body weight. (A) Experiment I: Group II, 2 g $NaNO_2$/L; Group III, 3 g $NaNO_2$/L; Mean body weights are expressed as %age of controls. Data for m&f pups are shown (no sex differences was confirmed); *$p<0.05$

Significant differences in mean body weights between control and Group III pups were seen from LD 6 and between control and Group II pups from LD13. By the second week of lactation, affected pups were pale, weak, and in generally poor condition with distended bellies. By LD21 mortality rates were 2% for controls, 10% for Group II and 30% for Group III offspring animals.

Hematological parameters of pups are shown in Table 2 below:

TABLE 2

HEMATOLOGICAL PARAMETERS OF PUPS—EXPERIMENT I

	Hemoglobin (g/dl)	RBC ($\times 10^6$/mm^3)	MCV (fl)	Methemoglobin (%)
Day 9				
Group I ($N = 4$)	9.85 ± 0.24^a	2.94 ± 0.21^a	94.5 ± 4.5	1.44 ± 0.46
Group II ($N = 3$)	6.70 ± 0.53^b	2.03 ± 0.13^b	86.7 ± 4.7	2.72 ± 1.36
Group III ($N = 4$)	6.13 ± 1.19^b	1.94 ± 0.18^b	81.0 ± 11.0	2.12 ± 1.47
Day 16				
Group I ($N = 4$)	8.28 ± 0.44^a	3.55 ± 0.27^a	69.5 ± 3.9^a	0.37 ± 0.57
Group II ($N = 7$)	5.83 ± 0.64^b	2.18 ± 0.32^b	61.7 ± 2.4^b	0.19 ± 0.38
Group III ($N = 4$)	5.95 ± 1.23^b	1.85 ± 0.31^b	62.3 ± 1.3^b	0.90 ± 0.81

[a,b] On each day within a column values with different superscripts are significantly different, $p < 0.05$. Values are $\bar{X} \pm$ SD.

Pups of both groups II and III were severely anemic on LD 9 and 16 with depressed hemoglobin levels and RBC counts, relative to control values, on both days, MCVs for both test groups were lower than that of the control group on LD16.

Plasma was negative for nitrite on LD9 and 16, but detected on LD19 and 20, when pups were directly consuming treated drinking water. Nitrite was detected in plasma of dams.

Experiment II

There were no differences in maternal weight gain between treated and untreated dams at GD18 and LD20 (see Table 1). Fluid consumption was lower only in Group VII (2 g $NaNO_2$/L) compared to control. At LD20 fluid consumption for Groups VI and VII was the same as that of the controls, in Group V consumption was greater than in controls (see Table 1).

Again, there were no significant differences in birth weight, litter size, and sex ratio amoung groups at parturition. The growth of pups in Group VII lagged behind controls with significant differences in pup body weights observed on LD17 and 20 (see Fig. 1B). No treatment-related pup mortality during lactation was observed.

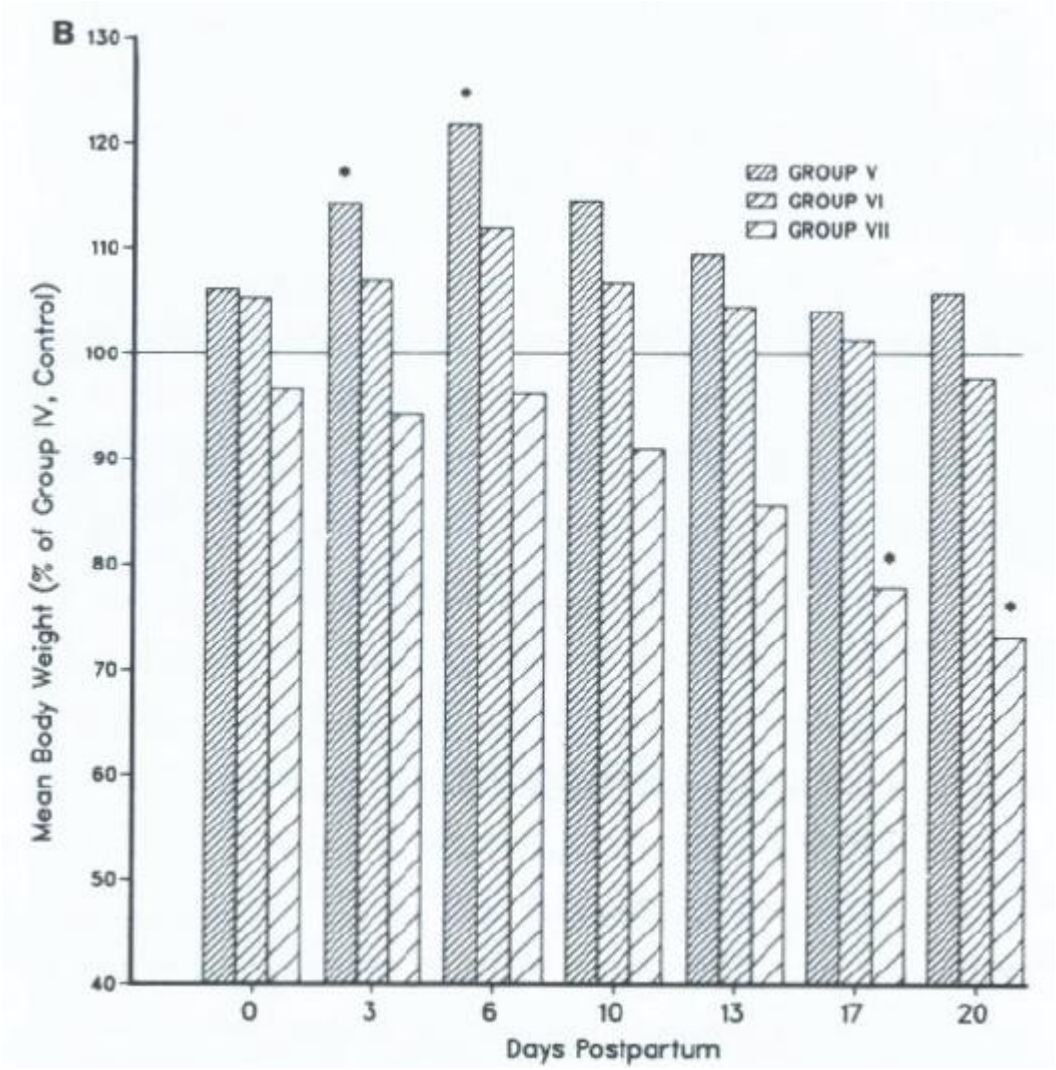


Fig. 1B: Effect of maternal consumption of sodium nitrite on pup body weight. (B) Experiment II: Group IV, 0.5 g $NaNO_2$/L; Group VI, 1 g $NaNO_2$/L; Group VII, 2 g $NaNO_2$/L. Mean body weights are expressed as %age of controls. Data for m&f pups are shown (no sex differences was confirmed); *$p<0.05$

Hematological parameters of offspring are shown in Fig 2 below:

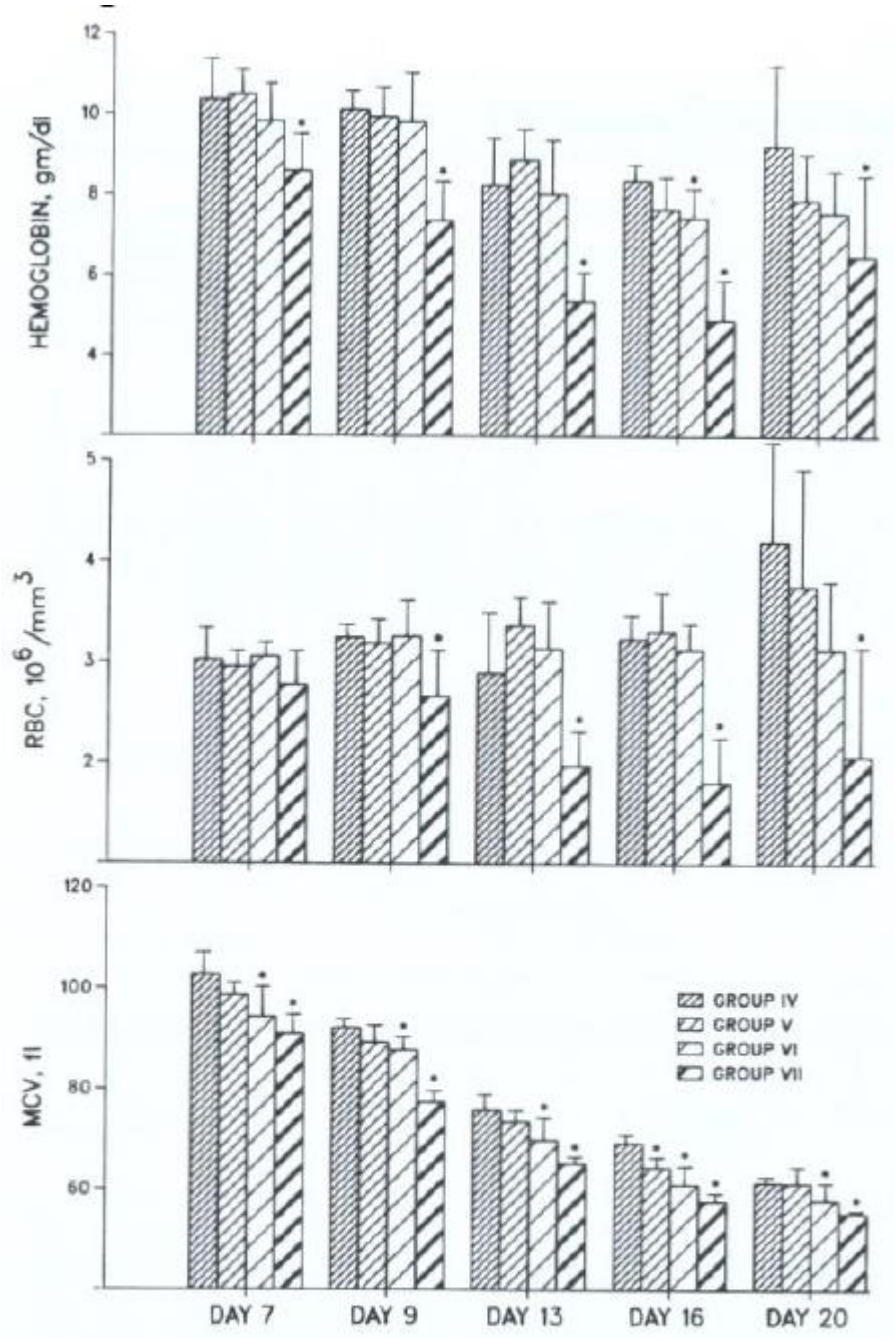


FIG. 2. Hematological parameters from Day 7 postpartum to weaning of pups from dams consuming 0, 0.5, 1.0, or 2.0 g $NaNO_2$/liter, (Experiment II). Group IV, control; Group V, 0.5 g $NaNO_2$/liter; Group VI, 1.0 g $NaNO_2$/liter; and Group VII, 2.0 g $NaNO_2$/liter. Bars indicate standard deviation. *Significantly different from control, $p < 0.05$.

For Group VII (2 g $NaNO_2$/L) hemoglobin levels were reduced from control values as early as LD7 and red blood cell counts were reduced by LD9. Group VI (1 g $NaNO_2$/L) had significantly reduced hemoglobin levels on LD16. MCV in Groups VI and VII were reduced on LD7 and Group V was reduced on LD16.

Experiment III

There were no significant differences between the control and treatment group in pup weight, litter size, and sex ratio at birth and no treatment-related postpartum mortality. Individual pup weights shwoed significant differences among groups beginning on LD5 when Group D (treated-treated) mean weight was reduced from that of the control. Fig. 3 below graphically depicts the mean pups weights of Groups B (treated-untreated), C (untreated-treated), and D (treated-treated) expressed as %age of Grop A (control-control).

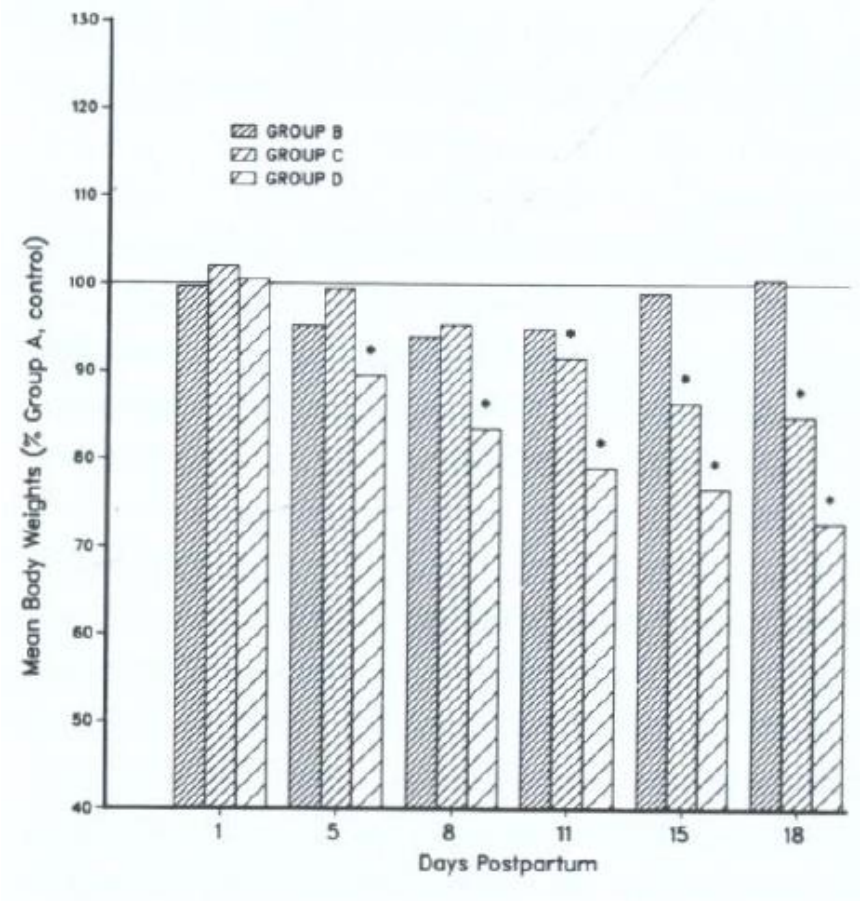


FIG. 3. Mean body weights of cross-fostered pups (Experiment III), expressed as percentage of control (Group A). Results of Tukey's multiple comparison of body weight means are as follows: Day 1, CDAB; Day 5, ACBD; Day 8, ACBD; Day 11, ABCD; Day 15, ABCD; Day 18, BACD (groups are listed in order of decreasing means and means of groups underlined with the same line are not significantly different, $p > 0.05$). Sex was not a significant variable. Group B: pups born to dams drinking 2 g $NaNO_2$/liter and suckled on dams drinking tap water. Group C: pups born to dams drinking tap water and suckled on dams drinking 2 g $NaNO_2$/liter. Group D: pups born to and suckled on dams drinking 2 g $NaNO_2$/liter. *Significantly different from control, $p < 0.05$.

By LD11 both groups weaned by dams treated with sodium nitrite weighed less than the controls, while Group B pups, exposed only prenatally, remained indistinguishable from the controls. A repeated measures analysis of variance investigating variables affecting weight showed that the combined gestational and

lactational treatmetn effect did not lead to significant differences (p>0.07); therefoe, gestational and lactational treatment effects could be considered separately. The effects of gestational treatment resulted in significant differences (p<0.008) as prenatally exposed pups (Groups B and D), weighted less than pups not prenatally exposed (Groups A and C). Postnatal treatment effect differences were highly esignificant (p<0.0001) with postnatally exposed pups (Groups C and D) weighing significantly less than pups not postnatally exposed (Groups A and B). The influence of prenatal treatment was predominant on days 1 through LD8 while postnatal treatmetn effects predominated on LD11 to 18.

Hematological parameters of pups on LD7, 14 and 21 are shown in Table 3:

TABLE 3

HEMATOLOGICAL PARAMETERS OF CROSS-FOSTERED PUPS

	Group A	Group B	Group C	Group D
MCV (fl)				
Day 7	$98.4 \pm 2.6^{a,b}$	101.4 ± 0.9^{a}	$93.8 \pm 7.5^{b,c}$	90.8 ± 5.3^{c}
Day 14	73.0 ± 2.7^{a}	70.4 ± 3.5^{a}	62.8 ± 1.0^{b}	62.8 ± 2.4^{b}
Day 21	61.4 ± 4.5^{a}	56.6 ± 2.9^{b}	56.6 ± 2.3^{b}	56.2 ± 3.1^{b}
RBC ($\times 10^6$/mm^3)				
Day 7	3.43 ± 1.6^{a}	3.30 ± 0.28^{a}	3.51 ± 0.33^{a}	3.49 ± 0.44^{a}
Day 14	3.66 ± 0.32^{a}	3.42 ± 0.27^{a}	2.87 ± 0.31^{b}	2.34 ± 0.39^{c}
Day 21	4.22 ± 0.33^{a}	4.22 ± 0.79^{a}	2.82 ± 0.38^{b}	1.79 ± 0.92^{c}
Hemoglobin (g/dl)				
Day 7	11.8 ± 0.6	11.5 ± 0.8	11.2 ± 0.9	10.4 ± 1.9
Day 14	9.2 ± 1.0^{a}	8.3 ± 1.0^{a}	6.7 ± 0.6^{b}	6.2 ± 0.7^{b}
Day 21	9.0 ± 0.9^{a}	8.4 ± 1.5^{a}	6.6 ± 0.5^{b}	5.3 ± 1.6^{b}

^{a,b,c} Values within a line with different superscript are significantly different, $p < 0.05$. Values are all $\bar{X} \pm$ SD for five or more animals.

Pups weaned by dams drinking sodium nitrite (Groups C and D) were anemic by LD14 with significant reductions in RBC, MCV and haemoglobin values as compared to control values. Lactational effects on hematological parameters were revealed on comparison of Group A values with those from Group C, and comparison of Group B values with Group D. Both comparisons showed differences in all parameters on LD14 and 21. In contrast, gestational exposure alone seemed to have little effect on subsequent hematological status.

Histopathological examination revealed mild centrilobular vacuolization of the liver (2/5) and decreased hematopoiesis in the spleen (1/5) in Group D on LD14. Group D animals at LD21 showed prominent centrilobular cacuolization of the liver (4/5) and decreased hematopoiesis of he spleen (2/5); a mild decrease of mature erythroid cells in femoral marrow was seen in 1/5. Blood smears of LD14 and 21 showed severe anisocytosis and hypochromasia in Group D. Mild anisocytosis and hypochromasia was observed in Groups B and C.

Conclusion

Offspring of dams treated with different concentrations of sodium nitrite, which causes iron deficiency and anemia, showed no effects on litter size, pup body weight or sex ratio. However offspring developed growth reductions during lactation of treated dams. Signs of offspring anemia was evident at LD 9 and 16 without detectable sodium nitrite in plasma, which indicates a secondary effect to maternal anemia. A cross-fostering experiment confirmed, that offspring anemia and body weight development is strongly correlated with lactational exposure via sodium-nitrite treated mothers. Decreased body weight development is only seen in lactating offspring of dams drinking sodium nitrite during lactation (Groups C and D), not with dams, drinking sodium nitrite only during gestation (Group B).

3.10.1.23 Study 23

Published lactating animal studies, rat
Study reference:

Roth & Smith (1988): Nitrite-induced iron deficiency in the neonatal rat. Toxicology and Applied Pharmacology 96, 43-51 (BASF DocID 1988/1003436)

Detailed study summary and results:

Test type
Maternally mediated, nitrite-associated iron-deficiency in neo-natal rats. Mechanistical iron replacement and iron status study, no guideline available.

Test substance
Test substance used is the equivalent to substance in CLH dossier: no, $NaNO_2$ (sodium nitrile)
Analytical purity: no data

Test animals

Species:	rat
Strain:	Long-Evans hooded
Sex:	male and female
Source:	Charles River Laboratories
No. of animals/sex/dose:	6 - 13 females
Weight at study initiation:	~ 200 g
Housing:	before mating: Male rats were single-housed and virgin females were maintained three to a cage until; at GD0: females were single-housed in plastic shoebox cages
Diet:	Purina starndard lab chow, containing ~ 250 ppm iron, *ad libitum*
Water:	tap water, *ad libitum*
Environmental conditions:	Photoperiod (h dark/h light): 12/12

Administration/exposure

Route of administration:	oral; by drinking water
Vehicle:	drinking water
Duration of treatment:	Experiment I: GD0 – LD21 Exeriments II/III: GD0 – LD15
Frequency of treatment:	daily
Dose levels:	Experiment I (iron replacement): 0; 0 + Fe (to pups only); 3 g $NaNO_2$/L; 3 g $NaNO_2$/L + Fe (to pups only) Experiment II/III (iron status): 0; 2 g $NaNO_2$/L
Control group and treatment:	yes, tap water. Experiment I: additional pup control group receiving the vehicle and Fe-supplement was introduced
Statistical analysis:	data were analysed using the ANOVA i.e. followed by Tukey'S mulpiple comparison or Duncan's post hoc analysis, Kruskal-Wallis test or Student's t-test

Observations and examinations performed and frequency:

Experiment I (iron replacement):

Two groups of females (six per group) were maintained throughout gestation and lactation on tap water (control) or 3.0 g $NaNO_2$/liter. On Days 0, 7, and 14 post-partum, half of each litter (five pups) was injected i.p. with 5, 10, and 20 µL, respectively of Imferon (Merrell Dow; 50 mg elemental iron/mL), equivalent to 0.25, 0.5, and 1.0 mg elemental iron. This resulted in four groups of pups: unsupplemented pups from dams on tap water (control); their iron-supplemented litter-mates (control/Fe); pups of dams drinking sodium nitrite with no intervention (nitrite); and their iron-supplemented litter-mates (nitrite/Fe). Pups selected across litters were sacrificed on Days 7, 14. and 21 post-partum, blood was taken, and tissues were preserved for histopathological examination.

Experiment II (maternal iron status):
Two groups of pregnant females were maintained from Day 0 gestation on: tap water control (10 animals) or 2.0 g $NaNO_2$/liter (13 animals). Also maintained concurrently were two groups of virgin females: control-unbred (5 animals) on tap water and nitrite-unbred (4 animals) on 2 g $NaNO_2$/liter. All dams and pups were sacrificed at Day 15 postpartum, blood was taken. and hematological parameters and plasma iron levels were determined.

Experiment III (pup iron status):
Pregnant females were maintained from Day 0 gestation on tap water (control, seven animals) or 2.0 g $NaNO_2$/liter (eight animals). All pups and dams were sacrificed on Day 15 post-partum, blood samples taken and hematological parameters, plasma iron levels and total plasma iron binding capacity (TIBC) determined. Heart, liver. and spleen were removed and weighed. Pup liver and spleen as well as milk, were retained for iron and copper analysis.

Results and discussion
Experiment I (iron replacement):
During gestation, females drinking 3.0 g $NaNO_2$/liter consumed significantly less fluid than did controls, but there were no statistically significant differences between groups in maternal gestational weight gain. At parturition, there were no differences in sex ratio or litter size, but pups of dams drinking 3.0 g $NaNO_2$/liter weighed significantly less than did control pups.
Without intervention, pups of dams drinking 3.0 g $NaNO_2$/liter developed severe microcytic anemia by the second week post-partum, showed poor weight gain, and suffered a 44% mortality rate by PND 20. Iron-supplemented litter-mates of these anemic pups showed normal hematological parameters, increased weight gain, and had no mortality before PND 20. Unsupplemented nitrite pups had hemoglobin, RBC, and MCV values all significantly lower than the values for control pups. Their iron-supplemented litter-mates, (nitrite/Fe pups) had hemoglobin and RBC values identical to control values, and MCVs that were greater than the unsupplemented nitrite pup value, although less than the control value. By PND 20, the MCV of nitrite/Fe pups was the same as the control value. Iron-supplemented litter-mates of control pups, control/Fe pups, had MCV and hemoglobin levels significantly elevated over unsupplemented control values. On PND 14, the mean body weight of control/Fe pups was not significantly different from that of control pups. Unsupplemented nitrite pups weighed 57% and their supplemented litter-mates (nitrite/Fe pups) 68% of control, both significantly different from control and each other. These relative differences in body weight persisted throughout the lactational period to PND 20.
Histological evaluation of pup tissues revealed decreased erythropoiesis in the liver on PND 7 in nitrite/Fe pups (2/6, minimal decrease) and nitrite pups (3/6, minimal to moderate decrease) and centrilobular cytoplasmic vacuolization of the liver in one nitrite pup. On PND 14, decreased spleen hematopoiesis was noted in two of six nitrite pups examined, and centrilobular cytoplasmic vacuolization of the liver was noted in five of six nitrite pups. Centrilobular cytoplasmic vacuolization was also observed in the livers of five of six nitrite pups and one of six control pups on PND 21. Evidence of decreased erythropoiesis was also seen in nitrite pups. This comprised a decrease in mature erythroid cells of the femur (3/6) and rib (l/6), combined with marrow hypoplasia and decreased erythropoiesis in the femur (l/6).

MATERNAL WEIGHT GAIN AND FLUID INTAKE

	Gestational weight gain (%)	Day 15 postpartum weight (g)	Fluid intake (ml/100 g body wt/day)	
			Gestation	Postpartum
Experiment I[a]				
Control	64.8 ± 10.3[b]	—	14.2 ± 1.5	—
3.0 g $NaNO_2$/liter	56.4 ± 9.4	—	10.7 ± 0.7*	—
Experiment II[a]				
Control	47.8 ± 8.0	311 ± 14	12.0 ± 5.1	17.8 ± 6.4
2.0 g $NaNO_2$/liter	45.4 ± 8.2	259 ± 16*	8.2 ± 0.9*	15.8 ± 2.3*
Control—unbred[c]	13.1 ± 14.8	239 ± 5	10.6 ± 1.1	10.1 ± 3.3
2.0 g $NaNO_2$/liter—unbred[c]	3.8 ± 3.5	227 ± 14	8.8 ± 1.4*	7.1 ± 0.9*
Experiment III[a]				
Control	53.5 ± 6.5	319.8 ± 23.7	15.7 ± 2.1	26.3 ± 3.4
2.0 g $NaNO_2$/liter	53.4 ± 5.4	275.9 ± 16.7*	10.3 ± 1.8*	20.5 ± 1.3*

[a] In each experiment female rats were maintained on tap water (control) or the indicated nitrite solution from Day 0 gestation. Body weights and fluid intake were monitored biweekly.
[b] Means ± SD.
[c] Maintained concurrently with mated females.
* Significantly different than the corresponding control, $p < 0.05$.

BIRTH PARAMETERS

	Mean litter size	Sex ratio (female/male)	Pup weight	
			Female	Male
Experiment I[a]				
Control	11.3	0.99	6.15 ± 0.15[b]	6.56 ± 0.11
3.0 g $NaNO_2$/liter	12.7	0.86	5.45 ± 0.15*	5.86 ± 0.15*
Experiment II[a]				
Control	11.2	0.98	6.49 ± 0.16	6.84 ± 0.17
2.0 g $NaNO_2$/liter	11.6	1.19	5.98 ± 0.17	6.47 ± 0.16
Experiment III[a]				
Control	10.1	0.90	6.30 ± 0.19	6.51 ± 0.20
2.0 g $NaNO_2$/liter	14.4*	0.90	5.60 ± 0.18*	5.88 ± 0.19*

[a] Mothers given either tap water (control) or nitrite solution in the water bottle throughout gestation.
[b] Means ± SD.
* Significantly different than the corresponding control, $p < 0.05$.

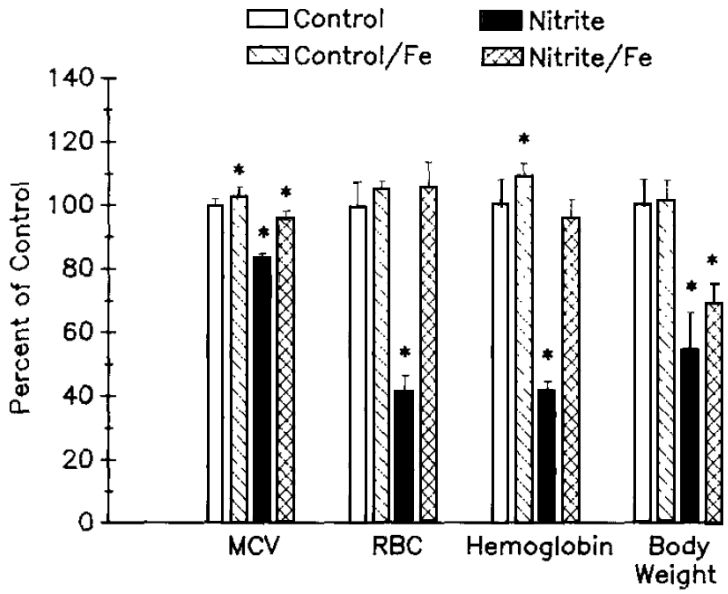


Figure 3: Hematological parameters and body weights of Experiment I pups on PND 14 expressed as percentage of the mean control value; control/Fe: iron-supplemented litter-mates of control pups; nitrite: unsupplemented pups of dams drinking 3.0 g $NaNO_2$/liter; nitrite/Fe: iron-supplemented litter-mates of the nitrite pups. Bars with asterisks are significantly different than control, $p < 0.05$.

Experiment II (maternal iron status):

Fluid consumption during gestation and lactation or in the unbred state was significantly decreased in adult females drinking 2 g $NaNO_2$/liter compared to respective controls. Gestational weight gain was unaffected by sodium nitrite, but the final body weight on Day 14 post-partum was significantly decreased in the treated mothers.

Birth weight, litter size, and sex ratio were unaffected by maternal consumption of 2 g $NaNO_2$/liter. Thereafter, pup growth was affected in these pups such that their PND 14 mean body weight was 75% of control weight. By PND 15, pups of dams drinking 2 g $NaNO_2$/liter developed microcytic, hypochromic anemia relative to control pups. Treatment group pups had MCVs and RBC and hemoglobin levels which were all significantly reduced from control values. Plasma iron values were also significantly reduced in pups of dams drinking 2 g $NaNO_2$/liter compared to control pups.

In mothers drinking 2 g $NaNO_2$/liter, microcytosis was found with hemoglobin levels and plasma iron values significantly reduced compared to control mothers. Maternal RBC counts were unaffected by treatment.

Nitrite treatment of unbred adult females had no effect on hematological parameters or plasma iron values compared to unbred females maintained on tap water. In comparison with the lactating mothers, RBC counts were higher in unbred females. Hemoglobin levels in unbred females were the same as in the untreated control mothers, however, MCVs and plasma iron levels were comparable to those of nitrite-treated mothers.

HEMATOLOGICAL PARAMETERS ON DAY 15 POSTPARTUM, EXPERIMENT II[a]

	Mean corpuscular volume (fl)	Red blood cell count (10^6/mm)	Hemoglobin (g/dl)	Plasma iron (μg/dl)
Pups				
Control	71.8 ± 2.6[b]	3.73 ± 0.39	9.61 ± 1.22	116 ± 43
2.0 g $NaNO_2$/liter	63.3 ± 2.6*	2.67 ± 0.49*	6.64 ± 0.89*	51 ± 25*
Dams				
Control	62.6 ± 2.9[c]	6.93 ± 0.28[c]	14.8 ± 0.50[c]	530 ± 81[c]
2.0 g $NaNO_2$/liter	54.8 ± 1.5[d]	7.05 ± 0.39[c]	13.4 ± 0.99[d]	232 ± 97[d]
Unbred adult females				
Control	54.4 ± 0.5[d]	7.96 ± 0.40[d]	15.6 ± 0.75[c]	223 ± 48[d]
2.0 g $NaNO_2$/liter	56.5 ± 2.1[d]	7.61 ± 0.21[d]	15.5 ± 0.85[c]	243 ± 41[d]

[a] Adult females maintained on tap water (control) or 2 g $NaNO_2$/liter from Day 0 gestation until death on Day 15 postpartum.
[b] Means ± SD.
[c,d] Mean values for all adults were compared by a one-way ANOVA, followed by Duncan's post hoc analysis. Means with the same superscript in a column are not significantly different, $p > 0.05$.
* Significantly different than control, $p < 0.05$.

Experiment III (pup iron status):

Fluid consumption was again decreased in dams drinking 2 g $NaNO_2$/liter compared to dams drinking tap water.

At parturition, there was no significant difference between groups in pup sex ratio. Litter size was significantly increased in the treatment group, and mean pup birth weight was concomitantly decreased. By PND 14, treatment group pups had gained less weight than had control pups. On PND 15, treated pups had significantly reduced hematological parameters compared to controls. Their plasma iron levels were reduced and TIBC values were increased.

Pups suckled by mothers drinking 2 g $NaNO_2$/liter had enlarged hearts and small spleens compared to control pups. Liver iron content was significantly reduced and liver copper content significantly increased in pups of treated mothers compared to controls.

The mothers drinking 2 g $NaNO_2$/liter had reduced plasma iron, elevated TIBC, and enlarged hearts and spleens. The iron content of their milk was significantly decreased compared to mothers maintained on tap water.

HEMATOLOGICAL PARAMETERS OF PUPS ON DAY 15 POSTPARTUM, EXPERIMENT III[a]

	Mean corpuscular volume (fl)	Red blood cell count (10^6/mm)	Hemoglobin (g/dl)	Serum iron (g/dl)	TIBC (g/dl)
Control	70.7 ± 2.7[b]	3.74 ± 0.36	10.13 ± 0.99	140 ± 17	493 ± 64
2 g $NaNO_2$/liter	59.6 ± 1.6*	1.97 ± 0.47*	5.94 ± 0.91*	55 ± 11*	666 ± 9*

[a] Mothers were maintained on tap water (control) or 2 g $NaNO_2$/liter from Day 0 gestation to Day 15 postpartum.
[b] Means ± SD.
* Significantly different than control $p < 0.05$.

PUP TISSUE WEIGHTS AND Fe AND Cu CONTENT ON DAY 15 POSTPARTUM

	Control pups[a]		2.0 g $NaNO_2$/liter pups[a]	
	Female	Male	Female	Male
Heart wt/body wt × 10^2	0.61 ± 0.07[b]	0.61 ± 0.07	0.94 ± 0.21*	0.93 ± 0.15*
Spleen wt/body wt × 10^2	0.49 ± 0.09	0.49 ± 0.07	0.35 ± 0.06*	0.33 ± 0.07*
Liver wt/body wt × 10^2	3.89 ± 1.03	3.64 ± 0.37	3.43 ± 0.92	3.67 ± 0.39
Spleen iron (μg/g dry wt)	453 ± 110	425 ± 79	412 ± 132	372 ± 117
Liver iron (μg/g dry wt)	106 ± 31	132 ± 49	79 ± 17*	79 ± 12*
Spleen copper (μg/g dry wt)	8.9 ± 2.0	9.6 ± 2.0	11.6 ± 2.8	11.9 ± 3.6
Liver copper (μg/g dry wt)	124 ± 64	125 ± 67	245 ± 78*	266 ± 104*

[a] Mothers of pups were maintained on tap water (control) or 2.0 g $NaNO_2$/liter from Day 0 gestation to Day 15 postpartum.
[b] Means ± SD.
* Significantly different than control, $p < 0.05$.

MATERNAL TISSUE WEIGHTS AND Fe AND Cu CONTENT OF MILK DAY 15 POSTPARTUM

	Control dams[a]	Treated dams[a]
Heart wt/body wt × 10^2	0.31 ± 0.03[b]	0.40 ± 0.07*
Spleen wt/body wt × 10^2	0.188 ± 0.04	0.227 ± 0.05*
Liver wt/body wt × 10^2	4.74 ± 0.51	4.80 ± 0.45
Milk Fe (μg/g dry wt)	34.2 ± 9.9	22.2 ± 9.9*
Milk Cu (μg/g dry wt)	8.7 ± 1.7	10.8 ± 2.7*
Plasma iron (μg/dl)	353 ± 28	165 ± 52*
TIBC (μg/dl)	583 ± 23	727 ± 37*

[a] Dams given either tap water (control) or 2 g $NaNO_2$/liter (treated) in the water bottle from Day 0 gestation until death on Day 15 postpartum.
[b] Means ± SD.
* Significantly different than control, $p < 0.05$.

Conclusion

Anaemic mothers were unable to transfer sufficient iron to offspring via the milk.

3.10.1.24 Study 24

Published lactating animal studies, rat
Study reference:

Anaokar and Garry (1981): Effects of maternal iron nutrition during lactation on milk iron and rat neonatal iron status. Am J Clin Nutr. 34(8):1505-12. (BASF DocID 1981/1001521)

Detailed study summary and results (see RAR (2017) Volume 3, Annex B-6, pp 211 – 214):

Report:	KCA 5.6.1/3 Anaokar S.G, Garry P.J. (1981; BASF DocID 1981/1001521)
Title:	Effects of maternal iron nutrition during lactation on milk iron and rat neonatal iron status. Am J Clin Nutr. 34(8):1505-12.
Guideline:	Not stated
Test substance:	none
Previous evaluation:	none
GLP:	Non-GLP
Relevance check:	Relevant
Reliability check:	Reliable (Klimish score: 2, the number of animals, the period of treatment before mating is lower than recommended in the relevant guideline)

32 pregnant and 13 nonpregnant Sprague-Dawley rats (77 day old) were maintained on control diet and deionized water until the third day of lactation.
On the third lactation day pups of 5 lactating dams were weighed and sacrificed. Blood samples of pups from the same litters were pooled. 16 hours later the five lactating dams were milked, blood samples were taken and the animals were sacrificed. Their livers were perfused with saline (0.9% NaCl), removed and kept frozen with plasma and milk samples until assayed. Five non-pregnant females were sampled in the same way.
From the third day of lactation 8 dams/group were fed iron-deficient diet (25 ppm iron), control diet (250 ppm iron), and high iron-content diet (2500 ppm iron).
A nonlactating group of dams (3 dams whose pups were sacrificed ater their birth) was fed with control diet. The non-pregnant rats were continued to be fed with control diet. All animals received deionized water, *ad libitum*.
On the 11^{th} day of lactation pups from 3 dams/ treatment group were weighed and sacrificed. Blood, plasma and liver samples were collected. Iron content of milk samples, ferritin content in plasma and liver samples and total iron binding capacity (TIBC) were determined with adequate methods.
Results are summarized in figures1-4 and tables 2-4.

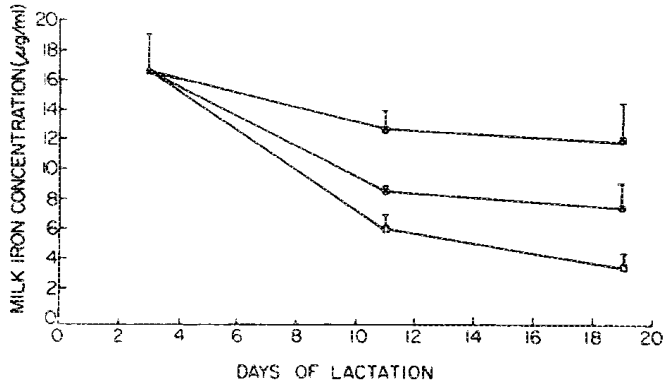


FIG. 1. Changes in milk iron concentration (μg/ml) during lactation of dams from the high-iron (▩), the control (●), and the iron-deficient (□) groups (the zero on the X-axis indicates the day of parturition). Each *point* represents the mean concentration and the *horizontal bars* indicate 1 SD.

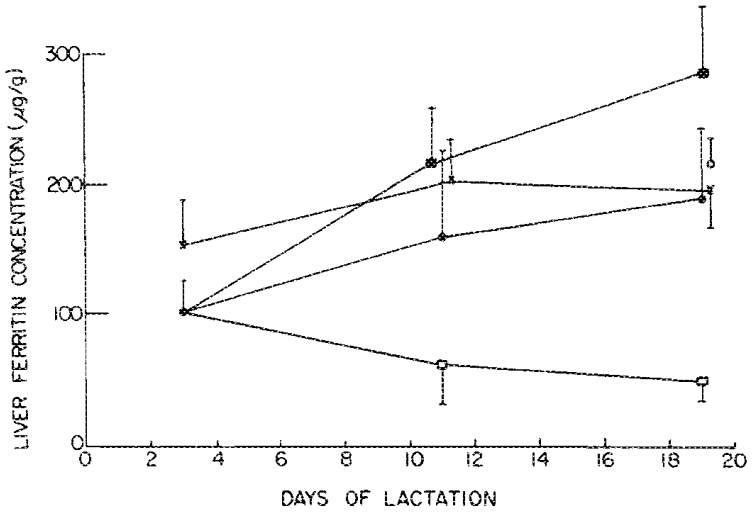


FIG. 2. Changes in liver ferritin concentration (μg/g) during lactation of dams from the high-iron (▩), the control (●), the iron-deficient (□), the nonpregnant (×), and the nonlactating (○) groups (the zero on the X-axis indicates the day of parturition). Each *point* represents the mean concentration and the *horizontal bars* indicate 1 SD.

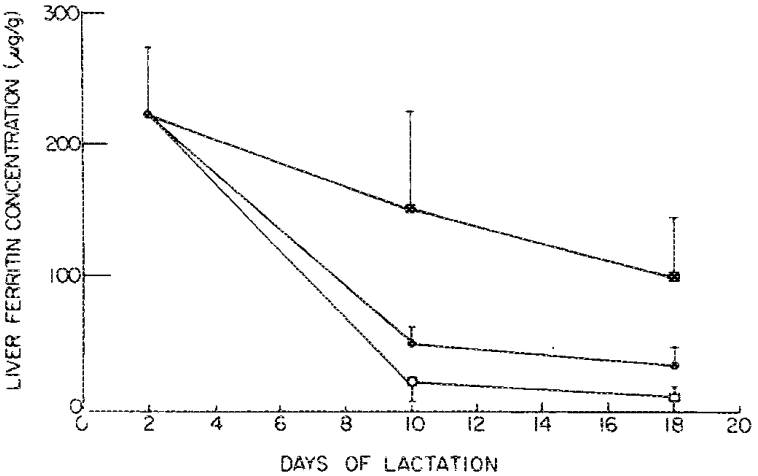


FIG. 3. Changes in liver ferritin concentration (μg/g) of pups of dams from the high-iron (▩), the control (●), and the iron-deficient (□) groups (the zero on the X-axis indicates the day of parturition). Each *point* represents the mean concentration and the *horizontal bars* indicate 1 SD.

TABLE 2
Hematological and biochemical changes in adult rats

Day of lactation*	Group	Hemoglobin	Plasma ferritin	Plasma iron	TIBC
		g/100 ml ± SD	*ng/ml ± SD*	*μg/100 ml ± SD*	*μg/100 ml ± SD*
4th	Lactation	14.3 ± 0.7	133 ± 64	270 ± 120	600 ± 80
4th	Nonpregnant	15.2 ± 0.6	168 ± 37	285 ± 40	500 ± 30
12th	Iron deficient	14.7 ± 0.4	123 ± 65	220 ± 59	676 ± 89
12th	Control	17.3 ± 0.9	338 ± 130	291 ± 50	575 ± 73
12th	High iron	17.3 ± 1.7	470 ± 120	429 ± 32	516 ± 66
12th	Nonpregnant	16.9 ± 0.6	213 ± 190	255 ± 21	563 ± 20
20th	Iron deficient	14.8 ± 0.7	90 ± 36	100 ± 45	690 ± 50
20th	Control	16.6 ± 1.2	230 ± 77	350 ± 100	590 ± 50
20th	High iron	16.5 ± 0.7	460 ± 212	400 ± 75	530 ± 60
20th	Nonpregnant	15.5 ± 0.7	270 ± 130	315 ± 42	450 ± 99
20th	Nonlactating	16.3 ± 0.9	268 ± 72	336 ± 96	550 ± 41

* The day of parturition was counted as the 1st day of lactation.

TABLE 3
Hematological and biochemical changes in pups

Day of lactation*	Group	Hemoglobin	Plasma ferritin	Plasma iron	TIBC
		g/100 ml ± SD	*ng/ml ± SD*	*μg/100 ml ± SD*	*μg/100 ml ± SD*
3rd		11.3 ± 1.3	1712 ± 300	225 ± 75	325 ± 75
11th	Iron deficient	8.5 ± 0.1	250 ± 160	60 ± 15	553 ± 230
11th	Control	11.5 ± 0.1	325 ± 125	195 ± 60	500 ± 85
11th	High iron	11.1 ± 0.6	560 ± 120	245 ± 50	500 ± 65
19th	Iron deficient	7.2 ± 0.5	87 ± 43	40 ± 10	630 ± 45
19th	Control	9.9 ± 0.4	134 ± 38	330 ± 100	575 ± 45
19th	High iron	11.8 ± 0.5	331 ± 52	420 ± 60	515 ± 40

* The day of parturition was counted as the 1st day of lactation.

MATERNAL DIET AND NEONATAL IRON STATUS 1511

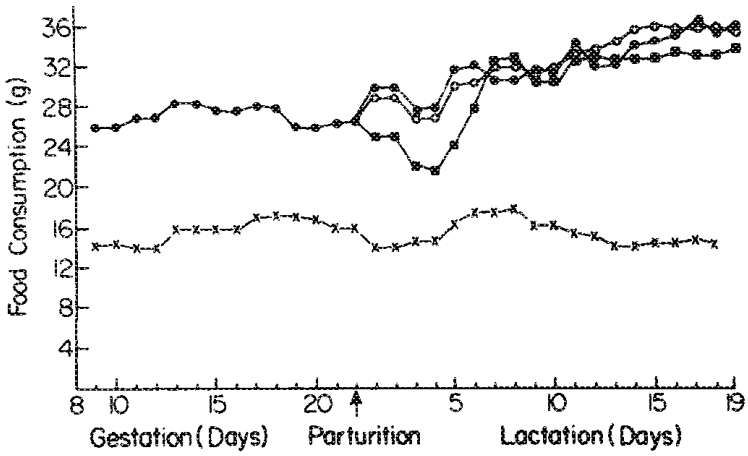


FIG. 4. Daily food consumption (g) during gestation of pregnant (●) and nonpregnant (×) rats, and during lactation of dams from the high-iron (■), the control (●), and the iron-deficient group (○), and nonpregnant rats (×).

TABLE 4
Weights of dams and pups during lactation

Group	Dams		Pups	
	Day of lactation*	Aveage wt of dams ± SD	Day of lactation*	Average wt of pups ± SD
		g		g
Pregnant	4th	242 ± 26	3rd	10 ± 1
Nonpregnant	4th	239 ± 7		
Iron deficient	12th	266 ± 28	11th	23 ± 3
Control	12th	282 ± 11	11th	18 ± 3
High iron	12th	278 ± 15	11th	21 ± 4
Nonpregnant	12th	262 ± 18		
Iron deficient	20th	263 ± 9	19th	35 ± 3
Control	20th	289 ± 8	19th	34 ± 3
High iron	20th	237 ± 10	19th	34 ± 5
Nonpregnant	20th	264 ± 10		
Nonlactating	20th	270 ± 40		

* The day of parturition was counted as the 1st day of lactation.

Conlusion (relevant to the assessment of dimoxystrobin):
Pups of iron deficient dams became iron deficient, indicated by their decreased ferritin, plasma iron and haemoglobin levels and increased TIBC. **Direct relationship was found between iron intake during lactation and milk iron level, milk iron level reduction and the duration of lactation and between milk iron level and neonatal iron status.**

3.10.2 Human data

No human data available.

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

No relevant in vitro data available.

3.11 Specific target organ toxicity – single exposure

Not relevant as no changes to the existing harmonized classification for dimoxystrobin are proposed.

3.12 Specific target organ toxicity – repeated exposure

Not relevant as no changes to the existing harmonized classification for dimoxystrobin are proposed.

3.13 Aspiration hazard

Not relevant as no changes to the existing harmonized classification for dimoxystrobin are proposed.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

[Study 1]

Study reference:
Werner (1999): Determination of the biodegradability of BAS 505 F in the manometric respirometry test according to GLP, EN 45001 and ISO 9002, 1999/10288, BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep

Detailed study summary and results (see also RAR (2017) – Volume 3 - B.8 (AS), chapter B 8.2.2.1):

Studies evaluated previously, DAR, 2003
Ready biodegradation of dimoxystrobin was studied in the Manometric Respiratory Test according to OECD guidelines (No. 301/F, 1992). This is EC method C.4-D.
Activated sewage sludge was used for the test incubation carried out with a dimoxystrobin concentration of 100 mg/l.
The degree of biodegradation (% biological oxygen demand/theoretical oxygen demand) over the 28 day test duration was in the range 0-10%. Hence, dimoxystrobin is classified as 'not readily biodegradable' under the conditions of the test.

(Werner, 1999a)

Summary and Assessment
Dimoxystrobin was classified as 'not readily biodegradable' (OECD guideline 301/F, 1992).

Studies submitted for the renewal of the active substance
No new biodegradability study was performed for the renewal of the active substance. The already peer-reviewed study is considered still valid. Dimoxystrobin was found to be not readily degradable according to OECD guideline 301 F.

4.1.2 BOD_5/COD

No study available

4.1.3 Aquatic simulation tests

4.1.3.1 [Study 1]

Study reference:

McKenna & Baucom (1997): Hydrolysis of BAS 505 F [benzyl-U-14C] in aqueous media, 1996/5244, BASF Corp. Agricultural Products Center; Research Triangle Park, NC; United States of America

Detailed study summary and results (see also RAR (2017) – Volume 3 - B.8 (AS), chapter B 8.2.1.1):

Studies evaluated previously, DAR, 2003

The hydrolytic stability of dimoxystrobin was studied according to EPA (Pesticide Assessment Guidelines, Subdivision N, Series 161-1, 1982) guidelines and EC method C7.

Sterile aqueous buffer solutions (pH 4 5, 7 and 9) were prepared containing [^{14}C-benzyl dimoxystrobin (1.3 mg a.s./litre). Samples (25 ml) of each treated buffer were then incubated at 25°C (pH 5,7 and 9) or 50°C (pH4,7 and 9) in flasks in the dark. At the times indicated in the table below samples of each buffer were analysed directly by HPLC with the identity of parent dimoxystrobin confirmed by MS.

The results of the hydrolysis study indicate that dimoxystrobin was stable at all pH-values. Only trace amounts of some unknown substances (always < 1.59% AR) were visible in the chromatograms.

Table 4.1.3.1-1 Characterisation of radioactivity in %AR during hydrolysis of [^{14}C]-benzyl-labelled dimoxystrobin (DAR, 2003)

pH	DAT	dimoxystrobin	others (no. components)	sum
50°C				
4	0	98.9	1.1 (3)	100.0
	1	99.3	1.1 (3)	100.43
	2	103.7	1.5 (3)	105.15
	3	99.5	1.3 (3)	100.81
	4	96.9	2.3 (3)	99.27
	5	99.6	1.1 (3)	101.8
7	0	98.5	1.5 (3)	100.0
	1	100.7	1.7 (3)	102.45
	2	96.8	2.4 (3)	99.19
	3	101.7	1.7 (3)	103.48
	4	102.1	2.1 (3)	104.25
	5	101.8	1.7 (3)	103.54
9	0	97.7	2.3 (3)	100.0
	1	92.9	2.3 (3)	95.18
	2	93.5	2.7 (4)	96.21
	3	98.6	1.9 (3)	100.54
	4	100.1	1.3 (3)	101.50
	5	98.6	2.2 (3)	100.77
25°C				
5	0	96.9	3.1 (4)	100.0
	15	96.4	2.8 (5)	99.22
	30	90.7	3.3 (5)	93.92
7	0	98.0	2.0 (3)	100.0
	15	94.8	1.6 (3)	96.45
	30	93.7	2.2 (5)	95.73
9	0	97.6	2.3(3)	100.0
	15	101.8	2.6 (3)	104.47
	30	97.8	3.2 (6)	100.52

(McKenna and Baucom, 1997a)

Summary and Assessment
Dimoxystrobin was stable to hydrolysis under sterile conditions following incubation at 25°C and 50°C in aqueous solutions buffered at pH 4, 5, 7, 9. Therefore, hydrolysis is unlikely to be a major route of degradation at environmentally relevant temperature and pH.

Studies submitted for the renewal of the active substance
No new experimental data on hydrolysis of Dimoxystrobin were produced for the renewal of the active substance. The old hydrolysis study is considered still valid. Dimoxystrobin proved to be stable at all tested pH values (pH 4-9) and temperatures (25 and 50°C).

4.1.3.2 [Study 2]

Study reference:
Yeomas (2014): [14C]-Dimoxystrobin (BAS 505 F): Aerobic mineralisation in surface water, 2014/1031018, Smithers Viscient (ESG) Ltd., Harrogate North Yorkshire HG3 1PY, United Kingdom

Detailed study summary and results (see also RAR (2017) – Volume 3 - B.8 (AS), chapter B 8.2.2.2):

Reference	Yeomans P., 2014a 14C-Dimoxystrobin (BAS 505 F): Aerobic mineralisation in surface water 2014/1031018 CA 7.2.2.2/1
Guidelines	OECD 309 (April 2004)
GLP	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
test item	Dimoxystrobin (BAS 505 F)
study duration	59 days
study completed	November 2014

Executive Summary
The purpose of this study was to determine the mineralisation and degradation rates of the fungicidal active substance dimoxystrobin (BAS 505 F) in an aquatic system under dark conditions. The study was performed according to the OECD guideline 309 (Aerobic mineralization in surface water – Simulation biodegradation test). The pelagic test system was chosen for this study (surface water only).

The test was performed at two different dimoxystrobin concentrations (10 µg L^{-1} and 90 µg L^{-1}), using two differently ^{14}C-labelled test items (phenyl and benzyl label). Sterile samples were tested for each label of the higher concentration. The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of 20 ± 2°C in the dark. Samples were taken at 0, 3, 7, 14, 21, 35 and 59 days after treatment.
The amount and nature of radioactivity in the water samples was determined by liquid scintillation counting (LSC) and chromatographic methods (radio-HPLC). Volatiles were trapped in 2 M sodium hydroxide and were also analysed by LSC. Parent substance and metabolite identification was done by co-chromatography with the corresponding reference items on HPLC.

From the obtained results it could be concluded that dimoxystrobin was not significantly degraded in the natural water environment provided in the test. After 59 days, 89 to 97% AR (applied radioactivity) was recovered as unchanged active substance.
Some trace amounts (<1%) of the known metabolites 505M08 and 505M09 were detected and assigned by retention time comparison on HPLC analysis. Other degradation products were detected only in minor amounts (≤1.5% AR, highest single value).
Radioactivity in the volatile traps did not exceed 1% AR indicating a low rate of mineralization. No differences in the test item behaviour were observed regarding radiolabel or concentration level.
Overall, the compound was stable in the test system. Degradation kinetics was not calculated as no significant degradation was observed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS-Code: BAS 505 F (Dimoxystrobin)
Chemical name: (E)-o-(2,5-dimethylphenoxymethyl)-2-methoxyimino-N-methylphenylacetamide
Molecular formula: $C_{19}H_{22}N_2O_3$
Molar mass: 326.4 g mol^{-1} (unlabelled)

Label 1 (phenyl label)
Label: phenyl-U-^{14}C
Batch No.: 597-1501
Specific radioactivity: 4.67 MBq mg^{-1}
Radiochemical purity: 99.9%
Chemical purity: 98.7%

Label 2 (benzyl label)
Label: benzyl-U-^{14}C
Batch No.: 596-3101
Specific radioactivity: 7.41 MBq mg^{-1}
Radiochemical purity: 99.0%
Chemical purity: 96.4%

2. Test system

Water and sediment were collected from The Lake at Studley Royal (Ripon, United Kingdom). Prior to use the sediment and water were stored together in the dark at 4 ± 2°C with free access to air. Water was filtered through a 0.1 mm sieve and sediment was passed through a 2 mm sieve prior to use and characterisation. The physico-chemical properties of the system are summarized in Table 4.1.3.2-1.

Table 4.1.3.2-1: Characterization of the water/sediment system

Designation **Origin**		**Fountains Abbey** **The Lake, Studley Royal, Ripon, UK**
Water		
Temperature	[°C]	4.8*
pH water	-	8.99*
Oxygen content	[%]	12.46*
Redox potential (Eh)	[mV]	134.1*
Hardness	[mg $CaCO_3$ L^{-1}]	90

Table 4.1.3.2-1: Characterization of the water/sediment system

Designation **Origin**		**Fountains Abbey** **The Lake, Studley Royal, Ripon, UK**	
Total organic carbon	[ppm]	8.15	
Total N	[%]	0.00375	
Total P	[mg L^{-1} PO_4^{3-}]	0.27	
Sediment			
Textural class		UK & BBA Particle Size Distribution	USDA
Sand	[%]	76	78
Silt	[%]	19	17
Clay	[%]	5	5
Soil type	-	Loamy sand	Loamy sand
pH	-	8.35*	
pH (H_2O)	-	8.1	
pH ($CaCl_2$)	-	7.5	
Redox potential (Eh)	[mV]	-182*	
Organic carbon	[%]	1.3	

* measured at sampling

B. STUDY DESIGN

1. Experimental conditions

A total of 94 test vessels were prepared for incubation: 18 test vessels for each radiolabel (phenyl and benzyl) and each nominal concentration (10 and 90 µg L^{-1}), 9 vessels for the sterile incubation (for both labels; 90 µg L^{-1}), 2 vessels as system control with sodium [^{14}C]-benzoate and 2 vessels with sodium [^{14}C]-benzoate plus treatment solvent.

The vessels were filled with about 100 mL test water, using sterile techniques where necessary. Appropriate amounts of the respective application solutions were pipetted to the water surface to achieve nominal application rates of 10 µg L^{-1} or 90 µg L^{-1}.

The systems were incubated at 20±2°C in a metabolism apparatus (incubator) with a gas flow system. Each test vessel was connected to a volatile trapping system of two gas washing bottles containing trapping solutions (2x NaOH) for the ^{14}C-volatiles to be expected. Test vessels containing sterile water were also aerated, however, the air stream was led through sterile filters to avoid contamination of the test system by airborne germs. Vessels were kept in the dark and were agitated by continuous stirring on magnetic stirrers throughout the incubation period.

2. Sampling

Test vessels, including the sterile groups, were sampled at 0, 3, 7, 14, 21, 35 and 59 days after treatment (DAT). For sampling, the flasks were removed from the rigs and the conductivity, O_2 content, pH and redox potential of the water were measured.

3. Description of analytical procedures

Water

The water in the test vessels was transferred into glass jars, the test vessels were rinsed with Milli-Q water which was added to the water from the test vessel and weighed prior to LSC. The test vessels were then washed (with sonication) with acetonitrile (25 mL).

Weighed aliquots of the water for LSC were mixed with fresh acetonitrile prior to addition of scintillant.

For higher concentration samples (90 µg L^{-1}), HPLC analysis was carried out without further work-up. For lower concentration samples (10 µg L^{-1}), sub-samples of the water were partitioned with dichloromethane. The dichloromethane was concentrated to dryness and the samples reconstituted in acetonitrile or acetonitrile : water (1:1, v/v) prior to chromatography. Procedural recoveries were checked by LSC and were found to be 90% or greater.

Volatiles

Throughout the test, traps were collected for sampled vessels. Remaining test samples had their traps collected and replenished with fresh solutions at 3, 7, 14, 21, 29, 35, 43 and 51 DAT. Reference vessels had traps collected at the same intervals. Volatiles trapped in sodium hydroxide were analysed by LSC.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The material balance and distribution of radioactivity are shown in Table 4.1.3.2-2 and Table 4.1.3.2-3.
The applied mass of test item per test vessel containing 100 mL of water was 9.2 µg (high concentration) and 1.0 µg (low concentration).
The material balance for the pelagic test ranged from 91.8 to 105.3% of the total applied radioactivity (TAR) in Lake water treated with the phenyl-^{14}C-labeled test item and from 93.1 to 102.3% TAR in Lake water treated with the benzyl-^{14}C-labeled test item. In the sterile vessels, the material balance ranged from 90.7 to 93.7 TAR (phenyl label) and from 91.2 to 97.5% TAR (benzyl label).
The radioactivity recovered from test vessels was found predominantly in the water. Adsorption to the inner test vessel surface was negligible, only up to 2% AR was found in the acetonitrile rinsing solution. At the end of the study (59 DAT) the water accounted for 92.2 to 98.6% TAR for the viable test vessels and 91.4 to 93.9% TAR for the sterilized vessels. For all test samples and sampling time points, the radioactivity in the volatile traps remained below 1.0% TAR indicating a low rate of mineralization.

Table 4.1.3.2-2: **Material balance (%) and distribution of radioactivity after application of [phenyl-U-^{14}C] dimoxystrobin to lake water**

Days after treatment	Percent of total applied radioactivity [% TAR]			
	Water	Vessel wash**	NaOH Trap (CO_2)	Material balance
Low concentration (10 µg L^{-1})*				
0	105.3	-	NA	105.3
3	99.1	-	-	99.1
7	95.2	0.9	0.1	96.2
14	99.5	1.4	0.3	101.1
21	97.0	0.8	0.2	97.9
35	96.0	-	0.5	96.4
59	98.1	0.7	0.1	98.8
High concentration (90 µg L^{-1})*				
0	92.0	0.3	NA	92.3
3	92.9	0.3	-	93.1
7	93.2	0.6	0.1	93.9
14	92.9	0.7	0.1	93.6
21	91.3	0.4	0.2	91.8
35	91.8	0.3	0.3	92.4
59	92.2	0.4	0.7	93.3
Sterilized (90 µg L^{-1})				
0	91.6	0.2	NA	91.8
3	93.7	-	-	93.7
7	91.9	0.3	-	92.2
14	90.2	0.5	-	90.7
21	91.5	0.2	-	91.7
35	91.3	0.2	0.7	92.2
59	91.4	0.3	-	91.7

NA not applicable
- not detected (or < 0.1%)
* mean of two replicates
** acetonitrile wash of the incubation vessel

Table 4.1.3.2-3: **Material balance (%) and distribution of radioactivity after application of [benzyl-U-^{14}C] dimoxystrobin to lake water**

Days after treatment	Percent of total applied radioactivity [% TAR]			
	Water	**Vessel wash****	**NaOH Trap (CO_2)**	**Material balance**
Low concentration (10 µg L^{-1})*				
0	102.3	-	NA	102.3
3	99.0	-	-	99.0
7	99.8	1.2	0.1	101.0
14	91.8	1.5	0.2	93.4
21	95.8	0.4	0.1	96.2
35	97.8	-	0.1	97.9
59	98.6	0.5	0.2	99.2
High concentration (90 µg L^{-1})*				
0	94.9	0.2	NA	95.1
3	92.8	0.2	0.1	93.1
7	92.8	1.1	0.1	93.9
14	92.8	0.7	0.1	93.6
21	92.8	0.5	0.1	93.4
35	93.4	0.6	0.1	94.1
59	93.7	0.5	0.2	94.4
Sterilized (90 µg L^{-1})				
0	95.5	0.3	NA	95.8
3	94.7	0.1	-	94.8
7	92.0	0.5	-	92.5
14	96.9	0.6	-	97.5
21	91.0	0.2	-	91.2
35	92.9	0.4	-	93.3
59	93.9	0.2	-	94.1

NA not applicable

\- not detected (or < 0.1%)

* mean of two replicates

** acetonitrile wash of the incubation vessel

B. TRANSFORMATION OF PARENT COMPOUND

Water

The results of radio-HPLC analysis are summarized in Table 4.1.3.2-4 and Table 4.1.3.2-5.

No significant degradation of dimoxystrobin was observed during the test. After 59 days, between 89 and 97% TAR could still be recovered as unchanged parent for the different concentrations and radiolabels. Additional peaks only appeared in trace amounts. Two of them could be assigned by retention time comparison to metabolites 505M08 and 505M09. However, none of the peaks exceeded 1.5% TAR at any sampling time.

The low amount of volatiles, metabolites and other degradation products detected indicate that only negligible microbial degradation took place.

Control samples with benzoic acid

The control vessels treated with [^{14}C]-sodium benzoate showed that the test system was microbially active both without and with the addition of acetonitrile. The total recoveries of trapped volatile radioactivity after 59 days were 96.3 and 97.8% TAR and the material balances were 100.5 and 102.2% TAR for the samples without and with acetonitrile, respectively.

A material balance was also established for intermediate sampling times, where water samples were radioassayed (after 7, 14 and 29 days). The material balance ranged from 96.3 to 100.3% TAR.

Sterilized samples

The very limited degradation observed in the viable test vessels resulted in no significant difference in test item concentration between the sterilized incubations and the viable vessels.

Table 4.1.3.2-4: **Metabolite overview for the water phase after application of [phenyl-U-^{14}C] dimoxystrobin to lake water**

Days After Treatment	Percent of total applied radioactivity [% TAR]					
	BAS 505 F	505M08	505M09	Unknown	Unresolved background	Total
Low concentration (10 µg L^{-1})*						
0	104.0	-	-	-	1.2	105.3
3	98.6	-	-	-	0.4	99.1
7	94.6	-	-	-	0.6	95.2
14	98.8	-	-	-	0.6	99.5
21	95.8	-	-	-	1.1	97.0
35	95.3	-	-	-	0.6	96.0
59	96.7	-	0.7	-	0.6	98.0
High concentration (90 µg L^{-1})*						
0	91.2	-	-	-	0.8	92.0
3	91.9	-	-	-	1.0	92.9
7	91.2	-	-	0.4	1.5	93.2
14	91.7	-	-	-	1.1	92.9
21	89.4	-	0.5	-	1.4	91.3
35	89.4	0.9	0.6	-	1.0	91.8
59	90.0	-	0.4	0.7	1.1	92.2
Sterilized lake water (90 µg L^{-1})						
0	90.4	-	-	-	1.3	91.6
3	92.3	-	-	-	1.4	93.7
7	90.1	-	-	-	1.8	91.9
14	88.7	-	-	-	1.5	90.2
21	90.2	-	0.5	-	0.8	91.5
35	90.2	-	-	-	1.1	91.3
59	89.2	-	-	0.8	1.5	91.4

- not detected (or < 0.1%)
* mean of two replicates

Table 4.1.3.2-5: Metabolite overview for the water phase after application of [benzyl-U-^{14}C] dimoxystrobin to lake water

Days After Treatment	Percent of total applied radioactivity [% TAR]					
	BAS 505 F	**505M08**	**505M09**	**Unknown**	**Unresolved background**	**Total**
Low concentration (10 µg L^{-1})*						
0	101.3	-	-	-	1.0	102.3
3	98.0	-	-	-	1.0	99.0
7	99.0	-	-	-	0.8	99.8
14	90.4	-	-	0.6	0.7	91.8
21	94.8	-	-	-	1.0	95.8
35	96.5	-	-	-	1.3	97.8
59	95.6	-	0.7	1.1	1.1	98.6
High concentration (90 µg L^{-1})*						
0	93.6	-	-	-	1.3	94.9
3	91.6	-	-	-	1.2	92.8
7	91.8	-	-	-	0.9	92.8
14	91.9	-	-	-	0.8	92.8
21	91.6	-	-	-	1.2	92.8
35	91.4	-	-	0.5	1.5	93.4
59	91.0	0.7	0.7	-	1.3	93.7
Sterilized lake water (90 µg L^{-1})						
0	95.2	-	-	-	0.3	95.5
3	93.9	-	-	-	0.8	94.7
7	91.7	-	-	-	0.3	92.0
14	96.5	-	-	-	0.4	96.9
21	90.5	-	0.4	-	0.1	91.0
35	90.6	-	-	0.6	1.7	92.9
59	92.0	-	-	0.9	1.0	93.9

- not detected (or < 0.1%)
* mean of two replicates

Degradation rates
Overall, the compound was stable in the test system. Degradation kinetics was not calculated as no significant degradation was observed.

III. CONCLUSION
From the obtained results it can be concluded that dimoxystrobin was not significantly degraded in the natural water environment provided in the test. After 59 days, 89 to 97% AR was recovered as the unchanged active substance. Some trace amounts (<1%) of the metabolites 505M08 and 505M09 were detected and identified by retention time comparison on HPLC analysis. Other degradation products were also detected only in minor amounts (always < 1.5% AR). Radioactivity in the volatile traps did not exceed 1.0% AR indicating a low rate of mineralization. No differences in the test item behaviour were observed regarding radiolabel or concentration level. Overall, the compound was stable in the test system. Degradation kinetics was not calculated since no significant degradation was observed.

RMS comment at renewal

Summary of the relevant information and conclusions on the biological degradation of dimoxystrobin in aquatic systems - ready biodegradability and aerobic mineralisation in surface water.

No new biodegradability study was performed for the renewal of the active substance. The already peer-reviewed study is considered still valid. Dimoxystrobin was found to be not readily degradable according to OECD guideline 301 F.

Aerobic mineralisation in surface water was tested in pelagic system and dimoxystrobin was not significantly degraded. After 59 days, 89 to 97% AR was recovered as unchanged active substance. Some trace amounts (<1%) of the known metabolites 505M08 and 505M09 were detected and assigned by retention time comparison on HPLC analysis. Other degradation products were detected only in minor amounts (≤1.5% AR, highest single value). The low amount of volatiles, metabolites and other degradation products detected indicate that only negligible microbial degradation took place.

4.1.4 Other degradability studies

4.1.4.1 [Study 1]

Study reference:
Ebert (2000), Degradation of BAS 505 F in aerobic aquatic environment, 2000/1000121, BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep.

Detailed study summary and results (see RAR (2017) – Volume 3 - B.8 (AS), chapter B 8.2.2.3):

Studies evaluated previously, DAR, 2003

a) Aerobic sediment/water studies were conducted according to SETAC (1995), BBA (Part IV,5-1, 1986) and EPA (Subdivision N, Series 162-4, 1982) guidelines
The distribution and degradation of dimoxystrobin was studied in two natural systems of water and sediment. The water/ sediment systems were taken from a pond (System A) and a pond-like side arm of a river (System B) respectively, both in Rhineland-Palatinate, Germany.
[^{14}C-Phenyl] and [^{14}C-benzyl]dimoxystrobin were used and applied separately to the test systems. Characteristics of the water/sediment systems are given in the table below. Test vessels contained 290ml (ca.6 cm depth) water and 190-200g wet weight (2-2.5cm depth) sediment. Dimoxystrobin was applied to the water at a rate of 0.103 mg a.s./l. Experiments under sterile conditions were also carried out in both water/ sediment systems. The test vessels were incubated in the dark at a temperature of 20 ± 2°C for up to 100 days. Aeration was achieved by a stream of CO_2 free air over the water surface. CO_2 and organic volatiles were trapped in NaOH and ethylene glycol respectively.

Table 4.1.4.1-1 Characterisation of the water/sediment systems (DAR, 2003)

Designation **Origin**		**System A** **Kellmetschweiher** **Rhineland-Palatinate, FRG**	**System B** **Berghäuser Altrhein** **Rhineland-Palatinate, FRG**
Sediment	sand [%]	83	47
	silt [%]	8	41
	clay [%]	9	12
	textural class (German scheme)	clayey sand / loamy sand	silty loamy sand
	pH ($CaCl_2$)	7.5	7.6
	organic C [%]	1.2	1.0
	total N [%]	0.11	0.11
	total P [%]	0.02	0.05
	CEC [mVal/100g]	16	15
	ATP [µg/kg]	52.8	469.6
	plate counts [cfu/g]		
	bacteria	2.9×10^6	2.3×10^7
	actinomycetes	1.4×10^4	4.4×10^5
	fungi	3.1×10^3	2.7×10^4
Water	pH	8.5	8.2
	hardness [mmol/l]	0.93	1.03
	Total organic C [mg/l]	12.7	4.6
	total N [mg/l]	1	1
	total P [mg/l]	<3	<3

ATP adenosine-tri-phosphate
CEC cation exchange capacity

Radioactivity in the water was quantified directly by LSC and analysed by HPLC and HPTLC. Dissolved oxygen, redox potential and pH were also measured. Sediment was extracted with acetonitrile/water then acetonitrile and radioactivity in the extracts was quantified by LSC and analysis by HPLC and HPTLC. Radioactivity in the extracted sediment was quantified by combustion and LSC. The unextracted sediment residue was characterised as associated with fulvic acid, humic acid and humins.
Aerobic conditions in the water phase were maintained, dissolved oxygen in the water phase was always > 28% and water redox potentials were positive. Sediment redox potentials were negative but there was no trend of increasing or decreasing redox potential during the studies. pH remained relatively constant.
The results from the two different radiolabels were comparable; therefore the average result from both radiolabels are summarised below. The distribution and recovery of radioactivity from water/sediment system A and B are shown in the tables below.
Most of the sediment unextracted radioactivity was located in the humic acids and humins. In the fulvic acid fraction, less than 4% AR could be detected in the 100 DAT sample.

Table 4.1.4.1-2 Material balance and distribution of radioactivity after application of [^{14}C]-dimoxystrobin to water/sediment system A (DAR, 2003)

				% AR							
DAT	**water**			**Sediment**						**CO_2**	**material balance**
	dimoxystrobin	**Dimoxystrobin Z isomer**	**others**	**extractable residues**				**unextracted residues**	**total**		
				dimoxystrobin	**Dimoxy-strobin Z isomer**	**others**	**total**				
0	88.3	4.5	0	3.0	0.0	0	3.0	0.1	3.1	n.d.	95.8
1	79.0	3.5	0.3	13.1	0.6	0.1	13.8	0.5	14.3	0.0	97.0
2	72.3	3.8	0.1	19.5	0.0	0	19.5	0.6	20.1	0.0	96.2
7	59.6	2.3	0	30.9	2.6	0	33.5	1.6	35.1	0.0	97.0
14	50.4	1.7	0	40.0	1.9	0	41.9	3.3	45.2	0.0	97.2
30	42.6	1.4	1.5	42.1	2.8	0	44.9	4.8	49.7	0.0	95.2
62	32.2	1.0	2.2	51.9	2.8	0	54.7	5.5	60.2	0.9	96.5
100	25.8	0.7	4.6	55.3	2.8	0.1	58.2	6.3	64.4	0.8	96.3
105 s	22.0	0.6	0	65.4	3.4	0.1	68.9	5.4	74.2	0.0	96.8

s = sterilised
n.d. = not determined

In system A metabolite 505M08 was identified but represented a maximum of 0.6% AR in the water phase (days 62 and 100). Metabolite 505M09 was also identified but represented a maximum of 3.5% AR in the water phase (day 100). These metabolites were not identified in the sediment extracts at any sampling time.

Table 4.1.4.1-3 Material balance and distribution of radioactivity after application of [^{14}C]-dimoxystrobin to water/sediment system B (DAR, 2003)

				% AR							
DAT	**water**			**Sediment**						**CO_2**	**material balance**
	Dimoxystrobin	**Dimoxystrobin Z isomer**	**others**	**extractable residues**				**Unextracted residues**	**total**		
				dimoxystrobin	**Dimoxystrobin Z isomer**	**others**	**total**				
0	84.8	4.5	0.4	3.3	0.0	0	3.3	0.1	3.4	n.d.	93.1
1	77.9	3.9	0	14.0	0.5	0	14.5	0.7	15.2	0.0	96.9
2	72.5	3.2	0.1	19.0	1.9	0.4	21.3	1.0	22.3	0.0	98.0
7	56.8	2.2	0	34.0	2.1	0.1	36.2	2.1	38.2	0.1	97.2
14	45.0	1.4	0	44.5	2.1	0	46.6	3.7	50.3	0.2	96.9
30	30.5	0.7	1.7	52.2	3.0	0	55.2	7.5	62.6	0.1	95.6
61	20.3	0.4	3.4	61.3	2.3	0	63.6	8.3	71.8	0.1	95.9
100	14. 1	0.0	7.8	59.0	2.5	0	61.5	10.7	72.2	2.1	96.1
105 s	19.6	0.2	0	68.7	3.4	0	72.1	4.4	76.5	0.0	96.3

s = sterilised
n.d. = not determined

In system B metabolite 505M08 was identified but represented a maximum of 2.5% AR in the water phase (day 100). Metabolite 505M09 was also identified but represented a maximum of 5% AR in the water phase (day 100). These metabolites were not identified in the sediment extracts at any sampling time.
The degradation of the test substance in the sterilised test vessels was slightly slower than in the viable samples. After 105 days, 91.4%AR (system A) and 91.9 %AR (system B) was still unchanged test substance. Metabolites were not detected at all and final degradation to CO_2 and formation of unextracted residues was very low.
Disappearance times could only be calculated for the active substance in the water phase. For this purpose, the applicant used a simple 2 compartment biphasic model for the two water/sediment systems which were used for parameter estimation by the computer program ModelMaker (v.3 0.4) using the MARQUARDT-NEWTON, least squares method. In the calculations the results from the two radiolabels were treated as replicates. For both models, a very good coefficient of determination of $r^2 = 0.99$ was achieved.
Using the two compartment model, the resulting 'statistical best fit graphical' DT_{50} of dimoxystrobin + dimoxystrobin Z-isomer in water phase of system A was determined to be 27 days, the DT_{90} estimated was >200 days and was not reported as the estimate is extrapolated significantly beyond the study duration. In system B, the DT_{50} of dimoxystrobin+dimoxystrobin Z isomer in the water phase was 15 days, the DT_{90} was estimated as 136 days (also extrapolated beyond the study duration). (Ebert, 2000)

Note: The DT_{50} and DT_{90} values calculated by the rapporteur using Timme *et al*. were in close agreement with the applicant's estimates above. For system A, water phase, the DT_{50} = 22 days and DT_{90} = >500 days (√1.5 order, r^2=1.0). For system B, water phase, the DT_{50} = 13 days and DT_{90} = 140 days ~(√1 order, r^2=1.0).

Summary and Assessment
Distribution and degradation of dimoxystrobin (benzyl and phenyl radiolabels) was further investigated using two natural water sediment systems ('A' & 'B'), incubated at 20 ±2°C in the dark for 100 days, (water depth 6 cm). The sediment layer, a clayey sand/loamy sand and silty loamy sand, (2-2.5 cm depth) were of similar pH (7.5-7.6) and organic carbon content (1-1.2) and the water used in both systems was of similar pH (8.2-8.5). However, ATP levels in the sediment of system 'B' were over eight times higher than in system 'A' suggesting greater microbial activity. There was little difference in partitioning to sediment between the two systems or the two radiolabels. No major metabolites were formed in the water or sediment phase of either system. Minor metabolites were 505M08 and 505M09 (at <10%AR), mostly in the water phase of both systems. Little if any mineralisation to CO_2 occurred until after 61 days, then reaching a maximum of 2.1% AR by day 100 (system 'B'). Unextracted residues in sediment were also low, rising to 6.3-10.7% AR by day 100. Overall recovery was acceptable throughout the study (> 93% AR).
Over 100 days, dimoxystrobin increased in the sediment phase to 55.3 and 59%AR in systems 'A' and 'B' respectively, (peaking at 61.3% AR at day 61, system 'B'). Over 100 days, in the water phase levels of dimoxystrobin declined to 25.8 and 14.1% AR in systems 'A' and 'B' respectively. The low levels of metabolites, mineralisation and unextracted residues support a lack of degradation, with dimoxystrobin mostly partitioning to the sediment phase. Samples that were sterilised showed slightly slower degradation of the test substance, indicating that at least some degradation of dimoxystrobin is microbially mediated. As residue levels in the sediment did not decline during the study, a DT_{50} for the sediment phase could not be calculated. The graphical 'best fit' DT_{50} and DT_{90} values for dimoxystrobin + dimoxystrobin Z-isomer in the water phase were estimated as detailed in a table later (Table B.8.2.2.4-8 Summary of first order half-life values in aquatic dissipation studies).

Studies submitted for the renewal of the active substance
No new water/sediment study was performed for the renewal of the active substance. The already peer-reviewed study is considered still valid, in which the main results were as follows:
The behaviour in water/sediment was characterized by a rather fast movement from the water to the sediment. The radioactivity in the water decreased within 100 days to 21.9 - 31.1% AR in the two tested systems, respectively. In the sediment, the radioactivity correspondingly increased and accounted to 64.4 - 72.2% AR at the end of the incubation period.
Mineralization was low in both systems (<2.5% AR) and no other volatile degradates were detected. The bound residues in the sediment were formed only to a low extent, reaching 6.3 - 10.7% AR. The bound residues in system B were fractionated into humins, humic acids and fulvic acids. Most of the radioactivity was located in the humic acids and humins. In the fulvic acid fraction, less than 4% AR could be detected in the 100 day sample.
Dimoxystrobin was the only major compound in both water/sediment systems. The metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) could be detected only in the water phase and only in very low amounts (≤ 5% AR). In the sediment, no metabolites could be detected at any sampling time.
The half-lives of dimoxystrobin were re-calculated according to the newest guidelines and guidance documents and listed in Table 4.1.4.3-4.

4.1.4.2 [Study 2]

Study reference:

Fendt (2001): Degradation and distribution of BAS 505 F in a water-sediment system under outdoor conditions, 2000/1014987, SLFA - Staatliche Lehr- und Forschungsanstalt fuer Landwirtschaft Weinbau und Gartenbau; Neustadt/Weinstrasse; Germany Fed.Rep.

Detailed study summary and results (see RAR (2017) - Volume 3 - B.8 (AS), chapter B 8.2.2.4)

Studies evaluated previously, DAR, 2003

a) An outdoor aerobic sediment/water study was conducted (natural light exposure) to GLP. There are no agreed guidelines available for a study design of this type.

The water/sediment system for this study was taken from Kellmetschweiher, the same site as for the water sediment studies. The water/sediment characteristics are summarised below. Test vessels were filled with about 2.0 cm sediment (about 400 g wet weight) and a water layer of about 20 cm height (1950 ml). The system was allowed to equilibrate for 9 days before treatment. The water surface was treated with [^{14}C-benzyl]dimoxystrobin at a rate of 140 µg per test vessel (ca. 71 µg/l).

The water/sediment systems were placed in large isolated plastic tanks, filled with water in order to simulate a bigger water body with respect to temperature. The tanks were located in an ambient outdoor lysimeter facility in Neustadt an der Weinstraβe, Germany with outdoor temperature and light conditions (treatment date 5th July, 2000). In order to protect the vessels from rainfall they were placed under a special plexiglass cover which allowed UV and visible light transmission. If no rainfall was forecasted the plexiglass cover was removed.

Samples were taken at 0, 1, 2, 7, 14, 30, 58, 103, and 120 days after treatment. One test vessel was worked up per sampling day. Dissolved oxygen, redox potential and pH were also measured. The water was analysed directly by HPLC. The identity of metabolites in water was confirmed by HPLC-MS/MS. The sediment was extracted with acetonitrile/water, acetonitrile/0.01 M (aq) $CaCl_2$ then acetonitrile and radioactivity in the extracts analysed by HPLC. The HPLC method used did not resolve dimoxystrobin from its Z isomer. Unextracted radioactivity was quantified by combustion /LSC. Volatiles were not trapped. Aerobic conditions in the water phase were maintained, dissolved oxygen in the water phase was always > 60% and water redox potentials were positive. Sediment redox potentials were negative but there was no trend of increasing or decreasing redox potential during the study. pH remained relatively constant (8-9).

The mean water temperature during the experiment was 18.1°C (8.6-28.5°C). Over the first 58 days these values were 21.6°C (15.8-28.5°C). The measured average daily global radiation during the experiment was in the range 25-300 w/m^2. Over the first 58 days the range of these values were 75-300 w/m^2.

Table 4.1.4.2-1 Characterisation of the water/sediment system Kellmetschweiher used for the water/sediment study under outdoor conditions (DAR, 2003)

water/sediment designation origin		Kellmetschweiher Schifferstadt, Rhineland Palatinate, Germany
water	pH at site of sampling	8.8
	Total organic C [mg/l]	16.8
	total hardness	1.04
	plate counts [cfu/ml]	
	bacteria	8.14×10^2
	fungi	2
	actinomycetes	0
sediment	textural class (German scheme)	sand / clayey sand
	clay [%]	12
	silt [%]	4
	sand [%]	84
	plate counts [cfu/g]	
	bacteria	4.77×10^6
	fungi	4.39×10^4
	actinomycetes	2.52×10^4
	organic C [%]	
	nitrogen total [%]	1.6
	phosphorus [mg/kg]	0.14
		150

The distribution of radioactivity and material balance in the water/sediment system is shown in the tables below.

Table 4.1.4.2-2 Distribution of radioactivity and material balance in the water/sediment system after application of ^{14}C-dimoxystrobin and incubation under outdoor light and temperature conditions (DAR, 2003)

time after treatment	**% AR**					
	water	**sediment extractable residues**	**sediment unextracted residues**	**Sediment total**	**material balance (water + sediment)**	**Balance proposed to be CO_2**
0 h	98.4	0.1	0.0	0.1	98.5	-
1 d	94.8	5.9	0.2	6.1	100.9	-
2 d	90.5	8.4	0.4	8.8	99.3	-
7 d	81.9	13.1	1.0	14.1	96.0	4.0
14 d	75.2	17.9	2.3	20.2	95.4	4.6
30 d	61.3	16.5	7.9	24.4	85.7	14.3
58 d	50.1	16.1	11.6	27.7	77.8	22.2
103 d	37.7	18.3	24.1	42.4	80.1	19.9
120 d	36.9	19.6	23.5	43.1	80.0	20.0

The sum of radioactivity in water and sediment declined to approximately 80% AR after 58 days which was considered by the applicant as indicative of mineralisation of approximately 20% AR.

No major metabolites (> 10% AR) were formed. One metabolite (505M96) reached 9.6% TAR after 30 days in the water phase, however, it degraded again reaching 1.9% AR at the end of the study. Numerous degradation and breakdown products were formed in the water during the study, most of them never reached more than 3.5% AR. In the sediment, only traces of metabolites could be detected.
Since the study was performed with the benzyl-label, corresponding breakdown products containing the phenyl-moiety were not identified. However, results of the aqueous photolysis and also soil photolysis studies (performed with both labels) indicate that no additional metabolites were found with the phenyl-label.

Table 4.1.4.2-3 HPLC analysis of the water samples and sediment extracts after application of ^{14}C-dimoxystrobin to a water/sediment system (20cm water depth, 1.6% oc sediment) and incubation under outdoor light and temperature conditions (DAR, 2003)

	% AR								
time after treatment	**total**	**Dimoxystrobin + Z-isomer**	**505M01**	**505M08**	**505M09**	**505M96**	**505M97**	**unknown**	**others***
water									
0 h	98.4	96.8	0.4	0.3	0.0	0.2	0.0	0.1	0.8
1 d	94.8	88.0	2.9	0.7	0.0	0.5	0.0	0.4	2.3
2 d	90.5	84.4	3.2	0.7	0.0	0.6	0.0	0.5	1.1
7 d	81.9	69.0	2.6	0.8	0.9	2.5	0.2	0.2	5.6
14 d	75.2	59.4	3.2	1.3	1.4	3.5	0.3	0.9	5.3
30 d	61.3	30.5	2.4	1.2	0.7	9.6	0.0	3.6	12.0
58 d	50.1	12.4	1.0	3.6	5.3	6.5	3.4	5.8	12.1
103 d	37.7	9.6	0.4	3.0	4.4	3.2	2.7	6.0	8.4
120 d	36.9	10.7	0.3	2.5	4.5	1.9	2.6	5.5	9.0
sediment									
0 h	0.1	0.0	0.0	0.0	0.0	-	-	-	0.0
1 d	5.9	5.9	0.0	0.0	0.0	-	-	-	0.0
2 d	8.4	8.0	0.0	0.0	0.0	-	-	-	0.0
7 d	13.1	12.0	0.2	0.0	0.0	-	-	-	0.9
14 d	17.9	15.9	0.4	0.0	0.0	-	-	-	1.7
30 d	16.5	10.9	0.2	0.3	0.5	-	-	-	4.6
58 d	16.1	8.5	0.2	0.7	1.1	-	-	-	5.6
103 d	18.3	8.5	0.2	0.8	1.2	-	-	-	7.7
120 d	19.6	11.8	0.1	0.6	1.0	-	-	-	6.0

* sum of up to 22 peaks (each individual peak < 3.5% AR)

For half-life time calculation, only the data of the first 58 days of incubation were used. The data show that after 58 days, the dissipation processes in the test vessels considerably slowed down. This is proposed by the applicant as being attributable to temperature and global radiation decreasing during the last two months of incubation which were quite late in the year (September – beginning of November).
The first order DT_{50} for dimoxystrobin+dimoxystrobin Z-isomer in water and sediment and for the metabolites 505M01 and 505M96 in the water were calculated with the computer program ModelMaker (v.4, MARQUARDT-NEWTON, least squares method) using the compartment model outlined in the figure below.

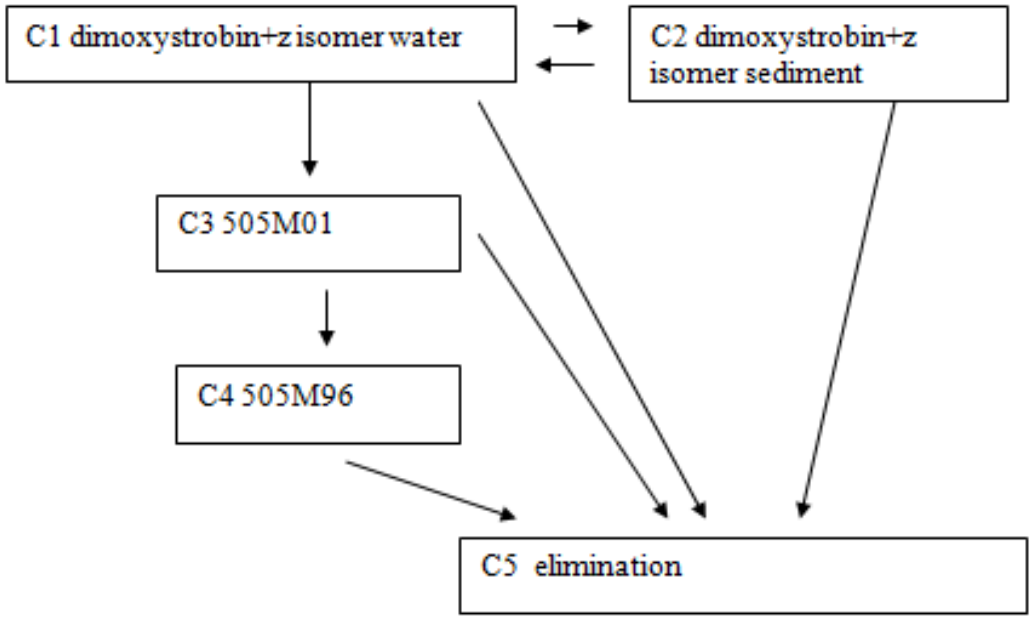


Figure 4.1.4.2-1 5-compartment model used in calculating DT50 for dimoxystrobin and its metabolites in an outdoor light exposed sediment/water system (DAR, 2003)

The coefficient of determination for the whole model was r^2 = 0.999. First order DT_{50} calculated for dimoxystrobin+dimoxystrobin Z-isomer and metabolites are shown in the table below.

Table 4.1.4.2-4 **First order DT_{50}-values of dimoxystrobin + dimoxystrobin Z isomer and metabolites in the water/sediment study (20cm water depth) under summer outdoor (mean temperature 21.6°C, range 15.8-28.5°C) conditions in Germany (DAR, 2003)**

substance			DT_{50} (first order) [days]
Dimoxystrobin+dimoxystrobin Z isomer	(water)		15.3
Dimoxystrobin+dimoxystrobin Z isomer	(sediment)		9.1
505M01	(water)		5.7
505M96	(water)		19.7

(Fent, 2001; Platz, 2000b)

b) An outdoor mesocosm study was conducted primarily to address the effects on aquatic organisms in accordance with SETAC-Europe (1992), SETAC-RESOLVE (1992), HARAP (1999) and Classic (1999) guidelines. (Full summary in the ecotox section). The pattern of dissipation measured in this study is evaluated here.

Dimoxystrobin was applied formulated as a 170 g/l SC as a spray on 4th May to the surface of outdoor mesocosm ponds (12), each with a diameter of 2.84 m, a water depth of 100 cm and an according water volume of 6.335 m^3. 4 control ponds received no applications. The amount of spray applied to the mesocosm was adjusted to reach the following nominal concentrations of dimoxystrobin: 1.7, 5, 15 and 45 µg a.s./l (corresponding to: 10, 31, 92, 277 µg/l SC formulated product). The highest test concentration reflects about 67% of an overspray situation (based on the single maximum application rate of 200 g a.s./ha), the lowest test concentration simulates approximately spray drift at a distance of about 1 m assuming 2.77% drift. The mesocosm site was located at Limburgerhof in Rheinland-Pfalz (southwest Germany).

The ponds were largely embedded in the ground. The bottom was covered by a 10 cm layer of natural sediment. The sediment used in this study was obtained from a rather oligotrophic lake (formerly used for gravel production). The water was collected from another lake (sediment was not collected at this site as it was a nature reserve). The lake water was diluted with deionized water (5:1 natural:deionized water). The lake where the water was derived from was described as supporting a large diversity of different species. The systems were homogenised and equilibrated for 6 months (water circulated continuously between the ponds before the test substance was applied in May 1999). The measured characteristics of the water and sediment after equilibration are outlined in the table below.

Table 4.1.4.2-5 Characterisation of the water/sediment in the Speyer / Neuhofener Altrhein outdoor mesocosm study located in south west Germany (DAR, 2003)

water/sediment designation origin		
water	pH	8.3*
	Total Organic C [mg/l]	4
	total hardness	2.1
sediment	textural class	Sand
	< 2 µm clay [%]	3
	2-20µm silt [%]	0
	20-200µm silt/sand mixed fraction[%]	40
	>200µm sand [%]	57
	pH	7.8
	organic C [%]	0.2
	nitrogen total [%]	0.02
	phosphorus [mg/kg]	100

*rose to pH 10.5 during study then stabilised at pH 8.5 - 9.5.

The test was performed under outdoor conditions. Mean daily water temperatures were in the range 15-27°C

Water samples were taken at the times indicated in the table below. Samples (500 ml) were concentrated using solid phase extraction before analysis for parent dimoxystrobin by HPLC-MS-MS using method 429. Procedural recoveries fortified at 0.05-5 µg/l were in the range 83-105%. The validated LOQ was 0.05µg a.s./l.

Sediment samples were taken at the times indicated in the table below. Samples (500 ml) were concentrated using solid phase extraction before analysis for. Samples (10 g from the top 0-2 cm) were extracted with methanol/water before concentration, solution in acetonitrile/water and analysis for parent dimoxystrobin by HPLC-MS-MS using method 426. Procedural recoveries fortified at 0.07 mg a.s./kg were in the range 65-82%. When fortified at 1.76 mg a.s./kg the range was 85-95%. The validated LOQ was 0.01 mg a.s./kg when recoveries were 85-90%.

Determination of test substance concentrations in the spraying solutions and directly in the water shortly after spraying confirmed the correct application and the nominal water concentrations of the active substance. The concentrations measured are set out in the table below. Note the study authors explain the observed increases in concentration measured in water at certain time points as a result of evaporation from the ponds which had to be topped up from time to time to replace evaporated water (i.e. there was not always a constant water volume).

Table 4.1.4.2-6 HPLC analysis of the water samples and sediment extracts after application of dimoxystrobin to outdoor mesocosm ponds (1m water depth, 0.2% oc content sediment) mean values from each replicated pond (3) (DAR, 2003)

time after treatment	1.7 µg dimoxystrobin /l	5 µg dimoxystrobin /l	15 µg dimoxystrobin /l	45 µg dimoxystrobin /l
Water	**Concentration µg a.s./l**			
0 h	1.305	4.212	13.334	39.155
2 d	1.466	4.696	13.522	44.582
7 d	1.606	5.187	16.637	47.272
14 d	1.291	3.817	11.698	37.746
21 d	1.050	3.331	10.493	34.457
28 d	1.088	2.898	10.815	33.712
42 d	0.785	2.934	8.241	26.066
57 d	0.525	1.566	5.176	16.492
70 d	0.678	2.326	7.181	23.020
84 d	0.498	1.902	5.649	19.116
98 d	0.432	1.698	4.969	17.966
126 d	0.339	1.140	3.397	11.431
154 d	0.275	0.891	2.357	9.704
Sediment	**Concentration mg a.s./kg in top 0-2cm.**			
2 d	0.0019	0.0003	0.0066	0.0217
28 d	0.0017	0.0004	0.0150	0.0374
154 d	0.0022	0.0004	0.0058	0.0107

The first order DT_{50} of dimoxystrobin in the water phase of the mesocosm study calculated by the rapporteur using linear regression are summarised in the table below.

Table 4.1.4.2-7 The first order DT_{50} of dimoxystrobin in the water phase of the mesocosm study calculated by the rapporteur using linear regression (DAR, 2003)

Dose	DT_{50} (first order) [days]	DT_{90} (first order) [days]
1.7 µg dimoxystrobin /l	60	200
5 µg dimoxystrobin /l	65	220
15 µg dimoxystrobin /l	60	200
45 µg dimoxystrobin /l	69	230
mean	63.5	212

(Dohmen, 2001e; Keller, 1998e,f)

Summary and Assessment

An additional higher tier outdoor water sediment study and an outdoor mesocosm in Germany were also used to investigate the aquatic dissipation of dimoxystrobin.

In the outdoor water-sediment study, [^{14}C-benzyl]-dimoxystrobin was applied in early July to water and sediment from the same source as that used in laboratory system 'A', (20 cm water depth, 2 cm sediment depth) and exposed to natural radiation, (measured average daily 25-300 w/m^2). As in the laboratory, no major metabolites were identified. Metabolites 505M01, 505M08, 505M09, 505M96 and 505M97 were only found at <10%AR (in water) and in trace amounts (in sediment). Volatile products were not trapped, but mineralisation was proposed by the applicant to be *ca*. 20-22% AR from day 58 to 103. Unextracted residues in sediment peaked at ca. 24% AR on day 103. The proposed metabolic pathway is shown in the figure below (Figure B.8.2.2.4-1).

The applicant postulates that dimoxystrobin on reaching water undergoes photolytical transformation to a number of breakdown products and polar degradates and is also simultaneously partitioned and adsorbed to the sediment phase. (The low water solubility and high adsorption co-efficient of dimoxystrobin were cited as supporting its rapid partitioning into sediment). Minor levels (<10%AR) of numerous metabolites (>25) were formed in the water phase and present in trace amounts in the sediment. The aqueous and soil photolysis studies using the [^{14}C-phenyl] label also showed that no major metabolites were found. Oxidation of both methyl groups at the phenyl ring is proposed to form 505M08 and 505M09 and to also occur with the breakdown products, giving benzoic acid derivatives.

Levels of dimoxystrobin + Z-isomer remaining in the water phase at 100 days were slightly lower (9.6-10.7% AR), than observed in the laboratory (14.1-26.5% AR). Levels in the sediment were lower (8.5-11.8% AR) than in the laboratory (58.1-61.5% AR). For the outdoor study degradation rates were estimated using a compartment model and assuming first order kinetics. The applicant made a case for basing half-life in the water phase on only the 0-58 DAT data, ignoring the last two sampling points (103 and 120 DAT). They claimed that the proposed application would be earlier than the time that application was made in the water sediment study (i.e. May-June instead of early July). Therefore, at the proposed time of application any dimoxystrobin in surface water would be exposed for a longer period to higher global irradiation and temperatures. The last 2 sampling points (equated to September-November) were excluded from the DT_{50} calculation, on grounds of being cooler with lower global irradiation than dimoxystrobin, carried by drift, would be exposed to in normal agricultural practice). There is some validity in this argument, but the proposed timing of application might be only slightly earlier than in this study, if applied at the beginning to end of June (i.e. 1-5 weeks earlier.)

DT_{50} values of 15.3 days and 9.1 days were calculated for dimoxystrobin + Z-isomer in water and sediment respectively (using 0-58 DAT data). These were similar to the degradation rate determined in the laboratory for system B, though faster than DT_{50} of 27 days obtained in the equivalent system A. The rapporteur calculated this first order DT_{50} in the water phase as slightly longer at 20 days using Timme et al (r^2 = 1.00), based on 0-58 DAT data.

In the mescosm study, dimoxystrobin was applied in early May to 2.84 m diameter ponds (1 m water depth, 10 cm sediment layer). The sediment consisted of sand obtained from an oligotrophic lake, with low 0.2% OC content. Samples were taken up to 154 DAA. In the water phase first order DT_{50} and DT_{90} values for dimoxystrobin were calculated as 60-69 days and 200-230 days, respectively.

Table 4.1.4.2-8 Summary of first order half-life values in aquatic dissipation studies (DAR, 2003)

	Water depth (cm)	Sediment depth (cm)	DT50 (days) water	DT90 (days) water	DT50 (days) Sediment
Laboratory System 'A'	6	2-2.5	27	>200*	n.c.
Laboratory System 'B'	6	2-2.5	15	136*	n.c.
Outdoor wat-sed study	20	2	15.3	n.c.	9.1
Mesocosm study	100	10	60-69	200-230	n.c.

* extrapolated beyond study duration

The DT_{50} of 15.3 days from the outdoor water-sediment study has been used in further assessment to predict concentrations in surface water. It is considered justified to use this outdoor study instead of the laboratory study in the dark because there was evidence that photolysis could contribute to degradation in practice. DT_{50} values for degradation in the soil were faster in the outdoor field dissipation trials than in the dark laboratory degradation studies. In the laboratory, there was greater degradation of dimoxystrobin in irradiated soil samples than in soil incubated under dark conditions. Two aqueous photolysis studies also showed faster dissipation of dimoxystrobin under irradiated conditions compared to in dark controls. In the outdoor water-sediment study exposed to natural light, dimoxystrobin degraded more quickly than in the 'dark' laboratory system 'A', in which the same sediment and water had been used. The sediment and water for the outdoor water-sediment study were taken from the worst-case laboratory system 'A' that showed the slowest dissipation rate. With deeper water in the outdoor study, it might have been expected that dissipation would have been slower, but an equivalent DT_{50} was obtained to that for the faster laboratory system 'B'.

The greater ratio of water to sediment in the outdoor water-sediment study, compared to the dark laboratory water-sediment study appeared to allow more transformation processes to take place, despite the deeper water depth, possibly as a result of exposure to UV light. Photolysis appears to have been a contributing factor in the outdoor and mesocosm studies.

It is considered justified to use the half-life of 15.3 days obtained from the outdoor water-sediment study in calculating PEC_{sw} values, even though a longer mean DT_{50} of 63.5 days was obtained in the mesocosm study. The water depth of 20 cm in the outdoor water-sediment study is closer to the standard water body depth of 30 cm usually assumed for PEC_{sw} calculations and possibly more representative of edge of field ditches than the water depth of 100 cm in the mesocosm study. The DT_{50} value in the water phase covers both degradation in water and dissipation through partitioning to sediment. Once partitioned to the sediment, bioavailability of dimoxystrobin for degradation is reduced. There was a deeper sediment layer in the mesocosm study and the organic carbon content of that sediment was very low (0.2%). Consequently, dissipation might be expected to have been longer. (However, the selection of a half -life of 15.3, 20, 27 or 63.5 days does not alter the initial PEC_{sw} obtained, as the proposed use is only for a single application per crop. See PECsw section.)

No metabolites were present at >10% AR in water or sediment, although 505M96 approached this in water at 9.6%AR. The ecotoxicological significance of 505M96 is addressed at the relevant section. None of the minor metabolites were still increasing at the end of the study (120 d).

Studies submitted for the renewal of the active substance

An irradiated water/sediment study was submitted and evaluated in the previous Annex I listing process. This study was initiated after it became obvious that the degradation of dimoxystrobin in water is considerably dependent on light conditions. In natural surface waters the two factors photolysis and adsorption to sediment do occur simultaneously, this additional study was designed where both factors were combined. One water/sediment system was used for this study and the test vessels were treated with benzyl-labelled test item on July 5th, 2000. Then the test vessels were placed outdoor and exposed to natural daily sunlight and temperature variations for 120 days. The results can be summarized as follows: When reaching water, dimoxystrobin undergoes a photolytical transformation forming many breakdown products and polar degradates. At the same time, adsorption to sediment takes place where dimoxystrobin is finally bound to the sediment matrix. In the water phase, one metabolite (505M96) reached 9.6% TAR after 30 days and degraded again to 1.9% at the end of incubation. The known photolysis product 505M01 (BF 505-4) was detected in max. amount of 3.2% TAR, and the carboxylic acid metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) reached max. amounts of 3.6 and 5.3% TAR, respectively. One further metabolite 505M97 was detected with max. 3.4% TAR in the water phase. In the sediment only traces of metabolites could be detected.
Overall, the understanding of the route of degradation of dimoxystrobin in surface waters (as shown in Figure 4.1.4.2-1) did not change.

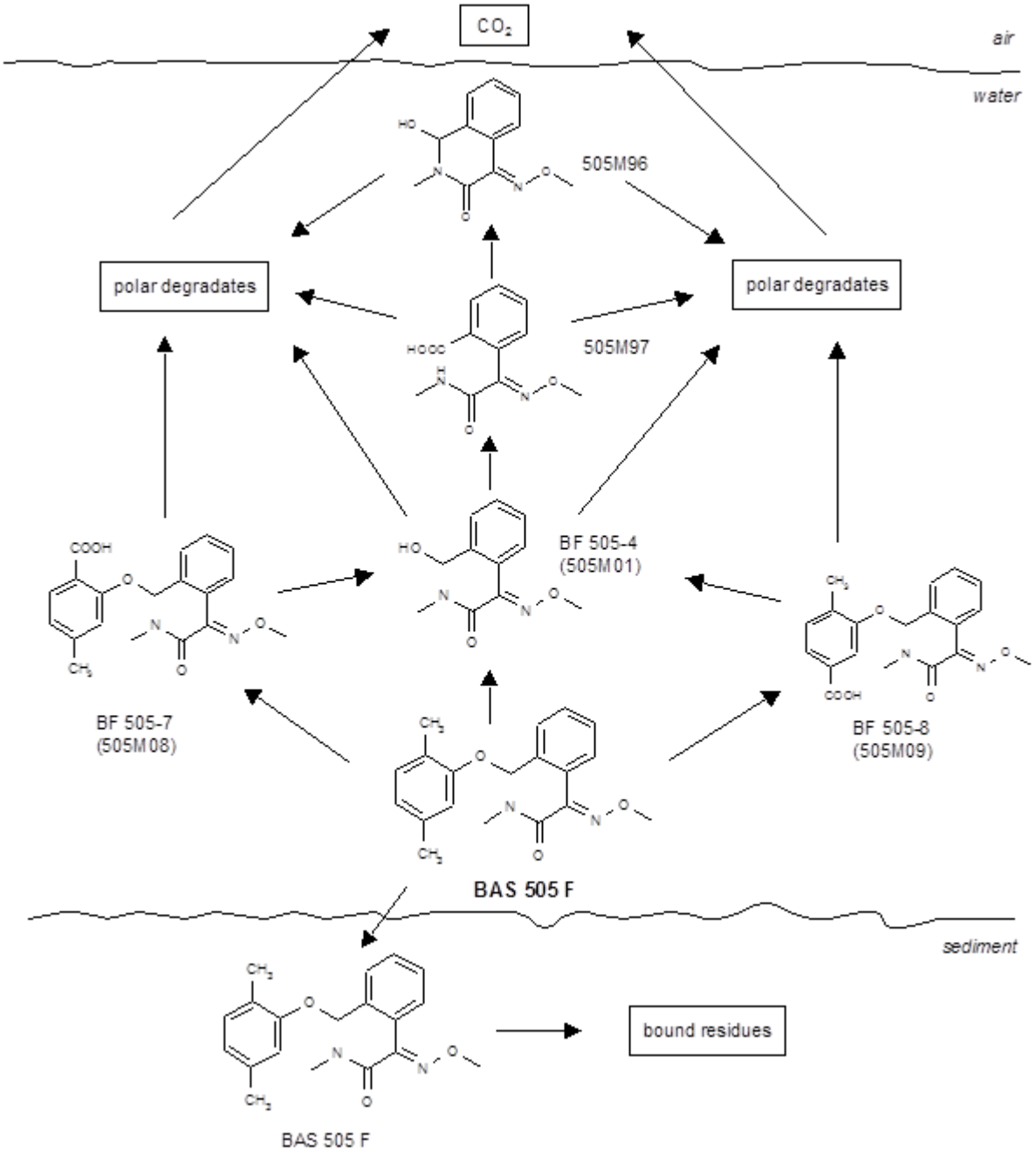


Figure 4.1.4.2-1: **Proposed route of degradation of dimoxystrobin in water and sediment under outdoor conditions (from dimoxystrobin dossier 2001)**

The half-lives of dimoxystrobin and metabolites (where feasible) in water/sediment under outdoor conditions were re-calculated according to the newest guidelines and guidance documents and listed in Table 4.1.4.1.1.1-2.

4.1.4.3 [Study 3]

Study reference:

Budde (2015): Kinetic evaluation of two water-sediment studies with BAS 505 F - dimoxystrobin according to FOCUS Degradation Kinetics, 2014/1133879, BASF SE, Limburgerhof, Germany Fed.Rep.

Detailed study summary and results (see RAR (2017) – Volume 3 - B.8 (AS), chapter B 8.2.2.4):

Reference	Budde E., 2015e Kinetic evaluation of two water-sediment studies with BAS 505 F - Dimoxystrobin according to FOCUS Degradation Kinetics 2014/1133879 CA 7.2.2.4/1
Guidelines	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP	-
test item	-
study duration	-
study completed	May 2015

Executive Summary

The aim of the study was to evaluate the kinetics of BAS 505 F - dimoxystrobin under aerobic aquatic conditions in three water/sediment systems in two studies [*BASF DocID 2000/1000121; BASF DocID 2000/1014987*]. In the first study, two different natural systems of water and sediment were treated with phenyl-[^{14}C] and benzyl-[^{14}C] labelled dimoxystrobin and incubated in the dark under aerobic conditions for 100 days. In a higher tier approach, a natural system of water and sediment was treated with [benzyl-U-^{14}C] labelled dimoxystrobin and incubated outdoor for 120 days under aerobic conditions and exposed to natural sunlight.

The kinetic analysis was carried out following the recommendations of the FOCUS workgroup on degradation kinetics to derive trigger endpoints and modelling endpoints.

In the outdoor test system, variations in temperature were observed over the study duration. To obtain endpoints suitable for use in environmental fate models, the data were time-step normalized to a reference temperature of 20°C in order to derive normalized modelling endpoints (DT_{50}).

Kinetic evaluation for the parent substance was performed at P-I level (one-compartment approach) as well as at P-II level (two-compartment approach: water and sediment).

Kinetic evaluation of metabolite dissipation/degradation was attempted for metabolites 505M96, 505M01, 505M08 and 505M09.

For the two laboratory systems, trigger $DegT_{50}$ values of 297.6 and 834.5 days in the total system and 13.6 and 25.3 days in the water compartment were calculated. For the outdoor system, trigger $DegT_{50}$ values of 26.7, 18.9 and 101.3 days were determined for the total system, the water compartment and the sediment phase, respectively.

Reliable modelling-$DegT_{50}$ values for the whole system were derived for system A (Kellmetschweiher) with 525.6 days, system B (Berghäuser Altrhein) with 297.6 days and outdoor system (Kellmetschweiher) with 35.2 days. For the water compartment, $DegT_{50}$ values of 81.3, 52.5 and 21.4 days were determined for System A, System B and the outdoor system, respectively. For P-II level no trigger or modelling endpoints could be calculated.

For metabolite 505M01, a trigger-$DisT_{50}$ of 31.2 days and a modelling-$DegT_{50}$ of 1.7 days was calculated, while for metabolite 505M96, a trigger-$DisT_{50}$ of 43.4 days and a modelling-$DegT_{50}$ of 16.9 days was calculated. No reliable endpoints could be derived for metabolites 505M08 and 505M09.

I. MATERIAL AND METHODS

Test systems

Laboratory study (dark study) [*BASF DocID 2000/1000121*]

The distribution and degradation of dimoxystrobin was studied in two natural systems of water and sediment. The water/sediment systems were taken from Kellmetschweiher (System A), and Berghäuser Altrhein, a pond-like side arm of a river (System B), both in Rhineland-Palatinate, Germany. The characteristics of the aquatic test systems Kellmetschweiher and Berghäuser Altrhein are described in Table 4.1.4.3-1.

Table 4.1.4.3-1: Characteristics of the test systems incubated in the dark

Designation origin	**System A** **Kellmetschweiher** **Rhineland-Palatinate, Germany**	**System B** **Berghäuser Altrhein** **Rhineland-Palatinate, Germany**
Sediment		
Sampling depth [cm]	~ 20	~ 20
Textural class (USDA)	loamy sand	sandy loam
Particle size distribution sand [%] silt [%] clay [%]	 83 8 9	 55 33 12
pH ($CaCl_2$)	7.5	7.6
Organic C [%]	1.2	1.0
Water		
pH	8.5	8.2
Hardness [mmol L^{-1}]	0.93	1.03
TOC [mg L^{-1}]	12.7	4.6
Total N [mg L^{-1}]	1	1
Total P [mg L^{-1}]	<3	<3

Two radiolabelled forms of dimoxystrobin, phenyl-[^{14}C] and benzyl-[^{14}C], were applied separately to the test systems. Dimoxystrobin was applied to the water at a rate of 30 µg a.s. per test vessel which corresponds to an application rate of 300 g a.s. ha^{-1}, when assuming direct overspray of a 30 cm deep water body. The systems were incubated in the dark at a temperature of 20 ± 2°C for 100 days. Aeration was achieved by a stream of air over the water surface.

Sampling intervals were at 0, 1, 2, 7, 14, 30, 62, and 100 days after treatment (DAT). The samples were worked up and measured by HPLC.

Dimoxystrobin was the only major compound in both water/sediment systems. The metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) could be detected only in the water phase and only in low amounts (≤ 5% TAR). In the sediment, no metabolites could be detected at any sampling time.

Higher-tier outdoor study [*BASF DocID 2000/1014987]*

The degradation of dimoxystrobin under aerobic conditions and exposed to natural sunlight was investigated in one natural water/sediment system. The water/sediment system was taken from a pond named Kellmetschweiher located in the south-western part of Germany. The characteristics of the Kellmetschweiher test system are described in Table 4.1.4.3-2.

Table 4.1.4.3-2: **Characteristics of the test system incubated under outdoor conditions**

Designation origin	**Kellmetschweiher** **Schifferstadt, Rhineland Palatinate, Germany**
Sediment	
Sampling depth [cm]	~ 20
Textural class (USDA)	loamy sand
Particle size distribution sand [%] silt [%] clay [%]	 84 4 12
Organic C [%]	1.6

Table 4.1.4.3-2: Characteristics of the test system incubated under outdoor conditions

Designation origin	**Kellmetschweiher Schifferstadt, Rhineland Palatinate, Germany**
Water	
pH	8.8
Total hardness [mmol L^{-1}]	1.04
TOC [mg L^{-1}]	16.8

The test systems were treated with [benzyl-U-^{14}C]-labelled dimoxystrobin at an application rate of 140 µg per test vessel, corresponding to a nominal application rate of 200 g a.s. ha^{-1}, when assuming direct overspray of a 30 cm deep water body.
The test systems were placed in big isolated plastic boxes filled with water to a distinct level to simulate a bigger water body with respective temperature compensation. Equilibration and subsequent incubation was carried out under outdoor conditions. Water temperature in one control vessel and air temperature above the test vessels were recorded with data loggers.
The vessels were exposed to natural light. In order to protect the vessels from rainfall, the vessels were placed under a plastic glass cover which was optimized concerning UV and visible light transmission. If no rainfall was forecasted the cover was removed.
Water temperatures during the test period were in the range from 9.9 to 26.5°C, with a mean temperature of 18.2°C. A decline towards the end of the study was observed, with temperature dropping to below 15°C after day 90.
Samples were taken at 0, 1, 2, 7, 14, 30, 58, 103, and 120 DAT. One test vessel was worked up per sampling date. The water was analysed by HPLC without further treatment. The sediment was extracted, and the extracts were analysed by HPLC. Volatiles could not be trapped.
Numerous metabolites were formed in the water phase, but most of them never reached more than 3.5% TAR. The metabolite 505M96 was observed with 9.6% TAR after 30 days in the water phase, while metabolites 505M01 (BF 505-4), 505M08 (BF 505-7) and 505M97 were detected with less than 5% TAR. Metabolite 505M09 (BF 505-8) reached 6.4%TAR in the total system at DAT 58, and did not decline significantly until the end of the study. In sediment, only traces of metabolites could be detected.

Kinetic modelling

The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The best-fit model was selected based on visual and statistical assessment, and corresponding DT_{50} and DT_{90} values are reported as *trigger endpoints*. Appropriate DT_{50} values for use in environmental fate models were derived depending on the kinetic model and are reported as *modelling endpoints*.

Experimental data of dimoxystrobin were analysed at the P-I level (one-compartment approach) for degradation in the whole system as well as dissipation from the water phase and from the sediment phase of the test systems. At the P-II level (two-compartment approach: water and sediment), the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.

Kinetic evaluation at level M-I (dissipation) was performed for dimoxystrobin metabolites 505M96 and 505M01 observed in the outdoor test system Kellmetschweiher, and based on metabolite decline from the maximum occurrence in the total system to derive trigger and modelling endpoints. Only these two metabolites had a suitable data set, i.e., more than 3 data points when starting from the observed maximum.

In addition, kinetic evaluation at level M-I (degradation) to derive modelling endpoints was performed for metabolites 505M96, 505M01, 505M08 and 505M09. Estimation of metabolite degradation at level M-I requires fitting the data of parent and metabolites in a combined fit. This was attempted for the metabolites with maximum occurrences >5% (505M09, 505M96) and metabolites which showed a pattern of formation, plateau and decline phase (505M01, 505M08). The metabolite 505M97 did not fulfil these criteria and was therefore not included in the pathway fit. Consequently, the degradation pathway was implemented in KinGUI according to Figure B.8.2.2.4-1, leaving out 505M97 (i.e., formation of 505M01, 505M08 and 505M09 from dimoxystrobin, and formation of 505M96 from 505M01).

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation reports.

Kinetic models included in the assessment

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC), and the biexponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling

Degradation in the whole system and dissipation from the water phase of the parent compound (level P-I) were evaluated starting on the day of treatment (i.e. 0 days after treatment, DAT 0).

At level P-I of the analysis, the measured initial concentration of the parent substance in the total system or in water was set to the material balance recovered at DAT 0 as recommended by the FOCUS kinetics guidance [*FOCUS (2006)*]. At the P-II level, the total recovered amount at DAT 0 was considered as the measured initial concentration in water, while the initial concentration of the sediment phase was assumed to be zero.

At Level M-I, estimation of metabolite dissipation requires kinetics to be fitted to the corresponding decline data for each compartment, starting from the maximum observed level of the metabolite in the compartment. The dissipation of the metabolite was thus evaluated starting at the day of maximum occurrence that was defined as 0 days after maximum concentration (0 DAMC). All later time points were adjusted accordingly as days after maximum concentrations (DAMC).
Estimation of metabolite degradation at level M-I requires fitting the data of parent and metabolites in a combined fit. This was performed for metabolites 505M96, 505M01, 505M08 and 505M09. The measured initial concentration of all metabolites was set to zero at DAT 0 as recommended by the FOCUS kinetics guidance.
Values below the quantification or detection limit for parent compound and metabolites were treated as recommended by the FOCUS workgroup [*FOCUS (2006), chapter 6.1.4 and chapter 8.3.1*].
Residue data obtained from using separate labelling forms of dimoxystrobin in the dark laboratory study [*BASF DocID 2000/1000121*] were treated as replicates, as the test substance showed similar behaviour with both labels.

Time-step normalization of outdoor study
To obtain endpoints suitable for use in environmental fate models, the data derived from the outdoor study were time-step normalized to derive normalized modelling endpoints. The normalization procedure was carried out by reducing or increasing day lengths depending on measured water temperature by means of a correction factor (f_{temp}). Daily water temperature values were available from the original raw data. The temperature normalization to a reference temperature of 20°C was performed using the temperature correction factor in Equation 4.1.4.3-1, and a default Q_{10} value of 2.58 was considered [FOCUS (2006)]. Daily correction factors for each day were calculated, and the cumulative time between sampling points was determined and used as input for kinetic evaluation.

Equation 4.1.4.3-1: Temperature correction factor calculation

$$f_{temp} = Q_{10}^{\frac{Tact - Tref}{10}}$$

with:
f_{temp} temperature correction factor [-]
T_{act} incubation temperature [°C]
T_{ref} reference temperature (20°C) [°C]
Q_{10} factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.58) [-]

The normalized day lengths were derived according to Equation 4.1.4.3-2a. Normalized sampling days (DATnorm) after application were calculated by cumulatively summing up normalized day lengths according to Equation 4.1.4.3-2b.

Equation 4.1.4.3-2: **Calculation of normalized day length based on temperature correction factors**

a)

$$D_{norm} = D * f_{temp}$$

b)

$$t_i = \sum_{t=1}^{i-1} D_{norm}$$

with:
t_i = Time from application till sampling at day i [d]
D_{norm} = Normalized day length (20°C) [d]
i = Time span between application and sampling [d]

c)

$$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact - Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

with:
D_{norm} = normalized day length (temperature and moisture) [d]
f_{temp} = temperature correction factor [-]
D = 1 day
T_{act} = actual water temperature [°C]
T_{ref} = reference temperature, 20 °C
Q_{10} = factor of increase of degradation rate with an increase in temperature of 10°C, Q_{10} = 2.58 [-]

The sampling days and the corresponding normalized (20°C) day lengths based on the measured data of daily water temperature are presented in Table 4.1.4.3-3.

Table 4.1.4.3-3: **Time-step normalized sampling days of the outdoor study**

DAT	D_{norm}
0	0
1	1.4
2	2.8
7	7.8
14	13.1
30	31.8
58	70.0
103	102.2
120	110.4

Software for kinetic evaluation

The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting [*Schafer, D., Mikolasch, M., Rainbird, P., Harvey, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. BASF DocID 2007/1062781*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

II. RESULTS AND DISCUSSION

For modelling endpoints, the initial fit was performed using SFO kinetics. If the fit was not satisfactory, FOMC, DFOP and HS kinetics were tested. For trigger endpoints, SFO and FOMC kinetics were tested in a first step; if SFO was not acceptable or worse than FOMC, DFOP and HS kinetics were tried in addition. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given in the original study reports.

Level P-I

An overview of the estimated trigger and modelling endpoints for dimoxystrobin from three water/sediment systems is given in Table 4.1.4.3-4.

Table 4.1.4.3-4: Summary of trigger and modelling endpoints for dimoxystrobin (Level P-I)

Test system, BASF DocID	**Trigger endpoints**				**Modelling endpoints**		
Total system	**Kinetic model**	**χ^2 error**	**$DegT_{50}$ [d]**	**$DegT_{90}$ [d]**	**Kinetic model**	**χ^2 error**	**$DegT_{50}$ [d]**
Kellmetschweiher (system A), 2000/1000121	HS	0.5	834.5	>1000	SFO	1.0	525.6[a]
Berghäuser Altrhein (system B), 2000/1000121	SFO	1.5	297.6	988.7	SFO	1.5	297.6[a]
Kellmetschweiher (outdoor), 2000/1014987	HS	4.4	26.7	>1000	SFO	7.4	35.2[c]
Water compartment	**Kinetic model**	**χ^2 error**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**χ^2 error**	**$DisT_{50}$ [d]**
Kellmetschweiher (system A), 2000/1000121	DFOP	2.4	25.3	213.9	DFOP	2.4	81.3[b]
Berghäuser Altrhein (system B), 2000/1000121	DFOP	3.0	13.6	126.9	DFOP	3.0	52.5[b]
Kellmetschweiher (outdoor), 2000/1014987	HS	4.5	18.9	140.4	SFO	6.6	21.4[d]
Sediment compartment	**Kinetic model**	**χ^2 error**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**χ^2 error**	**$DisT_{50}$ [d]**
Kellmetschweiher (outdoor), 2000/1014987	FOMC[f]	3.5	101.3	>1000	SFO[f]	9.7	87.9[e]

[a] much longer than the study period of 100 d; interpret with care
[b] $DisT_{50}$ calculated from DFOP slow phase ($DisT_{50}$ = ln2/k2)
[c] temperature-normalized value at reference temperature of 20°C (non-normalized value: 32.5 d)
[d] temperature-normalized value at reference temperature of 20°C (non-normalized value: 20.2 d)
[e] temperature-normalized value at reference temperature of 20°C (non-normalized value: 197.1 d)
[f] outlier removed

Level P-II

The kinetic evaluation resulted in acceptable SFO fits for the evaluated water/sediment systems, with back-transfer rates greater than zero. However, for Kellmetschweiher (system A of dark study) and the outdoor system, the F_{sed} test was passed, but at least one degradation rate in each system failed the t-test. For Berghäuser Altrhein (system B of dark study) and the time-step normalized outdoor system, the F_{sed} test failed. Consequently, no trigger or modelling endpoints were calculated. For modelling, endpoints should be set using a default approach according to FOCUS kinetics.

Level M-I

A summary of the trigger and modelling endpoints (dissipation) (Table 4.1.4.3-5) and modelling endpoints (degradation) (Table 4.1.4.3-6) for dimoxystrobin metabolites is given below.

Table 4.1.4.3-5: Summary of estimated trigger and modelling endpoints for the metabolites of dimoxystrobin (Level M-I dissipation*, total system)

Test system	Substance	Trigger endpoints				Modelling endpoints		
		Kinetic model	χ^2 error	$DisT_{50}$ [d]	$DisT_{90}$ [d]	Kinetic model	χ^2 error	$DisT_{50}$ [d]
Kellmetschweiher (outdoor), total system	505M01	SFO	4.4	31.2	103.6	SFO	3.5	34.5[a]
	505M96	SFO	4.2	43.4	144.2	SFO	11.4	43.8[a]

[*] dissipation from maximum occurrence

[a] temperature-normalized value

Table 4.1.4.3-6: Summary of estimated modelling endpoints for the metabolites of dimoxystrobin (Level M-I degradation*, total system)

Test system	Substance	Kinetic model	χ^2 error	Formation fraction [-]	Std. error of FF [-]	$DegT_{50}$ [a] [d]
Kellmetschweiher (outdoor), total system	505M01	SFO	18.8	0.8809[b]	0.0844	1.7
	505M08	SFO	21.0	no reliable endpoints derived		
	505M09	SFO	24.2	no reliable endpoints derived		
	505M96	SFO	21.4	0.3770[c]	0.0873	16.9

[*] considering formation and degradation in a linked model

[a] temperature-normalized value at reference temperature of 20°C

[b] from parent

[c] from 505M01

III. CONCLUSION

The dissipation and degradation of dimoxystrobin in three water/sediment systems was evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*] to determine trigger and modelling endpoints. In the first study, two different natural systems of water and sediment were incubated in the dark under aerobic conditions and in the second study, a natural system of water and sediment was incubated outdoor under aerobic conditions and exposed to natural sunlight.

For the two laboratory systems, trigger $DegT_{50}$ values of 297.6 and 834.5 days in the total system and 13.6 and 25.3 days in the water compartment were calculated. For the outdoor system, trigger $DegT_{50}$ values of 26.7, 18.9 and 101.3 days were determined for the total system, the water compartment and the sediment phase, respectively.

Reliable modelling-$DegT_{50}$ values for the whole system were derived for system A (Kellmetschweiher) with 525.6 days, system B (Berghäuser Altrhein) with 297.6 days and outdoor system (Kellmetschweiher) with 35.2 days. For the water compartment, $DegT_{50}$ values of 81.3, 52.5 and 21.4 days were determined for System A, System B and the outdoor system, respectively. For P-II level no trigger or modelling endpoints could be calculated.

For metabolite 505M01, a trigger-$DisT_{50}$ of 31.2 days and a modelling-$DegT_{50}$ of 1.7 days was calculated, while for metabolite 505M96, a trigger-$DisT_{50}$ of 43.4 days and a modelling-$DegT_{50}$ of 16.9 days was calculated. No reliable endpoints could be derived for metabolites 505M08 and 505M09.

4.1.4.4 [Study 4]

Study reference:

Singh (1998): Photolysis of 14C-BAS 505 F in aqueous media, 1997/5286, BASF Corporation Agricultural Products Center; Research Triangle Park, NC 27709; United States of America

Detailed study summary and results (see RAR (2017) – Volume 3 - B.8 (AS), chapter B 8.2.1.2):

Studies evaluated previously, DAR, 2003

a) The aqueous photolysis of dimoxystrobin in a sterile buffer was studied according to EPA guidelines. (Pesticide Assessment Guidelines, Subdivision N, Series 161-2, 1982).

The direct photolysis was performed because the absorption coefficients of dimoxystrobin for wavelengths above 290 nm were > 10 l/(mol x cm). The absorption coefficients (max. 1170 l/(mol x cm)) were used for the determination of the quantum yield and to calculate the photolytic half-life in the top layer of aqueous systems.

The direct photolysis was performed with both [^{14}C-benzyl] and [^{14}C-phenyl]dimoxystrobin. The study was performed in a pH 7 buffer. The concentration of the active substance in the sterile aqueous buffer solution was 2.03 mg/l for the benzyl-label and 1.87 mg/l for the phenyl-label.

For each label a separate experiment was performed. Sterilised glass vessels (20 ml volume) with quartz glass caps containing about 18 ml test solution were irradiated in a thermostated block. Each vessel had an air inlet and an air outlet. The incoming air was moistened, sterilised, and the CO_2 was removed. For each label, four vessels were filled with treated buffer solution and placed in one row in the thermostated block. A trapping system for volatiles was connected to each row. The thermostated vessels were located under a xenon lamp (spectral cut-off at 290 nm; 738 W/m² at 295-805 nm at soil layer level, 1664 µEinsteins/cm^2/sec). The duration of the experiment was 15 days with continuous irradiation. The study authors related the intensity of the lamp as equivalent to clear April / May Days *ca*. 36°N (North Carolina, USA).

Appropriate volumes of each test solution were stored in a climatic chamber to be used as dark control. The temperature was 22 ± 1°C during the experiments.

Samples were analysed by HPLC with analyte identity confirmation by MS. Trapped volatiles were quantified by LSC.

For the determination of the quantum yield of dimoxystrobin, a mixture of p-nitroacetophone and pyridine was used as chemical actinometer. During each irradiation experiment, one vessel with the actinometer solution was irradiated simultaneously with the test solutions.

During direct photolysis, a slow degradation of the active substance could be observed. The characterisation of the radioactivity is summarised below. In the dark control, no degradation was observed.

Table 4.1.4.4-1 Distribution of radioactivity after application of ^{14}C-dimoxystrobin in a laboratory (22°C, continuously irradiated, sterile, pH7) aqueous photolysis study (DAR, 2003)

DAT	% AR							
	dimoxystrobin	dimoxystrobin Z isomer	505M01	505M03	505M04	others*	CO_2	sum
benzyl-label								
0 d	98.0	1.2	0.9	-	0.0	0.0	n.m.	100.1
3 d	87.0	6.5	1.6	-	1.0	1.8	< 0.1	97.9
7 d	84.6	5.7	3.4	-	1.6	3.2	0.1	98.6
11 d	78.7	6.7	5.0	-	2.0	3.6	0.1	96.1
15 d	78.8	5.8	7.8	-	2.3	5.3	0.1	100.1
phenyl-label								
0 d	97.5	1.9	-	0.5	0.1	0.0	n.m.	100.0
3 d	94.0	5.3	-	1.4	0.7	0.4	0.3	102.2
7 d	87.5	6.8	-	2.1	1.1	3.0	0.4	100.9
11 d	87.3	6.6	-	1.8	1.5	3.8	0.9	102.0
15 d	76.6	6.7	-	2.3	1.0	8.7	1.2	96.5

n.m.: not measured

* each individual peak ≤3% AR at any sampling time

The DT_{50} of the active substance was estimated according to first order kinetics using the computer program ModelMaker (v.4, MARQUARDT-NEWTON, least squares method). The DT_{50} estimated was 64.8 test system days (r^2= 0.81). Note this value is extrapolated significantly beyond the duration of the study and the test system used continuous irradiation.

The quantum yield of dimoxystrobin was estimated to be 1.29×10^{-3}.

With quantum yield, absorption spectrum, and with the help of a program which uses the algorithms developed by FRANK and KLÖPFFER for the direct photochemical transformation of chemicals in water, the theoretical photolytic half-life of dimoxystrobin in the top layer of aqueous systems was calculated to be about twice the study duration. Actual numbers were not given in the report because values were considered not to be statistically valid.

(Scharf, 1998; Singh, 1998; Platz, 2000b)

4.1.4.5 [Study 5]

Study reference:

Goetz & Moss (1998): Natural water photolysis of BAS 505 F, 1997/5428, BASF Corporation Agricultural Products Center; Research Triangle Park, NC 27709; USA

Detailed study summary and results (see RAR (2017) – Volume 3 - B.8 (AS), chapter B 8.2.1.2):

b) The aqueous photolysis of dimoxystrobin in a natural surface water was studied according to EPA guidelines. (Pesticide Assessment Guidelines, Subdivision N, Series 161-2, 1982).
Natural water was obtained from a pond located in Holly Springs, CA, USA. The water had a pH of 8.6, organic matter content of 6 mg/l, and a nitrate content of 4 mg/l. The study was performed with non-labelled dimoxystrobin (purity 99.9%). The test substance was dissolved in 15 ml pond water at a concentration of 2 mg/l. A thermostated glass vessel with a quartz glass cap, (7.7 cm diameter, 3.9 cm quartz window, 12.7 cm height), filled with the test solution was irradiated under a xenon lamp (spectral cut-off at 290 nm; 738 W/m² at 295-805 nm at soil layer level, 1664 µEinsteins/cm²/sec). The study authors related the intensity of the lamp as equivalent to clear April/May Days *ca*. 36°N (North Carolina, USA). The temperature was kept at 22°C. After 0, 1, 2, 3, 6, 8, 10, 13, and 15 days of continuous irradiation, respectively, a 1 ml sample was taken from the glass vessel and analysed by HPLC-UV for parent dimoxystrobin. One dark control sample was analysed at the same times as the irradiated sample, respectively.
The results of the HPLC-analysis are shown below.

Table 4.1.4.5-1 HPLC measured loss of dimoxystrobin in a natural water laboratory (22°C, continuously irradiated, pH 8.6) aqueous photolysis study (DAR, 2003)

	% applied substance	
DAT	**irradiated**	**dark control**
0	96.4	96.4
1	90.5	99.0
2	90.3	103.3
3	83.7	100.6
6	71.2	96.2
8	64.9	98.2
10	55.1	97.6
13	53.0	95.3
15	46.9	98.7

The photolytic DT_{50} of dimoxystrobin in this natural water, calculated according to first order linear regression analysis was 14.1 test system days (r^2=0.98). Note the test system used continuous irradiation. Dimoxystrobin was stable in the dark control.

(Goetz and Moss, 1998)

Summary and Assessment

When exposed to continuous irradiation (xenon lamp, 738 W/m^2 wavelength > 290 nm, equated to clear April/May day at 36°N) in a sterile buffer (pH7) for 15 days at 22 ±1°C, some slow degradation of dimoxystrobin occurred (*ca*. 76-79%AR remaining at study end), compared to none in the dark. A DT_{50} was estimated as 64.8 'test system' days (1st order, r^2 =0.81). However, this was extrapolated beyond the study duration of 15 days. Although photolysis did occur, dimoxystrobin was not readily photodegraded under laboratory conditions. The Z isomer of dimoxystrobin was initially present in the water phase at <2%AR at day 0, but during the study was detected at a maximum of 6.7-6.8% AR for both radiolabels in the water phase, and remained around this level. No major photodegradates (>10% AR) were formed. Three minor metabolites (all <10%AR) were identified as 505M01 and 505M03 (benzyl and phenyl labels respectively) and 505M04 (both labels).

Quantum yield of dimoxystrobin was determined as 1.29 x 10^{-3}. Using the Frank and Klöpffer model, an environmental half-life for direct photolysis of dimoxystrobin was calculated to be about twice the study duration which was 15 days (i.e. not stated but approximately 30 days).

Non-radiolabelled dimoxystrobin was also exposed to continuous irradiation (xenon lamp, 738 W/m^2 wavelength > 290 nm, equated to clear April/May day at 36°N) in natural pond water (pH 8.6) for 15 days at 22°C. After 15 days, greater degradation had occurred in the irradiated samples than in the dark controls, (46.9 and 98.7% applied remained as dimoxystrobin in irradiated and dark samples, respectively). The DT_{50} was calculated as 14.1 test system days (1st order, r^2 = 0.98).

Table 4.1.4.5-2 First order DT_{50} values ('test system' days) calculated for aqueous photolysis of dimoxystrobin (DAR, 2003)

	Irradiated	**Dark**
Laboratory	64.8* (r^2 = 0.81)	Stable
Pond Water	14.1 (r^2 = 0.98)	Stable

* extrapolated beyond study end.

Studies submitted for the renewal of the active substance

No new experimental data on direct photolysis of dimoxystrobin was produced for the renewal of the active substance. The already peer-reviewed study is considered still valid.

Dimoxystrobin showed a very slow degradation under photolytic conditions. After 15 days irradiation, it still amounted to 77% (phenyl-label) and 79% AR (benzyl-label). Besides slight shift from E- to Z-isomer (max. 6.8% AR), the cleavage product 505M01 (BF 505-4) was formed in amounts of 7.8% AR. Most of the other photolysis products occurred only in trace amounts and none of them exceeded 4% AR at any sampling time. Although not used in risk assessment, new kinetic evaluations of the two direct aqueous photolysis experiments with the two radiolabels were performed.

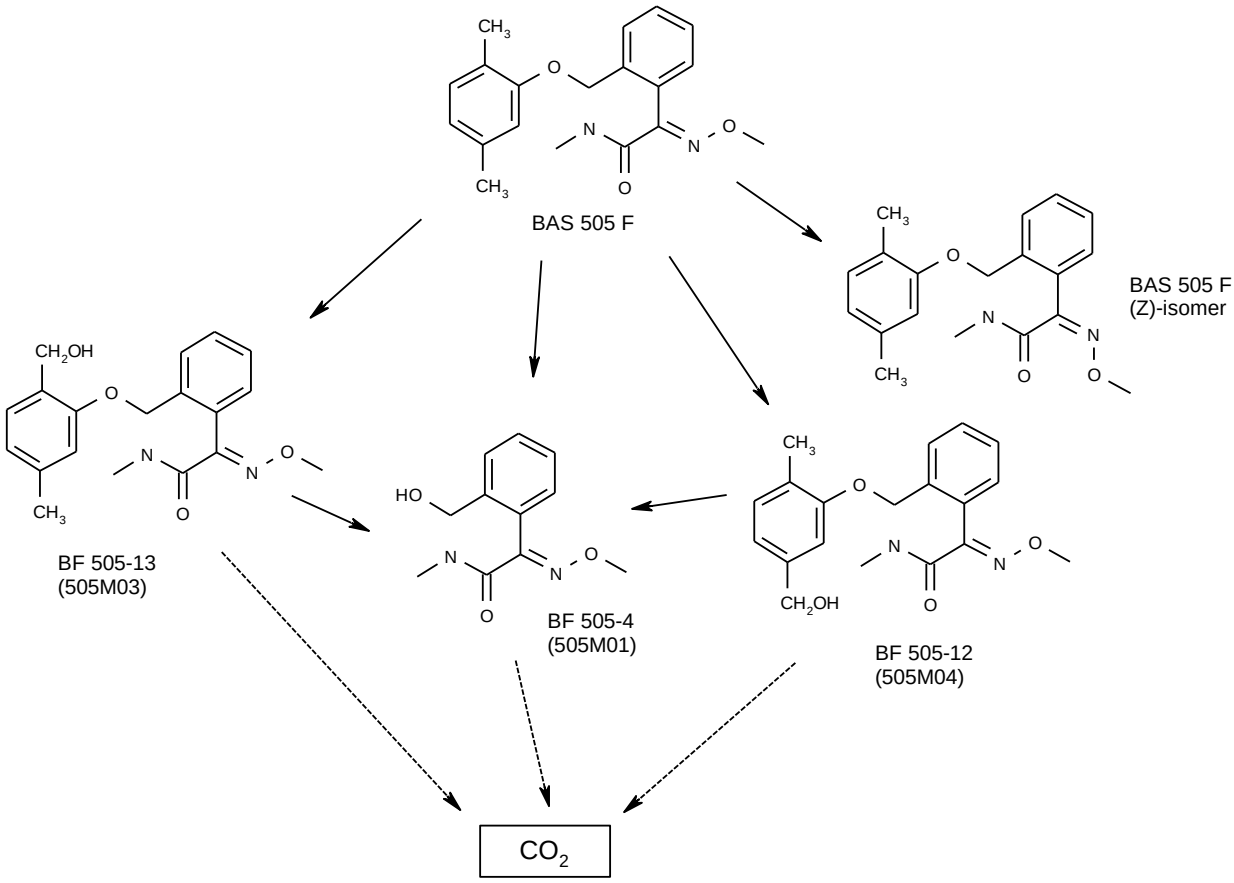


Figure 4.1.4.5-1: Proposed route of degradation of dimoxystrobin during aqueous photolysis (from dimoxystrobin dossier 2001)

4.1.4.6 [Study 6]

Study reference:

Budde (2014): Kinetic evaluation of two studies on aqueous photolysis of BAS 505 F - dimoxystrobin according to FOCUS Degradation Kinetics, 2014/1263218, BASF SE, Limburgerhof, Germany Fed.Rep.

Detailed study summary and results (see RAR (2017) – Volume 3 - B.8 (AS), chapter B 8.2.1.2):

Reference	Budde E., 2014b Kinetic evaluation of two studies on aqueous photolysis of BAS 505 F - Dimoxystrobin according to FOCUS Degradation Kinetics 2014/1263218 CA 7.2.1.2/1
Guidelines	FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0
GLP	-
test item	-
study duration	-
study completed	October 2014

Executive Summary

The aqueous photolysis of BAS 505 F - dimoxystrobin has been investigated in two studies, one of them performed in sterile aqueous buffer solution, the other one performed with natural water. The purpose of this evaluation was to analyse the degradation kinetics of dimoxystrobin observed in the studies, taking into account the current guidance of the FOCUS workgroup on degradation kinetics.

The best-fit $DegT_{50}$ values were 61.5 and 63.8 days in the sterile buffer solutions, and 14.0 days in the natural water system. In all test systems, the SFO kinetic model provided an appropriate fit to the measured data.

I. MATERIAL AND METHODS

The aqueous photolysis of dimoxystrobin was investigated in one study with a sterile buffer solution [*Singh, M. (1998): Photolysis of 14C-BAS 505 F in aqueous media. BASF DocID 1997/5286*] and in one study with natural water [*Goetz, A., Moss, I. (1998): Natural water photolysis of BAS 505 F. BASF DocID 1998/5428*].

In the study by Singh, the photolytic degradation of ^{14}C-labelled dimoxystrobin was investigated at pH 7 in sterile aqueous buffer solution, using benzyl- or phenyl-labelled test substance. The concentration of dimoxystrobin was 2.03 mg L^{-1} for the benzyl-label and 1.87 mg L^{-1} for the phenyl-label. The treated buffer solutions were continuously exposed to artificial sunlight for about 15 days (355 hours), while being maintained at 22±1°C. Dimoxystrobin degraded slowly, with 78.8%TAR and 76.6%TAR remaining after 355 hours (=15 days) in the benzyl- and phenyl-labelled systems, respectively. The experimental data of the irradiated systems used for kinetic analysis are given in the table below. The data of the dark control system were not evaluated, as dimoxystrobin proved to be stable.

Table 4.1.4.6-1 Data for kinetic evaluation of the aqueous photolysis study (irradiated systems)

Day [a]	Experimental data [%TAR]		Input data according to FOCUS [%TAR]	
	Benzyl label	Phenyl label	Benzyl label	Phenyl label
0	99.1	99.4	100.0 [b]	100.0 [b]
3	93.5	99.4	93.5	99.4
7	90.3	94.3	90.3	94.3
11	85.5	93.9	85.5	93.9
15	84.6	83.3	84.6	83.3

TAR = Total applied radioactivity

[a] reported sampling times in hours converted to days

[b] set to material balance

In the study by Goetz and Moss, the photolytic degradation of dimoxystrobin was investigated in natural water. The water had a pH of 8.6, an organic matter content of 6 mg L^{-1}, and a nitrate content of 4 mg L^{-1}. The study was performed with non-labelled dimoxystrobin at a concentration of 2 mg L^{-1}. Samples were irradiated for 15 days (357 hours) and maintained at 22°C during irradiation. Dimoxystrobin degraded moderately, with 46.9% TAR remaining at the end of the study. The experimental data of the irradiated system used for kinetic analysis are given in the following table. The data of the dark control system were not evaluated, as dimoxystrobin proved to be stable.

Table 4.1.4.6-2 Data for kinetic evaluation of the natural water photolysis study (irradiated system)

Day [a]	Irradiated system [%AD]
0	96.4
1	90.5
2	90.3
3	83.7
6	71.2
8	64.9
10	55.1
13	53.0
15	46.9

AD = Applied dose

[a] reported sampling times in hours converted to days

Kinetic modelling

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints). The appropriate kinetic model for deriving trigger endpoints was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate*

Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 436pp]. The best-fit model was selected based on visual and statistical assessment and the corresponding $DegT_{50}$ and $DegT_{90}$ values are reported as trigger endpoints.

Kinetic models included in the assessment

The kinetic models employed for this evaluation were described by the FOCUS workgroup on degradation kinetics: Single-First-Order (SFO); Box 5-1, in *FOCUS (2006)*, Gustafson and Holden (FOMC): Box 5-2, in *FOCUS (2006)*, Double first-order in parallel (DFOP); Box 5-4, in *FOCUS (2006)*.

The appropriateness of a distinct kinetic model to describe degradation can be tested with the following checks recommended by FOCUS [*FOCUS (2006)*, chapter 6.3.1]: visual assessment of goodness-of-fit, estimation of the error percentage at which the χ^2 test is passed (Equation 6-2 in *FOCUS (2006)*), t-test to evaluate whether estimated degradation parameters differ from zero.

A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 - error value is < 15% and the estimated degradation parameters differ from zero as outlined by FOCUS [*FOCUS (2006)*, chapter 6.3.1].

Data handling and software for kinetic evaluation

The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*]. The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting [*Schafer, D., Mikolasch, M., Rainbird, P., Harvey, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. BASF DocID 2007/1062781; Schmitt, W., Gao, Z., Meyer, H. (2011) KinGUII, Version 2.2012.320.1629 Bayer CropScience AG*]. The error tolerance and the number of iterations of the optimization tool were set to $1x10^{-6}$ and 100, respectively.

II. RESULTS AND DISCUSSION

The datasets for each system were analysed considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given in the original study report.

An overview of the estimated trigger endpoints for dimoxystrobin from the two studies is given in Table 4.1.4.6-3.

Table 4.1.4.6-3 Kinetic endpoints for the degradation of dimoxystrobin in the aqueous photolysis studies

Study matrix	Test system	Kinetic model	χ^2 error	k [d^{-1}]	Kinetic endpoints	
					$DegT_{50}$ [d]	**$DegT_{90}$ [d]**
Sterile buffer solution	benzyl label	SFO	1.2	0.0113	61.5	204.3
	phenyl label	SFO	2.0	0.0109	63.8	212.0
Natural water	irradiated	SFO	2.0	0.0495	14.0	46.5

III. CONCLUSION

The degradation of dimoxystrobin in two aqueous photolysis studies was investigated and evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics. The SFO model proved to be appropriate to derive kinetic endpoints for dimoxystrobin from the aqueous photolysis studies, with $DegT_{50}$ values ranging from 14.0 (natural water) to 63.8 days (sterile buffer) and $DegT_{90}$ values ranging from 46.5 (natural water) to 212.0 days (sterile buffer). For the evaluated systems, visual assessment and goodness-of-fit statistics of the SFO model indicate plausible fit and therefore, the resulting values can be considered reliable.

4.1.4.7 Indirect photochemical degradation

Studies submitted for the renewal of the active substance

No new experiments on indirect photochemical degradation of dimoxystrobin were performed for the renewal of the active substance. Since the existing and already peer-reviewed study with dimoxystrobin was done with non-labelled test item, no further information on the route of degradation in the aquatic environment can be given. The half-life was re-evaluated according to FOCUS (2006) and is reported in Table 4.1.4.6-3.

RMS comment at renewal

Summary of the relevant information and conclusions on the hydrolytic and photochemical degradation of dimoxystrobin

No new experimental data on hydrolysis of dimoxystrobin were produced for the renewal of the active substance. The old hydrolysis study is considered still valid. Dimoxystrobin proved to be stable at all tested pH values (pH 4-9) and temperatures (25 and 50°C).

The aqueous photolysis of dimoxystrobin in a sterile buffer was studied previously. The test system used continuous irradiation. The DT50 estimated was 64.8 test system days, which value is extrapolated significantly beyond the duration of the study (15 days).

The aqueous photolysis of dimoxystrobin in natural surface water (pond water) was studied under continuous irradiation previously also. The photolytic DT50 of dimoxystrobin in this natural water, calculated according to first order linear regression analysis was 14.1 test system days (the duration of the study was15 days).

No new experimental data on direct photolysis of dimoxystrobin was produced for the renewal of the active substance. The already peer-reviewed study is considered still valid.

Although not used in risk assessment, new kinetic evaluations of the two direct aqueous photolysis experiments with the two radiolabels were performed.

The SFO model proved to be appropriate to derive kinetic endpoints for dimoxystrobin from the aqueous photolysis studies, with DegT50 values ranging from 14.0 (natural water) to 63.8 days (sterile buffer) and DegT90 values ranging from 46.5 (natural water) to 212.0 days (sterile buffer).

4.1.4.7.1 Summary of occurrences and degradation rates of dimoxystrobin and its metabolites

Table 4.1.4.7.1-1: **Maximum occurrence of dimoxystrobin metabolites in water phase of water/sediment studies**

Metabolite	Matrix	BASF DocID	System	Incubation	Parent label	Maximum % AR
505M08 (BF 505-7)	water	2000/1000121 [1]	Kellmetschweiher	dark	phenyl benzyl	0.8 [2] 0.7 [2]
			Bergh. Altrhein	dark	phenyl benzyl	2.1 [2] 2.9 [2]
		2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	3.6
505M09 (BF 505-8)	water	2000/1000121 [1]	Kellmetschweiher	dark	phenyl benzyl	3.4 [2] 3.7 [2]
			Bergh. Altrhein	dark	phenyl benzyl	4.7 [2] 5.2 [2]
		2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	5.3
505M01 (BF 505-4)	water	2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	3.2
505M96	water	2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	9.6
505M97	water	2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	3.4

[1] already peer-reviewed during previous EU evaluation
[2] values taken from appendix of original report; for modelling, mean values as listed in the table section of the original report and former dossier tables are used.

Table 4.1.4.7.1-2: **Summary table on kinetic endpoints of dimoxystrobin and metabolites obtained in water/sediment studies**

Study BASF DocID	Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	DT_{50} whole system [d]	Kinetic model	DT_{50} water [d]	Kinetic model	DT_{50} sediment [d]	Kinetic model
dimoxystrobin										
2000/1000121 dark	Kellmetschw.	8.5	7.5	20	834.5	HS	25.3	DFOP	-	-
2000/1000121 dark	Berghäuser Altrhein	8.2	7.6	20	297.6	SFO	13.6	DFOP	-	-
2000/1014987 irradiated	Kellmetschw.	8.8	n.r.	outdoor[a]	26.7	HS	18.9	HS	101.3	FOMC
505M01										
2000/1014987 irradiated	Kellmetschw.	8.8	n.r.	outdoor[a]	31.2 [b] 1.7 [c]	SFO SFO	-	-	-	-
505M96										
2000/1014987 irradiated	Kellmetschw.	8.8	n.r.	outdoor[a]	43.4 [b] 16.9 [c]	SFO SFO	-	-	-	-

n.r. not reported in study
[a] mean water temperature over incubation period 18.1°C (min. 8.6°C, max. 28.5°C)
[b] Level M-I dissipation, total system ($DisT_{50}$ considering decline from time of maximum occurrence)
[c] Level M-I degradation, total system ($DegT_{50}$ considering simultaneous formation and degradation of metabolite)

RMS comment at renewal

Summary of information related to the water/sediment studies of dimoxystrobin

Laboratory studies (dark)

Previously evaluated laboratory dark aerobic sediment/water studies

Parameters: Water/ sediment systems from a pond (System A) and a pond-like side arm of a river (System B), ca.6 cm depth water and 2-2.5cm depth sediment, incubated at 20±2°C for up to 100 days. No major metabolites identified. Metabolites 505M08 and 505M09 <5% AR in the water phase (0.6% AR, 3.5% AR, respectively). No metabolites identified in the sediment.

Disappearance times could only be calculated for the active substance in the water phase: DT_{50} system A 27 days, DT_{90} >200 days, system B DT_{50} 15 days, DT_{90} 136 days (DT90 values extrapolated beyond the study duration). DT50/90sed could not be calculated (residue levels in the sediment did not decline during the study).

Study performed for the renewal of the active substance

No new water/sediment study was performed for the renewal of the active substance. The already peer-reviewed study is considered still valid. However, the half-lives of dimoxystrobin were re-calculated according to the newest guidelines and guidance documents.

Recalculated values: Trigger DT50water system A 25.3 and system B 13.6 days.

Modelling-$DegT_{50}$ values determined: whole system A 525.6 days, system B 297.6 days; water compartment, $DegT_{50}$ System A 81.3, System B 52.5.

Outdoor studies (irradiated)

Outdoor aerobic sediment/water study (natural light exposure) evaluated previously

Parameters: 2.0 cm sediment and a water layer of about 20 cm height, outdoor temperature and light conditions, treatment in July, in Germany, 120 days study duration. Mean water temperature in the first 58 days 21.6°C. Only the data of the first 58 days of incubation were used for half-life time calculation (then dissipation processes in the test vessels considerably slowed down in September-November). DT50water 15.3 days, DT50sed 9.1 days, DT50whole system 26.9 days.

In DAR, 2003 the RMS supported the use of the half-life value of 15.3 days obtained from the outdoor water-sediment study for PEC_{sw} calculation.

Recalculated values

Trigger $DegT_{50}$ total system 26.7, water compartment 18.9 and sediment phase 101.3 days. Modelling-$DegT_{50}$ whole system 35.2 days, water compartment 21.4 days.

For metabolite 505M01, a trigger-$DisT_{50}$ of 31.2 days and a modelling-$DegT_{50}$ of 1.7 days were calculated, while for metabolite 505M96, a trigger-$DisT_{50}$ of 43.4 days and a modelling-$DegT_{50}$ of 16.9 days were calculated. No reliable endpoints could be derived for metabolites 505M08 and 505M09.

Outdoor mesocosm study evaluated previously

Parameters: Water depth 100 cm, water from a lake, 10 cm layer of natural sediment, sediment from another lake, equilibrated for 6 months, 4 a.s. concentrations tested (the highest test concentration reflects about 67% of an overspray situation (based on the single maximum application rate of 200 g a.s./ha), the lowest test concentration simulates approximately spray drift at a distance of about 1 m assuming 2.77% drift), treatment in May, in Germany, mean daily water temperatures in the range 15-27°C. Dissipation pattern was evaluated. DT50water 60-69 days, mean 63.5 days, DT90 200-230 days, mean 212 days.

PPR Panel used the dissipation DT50 63.5 d calculated for the water phase from the outdoor pond mesocosm study, as conservative but realistic value for the exposure assessment (Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from EFSA related to the evaluation of dimoxystrobin, 2005).

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

4.2.1.1 Study 1 – Bioconcentration in rainbow trout

Report: CA 8.2.2.3/1
Anonymous, 1999 a
14C-BAS 505 F - Bioconcentration in rainbow trout
1999/11247

Guidelines: EEC 91/414, OECD 305, EPA 165-4, EEC 96/12

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

EXECUTIVE SUMMARY

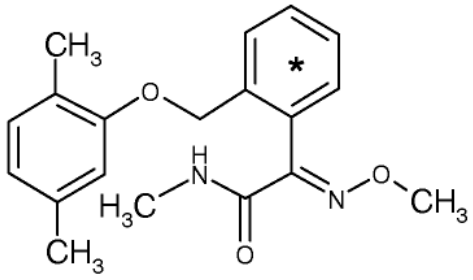
An experimental aquatic study to determine the bioconcentration potential (BCF) of dimoxystrobin (purity >97%) was conducted. The study used radiolabelled [benzyl-U-^{14}C]-BAS 505 F dissolved in N,N-dimethyl formamide (DMF), a flow-through system with Rainbow Trout (*Oncorhynchus mykiss*) and exposure to a single concentration of test substance at 0.5 µg a.i./L (nominal). A control experiment was conducted where fish were exposed to DMF and diluent water alone. The exposure period ran for 35 days followed by a 14-day depuration period. Total radioactivity was determined in tissue and water samples. Extracts of water, edibles and inedibles were analysed by radio-HPLC and/or radio-TLC. Unchanged parent compound and degradation products were identified by chromatographic comparison with reference compounds.

The depuration half-life (CTSO) in whole fish was 0.5 days. The time to reach 90% depuration (CT9o) is 1.6 days. The bioconcentration factor calculated directly from the ratio of the '4C-concentrations in water and tissue fractions (mean of Days 4 - 35) was 84 for whole fish, 47 for edible tissues and 110 for viscera [see Table 30]. The values were in good accordance with those obtained by kinetic modelling (whole fish: 91, edibles: 49, inedibles: 120). The compound was intensively metabolized to mainly hydroxylation products and their glucuronic acid conjugates. Due to the low bioconcentration factor and the rapid excretion of the active substance from fish it is concluded that there is no risk of bioaccumulation of dimoxystrobin.

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: The bioaccumulation in fish of BAS 505 F was investigated using [benzyl-U-^{14}C]-BAS 505 F.
[Benzyl-U-^{14}C]-BAS 505 F ([benzyl-U-^{14}C]-Reg. No. 285028), batch no. 596-1015, radiochemical purity > 97%.



B. STUDY DESIGN

Test species: Rainbow Trout (*Oncorhynchus mykiss*).

Test design: Flow through system.

Test concentrations: 0.5 µg a. i./L (nominal concentration).
The test concentration was derived from acute toxicity data of BAS 505 F for rainbow trout (ca. 1 % of the 96 h LC_{50}). Since this concentration was already very low, a test with a second lower concentration could not be conducted, as results would have been below analytical limits of determination.

Test conditions: The active substance was dissolved in N,N-dimethyl formamide (DMF) and this stock solution was pumped into a mixing vessel supplied with a diluent stream of water. From there, the treated water flowed through the tank at a rate of 1440 l/day to give a nominal concentration of 0.5 µg/l. Test fish (ca. 130 of ca. 4 – 6 cm size at day 0) were exposed for 35 days. Subsequently fish were exposed to a continuous flow of dilution water alone for either 14. Water from the main exposure tank flowed into an overflow tank (90 litres) into which additional fish of ca. 5 - 10 cm size were placed. These fish were to be used for the generation of metabolites if required. A control experiment with another 130 fish was conducted where fish were exposed to DMF and diluent water alone.

Analytics: Fish were sampled at suitable intervals (Days 0, 1, 2, 4, 7, 14, 21, 28, and 35 of exposure; Days 1, 3, 7, 10, and 14 of depuration) and dissected into edible (muscle) and inedible (viscera) tissue fractions. Tank water was collected from various parts of the tank. Total radioactivity was determined in tissue and water samples. Extracts of water, edibles and inedibles were analysed by radio-HPLC and/or radio-TLC. Unchanged parent compound and degradation products were identified by chromatographic comparison with reference compounds.

II. RESULTS AND DISCUSSION

Findings

During the uptake phase the actual concentration of total radioactivity in water was maintained at - 0.42 ± 0.02 µg/L. The concentration in water sampled at the first day of depuration and thereafter dropped to levels below the limit of determination. The only radioactive component in water was unchanged BAS 505 F.

Mean concentrations of radioactivity in the total fish reached a plateau concentration of about 0.04 µg/g after 14 days of exposure. Mean concentrations of radioactivity in the edible part reached a plateau of 0.02 - 0.03 µg /g from the first day of exposure. In the inedible fraction (viscera) a plateau of 0.05 µg/g from Day 14 onwards.

After the transfer to a tank containing untreated water, mean concentration decreased during Day 1 of depuration to 0.01 µg /g in total fish, 0.005 µg/kg in the edible fraction and 0.013 µg /g in the inedible fraction. After Day 3 the concentration dropped to levels below the limit of quantification (<0.001 mg/kg) which corresponds to <2.5% (whole fisch), <3.3% (edibles) and <2% (viscera) of the plateau concentration.

The bioconcentration factors and kinetic parameters based on total radioactivity concentrations were derived from Non-Linear-Regression Analysis using a 2-Compartment Model and are summarized below [see Table 4.2-1]. The depuration half-life (CTSO) in whole fish was 0.5 days.

Accordingly, the time to reach 90% depuration (CT9o) is 1.6 days. The bioconcentration factor calculated directly from the ratio of the '4C-concentrations in water and tissue fractions (mean of Days 4 - 35) was 84 for whole fish, 47 for edible tissues and 110 for viscera [see Table 4.2-2]. The values were in good accordance with those obtained by kinetic modelling (whole fish: 91, edibles: 49, inedibles: 120).

Nature of the radioactive residues

The nature of the residues in fish fractions from the steady-state period (Day 35 of exposure) is summarised below [see Table 4.2-3]. It appeared that BAS 505 F was initially metabolised to form the hydoxylated metabolites 505M03, 505M04, and BF 505-5 (505M02) which then were further metabolised to form glucuronic acid conjugates (for chemical structures cf. [see Table 4.2-4]). In total, six unknown components were observed which, however, were only present at levels of < 0.001 µg/g in the edible fraction and 0.003 µg/g or less in the inedible fraction.

From the concentrations of unchanged BAS 505 F in edibles (0.025 µg/g), inedibles (0.017 µg/g), whole fish (0.021 µg/g) and water (0.44 µg/I) at Day 35, the bioconcentration factors of unchanged BAS 505 F was calculated to be 57, 39, and 48 for edibles, viscera, and whole fish, respectively.

Table 4.2-1: Kinetic parameters

Parameter	Tissue fraction		
	Edibles	**Viscera**	**Whole fish**
Uptake rate constant k_1 [days $^{-1}$]	69.0	171.1	129.9
Depuration rate constant k_2 [days $^{-1}$]	1.419	1.426	1.421
Depuration half-life CT_{50} [days]	0.49	0.49	0.49
Time to reach 90% depuration CT_{90} [days]	-	-	1.62
Bioconcentration factor (BCF) k_1/k_2	48.6	120.0	91.4

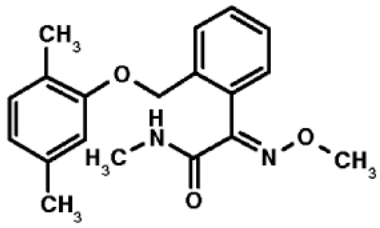
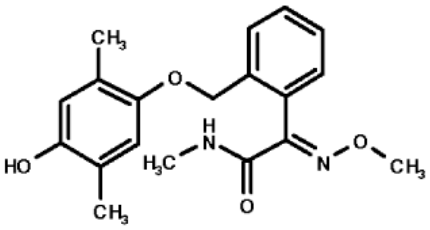
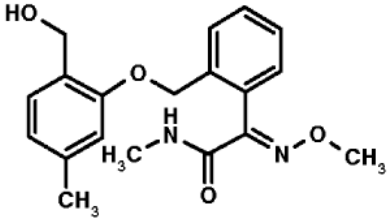
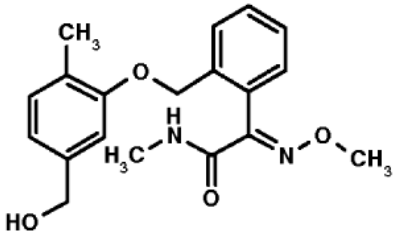
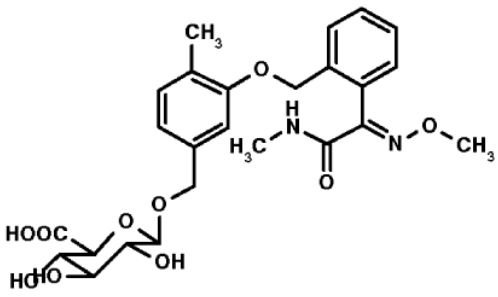
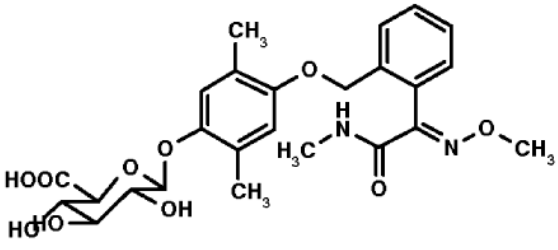
Table 4.2-2: Bioconcentration factors calculated directly from the ratio of total radioactivity concentrations in water and tissues

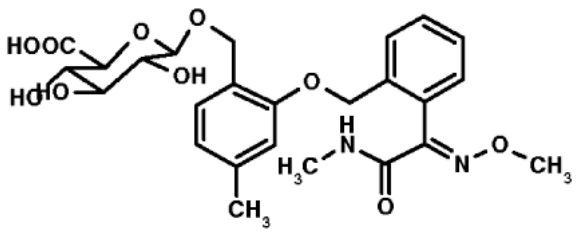
Uptake phase Day No.	Tissue fraction		
	Edibles	**Viscera**	**Whole fish**
1	36	71	57
2	50	180	133
4	35	102	72
7	37	95	72
14	40	116	86
21	60	126	98
28	49	105	84
35	61	116	93
Mean Days 4 – 35 (plateau)	47	110	84

Table 4.2-3: Summary of identified metabolites in fish tissues sampled at Day 35. Values in µg/g tissue and % of total tissue radioactivity (in parenthesis)

Metabolite	Tissue fraction	
	Edibles	**Inedibles**
BAS 505 F	0.025 (89.9)	0.017 (30.7)
BAS 505 F isomer	0.001 (2.9)	0.001 (1.3)
BF 505-5 (505M02)	≤ 0.001 (0.5)	0.003 (6.0)
505M03 / 505M04	< 0.001 (< 0.7)	0.001 (2.5)
505M49 / 505M78 / 505/M79	0.001 (5.3)	0.024 (44.0)

Table 4.2-4: Structures of identified metabolites

Metabolite	Structure
BAS 505 F	
BF505-5 (505M02)	
505M03	
505M04	
505M49	
505M78	

Metabolite	Structure
505M79	

4.2.2 Bioaccumulation test with other organisms

No other organisms were tested for bioaccumulation of BAS 505 F.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

4.3.1.1 Study 1 – Acute toxicity study in the rainbow trout

Report: CA 8.2.1/1
Anonymous, 1998 a
BAS 505 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a static system (96 hours)
1998/10601

Guidelines: EPA 72-1

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

EXECUTIVE SUMMARY

In a 96-hour flow-through acute toxicity laboratory study, rainbow trouts were exposed to a dilution water control, a solvent control and to dimoxystrobin at nominal concentrations of 0.01, 0.0147, 0.0215, 0.0316, 0.0464, 0.0681 and 0.1 mg a.s./L (corresponding to mean measured concentrations of 0.093, 0.0156, 0.0205, 0.0302, 0.0434, 0.0705 and 0.0898 mg a.s./L) in groups of 10 animals in glass aquaria containing. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

Based on the nominal concentrations the median lethal concentration LC_{50} (96 h) is about 0.0464 mg a.s./L. Mortality occurred first at the concentration of 0.0464 mg a.s./L. Behavioral symptoms such as apathy and tumbling were monitored at the test concentrations 0.0464 mg a.s./L. Other substance related effects included convulsions and narcotic-like state. These were observed in the test concentrations 0.0681 mg a.s./L and 0.1 mg a.s./L before the fish died. The NOEC (96 h) is about 0.0316 mg a.s./L (nominal). Based on the mean values of the analytically detected filtrated concentrations the median lethal concentration LC_{50} (96 h) is about 0.0434 mg a.s./L.

Based on the mean values of the analytically detected filtrated concentrations the median lethal concentration LC_{50} of BAS 505 F on the rainbow trout was 0.0434 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 505 F (Reg. No. 285 028); batch no. N 6 (lot no. 3004); purity: 98.8%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), mean body length 7.64 (6.0 - 9.9) cm; mean body weight 5.30 (2.8 - 10.9) g.

Test design: Static system (96 hours); 10 fish per aquarium (loading about 0.53 g fish/L) and per concentration.

Test concentrations: 0, 0*, 0^+, 0.01^+, 0.01^+, 0.0147, 0.0215, 0.0316, 0.0464, 0.0681 and 0.1 mg a.s./L (nominal).
* = solvent control
+ = added concentration

Test conditions: Temperature: 12 °C – 13 °C; pH 8.2 - 8.6; oxygen content: 9.0 - 11.2 mg/L; total hardness about 2.5 mmol/L; acid capacity about 5.5 mmol/L; photoperiod: 16 hours light : 8 hours dark.

Analytics: HPLC with UV-detection at 230 nm.

Statistics: Descriptive statistics; probit analysis.

II. RESULTS AND DISCUSSION

Analytical measurements: With the exception of the lowest nominal concentration (yielding to higher concentrations) the analytical results varied between 86.9% and 103.2% of the nominal values and confirmed the nominal contents. The analytical results obtained at the end of the test vary between 90.7% and 115.7% of the nominal contents and confirm the nominal concentrations. At the end of the study test compound was found in the control (0.0866 mg/L unfiltrated samples). No test substance was found at test initiation in the control
samples. These analytically detected values were above of the threshold for mortality, which was 0.0464 mg a.s./L (nominal). Since no fish died in the control or showed any toxic symptoms, these high analytically detected values are considered artifacts. The death of 10 fish at the lowest concentration (0.01 mg/L) was obviously not substance-related and was therefore not considered for the results. The biological results are based on the mean value of the analytically detected filtrated concentrations after 1 + 96 hours.

Biological results: Based on the nominal concentrations the median lethal concentration LC_{50} (96 h) is about 0.0464 mg a.s./L. Mortality occurred first at the concentration of 0.0464 mg a.s./L. Behavioral symptoms such as apathy and tumbling were monitored at the test concentrations 0.0464 mg a.s./L. Other substance related effects included convulsions and narcotic-like state. These were observed in the test concentrations 0.0681 mg a.s./L and 0.1 mg a.s./L before the fish died. The NOEC (96 h) is about 0.0316 mg a.s./L (nominal). Based on the mean values of the analytically detected filtrated concentrations the median lethal concentration LC_{50} (96 h) is about 0.0434 mg a.s./L (see Table 4.3-1).

Table 4.3-1: Acute toxicity (96 h) of BAS 505 F on rainbow trout (*Oncorhynchus mykiss*)

Concentration (nominal) [mg a.s./L]	**Control**	**0.01**	**0.0147**	**0.0215**	**0.0316**	**0.0464**	**0.0681**	**0.1**
Concentration (analytic) [mg a.s./L]	**Control**	**0.093**	**0.0156**	**0.0205**	**0.0302**	**0.0434**	**0.0705**	**0.0898**
Mortality [%]	0	0 p)	0	0	0	40	100	100
Symptoms	none	none	none	none	none	A, T	A	K, N
Endpoints [mg a.s./L]								
LC_{50} (1 + 96 h, analytic)	0.0434 (0.0464 nominal)							
NOEC (96 h, analytical)	0.302 (0.0316 nominal)							

Symptoms: A = apathy, K = convulsion, N = narcotic-like state, T = tumbling

p) The death of 10 fish was obviously not substance-related and was therefore not considered for the results.

III. CONCLUSION

Based on the mean values of the analytically detected filtrated concentrations the median lethal concentration LC_{50} of BAS 505 F on the rainbow trout was 0.0434 mg a.s./L.

4.3.1.2 Study 2 – Acute toxicity study in the sheepshead minnow

Report: CA 8.2.1/2
Anonymous, 2000 a
Flow-through acute toxicity of BAS 505 F to the sheepshead minnow, Cyprindodon variegatus
2000/5062

Guidelines: EPA 72-3(a), EPA 850.1075

GLP: yes
(certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

In a 96-hour flow-through acute toxicity laboratory study, juvenile sheepshead minnow were exposed to a dilution water control, a solvent control and to dimoxystrobin at nominal concentrations of 0.065, 0.110, 0.180, 0.300 and 0.500 mg a.s./L (corresponding to mean measured concentrations of 0.0576, 0.113, 0.189, 0.301 and 0.512 mg a.s./L) in groups of 10 animals in glass aquaria containing 15 L water. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control and at the lowest test item concentration of 0.0576 mg a.s./L, whereas 20%, 50%, 100% and 100% mortality was observed at test item concentrations of 0.113, 0.189, 0.301 and 0.512 mg a.s./L. In the solvent control, 5% mortality occurred. No sub-lethal effects were found in the control groups and in all test item treatments after 96 hours.

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of dimoxystrobin was 0.167 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0576 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. no.: 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*), juveniles; mean body length of control fish: 23.7 mm; mean wet weight of control fish: 0.19 g; supplied by "Aquatic BioSystems", Fort Collins, Colorado, USA.

Test design: Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control; 2 replicates per treatment; 10 fish per aquarium (loading 0.13 g fish/L); assessment of mortality and sub-lethal effects directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.065, 0.110, 0.180, 0.300 and 0.500 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of < Limit of Quantification (LOQ), < LOQ, 0.0576, 0.113, 0.189, 0.301 and 0.512 mg a.s./L.

Test conditions: 20 L glass aquaria, test volume: 15 L; dilution water: filtered natural seawater mixed with deionized water, salinity: 16 ‰; flow rate: 6.3 volume additions per 24 hours on average per test vessel; temperature: 21.3°C- 22.8°C; pH 7.9 - 8.1; photoperiod 16 h light : 8 h dark; light intensity: approx. 46 foot candles; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; probit method for calculation of the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of dimoxystrobin concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of dimoxystrobin ranged from 89.7% to 130.6% of nominal at test initiation and from 85.4% to 104.0% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control and at the lowest test item concentration of 0.0576 mg a.s./L, whereas 20%, 50%, 100% and 100% mortality was observed at test item concentrations of 0.113, 0.189, 0.301 and 0.512 mg a.s./L. In the solvent control, 5% mortality occurred. No sub-lethal effects were found in the control groups and in all test item treatments after 96 hours. The results are summarized in Table 4.3-2.

Table 4.3-2: Acute toxicity (96 h) of dimoxystrobin to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.065**	**0.110**	**0.180**	**0.300**	**0.500**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.0576**	**0.113**	**0.189**	**0.301**	**0.512**
Mortality [%] (96 h)	0	5	0	20	50	100	100
Symptoms (after 96 h)	none	none	none	none	none	n.d.	n.d.
Endpoints [mg dimoxystrobin/L] (mean measured)							
LC_{50} (96 h)	0.167 (95% confidence limits: 0.143 - 0.193)						
NOEC (96 h)	0.0576						

n.d. = not determined, all fish dead

III. CONCLUSION

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of dimoxystrobin was 0.167 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0576 mg a.s./L (mean measured).

4.3.1.3 Study 3 – Acute toxicity study in the rainbow trout

Report: CA 8.2.1/3
Anonymous, 2000
Flow-through acute toxicity of BAS 505 F to the rainbow trout, Oncorhynchus mykiss
2000/5125
Guidelines: EPA 850.1075; EPA 72-1
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a static acute toxicity laboratory study, juvenile rainbow trout were exposed to , 0.013, 0.022, 0.036, 0.06 and 0.1 mg dimoxystrobin/L (nominal) in groups of 20 animals in glass aquaria containing 15 L water with 2 replicates per concentration. Fish were observed for survival and symptoms of toxicity within 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations. After 96 hours of exposure no mortality and toxic effects were observed in the control and the solvent control and at concentrations of up to and including 0.022 mg dimoxystrobin/L, whereas 5%, 95% and 100% mortality were observed at the three highest test item concentrations of 0.036, 0.06 and 0.1 mg/L, respectively. Surviving fish showed sub-lethal effects (*e.g.* loss of equilibrium, erratic swimming, lethargy).

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) for dimoxystrobin was determined to be 0.0444 mg/L based on mean measured concentrations. The NOEC (96 h) was 0.0218 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimoxystrobin (BAS 505 F, Reg. No. 285 028), batch no. N 15, purity: 96.0%.

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*), mean body length 29.4 cm; mean body weight 0.19 g; supplied by Thomas Fish Company, Anderson, California, USA.
Test design:	Static system (96 hours); 20 fish per aquarium (loading 0.13 g fish/L), 2 replicates per concentration; assessment of mortality and symptoms of toxicity within 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, solvent control, 0.013, 0.022, 0.036, 0.06 and 0.1 mg dimoxystrobin/L (nominal).
Test conditions:	Glass aquaria (20 L), test volume 15 L, non-chlorinated, filtered tap water; temperature: 11.5 °C - 12.6 °C; pH 7.5 - 7.9; oxygen content: 10.4 mg/L - 11.4 mg/L; total hardness: about 44 mg $CaCO_3$/L; conductivity: 130 µmhos/cm - 150 µmhos/cm; photoperiod 16 h light : 8 h dark; no aeration, no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at test initiation and at the end of the test. Measured concentrations for dimoxystrobin ranged from 94.0% to 105.0% of nominal. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality and toxic effects were observed in the control and the solvent control and at concentrations of up to and including 0.022 mg dimoxystrobin/L, whereas 5%, 95% and 100% mortality were observed at the three highest test item concentrations of 0.036, 0.06 and 0.1 mg/L, respectively. Surviving fish showed sub-lethal effects (*e.g.* loss of equilibrium, erratic swimming, lethargy). The results are summarized in Table 4.3-3.

Table 4.3-3: Acute toxicity (96 h) of dimoxystrobin to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] nominal	**Control**	**Control[1)]**	**0.013**	**0.022**	**0.036**	**0.06**	**0.1**
Concentration [mg/L] analyzed	**Control**	**Control[1)]**	**0.014**	**0.022**	**0.035**	**0.056**	**0.099**
Mortality [%]	0	0	0	0	5	95	100
Symptoms *	none	none	none	none	D, E	D, E, L	n.d.
Endpoints [mg dimoxystrobin/L] (analyzed)							
LC_{50} (96 h)	0.0444 (95% limit: 0.035 - 0.056)						
NOEC (96 h)	0.0218						

* Symptoms: D = loss of equilibrium, E = erratic swimming, L = lethargy
n.d. = not determined; all fish dead
1) = solvent control

III. CONCLUSION

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) for dimoxystrobin was determined to be 0.0444 mg/L based on mean measured concentrations. The NOEC (96 h) was 0.0218 mg/L (mean measured).

4.3.1.4 Study 4 – Acute toxicity study in the bluegill

Report: CA 8.2.1/4
Anonymous, 1998
BAS 505 F - Acute toxicity study on the bluegill (Lepomis macrochirus RAF.) in a static system (96 hours)
1998/10620

Guidelines: EPA 72-1

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a static acute toxicity laboratory study, juvenile bluegills (approx. 11 months old) were exposed to 0.0147, 0.0215, 0.0316, 0.0464, 0.0681 and 0.1 mg dimoxystrobin/L (nominal) in groups of 10 animals in glass aquaria containing 100 L water with 2 replicates per concentration. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.

After 96 hours of exposure no mortality and toxic effects were observed in the control and at concentrations of up to and including 0.0464 mg dimoxystrobin/L, whereas 100% mortality was observed at the 0.0681 mg/L and 80% mortality at the highest concentrations of 0.1 mg/L. At a concentration of 0.1 mg dimoxystrobin/L surviving fish showed sub-lethal effects (*e.g.* apathy).

In a static acute toxicity study with bluegill sunfish the LC_{50} (96 h) for dimoxystrobin was determined to be 0.0512 mg/L based on mean measured values. The NOEC (96 h) was 0.0424 mg/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F, Reg. No. 285 028), batch no. N 6 (Lot - No. 3004), purity: 98.8%.

B. STUDY DESIGN

Test species: Bluegill (*Lepomis macrochirus* Raf.), approx. 11 months old; mean body length 5.33 cm (4.3 - 6.3 cm); mean body weight 1.98 g (1.1 - 3.3 g).; supplied by Osage catfisheries INC., Missouri, USA.

Test design: Static system (96 hours); 10 fish per aquarium (loading 0.2 g fish/L), 2 replicates per concentration; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: 0, 0*, 0+, 0.0147++, 0.0147++, 0.0215, 0.0316, 0.0464, 0.0681 and 0.1 mg dimoxystrobin/L (nominal).
* = solvent control
+ = added solvent control
++ = added concentration

Test conditions: Glass aquaria with stainless steel frames (80 x 35 x 46 cm), test volume 100 L, non-chlorinated, filtered tap water; temperature: 22 °C; pH 7.9 - 8.7; oxygen content: 1.1 mg/L - 8.3 mg/L; total hardness: about 250 mg $CaCO_3$/L; acid capacity: 5.5 mmol/L; photoperiod 16 h light : 8 h dark; no aeration, no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics; probit analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: The results for the samples at test beginning showed recoveries in the range from 90.5% - 204.0% of the nominal value. The results for the 0.1 mg a.s./L, 0.0215 mg a.s./L and the 0.0147 mg a.s./L samples could not be confirmed with subsequent sampling. The very small contents of 0.007 - 0.009 mg a.s./L test substance in the control samples cannot be explained and were not confirmed with the subsequent analyses of samples from identical aquaria. Additional samples were taken 24 hours after start from the aquarium with the nominal concentration 0.0147 mg a.s./L and were analyzed. The analytical values of these samples ranged from 78% to 82% of the nominal value. Furthermore the subsequent samples were analyzed before and after filtration. The samples taken 48 hours after start ranged from 73.3% to 95.3% of the nominal content. The results for the filtrated samples did not vary significantly from those which were not filtered, except the 0.1 mg a.s./L sample. The final samples yielded recoveries in the range from 83.8% - 101.8% of the nominal content. To investigate the fact that all fish died in the aquarium with the smallest concentration 0.0147 mg a.s./L an additional test was started. The results of this test were found to be in the range from 88.4% - 144.2% for the initial samples and from 76.2% - 101.0% for the final samples.

Biological results: After 96 hours of exposure no mortality and toxic effects were observed in the control and at concentrations of up to and including 0.0464 mg dimoxystrobin/L, whereas 100% mortality was observed at the 0.0681 mg/L and 80% mortality at the highest concentrations of 0.1 mg/L. At a concentration of

0.1 mg dimoxystrobin/L surviving fish showed sub-lethal effects (*e.g.* apathy). The results are summarized in Table 4.3-4.

Table 4.3-4: Acute toxicity (96 h) of dimoxystrobin to bluegill sunfish (*Lepomis macrochirus*)

Concentration [mg/L] nominal	**Control**	**Control[1])**	**Control[2])**	**0.0147**	**0.0147[3])**	**0.0215**	**0.0316**	**0.0464**	**0.0681**	**0.1**
Concentration [mg/L] mean measured	**Control**	**Control**	**Control**	**0.0138**	**0.0123**	**0.0267**	**0.0283**	**0.0424**	**0.0618**	**0.134**
Mortality [%]	0	0	0	0	0	0	0	0	100	80
Symptoms *	none	none	none	none	none	none	none	none	n.d.	A
	Endpoints [mg dimoxystrobin/L] (nominal)					**Endpoints [mg dimoxystrobin/L] (mean measured)**				
LC_{50} (96 h)	0.0562 [4)] (> 0.0464 < 0.0681)					0.0512 [4)] (> 0.0424 < 0.0618)				
NOEC (96 h)	0.0464					0.0424				

* Symptoms: A = apathy
n.d. = not determined; all fish dead
1) = solvent control with acetone
2) = added solvent control with acetone
3) = added concentration
4) = geometric mean

III. CONCLUSION

In a static acute toxicity study with bluegill sunfish the LC_{50} (96 h) for dimoxystrobin was determined to be 0.0512 mg/L based on mean measured values. The NOEC (96 h) was 0.0424 mg/L (mean measured).

4.3.1.5 Study 5 – Acute toxicity study in the bluegill sunfish

Report: CA 8.2.1/5
Anonymous, 2000 b
Flow-through acute toxicity of BAS 505 F to the bluegill sunfish, Lepomis macrochirus
2000/5092

Guidelines: EPA 850.1075; EPA 72-1

GLP: yes
(certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

In a static acute toxicity laboratory study, juvenile bluegills were exposed to 0.013, 0.022, 0.036, 0.06 and 0.1 mg dimoxystrobin/L (nominal) in groups of 20 animals in glass aquaria containing 15 L water with 2 replicates per concentration. Fish were observed for survival and symptoms of toxicity at 24, 48, 72 and 96 hours after start of exposure.

No mortality of fish was observed in the control. In the solvent control a survival of 95% occurred. No control or solvent control sublethal effects were noted during the exposure period. Fish exposed to 0.0579 mg dimoxystrobin/L exhibited a loss of equilibrium at 24 and 48 hours, lethargy or a loss of equilibrium at 72 hours and lethargy at 96 hours. Fish exposed to 0.0981 mg a.s./L exhibited a loss of equilibrium and a change of coloration at 24 hours.

In a static acute toxicity study with bluegill sunfish the LC_{50} (96 h) for dimoxystrobin was determined to be 0.052 mg/L based on mean measured values. The NOEC (96 h) was 0.036 mg/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F, Reg. No. 285 028), batch no. N 15, purity: 96.0%.

B. STUDY DESIGN

Test species: Bluegill (*Lepomis macrochirus* Raf.); mean body length 27.6 cm; mean body weight 0.21 g; supplied by Osage catfisheries INC., Missouri, USA.

Test design: Flow-through system (96 hours); 20 fish per aquarium (loading 0.14 g fish/L), 2 replicates per concentration; assessment of mortality and symptoms of toxicity at 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control, solvent control, 0.013, 0.022, 0.036, 0.06 and 0.1 mg dimoxystrobin/L (nominal).

Test conditions: Glass aquaria (20 L), test volume 15 L, non-chlorinated, filtered tap water; temperature: 21.2 °C - 22.8 °C; pH 7.4 - 7.7; oxygen content: 8.6 mg/L - 9.7 mg/L; total hardness: about 44 mg $CaCO_3$/L; conductivity: 130 µmhos/L - 180 µmhos/L; photoperiod 16 h light : 8 h dark; no aeration, no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: The analytically detected concentration of the samples were in the range of 89% - 99% of nominal values and concentrations were stable during the 96 hours testing period. Mean measured concentrations were used for all calculations.

Biological results: No mortality of fish was observed in the control. In the solvent control a survival of 95% occurred. No control or solvent control sublethal effects were noted during the exposure period. Fish exposed to 0.0579 mg dimoxystrobin/L exhibited a loss of equilibrium at 24 and 48 hours, lethargy or a loss of equilibrium at 72 hours and lethargy at 96 hours. Fish exposed to 0.0981 mg a.s./L exhibited a loss of equilibrium and a change of coloration at 24 hours. The results are summarized in Table 4.3-5.

Table 4.3-5: Acute toxicity (96 h) of dimoxystrobin to bluegill (*Lepomis macrochirus*)

Concentration [mg/L] nominal	**Control**	**Control[1)]**	**0.013**	**0.022**	**0.036**	**0.060**	**0.100**
Concentration [mg/L] mean measured	**Control**	**Solvent Control**	**0.012**	**0.021**	**0.036**	**0.058**	**0.098**
Mortality [%]	0	5	0	0	0	70	100
Symptoms *	none	none	none	none	none	D, L	n.d.
Endpoints [mg dimoxystrobin/L] (mean measured)							
LC_{50} (96 h)	0.052 (95% limit: 0.035 - 0.098)						
NOEC (96 h)	0.036						

* Symptoms: D = loss of equilibrium, L = lethargy
n.d. = not determined; all fish dead
1) = solvent control

III. CONCLUSION

In a static acute toxicity study with bluegill sunfish the LC_{50} (96 h) for dimoxystrobin was determined to be 0.052 mg/L based on mean measured values. The NOEC (96 h) was 0.036 mg/L (mean measured).

4.3.2 Short-term toxicity to aquatic invertebrates

4.3.2.1 Study 1 – Acute toxicity tp *Daphnia magna*

Report: CA 8.2.4.1/1
Dohmen P., 1999 a
Effect of BAS 505 F on the immobility of Daphnia magna STRAUS in a 48 hour static, acute toxicity test
1999/11525

Guidelines: OECD 202

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

EXECUTIVE SUMMARY

In a 48-hour static acute toxicity laboratory study, the effect of BAS 505 F on water flea neonates was investigated. Neonates less than 24 hours old were exposed to nominal concentrations of 2, 4, 8, 16, 32 and 64 µg a.s./L (nominal). Additionally, a dilution water control was set up. Daphnids were exposed in 4 replicates per concentration, containing 5 daphnids each. The daphnids were observed for immobility 24 and 48 hours after start of exposure.

The following biological results are based on nominal concentrations. Significant immobility of the daphnids was only observed at the two highest concentrations with 20% immobility at 32 µg a.s./L and complete immobility at 64 µg a.s./L after 48 hours; at 16 µg a.s./L one immobile daphnid was observed.

In a 48 hours static acute toxicity study with *Daphnia magna* the EC_{50} of BAS 505 F was determined to be 39.4 µg a.s./L, the NOEC was 8 µg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 505 F (Reg. No. 285 028); batch no. N 6; purity: 97.4%.

B. STUDY DESIGN

Test species: Waterflea (Daphnia magna STRAUS), neonates less than 24 hours old at test initiation; culture conditions in accordance with test conditions.

Test design: Static test (48 hours), 6 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility (and other effects) after 24 and 48 hours.

Test concentrations: Control, 2, 4, 8, 16, 32 and 64 µg a.s./L (nominal).

Test conditions: Dilution water "M4"; pH 8.03 - 8.06; oxygen content 8.5 - 8.7 mg/L; total hardness 2.46 mmol/L; conductivity 663 ~S/cm; glass vessels, test volume 50 mL, no feeding, no ventilation; temperature 20.7 °C - 25.8 °C; photo-period 16 hours light : 8 hours dark; light intensity < 1500 lux.

Analytics: Analytical verification of test substance concentrations was conducted using RP-HPLC with UV-detection.

Statistics: Standard procedures, log-log analysis.

II. RESULTS AND DISCUSSION

Analytical measurements: The measured values ranged from 106% to 118.6% of nominal at the beginning of the test and from 88.6% to 117.0% at the end of the test, confirming the nominal data. Therefore the following biological results are based on nominal concentrations.

Biological results: Significant immobility of the daphnids was only observed at the two highest concentrations with 20% immobility at 32 µg a.s./L and complete immobility at 64 µg a.s./L after 48 hours; at 16 µg a.s./L one immobile daphnid was observed (see Table 4.3-6).

Table 4.3-6: Effect of BAS 505 F on *Daphnia magna* immobility

Concentration (nominal) [µg a.s./L]	**Control**	**2**	**4**	**8**	**16**	**32**	**64**
Immobile (24 hours) [%]	0	0	0	0	5	10	30
Immobile (48 hours) [%]	0	0	0	0	5	20	100
Endpoints [µg a.s./L]							
EC_{50} (48 h)	39.4 (95% limits: 33.4 - 46.6)						
NOEC (48 h)	8.0						

III. CONCLUSION

In a 48 hours static acute toxicity study with *Daphnia magna* the EC_{50} of BAS 505 F was determined to be 39.4 µg a.s./L, the NOEC was 8 µg a.s./L (nominal).

4.3.2.2 Study 2 – Acute toxicity to *Americamysis bahia*

Report:	CA 8.2.4.2/1 Wyskiel D.C. et al., 2000 b Flow-through acute toxicity of BAS 505 F to the mysid, Americamysis bahia 2000/5124
Guidelines:	EPA 72-3(b), EPA 850.1035
GLP:	yes (certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

In a 96-hour flow-through acute toxicity laboratory study, saltwater mysids were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0065, 0.011, 0.018, 0.030 and 0.050 mg dimoxystrobin/L (nominal) (corresponding to mean measured concentrations of 0.00767, 0.0111, 0.018, 0.0299 and 0.0497 mg a.s./L) in two replicates per treatment containing 10 mysids each. Saltwater mysids were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item (measured over the 96 h study period). After 48 hours of exposure, no mortality were observed in the control, the solvent control and at concentrations of up to and including 0.018 mg/L, whereas 10% and 70% mortality was observed at the two highest test item concentrations of 0.0229 and 0.0497 mg a.s./L, respectively. After 48 hours, sub-lethal effects, observed as lethargy and/or erratic swimming, were noted at the two highest tested concentrations of dimoxystrobin.

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for dimoxystrobin was determined to be 0.0429 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. No. 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Americamysis bahia*), juveniles, age: less than 24 hours old; average wet weight of control mysids: 0.26 mg; source: in-house cultures.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates per treatment; 10 mysids per replicate (loading 0.00017 g mysid/L); assessment of mortality and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50} (48 h), mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.0065, 0.011, 0.018, 0.030 and 0.050 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of < Limit of Quantification (LOQ), < LOQ, 0.00767, 0.0111, 0.018, 0.0299 and 0.0497 mg dimoxystrobin/L.

Test conditions: Glass aquaria (20 L), test volume 15 L; test chambers: glass cylinders (8 cm in height and 8 cm in diameter) with mesh screen attached to the bottom; dilution water: filtered, sterilized and aerated natural seawater mixed with deionized water; flow rate: 6.6 volume additions per 24 hours on average; salinity: 16 - 17‰; temperature: 21.2°C - 22.8°C; pH 7.6 - 8.0; oxygen content: 6.4 - 8.6 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: 51 foot-candles; feeding: juvenile mysids were fed daily with live *Artemia salina* nauplii; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; probit method for calculation of the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test (96 h). The analytically determined concentrations of dimoxystrobin ranged from 96.7% to 100% of nominal concentrations at test initiation and from 97.3% to 114.9% of nominal at test termination. The following biological results are based on mean measured concentrations (measured over the 96 h study period).

Biological results: After 48 hours of exposure no mortality were observed in the control, the solvent control and in concentrations of up to and including 0.018 mg/L, whereas 10% and 70% mortality was observed at the two highest test item concentrations of 0.0229 and 0.0497 mg a.s./L, respectively. After 48 hours, sub-lethal effects, observed as lethargy and/or erratic swimming were noted at the two highest tested concentrations of dimoxystrobin. The results are summarized in Table 4.3-7

Table 4.3-7: Acute toxicity (48 h) of dimoxystrobin to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0065**	**0.011**	**0.018**	**0.030**	**0.050**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.00767**	**0.0111**	**0.018**	**0.0299**	**0.0497**
Mortality (48 h) [%]	0	0	0	0	0	10	70
Symptoms after 48 h *	none	none	none	none	none	A*	A*
Endpoints [mg dimoxystrobin/L] (mean measured)							
LC_{50} (48 h)	0.0429 (95% confidence limits: 0.0375 - > 0.0497)						

* Symptoms after 48 h: A = lethargy and/or erratic swimming

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for dimoxystrobin was determined to be 0.0429 mg a.s./L, based on mean measured concentrations.

4.3.2.3 Study 3 – Acute toxicity to *Crassostrea virginica*

Report: CA 8.2.4.2/2
Wyskiel D.C. et al., 2000 c
Flow-through mollusc shell depostion test with BAS 505 F
2000/5096
Guidelines: EPA 72-3(c), EPA 850.1025
GLP: yes
(certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

In a 96-hour acute toxicity laboratory study the effect of dimoxystrobin on shell deposition of eastern oysters was investigated under flow-through conditions. The eastern oysters were exposed to a dilution water control, a solvent control and to dimoxystrobin at nominal concentrations of 0.0033, 0.0055, 0.0090, 0.015 and 0.025 mg a.s./L (corresponding to mean measured concentrations of 0.00301, 0.00514, 0.00814, 0.0141 and 0.0237 mg a.s./L) in groups of 10 oysters per replicate with two replicates per treatment. Eastern oysters were observed for survival and symptoms of toxicity daily during the exposure period. Measurements of shell deposition for each oyster were made after 96 hours.

The biological results are based on mean measured concentrations of the test item. After 96 hours no mortality occurred at test item concentrations of up to and including 0.00514 mg a.s./L, whereas 5%, 10% and 30% mortality was observed at 0.00814, 0.0141 and 0.0237 mg a.s./L, respectively. After 96 hours, sub-lethal effects, observed as oysters exhibited a delayed reaction to gentle prodding (slowly closing shells) at the two highest concentrations of dimoxystrobin. Statistically significant inhibition of shell growth compared to the pooled control / solvent control was observed at the four highest tested concentrations

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for dimoxystrobin was 0.00842 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.00301 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. No. 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species: Eastern oyster (*Crassostrea virginica*), juveniles, height: 32 - 50 mm; source: "P. Cummins Oyster Company", Baltimore, Maryland, USA.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates for each test item concentration and the controls with 10 oysters per replicate (20 animals per treatment); initial and daily assessment of mortality and symptoms of toxicity; measurements of shell deposition 96 hours after start of exposure.

Endpoints:	EC_{50} and NOEC (96 h) for shell growth inhibition, mortality and symptoms of toxicity.
Test concentrations:	Control (dilution water: unfiltered seawater), solvent control (0.1 mL dimethylformamide/L); 0.0033, 0.0055, 0.0090, 0.015 and 0.025 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of < Limit of Quantification (LOQ), < LOQ, 0.00301, 0.00514, 0.00814, 0.0141 and 0.0237 mg a.s./L.
Test conditions:	20 L glass aquaria, test volume 15 L, natural unfiltered seawater, flow rate: average of 8.5 volume additions per 24 hours in each test vessel, 0.53 L per oyster per hour; salinity: 34‰ - 35‰; temperature: 20.0°C - 20.9°C; pH 7.8 - 8.0; oxygen content: 5.7 mg/L - 7.7 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: 54 foot candles; no aeration; live marine phytoplankton as supplement to existing food in unfiltered seawater used as dilution water.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.
Statistics:	Descriptive statistics; t-test ($\alpha = 0.05$) for comparison of shell deposition data in the control groups; standard statistical techniques for calculation of EC_{50}, ANOVA followed by Bonferroni's test ($\alpha = 0.05$) for shell deposition data of the test item treatments.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and at test termination. Mean measured concentrations for dimoxystrobin ranged from 88.9% to 96.0% of nominal concentrations at test initiation and from 89.1% to 96.0% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours no mortality occurred at test item concentrations of up to and including 0.00514 mg dimoxystrobin/L, whereas 5%, 10% and 30% mortality was observed at 0.00814, 0.0141 and 0.0237 mg a.s./L, respectively. After 96 hours, sub-lethal effects, observed as oysters exhibited a delayed reaction to gentle prodding (slowly closing shells) at the two highest concentrations of dimoxystrobin. Control and solvent control oysters deposited an average of 2.3 and 2.5 mm of new shell during the test, respectively. No statistically significant difference in shell deposition was observed between the control groups (t-test, $\alpha = 0.05$). Subsequent statistical analyses were performed by comparing the pooled control and solvent control data to the treatment data. Statistically significant inhibition of shell growth compared to the pooled control / solvent control was observed at the four highest tested concentrations (Bonferroni's test). The results are summarized in Table 4.3-8.

Table 4.3-8: Acute toxicity (96 h) of dimoxystrobin to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0033**	**0.0055**	**0.0090**	**0.015**	**0.025**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.00301**	**0.00514**	**0.00814**	**0.0141**	**0.0237**
Mortality after 96 h [%]	0	0	0	0	5	10	30
Shell growth after 96 h [% of control]	--	109	78	70*	57*	35*	9*
Symptoms (after 96 h) #	none	none	none	none	none	A	A
Endpoints [mg dimoxystrobin/L] (mean measured)							
EC_{50} (96 h)	0.00842 (95% confidence limits: 0.00654 - 0.0108)						
NOEC $_{\text{shell deposition}}$ (96 h)	0.00301						

Symptoms after 96 h: A = Affected oysters exhibited a delayed reaction to gentle prodding (slowly closing shells).
* Statistically significant difference compared to the pooled control / solvent control (Bonferroni's test, $\alpha = 0.05$).

III. CONCLUSION

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for dimoxystrobin was 0.00842 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.00301 mg a.s./L (mean measured).

4.3.2.4 Study 4 – Acute toxicity tp *Asellus aquaticus*

Report: CA 8.2.4.2/1
Janson G.-M.,Dohmen G.P., 2008a
Acute toxicity of BAS 505 F (Reg.No. 285028) to Asellus aquaticus in a 96 hour static test
2008/1055034

Guidelines: OECD 202

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

EXECUTIVE SUMMARY

The acute toxicity of dimoxystrobin to *Asellus aquaticus* was investigated in a 96 h static toxicity study. 10 replicates per treatment group were treated with nominal test concentrations of 0.030, 0.060, 0.120, 0.240 and 0.480 mg dimoxystrobin/L, plus a control and a solvent control. Animals were observed for mortality and other symptoms 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations. After 48 h of exposure, no mortality of the test organisms were observed in the controls and at up to and including the test item concentration of 0.120 mg a.s./L. At the two highest tested concentrations of 0.240 and 0.480 mg a.s./L, 30% and 50% mortality occurred, respectively. The effects in all test item treatments were not statistically significantly different compared to the controls. No sub-lethal impact of the test substance was observed.

In a 96 hours toxicity test with *Asellus aquaticus* the LC_{50} (48 h) of dimoxystrobin was determined to be 0.437 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F, Reg. No. 285028), batch FF 18850, purity 99.9%.

B. STUDY DESIGN

Test species: Freshwater isopod (*Asellus aquaticus*), the animals were collected from an uncontaminated pond (distant from industrial and agricultural sides) and maintained in-house (non-GLP) for an acclimation period of at least 19 days. The animals selected for the study were juvenile with a body length of approximately 4-7 mm.

Test design: Static system, 5 concentrations plus a control and a solvent control, 10 replicates for each treatment and controls, one animal/replicate; assessment of mortality and other symptoms 24, 48, 72 and 96 hours after start of exposure.

Endpoints: NOEC and LC_{50} (48 h), based on mortality and observations of sub-lethal symptoms.

Test concentrations: Control, solvent control, 0.030, 0.060, 0.120, 0.240 and 0.480 mg dimoxystrobin/L (nominal).

Test conditions: Glass vessels, test volume 50 mL, dilution water "M4" (Elendt medium), pH 7.94 - 8.05, oxygen content 8.6 – 9.0 mg/L, total hardness 2.28 mmol/L, conductivity 624 µS/cm (total hardness and conductivity at test initiation), temperature 20 - 21°C, light intensity 274 - 489 lux, 16 hours light : 8 hours dark, no feeding, no aeration.

Analytics: The test item concentrations were analyzed using HPLC with MS-detection and external calibration.

Statistics: Fisher's Exact Test ($p < 0.05$) for determination of NOEC, calculation of LC_{50} by probit analysis using "ToxRatPro Version 2.09".

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of dimoxystrobin was carried out in each test concentration at the beginning and at the end of the test. Measured values ranged from 98.8% to 104.9% of the nominal concentration at test initiation and from 112.0% to 115.8% at test termination. The following biological results are based on nominal test item concentrations.

Biological results: After 48 h of exposure, no mortality of the test organisms were observed in the controls and at up to and including the test item concentration of 0.120 mg a.s./L. At the two highest tested concentrations of 0.240 and 0.480 mg a.s./L, 30% and 50% mortality occurred, respectively. The measured effects were not statistically significantly different compared to the controls (Fisher`s Exact test, $p < 0.05$). No sub-lethal impact of the test substance was observed. The results are summarized in Table 4.3-9.

Table 4.3-9: Acute toxicity (48 h) of dimoxystrobin to *Asellus aquaticus*

Concentration [mg a.s./L] nominal	**Control**	**Solvent control**	**0.030**	**0.060**	**0.120**	**0.240**	**0.480**
Mortality (48 h) [%]	0	0	0	0	0	30	50
Endpoints [mg a.s./L] (nominal)							
LC_{50} (48 h)	0.437						

III. CONCLUSION

In a 96 hours toxicity test with *Asellus aquaticus* the LC_{50} (48 h) of dimoxystrobin was determined to be 0.437 mg a.s./L, based on nominal concentrations.

4.3.3 Algal growth inhibition tests

4.3.3.1 Study 1 – Growth inhibition with *Navicula pelliculosa*

Report: CA 8.2.6.2/1
Wyskiel D.C. et al., 2000 d
Growth and reproduction toxicity test with BAS 505 F and the freshwater alga, Navicula pelliculosa
2000/5128

Guidelines: EPA 123-2, EPA 850.5400

GLP: yes
(certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

In a 120-hour static toxicity laboratory study, the effect of dimoxystrobin on the growth of the freshwater diatom *Navicula pelliculosa* was investigated. The following nominal concentrations were applied: 0.00086, 0.0017, 0.0034, 0.0065 and 0.013 mg dimoxystrobin/L (corresponding to mean measured concentrations of 0.00122, 0.00177, 0.00359, 0.00607 and 0.0138 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24, 48, 72, 96 and 120 h after test initiation.

The biological results are based on mean measured concentrations of the test item. Statistically significant differences compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours.

In a 120 hour algae toxicity test with *Navicula pelliculosa,* the 120 h E_rC_{50} and E_bC_{50} values for dimoxystrobin were determined to be > 0.0138 mg a.s./L and 0.00264 mg a.s./L, respectively, based on mean measured concentrations. After 72 hours of exposure, the E_rC_{50} and E_bC_{50} values were determined to be 0.0078 mg a.s./L and 0.0025 mg a.s./L, respectively (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. No. 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species: Freshwater diatom, *Navicula pelliculosa*, strain UTEX 664, stock originally obtained from the "Culture Collection of Algae", University of Texas, Austin, USA.

Test design: Static system; test duration 120 hours; 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.

Endpoints: EC_{50} with respect to biomass and growth rate after exposure over 72 and 120 hours.

Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.00086, 0.0017, 0.0034, 0.0065 and 0.013 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of < Limit of Quantification (LOQ), < LOQ, 0.00122, 0.00177, 0.00359, 0.00607 and 0.0138 mg a.s./L.
Test conditions:	250 mL glass flasks; test volume 50 mL; sterile enriched medium supplemented with 0.2 g/L Na_2SiO_3 x 9 H_2O; pH 7.4 - 7.5 at test initiation and pH 7.6 - 8.7 at test termination; temperature: 23.2°C - 23.6°C; initial cell densities 3 x 10^3 cells/mL; continuous light at 4200 - 4300 lux; constant shaking at 100 rpm.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics; t-test ($\alpha = 0.05$) for comparison of cell densities and growth rate data in the control and the solvent control; weighted least squares non-linear regression analysis for determination of EC_x values. ANOVA followed by Bonferroni's test ($\alpha = 0.05$) for determination of the NOEC (120 h) values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of dimoxystrobin ranged from 85.1% to 112.8% of nominal concentrations at test initiation and from 101.2% to 173.3% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data after 120 hours (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Statistically significant differences of the cell numbers and average specific growth rates compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours (Bonferroni's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 4.3-10.

Table 4.3-10: Effect (72 h and 120 h) of dimoxystrobin on the growth of the freshwater diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Water control**	**Solvent control**	**0.00086**	**0.0017**	**0.0034**	**0.0065**	**0.013**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.00122**	**0.00177**	**0.00359**	**0.00607**	**0.0138**
Mean cell density (72 h) [% of water control] #	--	100	68	66	40	< 23	< 19
Growth rate (72 h) [% of water control] #	--	100	88	85	68	48	43
Mean cell density (120 h) [% of water control]	--	115	96	57	41	27	6
Growth rate (120 h) [% of water control]	--	102	100	91 *	87 *	80 *	57 *
Endpoints [mg dimoxystrobin/L] (mean measured)							
E_rC_{50} (72 h)	0.0078 (95% confidence limits: 0.00617 - 0.00986)						
E_bC_{50} (72 h)	0.0025 (95% confidence limits: 0.00182 - 0.00343)						
E_rC_{50} (120 h)	> 0.0138						
E_bC_{50} (120 h)	0.00264 (95% confidence limits: 0.00205 - 0.00339)						

* Statistically significantly different compared to the pooled control (ANOVA followed by Bonferroni's test, $\alpha = 0.05$).
\# Statistically significant differences were only determined after 120 h of exposure.

III. CONCLUSION

In a 120 hour algae toxicity test with *Navicula pelliculosa,* the 120 h E_rC_{50} and E_bC_{50} values for dimoxystrobin were determined to be > 0.0138 mg a.s./L and 0.00264 mg a.s./L, respectively, based on mean measured concentrations. After 72 hours of exposure, the E_rC_{50} and E_bC_{50} values were determined to be 0.0078 mg a.s./L and 0.0025 mg a.s./L, respectively (mean measured).

4.3.3.2 Study 2 – Growth inhibition test with *Anabaena flos-aquae*

Report: CA 8.2.6.2/2
Wyskiel D.C. et al., 2000 e
Growth and reproduction toxicity test with BAS 505 F and the freshwater alga, Anabaena flos-aquae
2000/5129
Guidelines: EPA 123-2, EPA 850.5400
GLP: yes
(certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

In a 120-hour static toxicity laboratory study, the effect of dimoxystrobin on growth of the blue-green alga *Anabaena flos-aquae* was investigated. The following nominal concentrations were applied: 0.13, 0.25, 0.50, 1.0 and 2.0 mg dimoxystrobin/L (corresponding to mean measured concentrations of 0.133, 0.257, 0.524, 1.07 and 2.06 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation.

The biological results are based on mean measured concentrations of the test item. Statistically significant differences compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours.

In a 120-hour algae toxicity test with *Anabaena flos-aquae*, the 72 h and 120 h E_rC_{50} values for dimoxystrobin was determined to be both > 2.06 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 0.960 and 1.13 mg a.s./L, respectively (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. No. 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species: Freshwater blue-green alga, *Anabaena flos-aquae* (UTEX 1444); stock originally obtained from the Culture Collection of Algae, University of Texas, Austin, USA; stock was maintained at test conditions for more than 14 days before the test; inocula 6 day old culture.

Test design: Static system (120 hours); 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.

Endpoints: EC_{50} with respect to biomass development and growth rate after exposure over 72 and 120 hours.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.13, 0.25, 0.50, 1.0 and 2.0 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of 0.133, 0.257, 0.524, 1.07 and 2.06 mg a.s./L.

Test conditions: 250 mL glass flasks; test volume: 50 mL; sterile enriched medium; pH 7.4 at test initiation and pH 7.5 - 7.8 at test termination; temperature: 24.5°C - 25.2°C; initial cell densities: 3 x 10^3 cells/mL; continuous light; light intensity: 2000 - 2200 lux, continuous shaking at 100 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics, weighted least squares non-linear regression for EC_{50} calculation; t-test ($\alpha = 0.05$) for comparison of cell densities and growth rate in the control and solvent control; ANOVA followed by Bonferroni's test ($\alpha = 0.05$) for determination of the NOEC (120 h) values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured concentrations of dimoxystrobin ranged from 102.3 to 107.0% of nominal concentrations at test initiation and from 101.6 to 106.0% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Statistically significant differences of the cell numbers and average specific growth rates compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours (Bonferroni's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 4.3-11.

Table 4.3-11: Effect (72 h and 120 h) of dimoxystrobin on the growth of the blue-green alga *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Water control**	**Solvent control**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.133**	**0.257**	**0.524**	**1.07**	**2.06**
Mean cell density (72 h) [% of water control] #	--	90	80	109	88	26	22
Growth rate (72 h) [% of water control] #	--	98	95	104	98	68	65
Mean cell density (120 h) [% of water control]	--	100	103	65*	64 *	48 *	44 *
Growth rate (120 h) [% of water control]	--	100	102	94 *	94 *	88 *	88 *
Endpoints [mg dimoxystrobin/L] (mean measured)							
E_rC_{50} (72 h)	> 2.06						
E_bC_{50} (72 h)	0.960 (95% confidence limits: 0.665 - 1.39)						
E_rC_{50} (120 h)	> 2.06						
E_bC_{50} (120 h)	1.13 (95% confidence limits: 0.741 - 1.80)						

* Statistically significantly different compared to the pooled control (ANOVA followed by Bonferroni's test, $\alpha = 0.05$).
\# Statistically significant differences were only determined after 120 h of exposure.

III. CONCLUSION

In a 120-hour algae toxicity test with *Anabaena flos-aquae*, the 72 h and 120 h E_rC_{50} values for dimoxystrobin was determined to be both > 2.06 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 0.960 and 1.13 mg a.s./L, respectively (mean measured).

4.3.3.3 Study 3 – Growth inhibition test with *Skeletonema costatum*

Report:	CA 8.2.6.2/3 Wiskiel D.C. et al., 2000 a Growth and reproduction toxicity test with BAS 505 F and the marine alga, Skeletonema costatum 2000/5127
Guidelines:	EPA 850.5400, FIFRA Subdivision J Series 123-2
GLP:	yes (certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

In a 120-h static toxicity laboratory study, the effect of dimoxystrobin on the growth of the marine diatom *Skeletonema costatum* was investigated. The following nominal concentrations were applied: 0.33, 0.66, 1.3, 2.5 and 5.0 mg dimoxystrobin/L, corresponding to mean measured concentrations of 0.330, 0.657, 1.29, 2.32 and 4.31 mg a.s./L. Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted directly after start of exposure and after 24, 48, 72, 96 and 120 h after test initiation.

The biological results are based on mean measured concentrations of the test item. After 120 hours of exposure statistically significant differences compared to the pooled control were observed for growth rate at the highest test item concentration.

In a 120 h algae test with *Skeletonema costatum*, the 72 h and 120 h E_rC_{50} were determined to be both > 4.31 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 1.28 mg a.s./L and > 4.31 mg a.s./L, respectively (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. no.: 285028), batch no. N15; purity: 96.0%.

B. STUDY DESIGN

Test species: Marine diatom, *Skeletonema costatum*, strain UTEX LB 2308, in-house culture; stock originally obtained from the "Culture Collection of Algae", University of Texas, Austin, USA.

Test design: Static system (120 hours); 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.

Endpoints: EC_{50} with respect to cell density and growth rate after exposure over 72 and 120 hours.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.33, 0.66, 1.3, 2.5 and 5.0 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of 0.330, 0.657, 1.29, 2.32 and 4.31 mg a.s./L.

Test conditions: 250 mL Erlenmeyer flasks; test volume: 50 mL; enriched marine media; pH 8.0 at test initiation and pH 8.6 - 9.4 at test termination; temperature: 19.4°C - 19.5°C; initial cell densities: 1 x 10^4 cells/mL; photoperiod: 16 hours light : 8 hours dark, light intensity: 4100 - 4300 lux, continuous shaking at 100 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC method with UV detection.

Statistics: Descriptive statistics, t-test ($\alpha = 0.05$) for comparison of cell densities and growth rate data in the control and the solvent control; weighted least squares non-linear regression estimation procedure for determination of EC_{50} values, ANOVA followed by Bonferroni's test ($\alpha = 0.05$) for determination of the NOEC (120 h) values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured concentrations of dimoxystrobin at test initiation ranged from 101.2% to 105.8% of nominal concentrations. At test termination measured concentrations of dimoxystrobin were between 71.0% and 94.5% of nominal. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects after 120 h of exposure. After 120 hours of exposure statistically significant differences compared to the pooled control were observed for growth rate at the highest test item concentration (ANOVA followed by Bonferroni's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 4.3-12.

Table 4.3-12: Effect (72 h and 120 h) of dimoxystrobin on the growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**Water Control**	**Solvent control**	**0.33**	**0.66**	**1.3**	**2.5**	**5.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.330**	**0.657**	**1.29**	**2.32**	**4.31**
Mean cell density (72 h) [% of control] #	--	75	50	65	55	22	19
Growth rate (72 h) [% of control] #	--	93	81	89	83	61	57
Mean cell density (120 h) [% of control]	--	92	90	80	78	73	59
Growth rate (120 h) [% of control]	--	98	98	96	96	94	90 *
Endpoints [mg dimoxystrobin/L] (mean measured)							
E_rC_{50} (72 h)	> 4.31						
E_bC_{50} (72 h)	1.28 (95% confidence limits: 0.551 - 2.96)						
E_rC_{50} & E_bC_{50} (120 h)	> 4.31						

* Statistically significantly different compared to the pooled control (ANOVA followed by Bonferroni's test, $\alpha = 0.05$).
\# Statistically significant differences were only determined after 120 h of exposure.

III. CONCLUSION

In a 120 h algae test with *Skeletonema costatum*, the 72 h and 120 h E_rC_{50} were determined to be both > 4.31 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 1.28 mg a.s./L and > 4.31 mg a.s./L, respectively (mean measured).

4.3.3.4 Study 4 – Growth inhibition test with *Pseudokirchneriella subcapitata*

Report: II A 8.2.6 / 1
Kubitza J. 1999
Effect of BAS 505 F on the growth of the green alga Pseudokirchneriella subcapitata
1999/11481
Guidelines: OECD 201
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

EXECUTIVE SUMMARY

In a 96-h static toxicity laboratory study, the effect of dimoxystrobin on the growth of the unicellular fresh water green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 4, 8, 16, 32, 64, 128, 256 µg a.s./L. Additionally, a dilution water control was set up. Assessment of growth was conducted 48, 72 and 96 h after test initiation.

The results are based on nominal concentrations. No morphological effects were observed at concentration up to 16 µg a.s./L. At the concentrations 32 µg a.s./L to 256 µg a.s./L a few cells exhibited morphological changes (the algal cells appeared rounder than those in the control). A test prolongation demonstrated quick recovery showing that the effects are algistatic rather than algicidal.

In a 96-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} of BAS 505 F was determined to be 152.6 µg a.s./L, the E_rC_{10} was 13.3 µg a.s./L. Effects can be rated algistatic rather than algicidal.

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 505 F (Reg. No. 285 028); batch no. N 6; purity: 97.4%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (syn.: *Selenastrum capricornutum)*, SAG 61.81; stock obtained from „Sammlung Algenkulturen“, Göttingen (FRG), cultures maintained in-house.

Test design: Static system; test duration 96 hours; 7 test concentrations, each with 5 replicates plus a solvent control with 10 replicates and a standard control with 5 replicates; daily assessments of growth beginning 48 hours after initiation.

Test concentrations: Control, 4, 8, 16, 32, 64, 128, 256 µg a.s./L (nominal).

Test conditions: OECD 201 nutrient solution, pH 8.0 at test initiation; glass Erlenmeyer dimple flasks; continuous shaking; initial cell densities 3 x 10^3 cells/mL; temperature 21 °C - 23 °C; continuous light (white fluorescent lamps), light intensity about 8000 lux.

Analytics: Analytical verification of the test concentrations were conducted using HPLC and UV-detection.

Statistics: Standard procedures, probit analysis, maximum-likelihood-method.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test substance was carried out in each test concentration at the beginning and at the end of the test after 96 hours. Average measured values ranged from 93.4% to 102.5% of nominal at the beginning and from 96.2% to 112.5% at the end of the test. Therefore the following results are based on nominal concentrations.

Biological results: No morphological effects were observed at concentration up to 16 µg a.s./L. At the concentrations 32 µg a.s./L to 256 µg a.s./L a few cells exhibited morphological changes (the algal cells appeared rounder than those in the control). The relative growth inhibition values at different concentrations of BAS 505 F are depicted below [see Table 4.3-13]. A test prolongation demonstrated quick recovery showing that the effects are algistatic rather than algicidal.

Table 4.3-13: Effect of BAS 505 F on the growth of *Pseudokirchneriella subcapitata*

Concentration [µg a.s./L]	Control	4	8	16	32	64	128	256
Inhibition (biomass) [%]	0	7.2	23.1	55.1	75.8	89.9	93.1	95.3
Inhibition (growth rate) [%]	0	0.9	3.6	13.2	23.6	40.1	47.5	52.9
	Endpoints [µg a.s./L]							
E_rC_{50} (96 h)	152.6 (95% limits: 139.9 – 166.5)							

E_rC_{10} (96 h)	13.3	(95% limits: 12.0 – 14.7)
E_bC_{50} (96 h)	17.0	(95% limits: 16.2 - 17.8)
E_bC_{10} (96 h)	3.5	(95% limits: 3.3 - 3.9)

III. CONCLUSION

In a 96-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} of BAS 505 F was determined to be 152.6 µg a.s./L, the E_rC_{10} was 13.3 µg a.s./L. Effects can be rated algistatic rather than algicidal.

4.3.4 *Lemna* sp. growth inhibition test

4.3.4.1 Study 1 – Growth inhibition test with *Lemna gibba*

Report:	CA 8.2.7/1 Wyskiel D.C. et al., 2000 f Growth and reproduction toxicity test with BAS 505 F and the duckweed, Lemna gibba G3 2000/5099
Guidelines:	EPA 123-2, EPA 850.4400
GLP:	yes (certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

In a 14-day static toxicity laboratory study, the effect of dimoxystrobin on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0.033, 0.065, 0.13, 0.25 and 0.50 mg dimoxystrobin/L (corresponding to initial measured concentrations of 0.0335, 0.0634, 0.132, 0.247 and 0.444 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of plant growth and other effects was conducted 2, 5, 7, 9, 12 and 14 days after test initiation. Percent growth inhibition relative to the control was calculated for each test concentration based upon biomass for the parameter frond number. Dry weight was determined at test termination.

The biological results are based on initial measured concentrations of the test item. The duckweed population in the control vessels showed sufficient growth. At the end of the test, chlorotic fronds were observed in the control, the solvent control and at the test item concentrations of 0.0335, 0.0634 and 0.444 mg a.s./L. Statistically significant effects on the number of non-chlorotic fronds and plant dry weight compared to the pooled control were observed at the four highest tested concentrations and at the three highest tested concentrations, respectively.

In a 14-day aquatic-plant test with *Lemna gibba,* the E_bC_{50} values of dimoxystrobin were determined to be 0.149 mg a.s./L based on frond number and 0.226 mg a.s./L based on dry weight (initial measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. no.: 285028), batch no. N15; purity: 96.0%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3); inoculum: 12 days old; cultures maintained in-house; stock obtained from "Climate Stress Laboratory", USDA, Beltsville, Maryland, USA.

Test design: Static system; test duration 14 days; 5 test item concentrations plus a control and a solvent control, 3 replicates for each test item concentration, the control and the solvent control; 4 plants with 3 fronds, total number of fronds at test initiation: 12 per replicate; assessment of growth and other effects on days 2, 5, 7, 9, 12 and 14; determination of dry weight at test termination.

Endpoints: EC_{50} with respect to biomass development after exposure over 14 days.

Test concentrations: Control, solvent control (0.1 mL dimethylformamide/L), 0.033, 0.065, 0.13, 0.25 and 0.50 mg dimoxystrobin/L (nominal), corresponding to initial measured concentrations of < limit of quantitation (LoQ), < LoQ, 0.0335, 0.0634, 0.132, 0.247 and 0.444 mg a.s./L.

Test conditions: 500 mL Erlenmeyer glass flasks, test volume: 200 mL, M-Hoagland's media without sucrose or EDTA, pH 5.0 at test initiation and pH 5.5 - 6.0 at test termination; temperature: 23.9°C - 24.4°C, continuous light, light intensity: about 510 - 520 foot-candles.

Analytics: Analytical verification of the test item was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics, t-test ($\alpha = 0.05$) for comparison of control and solvent control data, weighted least squares non-linear regression for determination of EC_x values based on frond no. and dry weight, ANOVA followed by Bonferroni's test for determination of the NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of dimoxystrobin ranged from 88.8% to 101.5% of nominal at test initiation and from 59.4% to 98.4% of nominal at test termination. The following biological results are based on initial measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. The duckweed population in the control vessels showed sufficient growth, increasing from an average of 12 fronds per vessel to an average of 250 fronds per vessel, corresponding to a 21 x multiplication. At the end of the test, chlorotic fronds were observed in the control, the solvent control and at the test item concentrations of 0.0335, 0.0634 and 0.444 mg a.s./L.

Statistically significant effects on the number of non-chlorotic fronds and plant dry weight compared to the pooled control were observed at the four highest tested concentrations and at the three highest tested concentrations, respectively (ANOVA followed by Bonferroni's test; α = 0.05). Effects on biomass development are summarized in Table 4.3-14.

Table 4.3-14: Effects of dimoxystrobin on growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.033**	**0.065**	**0.13**	**0.25**	**0.50**
Concentration [mg a.s./L] (initial measured)	**--**	**--**	**0.0335**	**0.0634**	**0.132**	**0.247**	**0.444**
Number of non-chlorotic fronds (14 d) [% of control]	--	--	92	84 *	45 *	35 *	24 *
Mean dry weight of fronds (14 d) [mg]	35.1	42.0	33.0	30.3	20.0 *	20.5 *	13.9 *
Endpoints [mg a.s./L] (initial measured)							
E_bC_{50} (14 d) based on frond no.	0.149 (95% confidence limits: 0.119 - 0.187)						
E_bC_{50} (14 d) based on dry weight	0.226 (95% confidence limits: 0.129 - 0.398)						

* Statistically significantly different compared to the pooled control (ANOVA followed by Bonferroni's test, α = 0.05).

III. CONCLUSION

In a 14-day aquatic-plant test with *Lemna gibba,* the E_bC_{50} values of dimoxystrobin were determined to be 0.149 mg a.s./L based on frond number and 0.226 mg a.s./L based on dry weight (initial measured).

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

4.4.1.1 Study 1 – FELS toxicity test with the rainbow trout

Report: CA 8.2.2.1/1
Anonymous, 1999 a
BAS 505 F - Early life-stage toxicity test on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792)
1999/10521

Guidelines: OECD 210, EPA 72-4 (a)

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

EXECUTIVE SUMMARY

The chronic toxicity of BAS 505 F to Rainbow trout (*Oncorhynchus mykiss*) embryos and fry was investigated in a 97-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and to BAS 505 F at nominal concentrations of 0.000316, 0.001, 0.00316, 0.01 and 0.0316 mg a.s./L. Hatchability, survival rate and behavior of sheepshead minnow embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

The biological results are based on nominal concentrations. In the highest test concentration 0.0316 mg a.s./L the embryos were killed nearly quantitatively at hatching. In the second highest concentration (0.01 mg a.s./L) the survival of the young fish was moderately impaired. There were no sublethal effects caused by the test compound in the two lowest concentration (0.000316 and 0.001 mg a.s./L). In the higher concentrations 0.00316 and 0.01 mg a.s./L there were clear compound-related sublethal effects such as abnormalities of the tail and/or body, swimming in circles as sequela of abnormal tail, narcotic-like state, retarded resorption of yolk sac and untypically extended yolk sac. The development of the mean body weight measured at the end of the study was impaired in the two highest dose groups with surviving fish. The mean body length at (97 d) was lower in the 3 highest concentrations with surviving, however, only to a very minor extent - 5% reduction - at 0.001 mg/L. Thus, there were apparent reductions in mean body weight (not statistically significant) and body length (statistically significant) at 0.001 mg/L. However, at the same time the numbers of surviving fish was higher in the 0.001 mg/L treatment. If the higher numbers of fish are taken into consideration and the total biomass or length per replicate (i.e the sum of the fish weights and length per replicate) is used than it becomes clear that there is no statistically or biologically significant difference to the control in the 0.001 mg/L treatment. The NOEC is thus (deviating from the report to the study) established at 0.001 mg/L, the LOEC with small but significant effects on biomass is 0.00316 mg/L.

In an early life stage study of BAS 505 F on rainbow trout an overall NOAEC of 0.001 mg a.s./L and an overall LOAEC of 0.00316 mg a.s./L has been determined.

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 505 F (Reg. No. 285 028); batch no. N 15; purity: 98.4%.

B. STUDY DESIGN

Test species: Rainbow trout (Oncorhynchus mykiss WALBAUM 1792), embryos (approx. 16 hours after fertilization).

Test design: Flow-through system (97 d); 4 replicates of 25 embryos per test vessel and per concentration; flow rate of the test solution 2.5 L/h/aquarium. The study was terminated on day 97, 58 days after completion of hatch (day 39).

Test concentrations: Control, 0.000316, 0.001, 0.00316, 0.01 and 0.0316 mg a.s./L (nominal).

Test conditions: Temperature 10°C - 12°C; pH 7.5 - 8.2; oxygen content 9.6 - 11.6 mg/L; total hardness 0.38 - 0.54 mmol/L; conductivity: 122 – 125 µS; photoperiod 16 hours light : 8 hours dark, light intensity 150 lux.

Analytics: Concentration control analyses have been performed using RP-HPLC.

Statistics: Dunnett"s test, log-rank test.

II. RESULTS AND DISCUSSION

Analytical measurements: The mean analytically determined concentration values of the test compound in the test water were within a range of 89.3% - 105.6% of the nominal concentrations. Therefore, the results are based on nominal concentrations.

Biological results: In the highest test concentration 0.0316 mg a.s./L the embryos were killed nearly quantitatively at hatching. In the second highest concentration (0.01 mg a.s./L) the survival of the young fish was moderately impaired. There were no sublethal effects caused by the test compound in the two lowest concentration (0.000316 and 0.001 mg a.s./L). In the higher concentrations 0.00316 and 0.01 mg a.s./L there were clear compound-related sublethal effects such as abnormalities of the tail and/or body, swimming in circles as sequela of abnormal tail, narcotic-like state, retarded resorption of yolk sac and untypically extended yolk sac. The development of the mean body weight measured at the end of the study was impaired in the two highest dose groups with surviving fish at the $p \leq 0.01$ level. The mean body length at (97 d) was lower in the 3 highest concentrations with surviving fish (0.001 mg a.s./L $p \leq 0.05$; 0.00316 and 0.01 mg a.s./L, $p \leq 0.01$) [see Table 4.4-1], however, only to a very minor extent - 5% reduction - at 0.001 mg/L. Thus there were apparent reductions in mean body weight (not statistically significant) and body length (statistically significant) at 0.001 mg/L. However, at the same time the numbers of surviving fish was higher in the 0.001 mg/L treatment. If the higher numbers of fish are taken into consideration and the total biomass or length per replicate (i.e the sum of the fish weights and lenght per replicate) is used than it becomes clear that there is no statistically or biologically significant difference to the control in the 0.001 mg/L treatment. The NOEC is thus (deviating from the report to the study) established at 0.001 mg/L, the LOEC with small but significant effects on biomass is 0.00316 mg/L.

Table 4.4-1: Early life stage test (97 d) of BAS 505 F on rainbow trout (*Oncorhynchus mvkiss*)

Concentration [mg a.s./L]	**Control**	**0.000316**	**0.001**	**0.00316**	**0.01**	**0.0316**
Start of hatch [day]	32-33	32-33	32-33	32-33	32-33	36
End of hatch [day]	39	39	39	39	39	--
Time to swim-up [day]	55	55	55	55	55	--
Young fish survival rate (0-97 d)/100 individuals (0 d) [%]	58	55	61	52	55	0*
Mean wet weight (97 d) [% of control]	100	96	89	63*	70*	--
Total wet weight [g]	12.99 (+/- 1.99)	11.82 (+/- 1.39)	12.13 (+/- 1.53)	7.38* (+/- 1.25	8.66* (+/- 2.26)	--
Mean body length [% of control]	100	97	95*	86*	87*	--
Total length [cm]	63.4 (+/- 11.1)	58.6 (+/- 8.9)	63.6 (+/- 8.4)	48.84 (+/- 3.2)	52.4 (+/- 6.1)	--
Endpoints [mg a.s./L]						
LOEC (97 d)	0.00316					
NOEC (97 d)	0.001					

Data = range for the 4 replicates [%]
* Statistically significantly different from the control (Dunnett's test $p < 0.05$)

III. CONCLUSION

In an early life stage study of BAS 505 F on rainbow trout an overall NOAEC of 0.001 mg a.s./L and an overall LOAEC of 0.00316 mg a.s./L has been determined.

4.4.1.2 Study 2 – FELS toxicity test with the fathead minnow

Report: II A 8.2.4/2
Anonymous, 2000
Early life stage toxicity of BAS 505 F to the fathead minnow, Pimephales promelas
2000/5156
Guidelines: OECD 210, EPA 72-4 (a)
GLP: yes
(certified by United States Environmental Protection Agency)

EXECUTIVE SUMMARY

In a 36 d flow-through toxicity study, embryos of *Pimephales promelas* were exposted to 2.2, 4.2, 8.0, 16 and 32 µg dimoxystrobin/L. Four replicates of 10 embryos per test vessel and per concentration were set up. The study was terminated on day 36, 32 days after completion of hatch and young fish survival, sublethal effects as well as effects on weight and length were assessed.

Analytical measurements: The mean analytically determined concentration values of the test item in the test water were within a range of 99% - 106% of the nominal concentrations and stable throughout the toxicity test. No insoluble material was observed in any vessel containing dimoxystrobin during the study.

Biological results: There was at least 95% hatch in each replicate control and solvent control vessel, the hatch in each control chamber was less than 1.6 times the hatch in the other control chambers, and the number of live, normal control and solvent control fish at 36 days of exposure (32 days post hatch) was at least 70% in each vessel. Control and solvent control fish had an average wet weight (blotted) of 89.3 mg and 92.3 mg, and average dry weight of 26.5 mg and 27.1 mg and an average total length of 24.6 mm and 24.1 mm, respectively, at the end of the test. The relative standard deviation of the weights of surviving fish in the control test chamber was less than 40%. Maximum control loading rate during the toxicity test was approximately 0.13 g/L at any time and 0.017 g/L/24 hours. Sublethal effects, observed as one fish exhibited a deformed tail, were noted at 32 µg/L on days 18 through 21. This effect was not observed at any other time during the test. No significant sublethal effects (other than size differences) were observed at the lower concentrations of dimoxystrobin at any time. The most sensitive measured biological endpoints were the survival of fish (particularly during time of hatching and shortly afterwards). A slight, but not statistically significant reduction in biomass was observed at the highest concentrations.

Exposure of embryonic, larval and juvenile fathead minnows to dimoxystrobin for 36 days resulted in an LOEC of 32 µg/L. A NOEC of 16 µg a.s./L has been determined.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F, Reg. No. 285 028); batch no. N 15; purity: 96%.

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), embryos less than 24 hours old; obtained from a commercial supplier (Aquatic Bio Systems, Inc., Fort Collins, Colorado, USA).

Test design: Flow-through system (36 d); 9 liter glass aquaria; 5 concentrations of the test item, plus a control and a dilution water control, 4 replicates of 10 embryos per test vessel and per concentration. The study was terminated on day 36, 32 days after completion of hatch (day 4).

Test concentrations: Control, solvent control, 2.2, 4.2, 8.0, 16 and 32 µg a.s./L (nominal).

Test conditions: Temperature: 24.2 °C - 26.6 °C; pH 7.4 - 7.9; oxygen content: 7.8 - 9.4 mg/L; conductivity: 130 - 190 µS; photoperiod: 16 hours light : 8 hours dark.

Analytics: Concentration control analyses have been performed using HPLC with UV-detection.

Statistics: Descriptive statistics, ANOVA, Bonferroni´s test.

II. RESULTS AND DISCUSSION

Analytical measurements: The mean analytically determined concentration values of the test item in the test water were within a range of 99% - 106% of the nominal concentrations and stable throughout the toxicity test. No insoluble material was observed in any vessel containing dimoxystrobin during the study.

Biological results: There was at least 95% hatch in each replicate control and solvent control vessel, the hatch in each control chamber was less than 1.6 times the hatch in the other control chambers, and the

number of live, normal control and solvent control fish at 36 days of exposure (32 days post hatch) was at least 70% in each vessel. Control and solvent control fish had an average wet weight (blotted) of 89.3 mg and 92.3 mg, and average dry weight of 26.5 mg and 27.1 mg and an average total length of 24.6 mm and 24.1 mm, respectively, at the end of the test. The relative standard deviation of the weights of surviving fish in the control test chamber was less than 40%. Maximum control loading rate during the toxicity test was approximately 0.13 g/L at any time and 0.017 g/L/24 hours. Sublethal effects, observed as one fish exhibited a deformed tail, were noted at 32 µg/L on days 18 through 21. This effect was not observed at any other time during the test. No significant sublethal effects (other than size differences) were observed at the lower concentrations of dimoxystrobin at any time. The most sensitive measured biological endpoints were the survival of fish (particularly during time of hatching and shortly afterwards). A slight, but not statistically significant reduction in biomass was observed at the highest concentrations [see Table 4.4-2].

Table 4.4-2: Early life stage toxicity test (36 d) of dimoxystrobin on fathead minnow (*Pimephales promelas*)

Concentration (nominal) [µg a.s./L]	**Control**	**Solvent control**	**2.2**	**4.2**	**8.0**	**16.0**	**32.0**
Concentration (analytic) [µg a.s./L]	**Control**	**Solvent control**	**2.34**	**4.25**	**7.9**	**16.5**	**32.8**
Start of hatch [day]	4	4	4	4	4	4	4
Young fish survival rate 32 d post hatch [%]	90	88	98	78	93	78	38
Sublethal effects 32 d post hatch [%]	none	none	none	none	none	None	D [1)]
Mean wet weight [mg]	89.3	92.3	82.3	97.6	81.7	76.5	71.4
Mean body length [mm]	24.6	24.1	23.6	24.8	23.9	23.5	22.4
	Endpoints [µg a.s./L]						
LOEC (36 d)	32						
NOEC (36 d)	16						

Data = range for the 4 replicates [%]

[1)] D = deformed tail (one fish only from day 18 - 21)

III. CONCLUSION

Exposure of embryonic, larval and juvenile fathead minnows to dimoxystrobin for 36 days resulted in an LOEC of 32 µg/L. A NOEC of 16 µg a.s./L has been determined.

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

No new studies available.

4.4.3 Aquatic Toxicity – Fish, juvenile growth test

No new studies available.

4.4.4 Chronic toxicity to aquatic invertebrates

4.4.4.1 Study 1 – 21-day test with *Daphnia magna*

Report:	CA 8.2.5.1/1 Jatzek H.-J., 2000 a Determination of the chronic effect of BAS 505 F on the reproduction of the water flea Daphnia magna STRAUS according to EEC guidelineXI/691/86, draft 4: and GLP, EN 45001 and ISO 9002 2000/1000120
Guidelines:	EEC XI/691/86, DIN 38412 (Entwurf 1981), OECD 202, EPA 660/3-75-009
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

EXECUTIVE SUMMARY

In a 21-day semi-static toxicity test, effects of BAS 505 F to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 1.56, 3.13, 6.25, 12.5, 25 and 50 µg a.s./L. Additionally, a dilution water control and a solvent control were set up. All treatment groups and the controls consisted of 10 replicates containing one daphnid. Assessment of parent mortality and reproduction was conducted daily.

The biological results are based on nominal concentrations. Parent mortality was observed only at 50 µg a.s./L, the highest concentration tested. The mean number of offspring per parent in the control and the solvent control was 95.7 and 144.6, respectively. No offspring was produced in the 50 µg a.s./L test concentration. A slight but statistically non-significant reduction in the number of offspring was observed at 25 µg a.s./L. First juveniles were observed at day 8 in each treatment. At 25 µg a.s./L some aborted eggs were observed, in addition a significant number of dead young were found in this variant.

In a 21-day semi-static chronic toxicity study with Daphnia magna the NOEC of BAS 505 F was 12.5 µg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 505 F (Reg. No. 285 028); batch no. 01171-55; purity: 99.7%.

B. STUDY DESIGN

Test species: Waterflea (*Daphnia magna* STRAUS), neonates collected from in house culture, age at test initiation less than 24 hours; culture conditions in accordance with test conditions.

Test design: Semi-static test (21 days), 6 test concentrations plus control, 10 replicates per concentration with 1 daphnid in each; daily assessment of mortality and reproduction (and other effects).

Test concentrations: Control, solvent control, 1.56, 3.13, 6.25, 12.5, 25 and 50 µg a.s./L (nominal).

Test conditions: Glass vessels; test volume 50 mL, reconstituted, synthetic water "M4"; pH 7.3 - 8.1; total hardness 2.2 - 3.2 mmol/L; oxygen content 5.1 - 9.2 mg/L; conductivity 550 – 650 µS/cm; temperature: 20.3°C - 20.9°C; photoperiod 16 hours light: 8 hours dark; light intensity: about 2 – 7 µE/(m^2 x s), wave length 400 – 700 nm; feeding with algae, no ventilation.

Analytics: Analytical verification of all test substance concentrations was conducted using HPLC with UV-detection.

Statistics: Duncan’s multiple range test.

II. RESULTS AND DISCUSSION

Analytical measurements: Concentration control analysis of BAS 505 F in the water samples at test initiation resulted in a range of 98.5% to 123.8% of the nominal concentrations. In the course of the study the nominal concentration of BAS 505 F was confirmed in general. At the end of the study recoveries of 87.3% to 141.7% were obtained.

Biological results: Parent mortality was observed only at 50 µg a.s./L, the highest concentration tested. The mean number of offspring per parent in the control and the solvent control was 95.7 and 144.6, respectively. No offspring was produced in the 50 µg a.s./L test concentration. A slight but statistically non significant reduction in the number of offspring was observed at 25 µg a.s./L. First juveniles were observed at day 8 in each treatment. At 25 µg a.s./L some aborted eggs were observed, in addition a significant number of dead young were found in this variant (see Table 4.4-3).

Table 4.4-3: Effects of BAS 505 F on *Daphnia magna* reproduction and parent mortality

Concentration (nominal) [µg a.s./L]	**Control**	**Solvent control**	**1.56**	**3.13**	**6.25**	**12.5**	**25**	**50**
Offspring/parent	96	145	175	177	175	157	103	0
Parent-mortality [%]	10	0	0	0	0	0	0	80
	Endpoints [µg a.s./L]							
LOEC (21 d)	25.0							
NOEC (21 d)	12.5							

III. CONCLUSION

In a 21-day semi-static chronic toxicity study with Daphnia magna the NOEC of BAS 505 F was 12.5 µg a.s./L (nominal).

4.4.5 Chronic toxicity to algae or aquatic plants

[See short-term toxicity]

4.5 Acute and/or chronic toxicity to other aquatic organisms

The following study is a 28 d flow-through toxicity study on rainbow trout. The study is not considered a suitable long-term study according to the Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.7b: Endpoint specific guidance, version 4.0, June 2017) as it does not evaluate effects off sensitive life stages. However, it is presented as additional information.

4.5.1 Study 1 – Long-term toxicity study in the rainbow trout

Report: II A 8.2.3/1
Anonymous, 1999
BAS 505 F - Sublethal toxic effects on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a flow-through system (28 days)
1999/10311

Guidelines: OECD 204

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

EXECUTIVE SUMMARY

In a 28 day flow-through toxicity study, juvenile rainbow trout (approx. 6 months old) were exposed to 0.000316, 0.001, 0.00316, 0.01 and 0.0316 mg dimoxystrobin/L (nominal) in groups of 20 animals in glass aquaria containing 60 L water. Fish were observed for survival and symptoms of toxicity daily.

Mortality occurred only in the highest substance concentration 0.0316 mg dimoxystrobin/L. It started on day 1 (10%) and increased to 55% on day 8. No further increase in mortality until day 28 (end of study) was noted. Compound-related toxic signs were observed only in the highest concentration (0.0316 mg dimoxystrobin/L) starting on day 26 in form of reduced or no food uptake and swimming near the bottom. The mean body weight and the mean body length at the end of the study were not statistically different from the control group. In the highest concentration there was however a marginal decrease of body weight gain (3.4%) and decrease in body length (3%) compared to the control.

In a flow-through chronic toxicity study with rainbow trout the NOAEC (28 d) for dimoxystrobin was determined to be 0.01 mg/L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F, Reg. No. 285 028); batch no. N 15; purity: 98.4%;

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), approx. 6 month; mean body length 5.4 cm (5.0 - 5.8 cm), mean body weight 1.6 g (1.4 - 1.9 g); supplied by Forellenhof Fredelsloh, Mohringen, Germany.

Test design: Flow-through system (28 days); 20 fish per aquarium; flow rate of the test solution 10 L/h/aquarium.

Endpoints: NOAEC, body weight gain and body length.

Test concentrations: Control, 0.000316, 0.001, 0.00316, 0.01 and 0.0316 mg dimoxystrobin/L (nominal).

Test conditions: Glass aquaria with stainless steel frames (60 x 35 x 40 cm), test volume 60 L, non-chlorinated, filtered tap water; temperature: 14 °C - 15 °C; pH 8.0 - 8.2; oxygen content: 6.3 mg/L - 10.4 mg/L; total hardness: about 2.25 - 2.4 mmol/L; photoperiod 16 h light : 8 h dark; no aeration, no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics; ANOVA followed by Dunnett's t-test.

II. RESULTS AND DISCUSSION

Analytical measurements: The values for the theoretical concentrations varied between 90.6% and 129.6% at test initiation and between 101.5% and 117.1% at the end of the study after 28 days. Hence the results are based on the nominal concentrations.

Biological results: Mortality occurred only in the highest substance concentration 0.0316 mg dimoxystrobin/L. It started on day 1 (10%) and increased to 55% on day 8. No further increase in

mortality until day 28 (end of study) was noted. Compound-related toxic signs were observed only in the highest concentration (0.0316 mg dimoxystrobin/L) starting on day 26 in form of reduced or no food uptake and swimming near the bottom. The mean body weight and the mean body length at the end of the study were not statistically different (Dunnett's t-test) from the control group. In the highest concentration there was however a marginal decrease of body weight gain (3.4%) and decrease in body length (3%) compared to the control. The results are summarized in Table 4.5-1.

Table 4.5-1: Sublethal toxicity (28 d) of dimoxystrobin on rainbow trout (*Oncorhynchus mykiss)*

Concentration [mg/L] nominal	**Control**	**0.000316**	**0.001**	**0.00316**	**0.01**	**0.0316**
Mortality [%]	0	0	0	0	0	55
Symptoms *	none	none	none	none	none	A, Ae; P, Z
MEAN WEIGHT [G] (28 D)	5.26	5.67	5.53	5.30	5.33	5.08
Mean length [cm] (28 d)	7.67	7.85	7.77	7.70	7.67	7.44
Endpoints [mg dimoxystrobin/L]						
NOAEC (28 d)	0.01					

* Symptoms: A = apathy, Ae = opacity (moderate to severe) of eyes both sides; Z = reduced or no feed consumption; P = swimming on the bottom

III. CONCLUSION

In a flow-through chronic toxicity study with rainbow trout the NOAEC (28 d) for dimoxystrobin was determined to be 0.01 mg/L based on nominal concentrations.

The following study is a study on the aquatic insect Chironomus riparius. For classification purposes only aquatic crustacean species are relevant according to Regulation (EC) No 1272/2008 (CLP Regulation). However, the study is presented as additional information.

4.5.2 Study 2 – Development and emergence toxicity study in *Chironomus riparius*

Report: II A 8.2.7 / 1
Dohmen G.P. 2001
Effects of BAS 505 F on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system
2000/1012491

Guidelines: OECD 204

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

EXECUTIVE SUMMARY

Groups of 25 individuals of *Chironomus riparius* (4 replicates) were exposed to five test concentrations (3, 10, 32, 100 and 316 µg a.s./L) of dimoxystrobin for 28 days under static conditions in a water-sediment system. A control group exposed to test water without test item as well as a solvent control were run concurrently. The invertebrates were observed daily behavioural differences and emergence of female and male midges.

The emergence rates were generally quite high. More than 70% - the required minimum for the untreated controls - of the chironomids emerged in all but the highest test concentration. Statistically significant effects were obtained at the three highest test concentrations. However, also the highest test concentration of 316 µg/L caused a reduction in emergence of less than 50% (39%). The EC_{50} for emergence rate is thus > 316

µg/L. There were no significant treatment effects on the development rate although the onset of emergence was 1-2 days later at the highest test concentration.

Based on slight effects on the emergence rate of *Chironomus riparius* at 32 µg/L BAS 505 F, the NOEC was determined to be 10 µg/L, the LOEC was 32 µg/L and the LC_{50} was > 316 µg/L.

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 505 F (Reg. No. 285 028); batch no. N 6; purity: 98.8% (re-analysis in 1998: 97.4%).

B. STUDY DESIGN

Test species: *Chironomus riparius*, collected from in house culture; larvae up to 3 days old

Test design: Static test; 5 concentrations each with three replicates plus a control with 2 replicates and a solvent control with 4 replicates; 25 *Chironomus riparius* larvae (< 3 days old) added to each vessel; assessment of emergence of male and female midges and of visual anomalies.

Test concentrations: 0 (control), 0 (solvent control), 3, 10, 32, 100 and 316 µg/L, nominal

Test conditions: Glass vessels, about 2 cm sediment layer (soil according to OECD 207), 1.8 L "M4" water (Elendt medium) according to a 16.5 cm water layer, pH 7.8 - 7.9, oxygen content 7.3 - 8.3 mg/L, total hardness 2.39 mmol/L, conductivity 695 µS/cm (all at test initiation, hardness and conductivity from a combined sample), feeding with TetraMin, slight ventilation, mean water temperature 20.65 ± 0.65 °C, light intensity 960 - 1140 lux, day: night-rhythm 16:8 h.

Analytics: HPLC with UV-detection at 230 nm.

Statistics: Standard procedures, analysis of variance, Dunnett´s-, Bonferroni-, and William´s-test.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical determinations of test substance shortly after addition to the system yielded recoveries in the water phase of 94.2% to 112.4% of the nominal concentrations, indicating the correct addition of test substance to the system. As was to be expected from the water-sediment study, the test substance dissipated rapidly from the water phase and significant quantities of BAS 505 F were detected in the sediment.

Biological results: The emergence rates were generally quite high. More than 70% - the required minimum for the untreated controls - of the chironomids emerged in all but the highest test concentration. Statistically significant effects were obtained at the three highest test concentrations. However, also the highest test concentration of 316 µg/L caused a reduction in emergence of less than 50% (39%). The EC_{50} for emergence rate is thus > 316 µg/L.

There were no significant treatment effects on the development rate although the onset of emergence was 1-2 days later at the highest test concentration.

Table 4.5-2: Effect of BAS 505 F on *Chironomus riparius* emergence and development rates

Concentration [µg/L]	Control + solvent	Control without	3	10	32	100	316
Emergence rate	0.99	0.96	0.93	0.89	0.76*	0.73*	0.61*
Development rate	0.073	0.073	0.075	0.069	0.069	0.079	0.069

* Statistically significant differences compared to the control (William's test, $\alpha = 0.05$)

III. CONCLUSION

Based on slight effects on the emergence rate of *Chironomus riparius* at 32 µg/L BAS 505 F, the NOEC was determined to be 10 µg/L, the LOEC was 32 µg/L and the LC_{50} was > 316 µg/L.