Bayer CropScience

Document Title

Summary of the ecotoxicological studies
Methiocarb FS 500 (500 g/L)

Data Requirements

EU Regulation 1107/2009 & EU Regulation 284/2013
Document MCP
Section 10: Ecotoxicological studies

According to the guidance document SANCO 10181/2013

for preparing dossiers for the approval of a chemical active substance

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Table of Contents

CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Methiocarb is an insecticide and repellent active substance and was included into Annex I of Directive 91/414 on 1st October 2007 (Directive 2007/5/EC).

This Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of methiocarb under Directive 91/414/EEC and which were therefore not evaluated during the first EU review. All data which were already submitted by Bayer CropScience (BCS) for the Annex I inclusion under Directive 91/414/EEC are contained in the DAR, its Addenda and are included in the Baseline Dossier provided by BCS. These data are only mentioned in the Supplementary Dossier for the sake of completeness and only general information (e.g. author, reference etc.) is available for these data. In order to facilitate discrimination between new data and data submitted during the Annex I inclusion process under Directive 91/414/EEC, the old data are written in grey typeface. For all new studies, detailed summaries are provided within this Supplementary Dossier.

Studies with the active substance methiocarb as well as the metabolites methiocarb-sulfoxide (MSO), methiocarb-phenol (MP), methiocarb-sulfoxide-phenol (MSOP), methiocarb-sulfone-phenol (MSOOP) and methiocarb-methoxy-sulfone (MMS) can be retrieved in the respective node and subnodes of CA 8 for the active substance.

The presented and submitted studies used different synonyms and codes for the active substance methiocarb.

Use pattern considered in this risk assessment

Table10- 1: Intended application pattern

Crop	Timing of application	Number of applications	Application rate product (mL/dt)	Max. seeding rate (kg/ha)	Max. application rate Methiocarb	
					[g a.s./ha]	[mg a.s./ seed] A
Maize	Seed treatment (BBCH 00)	1	1000	30	150	1.5

A Assuming a thousand grain weight of the seeds of 300 g

Definition of the residue for risk assessment

Justification for the residue definition for risk assessment is provided in MCA Sec.7, Point 7.4.1 and MCA Sec. 6, Point 6.7.1.

Table10- 2: Definition of the residue for risk assessment

Compartment	Compound / Code
Soil	methiocarb, methiocarb sulfoxide (M01), methiocarb sulfoxide phenol (M04), methiocarb sulfone phenol (M05), methiocarb methoxy sulfone (M10),*
Groundwater	methiocarb, methiocarb sulfoxide (M01), methiocarb sulfoxide phenol (M04), methiocarb sulfone phenol (M05), methiocarb methoxy sulfone (M10),*
Surface water	methiocarb, methiocarb sulfoxide (M01),methiocarb phenol (M03), methiocarb sulfoxide phenol (M04), methiocarb sulfone phenol (M05), methiocarb methoxy sulfone (M10)
Sediment	methiocarb, methiocarb sulfoxide (M01),methiocarb phenol (M03), methiocarb sulfoxide phenol (M04), methiocarb sulfone phenol (M05), methiocarb methoxy sulfone (M10)
Air	methiocarb

* The metabolite methiocarb phenol (M03) occurs in soil only under strictly anaerobic conditions. Under aerobic conditions methiocarb phenol (M03) is a metabolite detected in one soil with 2% on day 0 only and not detected at all in 4 further soils.
It was considered whether or not a calculation of predicted environmental concentrations in soil and groundwater was required for methiocarb phenol (M03) whenever prolonged strictly anaerobic conditions could be present shortly after application.
The intended use of methiocarb is a seed treatment in maize. Growth of the maize seed will be severely inhibited under anaerobic conditions due to shortage of oxygen. Sites where anaerobic conditions may occur during the early vegetation period of maize in late spring and summer will produce uneconomic yields and are consequently not used to grow maize. It is therefore extremely unlikely that metabolites which are only formed in an anaerobic environment occur under realistic use conditions. Therefore the metabolite methiocarb phenol (M03) is not considered relevant for soil and groundwater risk assessment.

CP 10.1 Effects on birds and other terrestrial vertebrates

The risk assessment has been performed according to "European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438), referred to in the following as "EFSA GD 2009".

CP 10.1.1 Effects on birds

Table 10.1.1- 1: Endpoints used in risk assessment

Test substance	Exposure	Species	Endpoint	Reference
Methiocarb	Acute risk assessment	Japanese quail	LD_{50} 5 mg a.s./kg bw	[redacted] (1983) M-012876-01-2 KCA 8.1.2.1
	Reproductive risk assessment	Mallard duck (1997)	NOED 4.91 mg a.s./kg b.w/d	[redacted] (1988) M-012909-01-1 KCA 8.1.1.3

Note:
- studies referring to KCA are filed in the dossier for the active substance

For birds feeding on treated seeds:

In case of a seed treatment the following generic focal species have to be used:

Table 10.1.1- 2: Type of seeds, corresponding generic focal species and their food intake rate per body weight

Type of seeds	Generic focal species	FIR/bw
'Large seeds' (maize, beans or peas)	Large granivorous bird	0.1
'Small seeds' (not maize, beans or peas)	Small granivorous bird	0.3

For birds feeding on crop seedlings:

The Tier-1 acute and reproductive risk assessments for birds feeding on crop seedlings from a seed treatment have to be carried out according to the shortcut value as shown below.

Table 10.1.1- 3: Generic focal species and corresponding shortcut values for assessment of residues present in newly emerged crop shoots

Generic focal species	Short-cut value (SV) for acute risk*
Small omnivorous bird	0.5 x NAR/5

*For the reproductive assessment, these shortcut values should be combined with appropriate time windows and default degradation/dissipation rates for residues (see equation above).
NAR = Nominal loading/application rate of active substance in mg/kg seed.

ACUTE DIETARY RISK ASSESSMENT

Birds feeding on treated seeds:

The tier 1 risk assessment was performed based on an application rate of 1000 mL product/100 kg seeds, corresponding to 5000 mg methiocarb/kg seeds.

Table 10.1.1- 4: Tier 1 acute TER calculation for birds feeding on treated seeds

Compound	Generic focal species	Toxicity [mg a.s./kg bw]	Exposure		TER_A	Trigger
			FIR/bw	NAR [mg a.s./kg seeds]		
Methiocarb	Large granivorous bird	5	0.1	5 000	**0.01**	10

Bold values do not meet the Tier 1 TER trigger
NAR = Nominal loading/application rate of active substance in mg/kg seed.

Birds feeding on crop seedlings

Table 10.1.1- 5: Tier 1 acute TER calculation for birds feeding on crop seedlings

Compound	Generic focal species	Toxicity [mg a.s./kg bw]	Exposure	TER_A	Trigger
			SV*		
Methiocarb	Small omnivorous bird	5	500	**0.01**	10

* SV = 0.5 x NAR/5
Bold values do not meet the Tier 1 TER trigger

The TER values for birds feeding on treated seeds or crop seedlings are lower as the required trigger of 10 for acute exposure. Accordingly, further refinement is necessary.

A. Refined acute risk assessment for granivorous birds

The two **main factors** which diminish the risk for birds are the **low exposure** to treated seeds and the **repellency of Methiocarb**. These relevant factors do not fit into the risk equation of the guidance document. Therefore a <u>"weight of evidence"</u> approach is considered appropriate to refine the risk assessment based on these factors.

Exposure of birds to treated maize seeds

Maize is precision drilled, with seeds placed deep in the soil and at a low density compared to cereals. This means that, provided a good seed bed preparation, the density of seeds left on the surface of a drilled field and the associated risk can be regarded as "very low" ([redacted] et al. 1995, M-042897-01-1). For the Netherlands: the percentage of maize seed remaining on the soil surface after drilling was 0.18% of the drilled amount, i.e. 0.02 seeds/m^2 (or 200 seeds per hectare). For Germany the generic avian field study on freshly drilled maize seeds ([redacted](2001a), M-031252-01-1) reported comparable seed exposure data: average number of seeds on the surface in the midfield and end row areas were 0.007 seeds/m^2 (= 0.1%) and 0.042 seeds/m^2 (=0.5%), respectively (n = 10 fields).
A low exposure to treated seeds is also reported in a monitoring study performed on maize seeds in Germany after drilling [redacted]2009, M-359439-01-1)

Results from monitoring study in Germany:

Application and exposure:

The maize drilling was always performed as precise drilling. On the 11 fields 5 different machines were used (Amazone, Horsch, Becker, Mascar and Kleine). The diversity of different seed types and batches was high as well.

Although the differences in the use of equipment and seed types were high, the exposure of seeds on the surface of the fields was always similar and in general low:

In midfield areas the mean number of maize seeds per m² amounted to 0.06 (SD 0.10); in endrow areas it amounted to 1.85 seeds per m² (SD 1.42).

As a worst case scenario an amount of 0.06 seeds per m² for midfield areas and 2 seeds per m² can be used.

Exposed bird species:

In another field monitoring study, [redacted] (2001a), document M-031252-01-1, observed in ten study fields located in Lower Rhineland in Germany the attractiveness of freshly drilled maize fields. Only large seed eating birds were observed eating maize: carrion crow (570 g bw), pheasant (950 - 1320 g bw), wood pigeon (490 g bw) (mean weights according to Cramp (1996, Birds of the western palearctic). Small seed eating birds only occasionally frequented the fields. Consumption of maize was not observed.

There was no evidence that maize seeds remaining on the soil after drilling or the dispersed maize seeds of the reference fields were of special attractiveness for seed eating birds.

The granivorous birds observed in the field study were in compliance with the EU-Guidance document, which requires risk assessment for treated maize seeds only for large granivorous birds.

Avoidance of treated seeds

Methiocarb is used as a repellent to protect the maize culture from damage by birds (mainly pheasants and crows), see Efficacy part of the dossier (section 7). The excellent repellency did not only protect the culture but as well the birds to get intoxicated. This repellent effect was confirmed by [redacted]; 1993; M-035103-01-2 and was also proved by several avoidance studies.

Pheasants:

7 peasants (3 males, 4 females) received treated maize seeds together with untreated maize seeds (25% of the daily food demand untreated) over a period of 5 days according to BBA 25.1. The treated seeds were almost completely avoided, even under increased starvation stress ([redacted] 1984, M-013213-01-2).

Crows:

Avoidance studies according BBA 11.1 were as well performed with 6 rooks (*Corvus frugilegus*) and one crow (*Corvus corone*) over 5 days by [redacted] (1992, M-013181-01-2). The mean consumption of Methiocarb treated wheat seeds (500g a.s./dt) amounted to 0.1 g/day/bird. Signs of intoxication were not observed. Even under this increasingly acute pressure situation, only a few grains of the test diet were ingested, whilst the untreated diet was always completely consumed.

Pigeons:

In an acceptance study according BBA 25.1. with domestic pigeon and maize seeds under aggravated conditions (8 hours on 3 consecutive days), no signs of intoxication or mortalities occurred. Moreover, a complete avoidance of the treated seeds was observed. Therefore, an exposure of birds as represented by the domestic pigeon to Methiocarb FS 500 treated maize seeds (5.2 g as/kg seeds) is not expected to pose a risk ([redacted] 2001b, M-048267-01-1).

Partridges:

Smaller European species like grey partridge or common quail usually prefer smaller food items than maize seeds but may ingest those occasionally. Pheasants are well known to feed on maize seeds and have been observed using freshly drilled maize fields as feeding ground in a field study ([redacted] 2001a M-031252-01-1).

In two cage trials the acceptance of maize seeds and especially of seeds treated with Methiocarb FS 500 by two gallinaceus bird species had been tested.

[redacted] (2001), M-039873-01-1, exposed 20 pen reared grey partridges to treated maize seeds (0.5 kg as/100 kg seeds). The partridges were housed in 4 groups of 5 individuals each in aviaries with a ground area of 2 m x 2 m and a height of 2 m. The food (exclusively maize seeds) was offered on trays. Due to difficulties in acclimating partridges on the consumption of maize seeds, found in pre-tests, a special method was chosen: On day –2 the birds received 150 untreated uncoloured wet maize seeds which were watered for at least 12 hours. The next day the birds received 150 untreated uncoloured watered maize seeds and 150 uncoloured dry maize seeds per aviary. On the day of exposure 2 groups received 150 dry Methiocarb FS 500 treated maize seeds, whilst the other 2 groups received 150 damp, Methiocarb FS 500 treated maize seeds. The exposure to maize seeds lasted from 8.00 to 16.00 on day –2 and –1 as well as on the exposure day (day 0). The consumed number of maize seeds was counted for each day. Between the exposure periods the partridges received no other food. On day –2 (exposure of untreated, uncoloured wet maize) in two groups maize were consumed to some extend while the other two groups refused it (overall mean intake per bird: 1.3 seeds). It was assumed that pioneer birds are necessary to start a group feeding. On day –1 the same two groups which ingested maize, were feeding on maize on a higher amount than the day before. They accepted dry and wet untreated maize seeds with a preference for the dry ones. The two groups who refused to consume maize the day before did not change their feeding behaviour. The overall mean intake per bird was 4.7 maize seeds. On the exposure day two scenarios were tested: one with dry treated maize and one with damped treated seeds. One group with proven maize eaters and one group which refused to ingest maize were assigned to each scenario. The results in both scenarios were the same: the proven maize eaters, although they were familiar with maize, avoided the treated seeds. The birds which refused maize did not test the treated maize despite of their increased starvation stress, documented by a mean weight loss of approx. 10% between day –4 and day 0. The overall (both scenarios) mean intake per bird was 0.15 treated seeds. No signs of intoxication were observed.

Therefore, partridges avoided the ingestion of maize seeds treated with Methiocarb FS 500 seed dressing, even when accustomed to maize as a part of their died and/or they are under starvation stress.

The avoidance studies with different granivorous birds verify the high repellency of methiocarb resulting in a margin of safety for the bird species.

Monitoring activities

In a generic field study ([redacted] (2001a), M-031252-01-1) the use of freshly drilled maize fields as feeding ground of large seed eating birds was investigated. Two exposure scenarios were considered, i.e. 3 fields commercially drilled with Methiocarb FS 500 treated seeds (drilling rate 2 units/ha) and 3 reference sites with untreated seeds dispersed in high exposure rate of 600 seeds/ha on the surface after ploughing and harrowing the soil. Birds were observed on the day of drilling and the following day, and birds species present, number of individuals and behaviour were recorded. Pheasant could be observed on all 6 fields. 45% of the food consumed by this species on the reference sites was maize seeds. On the fields drilled with Methiocarb FS 500 treated seeds, maize seeds accounted for only 0.5% of the observed uptakes by pheasants. No adverse effects on the birds following ingestion of treated seeds were observed. Obviously maize was a potential food for pheasants but was strongly avoided if treated with methiocarb, even though seed were available at the surface at mean rates of 70 (midfield) and 420 (endrow) seeds/ha.

These results were further verified by a monitoring study, performed on maize seeds in Germany after drilling ([redacted], R.; 2009; M-359439-01-1)

This study aimed to monitor the bird and mammals population in regard of potentially increased mortality after the drilling of maize, treated with Methiocarb FS 500.
This field monitoring was performed on fields in 11 areas in Northwest Germany.
From each field, a sample of treated seeds was collected, which was analysed on the loading with methiocarb.
The exposure of maize seeds on the soil surface was determined on the drilling day (day 0). On each field, 80 squares (1 m x 1 m) on eight transect lines of 50 m (4 in midfield area, 4 in endrow areas, per transect 10 squares) were randomly chosen, on which the number of remaining maize seeds was counted.
After the application, on each site 2 carcass searches for dead or impacted birds and mammals were performed (day +1 and +3). During the carcass search, a team of 2 – 4 people paced the test area. The team walked along the maize field in parallel rows.
Objective of the carcass search was to collect all carcasses and to determine them to species level. The place of finding, the circumstances of the finding and the conditions of the carcasses including signs of intoxication should be recorded. Appropriate carcasses should be submitted to residue analysis on methiocarb.
The efficiency of the search team was tested twice by disposing dummies. The activity of predatory birds and mammals which may influence the detection rate of carcasses by removing the carcasses was tested twice as well. On field 11 and on the fields 8 and 9 (relatively small fields which were not far away from each other) a defined number of dead quails were disposed for 24 h (field 11: 10 birds; field 8/9 15 birds) and then recollected.
On the application day, no carcass search was carried out in order not to chase the birds away. Instead of it, a bird observation of 2 hours was performed in the afternoon to scan for impacted birds. A further bird observation was carried out on day +1. During the bird observation all birds entering the field were recorded. Based on the results, the frequency of observance could be calculated for each species of concern.

Bird and mammals activities and indication of them (traces, de-husked seeds), which were detected during carcass searches, were recorded as well.

Bird observation:

The frequency of occurrence (FO) of the different birds is expressed in percentage related to all fields (n=11; FO_{field}) and related to all censuses (n=22; FO_{survey}): The ranking list of birds according to FO_{field} was as follows:

Carrion crow 90.9 %; wood pigeon: 72.7 %; blackbird and white wagtail: each 72.7 %; lapwing: 63.6 % and starling 54.5 %. Related to FO_{survey} the most frequent bird species were the same ones with little differences in the ranking order.

The abundance of birds was low. All observed birds behaved normally and were above any suspicion of being impacted by Methiocarb.

Overall conclusion

The tier 1 risk assessment indicated a high potential risk for granivorous birds feeding on treated maize seeds with Methiocarb FS 500.

Based on the results of field monitoring studies, four bird species of concern have been identified as relevant for refining the risk assessment: the pheasant, the pigeon, the crow and the partridge. Given the well documented repellency properties of methiocarb as indicated by several acceptance studies, there is clear evidence that the avoidance of the treated seed will be sufficient to avoid any severe intoxication.

Moreover, other field studies indicated a low availability of the treated seeds after sowing as well as a low attractivity of a freshly sown field.

Taking all this information into consideration, an acceptable acute risk to birds can be concluded.

B. Refined acute risk assessment for ingestion of seedlings

Residue level in maize seedlings

Residue studies with the Methiocarb FS 500 formulation applied at a rate of 1 L product/100 kg seeds or 5 g a.s./kg seeds have been carried out on maize in Germany, France, Belgium, Spain, Italy and Greece (please refer to section 6.3.4 of the active substance dossier, reports M-033763-01-1, M-034429-01-1; M-035447-01-1, M-032843-01-1). In samples from young maize plants collected 27-41 days after sowing traces of methiocarb (<0.01 mg/kg), methiocarb-sulfoxide (< 0.01 – 0.07 mg/kg) and methiocarb-sulfone (< 0.01 mg/kg) could be detected in the whole plant (without root) resulting in a calculated total residue between 0.03 and 0.09 mg/kg. In later samples, residues of methiocarb, methiocarb-sulfoxide and methiocarb-sulfone were below the limit of quantitation (LOQ = 0.01 mg/kg for each substance). The maximum value of 0.09 mg total residue/kg fresh plant material, summarising the residues of the parent compound and its two metabolites, will be used for refined risk assessment.

The refined risk assessment for the acute exposure to seedlings grown from seeds treated with methiocarb is conducted for a small omnivorous bird.

Table 10.1.1- 6: Refined acute TER calculation for birds feeding on crop seedlings – using the small omnivorous bird and maximum measured residue level in maize seedling

Compound	Focal species	Toxicity [mg a.s./kg bw]	FIR/bw	Residues [mg as/kg fresh wt.]	$TER_{A,ref}$	Trigger
Methiocarb	Small omnivorous bird	5	0.52*	0.09	107	10

* EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) – Appendix A (Tier 1 tables for birds, Maize: BBCH 10-29)

The refined risk assessment for small omnivorous birds does not indicate an unacceptable risk (TER_A > 10) posed by exposure to seedlings grown from seeds treated with methiocarb.

LONG-TERM REPRODUCTIVE ASSESSMENT

Birds feeding on treated seeds

The tier 1 risk assessment was performed based on an application rate of 1 000 mL product/100 kg seeds, corresponding to 5 000 mg methiocarb/kg seeds.

Table 10.1.1- 7: Tier 1 risk assessment for birds feeding on treated seeds

Compound	Generic focal species	Toxicity [mg/kg bw/d]	Exposure			TER_{LT}	Trigger
			FIR/bw	NAR [mg a.s./kg seeds]	f_{twa}		
Methiocarb	Large granivorous bird	4.51	0.1	5 000	0.53	**0.017**	5

Bold values do not meet the Tier 1 TER trigger

Birds feeding on crop seedlings

Table 10.1.1- 8: Tier 1 risk assessment for birds feeding on crop seedlings

Compound	Generic focal species	Toxicity [mg/kg bw/d]	Exposure		TER_{LT}	Trigger
			SV*	f_{twa}		
Methiocarb	Small omnivorous bird	4.51	500	0.53	**0.017**	5

Bold values do not meet the Tier 1 TER trigger
* SV = 0.5 x NAR/5

The TER_{LT} values for birds feeding on treated seeds and crop seedlings do not meet the trigger of 5 for long-term exposure. Accordingly, a refined risk assessment is needed.

Refined risk assessment

A. Ingestion of seeds

A granivorous bird exposed to Methiocarb FS 500 treated maize will not continue with the ingestion of higher amounts over several days or weeks, because of emergence of the seeds and the inherent repellency properties of the active substance. Therefore the same assumptions as for the refined acute assessment (see above) can be made: Low attractiveness of treated maize fields (bare fields) reduced number of seeds on the soil due to precise drilling.

B. Ingestion of seedlings

For the refined risk assessment, the maximum residue of 0.09 mg as/kg fresh weight determined in maize and small omnivorous birds are used (for more information see 10.1.1, refinement for ingestion of seedlings).

Table 10.1.1- 9: Refined chronic TER calculation for birds feeding on crop seedlings – using the small omnivorous bird and maximum measured residue level in maize seedling

Compound	Focal species	Toxicity [mg a.s./kg bw]	FIR/bw	Residues [mg as/kg fresh wt.]	f_{TWA}	$TER_{LT, ref}$	Trigger
Methiocarb	Small omnivorous bird	4.51	0.52*	0.09	0.53	182	5

* EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) – Appendix A (Tier 1 tables for birds, Maize: BBCH 10-29)

For small omnivorous birds the refined long-term TER value is above the a priori acceptability criterion (TERLT ≥ 5).

Overall conclusion on risks to birds

Within a very conservative and formal Tier I risk assessment the a priori acceptability criterions were demonstrated for scenarios where birds are feeding on plants growing on treated fields (in acute short- and long-term time scales).

Refining the acute oral and short/long-term dietary risk assessment for direct seed ingestion by considering more realistic studies and literature data, in relation to factors such as avoidance, repellency and bird behaviour and in which different bird species were exposed to rates of methiocarb according to the GAP showed no toxic effects. Therefore, the risk to birds from methiocarb treated maize seeds is expected to be low.

It can be concluded that the use of Methiocarb FS 500 as a maize seed treatment will not pose an unacceptable risk to avian wildlife under the conditions of good agricultural practice.

Acute risk assessment for birds drinking contaminated water from pools in leaf whorls

EFSA (2009, chapter 5.2.1) proposes to focus the risk assessment for birds and mammals on the dietary route of exposure. An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER greater than direct dietary consumption.

Long-term risk assessment for birds drinking contaminated water in puddles

An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER greater than direct dietary consumption.

RISK ASSESSMENT OF SECONDARY POISONING

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds if feeding on contaminated prey like fish or earthworms. A log P_{OW} > 3 is used to indicate a potential for bioaccumulation.

Table 10.1.1- 10: Log Pow values of methiocarb and its metabolites

Substance	log P_{OW}	compartment	Reference
Methiocarb	3.18	Soil	MCA, Section 2, point 2.7

For methiocarb, a log P_{OW} of 3.18 (pH 7, 20°C; see MCA, Section 2, point 2.7) was determined. Thus, bioaccumulation in bird prey like earthworms is considered possible. Therefore, a risk assessment for the active substance considering a generic earthworm eating bird is provided in the following.
As the compound is intended to be applied as seed treatment, the exposure of aquatic organisms to methiocarb will be very limited. Therefore, risk of bioaccumulation for fish eating birds exposed to methiocarb will be presented for information only.
Although, the log P_{OW} of Methiocarb-phenol is > 3, no secondary poisoning risk assessment was conducted because it has already been shown that possible effects of metabolites of methiocarb are covered by the risk assessment presented for the active substance methiocarb. Furthermore, a bioaccumulation study, presented in document MCA, Section 8.2.2.3, shows that due to the low bioconcentration factor, methiocarb-phenol (M03) is highly unlikely to accumulate in the aquatic food chain.

Table 10.1.1- 11: Avian generic focal species for the Tier 1 risk assessment of secondary poisoning

Generic avian indicator species	Body weight [g]	FIR [g]	FIR/bw
Earthworm eater	100	104.6	1.05
Fish eater	1000	159	0.159

Table 10.1.1- 12: BCF calculation for earthworms

parameter	Methiocarb
P_{OW}	3.18
K_{OC} [mL/g]	627
f_{OC}	0.02
BCF_{worm}	$BCF_{worm} = (0.84 + 0.012 \times P_{ow}) / f_{oc} \times K_{oc}$
	0.070

Long-term DDD and TER calculation for earthworm-eating birds

Table 10.1.1- 13: Tier 1 long-term DDD and TER calculation for earthworm-eating birds

Compound	Maize
Methiocarb	
BCF_{worm}	0.070
PEC_{soil} (twa, 21 d) [mg/kg]	0.123
PEC_{worm} [mg/kg]	0.009
FIR/bw	1.05
DDD [mg/kg bw/d]	0.009
NO(A)ED [mg/kg bw/d]	4.51
TER_{LT}	499
Trigger	5

The TER value for methiocarb is above the trigger of 5. Accordingly the risk to earthworm-eating birds following the use of the product in all relevant crops is acceptable. This is also in accordance with the study of [redacted]; 2015; M-535901-01-1

Long-term DDD and TER calculation for fish-eating birds

Table 10.1.1- 14: Tier 1 long-term DDD and TER calculation for fish-eating birds

Compound	Maize
Methiocarb	
BCF_{fish}	90
PEC_{SW} (twa, 21 d)[mg/L]	0.0008556
PEC_{fish} [mg/kg]	0.0770
FIR/bw	0.159
DDD [mg/kg bw/d]	0.01224
NO(A)ED [mg/kg bw/d]	4.51
TER_{LT}	368
Trigger	5

The TER value is above the trigger of 5. Accordingly the risk to fish-eating birds following the use of the product in all relevant crops is considered acceptable.

CP 10.1.1.1 Acute oral toxicity

Study already evaluated during the first Annex I inclusion (see Table 10.1.1- 1). No new studies were required.

CP 10.1.1.2 Higher tier data on birds

Report:	KCP 10.1.1.2/01[redacted].; 2001; M-031252-01-1
Title:	Attractiveness of freshly drilled maize fields for large seed eating birds
Report No.:	BAR/FS 005
Document No.:	M-031252-01-1
Guideline(s):	no specific guideline available
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Material and methods:

The study was performed in the Lower Rhineland in Germany on 10 study fields in a region where maize is widely cultivated (District of [redacted]). While all fields were evaluated to determine the number of seeds remaining on the soil surface after drilling, 5 fields were selected for bird observations (1 ha to 7 ha plots). Two exposure scenarios were tested, i.e. normal drilled fields and reference fields. On the latter, untreated seeds were dispersed on the surface of the ploughed and harrowed soil before drilling. Areas of 2 times 0.25 ha on field no. 1 (2x 150 seeds laid out) and 1 ha each on field no. 2 and 7 (600 seeds laid out) were designed as reference fields. As the seeds were uncovered, this represented a worst case in terms of exposure. The largest field (field no. 1) was used both as a reference field before drilling and for observation after drilling.

<u>Observations:</u>

Exposure of maize seeds after drilling was measured on Day 0 by counting all visible seeds within areas of 2500 m^2 (50 x 50 m) situated in both the midfield and endrow area. On 3 of the drilled and on 3 reference fields bird observations were carried out. It was investigated if the birds were particularly attracted by this increased exposure. Bird species present, number of individuals and behaviour (feeding rates and sort of food taken up) was recorded by means of „Scan-Sampling" (one observation interval every 5 minutes). However, observation sessions differed in their duration after drilling and on Day 1. Therefore, for the data analysis, the data sets were made comparable independent of the duration of each observation event. An index was calculated.

Observed birds were categorised by body weight (small and large birds (> 50 g bw)) and species which might take up seeds and those which do not feed on seeds. Observations were performed after dispersing/drilling of seed on Day 0 until dusk and on the following day for the whole daylight period.

For the feeding rates, on reference fields only food uptake within the designed parts was analysed. Single birds were observed for as long as possible and the frequency of food uptake as well as the kind of food was documented (maize seeds or other objects, as far as recognised). A pecking rate was calculated which reflects the number of food objects taken up per minute and includes both uptake of maize and other food items.

Results:
On all fields, the number of remaining seeds was higher in the end row areas than in the midfield. However, the amount of seeds was low. A maximum of 0.108 seeds/m^2 was counted in the endrow area and the mean was 0.042 seeds/m^2. In the midfield, a maximum of 0.024 seeds/m^2 and a mean of 0.007 seeds/m^2 was found. Due to the low exposure, pecking rates were low to moderate (0.1-18.5 food item/min.).

In total, 21 different bird species visited the test fields during the 2 observation days. Most of them were larger birds (17 species). Between 2 and 13 bird species were recorded per field. The number of individuals of a certain species was rather small. Two flocks were recorded, i.e. 31 woodpigeons on drilled field (no. 6 on Day 1) and 37 rock doves on reference field (no. 7 on Day 1). Carrion crow, pheasant and woodpigeon were most abundant on both types of fields and all belong to the group of large seed eating birds (> 50 g bw). Except on field no. 1 (Day 0 and 1), small seed eating species did not occur and in no case was a consumption of maize observed for the large seed eating birds, a difference in foraging was observed between the 2 types of field. The following table gives an overview about the percentage of large seed eating birds which were seen eating maize seeds.

Percentage of foraging large seed eating birds (in brackets number of species recorded)

	Drilled fields			Reference fields whole field		
Field	no. 1	no. 5	no. 6	no. 2	no. 7	
area observed	7 ha	1 ha	2.5 ha	5 ha	3 ha	
Day 0	55-100 (n=6)	0-60 (n=4)	50-100 (n=7)	0-29 (n=5)	50 (n=1)	0-100 (n=4)
Day 1	63-100 (n=6)	33-50 (n=2)	58-100 (n=7)	0-89 (n=6)	0-25 (n=2)	37-100 (n=6)
				re-designed part (% of all foraging individuals)		
area observed				0.5 ha	1 ha	1 ha
Day 0	-	-		100 (n=1)	-	4-33 (n=3)
Day 1	-	-		0 (n=0)	-	13-60 (n=6)

The higher foraging activity on drilled fields compared to reference fields was explained by the birds being used to finding food on a field after it has been worked on by a machine. Accordingly, the mere availability of maize seeds (as represented by the reference fields) is not a sufficient factor for attractiveness to seed eating birds.

From all large seed eating birds, only carrion crow, pheasant and woodpigeon were observed feeding on maize seeds. Thus, these 3 species only were considered for analysis of feeding behaviour in the table below.

Maximum number, resting time and food uptake of large seed eating birds (species considered were carrion crow, Jackdaw, pheasant, rock dove, stock dove and woodpigeon)

		Drilled fields			Reference fields designed part		
	Field	no. 1	no. 4	no. 6	no. 1	no. 2	no. 7
		maximum number (sum of all species)					
	Day 0	16	16	15	1	0	5
	Day 1	20	3	39	1	0	2
		resting time per group [min.]					
	Day 0	5-15	5-50	5-5[illegible]	5	-	5-1[illegible]
	Day 1	5-25	5-35	5-45	5	-	5-10
		food consumed (maize + other food items = total)					
Carrion crow	Day 0	25+16	0+128	5+3[illegible]	- / -	- / -	2+89 = 91
	Day 1	1+395	0+207	2+193	- / -	- / -	29+28 = 1[illegible]
Jackdaw	Day 0	- / -	- / -	0+12	- / -	- / -	- / -
	Day 1	- / -	- / -	- / -	- / -	- / -	6+15 = 21
Pheasant	Day 0	- / 3	0	4+667	12+5	- / -	1+19 = 20
	Day 1	- / -	0+148	0+613	1+2	- / -	35+41 = 76
Woodpigeon	Day 0	17+12	11+[illegible]3	- / -	- / -	- / -	7+25 = 34
	Day 1	18+93	- / -	20+397	- / -	- / -	7+42 = 49

In almost all cases, the amount of seeds consumed on the maize fields was significantly lower compared to other food items taken up. The figures for reference field no. 1 derived from only 1-2 pheasants observed. Maize seeds represented only 1-5% of the total food objects taken up by carrion crow, pheasant and woodpigeon. However, on reference field no. 7 and day 1, maize contributed to 25% of the total diet taken up from the birds on the maize fields.

The abundance of the species was different on Day 0 and 1 of the respective fields. Since the maximum number on a certain field was recorded on different days, it was not clearly related to the event of drilling. This applied for both types of field. Flocks of birds did not arrive immediately after drilling. Single birds were observed regularly, but they did not stay for long (resting time given per group). Obviously, there were enough other and richer feeding habitats.

Conclusion:
Freshly drilled maize fields do not increase the attraction to birds. Only large seed eating birds were observed eating maize. However, the portion of maize seeds of the total food taken up on the maize fields was rather low (mostly 1-5%, on one field and day 25%). Small seed eating birds were only seen on 2 days on one field, but they have not taken up any maize seeds.

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Report:	KCP 10.1.1.2/02[redacted],; 1993; M-035105-01-2
Title:	Feeding trial with dressed wheat seed to determine the repellent effect of Mesurol 500 FS (100 g a.i./dt) on rooks (*Corvus frugilegus*) and carrion crows (*Corvus corone*)
Report No.:	SBJ050/93
Document No.:	M-035105-01-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Material and methods:

Choice feeding tests were conducted in aviaries to determine the repellent effect of Mesurol-treated wheat seed (100 g as/dt) on rooks and crows. Six rooks (*Corvus frugilegus*) and one carrion crow (*Corvus corone*) were captured in the wild. The aviaries were each divided into two cages with a floor area of 2 m x 3 m, although in this trial they are intended for single occupancy. The side walls of the individual aviaries are fitted with screens. After a seven-day conditioning phase the birds were moved to single aviaries and offered a free choice of the test diet, a reference diet (Sibutol-Mordit-treated wheat seed) and untreated wheat seed. This feeding trial (acceptance test) extended over five days. At the beginning the birds were weighed and then returned to the aviaries they had occupied during conditioning (one bird per cage). Every day a selection of 50 g test diet (approximately equivalent to a crow's daily feed intake), 50 g reference diet and 15 g untreated feed (10 g wheat seed and 5 g minced meat) was provided between 08.00 and 16.00 hours. In order to compensate for side preferences the feeding bowls were switched every day. The birds had no access to feed from 4 p.m. to 8 a.m. Water was available ad libitum. As the amount of test and reference diet offered daily was approximately equivalent to a rook's daily feed ration, but the untreated feed supplied only 30% of this daily ration, a pressure situation developed which became more acute from day to day. The acceptance test was followed by a seven-day follow-up period. The birds remained in single cages during this time. The usual maintenance diet was provided (see above) and drinking water was available ad libitum.

Observations:

During the acceptance test with Mesurol-treated seed (repellent test), the birds were monitored during the day with a CCTV system. Feed consumption was measured daily. After completion of the trial the birds were weighed again. At the end of the follow-up period the birds were weighed again

Results:

The average daily quantities of Mesurol-treated wheat seed consumed were between 0 and 1.2 g. Some rooks ate up to 3.0 g of the test diet on one day. The mean feed intake per day and rook was 0.84 g (± 0.87). The carrion crow (No. 3 in Table 10.1.3/01) refused the treated seed almost completely; only on the fourth day did it eat just 03 g of the test diet. As the pressure became more acute, the amounts of test diet consumed remained consistently low. The carrion crow refused the test and reference diet almost completely. The birds lost weight during the conditioning phase and the feeding test. The average weight of the birds fell by a further 10.3 % during the acceptance test. All were apparently free of symptoms throughout the entire trial and displayed normal behaviour. The birds' bodyweight increased again during the follow-up period.

Feeding trial with individually caged rooks and crows to determine the repellent effect of Mesurol-treated wheat seed (100 g a.s./dt). Each bird was offered 50 g of Mesurol-treated wheat seed (ME) daily, 50 g of Sibutol-Morkit-treated wheat seed (MO reference diet) and 10 g of untreated wheat (UB) (+ 5 g minced meat). Feed intakes shown in g.

Bird No.	Day 1			Day 2			Day 3			Day 4			Day 5		
	ME	MO	UB	ME	MO	UB	ME	MO	UB	ME	MO	UB	ME	MO	UB
1 C.f.	0.4	0.3	10.0	0.1	0.0	10.0	0.1	0.0	10.0	0.5	0.0	10.0	0.0	0.2	10.0
2 C.f.	1.2	0.5	10.0	0.6	0.9	10.0	2.1	0.0	10.0	2.6	0.1	10.0	0.2	0.0	10.0
3 C.c.	0.0	0.0	10.0	0.0	0.0	10.0	0.0	0.0	10.0	0.3	0.0	10.0	0.0	0.0	10.0
4 C.f.	1.0	1.2	10.0	0.7	0.0	10.0	0.3	0.1	10.0	1.1	0.3	10.0	1.6	0.3	10.0
5 C.f.	0.2	0.4	10.0	0.8	0.0	10.0	0.3	0.0	10.0	0.5	0.3	10.0	0.0	0.0	10.0
6 C.f.	0.4	0.9	10.0	0.6	0.6	10.0	1.3	0.9	10.0	0.6	1.4	10.0	0.3	1.1	10.0
7 C.f.	2.3	0.5	3.6	0.3	0.6	10.0	3.0	0.3	10.0	0.5	1.2	10.0	0.2	0.0	10.0
M	0.8	0.5	9.1	0.4	0.3	10.0	1.0	0.2	10.0	1.2	0.5	10.0	0.8	0.4	10.0

C.c. = *Corvus corone* (carrion crow); C.f. = *Corvus frugilegus* (rook)
M = mean

Conclusion:
Mesurol 500 FS in the tested concentration of 100 g a.s./dt had a good repellent effect on rooks and crows.

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Report: KCP 10.1.1.2/03 [redacted]; 1992; M-013181-01-2
Title: Feeding trial with dressed wheat seed to determine the repellent effect of Mesurol 500 FS (500 g a.i./dt) on rooks (Corvus frugilegus) and carrion crows (Corvus corone)
Report No.: SBJ074/92
Document No.: M-013181-01-2
Guideline(s): -
Guideline deviation(s): -
GLP/GEP: **no**

Material and methods:
Choice feeding tests were conducted in aviaries to determine the repellent effect of Mesurol-treated wheat seed (500 g a.s./dt) on rooks and crows. Four rooks (*Corvus frugilegus*) and three carrion crows (*Corvus corone*) were captured in the wild. The aviaries were each divided into two cages with a floor area of 2 m x 2 m, although in this trial they are intended for single occupancy. After a seven-day conditioning phase the birds were moved to single aviaries and offered a free choice of the test diet, a reference diet (Sibutol-Morkit-treated wheat seed) and untreated wheat seed. This feeding trial (acceptance test) extended over five days. At the beginning the birds were weighed and then returned to the aviaries they had occupied during conditioning (one bird per cage). Every day a selection of 50 g test diet (approximately equivalent to a crow's daily feed intake), 50 g reference diet and 15 g untreated feed (10 g wheat seed and 5 g minced meat) was provided between 08.00 and 16.00 hours. In order to compensate for side preferences the feeding bowls were switched every day. The birds had no access to feed from 4 p.m. to 8 a.m. Water was available ad libitum. As the amount of test and

reference diet offered daily was approximately equivalent to a rook's daily feed ration, but the untreated feed supplied only 25% of this daily ration, a pressure situation developed which became more acute from day to day. The acceptance test was followed by a seven-day follow-up period. The birds remained in single cages during this time. The usual maintenance diet was provided (see above) and drinking water was available ad libitum.

Observations:
During the acceptance test with Mesurol-treated seed (repellent test) the birds were monitored during the day with a CCTV system. Feed consumption was measured daily. After completion of the trial the birds were weighed again. At the end of the follow-up period the birds were weighed again.

Results:
The average daily quantities of Mesurol-treated wheat seed consumed were between 0 and 0.3 g. The untreated seeds were consumed almost completely every day. Only carrion crow No. 3 refused to eat the untreated wheat on day 1 of the test. For the first three days of the trial the birds refused the Mesurol-treated wheat seed entirely. Small amounts of 0.1 g on average were only consumed on days 4 and 5. As the pressure became more acute, feed intakes remained consistently low. No difference was observed between the feeding behaviour of the rooks and that of the carrion crows. The birds lost weight during the conditioning phase and the feeding test. Six of the seven birds were apparently free of symptoms throughout the entire trial and displayed normal behaviour. The birds' bodyweight increased again during the follow-up period. One carrion crow developed a bacterial infection and died on day 7 of the follow-up.

Feeding trial with individually caged rooks and crows to determine the repellent effect of Mesurol-treated wheat seed (500 g as/dt). Each bird was offered 50 g of Mesurol-treated wheat seed (ME) daily, 50 g of Sibutol-Morkit-treated wheat seed (MO reference diet) and 10 g of untreated wheat (UB) (+ 5 g minced meat). Feed intakes shown in g.

Bird No.	Day 1			Day 2			Day 3			Day 4			Day 5		
	ME	MO	UB	ME	MO	UB	ME	MO	UB	ME	MO	UB	ME	MO	UB
1 C.c.	0.0	0.2	10.0	0.0	0.9	10.0	0.0	0.4	10.0	0.3	0.4	10.0	0.0	0.0	10.0
2 C.f.	0.0	0.0	10.0	0.0	0.0	10.0	0.0	0.0	10.0	0.5	1.3	10.0	0.3	0.0	10.0
3 C.c.	0.0	0.0	0.0	0.0	1.0	10.0	0.0	0.0	10.0	0.3	1.7	10.0	0.0	0.0	10.0
4 C.c.	0.0	0.2	10.0	0.0	0.7	10.0	0.0	0.9	10.0	0.2	0.2	10.0	0.1	0.0	10.0
5 C.f.	0.0	0.0	10.0	0.0	0.0	10.0	0.0	0.0	10.0	0.2	0.7	9.9	0.4	1.9	10.0
6 C.f.	0.0	0.0	10.0	0.0	0.0	10.0	0.0	0.9	10.0	0.8	3.0	10.0	0.7	2.3	10.0
7 C.f.	0.0	0.4	10.0	0.0	0.2	10.0	0.0	0.0	10.0	0.0	1.0	10.0	1.1	0.1	10.0
M	0.0	0.1	8.6	0.0	0.4	10.0	0.0	0.3	10.0	0.3	1.2	10.0	0.3	0.6	10.0

C.c. = *Corvus corone* (carrion crow) C.f. = *Corvus frugilegus* (rook)
M = mean

Conclusion:
Mesurol 500 FS in the tested concentration of 500 g as/dt had a good repellent effect on rooks and crows.

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Report:	KCP 10.1.1.2/04 [redacted]; 2001; M-039873-01-1
Title:	Acceptance of H 321 FS 500 treated maize seeds (0.5 kg methiocarb / 100 kg seeds) by grey partridges (*Perdix perdix*)
Report No.:	BAR/ANN 032
Document No.:	M-039873-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Material and methods:

In pre-tests singly caged partridges did not consume maize. Therefore, a special acclimatisation procedure was applied: The birds received wet maize seeds over an eight hour exposure period (day –2) and were housed in groups of five for the whole study. The following day (day –1) they were offered dry maize seeds as well as wet seeds, followed by another starvation period of 16 hours. Consumption was low and not all birds consumed maize seeds; exclusive feeding of maize caused severe reduction in body weights (mean about 10% in 3 days). Therefore the described acclimatisation was limited to 2 days prior the exposure.

On the exposure day (day 0) maize seeds, treated with H 321 FS 500 (0.5 kg as/100 kg seeds) were administered to 20 domestic grey partridges in 4 groups for 8 hours: Two groups of five partridges received 150 dry coloured treated maize seeds each, whilst the other two groups received 150 damp treated coloured maize seeds (moistured overnight).

Results:

Test substance:	Methiocarb FS 500
Test object:	Grey partridges *(Perdix perdix)* male, female
Exposure:	Coated maize seed (0.5 kg as/100 kg maize) (wet seeds and dry seeds)
Results and observations:	No mortality No signs of intoxication Low attractiveness of maize as food item Avoidance of treated seeds: total consumption of all birds decreased from 93 untreated seeds to 3 seeds (reduction of 97%), in the two seed eater groups from 92 to 0 (reduction of 100%).

Observations:

After the exposure the remaining maize seeds were removed and counted. The birds were observed for signs of intoxication as well as for effects on feed consumption and body weight. Body weight was measured at the beginning of the acclimation, the day of exposure and at the end of the study. Exposure was followed by a subsequent observation period of 3 days, during which only untreated standard seed diet was offered.

Conclusion:

Although maize seed is not a preferred food source for partridges, 2 groups of partridges could successfully be trained during the acclimatisation period to consume a limited amount of seed, whilst 2 other groups refused maize as food item. Maize seed treated with Mesurol FS 500 was almost completely avoided by all groups, including the maize eaters. This clearly demonstrates the repellency of methiocarb treated seeds. Based on the low attractiveness of maize seeds as a food item together

with a high repellency due to the treatment, the risk to ingest hazardous doses of methiocarb can be considered very low for seed eating birds as represented by partridges.

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Report:	KCP 10.1.1.2/05 [redacted] C; 2001; M-048267-01-1
Title:	Acceptance of H 321 FS 500 treated maize seeds (0.5 kg a.i./100 kg seed) by domestic pigeons (*Columba livia f. domestica*) under aggravated conditions
Report No.:	BAR/ANN 019
Document No.:	M-048267-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Material and methods:

After one week of acclimatization to the tested food item, maize seeds, treated with H 321 FS 500 (0.5 kg ai/100 kg seeds) were administered to 10 singly housed domestic pigeons for 8 hours, following a 16-hours starvation-period: 30 g treated maize and 10 g of standard food were spread out on plastic trays in each aviary. After the exposure the remaining food was removed and reweighed.

The birds were observed for signs of intoxication as well as for effects on feed consumption and body weight. The same exposure scenario was repeated on the next two days.

Body weight was measured at the beginning of the acclimatization, the day before exposure, after the last exposure period and at the end of the study. Exposure was followed by a subsequent observation period of 3 days, during which only untreated standard seed diet was offered.

Results:

Test substance:	H 321 FS 500
Test object:	Domestic pigeon (*Columba livia f. domestica*) m,f
Exposure:	Treated maize seed (521.24 g a.s./ 100 kg maize)
Results and observations:	No mortalities No symptoms of intoxication Complete avoidance of the treated seeds Reduction of body weight during the 3 exposure days at all pigeons

Conclusion:

The significant reduction of the body weights during the 3 exposure days demonstrates the severity of the exposure scenario. Since even under these aggravated test conditions not a single treated maize seed was consumed, the inherent repellent properties of H 321 has to be considered strong enough to protect large granivorous birds as represented by the domestic pigeon from an intake of a hazardous dose.

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Report:	KCP 10.1.1.2/06 [redacted]; 1984; M-013213-01-2
Title:	Aviary trial to determine the repellent effect of Mesurol 500 FS in maize against pheasants
Report No.:	V-84189
Document No.:	M-013213-01-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Material and methods:
Two aviary trials were conducted to determine the repellent effect of maize seed treated with Mesurol 500 FS (10 ml per 1 kg maize) on pheasants. The first trial was carried out with two males and one female pheasant, and the second trial with four females.

Four test aviaries were available; aviaries 1 and 4 had a floor area of 2.35 m x 1.70 m (= 4 m^2) and aviaries 2 and 3 a floor area of 2.80 m x 1.45 m (= 4 m^2). The aviaries were 2.00 m high. The aviary floor was constructed of plastered concrete and surrounded by 60 cm high walls, also concrete. The sides above the walls were wire mesh. The aviary floor was covered with a 5 mm layer of quartz sand (F 31, Frechener Quarzwerke). Each aviary had two perches: one in the right half and one in the left half of the aviary. The four aviaries were arranged in a row in a shady, quiet spot outdoors and protected from the elements by a transparent corrugated polyester roof.
The conditioning of the pheasants in the aviaries began 10 days before commencement of the trial. In order to ascertain any side preferences and to test the pheasants used to the diet, maize seed was offered in pricking out cups. Two small pricking out cups (9 x 9 x 5.5 cm) were placed alongside each other in a large grey pricking out tray (40 x 30 x 5 cm) and secured with small blue hose clips. The small cups were covered with wire netting (mesh size 2 mm) to prevent scattering of the grains. The netting was fixed at a height of 0.5 cm from the bottom of the cup. 200 maize seeds were placed into each cup. After an exposure time of 24 hours the remaining seeds were collected and counted. The cups were then replenished with the original amount of seed. Water and grit were available ad libitum throughout the trial. During the acceptance test that followed the conditioning phase the amount of untreated feed was restricted to 25 % of the normal daily feed ration, with the test diet providing 75 % of the normal feed ration. This created an increasingly acute pressure situation during the 5-day trial.. The daily feed ration for cereals stipulated in test guideline 25-1 is 70 g, and for hens 50 g. The locations of the feeding dishes in the aviary were switched daily.

Observations:
Feed consumption was measured by counting the grains. The birds were weighed at various times during the trial.

Results:
The untreated diet was always eaten completely, whereas only a few grains of the test diet were consumed. Even as the pressure grew more acute, especially on days 4 and 5 of the trial, the pheasants did not eat more of the treated grains than during the first few days.
This demonstrates the strong repellent effect of Mesurol, especially if one considers that the birds ingested only 25 % of their normal daily feed ration (= untreated feed).

During the trial and the subsequent follow-up period no adverse effects were observed in the pheasants.
The results are summarised in Tables 10.1.8/05 - 10.1.8/06.

Feeding results of aviary trial 1 to determine the repellent effect of Mesurol against pheasants (n = number of maize grains)

		Pheasant 1 ♂		Pheasant 2 ♂		Pheasant 3 ♀	
Day of trial	Untreated Treated	Dispensed (grains)	Eaten (grains)	Dispensed (grains)	Eaten (grains)	Dispensed (grains)	Eaten (grains)
1	Untreated	65	65	65	64	47	47
	Treated	200	0	200	1	200	1
2	Untreated	66	66	65	65	46	46
	Treated	200	0	200	2	200	6
3	Untreated	65	65	66	66	46	46
	Treated	200	1	200	14	200	0
4	Untreated	67	67	66	66	46	46
	Treated	200	1	200	15	200	0
5	Untreated	65	62	65	65	46	46
	Treated	200	1	200	0	200	0

Feeding results of aviary trial 2 to determine the repellent effect of Mesurol against pheasants (n = number of maize grains)

		Pheasant 4 ♀		Pheasant 5 ♀	
Day of trial	Untreated Treated	Dispensed (grains)	Eaten (grains)	Dispensed (grains)	Eaten (grains)
1	Untreated Treated	47 200	47 0	46 200	46 0
2	Untreated Treated	47 200	47 0	48 200	46 0
3	Untreated Treated	47 200	47 0	46 200	46 0
4	Untreated Treated	48 200	48 0	47 200	47 1
5	Untreated Treated	47 200	47 1	47 200	47 3

		Pheasant 6 ♀		Pheasant 7 ♀	
Day of trial	Untreated Treated	Dispensed (grains)	Eaten (grains)	Dispensed (grains)	Eaten (grains)
1	Untreated Treated	47 200	41 0	46 200	46 0
2	Untreated Treated	47 200	47 0	47 200	47 0
3	Untreated Treated	47 200	47 0	46 200	46 1
4	Untreated Treated	47 200	47 3	46 200	46 0
5	Untreated Treated	48 200	48 12	46 200	46 3

Conclusion:
In a 5-day aviary trial, maize seed treated with Mesurol FS 500 (10 ml per 1 kg maize) was avoided by pheasants in an increasingly acute pressure situation. The trial confirms the known repellent effect of the product.

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Report: KCP 10.1.1.04 [redacted]; 1995; M-042897-01-1
Title: Risks of granules and treated seeds to birds on arable fields
Report No.: Lit. 5346
Document No.: M-042897-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

Before a pesticide is approved for use in the Dutch market, an assessment must be made of the risk of its use to non-target organisms. This study considers the extent to which use of pesticides, in the form of granules or seed-treatment agents, constitutes a potential risk to birds. If treated seeds or granules remain on the field surface during drilling, they may be picked up by birds for two reasons: seeds may be taken for food, or granules and pelleted seeds may be taken for potential grit (small stones used by birds to grind down their food). There are presently a number of gaps in the knowledge required to assess the risk of using such granules and seed-treatment agents. First of all, nothing is known about the grit consumption of farmland birds in the Netherlands. In addition, it is unknown what proportion of seeds remains on the surface after drilling. This study therefore has a twofold aim:

1. to describe the grit particles consumed by farmland birds, establish their resemblance to granules and pelleted seeds, and assess the resulting risk to these birds, and
2. to estimate the number of treated seeds remaining available to birds at the soil surface after drilling various crops, establish factors of influence and incorporate these in a risk assessment procedure.

Part 1: Resemblance between grit and granules/pelleted seeds

In order to describe the grit in bird gizzards, the gizzard content of some 200 birds of varying size and diet (e.g. granivores and non-granivores) was examined. The grit particles in the gizzards were counted and the size, shape and colour of those particles larger than 0.5 mm were determined. The results show that the grit particles recovered from granivores differ in size from those recovered from all other groups. This group of birds have comparatively more grit particles in their gizzards. Small granivores such as sparrows and finches mainly consume particles somewhat larger than 1 mm. Large granivores such as woodpigeons and pheasants have particles of 2-3 mm in their gizzards. In the non-granivores large numbers of very small particles (< 0.5 mm) were found. Particles this size cannot possibly have been picked up individually. The shape of the grit particles was virtually the same for all bird groups, about 1.4 times longer than wide. No correlation was found between the colour (chroma) of the grit and the bird groups. However, large granivores were found to pick up lighter particles than the other bird groups. This may indicate selectivity in the case of these birds, or, alternatively, a correlation between grit size and the nature of the parent material.

The size, shape and colour of the grit found in bird's gizzards were compared with a number of granules and pelleted seeds in common use in the Netherlands. It was fount, that, in terms of size, small granules show a strong resemblance to the grit consumed by non-granivores and small granivores. The larger granules (pellets used to control slugs) show a stronger resemblance in size to the grit picked up by large granivores. The pelleted seeds investigated show only a slight overlap in size with the grit used by large granivores. On the basis of the resemblance between bird grit on the one hand and granules and pelleted seeds on the other, an estimate has been made of the potential risk to birds foraging on drilled fields. In doing so, the following factors were also given due consideration: dose and toxicity of the pesticides employed, availability of granules and pelleted seeds, number of particles consumed daily and foraging strategy employed. It was found that small granivores run the greatest risk. Of the pesticides, the small granules (approx. 1 mm) appear to be pose the greatest risk to small granivores, and the larger granules (slug pellets) to large granivores. The pelleted seeds also appear to pose a risk to large granivores, although to a lesser degree.

Part 2: Availability of treated seeds resembling natural food

Field research to establish the number of seeds remaining on the field surface was undertaken in nine arable crops in various districts of the Netherlands. These crops were drilled using various techniques (standard and precision) and the seeds were of various size. Sampling to establish the number of surface seeds post-drilling was performed at field centres and on headlands. Depending on the crop, counts were carried out in the spring or autumn. At a number of sites it was also investigated how long the seeds remain on the surface post-drilling.

The research results indicate that the greatest number of seeds remains on the field surface after drilling of a winter wheat crop (autumn sowing). Even after correcting for seed density, it is in this crop that the highest proportion of seeds remains on the surface. The main factors of influence on the number of surface seeds are drilling technique, soil condition (seed-bed quality) and position in the field: headland or field centre. In standard-drilled fields 4 times more surface seeds were found on average than in precision-drilled fields. In cereal crops an average of 13 times more surface seeds were found in the autumn than in spring, probably as a result of soil condition. On headlands, finally, an average of 4 times more surface seeds were found than at the field centre. The study also investigated the number of seed spill spots in fields, at places where drilling machines are filled, for example. It was found that in some fields the total number of seeds at such spill spots is comparable with the number of seeds remaining on the surface post-drilling. The number of surface seeds declines in the period post-drilling. In the autumn of 1994 it was found that 50% of the surface seeds had disappeared after about 6 days (in winter wheat). In the autumn of 1993 this period was more than 14 days, however. The results of the field study have been used to arrive at a risk estimate for several crop protection agents, crops and bird species. In doing so, optimal foraging theory has also been taken into consideration.

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Report:	KCP 10.1.1.2/56 [redacted] C; 2009; M-359439-01-1
Title:	Field monitoring of birds and mammals on maize seeds, treated with Methiocarb FS 500 (1.5mg a.s./seed) in Germany 2009
Report No.:	BAR/FS050
Document No.:	M-359439-01-1
Guideline(s):	The test was designed for the purpose of this study.
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The study aimed to monitor the bird and mammals population in regard of potentially increased mortality after the drilling of maize, treated with methiocarb FS 500.

Material and methods:

This field monitoring was performed on fields in 11 areas in Northwest Germany.

From each field, a sample of treated seeds was collected, which was analysed on the loading with methiocarb.

The exposure of maize seeds on the soil surface was determined on the drilling day (day 0). On each field, 80 squares (1 m x 1 m) on eight transect lines of 50 m (4 in midfield area, 4 in endrow areas, per

transect 10 squares) were randomly chosen, on which the number of remaining maize seeds was counted.
After the application, on each site 2 carcass searches for dead or impacted birds and mammals were performed (day +1 and +3). During the carcass search, a team of 2 – 4 people paced the test area. The team walked along the maize field in parallel rows.
Objective of the carcass search was to collect all carcasses and to determine them to species level. The place of finding, the circumstances of the finding and the conditions of the carcasses including signs of intoxication should be recorded. Appropriate carcasses should be submitted to residue analysis on methiocarb.
The efficiency of the search team was tested twice by disposing dummies. The activity of predatory birds and mammals which may influence the detection rate of carcasses by removing the carcasses was tested twice as well. On field 11 and on the fields 8 and 9 (relatively small fields which were not far away from each other) a defined number of dead quails were disposed for 24 hrs (field 11: 10 birds; field 8/9 15 birds) and then recollected.
On the application day, no carcass search was carried out in order not to chase the birds away. Instead of it, a bird observation of 2 hours was performed in the afternoon to scan for impacted birds. A further bird observation was carried out on day +1. During the bird observation all birds entering the field were recorded. Based on the results, the frequency of observance could be calculated for each species of concern.
Bird and mammals activities and indication of them (traces, de-husked seeds), which were detected during carcass searches, were recorded as well.

Results:

Test item	Maize seeds, treated with Methiocarb FS 500
Test object	Bird and mammal populations
Treatment related mortality	None

Methiocarb on maize seeds:
On the fields under monitoring a range of maize varieties and treatments were used. Based on the analytical results 7 of 11 fields fulfilled the requirement of the recommended methiocarb content. On 2 fields mixtures of different seed types were drilled, the main portion of the used seeds (pionier) fulfilled the requirement. In one field the loading rate was slightly below the 80 % value and on one field the maize contained not enough methiocarb for the purpose of the monitoring. Since the study provided also generic data, the findings of this field are as well reported.

Application and exposure:
The maize drilling was always performed as precise drilling). On the 11 fields 5 different machines were used (Amazone, Horsch, Becker, Mascar and Kleine). The diversity of different seed types and batches was high as well.
Although the differences in the use of equipments and seed types were high, the exposure of seeds on the surface of the fields was always similar and in general low:
In midfield areas the mean number of maize seeds per m² amounted to 0.06 (SD 0.10); in endrow areas it amounted to 1.85 seeds per m² (SD 1.42).
One spillage of ca. 250 seeds was detected on field 5, another one of ca. 100 seeds on field 7.

Bird observation:
The frequency of occurrence (FO) of the different birds is expressed in percentage related to all fields (n=11; FOfield) and related to all censuses (n=22; FOsurvey): The ranking list of birds according to FOfield was as follows:
Carrion Crow 90.9 %; Wood Pigeon: 72.7 %; Blackbird and White Wagtail: each 72.7 %; Lapwing: 63.6 % and Starling 54.5 %. Related to FOsurvey the most frequent bird species were the same ones with little differences in the ranking order.

The abundance of birds was low. All observed birds behaved normally and were above any suspicion of being impacted by Methiocarb.

Mammal observation:
Hills and burrows of Moles (*Talpa europaea*) and Northern Water Vole (*Arvicola terrestris*) were observed on the freshly drilled fields. On 11 places on field 2 we found de-husked maize seeds in areas with increased maize seed exposure. While the treated husk was remaining, the inner part with the germ was consumed. Since the husks contain the active ingredient, the de-husking is considered a successful strategy to avoid intoxication.

Carcass searches:
On each field 2 carcass searches were performed (day +1 and +3). In total, this activity took 27:55 hrs or 68:30 man hrs (hh:mm). No carcass was found. Some single feathers of the most abundant species (e.g. Wood pigeon, Rook) were regularly detected, but never a feather spot, which could be caused by predatory birds or mammals.

Conclusion:
The monitoring program aimed to describe and identify possible effects on birds and mammals after the drilling of maize seeds, treated with methiocarb.
Carcass searches and bird observation did not reveal any suspicion of intoxication or mortality of birds or mammals.
The exposure of maize seeds after precise drilling is low, even in endrow areas, where the number of seeds on the surface was slightly increased. Therefore the drilled field is not attractive for granivorous bird as demonstrated by the relative low abundance of birds on the fields.
Moreover methiocarb is known as an effective bird repellent. Since the birds of concern are large granivorous birds, ingestion of a single seeds is not sufficient to cause severe impacts on them but is adequate to initiate an avoidance reaction. The lack of findings at bird observation and carcass search are therefore not a surprise but verify the safe use of this product.
With the mammals, the small granivorous species are theoretically most at risk. Dead mice or other dead mammals were not found, but evidence for de-husking of treated maize seeds, which is considered to be a successful strategy to avoid intoxication.

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Report:	KCP 10.1.1.2/57[redacted]<; 2015; M-535901-01-1
Title:	Methiocarb FS 500 - A field study to evaluate residues of methiocarb, methiocarb-sulfoxide and methiocarb-sulfone in earthworms and carabids on bare soil drilled with methiocarb-treated maize seeds
Report No.:	S13-01825/EBMEN056
Document No.:	M-535901-01-1
Guideline(s):	based on the 'ISO Guideline 23611-1' (ISO, 2006) and the 'Technical recommendations for the update of the ISO earthworm field test guideline (ISO 11268-3)' (KULA et al., 2006)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Material and methods:

Test species: earthworms and carabids at the field site from soil to which Methiocarb FS 500-treated maize seeds were drilled once.

Test item: Methiocarb FS 500 (seeds dressed with Methiocarb FS 500, TOX-No. 10096-00, Batch-No. 2013-001632, nominal seed treatment rate per plot: 75 g Methiocarb/ 50,000 seeds; analysed seed treatment rate: 72.59 g Methiocarb/ 50,000 seeds).

The field study was carried out on bare soil in [redacted], Germany. The study consisted of one field trial: S13-01825-01 and one analytical trial: S13-01825-L1. The study included two treatment groups: One untreated control (C) and one test item group with methiocarb-treated maize seeds (T). The plot of 2,500 m^2 was defined as control plot before the drilling (application). Samplings were done 6 days before application, 2 days and 10 days after the application. Two different sampling methods were used: handsorting (earthworms) and pitfall trap sampling (carabids).

The climatic conditions during the trial compared to the long-term average (1961-1990) revealed slightly lower average temperatures for May and slightly higher temperatures for June. The rainfall at the field site was about 148 % of the long-term average in May and 105 % of the long-term average in June. The actual climatic conditions were recorded at a weather station approximately 25 m distance from the field site. Data of the long-term average were recorded at a weather station approximately 5.7 km distance from the field site.

Results:

The study was designed to determine the residue levels of methiocarb (MTC) and its metabolites (MTC-sulfoxide and MTC-sulfone) in earthworms and carabids over time following the drilling of methiocarb-treated maize seeds. For this purpose earthworms and carabids were caught once before the drilling of the methiocarb-treated maize seeds and two times (2DAA1 and 10DAA1) after the drilling. Different sampling methods were used to get earthworms and carabid samples for residue analysis.

Agricultural practices used for drilling of the methiocarb-treated maize seeds were according to good agricultural practice (drilling technique, row distance, seeding rate, field site preparation). The field trial of the study lasted from end of May 2013 until mid of June 2013.

The drilling (application) was performed on 04 June 2013. The drilling was performed using a commercial pneumatic drilling machine. The target rate was 100,000 seeds/ha, equivalent to 150.00 g a.i./ha (nominal). The deviation to the target drilling rate was +13.9 %.

Earthworm samples were taken once before the application and twice (2 DAA1 and 10 DAA1) after the application of methiocarb-treated maize seeds. No residues of methiocarb and the metabolites methiocarb-sulfoxide and methiocarb-sulfone could be detected in the sample taken before the application as well as in the samples taken at 2 DAA1 and 10 DAA1.

Carabid samples were taken once before the application and twice (2 DAA1 and 10 DAA1) after the application of methiocarb-treated maize seeds. No residues of methiocarb and the metabolites methiocarb-sulfoxide and methiocarb-sulfone could be detected in the sample taken before the application. Residues of methiocarb were found in the row in the sample of replicate d taken at 2 DAA1 (0.14 mg/kg) and in the sample of replicate b taken at 10 DAA1 (<LOQ). No further residues of methiocarb and the metabolites methiocarb-sulfoxide and methiocarb-sulfone could be detected in the samples taken in the rows. Regarding the carabid samples taken between the rows residues of methiocarb were found in the samples of the replicate c taken at 2 DAA1 (<LOQ), in the sample of replicate d taken at 2 DAA1 (0.13 mg/kg), in the sample of replicate b taken at 10 DAA1 (1.04 mg/kg) and in the sample of replicate a taken at 10 DAA1 (<LOQ). In the sample of replicate b taken at 10 DAA1 residues of methiocarb-sulfoxide (0.15 mg/kg) and methiocarb-sulfone (<LOQ) were detected as well.

Samples of dead earthworms and dead carabids were taken twice (2 DAA1 and 10 DAA1). Residues of methiocarb (13.10 mg/kg) and the metabolite methiocarb-sulfoxide (1.61 mg/kg) were found in the earthworm sample taken at 2 DAA1. No residues of methiocarb and its metabolites were found in the earthworm sample taken at 10 DAA1. 28.00 mg/kg methiocarb, 2.82 mg/kg methiocarb-sulfoxide and 0.36 mg/kg methiocarb-sulfone were found in the carabid sample taken at 2 DAA1. In the carabid sample taken at 10 DAA1 residues of methiocarb (5.28 mg/kg) and methiocarb-sulfoxide (0.53 mg/kg) were detected.

Conclusion:

No residues of methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone were found in active soil organism (earthworms) in samples taken in the seeding rows of the methiocarb-treated maize seeds as well as in the samples taken between the seeding rows. Single residues of methiocarb and once of methiocarb-sulfoxide were detected in the samples of active ground dwelling arthropods (carabids) taken in the seeding rows and between the seeding rows at 2 and 10 days after application. Residues of the metabolite methiocarb-sulfone was found once, but the residue value was below the LOQ (LOQ = 0.1 mg/kg).

In the dead soil organisms found on the soil surface residues of methiocarb and methiocarb-sulfoxide were found at 2 DAA1. Regarding the dead ground dwelling arthropods (carabids) high residues of methiocarb as well as residues of the metabolites methiocarb-sulfoxide and methiocarb-sulfone were found at 2 DAA1 and 10 DAA1 (no methiocarb-sulfone were found on 10 DAA1).

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Risk assessment for other terrestrial vertebrates

Reference is made to baseline and supplemental dossier KCA 5.2.1 and KCP 7.1.1

Table 10.1.2- 1: Endpoints used in risk assessment

Test substance	Exposure	Species/Origin	Endpoint		Reference
Methiocarb	Acute risk assessment	Rat	LD_{50}	19 mg a.s./kg bw	EFSA Scientific Report (2006)
	Long-term risk assessment	Rat	NO(A)ED	300 mg a.s./kg diet[1)] 15.0 mg a.s./kg bw/d	EFSA Scientific Report (2006)

[1)] Figures not lowest from mammalian toxicity data package but considered most appropriate for use in wild mammal risk assessment.

Note:
- studies referring to KCA are filed in the dossier for the active substance
- studies written in grey type are referring to studies in the corresponding Baseline-dossier, whereas studies in black type are studies of the Supplemental dossier

<u>For mammals feeding on treated seeds:</u>

In case of a seed treatment the following generic focal species have to be used:

Table 10.1.2- 2: Type of seeds, corresponding generic focal species and their food intake rate per body weight for risk assessment on Tier 1 level acc. to EFSA GD (2009)

Type of seeds	Generic focal species	FIR/bw
'Large seeds' (maize, beans or peas)	Small omnivorous mammal	0.24
'Small seeds' (not maize, beans or peas)	Small omnivorous mammal	0.24

<u>For mammals feeding on crop seedlings:</u>

The Tier 1 acute and reproductive risk assessments for mammals feeding on crop seedlings from a seed treatment have to be carried out according to the shortcut values as shown in the following table.

Table 10.1.2- 3: Generic focal species and corresponding shortcut values for assessment of residues present in newly emerged crop shoots for risk assessment on Tier 1 level acc. to EFSA GD (2009)

Generic focal species	Short-cut value (SV) for acute risk*
Small omnivorous mammal	0.24 x NAR/5

* For the reproductive assessment, these shortcut values should be combined with appropriate time windows and default degradation/dissipation rates for residues (see equation above).
NAR = Nominal loading/application rate of active substance in mg/kg seed.

Please note that the shortcut value depicted above is a conservative default value. More realistic data, which are based on residue studies, are to be considered in a refinement step.

ACUTE DIETARY RISK ASSESSMENT

Mammals feeding on treated seeds:

The tier 1 risk assessment was performed based on an application rate of 1000 mL product/100 kg seeds, corresponding to 5 000 mg methiocarb/kg seeds.

Table 10.1.2- 4: Tier 1 acute risk assessment for wild mammals feeding on treated seeds

Compound	Generic focal species	Toxicity [mg a.s./kg bw]	Exposure		TER_A	Trigger
			FIR/bw	NAR [mg a.s./kg seeds]		
Methiocarb	Small omnivorous mammal	19	0.24	5 000	**0.016**	10

Bold values do not meet the trigger
NAR = Nominal loading/application rate of active substance in mg/kg seed.

Mammals feeding on crop seedlings:

Table 10.1.2- 5: Tier 1 acute TER calculation for wild mammals feeding on crop seedlings

Compound	Generic focal species	Toxicity [mg a.s./kg bw]	Exposure	TER_A	Trigger
			SV*		
Methiocarb	Small omnivorous mammal	19	240	**0.079**	10

Bold values do not meet the trigger
* SV = 0.24 x NAR/5

The TER_A values for methiocarb are below the trigger of 10 for acute exposure. Accordingly, further refinement is necessary.

Refined risk assessment

A. Ingestion of seeds

The two **main factors** which diminish the risk for mammals are the **low exposure** to treated seeds and the **repellency of Methiocarb**. These relevant factors do not fit into the risk equation of the guidance document. Therefore a "weight of evidence" approach is considered appropriate to refine the risk assessment based on these factors.

Mammalian species of concern

For the target crop and the intended use pattern, the wood mouse (*Apodemus sylvaticus*) is regarded as the species of concern, as this species is common and widespread throughout Europe and has been

found to be consistently present in habitats next to arable fields. The wood mouse was the only species trapped inside the maize fields in a study investigating the exposure of mammals in maize fields ([redacted],; [redacted]; 2010; M-369149-01-1). However, their number was very low and none of them were trapped before emergence of maize. Other animals like the common vole and the greater white-toothed shrew were captured only outside the field. In rare observations, the European brown hare and the European rabbit has been observed, but only the hare was recorded to feed occasionally on maize plants (small sample sizes).

Results from acceptance test with treated maize seeds

In an acceptance test with house mice, the test animals almost completely avoided maize seeds treated with Methiocarb FS 500. Only two mice exhibited slight signs of intoxication during the first hour of exposure (reduced vigilance and discoordinated movement). This indicates that the repellency of methiocarb is sufficient to prevent mice from the uptake of a lethal dose ([redacted],; 2002; M-039893-01-1).

Exposure of mammals to treated maize seeds

Maize is precision drilled, with seeds placed deep in the soil and at a low density compared to cereals. This means that, provided a good seed bed preparation, the density of seeds left on the surface of a drilled field, and the associated risk, can be regarded as "very low", e.g. Leeuw et al. (1995, KIIIA 10.1.8/06) found a maximal exposure of 0.06 surface seeds per m² as the worst case, i.e. only one single seed is available on 16 m² field surface. Additionally, [redacted] ([redacted],; 2001; M-031252-01-1) reported comparable seed exposure data: average number of seeds on the surface in the midfield and endrow areas were 0.007 seeds/m² (= 0.1%) and 0.042 seeds/m² (=0.5%), respectively (n = 10 fields).

In addition to the previous studies, the dehusking of seeds before consumption was often observed for the wood mouse ([redacted], [redacted]; [redacted], R.; [redacted]; 2013; M-481178-01-1). Also, [redacted] ([redacted], R.; 2009; M-359439-01-1) found dehusked maize seeds in areas with increased maize seed exposure. Based on residues on seed husks and sand, [redacted] et al. (2011) calculated a dehusking factor of 0.41 for maize seeds (pigment analysis) indicating an exposure reduction of approximately 60% through the dehusking behaviour.

Information from field monitoring

A field monitoring of small mammals on maize fields drilled with Methiocarb FS 500 dressed seeds in Germany was conducted ([redacted]; [redacted]; [redacted]; 2003; M-077934-01-1). The use of Methiocarb FS 500 dressing on maize seeds had no effect on small mammals, neither on population nor on individual level. Due to the extremely low exposure of seeds after a drilling according to the use pattern (mean number of seeds on surface: 0.15 seeds/m²), and the very low attractiveness of freshly drilled maize fields to small mammals, the probability for small mammals to encounter treated seeds when foraging and consequently the risk of adverse effects can be regarded as very small, even when the loading of methiocarb on the seeds does not substantially decrease until plant emergence.

Conclusion

In summary it can be concluded that the exposure of small mammals to maize seeds, treated with Methiocarb FS 500 and drilled in spring according to GAP is very low because of the low exposure of

seeds on the surface and the extraordinarily low abundance of these species in this habitat. Even if a mouse or vole encounters a single seed, which is quite unlikely but may occur, e.g. in the field border area, the inherent repellent properties will prevent it from ingesting lethal doses of as.
Consequently the use of Methiocarb FS 500 as a maize seed treatment will not pose an unacceptable risk to small mammals, which was also demonstrated in the field trail of [redacted]

B. Ingestion of seedlings

The refined risk assessment for the acute exposure is conducted for herbivorous and small omnivorous mammals exposed to seedlings grown from maize seeds treated with methiocarb. Maximum methiocarb residues levels in seed are 0.09 mg as/kg fresh wt as described in KCP 10.1.1.

Table 10.1.2- 6: Refined acute TER calculation for mammals feeding on crop seedlings

Compound	Generic focal species	Toxicity [mg a.s./kg bw]	FIR/bw	Residues [mg a.s./kg fresh wt.]	$TER_{A,ref}$	Trigger
Methiocarb	Small omnivorous mammal	19	0.27	0.09	782	10
	Small herbivorous mammal		1.33*		159	

* EFSA Guidance Document on Risk Assessment for Birds & Mammals (2009) – Appendix A (Tier 1 tables for mammals, Maize: BBCH 10-29)

The $TER_{A,ref}$ for methiocarb exceed the trigger value for acceptable risk of 10. Accordingly, no risk is to be expected for mammals feeding on crop seedlings emerged from treated seeds.

LONG-TERM REPRODUCTIVE ASSESSMENT

Mammals feeding on treated seeds:

The tier 1 risk assessment was performed based on an application rate of 1000 mL product/100 kg seeds, corresponding to 5000 mg methiocarb/kg seeds.

Table 10.1.2- 7: Tier 1 long-term TER calculation for mammals feeding on treated seeds

Compound	Generic focal species	Toxicity [mg/kg bw/d]	Exposure			TER_{LT}	Trigger
			FIR/bw	NAR [mg a.s./kg seeds]	f_{twa}		
Methiocarb	Small granivorous mammal	13	0.24	5 000	0.53	**0.024**	5

The TER values for mammals feeding on treated seeds do not meet the required trigger of 5 for long-term exposure to methiocarb. Accordingly, a refined risk assessment is needed (see below).

Mammals feeding on treated seedlings:

Table 10.1.2- 8: Tier 1 long-term TER calculation for mammals feeding on treated seedlings

Compound	Generic focal species	Toxicity [mg/kg bw/d]	Exposure		TER_{LT}	Trigger
			SV*	f_{twa}		
Methiocarb	Small omnivorous mammal	15	240	0.53	**0.118**	5

* SV = 0.24 x NAR/5
[1)] This value is taken from the parent compound and represents an unrealistic worst-case scenario

Refined risk assessment

A. Mammals feeding on treated seeds

A mammal exposed to Methiocarb FS 500 treated maize will not continue with the ingestion of higher amounts over several days or weeks, because of emergence of the seeds and the inherent repellency properties of the active substance. Therefore the same assumptions as for the refined acute assessment (see above) can be made: Low attractiveness of treated maize fields (bare fields) reduced number of seeds on the soil due to precise drilling, dehusking.

B. Ingestion of seedlings

The refined risk assessment for the long-term exposure is conducted for small herbivorous and omnivorous mammals exposed to seedlings grown from maize seeds treated with methiocarb. Maximum methiocarb residues levels in seed are 0.09 mg as/kg fresh wt., as described in MCP 10.1.1.

Table 10.1.2- 9: Refined long-term TER calculation for mammals feeding on crop seedlings

Compound	Generic focal species	Toxicity [mg a.s./kg bw]	FIR/bw	Residues [mg a.s. /kg fresh wt.]	f_{TWA}	$TER_{LT, ref}$	Trigger
Methiocarb	Small omnivorous mammal	15	0.27	0.09	0.53	1165	10
	Small herbivorous mammal		1.33			236	

The $TER_{LT,ref}$ for methiocarb exceed the trigger value for acceptable risk of 10. Accordingly, no risk is to be expected for mammals feeding on crop seedlings emerged from treated seeds.

Overall conclusion on risks to mammals

Within a very conservative and formal Tier I risk assessment, the *a priori* acceptability criterions were demonstrated for scenarios where mammals are feeding on plants growing on treated fields (in acute short- and long-term time scales).

Refining the acute oral and short/long-term dietary risk assessment for direct seed ingestion by considering more realistic studies and literature data, in relation to factors such as avoidance,

repellency and mammal behaviour and in which different mammal species were exposed to rates of methiocarb according to the GAP showed no toxic effects. Therefore, the risk to mammals from methiocarb treated maize seeds is expected to be low.

It can be concluded that the use of Methiocarb FS 500 as a maize seed treatment will not pose an unacceptable risk to mammalian wildlife under the conditions of good agricultural practice.

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Acute risk assessment for mammals drinking contaminated water

EFSA (2009, chapter 5.2.1) proposes to focus the risk assessment for birds and mammals on the dietary route of exposure. An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER greater than direct dietary consumption.

Long-term risk assessment for mammals drinking contaminated water

An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER greater than direct dietary consumption.

RISK ASSESSMENT OF SECONDARY POISONING

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log $K_{ow} > 3$ is used to trigger an in-depth evaluation of the potential for bioaccumulation.

As presented in Table 10.1.1- 10, the log P_{ow} value is above the trigger value indicating a risk of secondary poisoning.

The risk assessment of secondary poisoning for wild mammals is performed following the principles developed in the secondary poisoning risk assessment for birds.

Risk assessment for bioaccumulation and food chain behaviour for mammals

The following generic focal species have to be addressed in the Tier 1 risk assessment.

Table 10.1.2- 10: Mammalian generic focal species for the Tier 1 risk assessment of secondary poisoning

Generic focal species	Body weight [g]	FIR [g]	FIR/bw
Earthworm eater	10	12.8	1.28
Fish eater	3000	425	0.142

Long-term DDD and TER calculation for earthworm-eating mammals

Table 10.1.2- 11: Tier 1 long-term DDD and TER calculation for earthworm-eating mammals

Compound	Maize
Methiocarb	
PEC_{worm} [mg/kg] [a)]	0.009
FIR/bw	1.28
DDD [mg/kg bw/d]	0.011
NO(A)EL [mg/kg bw/d]	15
TER_{LT}	1316
Trigger	5

[a)] calculation of PEC_{worm} see Table 10.1.1- 13

The TER value for compound 1 is above the trigger of 5. Accordingly the risk to earthworm-eating mammals from the use of the product in all relevant crops is acceptable.

Long-term DDD and TER calculation for fish-eating mammals

Table 10.1.2- 12: Tier 1 long-term DDD and TER calculation for fish eating mammals

Compound	Maize
Methiocarb	
PEC_{fish} [mg/kg] [a)]	0.0770
FIR/bw	0.142
DDD [mg/kg bw/d]	0.01093
NO(A)EL [mg/kg bw/d]	15
TER_{LT}	1372
Trigger	5

[a)] calculation of PEC_{fish} see Table 10.1.1- 14

The TER value is above the trigger of 5. Accordingly the risk to fish-eating mammals from the use of the product in all relevant crops is acceptable.

CP 10.4.2.1 Acute oral toxicity to mammals

Please refer to MCP 7.1.1 where a summary of the formulation study (rat, acute oral; [redacted]; 2005; M-261963-01-1) is presented.

Test item	Species	Endpoint	Reference
Methiocarb FS 500	Rat (males+females)	LD_{50}: 200 mg/kg bw	[redacted] 2005 M-261963-01-1

CP 10.1.2.2 Higher tier data on mammals

Report:	KCP 10.1.2.2/01 [redacted] B; 2002; M-039893-01-1
Title:	Acceptance of Mesurol FS 500 treated maize seeds (ai. Methiocarb) by house mice (*Mus musculus*), no choice test
Report No.:	BAR/ANN 034
Document No.:	M-039893-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Material and methods:

After 7 days of acclimatisation (choice in time-procedure: 4 hrs untreated maize seed / 20 hrs. standard food) 10 singly caged house mice were exposed to maize seeds treated with Mesurol FS 500 for a 4 hours lasting exposure period under no choice conditions. A control group of 5 mice received untreated maize seeds.

During the acclimatisation, a restricted amount of standard diet (4g) was offered in order to force the animals to ingest maize seeds. After the exposure standard diet was fed ad libitum (50g).

Food consumption was measured from day –3 to day +2. Body weight was determined on day –7, 0 and +3. All mice were observed on signs of intoxication and behavioural changes.

Results:

Test substance:	Methiocarb
Test object:	House mouse/*Mus musculus*/ f
Exposure:	Mesurol FS500 treated maize
Results and observations:	Signs of intoxication: reduced vigilance and discoordinated movement of two mice within the first hour of exposure. All other mice were free of symptoms. Almost complete avoidance of the treated maize seed.

Conclusion:

Under the test conditions the repellency of methiocarb was strong enough to prevent mice from the uptake of a lethal dose.

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Report:	KCP 10.1.2.2/09 [redacted],; [redacted]; [redacted]; 2003; M-077934-01-1
Title:	Field monitoring of small mammals on maize fields drilled with Mesurol FS 500 dressed seeds in Germany
Report No.:	WFC/FS 06
Document No.:	M-077934-01-1
Guideline(s):	Pesticides and Wildlife - Field Testings: Recommendations of an international workshop on terrestrial field testing of pesticides, attached to Pesticide Effects on Terrestrial Wildlife, Somerville & Walker (ed.), Taylor & Francis, London 1990
Guideline deviation(s):	--
GLP/GEP:	**yes**

Material and methods:

The field monitoring was conducted on four study fields and their surroundings near [redacted], North-Rhine-Westphalia, Germany. Two of these fields served as test fields, two fields were used as a control (no insecticide seed dressing used). All sites were commercially cultivated maize fields. The test material was maize seed, dressed with Methiocarb FS 500 (nominal 500 g a.s. per 100 kg seeds, commercially treated and supplied). Monitoring activities focused on exposure of seeds at the soil surface after drilling, the a.s. content and its change over time, on mammalian activities and their abundance on these fields and in the surroundings and on the occurrence of treatment-related effects to wild vertebrates. On a fifth (additional) field only the initial exposure of seeds on the soil surface and as content of seeds were measured.

Exposure of seeds remaining on the soil surface after drilling was measured by transect counts of surface seeds every third day (from day +1 after drilling) at six transects (100 m² each) on each field.

In order to analyse the dissipation of as from the seeds under field conditions, samples of seeds remaining on the soil surface were collected at specifically created plots every third day from the day 1 on each treatment field to evaluate the decrease of the as content per seed within the exposure period. The analytical method used for determination of the active substance was method no. 2201-0114604-98 (BCS-D-FT). The formation of main metabolites (methiocarb-sulfone, methiocarb-sulfoxide, both carrying the toxophor of the as) was followed using the LC-MS working method no. MSD 0086.

Small mammal species and their abundance were recorded and the spatial and temporal activities of the most abundant rodent species were monitored by means of capture-mark-recapture trapping and radio telemetry. Therefore, one trapping grid (1 ha, 100 traps each) was established on each study field.

In order to quantify site-specific mortalities, carcass searches were performed on the study fields and their surroundings on transect routes every 3rd day after the drilling.

Any mammal carcass found was inspected for injuries or any indications for the cause of death. Each carcass suitable for further examinations was collected for gross pathological examinations and for residue analyses due to method 00741 (MR-034/02) of the performing laboratory, BCS-D-ROCS.

Completion of analyses: 2002-09-18

Results:

Effects of Methiocarb FS 500 dressed seeds on small mammals under field conditions

<table>
<tr><td colspan="6">Test substance</td></tr>
<tr><td colspan="6">maize seeds treated with Methiocarb FS 500 (500 g a.s. / 100 kg maize seeds)</td></tr>
<tr><td colspan="6">Test object</td></tr>
<tr><td colspan="6">natural mammal community on two experimental fields and two control fields,
changes of the number of surface seeds and as loading over time on these fields
and initial values of exposure and as loading on one additional treated field</td></tr>
<tr><td colspan="6">Exposure</td></tr>
<tr><td colspan="2" rowspan="3">mean number of seeds on the soil surface [seeds/100 m²]</td><td colspan="2">initial number,
mean of all 5 fields</td><td colspan="2">1.53 (range: 0.00 to 6.33)</td></tr>
<tr><td colspan="2" rowspan="2">number 9 days after drilling
(in brackets: mean per single field)
[in square brackets: % of the mean initial exposure]</td><td>2 treated fields</td><td>0.58 (0.00, 1.17)
[>100%]</td></tr>
<tr><td>2 control fields</td><td>0.16 (0.00, 0.33)
[5%]</td></tr>
<tr><td colspan="6">dissipation of. a.s. from seeds exposed on the soil surface</td></tr>
<tr><td colspan="4">mean initial a.s. content per treated seed [mg/seed]</td><td colspan="2">0.82</td></tr>
<tr><td colspan="4">mean a.s. content per treated seed after 7 days of exposure [mg/seed]
(% of initial content)</td><td colspan="2">0.53 (66.85%)</td></tr>
<tr><td colspan="4">mean a.s. content per treated seed after 22 days of exposure [mg/seed]
(% of initial content)</td><td colspan="2">0.46 (59.54%)</td></tr>
<tr><td colspan="6">Small mammal monitoring</td></tr>
<tr><td colspan="2" rowspan="5">small mammal species recorded
(field and/or adjacent habitats)</td><td colspan="2" rowspan="3">rodent species</td><td colspan="2">Clethrionomys glareolus</td></tr>
<tr><td colspan="2">Apodemus sylvaticus</td></tr>
<tr><td colspan="2">Microtus arvalis</td></tr>
<tr><td colspan="2" rowspan="2">insectivorous species</td><td colspan="2">Sorex spec.</td></tr>
<tr><td colspan="2">Crocidura spec.</td></tr>
<tr><td colspan="2">number of individuals marked</td><td colspan="4">61</td></tr>
<tr><td rowspan="4">observed maximum density of rodent species (MNA: minimum number alive) [Ind./ha]</td><td>species</td><td>pl1 (treated)</td><td>pl2 (treated)</td><td>pl3 (control)</td><td>pl4 (control)</td></tr>
<tr><td>C. glareolus</td><td>7</td><td>-</td><td>-</td><td>1</td></tr>
<tr><td>A. sylvaticus</td><td>1</td><td>3</td><td>3</td><td>5</td></tr>
<tr><td>M .arvalis</td><td>-</td><td>1</td><td>16</td><td>-</td></tr>
<tr><td colspan="2" rowspan="3">preference of maize fields
(Jacobs Index:
-1=avoidance; +1=preference)</td><td colspan="2">Clethrionomys glareolus</td><td colspan="2">-1</td></tr>
<tr><td colspan="2">Apodemus sylvaticus</td><td colspan="2">-0.992 to +0.048</td></tr>
<tr><td colspan="2">Microtus arvalis</td><td colspan="2">-1 to +0.746</td></tr>
<tr><td colspan="4">number of dead rodents found during 60 hours of systematic carcass search on and around the treatment fields</td><td colspan="2">none</td></tr>
<tr><td colspan="4">marked animals, which possibly died treatment-related</td><td colspan="2">none</td></tr>
<tr><td colspan="4">differences in survival rates between treatment and control plots</td><td colspan="2">no difference caused by Methiocarb FS 500 seed dressing</td></tr>
<tr><td colspan="4">differences in population dynamics between treatment and control plots</td><td colspan="2">no difference caused by Methiocarb FS 500 seed dressing</td></tr>
</table>

Conclusion:

The use of Methiocarb FS 500 on maize seeds had no effect on small mammals, neither on population nor on individual levels. Due to the extremely low exposure of seeds after a drilling according to GAP and the very low attractiveness of freshly drilled maize fields to small mammals, the probability for foraging small mammals to encounter with treated seeds and consequently the risk of adverse effects

can be regarded as very small, even when the loading of as on the seeds does not substantially decrease until plant emergence.

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Report:	KCP 10.1.2.2/24 [redacted],; [redacted]; 2010; M-369149-01-1
Title:	Exposure of mammals in maize fields in France - Attractiveness of maize fields and relevant species
Report No.:	R09-012-2
Document No.:	M-369149-01-1
Guideline(s):	No official test guideline(s) available at present. The study was conducted under consideration of the Scientific Opinion of the Panel on Plant protection products and their residues on risk assessment for birds and mammals Anonymous 2008).
Guideline deviation(s):	none
GLP/GEP:	**yes**

Report:	KCP 10.1.2.2/25 [redacted]s; 2010; M-369666-01-1
Title:	Letter of access for generic behavioural ecology data - Study report: RIFCon report No. R09012-2, Syngenta study no. TK0003853 - Crop grouping: Maize, pre-emergence (seed treatments) and post-emergence: Exposure of mammals in maize fields in France - Attractiveness of maize fields and relevant species
Report No.:	M-369666-01-1
Document No.:	M-369666-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Objective:

This study aimed at obtaining information about the occurrence of wild mammals in maize fields in Southern Europe in order to define the focal species in this crop between drilling and BBCH growth stage 16.

Study site: The study was conducted in Southern France in a typical maize growing region south of [redacted] in the departments [redacted] and [redacted] (region Midi-Pyrénées).

Material and Methods:

The study was conducted in spring 2009. The occurrence of mammals in drilled maize fields was assessed by small mammal live trapping and scan sampling.

The live trapping of small mammals was carried out according to a 'Capture-Mark-Recapture (CMR)' design and was used to generate a list of small mammal species and their abundance in freshly drilled maize fields. This implicated individual marking of the captured animals with a passive integrated transponder (PIT). Data derived using this methodology enabled the abundance of mammals on the study fields to be estimated according to the 'Minimum Number Alive' (MNA) approach described by [redacted] (1989). Trapping was carried out from 27 April until 27 May 2009 on four different maize

fields with a trapping effort of 1,488 trap nights[1], 1 per field, with 25% of the traps set up in the adjacent off-crop habitat.

In order to identify and quantify the occurrence of nocturnal mammals in maize fields 'thermographic scan sampling' observations were carried out in four fields, using a thermographic camera (InfraTec VarioCam, 4x zoom) which is suitable for the detection of nocturnal mammals ([redacted]; 1994; M-549608-01-1 and [redacted].,;[redacted];[redacted]; [redacted]; 2001; M-549609-01-1). To quantify the abundance and to characterise the behaviour of diurnal mammals on drilled maize fields, ten study fields were observed by scan sampling for mammal activity.

With the purpose to obtain more detailed information about the foraging behaviour of mammals on maize fields (period: after drilling until BBCH 16), individual mammals with a focus on medium-sized herbivores (hares) were visually observed.

Live trapping, thermographic scan sampling, diurnal scan sampling and monitoring of foraging behaviour was done at three different times according to crop stages of the maize plants: shortly after drilling (BBCH 0), after emergence of maize seedlings (BBCH 10-11) and after emergence of leaves (BBCH 12-16).

In order to record any foraging damage to the maize crop potentially caused by mammals, a sample of maize seedlings was inspected twice after emergence of the crop. The first inspection was carried out shortly after the emergence of the seedlings and the second in the period of BBCH growth stages 12-16.

For the purpose of quantifying the exposure of maize seeds on the soil surface, counts were carried out within 24 hours after drilling was finished. This exposure assessment was conducted on ten maize fields.

Results:

Small mammal species in maize fields and their surroundings:

The most abundant small mammal species found was the wood mouse (*Apodemus sylvaticus*). Besides the wood mouse, the common vole (*Microtus arvalis*) and the greater white-toothed shrew (*Crocidura russula*) were captured. A comparison of trapping efficiencies for field and surrounding habitat evidently showed that small mammals were chiefly captured in the off-crop habitat.

Monitoring of diurnal and nocturnal mammal behaviour and activity:

Besides the wood mouse, the European brown hare (*Lepus europaeus*) and the European rabbit (*Oryctolagus cuniculus*) were the relevant species monitored as potentially foraging during thermographic scan sampling sessions. The hare was the only mammal species observed during daylight scan sampling. overall mammals showed low abundances.

[1] The parameter 'trapnights' is a measure of trapping effort taking the number of traps set and the number of checks into account: 1 trapnight = 1 trap set for 1 night

Monitoring of individual mammals foraging on maize seeds or seedlings:
The European brown hare was the only mammal species being observed during feeding observations. In rare observations, hares fed occasionally on maize plants. Although the sample size was small, a feeding rate for maize leaves was calculated.

Damage assessment:
Due to ambiguous damage patterns no useful results were derived from this approach.

Exposure assessment:
The number of seeds found on the soil surface of maize fields was low.
The following table gives an overview of the key results.

Overview of key results

Small mammal trapping				
Species	Mean trapping efficiency [captures/100 trapnights]			
	Field (based on 1,116 trapnights)	Off-crop (based on 372 trapnights)	Captures in the field [% of total captures]	
Wood mouse (*Apodemus sylvaticus*)	0.35	15.40	6.56	
Greater white-toothed shrew (*Crocidura russula*)	0.00	6.60	0.00	
Common vole (*Microtus arvalis*)	0.00	1.04	0.00	
Diurnal and nocturnal mammal monitoring				
Thermographic scan sampling				
Species	Abundance [ind./ha]	Foraging Individuals [%]		FOfield [%]
Wood mouse (*Apodemus sylvaticus*)	0.31	57.14	3.43	100
European brown hare (*Lepus europaeus*)	0.04	45.83	9.80	75
European rabbit (*Oryctolagus cuniculus*)	0.02	46.15	5.39	50
Diurnal scan sampling				
European brown hare (*Lepus europaeus*)	0.004	61.11	2.63	40
Exposure assessment				
	Mean density of exposed seeds [seeds/m2] (SD)	Average number of seeds per ha		
headland	0.16 (0.21)	1600		
midfield	0.06 (0.10)	600		

Conclusion:
Three small mammal species occurred in off-crop habitats adjacent to maize fields: the wood mouse (*Apodemus sylvaticus*), the common vole (*Microtus arvalis*) and the greater white-toothed shrew (*Crocidura russula*). Only the wood mouse was found inside maize fields and then only in very small numbers after emergence of maize.
In addition to the wood mouse, the European brown hare (*Lepus europaeus*) and the European rabbit (*Oryctolagus cuniculus*) were also observed in maize fields.

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Report:	KCP 10.1.2.2/26 [redacted]; [redacted]; [redacted]; [redacted]; 2013; M-481178-01-1
Title:	Exposure reduction of seed treatments through dehusking behaviour of the wood mouse (Apodemus sylvaticus).
Report No.:	M-481178-01-1
Document No.:	M-481178-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Objective:

Seed treatments are widely used on cereals and other annual crops throughout Europe. Most of the formulated pesticide is found on the outside of the seed, the husk. Risk assessments of seed treatments are especially needed for granivorous mice living in the agricultural landscape e.g. for registration using the guidance for risk assessment for birds and mammals (EFSA 2009). The dehusking of seeds before consumption is a known behaviour of these mammals, but so far, no quantitative data on the reduction of exposure of seed treatments by dehusking were published. Therefore, we aimed at providing a first quantitative estimate of this behaviour-related exposure reduction for the wood mouse (*Apodemus sylvaticus*) with different seed types.

Material and methods:

We evaluated the efficiency of dehusking behaviour of 20 wood mice captured in the wild for four different seeds (wheat, barley, maize and sunflower). One experimental setup used a fungicide (prothioconazole 100 FS) seed treatment where the remaining seed husks of consumed seeds were analysed with a HPLC-MS/MS technique. In the second setup, we measured generic pigment present in a blank seed treatment formulation and determined the leftover pigment in the husks with a photometric technique.

Results:

The exposure reduction was similar for the fungicide and the pigment design where the same seed types were studied. We could demonstrate exposure reductions ranging from 60 percent for cereals to almost 100 percent for sunflower seeds as a result of the dehusking behaviour.

Since exposure reduction was similar in both approaches, working with pigments would be a generic way to estimate the impact of dehusking behaviour on seed treatment exposure. This behaviour can result in a substantial exposure reduction and should, therefore, be considered in a seed-type specific way in the risk assessment of pesticide seed treatments.

Conclusion:

It is proposed to include a seed-specific dehusking factor in the calculations of estimated theoretical exposure of seed treatments for granivorous mice. The approach of accounting for a dehusking-related exposure reduction by field relevant wild mammal species seems a more promising way to advance the risk assessment instead of using generic species and neglecting behavioural traits. The pigment approach could be used to gather data for exposure reduction for other species and seed types. Its

advantage is that it is harmless to the test species and comparatively cheap since no chemical analysis is involved.

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

Please refer to Point 8.2.8 of the MCA 8 in the active substance dossier.

CP 10.2 Effects on aquatic organisms

The risk assessment is based on the current guidance: EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp.

Only endpoints used for the risk assessment are presented here. For an overview of all available endpoints on methiocarb and its metabolites please refer to the respective section of the MCA document.

Risk assessment for aquatic organisms

Ecotoxicological endpoints used in risk assessment

Table 10.2- 1: Endpoints used in risk assessment

Test substance	Test species	Endpoint		Reference
Methiocarb FS 500 G	Invertebrate, acute *Daphnia magna*	EC_{50}	0.0292 mg prod./L (nom) 0.013 mg a.s./L	[redacted] (2007) M-289429-01-1
	Invertebrate, chronic *Daphnia magna*	NOEC	3 x 0.0179 mg prod./L (nom) 3 x 0.008 mg a.s./L	[redacted] (2007) M-295095-01-1
Methiocarb	Fish, acute *Lepomis macrochirus*	LC_{50}	0.35 mg a.s./L (nom)	[redacted] (2000) M-021382-01-1 KCA 8.2.1
	Fish, chronic *Oncorhynchus mykiss*	NOEC	0.05 mg a.s./L (nom) [A]	[redacted] (1985) M-012845-01-1 KCA 8.2.2.1/01
	Invertebrate, acute *Daphnia magna*	EC_{50}	0.0077 mg a.s./L (mm)	[redacted] (2000) M-034439-01-1 KCA 8.2.4.1
	Invertebrate, chronic *Daphnia magna*	NOEC	0.0001 mg a.s./L (mm)	[redacted] (1988) M-012825-01-1 KCA 8.2.5.1
	Chironomid, chronic *Chironomus riparius* (spiked water)	NOEC (emergence)	0.160 (nom)	[redacted] (2006) M-268292-01-1 KCA 8.2.5.3
	Algae, growth inhibition *Desmodesmus subspicatus*	E_rC_{50}	2.2 mg a.s./L (mm)	[redacted] (2000) M-024134-01-1 KCA 8.2.6.1
Methiocarb sulfoxide (MSO)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	6.6 mg p.m./L (mm)	[redacted] (2000) M-022381-01-1 KCA 8.2.1
	Invertebrate, acute *Daphnia magna*	EC_{50}	0.056 mg pm/L (nom)	[redacted] (2001) M-079738-01-1 KCA 8.2.4.1

Test substance	Test species	Endpoint		Reference
	Invertebrate, chronic *Daphnia magna*	NOEC	0.00652 mg p.m./L (mm)	[redacted] (2008) M-300223-01-1 KCA 8.2.9
	Algae, growth inhibition *Desmodesmus subspicatus*	E_rC_{50}	2.75 mg p.m./L (mm)	[redacted] & [redacted] (2005) M-073140-01-1 KCA 8.2.6.1
Methiocarb-phenol (MP)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	3.2 mg p.m./L (nom)	[redacted] (1999) M-016605-01-1 KCA 8.2.1/04
	Invertebrate, acute *Daphnia magna*	EC_{50}	9.8 mg p.m./L (nom)	[redacted] (1999) M-016597-01-1 KCA 8.2.4.1
	Algae, growth inhibition *Desmodesmus subspicatus*	E_rC_{50}	11 mg p.m./L (nom)	[redacted] (1999) M-016599-01-1 KCA 8.2.6.1
Methiocarb-sulfoxide-phenol (MSOP)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	> 106 mg p.m./L (mm)	[redacted] & [redacted] (2001) M-056170-01-1 KCA 8.2.1
	Invertebrate, acute *Daphnia magna*	EC_{50}	157 mg p.m./L (nom)	[redacted] (2001) M-049549-01-1 KCA 8.2.4.1
	Algae, growth inhibition *Desmodesmus subspicatus*	E_rC_{50}	> 100 mg p.m./L (nom)	[redacted] & [redacted] (2001) M-073301-01-1 KCA 8.2.6.1
Methiocarb-sulfone-phenol (MSOOP)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	3.7 mg p.m./L (mm)	[redacted] & [redacted] (2001) M-021598-01-1 KCA 8.2.1
	Invertebrate, acute *Daphnia magna*	EC_{50}	7 mg p.m./L (nom)	[redacted] (2001) M-047970-01-1 KCA 8.2.4.1
	Algae, growth inhibition *Desmodesmus subspicatus*	E_rC_{50}	120 mg p.m./L (nom)	[redacted] & [redacted] (2001) M-073309-01-1 KCA 8.2.6.1
Methiocarb-methoxy-sulfone (MMS)	Fish, acute *Oncorhynchus mykiss*	LC_{50}	26.8 mg p.m./L (mm)	[redacted] & [redacted] (2001) M-057313-01-1 KCA 8.2.1
	Invertebrate, acute *Daphnia magna*	EC_{50}	> 180 mg p.m./L (nom)	[redacted] (2001) M-049570-01-1 KCA 8.2.4.1
	Algae, growth inhibition *Desmodesmus subspicatus*	E_rC_{50}	137 mg p.m./L (nom)	[redacted] & [redacted] (2001) M-054813-01-1 KCA 8.2.6.1

a.s. = active substance, pm = pure metabolite, prod. = product

[A] NOEC based on clinical signs of intoxication; all other NOEC and LOEC-values, based on weight, time to swim-up, hatching and survival were ≥ 0.100 mg/L.

Note:

- Studies referring to KCA are filed in the dossier for the active substance.

Selection of algae and macrophytes endpoints for risk assessment

Processes in ecosystems are dominantly rate driven and therefore, the unit development per time (growth rate) is more suitable to measure effects in algae and macrophytes. Also, growth rates and their inhibition can easily be compared between species, test durations and test conditions, which is not the case for yield or biomass based endpoints. Following current state of science, the test guidelines OECD TG 201 and 221, the EU-Method C3, the EC regulation for Classification and Labeling (EC regulation 1272/2008), the PPR Opinion (EFSA Journal 461, 1-44; 2007) and also the EFSA Aquatic Guidance Document (AGD, 2013, noted by SCFCAH on July 10-11th, 2014) list growth rate as the relevant endpoint of the algae and the *Lemna* growth inhibition test. The previous Guidance Document on Aquatic Toxicology (SANCO/3268/2001 rev. 4) still stated that "As there is no clear evidence available to indicate which is the most relevant endpoint for the field situation, the lower figure should be used in the risk assessment". As this statement is clearly superseded by recent scientific and regulatory developments toxicity-exposure-ratios in this assessment were based on the E_rC_{50}, when available.

Predicted environmental concentrations used in risk assessment

Table 10.2- 2: Initial max PECsw values – FOCUS Steps 1 and 2

Compound	FOCUS Scenario	Maize 1 × 150 g a.s./ha PEC$_{sw, max}$ [µg/L]
Methiocarb	STEP 1	27.23
	STEP 2 – North	1.17
	STEP 2 - South	2.33
Methiocarb sulfoxide	STEP 1	30.24
	STEP 2 – North	3.51
	STEP 2 - South	7.02
Methiocarb sulfoxide phenol	STEP 1	29.40
	STEP 2 – North	2.40
	STEP 2 - South	4.80
Methiocarb sulfone phenole	STEP 1	10.10
	STEP 2 – North	1.26
	STEP 2 - South	2.51
Methiocarb methoxy sulfone	STEP 1	5.06
	STEP 2 – North	0.91
	STEP 2 - South	1.83
Methiocarb phenol	STEP 1	7.13
	STEP 2 – North	0.31
	STEP 2 - South	0.61

Table 10.2- 3: Initial max PECsw values – FOCUS Step 3

Compound	FOCUS Scenario	Maize 1 × 150 g a.s./ha PEC$_{sw, max}$ [µg/L]
Methiocarb	D3 (ditch, 1st)	<0.001
	D4 (pond, 1st)	<0.001
	D4 (stream, 1st)	<0.001
	D5 (pond, 1st)	<0.001
	D5 (stream, 1st)	<0.001
	D6 (ditch, 1st)	<0.001
	R1 (pond, 1st)	<0.001
	R1 (stream, 1st)	<0.001
	R2 (stream, 1st)	<0.001
	R3 (stream, 1st)	<0.001
	R4 (stream, 1st)	<0.001
Methiocarb sulfoxide	D3 (ditch, 1st)	<0.001
	D4 (pond, 1st)	<0.001
	D4 (stream, 1st)	<0.001
	D5 (pond, 1st)	<0.001
	D5 (stream, 1st)	<0.001
	D6 (ditch, 1st)	<0.001
	R1 (pond, 1st)	<0.001
	R1 (stream, 1st)	<0.001
	R2 (stream, 1st)	<0.001
	R3 (stream, 1st)	<0.001
	R4 (stream, 1st)	<0.001

Risk assessment for aquatic organisms

The risk assessment is based on Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC, SANCO/3268/2001, rev 4 final, 17 October 2002.
Toxicity exposure ratios (TER values) are calculated based on the most sensitive species and worst-case PEC$_{sw}$ values.
The TER-values have been calculated based on the following equations:

$TER_A = LC_{50}$ or EC_{50} / initial PEC_{sw}
$TER_{LT} = E_rC_{50}$ / initial PEC_{sw}
TER_{LT} = chronic NOEC / long-term PEC_{sw}

The risk is considered acceptable if the TER_A values are ≥ 100, and the TER_{LT} values ≥ 10.

According to the new Aquatic Guidance Document (EFSA PPR Panel guidance, 2013), the risk to aquatic organisms is evaluated based on the derivation of Regulatory Acceptable Concentrations (RACs) as follows:

Acute risk assessment:
$RAC_{sw, ac} = LC_{50}$ or EC_{50} / 100
The risk is considered acceptable, if the $PEC_{sw, max} \leq RAC_{sw, ac}$

Chronic risk assessment:
$RAC_{sw, ch}$ = NOEC or EC_{10} / 10
$RAC_{sw, ch}$ = E_rC_{50} / 10
The risk is considered acceptable, if the $PEC_{sw, max} \leq RAC_{sw, ch}$
The risk is considered acceptable, if the $PEC_{sw, twa} \leq RAC_{sw, ch}$ (in case risk assessment is based on time weighted average concentrations).

To summarise, these abbreviations are used in subscript following the term PEC or RAC:

ac: acute
ch: chronic
sw: surface water
max: maximum

For the transition phase, BCS decided to present both approaches, the TER as well as the RAC, in order to facilitate the implementation of the new Aquatic Guidance Document (EFSA PPR Panel guidance, 2013). Both the results based on TER and on RAC approach are given below in the summary table.

Summary of calculated TER and RAC values for aquatic organisms

Table 10.2- 4: Summary of all TER and RAC[#] calculations as given under points 10.2.1.1 to 10.2.1.11 (based on most relevant endpoints)

Compound	Species	FOCUS Step	TER	Trigger	RAC[#] [µg/L]	$PEC_{sw} \geq$ RAC[#]	Mitigation
Maize							
Methiocarb	Fish, acute	2	279	100	6.5	no	-
	Fish, chronic	2	22	10	5.0	no	-
	Invertebrate, acute	3	>7700	100	0.1	no	-
	Invertebrate, chronic	3	>100	10	0.01	no	-
	Sediment dweller	2	69	10	16	no	-
	Green algae, chronic	2	944	10	220	no	-
Methiocarb sulfoxide	Fish, acute	2	940	100	66.0	no	-
	Invertebrate, acute	3	>56000	100	0.6	no	-
	Invertebrate, chronic	3	>6520	10	0.7	no	-
	Green algae, chronic	2	392	10	275	no	-
Methiocarb sulfoxide phenol	Fish, acute	2	> 22083	100	>1060	no	-
	Invertebrate, acute	2	32708	100	1570	no	-
	Green algae, chronic	2	> 20833	10	>10000	no	-
Methiocarb sulfone phenole	Fish, acute	2	27371	100	687	no	-
	Invertebrate, acute	2	21514	100	540	no	-
	Green algae, chronic	2	47809	10	12000	no	-
Methiocarb methoxy sulfone	Fish, acute	2	14645	100	268	no	-
	Invertebrate, acute	2	> 98361	100	>1800	no	-
	Green algae, chronic	2	74863	10	13700	no	-
Methiocarb phenol	Fish, acute	2	5246	100	32	no	-
	Invertebrate, acute	2	11148	100	68	no	-
	Green algae, chronic	2	1803	10	110	no	-

[#] The new EFSA aquatic guidance document ("EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 186 pp. doi:10.2903/j.efsa.2013.3290 ") which has been noted and may be implemented by member states during 2015 requires the reporting of the RAC which is compared directly with the PEC_{sw}. The RAC is obtained by considering the toxicity value and dividing it by the "trigger" of 100 /10 for the acute/chronic risk assessment. Therefore the risk is acceptable if the RAC is ≥ PEC_{sw}. Under the regulations applicable until end of December 2015 reporting of TER values is required. Therefore BCS has included both TER and RAC/PEC_{sw} comparisons in the over-view table in Section CP 10.2.

ACUTE RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 5: TER_A calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Maize						
Methiocarb	Fish, acute	LC_{50}	650	2.33	279	100
	Invertebrate, acute	EC_{50}	7.7		**3.3**	
Methiocarb sulfoxide	Fish, acute	LC_{50}	6600	7.02	940	
	Invertebrate, acute	EC_{50}	56		**8**	
Methiocarb sulfoxide phenol	Fish, acute	LC_{50}	> 106000	4.80	> 22083	
	Invertebrate, acute	EC_{50}	157000		32708	
Methiocarb sulfone phenole	Fish, acute	LC_{50}	68700	2.51	27371	
	Invertebrate, acute	EC_{50}	54000		21514	
Methiocarb methoxy sulfone	Fish, acute	LC_{50}	26800	1.83	14645	
	Invertebrate, acute	EC_{50}	> 180000		> 98361	
Methiocarb phenol	Fish, acute	LC_{50}	3200	0.61	5246	
	Invertebrate, acute	EC_{50}	6800		11148	

Bold values do not pass the risk assessment

CHRONIC RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 6: TER_{LT} calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]		$PEC_{sw.max}$ [µg/L]	TER_{LT}	Trigger
Maize						
Methiocarb	Fish, chronic	NOEC	50	2.33	22	10
	Invertebrate, chronic	NOEC	0.1		**0.04**	
	Sediment dweller	EC_{15}	160		69	
	Green algae, chronic	E_rC_{50}	2200		944	
Methiocarb sulfoxide	Invertebrate, chronic	NOEC	6.52	7.02	**0.9**	
	Green algae, chronic	EC_{50}	2750		392	
Methiocarb sulfoxide phenol	Green algae, chronic	E_rC_{50}	> 100000	4.80	> 20833	
Methiocarb sulfone phenole	Green algae, chronic	E_rC_{50}	120000	2.51	47809	
Methiocarb-methoxy-sulfone	Green algae, chronic	E_rC_{50}	137000	1.83	74863	
Methiocarb-phenol	Green algae, chronic	E_rC_{50}	1100	0.61	1803	

Bold values do not pass the risk assessment

The TER_A and the TER_{LT} values for invertebrates do not meet the respective trigger values and further assessment is necessary.

Refined assessment for *Daphnia* exposed to methiocarb and methiocarb sulfoxide

As the TER_A and TER_{LT} values for daphnids do not meet the respective trigger value refined risk assessment for methiocarb and the metabolite methiocarb sulfoxide based on FOCUS Step 3 values is presented below.

Table 10.2- 7: Refined TER calculations for methiocarb and methiocarb-sulfoxide using PEC_{sw} values based on FOCUS Step 3

Compound	Species	Endpoint [µg/L]	FOCUS scenario	$PEC_{sw,max}$ [µg/L]	TER	Trigger
Maize						
Methiocarb	Invertebrate, acute	EC_{50} 7.7	D3 (ditch, 1st)	<0.001	>7700	100
			D4 (pond, 1st)	<0.001		
			D4 (stream, 1st)	<0.001		
			D5 (pond, 1st)	<0.001		
			D5 (stream, 1st)	<0.001		
			D6 (ditch, 1st)	<0.001		
			R1 (pond, 1st)	<0.001		
			R1 (stream, 1st)	<0.001		
			R2 (stream, 1st)	<0.001		
			R3 (stream, 1st)	<0.001		
			R4 (stream, 1st)	<0.001		
	Invertebrate, chronic	EC_{50} 0.1	D3 (ditch, 1st)	<0.001	>100	10
			D4 (pond, 1st)	<0.001		
			D4 (stream, 1st)	<0.001		
			D5 (pond, 1st)	<0.001		
			D5 (stream, 1st)	<0.001		
			D6 (ditch, 1st)	<0.001		
			R1 (pond, 1st)	<0.001		
			R1 (stream, 1st)	<0.001		
			R2 (stream, 1st)	<0.001		
			R3 (stream, 1st)	<0.001		
			R4 (stream, 1st)	<0.001		
Methiocarb sulfoxide	Invertebrate, acute	EC_{50} 56.0	D3 (ditch, 1st)	<0.001	>56000	100
			D4 (pond, 1st)	<0.001		
			D4 (stream, 1st)	<0.001		
			D5 (pond, 1st)	<0.001		
			D5 (stream, 1st)	<0.001		
			D6 (ditch, 1st)	<0.001		
			R1 (pond, 1st)	<0.001		
			R1 (stream, 1st)	<0.001		
			R2 (stream, 1st)	<0.001		
			R3 (stream, 1st)	<0.001		
			R4 (stream, 1st)	<0.001		

Compound	Species	Endpoint [µg/L]	FOCUS scenario	$PEC_{sw,max}$ [µg/L]	TER	Trigger
	Invertebrate, chronic	EC_{50} 6.52	D3 (ditch, 1st)	<0.001	>6520	10
			D4 (pond, 1st)	<0.001		
			D4 (stream, 1st)	<0.001		
			D5 (pond, 1st)	<0.001		
			D5 (stream, 1st)	<0.001		
			D6 (ditch, 1st)	<0.001		
			R1 (pond, 1st)	<0.001		
			R1 (stream, 1st)	<0.001		
			R2 (stream, 1st)	<0.001		
			R3 (stream, 1st)	<0.001		
			R4 (stream, 1st)	<0.001		

All TER_A and TER_{LT} values meet the required trigger of 100 and 10, respectively, using FOCUS Step 3 values.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Report: KCP 10.2.1/02 [redacted]; 2007; M-289429-01-1
Title: Acute toxicity of methiocarb SC 500 to the waterflea *Daphnia magna* in a water-sediment system
Report No.: DOM 26016
Document No.: M-289429-01-1
Guideline(s): Performed under principle consideration to the procedures described by OECD-Guideline No. 202 (2004)
Guideline deviation(s): Exposure will occur in a water-sediment system similar to OECD Guideline 219 "Sediment-Water Chironomid Toxicity Test using Spiked Water"(2004).
Basins containing readily prepared test solutions will not be covered during any part of the study.
The water body of each study group will be artificially aerated during exposure. Enclosures, fitted with stainless-steel grid-bottoms prevent animals from contact with air bubbles.
GLP/GEP: yes

Objective:

The 48 hour (h) acute exposure is to evaluate possible effects on viability of *Daphnia magna* caused by the test item during static exposure in a water-sediment system.
If possible, the acute NOEC and the median effective concentration (EC_{50}) for a possible immobilisation of *Daphnia magna* caused by the test item will be derived from the recorded effects.

Material and methods:

Methiocarb SC 500 ,batch ID: PF90060209, article No.: 04212746, a.s.-content:: 513 g/L (D = 1.146 g/mL), TOX-07758-00; *Daphnia magna* (1st instars < 24 h old, 6 x 5 animals per treatment group and control), exposed in a static test system for 48 hours (without feeding) to the nominal initial

concentrations of 11.2, 22.3, 44.7, 89.4 and 179 µg form./L (corresp. to 5, 10, 20, 40 and 80 µg a.s./L), freshly prepared and admixed to the overlying water at start of exposure only.
Exposure concentrations of methiocarb were measured only at start of the 48 hours exposure period in the overlying water phase of the whole water-sediment test system.

Results:

Study Validity:

Sensitivity of the daphnid breeding-strain used is located within the required range as verified by periodically performed acute reference substance testing.
An immobilisation of 6.7% as observed for untreated control animals ranged well below the 10 % value which is regarded to represent the limit for natural mortality under such test conditions.
For water quality monitoring, temperatures, pH values and O_2 concentrations, conductivity, hardness and alkalinity of the test solutions, were regularly controlled throughout the study as recommended by the underlying guidelines. Dissolved oxygen concentrations ranged in the water phase from 7.8 to 8.6 mg O_2/L (8.0 mg O_2/L= 90 % O_2 -saturation), the water pH values ranged from 8.5 to 8.6 and the water temperature ranged from 19.3°C to 19.4°C measured in the overlying water of each test concentration day 0 and day 2.
As measurements show, the physical / chemical properties corresponded to the recommended values.
The sediment parameters measured directly after preparation, before start of equilibration time (day -18) fulfilled the guideline requirements with a water content of 30.9%, pH value of 7.0 and an organic carbon content of 2.3 %.

Analytical results:

The chemical analysis of methiocarb spiked in the overlying water of the basins at test initiation ranged between 103.6 % and 108.5 % (mean: 105.4 %) of the corresponding nominal concentrations, thus all results are based on nominal initial concentrations.

Biological results:

Toxicity to *Daphnia magna* (based on nominal initial concentrations)

Test Concentration		Exposed daphnids (t=100%)	Immobilised daphnids after 48 h of exposure	
µg a.s./L	µg form./L		n	%
Control (0)	0	30	2	6.7
5	11.2	30	2	6.7
10	22.3	30	12	40.0
20	44.7	30	25	83.3
40	89.4	30	28	93.3
80	179	30	30	100

Conclusions:

The EC_{50} for immobility after 48 hours of static exposure in a water sediment test system is, 29.2 µg form./L, corresponding to 13.1 µg a.s./L.

Statistical significant differences compared to control findings (α = 0.05) were established for test concentrations from 22.3 to 179 µg form./L, resulting in a NOEC of 11.2 µg form. /L, corresponding to 5 µg a.s./L.
Observations on sub-lethal effects revealed abnormal behaviour of the exposed daphnids from test concentrations of 22.3 to 89.4 µg form./L.

*****

Report:	KCP 10.2.1/03 [redacted]., 2007; M-295095-01-1
Title:	Influence of methiocarb SC 500 on development and reproductive output of the waterflea *Daphnia magna* in a static water-sediment test system after multiple spiking
Report No.:	DOM 26017
Document No.:	M-295095-01-1
Guideline(s):	Performed under principal consideration of the procedures described by OECD-Guideline No.211, "OECD-Guideline for Testing of Chemicals", adopted: 21st September 1998 "Daphnia magna, Reproduction Test".
Guideline deviation(s):	Exposure occurred in a water-sediment system (similar to OECD Guideline 219 "Sediment-Water Chironomid Toxicity Test using Spiked Water") taking into account environmental fate properties of the test item after single or repeated applications. - Basins containing readily prepared test solutions were not covered during any part of the study. - Initial exposure concentrations for chronic exposure were prepared and brought into the water phase at the start of the 21 days lasting exposure period and again after 7 and 14 days of exposure by multiple spiking (to simulate repeated application rates). - Served daily food amounts were adapted to the actual need of the test animals, but were identically for all replicates. - The water body of each study group was artificially aerated during exposure. Enclosures (cylinders) were fitted with stainless-steel gridbottoms to prevent animals from contact with air bubbles, but to permit intensive exchange between enclosure medium and medium of the entire treatment group (basin).
GLP/GEP:	**yes**

Material and methods:
Test item: Methiocarb SC 500, batch ID: PF90060209, material No.:04212746, purity: 513 g/L (density = 1.146 g/mL), TOX-07758-00.
Daphnia magna (1st instars < 24 h old, 10 x 1 animal per test level), exposed in a static test system for 21 days to a total of three water spikes of three nominal initial concentrations of 1.12, 4.47 and 17.9 µg form./L (corresponding to 0.5, 2.0 and 8.0 µg a.s./L), freshly prepared and repeatedly admixed to the overlying water in 7 days intervals (on study day 0, 7 and 14).
During the study, the measured concentrations of the test item in the overlying water were analysed four times on days 0, 7, 14 and 21 at all test concentrations and the control. The samples were taken from freshly prepared media (< 1 hour after application) and aged test solutions (7 days after the last application), additional samples were chosen at the end of the selected exposure interval (day 21).
The results of chemical analysis of methiocarb in the freshly prepared test solutions directly after application on day 0, 7 and 14 ranged between 97% and 104% (mean: 100%) of nominal for day 0, 102% to 112% (mean: 108%) of nominal for day 7 and 110% to 111% (mean: 110%) of nominal for

day 14. Additionally measurements of the freshly prepared application stocks on day 0, 7, 14 were 107, 108 and 109 % of nominal (on average).

Due to the analytical findings, all results are based on nominal concentrations of the test item in the overlying water for each spike.

The corresponding concentrations of the aged test solutions on day 7, 14 and 21 ranged between 8% and 9% (mean: 9%), 4% and 6% (mean: 5%) and 6% of nominal. The partitioning of the active ingredient between water and sediment is known from water/sediment studies done under comparable conditions. The recoveries in this study correspond to a half-life of the compound in the water phase of 1.8d. The analysed concentrations of the aged exposure solutions reflect the expected fast dissipation of the active ingredient from the water body and correspond to a geometric mean measured concentration of 2.14 µg/L in the highest concentration.

No contaminations of methiocarb were detected in samples from the untreated control.

Results:

Validity of the study:

Sensitivity of the daphnid breeding-strain used is located within the required range as verified by periodically performed acute reference substance testing.

An immobilisation of 20% as observed for untreated control animals fulfilling the study protocol quality criteria of 20 % value which is regarded to represent the limit for natural mortality of this special test design.

The reproductive output as recorded for untreated control fulfilled the required minimum value of > 60 neonates per female during 21 days.

For water quality monitoring, temperatures, pH values and 02 concentrations, conductivity, hardness and alkalinity of the test solutions, were regularly controlled throughout the study as recommended by the underlying guidelines. As measurements show, the physical / chemical properties corresponded to the recommended values.

During the study, the measured concentrations of the test item in the overlying water were analysed four times on days 0, 7, 14 and 21 at all test concentrations and the control. The samples were taken from freshly prepared media (< 1 hour after application) and aged test solutions (7 days after the last application), additional samples were chosen at the end of the selected exposure interval (day 21).

Biological findings:

Toxicity of methiocarb SC 500 to *Daphnia magna*, based on nominal concentrations of the formulation after each spike

nominal initial treatment (µg form./L)	parental endpoints		reproductive endpoints		
	body length (mm)	survival (%)	cumulative offspring per parent animal (n)	parentage at first offspring emergence (days)	neonates behaviour (% unaffected neonates)
Water control	5.0	80	298	9.3	100
3 x 1.12	4.8	90	253	10.1	100
3 x 4.47	5.0	80	292	9.4	100
3 x 17.9	5.0	90	283	9.6	100

The biological endpoints, as recommended by the underlying Guidelines, revealed the following results under more realistic exposure conditions (based on nominal concentrations after each spike):

- for the cumulative offspring per surviving parent animals:
- for immobilisation of the parent animals:
- for the parental life age at first offspring emergence:
- no observed effect concentration
- lowest observed effect concentration
- for final body length of surviving parental animals:

no observed effect concentration (NOEC)	> 3x 17.9 µg form./L
lowest observed effect concentration (LOEC)	> 3x 17.9 µg form./L

Conclusion:
The overall chronic NOEC for 21 days of static exposure after multiple spiking (3 times) of methiocarb SC 500 in a water- sediment - system to *Daphnia magna* expressed as nominal test concentration is > 3 x 17.9 µg form./L (corresponding to a nominal concentration of > 3 x 8 µg a.s./L.). The geometric mean measured concentration throughout the study period of 21d is 2.14 µg/L. Due to the absence of treatment related effects at the highest tested concentration of 3 x 17.9 µg form./L (nominally), the corresponding LOEC could not be determined.

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No new studies were necessary based on the current data requirements. See the respective summary MCA 8 in the active substance dossier.

CP 10.2.3 Further testing on aquatic organisms

No studies were necessary based on the current data requirements. See the respective MCA document.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

The risk assessment has been performed according to the existing guidance in force at the time of the preparation and submission of this dossier namely the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/ 10329/2002 rev 2) and EPPO Standard PP 3/10 (3) Environmental Risk Assessment Scheme for Plant Protection Products - Chapter 10: honey bees.

Commission Regulations (EU) 283/2013 and 284/2013 require where bees are likely to be exposed, testing by both acute (oral and contact) and chronic toxicity, including sub-lethal effects, to be conducted. Consequently in addition to the standard toxicity studies performed with adult bees (OECD 213 and 214) the following additional studies are also provided:

- Acute contact toxicity to adult bumble bees under laboratory conditions
- Chronic 10 day toxicity to adult honeybees under laboratory conditions
- Acute toxicity to larva honeybees under laboratory conditions
- Semi-field feeding studies according to Testing Method with special design. One tunnel test with honey bee colonies exposed to dressed maize seeds at 5.2 g a.s./kg seeds and the other tunnel to fortified maize pollen up to 3.1 µg a.s./kg.
- Field studies simulating a dust drift exposure scenario for honey bees in flowering Phacelia at the maximum application rate for the approval renewal of methiocarb and evaluating flight intensity, mortality and colony development.
- Field study according to a tailor made study design. Honey bee colonies were exposed to guttation fluid of treated maize seeds at 1.5 mg a.s./seed and investigated in terms of mortality, colony development and subsequent overwintering performance.

Supporting study

- Semi-field studies following OEPP/EPPO Guideline No. 170(4) exposing honey bees to methiocarb-treated pollen at 48 µg a.s./kg and to treated sugar solution at 20 µg a.s./kg and evaluating flight intensity, mortality and colony development.

Details of the honey bee testing with methiocarb and ecotoxicological endpoints are presented in MCA, Section 8.3.1, Document MCP, Section 10.3.1, as well as within the existing EFSA Scientific Report (2006) 79, 1-82. The tunnel and field tests with the representative formulation Methiocarb FS 500 are presented in this document (MCP Point 10.3.1). Study finding and endpoints are presented and discussed in the following section. Values highlighted in bold are used in the risk assessment.

Acute toxicity to adult honey bees

Findings from the studies on the acute toxicity of the active substance are presented in Table 10.3.1-1 and for formulated (FS500) product in Table 10.3.1-2. Overall the formulated product was of lower acute toxicity to bees compared to the active substance.

Table 10.3.1- 1: Acute toxicity of methiocarb (a.s.) to bees

Test substance	Test species/study design	Endpoint	EU agreed endpoint (EFSA Scientific Report (2006) 79)	Reference
Methiocarb tech.	Honey bee, 48 h	LD_{50} – oral 0.47 µg a.s./bee LD_{50} – contact **0.23 µg a.s./bee**	yes	[redacted] (1995) M-013166-01-1 KCA 8.3.1.1 KCA 8.3.1.1.2
Methiocarb tech.	Honey bee, 48h	LD_{50} – oral **0.08 µg a.s./bee** LD_{50} – contact 0.43 µg a.s./bee	New study	[redacted] (2009) M-308072-01-1 KCA 8.3.1.1.1

Bold values: endpoints used for risk assessment

Table 10.3.1- 2: Acute toxicity of formulated Methiocarb FS 500 to honey bees

Test substance	Test species/study design	Endpoint	EU agreed endpoint (EFSA Scientific Report (2006) 79)	Reference
Methiocarb FS 500	Honey bee, 48 & 72h	LD_{50} – oral **0,11 µg a.s./bee** LD_{50} – contact **0.38 µg a.s./bee** (72h)	New study	[redacted] (2009) M-357085-01-1

Bold values: endpoints used for risk assessment

Acute toxicity to adult bumble bees

Currently there are no testing requirements for any bee other than the honey bee within Regulation EU 1107/2009. The following study is presented as additional information.

There is currently no harmonized and ring tested test guideline available in Europe to assess the acute toxicity to bumble bees; this is particularly true for the oral route of exposure, as bumble bees do not share their food through trophallaxis. However, there is now sufficient experience within the European bee testing community to provide some experimental evidence on the acute toxicity to bumble bees although the official OECD ring test will not be run until 2016. For the determination of the contact and oral toxicity of methiocarb to bumble bee methods in line with the current ring test have been employed. The findings indicate that the bumble bee is not more sensitive to methiocarb compared to the honey bee.

Table 10.3.1- 3: Acute toxicity of methiocarb to Bumble bees (a.s.)

Test substance	Test organism	Ecotoxicological Endpoints:		Reference
Methiocarb technical (98.2 % w/w)	Bumble bee	48 h -LD_{50} oral	**19.3 µg a.s./bumble bee**	[redacted] (2014) M-479538-01-1 KCA 8.3.1.1.2

Chronic toxicity to adult honey bees

There is currently no harmonised and ring tested test guideline available in Europe to assess the chronic risk to adult honey bees. Nonetheless, there is to date some experience within the European honey bee testing community on conducting chronic studies in adult honey bees, by exposing honey bees orally to a treated 50% (w/v) sugar solution as an exclusive food source for a period of 10 consecutive days by continuous and ad libitum feeding. An OECD ring test for the method is planned for 2016 and the protocol followed is based on the proposed ring test method.
As the observed chronic toxicity endpoint is well above the acute endpoint (LD_{50} oral 48h = 0.11 µg a.s./bee there is no evidence for increased toxicity due to chronic exposure compared to acute exposure.

Table 10.3.1- 3: Chronic toxicity of Methiocarb FS 500 to adult honey bees (a.s)

Test substance	Test species/study design	Endpoint	Reference
Methiocarb FS 500	10 d chronic adult feeding study	NOEC 420 µg a.s./kg LC_{50} 1104.9 µg a.s./kg	[redacted] (2015) M-540431-01-1 KCA 8.3.1.2

Effects on honeybee development and other honeybee life stages

In order to address the potential toxicity of honeybee development brood stages, a laboratory *in vitro* study with the technical active substance was conducted according the OECD Guideline No.237. The findings, shown in table 10.3.1-1, indicate that under worst-case in vitro exposure honeybee larvae are not more sensitive that adults.

Table 10.3.1- 1: Honey bee larva in-vitro test

Test substance	Test organism	Ecotoxicological Endpoints:	EU agreed endpoint (EFSA Scientific Report (2006) 79)	Reference
Bee brood feeding test				
Methiocarb tech.	Honey bee brood (in vitro) *Apis mellifera* 72h	NOED 0.064 µg a.s./larva LD_{50} 0.547 µg a.s./larva	New study	[redacted] (2015) M-514260-01-1

Tunnel/cage and field tests

Findings from semi-field and field studies on the acute toxicity of the active substance are presented in Table 10.3.1-1 and for formulated (FS500) product in Table 10.3.1-2.

Table 10.3.1- 4: Semi-field and field tests of formulated Methiocarb FS500 to honey bees

Test substance	Test species/study design	Endpoint	Reference
Semi-field (tunnel) and field tests			
Methiocarb FS 500	Cage test with small bee colonies (approx. 1'500 bees), 35 d, dressed maize seeds, 5.2 g a.s./kg seeds	No treatment related effects on mortality, foraging activity, food consumption, hive weight increase, comb cell production, honey storage, population development, breeding activity and success due exposure to dressed maize seeds under tunnel conditions at 5.2 g a.s./kg seeds.	[redacted] (2003) M-088296-01-1
Methiocarb FS 500	Tunnel study with small bee colonies (approx. 500 bees), 52 d, fortified maize pollen, 3.1, 6.2 and 12.4 µg a.s./kg pollen	No treatment related effects on mortality, foraging activity, food consumption, hive weight increase, comb cell production, honey storage, population development, breeding activity and success due exposure to fortified maize pollen under tunnel conditions up to 3.1 µg a.s./kg pollen.	[redacted] (2002) M-059860-01-1
Methiocarb FS 500	Field, dressed maize seeds, 26 d, 150 g a.s./ha (1.5 mg a.s./seed)	No effects on the survival of adult bees and bee pupae, foraging activity, behaviour, colony development and colony strength, on the bee brood and the hibernation success at 150 g a.s./ha	[redacted] (2015) M-534762-01-1
Methiocarb FS 500	Field, dressed maize seeds, honeybee colonies (approx. 10'000 bees), ~12 month with overwintering, 150 g a.s./ha (1.5 mg a.s./seed	No adverse acute, short-term or long-term effects on colony strength and development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality, colony health, or on overwintering performance at 1.5 mg a.s./seed	[redacted]. (2015) M-534766-01-1 KCA 8.3.1.3

Supporting study

Table 10.3.1- 5: Supporting study generated with formulated methiocarb

Test substance	Test species/study design	Endpoint	Reference
Bee brood feeding test			
Methiocarb FS 500	Semi-field honey bee study (OEPP/EPPO Guideline No. 170(4); forced exposure conditions) to treated diet (sugar solution and pollen), set-up on a bare soil field	No adverse effects on honey bee mortality, colony strength, colony- and brood development, food storage and overall colony vitality up to and including about 20 µg a.s./kg in sugar solution (nectar) and up to and including about 48 µg a.s./kg in pollen.	[redacted] (2015) M-539746-01-1

Risk assessment for bees

The risk assessment for bee is based on the maximum single application rate of methiocarb applied as a seed treatment for maize at 1.5 mg a.s./seed (150 g a.s./ha) using the critical endpoints (LD_{50} values)

in bold in the preceding tables for Methiocarb of 0.08 and 0.23 µg a.s./bee for oral and contact toxicity respectively.

Hazard Quotients

The risk assessment is based on Hazard Quotient approach (Q_H) by calculating the ratio between the application rate (expressed in g a.s./ha or in g total substance/ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s./bee or in µg total substance/bee).

Q_H values can be calculated using data from the studies performed with the active substance and with the formulation. Q_H values higher than 50 indicate the need of higher tiered activities to clarify the actual risk to honey bees.

Hazard Quotient, oral:
$$Q_{HO} = \frac{\text{maximum application rate}}{LD_{50}\ \text{oral}} = \frac{[\text{g a.s./ha or g total substance/ ha}]}{[\text{µg a.s./bee or µg total substance/ bee}]}$$

Hazard Quotient, contact:
$$Q_{HC} = \frac{\text{maximum application rate}}{LD_{50}\ \text{contact}} = \frac{[\text{g a.s./ha or g total substance/ ha}]}{[\text{µg a.s./bee or µg total substance/ bee}]}$$

Table 10.3.1- 6: Hazard quotients for bees – oral exposure

Compound	Oral LD_{50} [µg a.s./bee]	Max. application rate [g a.s./ha]	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Methiocarb FS 500	0.11	150	1364	50	no
Methiocarb	0.08	150	1875	50	no

The hazard quotients for oral exposure are above the validated trigger value for higher tier testing (i.e. $Q_{HO} < 50$).

Table 10.3.1- 7: Hazard quotients for bees – contact exposure

Compound	Contact LD_{50} [µg a.s./bee]	Max. application rate [g a.s./ha]	Hazard quotient Q_{HC}	Trigger	*A-priori* acceptable risk for adult bees
Methiocarb FS 500	0.38	150	395	50	no
Methiocarb	0.23	150	652	50	no

The hazard quotients for contact exposure are above the validated trigger value for higher tier testing (i.e. $Q_{HC} < 50$).

Further considerations for the risk assessment

Based on this initial simplistic approach it is indicated that there may be a risk to bees. However, as the HQ has not been fully validated for seed treatment applications further consideration of the risk to bees due to the use of Methiocarb FS 500 as a seed treatment in maize is necessary.
As the risk assessment scheme for honeybees to be applied according to the Terrestrial Guidance Document (SANCO/ 10329/2002 rev 2) is recognized not to be fully sufficient to cover the specificities of soil-systemic pesticide uses, the risk assessment for the use of Methiocarb FS 500 as a seed treatment in maize was conducted to EPPO PP 3/10 (3), 2010 (M-403368-01-1). This is the currently valid and risk assessment scheme in force at the time of the submission of this dossier. However, this document does not specifically address exposure to dust, consequently product specific data on exposure are provided and the risk assessment used follows that of SANCO/ 10329/2002 rev 2 using the Hazard Quotient (HQ) approach using exposure levels estimated from a comprehensive data set of dust drift field trials.
Furthermore, data on the contact toxicity of technical methiocarb indicated that based on laboratory toxicity data there is no evidence to suggest that non-*Apis* bees, represented by bumblebees in this case, are at greater risk. Consequently the risk assessment for honey bees was considered to protect other bees.

For maize, seed treatment applications may result in bees being exposed to test substance via the following routes of exposure ([redacted]; 2011; M-504620-01-1; [redacted]; 2011; M-549027-01-1)

- Dust emitted from seed drilling equipment at the time of sowing
- Guttation water during the early growth stage of the plants
- Consumption of residues in pollen

The relevance of each point will be discussed below and where necessary a risk assessment provided.

Risk to bees due to exposure to dust emitted from seed drilling equipment at the time of sowing

During the drilling of maize seed treatment dust might be abraded and released in the environment. As the field is bare at the time of drilling any potential exposure would be due to the deposition of dust onto adjacent flowering areas.

Given that there is currently no EU-agreed guidance for performing a risk assessment for bees due to exposure from dust, no specific risk assessment covering this question will be presented here. Nevertheless, available higher tier studies related to dust exposure will be presented below for information only.

To determine dust drift rates for Methiocarb FS500 treated maize seeds a monitoring study ([redacted]; 2009; M-355846-02-1) analytical part conducted under GLP) has been conducted in April 2009 which included the dust drift measurements on 20 commercially operated maize fields in Germany (6 fields in Bavaria, 3 in Baden-Württemberg, 4 in Lower Saxony, 4 in North Rhine-Westphalia, 1 in Saxony, 1 in Brandenburg and 1 in Schleswig-

Holstein). The commercially treated maize (20 different seed batches including 19 different maize varieties; treated with Mesurol®; nominal seed dressing rate 1.5 mg methiocarb a.s./kernel) was sown by the respective farmer of the field. Eighteen fields were sown with deflected pneumatic sowing machines, 2 fields were sown with mechanical sowing machines. Overall, 14 different sowing machines were used. On each field 10 Petri-dishes filled with glycerol/water mixture were placed in at a distance of 1, 3 and 5 m (in total 30 Petri-dishes per field) at the down-wind border of the fields. The overall 90th -percentile of the ground deposition of methiocarb in 1 m distant from the field was 0.332 g a.s. methiocarb/ha.

To investigate the deposition of dust drift on vertically installed sampling devices a 3D-method trial has been conducted ([redacted],;[redacted]; 2010; M-362242-01-1). The outcome of this 3D-method trial revealed that vertically installed gauze netting can be considered to be the most appropriate and conservative surrogate sampling device for measuring vertical deposition in natural 3D off-crop structures. Based on a 90th percentile ground deposition value of 0.332 g a.s. methiocarb/ha, for maize the 3D off-field exposure for honey bees can be calculated as follows: 0.332 g a.s.× 6 × 1/3 = 0.664 g a.s./ha. This value is calculated under the consideration that the seed treatment quality of the treated seeds meets the minimum quality criterion of a Heubach value of ≤ 0.75 g dust/100,000 seeds and that vacuum pneumatic drillers are equipped with an appropriate deflector. Therefore, the obtained value is a conservative one.

In a realistic field test ([redacted]; 2015; M-534762-01-1), dust drift measurements were made during the sowing operation of methiocarb-treated maize seeds on the treatment fields (1.5 mg a.s./seed). The maximum vertical dust deposition, as measured by vertically erected gauze-netting units, directly adjacent to the maize sowing area, corresponded to a maximum drift rate of 0.41 g a.s./ha (mean values per sampling plot). Potential effects on honeybee colonies were assessed during and after vacuum-pneumatic sowing operation of maize seeds, sown directly adjacent to full-flowering *Phacelia tanacetifolia*. The application of Methiocarb FS 500 G did not cause any effects on the survival of adult bees and bee pupae, foraging activity, behaviour, colony development and colony strength as well as on the bee brood and the hibernation success.

Risk to bees due to exposure to guttation water

Honey bees are specific in their requirement for water to cool the hive and also to dilute concentrated honey stores. Other bees do not require water for these purposes and get their water from their diet (nectar). The occurrence of guttation droplets is highly dependent upon systemic properties, soil and air humidity and the type of crop.

In order for honey bees to be exposed to methiocarb residues in guttation water droplets the following conditions must occur, (i) methiocarb must be highly systemic and mobile within the plant, (ii) there must be the correct environmental and soil conditions during the early plant growth stage for guttation to occur and (iii) honey bees must be present, placed close to the field and collect guttation water in preference to other water sources such as puddles, dew, and water from the off-field area.

Methiocarb has very low to negligible systemic properties. This is evidenced from the findings from residue studies with the Methiocarb FS 500 formulation applied at a rate of 1 L product/100 kg seeds or 5 g a.s./kg seeds have been carried out on corn in Germany, France, Belgium, Spain, Italy and Greece (see baseline dossier KCA document section 6.3.4: report no. M-033763-01-1, M-034429-01-1; M-035447-01-1, M-032843-01-1). In samples from young corn plants collected 27-41 days after sowing, which coincides with the period of time guttation is most likely to occur in maize, only traces of methiocarb (< 0.01 mg/kg), methiocarb-sulfoxide (< 0.01 – 0.07 mg/kg) and methiocarb-sulfone (< 0.01 mg/kg) could be detected in the whole plant (without root) resulting in a calculated total residue between 0.03 and 0.09 mg/kg. In later samples, residues of methiocarb, methiocarb-sulfoxide and methiocarb-sulfone were below the limit of quantitation (LOQ = 0.01 mg/kg for each substance).

This is in accordance with the recent field study in maize, grown from seeds treated with the maize seed-treatment product Methiocarb FS 500 G ([redacted] et al.; 2015; KCA 8.3.1.3; M-534766-01-1). Residue analysis of guttation fluid revealed that methiocarb, methiocarb-sulfoxide and methiocarb-sulfone-residues were generally highest at the beginning of the assessment phase. Residues of methiocarb, methiocarb-sulfoxide and methiocarb-sulfone declined throughout the assessment phase until its end. The maximum residue level of methiocarb was 0.066 mg/L. The maximum residue level of methiocarb-sulfoxide was 35.1 mg/L and the maximum residue level of methiocarb-sulfone was 1.1 mg/L.

Overall, due to the lack of systemic activity, bees are unlikely to be exposed to methiocarb via guttation water.

Although honey bees are observed to collect guttation water due to the short period of time a guttation event may occur and the proportion of bees exposed means that this is not considered a significant route of exposure for the colony (however individual bees may be affected) and in any case the lack of systemic properties mean that there will be negligible levels of methiocarb present. In addition, it is good beekeeping practice to ensure an adequate supply of clean fresh water for colonies.

It is concluded that the risk of exposure for bees to methiocarb via guttation water is therefore low ([redacted]). Consequently no risk assessment is necessary for the use of Methiocarb FS 500 as a seed treatment in maize and the risk posed to bees is low.

Risk to bees due to consumption of residues in pollen

Methiocarb has low systemic properties and therefore the potential route of exposure to a seed-dressing product for honeybees via ingestion of pollen from seed-treated crop plants is negligible. Nevertheless, information about residues of methiocarb and its metabolites in pollen of maize and potential effects on bees are provided in the following table.

Table 10.3.1- 8: Residue levels of methiocarb and its metabolites methiocarb-sulfone and methiocarb-sulfoxide in pollen of seed-dressed maize under semi-field and field conditions

Study location Report no.	Residue concentration [mg/kg]		
	methiocarb	methiocarb metabolites	
		methiocarb-sulfone	methiocarb-sulfoxide
Brazil M-040031-01-1	< 0.001	< 0.001	< 0.001
Germany M-088296-01-1	< 0.001	< 0.001	< 0.001
Germany M-534966-01-1	< 0.002	< 0.002	< 0.002
Germany M-494337-01-1	< 0.005	< 0.005	< 0.005

In two residue trials with pollen from maize plants grown from treated seeds at 500 g a.s./100 kg seeds no residues of methiocarb and its degradation products methiocarb-sulfone and methiocarb-sulfoxide above the limit of detection (LOD) of 0.001 mg/kg could be detected in the treated pollen samples (Table 10.3.1- 8, M-040031-01-1, M-088296-01-1). Additionally, in the residue trial with pollen from *Phacelia tanacetifolia* grown from treated seeds at 15 g a.s./ kg seeds (equivalent to 150 g a.s./ha) no residues of methiocarb and its degradation products methiocarb-sulfone and methiocarb-sulfoxide above the limit of detection (LOD) of 0.002 mg/kg could be detected in the treated pollen samples (Table 10.3.1- 8, M-534966-01-1). This is in accordance with the residue trial with pollen from maize plants grown from treated seeds at 1.5 mg a.s./seed, where no residues of methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone were found in 119 of 120 maize pollen samples above the limit of detection (LOD) of 0.005 mg/kg (Table 10.3.1- 8, M-494337-01-1). In one single maize pollen sample, residues of methiocarb at the LOQ-level of 0.01 mg/kg were found (there were no detectable residues of methiocarb-sulfoxide and methiocarb-sulfone. Unfortunately it was not possible to verify or falsify the residues found in this sample.

Therefore, no exposure to honey bees from maize seed treatments with methiocarb according to the use pattern is anticipated and the calculation of hazard quotients is not appropriate.

Pollen exposure assessment

The lack of risk due to negligible exposure can be demonstrated by assuming a worst case situation where residues are assumed to be present in pollen at the level of the LOD of 0.001 mg /kg and comparing them to the extreme worse case situation for honey bees assumed to be feeding exclusively on maize pollen. Honey bees do not exclusively feed on maize pollen which is only collected as a protein source when there are no other pollen sources available.

Information on the use and consumption of pollen as a food source by honey bees is provided by several authors ([redacted]; 1955; M-504617-01-1, [redacted]; 2004; M-504603-01-1, [redacted]; 2005; M-292299-01-1). Pollen is the only natural protein source available to honey bees and is

used to feed larvae and is also consumed in the largest amounts by adult nurse bees that tend and fed the larvae in the colony. Forager bee pollen consumption levels are negligible. Consequently the risk to honey bees due to the consumption of pollen can be covered by considering the exposure to nurse bees and larvae. Pollen consumption levels for nurse bees and larvae are presented below:

Table 10.3.1- 9: Pollen consumption levels

Type of Honey bee	Location	Pollen consumption	Notes
Nurse bee	Within the colony	65 mg pollen / 10 days 6.5 mg pollen / day	May consume up to 12 mg pollen in one day
Larva (worker)	Within the colony	5.4 mg pollen total 1.3 mg on day 4 3.6 mg on day 5	On days 1-3 larvae are fed royal jelly. Pollen (and nectar) are fed on day 4 and 5 only

In the possible field situation where residues are assumed to be present in pollen at the level of the LOD of 0.005 mg /kg (i.e. 0.005 µg/g) and assuming pollen consumption rates as described in table 10.3.1-10, the following worst case risk assessment scenarios which cover the risk to bees due to the use of Methiocarb FS 500 as a seed treatment for maize cultivation are calculated (see table 10.3.1-11). Like that estimated theoretical doses lay between 27 and 60 picogrammes per bee. It should be stressed that these extremely low levels are impossible to measure analytically or to test under laboratory conditions with the available analytical methods.

Table 10.3.1- 10: Worst case theoretical exposure levels

Type of honey bee	Pollen consumption (g)	Residue level	Dose (µg/bee)
Nurse bees (acute risk)	0.012 g	0.005 µg/g	0.00006 µg/bee
Nurse bees	0.0065 g /day		0.000033 µg/bee/day
Larva (worker)	0.0054g (on day 4 and 5)		0.000027 µg/bee

Risk assessment for bees due to exposure to pollen

Although these levels of exposure are unlikely to cause adverse effects to honey bees the level of safety implied can be calculated using the acute oral toxicity endpoint of 0.08 µg/bee. Due to the negligible exposure level further testing of adults under chronic exposure conditions and of larval is not deemed necessary. The risk to bees due to the consumption of pollen containing the worst case theoretical residues of methiocarb is presented below. According to EPPO 2010 a Toxicity Exposure Ratio trigger of 10 is applied to acute endpoints (LD_{50}).

Table 10.3.1- 11: Methiocarb FS 500 seed treatment: Systemic risk to bees via pollen consumption

Type of honey bee	Risk	Endpoint	Exposure	Toxicity Exposure Ratio (TER)	EPPO (2010) Trigger
Nurse bee	Acute	LD_{50}: 0.08 µg a.s./bee	0.00006 µg/bee	1333	10
	Chronic	LDD_{50}: 0.0415 µg a.s./bee/day	0.000033 µg/bee/day	1277	10
Larva (worker)	Dietary	LD_{50}: 0.547 µg a.s./larva	0.000027 µg/bee	6204	10

The estimated toxicity values used, are within the bounds of normal expectation for methiocarb and are only used *illustratively* to indicate the lack of risk due to the negligible exposure levels. The calculated TER values range from 1277 to 6204; these margins of safety are high and exceed the EPPO 2010 triggers by several orders of magnitude.

As illustrated in the calculations above, due to the lack of exposure of honey bees from maize seeds treated with methiocarb no risk is anticipated for honey bees. Nevertheless, the long-term risk of residues of methiocarb in maize pollen on honeybees was examined under semi-field conditions (see Table 10.3.1- 8).

In a cage test (M-088296-01-1,) small honeybee colonies (approx. 1500 honeybees) were confined on oat plots in 16 m^2 tents and fed with maize pollen from plants grown from seeds dressed with methiocarb. The bee colonies were examined for treatment-related effects over a period of 35 days. The endpoints listed in Table 10.3.1- 2 were assessed. The residue levels of methiocarb and its metabolites methiocarb-sulfone and methiocarb-sulfoxide were below the limit of detection (as described above, see KCP 10.3.15). The results of the study show that there is no risk to honeybees by foraging on and consumption of maize pollen of plants originating from seeds dressed with methiocarb at rates up to 5.2 g a.s./kg seeds.

In a tunnel test (M-059860-01-1, KCP 10.3.1.5) small bee colonies (approx. 500 honeybees), which were confined on oat plots in 50 m^2 tents, were examined for treatment-related effects over a period of 52 days. The endpoints described in Table 10.3.1- 2 were assessed. The study showed that methiocarb residues up to 3.1 µg as/kg in pollen - an unrealistic high concentration since exposure to methiocarb residues in pollen is obviously low or negligible (< 0.001 mg/kg, see KCP 10.3.1.5) - do not pose a risk to honeybees.

It is concluded that the risk of exposure for bees to methiocarb via pollen from treated seeds is therefore low. This is demonstrated by the lack of exposure and confirmed experimentally in cage and tunnel tests with commercial bee hives

Overall conclusions for bees

The calculated Hazard Quotients based on the empirical exposure level of 150 g a.s./ha for technical and formulated methiocarb were above the validated trigger value for higher tier testing (i.e. Q_{HC} < 50).

However, this kind of risk assessment was considered of poor relevance to fully cover all concerns for a product applied as seed treatment. Other routes of exposure such as dust emitted from seed drilling equipment at the time of sowing, exposure to guttation water and consumption of residues in pollen may be investigated. In the absence of currently EU-agreed guidance for performing a risk assessment due to exposure from dust, no quantitative risk assessment related to this question has been performed. Nevertheless, available higher tier studies related to dust exposure have been presented for information. The outcome of these studies indicates an acceptable risk to bees due to exposure from dust. As methiocarb is not systemic the exposure via guttation water and pollen is low/negligible. No quantitative risk assessment was considered necessary for guttation water. An illustrative worst case risk assessment due to the theoretical consumption of maize pollen was conducted and indicated a high margin of safety to honey bees. This was also confirmed experimentally in cage and tunnel tests.

Overall, it can be concluded that methiocarb, when applied at the maximum application rate of 1.5 mg a.s./seed for maize, equivalent to 150 g a.s./ha does not pose an unacceptable risk to honey bees and honey bee colonies. Additionally there is no evidence to suggest that non-*Apis* bees are at greater risk.

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Report:	KCP 10.3.1/01 [redacted]; 2002; M-040031-01-1
Title:	Analysis of incurred residues of methiocarb, methiocarb-sulfone and methiocarb-sulfoxide in pollen by HPLC-MS/MS
Report No.:	MR-022/02
Document No.:	M-040031-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Material and methods:

The pollen was collected in Brazil in an area where maize form seeds dressed with Mesurol 500 FS was cultivated. Extraction, sample clean up and analytical determination of methiocarb, methiocarb-sulfone and methiocarb-sulfoxide by HPLC-MS/MS were performed according to method 00616/E001.

The Limit of Quantitation (LOQ) for methiocarb, methiocarb-sulfone and methiocarb-sulfoxide was set at the lowest standard concentration, which had been successfully validated. The LOQ for methiocarb, methiocarb-sulfone and methiocarb-sulfoxide in pollen was accordingly 0.025 mg/kg each. The LOQ for the total residue of methiocarb was set at 0.075 mg/kg.

The LOD was set at the lowest standard concentration of standard in matrix at which a clearly visible peak was obtained in the HPLC-MS/MS measurement. In the original method, the LOD for methiocarb, methiocarb-sulfone and methiocarb-sulfoxide accordingly had been set at 0.00025 mg/L

in the analytical solution, corresponding to 0.005 mg/kg (20% LOQ) for methiocarb, methiocarb-sulfone and methiocarb-sulfoxide in pollen. In the course of this study, the LOD of the HPLC-MS/MS-instrument used was re-evaluated using also lower standard concentrations. As a consequence, an LOD of 0.1 µg/L, corresponding to 0.001 mg/kg (4% LOQ) could be set for methiocarb, methiocarb-sulfone and methiocarb-sulfoxide for this study.
The analytical method was validated prior to analysis by running recovery sets at the LOQ and at the two- and tenfold LOQ in the course of validation of method 00616/E001. The results of these validation recoveries are allocated to study P602011006 and are not included in this report. In addition, during analysis of the samples of this study, concurrent recovery experiments were performed by spiking control samples with methiocarb, methiocarb-sulfone and methiocarb-sulfoxide. All results of the method validation are in accordance with the general requirements for residue analytical methods, therefore the method was validated successfully.

Results:
No residues of methiocarb and its degradation products above the LOD of 0.001 mg/kg could be detected in the treated pollen samples.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

For information on studies already evaluated for the Annex I inclusion of methiocarb under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience.

Report:	KCP 10.3.1.1.1/01; [redacted],; 2009; M-357085-01-1
Title:	Effects of methiocarb FS 500 G (MTC FS 500 G) (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.:	51241035
Document No.:	M-357085-01-1
Guideline(s):	OECD 213 and 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Material and Methods:
Test item: Methiocarb FS 500 G (active substance methiocarb (H 321); Specification No.: PF90144715; Density: 1.128 g/mL; Content of a.s.: 45.1% w/w, 508.7 g/L.
Thirty worker bees per treatment were exposed for 72 hours to doses of 1.0, 0.5, 0.25, 0.13 and 0.062 µg a.s./bee for topical application (contact) and for 48 hours to doses of 0.30, 0.15, 0.084, 0.041 and 0.021 µg a.s./bee for feeding (oral, value based on the actual intake of the test item). Due to increasing mortality between 24 and 48 hours the contact test was prolonged for further 24 hours up to 72 hours.

Results:

Contact test

Dose levels of 1.0, 0.5 and 0.25 µg a.s./bee led to mortality ranging from 96.7 to 20% at the end of the test (72 hours). No mortality occurred at 0.13 and 0.021 dose levels as well as in the control group (water + 0.5% Adhaesit). During the first 48 hours coordination problems, apathy or vomiting were observed in the two highest dose levels (1.0 and 0.5 µg a.s/bee). During the 72 hours assessment one bee in the 1.0 and 0.25 µg a.s./bee dose groups behaved abnormally. No behavioural abnormalities were found in the other dose levels at any time.

Oral Test

Oral doses of 0.30, 0.15, 0.084 and, 0.041µg a.s./bee resulted in mortality ranging from 93.3% to 3.3% at the end of the test (48 hours after application). No mortality occurred in the 0.021 µg a.s./bee group. Control mortality was 0.0%. During the 4 hours assessment movement coordination problems and/or apathy were observed in the 0.30, 0.15 and 0.084 µg a.s./bee dose groups. After 24 hours one bee showed uncoordinated movement in the 0.3 µg a.s./bee dose group. No behavioural abnormalities were found in the 0.41 and 0.021 µg a.s./bee dose groups at any time.

Toxicity to honey bees in a laboratory tests with Methiocarb FS 500 G

Test Item	Methiocarb FS 500 G	
Test object	*Apis mellifera*	
Application rate µg a.s./bee	1.0, 0.5, 0.25, 0.13 and 0.062	0.30, 0.15, 0.084, 0.041 and 0.021
Exposure	contact (solution in Adhaesit (0.5%)/water)	oral (sugar solution)
LD_{50} µg a.s./bee	24 hours: 0.52; 48 hours: 0.44; 72 hours: 0.38	24 hours: 0.11; 48 hours: 0.11

The contact and oral LD50 (24 h) values of the reference item (dimethoate) were calculated to be 0.16 and 0.15 µ.g a.i./bee, respectively.

Conclusion:

The toxicity of Methiocarb FS 500 G was tested in both an acute contact and oral toxicity test on honey bees. The LD_{50} (24 h + 48 h) was 0.11 µg a.s./bee in the oral toxicity test.
The LD_{50} (24, 48 + 72 h) of Methiocarb FS 500 G was determined to be 0.52, 0.44 and 0.38 µg a.s./bee in the contact toxicity test.

CP 10.3.1.1.2 Acute contact toxicity to bees

In the study by [redacted] F; 2009; M-357085-01-1 the acute and contact toxicity was assessed together (KCP 10.3.1.1.1)
Additionally, an acute contact toxicity study was conducted on bumble bees with methiocarb. The corresponding summary is provided in Document MCA, Section 8.3.1.1.2 ([redacted].; 2014; M-479538-01-1).

CP 10.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study was conducted with the active substance methiocarb ([redacted], A.; 2015; M-540431-01-1) and is included in the MCA document, Section 8.3.1.2.

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A honey bee colony study according to a tailor made study design ([redacted] et al.; 2015; M-534766-01-1) has been conducted with Methiocarb FS 500 and is included in Document MCA, Section 8.3.1.3.

CP 10.3.1.4 Sub-lethal effects

There is no particular study design / test guideline to assess "sub-lethal effects" in honey bees. However, in each laboratory study as well as in any higher-tier study, sub-lethal effects, if occurring, are described and reported.

CP 10.3.1.5 Cage and tunnel tests

For information on studies already evaluated for the Annex I inclusion of methiocarb under Directive 91/414/EEC, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the DAR.

Report:	KCP 10.3.1.5/01 [redacted],; [redacted]; 2003; M-088296-01-1
Title:	Evaluation of the effects of residues of Methiocarb in maize pollen from dressed seeds on honeybees (*Apis mellifera*) in the semifield
Report No.:	MAUS/AM 025
Document No.:	M-088296-01-1
Guideline(s):	special design, no standard guideline available
Guideline deviation(s):	none
GLP/GEP:	**yes**

Material and methods:

Test substance: maize pollen from plants grown from seeds which had been dressed with Methiocarb FS 500 (seeds dressed under non-GLP conditions with Methiocarb FS 500, TOX-No. 6047-00, Article No. 000441l935, Batch-No. 233025054, loading rate according to analysis of dressed seeds TOX-No. 06048-00: 521.32 g Methiocarb/ 100 kg seeds).

Small honeybee colonies (approx. 1.500 honeybees) were confined on oat plots (16 m²) in tents and fed with maize pollen from plants grown from seeds which had been dressed with Methiocarb FS 500 or untreated control pollen. For treatment and control, three replicates were set up each. Sunflower honey was provided as carbohydrate source. The small bee colonies were examined for treatment-related effects over a period of 35 days. In particular, the endpoints mortality, foraging activity and pollen stores were evaluated. Likewise, comb cell production, honey and pollen consumption, honey stores, egg laying activity, breeding activity, colony strength and hive weight development were assessed and statistically analysed using a t-Test.

Behavioural anomalies of the honeybees were also assessed.

Observations: There were no significant differences between control and treatment in comb cell production (t=-0.918, p=0.385), honey consumption (t=-0.771, p=0.484), pollen consumption (t=-1.455, p=0.219), honey stores (t=-0.186, p=0.857), hive weight increase (t=0.510, p=0.637), egg deposition (t=1.228, p=0.255), larval abundance (t=0.483, p=0.642), pupal abundance (t=-0.671, p=0.521) and abundance of adult bees (t=0.549, p=0.598). Foraging activity at the pollen feeder and the honey feeder was comparable in control and treatment. There were no pollen stores in the control and only small stores in the treatment. Due to the few data no statistical analysis could be carried out for this endpoint. Mortality was comparable in control and treatment, although slightly higher in the control but well in the usual range of bee mortality in both control and treatment. Towards study end is was noticed by the bee keeper that the bee colonies started with preparations overwintering in one replicate of the control and treatment.
The residue levels of methiocarb and its Sulfone- and Sulfoxide-metabolites determined in the pollen used for feeding which originated from seeds dressed with Methiocarb FS 500 were below the limit of detection (methiocarb, methiocarb-sulfone and methiocarb-sulfoxide: LOQ=0.025 mg/kg, LOD=0.001 mg/kg).

Results:

Effects of residues of Methiocarb FS 500 in pollen on small honeybee colonies

Testing Endpoint	Control 1A	Control 1B	Control 1C	Treatment 2A	Treatment 2B	Treatment 2C
Mortality (Total No. of dead bees in front of the bee hives) [n]	4	2	13	3	3	2
Cumulative comb cell production at study termination [cm²]	573	551	414	411	498	551
Cumulative honey collected [g]	699	671	447	621	709	681
Cumulative pollen collected [g]	35.5	53.7	24.0	43.4	55.6	56.2
Honey storage area at study termination [cm²]	329	272	227	285	240	363
Pollen storage area at study termination [cm²]	0	0	0	0	0	4
Egg laying activity [cm² comb area containing cells with eggs at study termination]	27	16	16	16	22	25
Larval abundance [cm² comb area containing cells with larvae at study termination]	5	0	0	0	0	0
Pupal abundance [cm² comb area containing cells with pupae at study termination]	0	49	11	11	54	46
Colony strength [cm² comb area covered with bees at study termination]	325	316	105	294	312	308
Hive weight increase [%]	2.6	-1.4	-10.4	-1.9	0.5	-1.8
Foraging activity [Average No. of bees at the pollen feeder / assessment]	0.5	1.6	0.6	1.2	1.1	1.5
Foraging activity [Average No. of bees at the honey feeder / assessment]	6.6	7.0	5.9	6.2	7.1	6.9

Conclusion:

The results of the study show that there is no risk to honeybees by foraging on and consumption of maize pollen of plants originating from seeds dressed with Methiocarb FS 500 at rates up to 521.32 g and Methiocarb/100 kg seeds.

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Report:	KCP 10.3.1.5/02[redacted]; 2002; M-059860-01-1
Title:	Evaluation of the effects of residues of Methiocarb FS 500 in fortified maize pollen on honeybees (*Apis mellifera*) in the semi-field
Report No.:	MAUS/AM 019
Document No.:	M-059860-01-1
Guideline(s):	Internal Testing Method
Guideline deviation(s):	--
GLP/GEP:	**yes**

Material and general methods:

Test substance: maize pollen fortified with Methiocarb FS 500, article No.: 0004411935, batch No. 233825178, TOX-No. 5206-01. Small honeybee colonies (approx. 500 honeybees) were confined on oat plots (50 m², drilled on 2001-05-03) in tunnels and fed with untreated control pollen and maize pollen fortified with Methiocarb FS 500. There were three replicates set up for the control and one replicate for each fortification level. Sunflower honey was provided as energy source. The small bee colonies were examined for treatment-related effects over a period of 52 days. In particular, the following endpoints were assessed: comb cell production, food consumption, pollen and honey storage behaviour, egg laying activity, breeding success, colony strength, hive weight development, foraging intensity. Behavioural anomalies were also assessed.

The residue levels in the control pollen were below the limit of detection (LOD=0.005 mg/kg) for methiocarb, methiocarb-sulfone and methiocarb-sulfoxide (analysis carried out 2002-04-11). After fortification of untreated pollen with Methiocarb FS 500 the actually achieved concentrations were 12.4µg/kg (treatment 4c), 6.2µg/kg (treatment 4b), 3.1µg/kg (treatment 4a) (analysis carried out 2001-07-18, 2001-07-19).

Results:

Effects of residues of Methiocarb FS 500 in fortified maize pollen on small honeybee colonies

Testing Endpoint	Control 1a	Control 1b	Control 1c	3.1 µg/kg	6.2 µg/kg	12.4 µg/kg
Mortality (Total No. of dead bees in front of the bee hives) [n]	1	1	0	3	3	12
Mortality (Total No. of dead bees at the tunnel edge) [n]	28	31	25	12	58	36
Total comb cell production at study termination [cm²]	768	708	675	656	638	636
Cumulative honey collected [g]	702	694	677	686	661	673
Cumulative pollen collected [g]	12.2	8.9	9.6	17.2	10.8	5.8
Average honey storage area per assessment [cm²]	226	231	179	202	209	192
Average pollen storage area per assessment [cm²]	7	4	6	3	6	4
Brood* in cm² comb area containing cells with brood at study termination]	382	260	358	320	75	111
Colony strength [cm² comb area covered with bees at study termination]	266	183	183	174	155	77
Hive weight increase [%]	25.5	27.6	22.1	25.4	17.1	25.0
Foraging activity [Average No. of bees at the pollen feeder / assessment]	0.7	0.7	0.7	0.6	0.4	0.4
Foraging activity [Average No. of bees at the honey feeder / assessment]	7.3	8.1	7.7	7.2	7.1	7.3
Foraging activity [Average No. of bees at the tent roof / assessment]	2.8	3.5	2.3	3.3	3.5	3.2

Observations:

There were no apparent differences found between control and any treatment in the endpoints mortality, foraging activity, comb cell production, the amount of honey collected, honey stores and hive weight development.

Differences were observed between control and the 12.4 µg/kg treatment group in the cumulative amount of pollen collected, which might be indicative for an avoidance response.

Likewise, colony strength at study termination was strongly reduced in the 12.4 µg/kg treatment compared with the control and the 6.2 µg/kg and 3.1 µg/kg treatments.

In the 6.2 µg/kg and 12.4 µg/kg treatments, abundance of pre-imaginal stages decreased towards the end of the study in comparison with the control and the 3.1 µg/kg treatment.

Conclusion:

There was no indication for a treatment related effect on mortality, foraging activity, comb cell production, the amount of honey collected, honey stores and the weight of the test colonies.

The amount of collected pollen was lower in the group exposed to 12.4 µg/kg test substance pollen than in the other groups. This may indicate an avoidance response.

Breeding performance was obviously lower towards study termination in the two higher treatment groups than in the control and in the 3.1 µg/kg pollen-treatment group, as it was the colony strength in the highest treatment group. However, the great fluctuations of these endpoint data during the test

make it difficult to reliably relate these differences to the treatments. What seems conclusive, however, from the results of this study, is that residues of the test item in pollen up to levels of at least 3.1 µg/kg do not pose an unacceptable risk to honey bees.

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Report:	KCP 10.3.1.5/03 [redacted],; 2015; M-539746-01-1
Title:	Methiocarb FS 500G: A semi-field study to evaluate the effects of spiked pollen and spiked sugar solution to the honey bee *Apis mellifera carnica* L. (Hymenoptera: Apidae) in Germany 2009
Report No.:	S09-00463
Document No.:	M-539746-01-1
Guideline(s):	OEPP/EPPO Guideline No. 170 (3), 2001, with modifications
Guideline deviation(s):	The target concentrations of the test item in pollen and sugar solution were not achieved. No analytical certificate of the spiked pollen was generated. No rainfall measured on DAS1. The outside of the hive pollen uptake could not be determined exactly.
GLP/GEP:	**yes**

Material and Methods:

Test item: Methiocarb FS 500 G; active ingredient: methiocarb (development code: H 321), 500 g a.s./L; nominal; Batch-ID: PF90144715.

The study was carried out by following the general provisions of the OEPP/EPPO Guideline No. 170 (3): Guideline for the efficacy evaluation of plant protection products – Side effects on honeybees (OEPP/EPPO, 2001), with modifications. The effects of pollen and sugar solution, both spiked with methiocarb (via Methiocarb FS 500G), were evaluated on small honey bee colonies under confined conditions in the semi-field, by exposing the honey bee colonies in the test item treatment groups exclusively to methiocarb-treated diet, in tunnels, set-up on a bare soil field. The study comprised one control group (C) and three test item treatment groups (T1, T2, T3); each group was replicated three times. The target concentration of methiocarb in the test item treatment groups was 10 µg methiocarb a.s./kg (= treatment level: T1), 30 µg a.s./kg (= treatment level: T2) and 80 µg a.s./kg (= treatment level: T3) in both, pollen and sugar solution, respectively.

In all of the tunnels, either methiocarb-treated pollen (i.e. T1-, T2-, T3-level) or untreated (i.e. control) pollen was offered inside and outside of the hives, on one Petri dish, respectively. The offered amount of pollen on each Petri dish was 25 g/day. During the 10 day exposure period under confined conditions, the pollen of the previous day was removed and renewed on a daily basis. On the day of the set-up of the hives, 2.5 kg methiocarb-treated (i.e. T1-, T2-, T3-level) or 2.5 kg untreated (i.e. control) sugar solution was offered per hive in a hive-feeder insert. Start of feeding (i.e. on DAS1) was the morning after set-up of the hives in their respective tunnels (which was accomplished the day before, on DAS-1).

Just before the set-up of the hives in the tunnels (i.e. before the first colony assessment on DAS -1), most of the pollen/bee-bread and nectar/honey inside the hives was removed, in order to guarantee full exposure of the colonies to the methiocarb-treated sugar solution and the methiocarb-treated pollen.

Two days before set-up of the colonies in their respective tunnels, the respective queens were fixed on a trapping comb within their respective hives. This assured that the brood on that particular comb had an almost uniform age and was fed to a large extent with the test item treated food (in the test item treatment groups). On DAS-1, i.e. just before the start of exposure, the queens were released from their respective trapping comb (and existing food stores (nectar/honey and pollen/bee-bread) were largely removed from the combs; see above). The development of the brood on the former trapping comb was thereafter observed separately.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment groups with the corresponding results as obtained in the control group. The following endpoints were assessed:

- Mortality in front of the hives and in the bee traps
- Flight activity (number of forager bees entering and leaving the hive)
- Food consumption
- Condition of the colonies, as assessed via colony strength, development of the bee brood and development of the in-hive food stores (all: once before exposure and four times after exposure)

Dates of work (biological phase): 14 APR 2009 to 21 MAY 2009

Results:

Test item	Methiocarb FS 500G			
Test object	*Apis mellifera*			
Exposure groups	T1, T2, T3: Feeding of honey bees with spiked (methiocarb-treated) pollen (offered inside and outside the hive) + spiked (methiocarb-treated) sugar solution (offered inside the hive) C (Control): Feeding of honey bees with untreated (control) pollen (offered inside and outside the hive) + untreated (control) sugar solution (offered inside the hive)			
Duration of confined exposure	10 consecutive days			
Code of exposure group	T1	T2	T3	C
Target concentration [µg a.s./kg]	10	30	80	N.A.
Mean actual concentration in pollen [µg a.s./kg]	≈6.3*	18.4	47.8	<LOD
Mean actual concentration in sugar solution [µg a.s./kg]	≈2.7*	≈7.3*	20.3	N.A.
Mean mortality during confined exposure [dead bees/day]	10.0	10.3	17.3	12.4
Mean daily flight activity (in / out) [bees]	7.3	6.4	6.9	5.3
Mean daily consumption of pollen in-/outside per hive [g]	1.4 / 4.7	1.0 / 6.8	0.3 / 2.0	3.1 / 13.4
Mean total consumption of sugar solution per hive [g]	2500	2500	2333	2458

N.A.: not applicable; LOQ (limit of quantification) = 10 µg/kg, LOD (limit of detection) = 2.5 µg/kg

* As the actual limit of quantification was 10 µg/kg, numerically derived values below 10 µg/kg are considered to be of approximate nature

Observations:

Honey bee mortality:

On the first day of confined exposure (DAS1), the mean mortality was comparable between all exposure groups (C: 5.3, T1: 5.0, T2: 2.3, T3: 2.3 dead bees). The number of dead bees increased on the following day due to bad weather conditions (DAS2) in both, in the test item treatment groups and in the control group (C: 29.7, T1: 34.3, T2: 32.7, T3: 66.7 dead bees), respectively. The somewhat higher mortality in the T3 group (2 times higher than C) was due to a higher mortality in one of the three replicates (3T3); however, there is no indication that this increased mortality in one of the three T3-replicates was correlated with a higher consumption of methiocarb-treated diet, neither within the T3-treatment level nor within any other treatment level or control. On the third day of exposure (DAS3), mortality was higher in the control group compared to the test item treatment groups (C: 22.3, T1: 12.0, T2: 15.3, T3: 11.0 dead bees; mean values), with mortality being lowest in the T3 group (2 times lower than C). During the period DAS5 to 10, the mean mortality of all exposure groups was similar and always below 20 dead bees per day. Overall, mortality rates in all test item treatment groups were comparable to the control group, showing a typical level of variability, with mean mortality rates during the entire exposure period (DAS1 to 10) of 12.4, 10.0, 10.3, and 17.3 dead bees/day for C, T1, T2, and T3, respectively.

Thus, no test-item related adverse effects on mortality were found.

Honey bee flight intensity:

With exception of the test item group T2, the flight activity on the first day after set up (DAS1) was the lowest during the study period. With a few exceptions, the flight activity increased constantly during the period DAS1 to 9, where the flight activity of all exposure groups was the highest. On the last day of exposure (DAS10), the flight activity decreased, but was still higher compared to the period DAS1 to 5. Overall, there were no distinct differences in the mean flight intensity between the control and the test item treatment groups, with mean flight intensity in the test item treatment groups being higher or equal compared to control on 6 of 10 days during the confined exposure period.

Thus, no test-item related adverse effects on flight intensity were found.

Food consumption:

During the confined exposure period, the mean daily consumption of pollen offered outside the hives in the control group was 13.4 g pollen/day.

Regarding pollen offered inside the control hives, the corresponding mean daily consumption was 3.1 g/day.

In the tunnels of the test item treatment group T1, T2 and T3, the mean daily consumption of pollen offered during the confined exposure period outside the hives was 4.7, 6.8 and 2.0 g pollen/day, respectively; regarding pollen offered inside the T1-, T2- and T3-hives, the corresponding mean daily consumption was 1.4, 1.0 and 0.3 g/day.

The re-weighing of the pollen offered outside of the hive was generally difficult. Difficulties associated with the outside offered pollen were air movement caused by the bee-wings (during bees flying over the Petri dishes), which sometimes moved pollen out of the Petri dish during pollen-uptake, and dirt, which was partly found in the Petri dishes (could not always be removed completely before re-weighing). Both, inside and outside of the hives, the pollen became sticky until re-weighing, potentially caused by bee activity and/or by air humidity, which could also be the reason for the sometimes negative values of pollen consumption (as determined by re-weighing). It can therefore be assumed that the values measured by pollen re-weighing represent approximate values of consumed pollen.

The consumption of pollen offered inside the hives decreased with the increase of the test item concentration (C>T1>T2>T3). The consumption of pollen offered outside the hives was the highest for C, followed by T2, T1, and finally T3. In general, the consumption of pollen offered outside the hives was higher than that of the pollen offered inside the hive. The control colonies showed the highest pollen consumption, T3 showed the lowest pollen consumption (both, inside and outside the hive).

The offered sugar solution (sugar solution was only offered inside the hive) was generally well accepted by the bees. Two control colonies consumed the sugar solution completely (i.e. 2500 g, respectively), one control colony left 125 g sugar solution. The test item exposed colonies of T1 and T2 consumed the total offered sugar solution. In the test item treatment group T3, one colony consumed the sugar solution completely (i.e. 2500 g), two colonies left 180 g and 320 g, respectively.

Thus, the lower consumption of methiocarb-treated diet suggests a test-item related repellence effect, which becomes particularly apparent related to methiocarb-treated pollen and in the highest test item treatment group T3.

Condition of the colonies:

The mean number of bees per colony at the colony assessment before set-up (DAS-1) was comparable between all (future) exposure groups (C: 3231, T1: 3273, T2: 3252, T3: 3252 bees/hive). At the second colony assessment, directly after the end of the 10 day confined exposure period (DAS11), the mean number of bees per colony in all exposure groups, treatments and control, respectively, had slightly increased compared to the first assessment (C: 3981, T1: 3878, T2: 4149, T3: 3689 bees/hive). The three following assessments (DAS20, DAS27, DAS35) revealed increasing numbers of bees in all exposure groups, at the last assessment (DAS35), the mean number of bees per colony was the highest throughout the study period (C: 6816, T1: 8004, T2: 9818, T3: 8506 bees/hive). The number of bees of the control group decreased slightly between the third and fourth assessment. In general, the mean number of bees at the last two assessments was higher in the colonies of the test item treatment groups when compared to the control group.

The mean number of total brood per colony (i.e. cells filled with eggs, larvae or pupae) at the assessment before set-up (DAS-1) was comparable between all (future) exposure groups (C: 10600, T1: 11933, T2: 14200, T3: 13667 cells/hive). At the second assessment, directly after the end of the 10 day confined exposure period (DAS11), the mean number of cells with brood in all exposure groups had either slightly increased or slightly decreased compared to the first assessment (C: 10400, T1: 13733, T2: 15200, T3: 12933 cells/hive), showing a typical level of variability. Between the 2nd until the 5th assessment, the mean number of brood of all colonies increased to its highest mean values at the last brood assessment on DAS35 (C: 24867, T1: 28467, T2: 29333, T3: 28067 cells/hive).

The mean number of food cells per colony (i.e. the number of cells filled with nectar/honey or pollen/bee-bread) at the assessment just before set-up of the colonies within the respective tunnels (DAS-1) was comparable between all (future) exposure groups (C: 6600, T1: 5667, T2: 7667, T3: 5067 cells/hive). At the second assessment, directly after the end of the 10 day confined exposure period (DAS11), the mean number of food cells per colony had increased in all exposure groups compared to the first assessment (C: 9133, T1: 7867, T2: 9333, T3: 6133 cells/hive), which shows that methiocarb-treated diet was not only consumed but also stored inside the hive. At the following assessments, the mean number of food cells per colony showed some variability, as typical for free ranging honey bee colonies, with a strong increase at the last (DAS35) assessment in all exposure groups (C: 26533, T1: 22333, T2: 25533, T3: 22800 cells/hive).

Thus, no test-item related adverse effects on colony strength (i.e. number of bees), brood- (i.e. cells filled with eggs, larvae or pupae) or food development (i.e. the number of cells filled with nectar/honey or pollen/bee-bread) were found.

<u>Development of brood on the trapping combs:</u>

The mean number of egg cells per colony at the 1st assessment, just before set-up (DAS-1; two days after fixing of the queen) was comparable between the treatment groups with values between 2133 and 3000. The number of eggs between the 1st and the 2nd assessment (i.e. directly after the end of the confined exposure period) decreased in all exposure groups (i.e. C, T1, T2, T3), whereas the number of pupae and larvae as well as the total number of brood cells had increased. This reflects the natural development of honey bees: approximately 3 days after egg laying the development of larvae started. Five days after hatching the larvae capped their cells and the development of the pupae began. In the majority of cases, the queen did not continue laying eggs on the former trapping comb after being released, but on the other combs of the colony. This observation is supported by the findings of the 2nd colony assessment (i.e. directly after the end of the confined exposure period), where the average number of eggs per colony was similar to the average number of eggs per colony as determined at the 1st colony assessment.

The mean number of total brood on the respective trapping comb per colony, at the assessment just before set up (DAS-1; i.e. at this stage, total brood only comprised cells filled with eggs), was comparable between all exposure groups (C: 2133, T1: 2200, T2: 2533, T3: 3000 cells/hive). At the second assessment, directly after the end of the confined 10 day exposure period (DAS11), the mean number of total brood on the respective trapping comb per colony had increased in all exposure groups compared to the first assessment (C: 2600, T1: 2400, T2: 3333, T3: 3733 cells/hive), showing a typical level of variability. At the following assessments, the number of total brood increased or decreased, coherently in all exposure groups (i.e. C, T1, T2, T3), with comparable values at the last (DAS35) assessment (C: 3733, T1: 4400, T2: 4000, T3: 3867 cells/hive).

Thus, consistent to the findings as obtained by the assessment of the entire brood status of the colonies (i.e. by considering total brood on all combs, see above), also no test-item related adverse effects on brood development were found when assessing the findings on the trapping comb separately.

Conclusion:

Overall, it can be concluded that a forced exposure of honey bee colonies under confined conditions with no alternative food source other than methiocarb-treated sugar solution and methiocarb-treated pollen, had no adverse effects on honey bee mortality, colony strength, colony- and brood

development, food storage and overall colony vitality up to and including about 20 ppb [µg a.s./kg] in sugar solution (nectar) + up to and including about 48 ppb [µg a.s./kg] in pollen.

CP 10.3.1.6 Field tests with honeybees

Report:	KCP 10.3.1.6/01 [redacted],; 2015; M-534762-01-1
Title:	Assessment of potential impacts on honeybee colony development, their hibernation performance and concurrent monitoring of aerial dust drift during the sowing operation of methiocarb FS 500 G - Treated maize with typical commercial vacuum-pneumatic sowing technology, directly adjacent to full-flowering *Phacelia tanacetifolia* in Germany
Report No.:	R12261
Document No.:	M-534762-01-1
Guideline(s):	ENV/MC/Chem(98)17 ENV/JM/MONO(2002)9 ENV/JM/MONO(99)22
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:

This study aimed to assess potential effects on honeybee colonies during and after vacuum-pneumatic sowing operation of maize seeds, sown directly adjacent to full-flowering *Phacelia tanacetifolia*. Dust drift deposits were concurrently measured during the sowing of dressed maize seeds with Methiocarb FS 500 G.

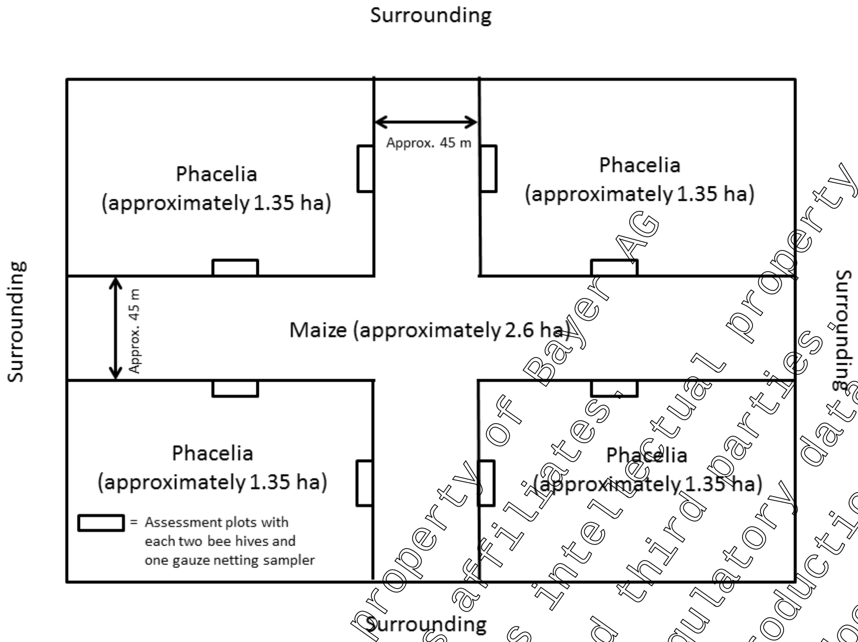
Material and Methods:

Test item:

Conventional maize seeds, dressed with Methiocarb FS 500 G, at a nominal treatment rate of 1.50 mg a.s. methiocarb/seed. The seeds received a conventional seed treatment and were dressed in addition to Methiocarb FS 500 G also with the standard fungicide Thiram® SC 700 (active substance: thiram) while maize seeds dressed with Thiram SC 700 only were drilled on the control fields.

Study sites and GLP-sowing:

The study was conducted in the vicinity of [redacted], Eastern Germany, on four different study fields, two treatment fields and two control fields, all of similar size. To ensure exposition of the honey bees to the potential arising dust drift deposits after the sowing operation, each of the maize fields was surrounded by approximately 5.4 ha flowering *Phacelia tanacetifolia*, a highly bee attractive crop. The dimension of the maize-drilled area inside the *Phacelia tanacetifolia* fields on each individual field was approximately 2.6 ha (actual 2.46 to 2.66 ha, Figure below). The target drilling rate was 100,000 seeds/ha (actual 97,482 to 98,900 seeds/ha on the treatment fields) which corresponded to nominally 150 g methiocarb/ha (actual 146.22 to 148.35 g methiocarb/ha).



Schematic design of the study fields (approximately 8 ha). Maize (approximately 2.6 ha) was sown in a crosswise manner within a *Phacelia* field (approximately 5.4 ha).

Prior sowing, mortality and behaviour were assessed daily for eight days (29 June 2013 to 06 July 2013) and the population strength once (01/02 July 2013). After the sowing operation in each field, a period of exposure, the honey bee hives were monitored for 17 days (07 July 2013 to 23 July 2013). During this period mortality and behaviour were assessed daily and the population strength and development once (22/23 July 2013).

After the exposure period the honey bees were relocated to three monitoring sites for further monitoring and hibernation in a region of North-Rhine-Westphalia near [redacted], with no intensive agricultural activities and no major crop in the flowering period. The 64 honey bee hives were set up evenly distributed (one third of the hives of each study field randomly selected to each hibernation location) on three hibernation locations at the monitoring site to avoid potential impacts due to a high density of honey bee hives, like a lack of food due to food concurrence or *Varroa destructor* infestation. To avoid local factors influencing the results of this study, honey bee hives from the study fields were relocated randomly to the monitoring sites.

Set-up of honey bee hives:

In total 64 honeybee colonies were monitored in the study, 16 on each study field. The honeybee colonies were placed in the assessment plots on 27 June, 2013 approximately 3 m from the edge of the maize field (sowing area). The entrance of each hive was directed to the *Phacelia* areas to recreate the regular apicultural practise. The hives were relocated to the monitoring and hibernation sites in the night between 23 July 2013 and 24 July 2013

Honey bee mortality and behaviour assessments:

The mortality of honeybees (e.g. workers, pupae, drones) was recorded daily for 17 days using dead bee traps during the time of exposure (07 July 2013 to 23 July 2013) and a period of eight days prior to the exposure period (29 June 2013 to 06 July 2013) in which the hives were located at the study fields. If on an assessment day ten or more dead bees were found in one dead bee trap of a hive during the exposure period, they were placed in a sample bottle and labelled individually (colony number, date) to preserve the possibility of further residue analysis. Although there were some colonies with more than ten dead bees on single days the mortality was generally inconspicuous and therefore no such analysis was performed. In parallel, observations on behavioural abnormalities of the honeybees were recorded at the entrance hole of the hives during the mortality assessments. When a queen died or showed significant reduced egg laying capacity, it was replaced by another sister queen. This happened altogether six times (four times in colonies of the control group and two times in colonies of the treatment group).

Honey bee colony strength and health assessment:

Population strength and development (number of cells filled with eggs, larvae or capped brood) as well as food stores (i.e. pollen and nectar) were assessed using the estimation method developed by the Bee Institute [redacted] (Imdorf, Buehlmann et al 1987) The first colony assessment was done shortly after the hives were set up on the edge of the fields but before sowing. This first colony assessment (pre-assessment) defined the starting conditions of the hives before exposure. Three weeks after the pre-assessment, the next colony assessment took place at the end of the exposure period on the study fields. After this assessment, the hives were relocated to the monitoring sites, where four further colony assessments were done before hibernation every three weeks until mid of October 2013. In March 2014, the last colony assessment took place to evaluate the hibernation success of the honey bee hives.

Sampling method:

At the time of bagging of the maize seeds at the Seed Treatment Application Centre of Bayer CropScience AG in D-[redacted], Germany, seed samples for Heubach analysis (non-GLP) and seed loading (non-GLP) were taken (non-GLP).

To measure aerial drift deposits vertically erected gauze-netting-samplers were set up on each assessment plot at the treatment fields. Each sowing operation per row was only performed when the wind speed was below 5 m/s, measured in the middle of the respective study field.

A total of eight units of gauze-netting-samplers (effective sampling area of 2 m x 3.3 m (6.6 m^2) each, were set up alternately at a distance of approx. 3 m from the zero line. Shortly before the beginning of the sowing the gauze-netting-samplers were wetted with a 1:1 (v/v) glycerol/water mixture. Soil samples for water content (non-GLP) and soil characterisation (non-GLP) were taken shortly before sowing.

30 minutes after the completion of sowing, the gauze samples (five 50 x 50 cm squares, 0.25 m^2 each) were cut out of each netting unit and immediately transferred into separate polyethylene flasks.

<u>Residue analysis:</u>

Methiocarb residues in the gauze samples were determined at the Analytical Test Site Bayer CropScience AG.

Dates of Work: 27th June to 24th July 2013 (sowing: 06th July)

Results:

<u>Honey bee mortality:</u>

In both control and treatment groups, honey bee mortality was on the same low level. In average ten dead bees per day were found during the assessments. Regarding to the mortality, no test item related adverse effect could be detected during the whole field phase. The mortality of the brood was on a very low level (mean control group: 0.52 ± 1.91; mean treatment group: 0.45 ± 1.08). On most days, no dead pupae or larvae was found in the dead bee trap.

<u>Honey bee colony development:</u>

Honey bee colony strength showed a similar development in control and treatment group. It was constant during the first three weeks after setup of the bee colonies on the study fields, both in control and treatment group. The amount of brood increased in the same period. This led to a strong increase of the colony strength from the first to the second colony assessment, in colonies of both control and treatment group. From the second assessment (mid of August), the colony strength decreased towards winter and stagnated on a stable level at the 4th and 5th colony assessment. Due to the normal reduction of the breeding activity during winter, the number of worker bees reduced towards spring. Throughout the Field Phase, no significant difference between the mean colony strength of the control and the treatment groups was observed. The slightly, but not significant higher colony strength observed in the control group can be explained by the influence of one single hive (colony 90), that developed to a much larger colony size (up to 50,565 worker bees) than the mean colony size (up to 25,289 worker bees (control group, 2nd Assessment on 13/14 August 2013)).

The mean amount of honey bee brood in both treatment groups was in all assessments on the same level. After an increase between the pre- and first assessment the amount of brood decreased rapidly in all hives in both groups to a very low level at the last assessment (shortly before winter). This is a normal development for honey bee colonies, which typically reduce their brood amount towards winter.

<u>*Varroa destructor* infestation:</u>

The infestation with *Varroa* mites was on approximately the same level in all colonies of both control and treatment group. Statistical analysis (Kruskal-Wallis-test, followed by Mann-Whitney U-test) revealed significant differences regarding the number of dead mites after both formic acid and the first oxalic acid treatment between the hibernation locations with each 20 to 22 hives, randomly selected from both groups. There were no significant differences between the locations [redacted]1 and [redacted]2, but between these two locations and the location [redacted] in almost all cases.

Since all honey bee colonies that did not survived the winter (three in the control group, one in the treatment group), were located at the location [redacted], it can be concluded that the losses were based on local factors like different *Varroa* infestation and not by test item related factors.

Residues:

No residues were found in the control gauze samples (no fortification). In the field spike samples, the mean recovery at study field T1 was 94 % ± 1.6 for 1 µg methiocarb/gauze sample and 98 % ± 1.6 for 100 µg methiocarb/gauze sample. At study field T2 the mean recovery was 89 % ± 0.0 for 1 µg methiocarb/gauze sample and 99 % ± 2.3 for 100 µg methiocarb/gauze sample.

The Limit of Quantification (LOQ) referring to the determination of methiocarb from gauze netting samples was 1 µg methiocarb/L on/from gauze netting samples, equivalent to 0.04 g a.s./ha. The corresponding Limit of Detection (LOD) was 0.1 µg methiocarb/L on/from gauze netting sample, equivalent to 0.004 g a.s./ha.

On study field T2, a clear wind-depending distribution of residues could be shown. On downwind assessment plots (i.e. assessment plot 1, 2 and 7, main wind direction northeast) the residues on the gauze samples (up to average 10.34 µg methiocarb/0.25 m², equivalent to 0.41 g a.s./ha) were distinctly higher compared to those determined on the upwind assessment plots. Due to changing wind conditions, no clear association of the assessment plots at study field T1 to upwind and downwind was possible. This was also demonstrated by relatively uniform residues on most assessment plots.

Conclusion:

To assess the potential effects of a sowing operation of Methiocarb FS 500 G-treated maize seeds on the colony development of honeybees (*Apis mellifera* L.), Methiocarb FS 500 G – treated maize seeds (1.5 mg methiocarb a.s./seed) were sown during bee flight in summer 2013. To increase the possible exposition of the bees to dust, the maize was sown inside adjacent areas of flowering *Phacelia tanacetifolia*, a highly bee attractive crop, were bees were actively foraging.

The dust drift measurements made during the sowing operation of methiocarb-treated maize seeds on the treatment fields (1.5 mg methiocarb a.s./kernel) indicate that seed-treatment dust, abraded and released during the sowing operation with modified (deflected) vacuum-pneumatic sowing equipment, resulted in a measurable off-crop exposure, which was distinctly higher at the downwind borders of the maize sowing area as compared to the corresponding upwind borders. The maximum vertical dust deposition, as measured by vertically erected gauze-netting units, directly adjacent to the maize sowing area, corresponded to a maximum drift rate of 0.41 g a.s./ha (mean values per sampling plot).

The application of Methiocarb FS 500 G did not cause any effects on the survival of adult bees and bee pupae, foraging activity, behaviour, colony development and colony strength as well as on the bee brood and the hibernation success.

CP 10.3.2 Effects on non-target arthropods other than bees

The risk assessment was performed according to Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).

Note: Given that there is currently no EU-agreed guidance for performing a risk assessment for NTA due to exposure from dust, no specific risk assessment covering this question will be presented here. Nevertheless, available higher tier studies related to dust exposure will be presented for information only.

Table 10.3.2- 1: Methiocarb FS 500 (current representative formulation)

Test species, Dossier-file-No., reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Aphidius rhopalosphi M-476014-01-1 [redacted]. 2013	Methiocarb FS 500 Extended lab., Seed treatment dust abraded from maize seeds; exposure on detached bean leaves	LR_{50}: 8.5 g a.s./ha ER_{50}: >6.3 g a.s./ha Corr. Mortality [%] — Effect on Reproduction [%] control — 7.1 — -14.6 A 1.9 g a.s./ha — 0 — -13.9 A 3.4 g a.s./ha — 3.6 — -26.4 A 6.3 g a.s./ha — 19.6 — -27.0 A 11.8 g a.s./ha — 85.7 — n.a.
Typhlodromus pyri M-473003-01-1 [redacted]. 2013	Methiocarb FS 500 Extended lab., Seed treatment dust abraded from maize seeds; exposure on detached bean leaves	LR_{50}: >40.9 g a.s./ha ER_{50}: >40.9 g a.s./ha Corr. Mortality [%] — Effect on Reproduction [%] 3.6 g a.s./ha — 0.0 — 19.6 6.4 g a.s./ha — -3.3 — 12.6 11.1 g a.s./ha — 3.3 — 15.1 21.5 g a.s./ha — -1.1 — 30.9 40.9 g a.s./ha — 4.3 — 35.3
Chrysoperla carnea M-476348-01-1 [redacted]. 2013	Methiocarb FS 500 Extended lab., Seed treatment dust abraded from maize seeds; exposure on detached bean leaves	LR_{50}: 21.2 g a.s./ha No effect on reproduction Corr. Mortality [%] — Eggs/Female/Day — Hatching [%] control — - — 26.1 — 79.7 3.4 g a.s./ha — 0.0 — 26.3 — 76.1 6.6 g a.s./ha — -7.1 — 28.8 — 81.9 11.8 g a.s./ha — 21.4 — 26.1 — 86.2 21.2 g a.s./ha — 42.9 — 27.1 — 88.1 40.9 g a.s./ha — 89.3 — n.a. — n.a.
Coccinella septempunctata M-476374-01-1 [redacted]. 2013	Methiocarb FS 500 Extended lab., Seed treatment dust abraded from maize seeds; exposure on detached bean leaves	LR_{50}: 5.3 g a.s./ha No effect on reproduction Corr. Mortality [%] — Eggs/Female/Day control — - — 11.3 1.9 g a.s./ha — 3.7 — 12.1 3.5 g a.s./ha — 33.3 — 19.2 6.3 g a.s./ha — 48.1 — 34.7 11.7 g a.s./ha — 100 — n.a. 21.8 g a.s./ha — 100 — n.a.

Test species, Dossier-file-No., reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Pardosa spec. M-070496-01-1 [redacted] 2001	Methiocarb FS 500 Extended lab., dressed maize seeds in standard soil (LUFA 2.1) 677 g a.s./ha	Corr. Mortality [%] 2.9 Effect on Feeding Rate [%] -5.3 [B]
Poecilus cupreus, **adults** M-033330-01-1 [redacted], 2001	Methiocarb FS 500 Extended lab., dressed maize seeds in standard soil (LUFA 2.1) 144 g a.s./ha	Corr. Mortality [%] 0 Effect on Feeding Rate [%] 5.7
Poecilus cupreus, **larvae** M-012921-01-1 [redacted], 1992	Methiocarb FS 500 Extended lab., dressed maize seeds in natural soil, 508 g a.s./100kg seeds, 50 units/ha 3750 g a.s./ha	Corr. Mortality [%] 100
Aleochara bilineata M-012919-01-1 [redacted], 1993	Methiocarb FS 500 Extended lab., dressed maize seeds in natural soil 2214 g a.s./ha	Effect on Reproduction [%] 2.4

[A]: A negative value indicates a lower mortality in the treatment than in the control
[B]: A negative value indicates a higher feeding rate in the treatment than in the control.
n.a. = not assessed

Risk assessment for other non-target arthropods

Toxicity tests on non-target arthropods were conducted with dust abraded from maize seeds treated with Methiocarb FS 500. The following 4 species have been tested: *Typhlodromus pyri*, *Aphidius rhopalosiphi* , *Chrysoperla carnea*, and *Coccinella septempunctata*. Further data are available for ground dwelling arthropods that were exposed to maize seeds treated with Methiocarb FS 500. A summary of the results is provided in Table 10.3.2- 1.

Risk assessment procedures

According to the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev 2 final, 17 October 2002) it is recommended that a test with *Folsomia* shall be conducted for the seed dressing product to address the risk for non-target arthropods. The results of the test with *Folsomia* are presented and evaluated in chapter 10.4.2.

There is no EU agreed procedure for the risk assessment of non-target arthropods in off-field habitat following the exposure to dust that might be released during the drilling process. Such a risk assessment should be conducted if an agreed and adopted EU guidance is available.

Soil-dwelling arthropods are exposed to methiocarb in the in-field area following the drilling of Methiocarb FS600 treated seeds. A study with *Poecilus cupreus* larvae at an exaggerated rate of 3750 g a.s./ha which is equivalent to 25 times the intended application rate resulted in 100% mortality.

Studies on the soil dwelling arthropod species *Poecilus cupreus* (adults) at 144 g a.s./ha indicated no effect on mortality and no relevant effect on the feeding rate. A study with *Aleochara bilineata* at an exaggerated application rate of 2214 g a.s./ha indicated no adverse effect on reproduction. Furthermore showed the study on *Pardosa* spec. at exposure rates of 677 g a.s./ha no adverse effects on the mortality or feeding rate. The data indicate that under exposure conditions representative for the intended use of methiocarb (150 g a.s./ha) no unacceptable adverse effects are to be expected on non-target arthropods in the in-field area.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

No new studies are required.

Report:	KCP 10.3.2.1/01 [redacted]; 1993; M-012919-01-1
Title:	Effects of Mesurol FS 500 on the life cycle of rove beetles (*Aleochara bilineata*) under laboratory conditions
Report No.:	SXR/AL 04
Document No.:	M-012919-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Material and methods:

Mesurol FS 500 (active substance: methiocarb, batch No.: 274-17010, content: 508.0 g as/L (analysed). Three plastic boxes (17 x 12.5 x 6 cm), filled with natural soil, served as replicate test chambers for each treatment. Ten female and 10 male rove beetles (*Aleochara bilineata*) were placed in each test box and exposed to either the test material as corn seed coating or the reference substance for 37 days. The test substance, Mesurol FS 500 treated corn seed, were planted approximately 2 cm deep into the soil (21 Units/ha). A carbofuran insecticide granular formulation (Curaterr GR 5) was used as reference substance and applied to the soil at a rate of 20 kg/ha. After the second week, hostfly pupae of *Delia antiqua* were added weekly following application to encourage parasitation. After the 37 day exposure, all the host pupae were sieved from the soil. The host pupae were then maintained in the same conditions and emerging offspring were counted and removed on every work day.

Results:

The total number of beetles that emerged from pupae in the control chambers (n = 2364) is used as a basis for comparison and assumed to be 100%. The offspring emergence rates for Mesurol FS 500 (Test) and Curaterr GR 5 (Reference) treatments were 97.6% (n = 2308) and 27.8% (n = 658), respectively.

Toxicity of Methiocarb FS 500 to the rove beetle (based on nominal concentration)

Test substance	Mesurol FS 500
Test species	*Aleochara bilineata*.
Exposure	37 days
	natural field soil
Application rate	4.36 kg product/ha (2.18 kg as/ha)
Reproduction relative to the control [%]	97.6
Effect on reproduction [%]	2.4
Reference	[redacted] (1999)

Conclusions:

The above results show that even at a seed drilling rate of 21 Units/ha (1 Unit = 50000 seeds) which is 20 times higher as under actual farming conditions, no adverse effects on rove beetles, as represented by *Aleochara bilineata*, are anticipated from an application of Mesurol FS 500 dressed corn seeds up to the proposed dressing rate (1 L product/dt). Under similar laboratory conditions, on the other hand, a reference treatment proved to be very effective at reducing the number of viable offspring.

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Report:	KCP 10.3.2.2/01 [redacted]; 2001; M-070496-01-1
Title:	Methiocarb FS 500 (treated corn seeds): Extended laboratory study to evaluate the effects on the spider, *Pardosa* spp. (Araneae, Lycosidae)
Report No.:	20011067/01-NEPa
Document No.:	M-070496-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	yes

Material and methods:

The effect of corn seeds coated with Mesurol FS 500 (active substance: methiocarb) (521.36 g as (analysed)/100 kg seeds, Tox-No. 05446-01) on lycosid spiders of the genus Pardosa was determined in the laboratory.

Test substance used: Mesurol FS 500, article-no. 04411935, batch-no. 233825178, Tox-No. 5206-00, content of as (H 321) analysed: 504.0 g/L.

Application rate: 677.25 g as/ha on coated seeds effective, based on analysed content of as/100 kg seeds (i.e. 521.36 g as/100 kg seeds) and an effective drilling rate of 129.9 kg seeds/ha, which is approximately 3.71 times the field seed rate (35 kg seeds/ha).

Spiders (24 per treatment group) were individually exposed in test units filled with moist natural soil (LUFA 2.1). One coated corn seed per exposure unit (177 cm²) was incorporated into the soil, which is equivalent to a drilling rate of 129.9 kg seeds/ha (approximately 3.71 times of the recommended drilling rate). Deionised water was used for the control treatment. Perfekthion (as dimethoate) was applied at 800 g as/ha (analysed) in the toxic reference treatment. Test duration was 14 days. During the exposure time the spiders were fed with Drosophila flies (strain unable to fly). Mortality and feeding rate were assessed. Mortality in the toxic reference treatment was 100%.

Results:

Test substance	Corn seeds coated with Methiocarb FS 500
Test species	*Pardosa* spp.
Exposure	standard soil (LUFA 2.1)
Application rate (coated seeds)	677.25 g as/ha (actual)
Corrected mortality [%] after 14 days	2.8
Feeding rate relative to the control [%]	105.3
Effect on feeding rate [%]	-5.3

Observations: No abnormal behavioural effects were observed in the control and in the Mesurol FS 500 treatment groups.

Conclusions:
Corn seeds dressed with Mesurol FS 500 applied at an application equivalent to 677.25 g a.s./ha have no adverse effects on lycosid spiders of the genus *Pardosa*.

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Report: KCP 10.3.2.2/02 [redacted] R, 2001; M-033330-01-1
Title: Acute effects of maize seeds treated with methiocarb FS 500 on carabid beetles (*Poecilus cupreus*) under extended laboratory test conditions
Report No.: MAUS/PC/07
Document No.: M-033330-01-1
Guideline(s): HEIMBACH (1991) (BBA VI, 23 -2.3 from June 1991); HEIMBACH et al. (in prep.)
Guideline deviation(s): none
GLP/GEP: **yes**

Material and methods:
Methiocarb FS 500 (active substance: methiocarb), batch no. 233825178, content: 504 g a.s./L (analysed)
Corn seeds were dressed in a dressing machine at a rate of 536.5 g a.s./100 kg seeds (weight of 1000 seeds: 238 g), allowed to air dry and stored until study initiation. One corn seed was sown per test container (polystyrene box with a surface area of 855 cm², rate equivalent to 144 g a.s./ha). Each box was filled with 3.5 kg of natural soil (Lufa 2.1) and set up 3 days before initiation of the test. Deionised water corresponding to 40% of the soil water-holding capacity. Thirty adult carabid beetles (3 males plus 7 females of *Poecilus cupreus*, 5-8 weeks old) were randomly assigned to each test box (5 replicates). The animals were deprived from food until treatment. Immediately after treatment, the beetles were fed with 1 pupa of house fly (*Musca domestica*) and when the feeding activity was recorded. The study duration was 14 days. During the acclimatisation and the exposure period, the test containers were maintained under a controlled temperature of 19-20°C, a relative humidity of 80-85% and a 16 hour photoperiod.

The reference substance (methyl-parathion) was applied as a bait formulation at a rate equivalent to 74.45 kg/ha. This treatment resulted in a mortality rate of 96.7% and a reduction of the feeding capacity of 44.3% relative to control.

Results:

Test substance	Corn seeds coated with Methiocarb FS 500
Test species	*Poecilus cupreus*
Exposure	standard soil (LUFA 2.1)
Application rate (dressed maiz seeds)	144 g as/ha
Corrected mortality [%] after 14 days	0
Feeding rate relative to the control [%]	94.3
Effect on feeding rate [%]	5.7

In the control group and in the Methiocarb FS 500 treatment group, no mortalities and no behavioural abnormalities were recorded. The control beetles have eaten on average 0.35 fly pupae per viable beetle and day. The beetles exposed to treated corn seeds have eaten on average 0.33 fly pupae per viable beetle and day which is not statistically significantly different to the control.

Observations: Behavioural impacts and survival rates were monitored 2, 4 and 6 hours after treatment and on Day 1, 2, 4, 7, 10, and Day 14. The number of pupae consumed was recorded on Day 2, 4, 7, 10, and Day 14. Differences in mortality rates were tested with the CHI²-test. The number of pupae consumed per living beetle was evaluated by the Mann & Whitney test.

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Report:	KCP 10.3.2.2/05 [redacted].; 2013; M-476014-01-1
Title:	Exposure of the parasitoid wasp *Aphidius rhopalosiphi* to seed treatment dust of methiocarb FS 500 g/L in an extended laboratory test on bean
Report No.:	CW13/047
Document No.:	M-476014-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable MEAD-BRIGGS ET AL. (2000) modified: Use of natural substrate (bean leaf) fixed in a glass cage; application of the test item as dust instead of spray application CANDOLFI ET AL. (2001)
Guideline deviation(s):	Use of natural substrate (bean leaf) fixed in a glass cage; application of the test item as dust instead of spray application
GLP/GEP:	**yes**

Material and methods:

Test item: Seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L, sieved dust fraction < 200 µm, was tested, specified by sample description: TOX 10106-00; specification no.: 102000007167-03; batch ID: 2013-001947 [analysed content of active ingredient: Methiocarb: 53.6% w/w].

The test item was evenly distributed over detached bean leaves (*Phaseolus vulgaris*) at rates of 1.0, 1.9, 3.4, 6.3 and 11.8 g a.s./ha and the effects on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a control of untreated soil (sieved to a fraction of < 200 µm). A toxic reference (active substance: Dimethoate) applied as spraying solution at 3.0 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system.
Mortality of 56 adult wasps, not older than 48 h at study start (4 replicates with 14 wasps per test group), was assessed 2, 24 and 48 h after application.
From the control and all test item rates, 15 impartially chosen females per treatment were each transferred to a cylinder containing untreated barley seedlings infested with *Rhopalosiphum padi* for a period of 24 h. The number of mummies was assessed 12 days later.
The climatic test conditions during the study were 19.0 - 22.0 °C temperature and 61 - 87% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 666 - 793 Lux in the mortality phase, 544 - 840 Lux in the parasitation phase and 14050 - 18490 Lux in the reproduction phase of the study.

Dates of experimental work: September 02, 2013 to September 17, 2013

Results:

Test item:	**Seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L**						
Test organism:	*Aphidius rhopalosiphi*						
Exposure on:	Detached bean leaves						
	Mortality after 48 h [%]				Reproduction		
Treatment	**g a.s./ha**	**Uncorr.**	**Corr.**	**P-Value(*)**	**Rate (mummies per female)**	**Red. rel. to control [%]**	**P-Value(#)**
Control	0	0.0			30.6		
Test item	1.0	7.1	7.1	0.177 n.sign.	35.1	-14.6	0.709 n.sign.
Test item	1.9	0.0	0.0	1.000 n.sign.	34.9	-13.9	0.386 n.sign.
Test item	3.4	3.6	3.6	0.495 n.sign.	38.7	-26.4	0.701 n.sign.
Test item	6.3	19.6	19.6	0.001 sign.	38.9	-27.0	0.813 n.sign.
Test item	11.8	85.7	85.7	<0.001 sign.	n.a.	n.a.	
Reference item	3.0	100.0	100.0		n.a.	n.a.	

LR_{50}: 8.5 g a.s./ha; 95 % Confidence Interval: 7.3 - 9.6 (calculated with Probit analysis)
ER_{50}: > 6.3 g a.s./ha
* Fisher`s Exact test, one-sided, p-values are adjusted according to Bonferroni-Holm
# Wilcoxon test (one-sided), p-values are adjusted according to Bonferroni-Holm
n.a. not assessed n.sign. not significant sign. significant

Conclusion:

In this extended laboratory test the effects of seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L on the survival of the parasitoid wasp *Aphidius rhopalosiphi* were

determined at the test item rates of 1.0, 1.9, 3.4, 6.3 and 11.8 g a.s./ha applied to detached bean leaves (*Phaseolus vulgaris*).

At the lowest test item rate of 1.0 g a.s/ha, a corrected mortality of 7.1% was found. No mortality could be observed at the 1.9 g a.s./ha rate. At the rates of 3.4 and 6.3 g a.s./ha, the corrected mortality was 3.6% and 19.6%, respectively. In the highest test item rate of 11.8 g a.s./ha, a corrected mortality of 85.7% was observed.

The LR_{50} was calculated to be 8.5 g a.s./ha.

Reproduction was assessed for all test item rates except for the highest rate of 11.8 g a.s./ha. No reduction in reproductive success relative to the control occurred at all rates tested.

The ER_{50} was estimated to be > 6.3 g a.s./ha.

The figures obtained fulfil the validity criteria of the laboratory method for the exposure on glass plates.

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Report:	KCP 10.3.2.2/06 [redacted],; 2014; M-476003-01-1
Title:	Exposure of the the predatory mite *Typhlodromus pyri* to seed treatment dust of methiocarb FS 500 g/L in an extended laboratory test on bean
Report No.:	CW13/046
Document No.:	M-476003-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable BLÜMEL ET AL. (2000) modified; CANDOLFI ET AL. (2001)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Material and methods:

<u>Test item:</u> Seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L, sieved dust fraction < 200 µm, was tested, specified by sample description: TOX 10106-00; specification no.: 102000007167-03; batch ID: 2013-001947 [analysed content of active ingredient: Methiocarb: 53.6% w/w].

The test item was evenly distributed over detached bean leaves (*Phaseolus vulgaris*) at rates of 3.6, 6.4, 11.1, 21.5 and 40.9 g a.s./ha and the effects on the predatory mite *Typhlodromus pyri* were compared to those of a control of untreated soil (sieved to a fraction of < 200 µm). A toxic reference (active substance: Dimethoate) as spraying solution at 20.0 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 100 predatory mites, protonymphs at study start (10 replicates with 10 individuals per test group), was assessed 1, 4, 7, 10, 12 and 14 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.

The reproduction rate of surviving mites was then evaluated from day 7 until day 14 after treatment by counting the total number of offspring (eggs and larvae) produced.

The climatic test conditions during the study were 24.0 - 25.5 °C temperature and 60 - 72% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 435 - 1165 Lux.

Dates of experimental work: September 06, 2013 to September 20, 2013

Results:

The mortality / escaping rate in the control exposure units up to day 7 after treatment was 8.0%. The mean corrected mortality of the mites and the mean reproduction rate of the surviving females exposed to the test item and the toxic reference is given below:

Test item:		**Seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L**					
Test organism:		*Typhlodromus pyri*					
Exposure on:		Detached bean leaves					
		Mortality after 7 days [%]			Reproduction		
Treatment	**g a.s./ha**	**Uncorr.**	**Corr.**	**P-Value(*)**	**Rate (eggs per female)**	**Red. rel. to control [%]**	**P-Value(#)**
Control	0	8.0			9.7		
Test item	3.6	8.0	0.0	1.000 n.sign.	7.8	19.6	
Test item	6.4	5.0	-3.3	1.000 n.sign.	8.5	12.6	
Test item	11.1	14.0	3.3	1.000 n.sign.	8.3	15.1	
Test item	21.5	7.0	-1.1	1.000 n.sign.	6.7	30.9	
Test item	40.9	12.0	4.3	1.000 n.sign.	6.3	35.3	
Reference item	20.0	98.0	97.8		n.a.	n.a.	

LR_{50}: > 40.9 g a.s./ha
ER_{50}: > 40.9 g a.s./ha
* Fisher`s Exact test, one-sided, p-values are adjusted according to Bonferroni-Holm
# one-way ANOVA, Williams test (one-sided)
n.a. not assessed n.sign. not significant sign. significant

Conclusions:

In this extended laboratory test the effects of seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L on the survival of the predatory mite *Typhlodromus pyri* were determined at the rates of 3.6, 6.4, 11.1, 21.5 and 40.9 g a.s./ha applied to detached bean leaves (*Phaseolus vulgaris*).

In all test item rates the corrected mortality was below 4.5%.

The LR50 was estimated to be > 40.9 g a.s./ha.

Reproduction was assessed for all test item rates. At the rates of 3.6, 6.4 and 11.1 g a.s./ha, the reproduction was reduced by 19.6%, 12.6% and 15.1%, respectively. A reduction of 30.9% and 35.3%, respectively, was found at the highest test item rates of 21.5 and 40.9 g a.s./ha.

The ER50 was estimated to be > 40.9 g a.s./ha.

The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

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Report:	KCP 10.3.2.2/07[redacted]; 2013; M-476348-01-1
Title:	Exposure of the green lacewing *Chrysoperla carnea* to seed treatment dust of methiocarb FS 500 g/L using an extended laboratory test on bean
Report No.:	CW13/048
Document No.:	M-476348-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable CANDOLFI ET AL. (2001); VOGT ET AL. (2000) modified
Guideline deviation(s):	Use of natural substrate (detached bean leaf) instead of glass plate; application of the test item as dust instead of spray application;
GLP/GEP:	**yes**

Material and methods:

Test item: Seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L, sieved dust fraction < 200 µm, was tested, specified by sample description: TOX 10106-00; specification no.: 102000007167-03; batch ID: 2013-001947 [analysed content of active ingredient: Methiocarb: 53.6% w/w].

The test item was evenly distributed over detached bean leaves (*Phaseolus vulgaris*) at rates of 3.4, 6.6, 11.8, 21.2 and 40.9 g a.s./ha and the effects on the green lacewing *Chrysoperla carnea* were compared to those of a control of untreated soil (sieved to a fraction of < 200 µm). A toxic reference (active substance: Dimethoate) applied as spraying solution at 28.0 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system.

The preimaginal mortality of 30 larvae, 2 days old at study start (per test group), was assessed till the hatch of the imagines (up to 19 days). The fertility and fecundity of the surviving hatched adults were then evaluated over the period of one week.

The climatic test conditions during the study were 23.5 - 27.0 °C temperature and 60 - 78% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 1295 - 2830 Lux in the mortality phase and 2051 - 2402 Lux in the reproduction phase of the study.

Dates of experimental work: August 29, 2013 to October 04, 2013

Results:

Test item:		**Seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L**				
Test organism:		*Chrysoperla carnea*				
Exposure on:		Detached bean leaves				
		Preimaginal mortality [%]			Reproduction	
Treatment	**g a.s./ha**	**Uncorr.**	**Corr.**	**P-Value(*)**	**Eggs per female and day**	**Fertility [hatching rate in %]**
Control	0	6.7			26.1	79.7
Test item	3.4	6.7	0.0	1.000 n.sign.	26.3	76.1
Test item	6.6	0.0	-7.1	1.000 n.sign.	28.8	81.9
Test item	11.8	26.7	21.4	0.120 n.sign.	26.1	86.2
Test item	21.2	46.7	42.9	0.002 sign.	27.1	88.1
Test item	40.9	90.0	89.3	<0.001 sign.	n.a.	n.a.
Reference item	28.0	63.3	60.7		n.a.	n.a.

LR_{50}: 21.2 g as/ha; 95 % Confidence Interval: 17.5 - 25.7 (calculated with Probit analysis)
No effect on reproduction
* Fisher`s Exact test, one-sided, p-values are adjusted according to Bonferroni-Holm
n.a. not assessed   n.sign. not significant   sign. significant

Conclusions:

In this extended laboratory test the effects of seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L on the survival of the green lacewing *Chrysoperla carnea* were determined at the rates of 3.4, 6.6, 11.8, 21.2 and 40.9 g a.s./ha applied to detached bean leaves (*Phaseolus vulgaris*).

In the lowest test item rates of 3.4 and 6.6 g a.s./ha, no corrected mortality was found (0% and -7.1%, respectively). In the 11.8 g a.s./ha rate, the corrected mortality was 21.4%. A corrected mortality of 42.9% was found in the 21.2 g a.s./ha rate. In the highest test item rate of 40.9 g a.s./ha, a corrected mortality of 89.3% was observed.

The LR_{50} was calculated to be 21.2 g a.s./ha.

Reproduction was assessed for all test item rates except for the highest rate of 40.9 g a.s./ha. There were no adverse effects of the test item on the reproductive performance. The mean number of eggs/female/day was above the lower limit given as validity criterion for the glass plate method (mean number of eggs/female/day: ≥ 15, mean hatching rate: ≥ 70%).

The figures obtained fulfil the validity criteria of the laboratory method for the exposure on glass plates.

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Report:	KCP 10.3.2.2/08 [redacted],; 2013; M-476374-01-1
Title:	Exposure of the ladybird beetle *Coccinella septempunctata* to seed treatment dust of Methiocarb FS 500 g/L in an extended laboratory test on bean
Report No.:	CW13/049
Document No.:	M-476374-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable CANDOLFI ET AL. (2001); SCHMUCK ET AL. (2000) modified
Guideline deviation(s):	Use of natural substrate (detached bean leaves) instead of glass plate; application of the test item as dust instead of spray application;
GLP/GEP:	**yes**

Material and methods:

Test item: Seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L, sieved dust fraction < 200 µm, was tested, specified by sample description: TOX 10106-00; specification no.: 102000007167-03; batch ID: 2013-001947 [analysed content of active ingredient: Methiocarb: 53.6% w/w].

The test item was evenly distributed over detached bean leaves (*Phaseolus vulgaris*) at rates of 1.9, 3.5, 6.3, 11.7 and 21.8 g a.s./ha and the effects on the ladybird beetle *Coccinella septempunctata* were compared to those of a control of untreated soil (sieved to a fraction of < 200 µm). A toxic reference (active substance: Dimethoate) applied as spraying solution at 12.0 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system.

The preimaginal mortality of 30 larvae, 4 days old at study start (per test group), was assessed till the hatch of the imagines (up to 15 days). The fertility and fecundity of the surviving hatched adults were then evaluated over the period of 17 days.

The climatic test conditions during the study were 23.5 - 27.0 °C temperature and 61 - 75% relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 1620 - 4638 Lux during the study.

Dates of experimental work: August 22, 2013 to October 01, 2013

Results:

Mortality and reproduction in each of the treatments are summarized below.

Test item:		Seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L			
Test organism:		*Coccinella septempunctata*			
Exposure on:		Detached bean leaves			
		Preimaginal mortality [%]			Reproduction
Treatment	**g a.s./ha**	**Uncorr.**	**Corr.**	**P-Value(*)**	**Eggs per female and day**
Control	0	10.0			11.3
Test item	1.9	13.3	3.7	0.500 n.sign.	12.1
Test item	3.5	40.0	33.3	0.015 sign.	19.2
Test item	6.3	53.3	48.1	<0.001 sign.	34.7
Test item	11.7	100.0	100.0	<0.001 sign.	n.a.
Test item	21.8	100.0	100.0	<0.001 sign.	n.a.
Reference item	12.0	100.0	100.0		n.a.
LR_{50}: 5.3 g as/ha; 95 % Confidence Interval: 3.7.5 - 6.5 (calculated with Probit analysis) **No effect on reproduction** * Fisher`s Exact test, one-sided, p-values are adjusted according to Bonferroni-Holm n.a. not assessed n.sign. not significant sign. significant					

Conclusions:

In this extended laboratory test the effects of seed treatment dust abraded from maize seeds treated with Methiocarb FS 500 g/L on the survival of the ladybird beetle *Coccinella septempunctata* were determined at the rates of 1.9, 3.5, 6.3, 11.7 and 21.8 g a.s./ha applied to detached bean leaves (*Phaseolus vulgaris*).

At the test item rates of 1.9, 3.5 and 6.3 g a.s./ha, a corrected preimaginal mortality of 3.7%, 33.3% and 48.1% has been observed, respectively. At the highest rates of 11.7 and 21.8 g a.s./ha a corrected preimaginal mortality of 100% each was found.

The LR50 was calculated to be 5.3 g a.s./ha.

Reproduction was assessed for the three lowest test item rates 1.9, 3.5 and 6.3 g a.s./ha. The mean number of fertile eggs per female and day was 11.3 in the control treatment and 12.1, 19.2 and 34.7 in the test item rates of 1.9, 3.5 and 6.3 g a.s./ha, respectively. Since the reproductive performance was within the range of the historical data base for control beetles (≥ 2 fertile eggs per female and day), this parameter is considered as not affected at the tested test item rates.

The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

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Report:	KCP 10.3.2.2/09 [redacted],; 1992; M-012921-01-1
Title:	Effects of Mesurol FS 500 on carabid larvae (*Poecilus cupreus*) under laboratory conditions
Report No.:	SXR/CA 101
Document No.:	M-012921-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Material and methods:

Methiocarb FS 500 (active substance: methiocarb), 274 167 010 (TOX No.: 3245-00), content: 508 g a.s./L (analysed); effects of the seed dressing Methiocarb FS 500 on carabid larvae were tested under laboratory conditions using dressed corn seeds (1 L of product per 100 kg of seed). There were 40 chambers for each treatment. One corn seed, dressed with Methiocarb FS 500, was sown in each replicate test box (40 cm²) at a depth of approximately 2 cm (corresponding to a seed drilling rate of 50 units/ha; 1 unit = 50 000 corn seeds). This application rate corresponds to an application rate of 3750 g a.s./ha.

Each box was filled with 130 g of natural soil (0.71% organic carbon). Deionised water corresponding to 65% of the water-holding-capacity, was added to the soil at the start of the test.

Curaterr GR 5 was used as the reference and applied at a nominal concentration of 1 g per running metre into a 2 cm deep seed furrow. No dummy formulation was used in the controls. One laboratory-bred *Poecilus cupreus* larvae was added to each cup and mealworm larvae (*Tenebrio molitor*) cut into halves, were provided as food until larvae entered the pupal stage.

Results:

Test substance	Corn seeds coated with Methiocarb FS 500
Test species	*Poecilus cupreus* (larvae)
Exposure	natural soil
Application rate (dressed maiz seeds)	3750 g as/ha
Mortality [%]	100

In the control boxes, 36 out of the 40 larvae successfully completed their metamorphoses. On average, larvae entered the pupal stage at day 26 and completed metamorphoses 13 days later. The mean body weight of the descendants was 71.1 mg (51.5 - 91.9 mg). One of the descendants exhibited wing deformations.

After exposure to either corn seed dressed with Methiocarb FS 500 or the reference treatment Curaterr GR 5, all beetle larvae development was arrested. None out of the 40 exposed larvae entered the pupal stage in either the reference or test treatments.

Observations: Behavioural impairments and survival rates of the carabid larvae were monitored until completion of metamorphosis. The remaining test boxes were emptied on day 43 and the soil was screened for surviving animals. Differences in mortality rates were tested with the CHI²-test. The rate of larval development (time between starting the experiment and pupation, time between pupation and

metamorphoses) were compared for each treatment type using the Kolmogorov-Smirnov statistical test.

Conclusion:
These results show that Mesurol FS 500 seed dressing may impact carabid larvae. In real farming conditions, the seed drilling rate is only 2 units per hectare. The probability of contact is, therefore, strongly diminished under field conditions compared with the laboratory exposure situation of our study (seed drilling rate: 50 units per hectare). Moreover, in a real field situation, the frequency of contact may be diminished due to repellency effects and/or a possible treatment-related shortage of prey within the treated seed furrows.

CP 10.3.2.3 Semi-field studies with non-target arthropods

No new semi-field studies were deemed necessary.

CP 10.3.2.4 Field studies with non-target arthropods

No new field studies were deemed necessary.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

No relevant exposure of non-target arthropods is expected by other routes of exposure.

CP 10.4 Effects on non-target soil meso- and macrofauna

The risk assessment procedure follows the requirements as given in the Council Directive 91/414/EEC (Annex III), Council Directive 97/57/EC (Annex VI) and the Guidance Document on Terrestrial Ecotoxicology.

Predicted environmental concentrations used in risk assessment

The PEC_{soil} values below are taken from MCP Sec.9, Point 9.1.3.

Table 10.4- 1: Initial max PEC_{soil} values

Compound	Maize
	$PEC_{soil, max}$ [mg/kg]
Methiocarb FS 500	0.45 [A]
Methiocarb	0.200
Methiocarb-sulfoxide-phenol	0.059
Methiocarb-sulfoxide	0.126
Methiocarb-methoxy-sulfone	0.025
Methiocarb-sulfone-phenol	0.035

[A] Calculated for a soil depth of 5 cm, a soil density of 1.5 g/mL and a product density of 1.125 g/mL

The tier 1 risk assessments are based on the worst case PEC_{soil} values for the application as a seed treatment in maize.

CP 10.4.1 Earthworms

Table 10.4.1- 1: Endpoints used in risk assessment

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Methiocarb FS 500	*Eisenia fetida* reproduction 56 d, mixed	**NOEC**	**≥1 mg prod./kg dws [A]** **≥0.447 mg a.s./kg dws [A]**	[redacted] (2013) M-465336-01-1 KCA 8.4.1
Methiocarb FS 500	*Eisenia fetida* reproduction 56 d, treated seeds	NOEC	≥500,000 treated seeds/ha ≥1.983 mg a.s./kg [B]	[redacted] (2001) M-038648-01-1
Methiocarb-sulfoxide-phenol	*Eisenia fetida* reproduction 56 d,	**NOEC**	**≥100 mg pm/kg dws**	[redacted] (2013) M-474567-01-1 KCA 8.4.1
Methiocarb-sulfoxide	*Eisenia fetida* reproduction 56 d,	**NOEC**	**1.12 mg pm/kg dws [C]**	[redacted] (2013) M-469958-01-1 KCA 8.4.1
Methiocarb-methoxy-sulfone	*Eisenia fetida* reproduction 56 d,	**NOEC**	**≥100 mg pm/kg dws**	[redacted] (2013) M-474553-01-1 KCA 8.4.1
Methiocarb-sulfone-phenol	*Eisenia fetida* reproduction 56 d,	**NOEC**	**≥100 mg pm/kg dws**	[redacted] (2013) M-474560-01-1 KCA 8.4.1

dws = dry weight soil; a.s. = active substance; pm = pure metabolite; prod. = product;
Bold values: endpoints used for risk assessment
[A] corrected by a factor of 2 to address log P_{ow} >2 of methiocarb and the high peat content of 10% in artificial soil

[B] calculated based on test substrate of 3 kg dry weight per test vessel, maximum test rate of 5 treated corn seeds per test vessel and actual loading rate of 1.19 mg a.s./corn seed
[C] Study endpoint derived from 28-d biomass endpoint

Risk assessment for earthworms

Table 10.4.1- 2: TER calculations for earthworms

Compound	Species, study type	Endpoint [mg/kg]	worst case $PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Methiocarb FS 500	Earthworm, reproduction	NOEC ≥ 1 [A]	0.450	**≥ 2.2**	5
Methiocarb (tech.)	Earthworm, reproduction	NOEC ≥ 0.447 [A,B]	0.200	**≥ 2.2**	5
Methiocarb-sulfoxide-phenol	Earthworm, reproduction	NOEC ≥ 100	0.059	≥ 1695	5
Methiocarb-sulfoxide	Earthworm, reproduction	NOEC 1.12 [C]	0.126	8.9	5
Methiocarb-methoxy-sulfone	Earthworm, reproduction	NOEC ≥ 100	0.025	≥ 4000	5
Methiocarb-sulfone-phenol	Earthworm, reproduction	NOEC ≥ 100	0.035	≥ 2857	5

Bold values do not meet the trigger
[A] corrected by a factor of 2 to address log P >2 of methiocarb and the high peat content of 10% in artificial soil
[B] The NOEC of MTC tech. given in mg a.s./kg soil was recalculated from the MTC FS 500 study
[C] Study endpoint derived from 28-d biomass endpoint

The TER values calculated with the worst case $PEC_{soil,\ max}$ values for the methiocarb metabolites methiocarb-sulfoxide-phenol, methiocarb-sulfoxide, methiocarb-methoxy-sulfone and methiocarb-sulfone-phenol clearly exceed the trigger value of 5. However, the TER value for Methiocarb FS 500 and methiocarb is below the trigger of concern, indicating a potential risk for earthworms. Therefore, further refinement is necessary.

Refined risk assessment

The effect of Methiocarb FS 500 (513 g a.s./L) dressed seed on the reproduction of earthworms (*Eisenia fetida*) was assessed in a subchronic laboratory study ([redacted] 2001, M-038648-01-1). Test item application were 100 000, 200 000 and 500 000 dressed maize seeds per ha (1, 2, and 5 seeds per vessel). The concentration in the highest rate is calculated being 1.983 mg a.s./kg, taking into account the surface of the test vessel, the loading of seeds of 1.18956 mg a.i./seed, 3 kg dw soil per test vessel, and an application rate of 5 seeds/vessel. Adult worms were exposed for 4 weeks (first part) and then removed from the test containers. The number of surviving animals and their body weight were determined. Cocoons and juvenile earthworms remained in the artificial soil for another 4 weeks (second part). Overall, no adverse effects on earthworms were observed. Methiocarb FS 500 applied as a seed dressing at up to 5-fold normal seeding rate of 500 000 seeds/ha revealed no effects earthworms. No effects at the 5-fold seeding rate and a TER of 10 (NOEC of 1.98 mg a.s./kg divided by a PEC_{soil} of 0.2 mg a.s./kg) demonstrate an acceptable risk to earthworms. In the past EFSA conclusion from 12th of May 2006 this study was already considered acceptable and a low risk to earthworms was concluded following the use of Methiocarb FS500 at a recommended application rate of 150 g a.s./ha.

CP 10.4.1.1 Earthworms sub-lethal effects

Studies are provided in KCA 8.4.2.1.

CP 10.4.1.2 Earthworms field studies

In view of the results presented above, no field studies were necessary.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Table 10.4.2- 1: Endpoints used in risk assessment

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Collembola, reproduction				
Methiocarb FS 500	*Folsomia candida* reproduction 28 d, mixed	NOEC	84.7 mg prod./kg dws **37.5 mg a.s./kg dws**	[redacted] (2002) M-062852-01-1 KCA 8.4.2.1
Methiocarb-sulfoxide-phenol	*Folsomia candida* reproduction 28 d, mixed	NOEC	**≥100 mg p.m./kg dws**	[redacted] (2001) M-061346-01-1 KCA 8.4.2.1
Methiocarb-sulfoxide	*Folsomia candida* reproduction 28 d, mixed	NOEC	**10 mg p.m./kg dws**	[redacted] (2001) M-075368-01-1 KCA 8.4.2.1
Methiocarb-methoxy-sulfone	*Folsomia candida* reproduction 28 d, mixed	NOEC	**10 mg p.m./kg dws**	[redacted] (2001) M-088567-01-1 KCA 8.4.2.1
Methiocarb-sulfone-phenol	*Folsomia candida* reproduction 28 d, mixed	NOEC	**≥1000 mg p.m./kg dws**	[redacted] (2001) M-087513-01-1 KCA 8.4.2.1
Soil mites, reproduction				
Methiocarb FS 500	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	45 mg prod./kg dws **20.12 mg a.s./kg dws**	[redacted] (2013) M-469819-01-1 KCA 8.4.2.1
Methiocarb-sulfoxide-phenol	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg p.m./kg dws**	[redacted] (2013) M-469826-01-1 KCA 8.4.2.1
Methiocarb-sulfoxide	*Hypoaspis aculeifer* reproduction 14 d,	**NOEC**	**10 mg p.m./kg dws**	[redacted] (2013) M-469961-01-1 KCA 8.4.2.1
Methiocarb-methoxy-sulfone	*Hypoaspis aculeifer* reproduction 14 d,	**NOEC**	**≥100 mg p.m./kg dws**	[redacted] (2013) M-469618-01-1 KCA 8.4.2.1
Methiocarb-sulfone-phenol	*Hypoaspis aculeifer* reproduction 14 d, mixed	**NOEC**	**≥100 mg p.m./kg dws**	[redacted] (2013) M-469625-01-1 KCA 8.4.2.1

dws = dry weight soil; a.s. = active substance; pm = pure metabolite, prod. = product
grey script: study is part of the Baseline Dossier (Annex I inclusion)
Bold values: endpoints used for risk assessment

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

Table 10.4.2- 2: TER calculations for other non-target soil meso- and macrofauna

Compound	Species	Endpoint [mg/kg]		$PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Methiocarb FS 500	*Folsomia candida*	NOEC	84.7	0.450	188	
	Hypoaspis aculeifer	NOEC	45		100	
Methiocarb tech.	*Folsomia candida*	NOEC	37.5 [A]	0.200	188	
	Hypoaspis aculeifer	NOEC	20.12 [B]		101	
Methiocarb-sulfoxide-phenol	*Folsomia candida*	NOEC	≥ 100	0.059	≥ 1695	
	Hypoaspis aculeifer	NOEC	≥ 100		≥ 1695	
Methiocarb-sulfoxide	*Folsomia candida*	NOEC	50	0.126	397	
	Hypoaspis aculeifer	NOEC	10		79	
Methiocarb-methoxy-sulfone	*Folsomia candida*	NOEC	10	0.025	400	
	Hypoaspis aculeifer	NOEC	≥ 100		≥ 4000	
Methiocarb-sulfone-phenol	*Folsomia candida*	NOEC	≥ 100	0.035	≥ 2857	
	Hypoaspis aculeifer	NOEC	≥ 100		≥ 2857	

[A] The NOEC of MTC tech. is given in mg a.s./kg soil in the MTC FS 500 study
[B] The NOEC of MTC tech. given in mg a.s./kg soil was recalculated from the MTC FS 500 study

All TER values calculated with the worst case $PEC_{soil, max}$ values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on soil macro-organisms are to be expected from the intended use of Methiocarb FS 500 G.

CP 10.4.2.1 Species level testing

Studies are provided in KCA 8.4.2.1.

CP 10.4.2.2 Higher tier testing

In view of the results presented above, no further testing is necessary.

CP 10.5 Effects on soil nitrogen transformation

Risk assessment for Soil Nitrogen Transformation

Table 10.5- 1: Endpoints used in risk assessment

Test item	Test design	Endpoint		References
N-transformation				
MTC FS 500 G	Study duration 28 d	no unacceptable effects	**≥3.9 mg prod./kg dws** **≥1.7 mg a.s./kg dws**	[redacted] (1988) M-013195-01-2 KCA 8.5
Methiocarb-sulfoxide-phenol	Study duration 28 d	no unacceptable effects	**≥1.09 mg/kg dws**	[redacted] (2000) M-023228-01-1 KCA 8.5
Methiocarb-sulfoxide	Study duration 28 d	no unacceptable effects	**≥1.47 mg/kg dws**	[redacted] (2000) M-026518-01-1 KCA 8.5
Methiocarb-methoxy-sulfone	Study duration 28 d	no unacceptable effects	**≥1.33 mg/kg dws**	[redacted] (2000) M-026516-01-1 KCA 8.5
Methiocarb-sulfone-phenol	Study duration 28 d	no unacceptable effects	**≥1.20 mg/kg dws**	[redacted] (2001) M-033536-01-1 KCA 8.5

Bold values: endpoints used in the risk assessment
grey script: study is part of the Baseline Dossier (Annex I inclusion)

Risk assessment for Soil Nitrogen Transformation

Table 10.5- 2: Risk Assessment for soil micro-organisms

Compound	Species	Endpoint [mg/kg]	$PEC_{soil,max}$ [mg/kg]	Refinement required
MTC FS 500 G	Soil micro-organisms	≥3.9	0.450	No
Methiocarb tech.	Soil micro-organisms	≥1.7 [A]	0.200	No
Methiocarb-sulfoxide-phenol	Soil micro-organisms	≥1.09	0.059	No
Methiocarb-sulfoxide	Soil micro-organisms	≥1.47	0.126	No
Methiocarb-methoxy-sulfone	Soil micro-organisms	≥1.33	0.025	No
Methiocarb-sulfone-phenol	Soil micro-organisms	≥1.20	0.035	No

[A] The endpoint of MTC tech. is given in mg a.s./kg soil in the MTC FS 500 study

According to regulatory requirements the risk is acceptable, if the effect on nitrogen transformation at the maximum PEC_{soil} values is < 25% after 100 days. In no case, deviations from the control exceeded 25% after 28 days, indicating low risk to soil micro-organisms.

CP 10.6 Effects on terrestrial non-target higher plants

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area. Spray drift from the treated areas may lead to residues of a product in off-crop areas.

As Methiocarb FS 500 is used as seed treatment, an exposure of non-target plants in adjacent fields due to spray drift is out of concern. Therefore, a risk assessment and tests on non-target plants are not required.
However, screening studies with the representative formulation Methiocarb FS 500 have been submitted during Annex I inclusion and are presented in the table below. For details reference is made to the corresponding section in the DAR (2005). Additionally, tier-1 limit tests have been conducted with the formulation Methiocarb SC 500. An overview is presented in the table below and study summaries can be found in CP 10.6.2.

Table 10.6- 1: Ecotoxicological effects for non-target terrestrial plants

Test item	Study type	Test duration	Lowest ER_{50}	Most sensitive species	References
Methiocarb FS 500	Pre-emergence screening; 11 species	21 days	> 240 g a.s./ha	No effect on any species tested	[redacted] (2001) M-090032-01-1 KCA 8.6.1
Methiocarb SC 500	Seedling emergence tier-1; 10 species	14 days	> 2 L prod./ha	Oilseed rape (38.9% red. of emergence)	[redacted] (2007a) M-288173-01-1
Methiocarb FS 500	Post-emergence screening; 11 species	17 days	> 240 g a.s./ha	No effect on any species tested	[redacted] (2001) M-090032-01-1 KCA 8.6.1
Methiocarb SC 500	Vegetative vigour tier-1; 10 species	21 days	> 2 L prod./ha	Tomato (32.8% red. of dry weight)	[redacted] (2007b) M-288172-01-1

Note: Studies written in **grey** font are referring either to studies which have been submitted for Annex I inclusion; whereas studies in **black** font are studies submitted for Annex I renewal.

CP 10.6.1 Summary of screening data

For information on studies already evaluated during Annex I inclusion of this compound, please refer to the corresponding section in the DAR and in the baseline dossier.

CP 10.6.2 Testing on non-target plants

Report:	KCP 10.6.2/01 A; ; 2007; M-288173-01-1
Title:	Non-target terrestrial plants: an evaluation of the effects of Methiocarb SC 500 in the seedling emergence and growth test (Tier 1)
Report No.:	SE07/01
Document No.:	M-288173-01-1
Guideline(s):	OECD 208 (July 2006): seedling emergence and growth test (Tier 1)
Guideline deviation(s):	none
GLP/GEP:	**no**

Material and methods:

Test item was Methiocarb SC 500, sample description: TOX07758-00, batch ID: PF90060209, content for release: 44.8% w/w methiocarb, appearance: white suspension, approved until: 2008-10-11.

Ten species of terrestrial non-target plants (3 monocots and 7 dicots) were treated at an application rate of 2 L product/ha. The species tested were maize (*Zea mays*), oat (*Avena sativa*), ryegrass (*Lolium perenne*), cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), sugar beet (*Beta vulgaris*), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum*) and buckwheat (*Fagopyrum esculentum*).

All seeds were planted on the day of application and test duration was 14 days after 70% emergence of the seedlings in the controls for each species.

Spray treatments were applied once at test initiation to the soil surface with a sprayer set at the nominal spray volume of 200 L/ha. Control pots were sprayed with deionised water. Four replicates with five seeds per pot were tested for each species. All pots were individually contained in saucers and retained on benches within a greenhouse. Assessment of emergence, survival and phytotoxicity were conducted on days 7 and 14. At study termination, endpoint determinations were performed for plant dry weights.

Results:

A summary of the effects (at study termination) of 2 L/ha Methiocarb SC 500 on the seedling emergence and growth of the 10 plant species tested is presented in the table below:

	cucum-ber	oilseed rape	soy bean	sugar beet	sun-flower	tomato	buck-wheat	maize	oat	rye-grass
Germination (% inhibition*)	0	38.9	(5.6)	11.1	(5.3)	(5.9)	0	10.0	12.5	(5.3)
Survival (% inhibition*)	0	0	0	0	0	0	0	0	0	0
Phytotoxicity	0	0-C	0	0	0	0	0	0	0	0
Dry Weight (% inhibition*)	12.6	32.7	(17.4)	17.2	14.9	21.6	21.9	5.1	18.3	(73.0)

* % inhibition compared to the untreated control

Phytotoxicity rating scale: C = severe symptom(s) throughout the whole plant with younger or newly developed leaves growing normally

() Figures in parentheses indicate that there was an increase when compared to the control

Statistical analysis was carried out using the Pairwise Mann-Whitney-U-test (one sided smaller).
There was no adverse effect of Methiocarb SC 500 on the survival of the ten species tested.
Severe phytotoxicity (stunting) was occasionally observed in oilseed rape only. No symptoms of phytotoxicity were observed for any other species tested.
Germination was inhibited in oilseed rape, sugar beet, maize and oat by 38.9%, 11.1%, 10% and 12.5%, respectively. Germination was increased in soybean, sunflower, tomato and ryegrass by 5.6%, 5.3%, 5.9% and 5.3%, respectively.
Biomass was reduced in cucumber, oilseed rape, sugar beet, sunflower, tomato, buckwheat, maize and oat by 12.6%, 32.7%, 17.2%, 14.9%, 21.6%, 21.9%, 5.1% and 18.3%, respectively. Biomass was increased in soybean and ryegrass by 17.4% and 73.0%, respectively.
None of these differences were significant at the 95% confidence limits. None of these differences reached or exceeded 50% to trigger further testing.

Conclusion: A nominal product application rate of 2 L/ha Methiocarb SC 500 showed no significant adverse effects greater than 50% for all the tested species in the seedling emergence test.

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Report:	KCP 10.6.2/02 [redacted], [redacted]; 2007; M-288172-01-1
Title:	Non-target terrestrial plants: an evaluation of the effects of Methiocarb SC 500 in the vegetative vigour test (Tier 1)
Report No.:	VV07/01
Document No.:	M-288172-01-1
Guideline(s):	OECD 227 (July 2006); vegetative vigour test (Tier 1)
Guideline deviation(s):	none
GLP/GEP:	**no**

Material and methods:
Test item was Methiocarb SC 500, sample description: TOX07758-00, batch ID: PF90060209, content for release: 44.8% w/w methiocarb, appearance: white suspension, approved until: 2008-10-11.
Ten species of terrestrial non-target plants (3 monocots and 7 dicots) were treated at an application rate of 2 L product/ha. The species tested were maize (*Zea mays*), oat (*Avena sativa*), onion (*Allium cepa*), cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max*), sugar beet (*Beta vulgaris*), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum*) and buckwheat (*Fagopyrum esculentum*).
Plants were treated at the 2-4-leaf stage with a single foliar spray application at test initiation, with a sprayer set at the nominal spray volume of 200 L/ha. Control pots were sprayed with deionised water. Four to five replicates with four to five plants per pot were tested for each species. All pots were individually contained in saucers and retained on benches within a greenhouse. Assessment for survival and phytotoxicity was conducted on days 7, 14 and 21 after application. At study termination, endpoint determinations were performed for plant dry weights.

Results:

A summary of the effects (at study termination) of 2 L/ha Methiocarb SC 500 on the vegetative vigour of the 10 plant species tested is presented in the table below:

	cucum-ber	oilseed rape	soy-bean	sugar beet	sun-flower	tomato	buck-wheat	maize	oat	onion
Survival (% inhibition*)	0	0	0	0	0	0	0	0	0	0
Phytotoxicity	0-B	0	0	0	0	0	0	0	0	0
Dry Weight (% inhibition*)	20.5	(9.1)	8.0	(13.5)	**18.9**	**32.8**	9.0	19.4	20.1	(46.3)

* % inhibition compared to the untreated control
Phytotoxicity rating scale: B = moderate symptom(s) throughout the whole plant or severe symptom(s) on a limited area, i.e. one-two leaves
() Figures in parentheses indicate that there was an increase when compared to the control
Bold figures are significant at the 95% confidence limits

Statistical analysis was carried out using the Pairwise Mann-Whitney-U-test (one sided smaller).
There was no adverse effect of Methiocarb SC 500 on the survival of the ten species tested.
Moderate phytotoxicity (chlorosis, stunting) was occasionally observed in cucumber only. No symptoms of phytotoxicity were observed for any other species tested.
Biomass was reduced in cucumber, soybean, sunflower, tomato, buckwheat, maize and oat by 20.5%, 8.0%, 18.9%, 32.8%, 9.0%, 19.4% and 20.1% respectively. Biomass was increased in oilseed rape, sugar beet and onion by 9.1%, 13.5% and 46.3% respectively. Differences were significant for sunflower and tomato at the 95% confidence limits.
None of these differences reached or exceeded 50% to trigger further testing.

Conclusion:

A nominal product application rate of 2 L/ha Methiocarb SC 500 showed no adverse effects greater than 50% for all the tested species in the vegetative vigour test.

CP 10.6.3 Extended laboratory studies on non-target plants

No extended laboratory studies have been conducted. Higher tier studies on non-target plants are not necessary given that Methiocarb FS 500 is used as seed treatment and therefore the exposure of non-target plants in adjacent fields is out of concern.

CP 10.6.4 Semi-field and field tests on non-target plants

No semi-field or field tests have been conducted. Higher tier studies on non-target plants are not necessary given that Methiocarb FS 500 is used as seed treatment and therefore the exposure of non-target plants in adjacent fields is out of concern.

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

No studies are required based on current data requirements.

CP 10.8 Monitoring data

Report:	KCP 10.8/01 [redacted],; [redacted]; [redacted]; 2009; M-355846-02-1
Title:	Monitoring of dust drift deposits during and after the commercial sowing of Mesurol® treated maize seeds in Germany
Report No.:	R09-079
Document No.:	M-355846-02-1
Guideline(s):	No official test guideline(s) available at present
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:

The aim of the current study was to determine the residue levels of methiocarb in dust drift deposits which can occur in commercial agricultural practice during and after the sowing of maize, commercially treated with the insecticidal seed treatment product Mesurol® (Methiocarb FS 500). Samples were taken using Petri-dishes filled with a solvent at the border of 20 fields in Germany during the sowing operation and for a 24h-period after sowing was completed. Residue levels of methiocarb were determined analytically.

Material and methods:

Test item: Maize, commercially treated with Mesurol® (Methiocarb FS 500; a.s. nominal: 500 g methiocarb/L). Dressing rate: nominal 150 mL Mesurol® / U (1 U = per 50,000 maize seeds; 1.5 mg methiocarb a.s./kernel).

Study sites and sowing: The study was conducted at various locations throughout Germany. Twenty commercially operated fields (maize sowing) were selected: six fields in Bavaria, three in Baden-Württemberg, four in Lower Saxony, four in North Rhine-Westphalia, one in Saxony, one in Brandenburg and one in Schleswig-Holstein.

The test field sizes sown with Mesurol® treated maize varied between 0.8 and 14.0 ha. Eighteen maize varieties were sown with seeding rates between 1.5 and 2 Units/ha (1 Unit = 50,000 maize seeds) resulting in effective application rates between 112 and 150 g methiocarb a.s./ha. Eighteen fields were sown with pneumatic sowing machines, 2 fields were sown with mechanical sowing machines. Overall, fourteen different sowing machines were used.

Sampling method during sowing: At each commercial field, maize seeds commercially treated with Mesurol® were sown by the respective farmer. Shortly before sowing the wind direction was determined and ten Petridishes were placed in groups of two at a distance of 1, 3 and 5 m (in total 30 Petri-dishes) at the down-wind border of the field. If the surroundings of the fields did not allow Petri-dishes to be set up at the described positions (e.g. because of hedges, bushes, streets, paths or other

obstacles) the position of the dishes was adjusted. Each Petri-dish was filled with 70 to 80 mL of a 1:1 (v/v) glycerol/water mixture. The Petri-dishes were arranged horizontally using metal racks approximately 1.5 to 2 cm above the soil or at the height of the vegetation surface, depending on the field boundary morphology. If needed, the vegetation at the field border was cut down. Once the farmer finished sowing, an additional waiting period of 15 minutes was allowed to elapse before the aqueous solutions of the respective Petri-dishes were quantitatively transferred into separate polyethylene flasks.

Sampling method after sowing: To monitor a potential dust drift during a 24h-period after sowing, ten new Petri-dishes were placed in pairs at the approximate middle of each field side at a distance of 1 m to the field borders. If the surroundings of the fields did not allow the Petri-dishes to be set up at this distance, the position of the dishes was adjusted. Handling of the Petri-dishes was carried out as described above. After 24 hours the entire content of each Petri-dish was quantitatively transferred into a separate polyethylene flask, respectively.

Residue analysis: Methiocarb residues were determined by Bayer CropScience AG.

Results:

Overall, 7 samples were destroyed/affected in the field (e.g. lost or overthrown resulting in nearly no volume left). Thus, a total of 1,393 samples were collected at the fields sown with maize during the field sampling phase of the study (normally 70 per field, resulting in nominally 1,400 samples, as 20 fields have been monitored) which were qualified for further considerations.

Of these 1,393 samples, 759 samples (54.5%) were found to contain no quantifiable residues (LOQ: 0.014 g a.s./ha) including 500 samples (35.9%) with no detectable residues (LOD1: 0.004 g a.s./ha). A total of 634 samples (45.5%) were found to contain residues above the limit of quantification (LOQ: 0.014 g a.s./ha); of these 634 samples, 501 were taken at the time of sowing, the remaining 133 were collected in the 24 hour post-sowing period. The maximum observed residue level was 2.483 g a.s./ha (Table S1).

For the mathematical processing of the 1,393 residue data, any residue value below the limit of detection (LOD: 0.004 g a.s./ha) was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification (LOQ: 0.014 g a.s./ha) was conservatively set to equal the LOQ. The calculated average residue values for samples collected during the sowing operation were 0.118 g a.s./ha for samples in a distance of 1 m to the sowing border, 0.106 g a.s./ha for samples in a distance from >1 to 3 m, 0.108 g a.s./ha for samples in a distance of 5 m and 0.074 g a.s./ha for samples in a distance of >5 m. For the samples collected during a 24h-period after sowing the average residue value was 0.015 g a.s./ha. The 90th%ile residue values during the sowing operation were 0.332 g a.s./ha, 0.238 g a.s./ha, 0.200 g a.s./ha and 0.116 g a.s./ha for a distance of 1 m, >1 to 3 m, 5 m and >5m, respectively. For the samples collected during a 24h-period after sowing the 90th%ile residue value was 0.024 g a.s./ha.

These results indicate that the dust drift deposits produced during and after the sowing of Mesurol®-treated maize seeds with deflected vacuum-pneumatic-, mechanical- and compressed-air-operated maize sowing machines, are limited.

The results of the residue analysis of the dust drift samples are summarised in the table below.

Summary of methiocarb residues at respective distances to the sowing borders

	During Sowing				24 h-sampling	Total
Nominal distance (actual distance)	**1 m (1m)**	**3 m (>1 to 3 m)**	**5 m (5 m)**	**n. a. (>5 m)**	**1 m (0 to 3 m)**	
No. of samples analysed	180	190	189	40	794	1,393
No. of samples destroyed / affected in the field and as such excluded from the evaluation **	0	0	1	0	6	7
Residue level	**Number of samples with residues levels [n]**					
< LOQ	27	35	36	0	661	759
0.014-0.050 g a.s./ha	39	64	84	5	95	287
0.051-0.100 g a.s./ha	62	57	34	28	20	201
>0.100 g a.s./ha	52	34	35	7	18	146
	Residue levels of methiocarb [g a.s./ha]					
Average *	0.118	0.106	0.108	0.074	0.015	n.a.
90tho%ile *	0.332	0.238	0.200	0.116	0.024	
Maximum *	1.203	2.483	1.893	0.141	0.597	

LOD = 0.004 g a.s./ha; LOQ = 0.014 g a.s./ha; n.a. = not applicable
° in some cases the position of the Petri-dishes had to be adjusted from the intended distance due to the surrounding structures of the field;
* calculated from the respective number of analysed samples; any residue value below the limit of detection was conservatively set to equal the LOD and any residue value above the LOD and below the limit of quantification was conservatively set to equal the LOQ;
** in total two samples were lost and no liquid could be recovered in the field after the sampling period; in addition, five samples revealed nearly no liquid after the sampling period and as such, these five samples were excluded from the evaluation, giving a total of 1,393 samples for further considerations

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Report:	KCP 10.8/02[redacted].,[redacted]; 2010; M-362242-01-1
Title:	Comparison of measurement methods to assess off-crop drift deposition patterns of seed treatment particles abraded from dressed maize seeds, emitted during sowing with a deflector modified pneumatic machine
Report No.:	IVADUST1
Document No.:	M-362242-01-1
Guideline(s):	Special designed study protocol, considering recommendations of the BBA Drift Guideline Part VII, 2-1.1, 1992
Guideline deviation(s):	none
GLP/GEP:	**no**

Objective:

The aim of the study was to compare different methods to assess the off-crop drift deposition of seed treatment particles.

Material and methods:

Test item: maize seeds treated with a seed treatment formulation provided by BASF SE. For confidentiality reasons, the name of the seed treatment product and the contained active ingredient

were not disclosed to the CWFG (Sponsor) and the other involved industry companies. Within this study report the seed treatment product and its active ingredient will be referred to as of "PRODUCT" and "COMPOUND", respectively. Seeds were intentionally treated twice without the use of a sticker to increase the potential dust release during drilling. The Heubach value at the time of drilling was 1.23 g/100,000 seeds.

The aim of the study was to gain experience with technical options to quantify aerial dust drift and deposition from the sowing of treated seeds in future drift trials. Therefore, the capture efficiency of several types of artificial, vertically oriented sampling devices and a semi-natural hedge were compared for the assessment of aerial dust drift occurring during sowing of PRODUCT treated maize seeds with a [redacted] ([redacted], Germany) approved modified pneumatic drilling machine. Samplers were located downwind from the drilled area at different heights above the ground. In order to distinguish between direct, secondary and long-term drift, different sampling times were considered in the test design.

Discussion and conclusion:

Dust deposition decreases with increasing height of sampling, indicating that the relevant sampling zone is less than 2 m above ground. In comparison to the primary drift the secondary drift was at least an order of magnitude lower.

Based on the vertical projection area the BSNE samplers, the gauze netting, and the pipe cleaners collected more dust than the glycerol/water treated semi-natural proxy hedge. Dust measurements with these samplers give therefore a conservative estimate for a projection area related exposure estimation of natural vegetation.

It was concluded that gauze netting provides the largest sampling area of all artificial samplers, supporting the generation of robust data in circumstances of low exposure. It may also show an aerodynamic behaviour which, amongst the tested samplers, is closest to a natural hedge.

Additionally by analysing these available comparative 2D- and 3D-data, it was found that on average 4.9 times (median: 5.8 times) more active substance deposited on the 3D dust samplers (gauze netting) as compared to the Petri-dishes.

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Report:	KCP 10.8/03 [redacted]; 2015; M-534966-01-1
Title:	Determination of residues of methiocarb in nectar, pollen and flowers of Phacelia tanacetifolia after sowing of methiocarb FS 500 G treated seeds in a semi-field residue study with honeybees (*Apis mellifera* L.) in Germany 2014 - Final report
Report No.:	S14-02127
Document No.:	M-534966-01-1
Guideline(s):	OEPP/EPPO Guideline No. 170(4), 2010; SANCO/3029/99 rev.4
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective:

The objective of the study was to determine methiocarb residues in nectar, pollen and flowers from *Phacelia tanacetifolia*, grown from seeds, seed-treated with different rates of Methiocarb FS 500 G, under confined semi-field conditions in Germany in 2014. In all test item treatment groups, the nominal sowing rate was 10 kg treated seeds/ha.

Materials and Methods:

Phacelia-flowers were collected directly from the flowering crop, Phacelia-nectar was pepared/sampled from forager bees and Phacelia-pollen was sampled from pollen traps, all during confined exposure of *Apis mellifera* L. to flowering *Phacelia tanacetifolia*, which was grown from seeds, seed-treated with Methiocarb FS 500 G at four different rates. The study was conducted under confined semi-field exposure conditions (gauze tunnels) by following the principal provisions of the OEPP/EPPO Guideline No. 170(4), 2010 and SANCO/3029/99 rev. 4.

Honeybees and honeybee colonies were exclusively used as a sampling device for nectar and pollen and no honey bee effect assessment was conducted.

The study was conducted near Pforzheim in Baden-Wuerttemberg, Southern Germany in 2014.

The study comprised one untreated control group (C) and four test item treatment groups (T1-T4), with flowering Phacelia-plants grown from seeds, seed-treated at different rates (1 replicate = 1 tunnel with two bee hives for the untreated control group (C) and 2 replicates = 2 tunnels per test item treatment group (T1-T4), respectively, with two bee hives each).

In all test item treatment groups (T1-T4) and in the untreated control group (C), the nominal (target) sowing rate was 10 kg seeds/ha. Phacelia-seeds were sown on the same day (21 May 2014) in the untreated control group and in the four test item treatment groups. Sowing started in the untreated control group (C) and continued from T1 to T4.

The employed row distance was 12.5 cm with a seeding depth of 2 cm. The sowing was performed on an area of 1044 m^2 per plot and treatment group (C, T1-T4). The target sowing rate was 1.04 kg Phacelia-seeds per plot. The employed *Phacelia tanacetifolia* seeds were of the same variety and either untreated (C) or seed-treated with Methiocarb FS 500 G, for the test item treatment group T1 at a nominal rate of 7.5 g a.s./kg Phacelia-seeds (= 75 g a.s./ha, nominally), for the test item treatment group T2 at a nominal rate of 15 g a.s./kg Phacelia-seeds (= 150 g a.s./ha, nominally), for the test item treatment group T3 at a nominal rate of 30 g a.s./kg Phacelia-seeds (= 300 g a.s./ha, nominally) and for the test item treatment group T4 at a nominal rate of 75 g a.s./kg Phacelia-seeds (=750 g a.s./ha, nominally).

The respective tunnels were set up shortly before flowering and the bee colonies were placed in the respective tunnels at the beginning of the flowering period (BBCH 61). Overall, seven samplings were performed within a time interval of eight days, from beginning of flowering to peak of flowering (full-bloom). On each sampling day, an A-sample (=actual sample) and a R-sample (=retain sample) from each replicate and treatment group, consisting of approximately 300 forager bees, respectively, was taken. Regarding pollen samples, on each sampling day, samples of at least 0.5 g for A and R-samples were taken from each replicate and treatment group, respectively. Regarding flower samples, on each sampling day, samples of at least 10 g of Phacelia-flowers were collected from each replicate and

treatment group, respectively, and divided into two sub-samples samples (A and R) of at least 5 g, each.

The collected flower, nectar and pollen samples were analysed for residues of methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone at Bayer CropScience AG, [redacted], Germany by using High Performance Liquid Chromatography (HPLC), coupled with electrospray and tandem mass spectrometry (MS/MS) detection.

Results:

All results of the method validation were in accordance with the general requirements for residue analytical methods; therefore, the employed method was validated successfully.

Analysis of flowers, nectar and pollen followed the provisions of the Bayer CropScience method 00616/M001 (methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone) with modifications. The Limit of Quantitation (LOQ), defined as the lowest validated fortification level, was 0.010 mg/kg (= 10 µg/kg = 10 ppb) for all analytes (= methiocarb, methiocarb-sulfoxide and methiocarb-sulfone) and all investigated matrices (nectar, pollen and flowers). The corresponding Limit of Detection (LOD), defined as the linearity response data of the lowest-concentration standards, was 0.002 mg/kg (= 2 µg/kg = 2 ppb) for all analytes and matrices.

A summary of the analytical results is provided in the following table.

Residues of Methiocarb, Methiocarb-sulfoxide and Methiocarb-sulfone in Samples of Nectar, Pollen and Flowers

Sample	Trial No.	Test Item	Residue [µg/kg]		
			Methiocarb	Methiocarb-sulfone	Methiocarb-sulfoxide
Nectar	T1 (75g a.s./ha)	Methiocarb FS 500 G seed treated *Phacelia* seeds	< LOD	< LOD	< LOD
	T2 (150g a.s./ha)		< LOD	< LOD	< LOD - < LOQ
	T3 (300g a.s./ha)		< LOD	< LOD	< LOD - < LOQ
	T4 (750g a.s./ha)		< LOD	< LOD	< LOD - < LOQ
Pollen	T1 (75g a.s./ha)		< LOD	< LOD	< LOD
	T2 (150g a.s./ha)		< LOD	< LOD	< LOD
	T3 (300g a.s./ha)		< LOD	< LOD	< LOD - < LOQ
	T4 (750g a.s./ha)		< LOD	< LOD	< LOD - < LOQ
Flowers	T1 (75g a.s./ha)		< LOD	< LOD	< LOD - < LOQ
	T2 (150g a.s./ha)		< LOD	< LOD	< LOD - < LOQ
	T3 (300g a.s./ha)		< LOD	< LOD	< LOD - LOQ
	T4 (750g a.s./ha)		< LOD	< LOD	< LOD - LOQ

LOQ = Limit of Quantitation = 10 µg/kg in nectar, pollen and flowers (methiocarb, methiocarb-sulfoxide and methiocarb-sulfone)
LOD = Limit of Detection = 2 µg/kg in nectar, pollen and flowers (methiocarb, methiocarb-sulfoxide and methiocarb-sulfone)

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Report:	KCP 10.8/04[redacted],; 2014; M-494337-01-1
Title:	Determination of methiocarb residues in winter oil seed rape flowers, grown on fields treated with Mesurol Schneckenkorn (methiocarb RB 2) and in maize pollen, grown from seeds commercially seed-treated with Mesurol fluessig (methiocarb FS 500) in Germany in 2013
Report No.:	192
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Guideline(s):	not applicable - special design study
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective:

According to the Regulation (EC) 1107/2009 the potential side-effects of crop protection products on honeybees have to be assessed. This study aimed to determine residues of methiocarb in winter oil seed rape (WOSR)-flowers and in maize pollen during spring/early summer 2013, to which honey bees may potentially get exposed to. WOSR was grown on commercial fields, treated with "Mesurol Schneckenkorn" (=Methiocarb RB 2) at a nominal rate of 3.0 to 10.0 kg product/ha (= 60 - 200 g a.s./ha) in autumn 2012, i.e. at the typical time of slug pellet application in WOSR. Maize plants were grown from seeds, commercially seed-treated with "Mesurol fluessig" (=Methiocarb FS 500) at a nominal rate of 1.5 mg a.s./seed; the commercial maize planting occurred during springtime 2013. Rape flowers were sampled from flowering winter oil seed rape and pollen from flowering maize plants.

Material and Methods:

Test item: "Mesurol Schneckenkorn" (=Methiocarb RB 2; a.s. methiocarb) used in WOSR fields (commercial, non-GLP application) and "Mesurol fluessig " (=Methiocarb FS 500; a.s. methiocarb) used as a seed-treatment product on maize seeds (commercial, non-GLP application). WOSR was grown on commercially operated fields treated with "Mesurol Schneckenkorn" (Methiocarb RB 2), maize plants were grown from seeds, commercially seed-treated with "Mesurol fluessig" (Methiocarb FS 500).

Study sites: The study was conducted on 22 commercial WOSR fields and on 44 commercial maize fields or varieties located in several regions in Germany (see Deviations, chapter 9.2 and 9.3).
The exact location (GPS coordinates) of the study fields and the BBCH stages of the crop were recorded. Information of the respective study field (non-GLP, e.g. field size, sowing dates, sowing density (seeds/kernel or units/ha), application rate, crop variety, soil type, certification number) were obtained by the respective farmer of the field.
Sampling method
At each study field, three equally distributed (for exception see Deviations) study plots were selected where sampling took place. The size of the study plots was adapted to the availability of flowering plants. The locations of the study plots were chosen equally distributed in the respective study field; their position was be recorded by GPS.

Samples were taken at dry weather conditions (for exception see Deviations). At each study plot, one sample for analysis (A-sample) and one retention sample (B-sample) were taken (if possible, see Deviations), resulting in a maximum of six samples/study field (three samples for analytics, three retention samples). If the envisaged number of samples could not be achieved (e.g. progressed BBCH, unfavourable weather condition, low amount of plants in a variety trial), priority was given to complete the A-samples (see Deviations). The GPS coordinates of each study plot were recorded. Each sample was double bagged in at least two proper containers (e.g. plastic bag, wide mouth bottle). The samples were labelled with the following information: GLP study number, sample-ID, sampling date, study field and study plot number, matrix type, A- or B-sample and GLP-ID of the sampling personnel.

The equipment used for sampling was either unused or cleaned with ethanol before and after each sampling.

After the sampling procedure at each study field was completed, the samples were stored with a datalogger recording the temperature deep-frozen on dry ice until storage at ≤ -18.0 °C in a freezer at the Test Facility (temperature recording by a datalogger).

Residue analysis: The residues of methiocarb within the collected WOSR flowers as well as within the collected maize pollen were analysed on the premises of the Analytical Test Site Bayer CropScience AG. All samples were investigated for residues of methiocarb by using the Bayer CropScience method 00616/M001: Modification M001 to the Analytical Method 00616 for the determination of residues of methiocarb, methiocarb-sulfoxide, methiocarb-sulfone in/on matrices of plant origin by HPLC-MS/MS.

Results:

The Limit of Detection (LOD) for all analytes (i.e. methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone) in WOSR flowers was 2 µg/kg, in maize pollen, the LOD was 5 µg/kg for all analytes. The Limit of Quantitation (LOQ) for all analytes was 10 µg/kg in both matrices.

A total of 66 WOSR flower samples from overall 22 WOSR fields located at several locations in Germany were analysed. No detectable residues of methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone were found in any of the WOSR flower samples under investigation (i.e. all residues <LOD).

A total of 120 maize pollen samples from overall 44 maize fields or variety trials located at several locations in Germany were analysed. In 119 of 120 maize pollen samples, no detectable residues of methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone were found (i.e. all residues <LOD). In one single maize pollen sample (Study Field Maize-24, sample 1A), residues of methiocarb at the LOQ-level were found (there were no detectable residues of methiocarb-sulfoxide and methiocarb-sulfone). Unfortunately, only one sample (Maize 24-1A) could be taken at the respective study field and therefore it was impossible to verify or falsify the result.

Summary of methiocarb, methiocarb-sulfoxide and methiocarb-sulfone residues in WOSR flowers and in maize pollen

Sample	Test Item	Residue [µg/kg]		
		Methiocarb	Methiocarb-sulfoxide	Methiocarb-sulfone
WOSR flowers	Methiocarb RB 2	< LOD	< LOD	< LOD
Maize pollen	Methiocarb FS 500	< LOD – LOQ	< LOD	< LOD

LOQ = Limit of Quantitation = 10 µg/kg in WOSR flower and maize pollen (methiocarb, methiocarb-sulfoxide and methiocarb-sulfone)
LOD = Limit of Detection = 2 µg/kg in WSOR flower and 5 µg/kg in maize pollen (methiocarb, methiocarb-sulfoxide and methiocarb-sulfone)

Conclusion

Samples of WOSR-flowers, collected on commercially operated fields treated with "Mesurol Schneckenkorn" (Methiocarb RB 2), as well as samples of maize pollen collected from maize on commercially operated fields, which were grown from maize-seeds, commercially seed-treated with "Mesurol fluessig" (Methiocarb FS 500), were investigated for potential residues of methiocarb and its plant metabolites methiocarb-sulfoxide and methiocarb-sulfone.

WOSR flowers

No detectable residues of methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone were found in any of the investigated 66 flower samples, collected from 22 different WOSR fields which were located at several locations in Germany.

Maize pollen

No detectable residues of methiocarb and its metabolites methiocarb-sulfoxide and methiocarb-sulfone were found in 119 of 120 maize pollen samples, collected from 44 maize fields or variety trials located at several locations in Germany. In one single maize pollen sample, residues of methiocarb at the LOQ-level were found (there were no detectable residues of methiocarb-sulfoxide and methiocarb-sulfone).