HSE

Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

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

Metalaxyl-M

Volume 3 – B.9 (AS)

Ecotoxicology

GB Article 7 Amendment

Great Britain

January 2024

Version History

When	What
January 2024	HSE assessment

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B.9. ECOTOXICOLOGY DATA

B.9.0 INTRODUCTION AND BACKGROUND

Background:

As a result of the EU renewal review of metalaxyl-M[1], the following was concluded regarding the risk to birds and mammals:

> *For the use as seed treatment, EFSA highlighted a risk to birds and mammals from exposure via the consumption of treated seeds after a higher tier assessment. Therefore, in order to ensure the protection of birds and mammals, seeds treated with metalaxyl-M shall only be sown in greenhouses.*
>
> *However, the risk to birds and mammals from consumption of seedlings was considered acceptable for the two representative crops considered (sunflowers and spinach). For the use on spinach, residue data allow to conclude that seedlings can be planted in the field after 21 days. For other crops, such data are not available.*

In addition, to the above, concern was raised regarding the risk from metabolites:

> *Concerning metabolites, the rapporteur Member State considered that the information provided by the applicant in the dossier was sufficient to exclude a risk to birds and mammals from exposure to metabolites of metalaxyl-M. The metabolites all have low log Kow values indicating low potential for bioaccumulation. Member States will in any case need to take into account the risk from exposure to metabolites when assessing plant protection products.*
>
> *The RMS included the applicants risk assessment in the revised RAR for 'Ridomil Gold MZ WG' and 'Apron XL' and agreed that no concern is identified from exposure to metabolites. EFSA considered that the argumentation does not transparently address the risk to birds and mammals from major plant metabolites and accordingly set a data gap for this point. However, taking into account the information available on the metabolites and parent substance this point can be considered addressed*.

Therefore, due to the risks identified to birds and mammals, the implementing Regulation for the renewal of metalaxyl-M[2] specifies that when used for seed treatment, only the treatment of seeds intended to be sown in greenhouses may be authorised. In order to address risks to birds and mammals via consumption of seedlings, a minimum period before transplanting outdoors seedlings grown from treated seed (21 days for spinach).

[1] Metalaxyl-M SANTE/11112/2019 Rev 5 24 March 2020 Final Review report for the active substance metalaxyl-M finalised by the Standing Committee on Plants, Animals, Food and Feed on 24 March 2020 in view of the renewal of the approval of metalaxyl-M as an active substance in accordance with Regulation (EC) No 1107/20091

[2] Commission Implementing regulation (EU) 2020/617, 5 May 2020 (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R0617&from=EN)

Under this Article 7 application, the applicant is seeking to amend the conditions of approval - removing the restriction that only the treatment of seeds intended to be sown in greenhouses may be authorised. New data and risk assessments have been provided in order to demonstrate that outdoor sowing of seed treated with metalaxyl-M will not result in unacceptable impacts on birds and mammals. These data and risk assessment will be reviewed by the UK HSE in this assessment report.

Representative uses:

The representative uses evaluated in the EU renewal for metalaxyl-M were for treatment of sunflower and spinach seeds. These crops are considered of limited relevance for GB. Under this Article 7 application the applicant has requested consideration of different representative uses of metalaxyl-M. The newly proposed representative uses are as seed treatment on sugar/fodder beet and vining peas. New representative formulations have also been proposed – Vibrance SB (also known as A20607B) and Wakil XL (also known as A9873C). In this assessment report, HSE will consider risks to birds and mammals from the new representative uses of metalaxyl-M on sugar/fodder beet and vining peas. The new representative uses assessed are detailed in table B.9.0-1 and B.9.0-2 below. It should be noted that if it is considered that the restriction can be removed, then the use of metalaxyl-M on other seed types would still need to be assessed by HSE. The relevance of the following assessment, to any future assessment is unknown.

The new representative formulation Vibrance SB A20607B is a flowable concentrate (FS) formulation for use as a seed treatment to control various diseases (*Pleospora betae, Thanatephorus cucumeris / Rhizoctonia solani*) and *Pythium* damping-off diseases on sugar and fodder beet. The other new representative formulation Wakil XL is a wettable granule (WG) formulation for use as a as seed treatment to control various diseases (*Peronospora viciae, Ascochyta complex: Ascochyta pisi, Mycosphaerella pinodes, Phoma medicaginis var. pinodella*) and *Pythium* damping-off diseases on a wide range of crops.

Vibrance SB contains the active substances fludioxonil (22.5 g/L) and sedaxane (15 g/L), in addition to metalaxyl-M (14.4 g/L). Similarly, Wakil XL also contains the active substances cymoxanil (100 g/kg) and fludioxanil (50 g/kg), in addition to metalaxyl-M (169.9 g/kg). Risks to birds and mammals from these other active substances in the representative formulations are outside the scope of this Article 7 application and will not be considered in this document. However, they will need to be evaluated in order to fully assess the risks to non-target organisms from these products in order to support their authorisation.

Table B.9.0-1: Intended uses of Vibrance SB (A20607B)

Crop	Seed rate (g a.s. /100 kg seed)	TGW (g)	NAR (mg a.s./kg seed)	Drilling rate (seed/ha)*	Field rate (g a.s. /ha)	Max µg a.s./seed
Sugar and fodder beet	1) 31.22 2) 19.98 3) 20.81	24-33 g/1000 seeds	1) 312.2 2) 199.8 3) 208.1	100000-130000	1) 0.97 2) 0.62 3) 0.65	1) 7.49 2) 4.80 3) 5.00

1) Fludioxonil
2) Metalaxyl-M
3) Sedaxane
*Equivalent to a sowing rate of 3.12-3.3 kg seeds/ha

Table B.9.0-2: Intended uses of Wakil XL (A9873C)

Crop	Seed rate (g a.s. /100 kg seed)	TGW (g)	NAR (mg a.s./kg seed)	Drilling rate (seed/ha)*	Field rate (g a.s. /ha)	Max µg (a.s./seed)
Peas (vining)	1) 20.00 2) 10.00 3) 33.92	225	1) 200.00 2) 100.00 3) 339.2	1 000 000	1) 45.00 2) 22.50 3) 76.32	1) 45 2) 22.5 3) 76.32

1) Cymoxanil
2) Fludioxonil
3) Metalaxyl-M
*Equivalent to a sowing rate of 225 kg seeds/ha

The appropriateness of the sowing rates assessed by the applicant have been checked for GB conditions. For sugar beet grown in GB a typical sowing rate of around 115000 seeds/ha is expected. This value is within the 100000-1300000 seeds/ha range proposed by the applicant. For vining peas, a typical sowing rate in the range 211-267 kg seeds/ha is expected in GB. The 225 kg seeds/ha figure used by the applicant is within this range. Therefore, the sowing rates for sugar/fodder beet and vining peas used by the applicant in their assessment are considered relevant for GB conditions. It is anticipated that in GB sugar (and fodder) beet seeds would generally be drilled in March-April, while vining pea seeds would be drilled from early February to mid-April.

Bioaccumulation:

According to EFSA (2015)[3] the ‘risk assessment for birds and mammals from bioaccumulation in earthworms and from major plant metabolites is not finalised’. The Final Review Report discusses both areas and this is presented below:

[3] EFSA (European Food Safety Authority), 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance metalaxyl-M. EFSA Journal 2015;13(3):3999, 105 pp. doi:10.2903/j.efsa.2015.3999

The risk assessment for birds and mammals from bioaccumulation in earthworms and from major plant metabolites is not finalised.

In the EFSA Conclusion it is stated that the log Kow for metalaxyl-M is less than 3 (1.71) and therefore according to the Guidance Document on birds and mammals (EFSA, 2009) a quantitative risk assessment is not required. However, a published paper indicated that there may be a potential for bioaccumulation in earthworms. The applicant provided a calculation of the BCF for metalaxyl-M according to the equation in the EFSA showing that it is far below 1000 and therefore the concern for bioaccumulation is low.

Concerning metabolites, the rapporteur Member State considered that the information provided by the applicant in the dossier was sufficient to exclude a risk to birds and mammals from exposure to metabolites of metalaxyl-M. The metabolites all have low log Kow values indicating low potential for bioaccumulation. Member States will in any case need to take into account the risk from exposure to metabolites when assessing plant protection products.

The RMS included the applicants risk assessment in the revised RAR for 'Ridomil Gold MZ WG' and 'Apron XL' and agreed that no concern is identified from exposure to metabolites. EFSA considered that the argumentation does not transparently address the risk to birds and mammals from major plant metabolites and accordingly set a data gap for this point. However, taking into account the information available on the metabolites and parent substance this point can be considered addressed.

HSE has not considered the above issues in detail but notes that published papers have been submitted regarding the potential for bioaccumulation (see Appendix 1 for details of the literature review). Furthermore, it is unclear from the above, how the risk from plant metabolites was addressed subsequent to the statement in EFSA (2015). Since the applicant has not addressed risks from metabolites to birds and mammals in this submission, this issue is not considered further. Issue does not affect seed restrictions and data gaps the applicant has applied to lift and should be addressed during active substance renewal.

Published literature:

The applicant has conducted a literature search in order to determine whether there are publicly available data that should be taken into account in the review of metalaxyl-M. The literature search was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009". This literature review is evaluated by HSE in Appendix 1 (below). While there are aspects of the relevance assessment provided by the applicant that are considered deficient, the literature review does not identify any data that require further consideration in this amendment evaluation regarding risks to terrestrial vertebrates. It is noted that this literature review was first submitted to HSE in 2020, therefore, while it includes published data from a 10-year time period, it does not cover the full 10-year period prior to the current Article 7 application. Issue does not affect seed restrictions and data gaps the applicant has applied to lift.

EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

Avian toxicity endpoints from the EU review of metalaxyl-M are reported in the following table.

Table B.9.1.1-1: Avian endpoints for Metalaxyl-M

Species	Substance	Exposure system	Results	Reference
Bobwhite quail (*Colinus virginianus*)	Metalaxyl-M	Acute toxicity	LD_{50} = 1419 mg/kg bw	EFSA Journal 2015;13(3):3999[4] [redacted], 1995; CGA329351/0310
Bobwhite quail (*Colinus virginianus*)	Metalaxyl-M	Acute toxicity	LD_{50} = 981 mg/kg bw	EFSA Journal 2015;13(3):3999 [redacted] *et al.*, 1995; CGA329351/0301
Geometric mean Bobwhite quail		Acute toxicity	**LD_{50} = 1180 mg/kg bw**	EFSA Journal 2015;13(3):3999
Mallard duck (*Anas platyrhynchos*)	Metalaxyl	Acute toxicity	LD_{50} = 1466 mg/kg bw	EFSA Journal 2015;13(3):3999; [redacted], 1977; CGA48988/0149
Geometric mean – applicant's proposal		Acute toxicity	LD_{50} = 1315 mg/kg bw	-
Bobwhite quail (*Colinus virginianus*)	'Ridomil Gold'	Acute toxicity (extrapolated)	LD_{50} = 3228 mg/kg bw	EFSA Journal 2015;13(3):3999; [redacted] & [redacted], 2005; CGA329351/2154
Bobwhite quail (*Colinus virginianus*)	Metalaxyl-M	Dietary 8 d Short-term	LD_{50} > 5620 mg/kg food (> 2631 mg/kg bw/d)	EFSA Journal 2015;13(3):3999; [redacted] *et.al*, 1995a; CGA329351/0302
Japanese quail (*Coturnix japonica*)	Metalaxyl		LD_{50} > 10 000 mg/kg food	EFSA Journal 2015;13(3):3999; [redacted], 1976; CGA48988/0154

[4] EFSA (European Food Safety Authority), 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance metalaxyl-M. EFSA Journal 2015;13(3):3999, 105 pp. doi:10.2903/j.efsa.2015.3999

Bobwhite quail (*Colinus virginianus*)	Metalaxyl		LD_{50} > 10 000 mg/kg food	EFSA Journal 2015;13(3):3999; [redacted], 1977a; CGA48988/0147
Mallard duck (*Anas platyrhynchos*)	Metalaxyl		LD_{50} > 10 000 mg/kg food	EFSA Journal 2015;13(3):3999; [redacted], 1977b; CGA48988/1998
Bobwhite quail (*Colinus virginianus*)	Metalaxyl-M	Dietary Reproductive toxicity	NOEL = 900 mg/kg food (84 mg/kg bw/d)	EFSA Journal 2015;13(3):3999; [redacted] and [redacted], 1998; CGA329351/1071
Mallard duck (*Anas platyrhynchos*)	Metalaxyl-M		NOEL = 900 mg/kg food (117.3 mg/kg bw/d)	EFSA Journal 2015;13(3):3999; [redacted] and [redacted], 1998a; CGA329351/1072
Bobwhite quail (*Colinus virginianus*)	Metalaxyl		NOEL = 900 mg/kg food (no mg/kg bw/day figure presented in EFSA (2015))	EFSA Journal 2015;13(3):3999; [redacted], 1980a; CGA48988/0151
Mallard duck (*Anas platyrhynchos*)	Metalaxyl		NOEL = 300 mg/kg food (**24.6 mg/kg bw/d**)	EFSA Journal 2015;13(3):3999; [redacted], 1980b; CGA48988/0152

Acute toxicity:

According to EFSA (2015), the key acute endpoint is one based on the geometric mean of the available studies on bobwhite quail, i.e. an LD50 of 1180 mg a.s./kg bw. The applicant has proposed changing this and has referenced EFSA (2009), and in particular points 2.4.1 and 2.4.2, where the guidance document proposes that when multiple studies are available with different species an overall geometric mean can be determined. In situations when more than one study is available with a single species (as is the situation for the bobwhite quail) a geometric mean is calculated for that species and used as a single input in determining an overall geometric mean for all species tested.

In addition to studies with the bobwhite quail, a study was conducted with the Mallard duck (*Anas platyrhynchos*) in which an LD_{50} of 1466 mg/kg bw/day was determined. Using these two endpoints (i.e. LD_{50} of 1180 mg/kg bw and LD_{50} of 1466 mg/kg bw), and in accordance with the EFSA guidance (2009), the applicant proposes that an overall geometric mean of 1315 mg/kg bw/day should be used in the acute risk assessment (see Appendix 2 for further details).

It is noted in the RAR, volume 3 CA B9, that the Mallard Duck study was considered to be 'generally in accordance with the SETAC recommendations', it is further noted that the 'description of the dosage procedure is confused'. In light of this, the RMS considered that this study was supplemental information and that it was not necessary for the evaluation of metalaxyl-M. It is assumed that due to the shortcomings, the RMS and peer review process did not consider it appropriate to combine this endpoint and hence determine a toxicity endpoint as proposed by the applicant. Whilst the endpoint is considered supplemental, it is listed in EFSA (2015).

HSE has not revisited the Mallard Duck study[5] and will retain the EU agreed acute toxicity endpoint, i.e. the bobwhite quail geometric mean **LD_{50} = 1180 mg a.s./kg bw**, for use in the risk assessment.

Short-term dietary toxicity studies are also available with metalaxyl-M. Given the resulting LD_{50} values are unbound 'greater than' values, this not considered to impact the selection of toxicity endpoint for the acute risk assessment.

Long-term/reproductive toxicity:

When metalaxyl-M was originally peer reviewed (see SANCO 2002) [6] two avian reproduction studies were submitted, one on the Bobwhite Quail ([redacted] (1980a)) and one on the Mallard Duck ([redacted] (1980b)). Both studies were deemed acceptable at the time of evaluation by the RMS and the peer review process. As a result, and as reported in in ECCO 98 (2000), a NOEC of 300 mg a.s./kg was agreed, and the RMS was requested to update the list of endpoints (LoEP). Subsequent to this an addendum was requested by the Commission. HSE has not been able to obtain a copy of the addendum, furthermore it is noted that according to SANCO (2002), the agreed avian reproduction endpoint is stated to be 900 mg a.s./kg diet. It is noted that the studies by [redacted] and [redacted] are listed in SANCO (2002); however, it is not clear which study was used in setting the final endpoint of 900 ppm. The reason for this is the lack of the addendum.

Metalaxyl vs metalaxyl-M

It was noted by the RMS that whilst the studies by [redacted] were done on metalaxyl, studies on the R-enantiomer, i.e. metalaxyl-M were not required as the available acute and dietary toxicity studies performed with metalaxyl and metalaxyl-M indicated that both compounds were of similar toxicity to birds.

Evaluations of the studies by [redacted] are presented below; it should be noted that these are more detailed study summaries than originally considered by the EU review process.

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[5] The Mallard duck study was not resubmitted with this application, neither was a case made to address the shortcomings identified by the RMS.

[6] Metalaxyl-M SANCO/3037/99-final 18 September 2002 COMMISSION WORKING DOCUMENT - DOES NOT NECESSARILY REPRESENT THE VIEWS OF THE COMMISSION SERVICES Review report for the active substance Metalaxyl-M Finalised in the Standing Committee for the Food Chain and Animal Health at its meeting on 19 April 2002 in view of the inclusion of Metalaxyl-M in Annex I of Directive 91/414/EEC.

It is noted that the same studies were also considered for the EU assessment of **metalaxyl** and both studies were considered acceptable. The original DAR for **metalaxyl** stated the following:

> *Reproduction studies were conducted on the Bobwhite quail and Mallard duck ([redacted], 1980a; 1980b). In both studies, mature (5 to 6 months old) birds were fed a range of single concentrations of metalaxyl at levels up to 900 mg a.s./kg feed in the diet during the pre-laying period (8 and 10 weeks) followed by feeding on untreated diet until the end of the tests (18 weeks). Several reproduction parameters were monitored.*
>
> *The bobwhite quail did not show any susceptibility to metalaxyl since no compound related impairment on reproduction was observed. Thus a NOEC of 900 mg a.s./kg feed was established. The mallard duck showed more susceptibility to metalaxyl with statistically significant effects on mean eggshell thickness and live 3 week embryos at concentrations of 900 mg a.s./kg feed. The mean chick body weight at hatching was also statistically affected at levels at and above 300 mg a.s./kg feed. A NOEC (18 weeks) was established as 300 mg a.s./kg feed.*

As a result of the above, the agreed endpoint for metalaxyl was 300 mg a.s./kg diet[7] which was from Mallard duck study carried out by [redacted] (1980b).

Renewal of metalaxyl-M

When metalaxyl-M underwent renewal (see EFSA (2015)[8]), four avian reproduction studies were assessed, [redacted] (1980a), [redacted] (1980b), [redacted] and [redacted] (1998a) and [redacted] and [redacted] (1998b). During the renewal process, the following was reported in the report of the EFSA peer review meeting[9]:

> *All the 4 available studies with birds need to be discussed. The endpoint which was used in the risk assessment by the RMS showed some drawbacks in the control.*
>
> *The studies of* [redacted] *with metalaxyl were included in the original DAR. The studies of* [redacted] et al *were included in an addendum linked to the original DAR.*[10] *The studies of* [redacted] et al *were on Metalaxyl-M. In one of the study of* [redacted] et al *there were some problems in the control and there was also a data requirement for historical control data which were not provided. These data would have given more robustness to the study results.*

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[7] Metalaxyl SANCO/10476/2010 – rev.1 12 March 2010 FINAL Review report for the active substance metalaxyl Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 12 March 2010 in view of the inclusion of metalaxyl in Annex I of Directive 91/414/EEC

[8] EFSA (European Food Safety Authority), 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance metalaxyl-M. EFSA Journal 2015;13(3):3999, 105 pp.

[9] Pesticides Peer Review Meeting 119 (23 – 25 September 2014) Metalaxyl-M 2 Appendix 1: Discussion Table

[10] HSE has not been able to obtain a copy of the addendum.

The RMS proposed not to use the endpoints from the studies of [redacted] (1980a,b) but to use the endpoint from [redacted] et al *1998a in the risk assessment, even if historical control data were missing.*

Some experts argued that the endpoints related to Metalaxyl-M have to be used in the risk assessment but it has also to be considered that the study of [redacted] *1980b gives a lower endpoint. In addition the study of* [redacted] et al *(1998a) from which the endpoint was derived showed problems in the control.*

In the studies of [redacted] *(1980a,b) the chemical purity of the test item was missing. Other experts would not agree to reject a study only because some information (purity) is missing especially if that study gives the lowest endpoint.*

RMS reported that the US-EPA in their assessment did not use the endpoints from [redacted] *(1980a,b) because of deviations in the methodology. The RMS mentioned 2 additional studies considered by US-EPA which were not part of the dossier but were summarised in the revised RAR as additional information (Studies from* [redacted]*) but were considered in the assessment by the US-EPA. However, experts did not agree to use this additional information as the studies were not available and peer-reviewed (only EPA assessment was available).*

The endpoint suggested in the RAR from the study of [redacted] *1980b was a NOEC of 300 ppm. However, at 100 ppm some effects in the normal hatchling of live 3 week embryos (not statistically significant) were also observed. The NOEC of 100 ppm would approximately give a NOAEL of about 13 mg/kg bw.*

Some experts suggested to compare the response of that parameter in the study of [redacted] *1980 b and the study of* [redacted] et al *testing the same species. A comparable trend could be observed in both studies.*

Based on a weight of evidence and considering all the available studies, the majority of experts agreed on the NOEC of 300 ppm to be used in the risk assessment as a conservative approach instead of 900 ppm. This endpoint in future could be revised based on e.g. historical control data from [redacted] et al *study. The endpoint has to be converted in the corresponding NOAEL and the risk assessment needs to be updated.*

The applicant has raised concerns regarding the endpoint and maintains the position that the NOEC of 84 mg a.s./kg bw/day obtained from the Bobwhite quail study ([redacted] and [redacted] 1998a) is the appropriate endpoint to use in the regulatory risk assessment as it is a robust study and in line with the relevant guideline (OECD 206). The applicant has highlighted that this position was supported by the RMS (Belgium) during the evaluation for the renewal (peer review expert meeting in November 2014).

In light of the above concerns regarding the appropriate long-term/reproductive endpoint, the applicant has submitted further work, notably work on historical control data (HCD) and a reconsideration of the original studies (see Appendices 2 and 3). Summaries of the four studies are presented below – it should be noted that these are more detailed than originally presented and considered during the EU review of metalaxyl-M. In addition, associated cases and data on the HCD are also presented below. Following the evaluation is a consideration of the applicant's case regarding the original Mallard duck study and the use of HCD to address the concerns raised during the review.

Reproduction studies with metalaxyl and metalaxyl-M

[redacted] (1980a). One-generation reproduction study – bobwhite quail, CGA 48988 technical.

Guidelines: Not specified in the report.

GLP: No – conducted prior to the requirement to conduct ecotoxicological studies to GLP.

Material and Methods:

Test substance: metalaxyl, CGA-48988 chemical purity: not given, described as "a brown material with large chunks, distinct odor (sic)"

Test species: Bobwhite quail (*Colinus virginianus*)

Sex, weight, age: 12 pens of 1 male and 2 female birds/treatment group, 197 g, 5-month old. Body weights were recorded at initiation and on weeks 2, 4, 6, 8 and at termination of the study.

Applied concentrations: untreated control, 100, 300, 900 mg metalaxyl/kg in the feed

Type of application: dietary application, the technical material was mixed with corn oil and incorporated into aliquots of basal diet. The basal diet was 19.4% protein, 6.7% fat and 3.8% fibre. The study report states that samples of the control diet and each of the treated diets were sent for analysis, however the outcome of this was not included in this study report.

Time of exposure: Test birds were kept indoors and fed treated diet *ad libitum* for 10 weeks during the pre-egg laying period and for the 8-week egg production period. Control birds were fed untreated basal diet throughout. The photoperiod for the first six weeks of the study was 8 hours light per day, the photoperiod was then increased to 17 hours of light per day and maintained at that duration until the termination of the study.

Study methodology: Eggs were collected daily and stored at 13.3±0.5°C and 87% humidity. At weekly intervals, the eggs were placed for incubation at 37.5°C, immediately prior to incubation all eggs were candled for eggshell cracks. All eggs were removed from the hatcher on Day 25 of incubation, the average bodyweight of representative hatchlings was determined. For each treatment level, fifty hatchlings from each week lot were randomly chosen for rearing. Hatchlings were housed in the appropriate parental grouping until 14-days of age. One egg from every other pen in each experimental group and the control was randomly selected for egg weigh and eggshell thickness. The analysis of the data was subject to analysis of variance, whilst the analysis of the egg data and other count variable was based on Cochran's concept of extraneous variability for the binomial distribution. Cochran's analysis was used on the following parameters: eggs laid, eggs cracked, eggs set, viable embryos, live three-week embryos, hatchlings, body weight hatchlings, 14-day old survivors, body weight, egg weight, and eggshell thickness.

Findings:

Table B.9.1.1-2: Effects of metalaxyl observed during the reproduction study of Bobwhite quail

Endpoints	Concentrations (mg a.s./kg feed)			
	0	100	300	900
Adults				
Mortality	2 females	2 females	0	1 female
	No gross compound related abnormalities were noted at necropsy.			
Observations	One female had a lacerated scalp and two showed head picking.	One male had scalp lacerations, a ruffled appearance, wing droop and lethargy. One female had depression and reduced reaction to external stimuli, wing	One female had scalp lacerations, one had toe picking. One male was noted to have tail pulling.	Two female had scalp lacerations, one exhibited lethargy and reduced reaction to external stimuli.

Endpoints	**Concentrations (mg a.s./kg feed)**			
	0	**100**	**300**	**900**
		droop, loss of coordination. One had a reproductive tract infection.		
Body weight		Statistically significant lower body weight in adult birds in all treatments in comparison with control (p<u><0.01</u>). The maximum actual mean difference was only 8 g. Lower body weights were observed throughout the study. A statistically significant difference (p<0.01) in the body weight of hatchling was observed in the 100 and 300 mg a.s./kg groups, and a less significant (p <0.04) effect was observed in the 900 mg/kg treatment group. The difference in body weights was 0.3 g.		
Food consumption	no compound related difference in food consumption			
Reproduction Parameters				
Number of eggs laid Mean egg weight Eggs cracked Eggs cracked of eggs laid (%) Mean egg shell thickness (mm) Eggs set Viable embryos Viable embryos of eggs set (%) Live 3-week embryos	814 9.9 35 4 0.220 734 635 87 621	803 9.8 74 9 0.211 684 607 89 596	834 9.8 84 10 0.217 704 492 70 490	834 10.0 52 6 0.215 734 633 86 623

Endpoints	Concentrations (mg a.s./kg feed)			
	0	100	300	900
Live 3-week embryos of viable embryos (%) Normal hatchlings Hatchling reared Normal hatchlings of live 3-week embryos (%) 14-day old survivors 14-day old survivors of hatchlings reared (%) 14-day old survivors per female*** Mean chick body weights at hatching (g) Mean chick body weights at 14 days (g)	98 386 304 62 286 94 15 6.6 19	98 424 322 71 286 89 16 6.3** 20	99.6 343 287 70 258 90 13 6.3** 19	98 429 324 69 271 84* 15 6.3* 19
Eggs laid/hen in 8 weeks	34	33	35	35

* significant ($p < 0.05$); ** highly significant ($p < 0.01$), *** adjusted by % of 14-day old survivors.

HSE Conclusions:

When assessed by the RMS for the first review of metalaxyl-M and the peer review process the study was deemed to be acceptable. It was noted by the RMS that no reproduction parameter showed compound related differences which were considered to be biologically relevant and the NOEC was the top concentration tested, i.e. 900 mg metalaxyl/kg feed. There was no consideration of the statistically significant decrease at 900 mg metalaxyl/kg feed in the 14-day old survivors of hatchlings reared (%), nor the statistically significant lower body weights of chicks at hatching in all concentrations, nor the lower body weights of adults. During the latest peer review, clarification was requested as to why the effects seen were not considered to be biologically relevant.

This study (including the concerns raised) is considered in further detail below.

[redacted] (1980b). One-generation reproduction study – Mallard duck, CGA 48988 technical.

Guidelines: Not specified in the report.

GLP: No – conducted prior to the requirement to conduct ecotoxicological studies to GLP.

Material and Methods:

Test substance: metalaxyl, CGA-48988 chemical purity: not given, described as “a brown material with large chunks, distinct odor (sic)”

Test species: Mallard duck (*Anas platyrhynchos*)

Sex, weight, age: 5 pens of 2 male and 5 female birds/treatment group, 1165 g, 6-month old. Body weights were recorded at initiation on weeks 2, 4, 6, 8 and at termination of the study.

Applied concentrations: untreated control, 100, 300, 900 mg metalaxyl/kg in the feed

Type of application: dietary application, the technical material was mixed with corn oil and incorporated into aliquots of basal diet. The basal diet was 19.4% protein, 6.7% fat and 3.8% fibre. The study report states that samples of the control diet and each of the treated diets were sent for analysis, however the outcome of this was not included in this study report.

Time of exposure: Test birds were kept indoors and fed treated diet *ad libitum* for 10 weeks during the pre-egg laying period and for the 8-week egg production period. Control birds were fed untreated basal diet throughout. The photoperiod for the first six weeks of the study was 8 hours light per day, the photoperiod was then increased to 17 hours of light per day and maintained at that duration until the termination of the study.

Study methodology: Eggs were collected daily and stored at 13.3°C±0.5°C and 87% humidity. At weekly intervals, the eggs were placed for incubation at 37.5°C, immediately prior to incubation all eggs were candled for eggshell cracks. All eggs were removed from the hatcher on Day 28 of incubation, the average bodyweight of representative hatchlings was determined. For each treatment level, forty hatchlings from each week lot were randomly chosen for rearing. Hatchlings were housed in the appropriate parental grouping until 14-days of age. One egg from every other pen in each experimental group and the control was randomly selected for egg weigh and eggshell thickness. The analysis on the data consisting of body weight and other measurement variables was subject to analysis of variance, whilst the analysis of the egg data and other count variable was based on Cochran’s concept of extraneous variability for the binomial distribution. Cochran’s analysis was used on the following parameters: eggs laid, eggs cracked, eggs set, viable embryos, live three-week embryos, hatchlings, body weight hatchlings, 14-day old survivors, body weight, egg weight, and eggshell thickness.

Findings:

Table B.9.1.1-3: Effects of metalaxyl observed during the reproduction study of Mallard duck

Endpoints	Concentrations (mg a.s./kg feed)			

	0	100	300	900
Adults				
Mortality	One male and two females	One male	-	One male and one female.
	All mortalities were the result of fighting between drakes or occurred during the stress of egg production. No compound related abnormalities were noted.			
Observations	Fighting was noted between drakes in the control and all treatments.			
Body weight, at termination (g)	1223	1206*	1287	1220
Food consumption	no compound related difference in food consumption			
Reproduction Parameters				
Number of eggs laid	666	786	818	705
Mean egg weight	59.5	60.6	61.7	59.0
Eggs cracked	27	18	39	47
Eggs cracked of eggs laid (%)	4	2	5	7
Mean egg shell thickness (mm)	0.365	0.372	0.362	0.346*
Eggs set	591	722	731	616
Viable embryos	544	662	660	570
Viable embryos of eggs set (%)	92	92	90	93
Live 3-week embryos	537	651	649	546
Hatchling reared	300	306	318	259
Live 3-week embryos of viable embryos	99	98	98	96**
(%)	379	473	387	319
Normal hatchlings	71	73	60	58*
Normal hatchlings of live 3-week	292	300	313	255
embryos (%)	27	31	33	28
14-day old survivors	97	98	98	98
Eggs laid/hen in 8 weeks (25 hens)	15	19	15	13
14-day old survivors of hatchlings	35	35	34*	33**
reared (%)	213	215	211	207
14-day old survivors per female				
Mean chick body weights at hatching (g)				
Mean chick body weights at 14 days (g)				

* significant ($p < 0.02$); ** highly significant ($p < 0.01$)

HSE Conclusions:

When originally assessed by the RMS and the peer review process the study was deemed to be acceptable and the NOEC was stated to be 300 mg metalaxyl/kg due to the presence of effects at 900 mg metalaxyl/kg for the following reproduction parameters:

- live 3-week embryos of viable embryos (%)
- normal hatchlings of live 3-week embryos (%)

There was no apparent consideration of either the statistically lower eggshell thickness at 900 mg metalaxyl/kg feed or the lower chick body weights at hatching at the 300 mg metalaxyl/kg feed concentration.

During the EU renewal of Metalaxyl-M, the following concern was raised:

> *There was a 11% decrease in the percentage of normal hatchlings of live 3 week embryos in the test group dosed at 300 mg a.s./kg feed. The level of effect was not statistically significant (although a 13% effect at 900 mg a.s./kg feed was) but it should be considered whether this level of effect is biologically relevant given the dose response observed*.

This study was discussed as part of the Expert's consultation during the renewal and it was concluded that the NOEC (*Anas platyrhynchos*, 18 weeks) was 300 mg metalaxyl/kg feed. This endpoint was determined to be to 24.6 mg a.s./kg bw/day based on a mean body weight of 1228 g and a mean food consumption of 100.5 g/bird/day.

It is noted that mortality occurred in both control and treatment groups, which was attributed to aggression between drakes. This is far from ideal and it would have been preferable to only include one drake per pen, as recommended in the study guideline. The aggression between males could have increased the stress level experienced by birds in the study. However, given that this issue was common to all groups and the overall NOEC from the study was identified on the basis of live 3-week embryos of viable embryos (%) and normal hatchlings of live 3-week embryos (%), any stress due to male aggression is considered unlikely to have impacted the key parameters in this study.

This study (including the concerns raised) is considered in further detail below.

Report:	**[redacted] & [redacted] (1998a), The Reproductive Toxicity Test of CGA-329351 with the Northern bobwhite (*Colinus virginianus*)**

Guideline(s):	Series 71-4 of the EPA Registration Guidelines, Pesticide Assessment Guidelines, Subdivision E. Hazard Evaluation: Wildlife and Aquatic Organisms (EPA, 1982) ASTM Standard Practice for Conducting Avian Reproduction Test, Designation: E 1062-86
Deviations:	No

GLP:	Yes
Test Material	CGA329351
Lot/Batch #:	FL951095 was used for the study, whilst Lot No FL980418 was used for analytical purposes.
Purity:	Lot no FL 951095 had 97.8% purity[11], whilst Lot no FL980418 had 95.6% purity.
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	13 June 1998
Treatments	
Test rates:	Control, 100 ppm, 300 ppm and 900 ppm
Food:	Purina Game Bird Ration (Layena)
Water:	Water was provided *ad libitum*
Chemical analysis:	Yes, at 100, 300 and 900 ppm via GC-NPD
Test organisms	
Species:	Northern bobwhite *(Colinus virginianus)* 28 weeks and 5 days of age at the start of the study
Source:	[redacted]
Acclimatisation period:	28 days
Treatment for disease:	None
Weight:	172.1-234.7 g
Test design	
Test cage description:	weld-wire cages measuring 51 cm (d) x 25 cm (w) x 20.5-25 cm (h)
Replication:	20
No. of birds/pen:	2
Duration of test:	204 days
Environmental test conditions	
Temperature:	18.3 to 28.3 °C
Humidity:	22 to 70% (mean 42.3% SD 10.6%)
Photoperiod:	7 hours light for 8 weeks, increased over a period of 6 days

[11] Re-analysis purity was 98.3%

to 17 hours light for remainder of the treated feed portion of the study.

Study Design and Methods

Experimental dates: 19 November 1997 – 11 June 1998

Mature Northern bobwhite received CGA329351 technical at nominal dietary concentrations of 100, 300 and 900 ppm for 23 weeks and 5 days. A control group receiving basal diet was maintained concurrently with the treatment groups.

The test item was provided at a treated diet. Purina game bird ration was used for feed and was provided *ad libitum* during acclimation and the test period.

Birds were kept in pairs one male and one female per cage. The cage dimensions were 51 cm deep, 25 cm wide and 20.5-25 cm in height, with a floor slope so eggs roll forward and out of the cage on to a collection tray.

Body weight was measured 4 times during acclimation and the definitive test. Feed consumption was measured weekly for each pair of birds. Birds where observed daily for signs of behavioural abnormality and mortality. Gross pathological examinations were performed on all birds succumbing prior to adult termination and on all birds surviving the test.

Eggs were collected on a daily basis and marked. Eggs were candled prior to being placed in an incubator. Cracked eggs were recorded as cracked and discarded. Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered cages during even numbered weeks and from each of the even numbered changes on odd numbered weeks. The eggs were cleaned of their contents, eggshells were then allowed to air dry measured at 5 points around the equator.

On day 14 of incubation, eggs were candled for fertility. Those that were not fertile were discarded. On day 21 of incubation eggs were candled for viability, those deemed not viable were discarded.

Upon hatch chicks were weighed and banded with a unique wing tag. Chicks were housed in caging units measuring 40 cm long x 80 cm wide x 26 cm high. Chicks were fed Purina Startena game ration *ad libitum*. Chicks were observed daily for behaviour and mortality. Surviving chicks were euthanized on the 14th day and weighed.

Statistical analysis was performed using TOXSTAT (1994). Data sets were tested for normality using a chi-square test and for homogeneity of variance using a Bartlett’s test or Levene’s test. Proportional data were arcsine transformed. If the data were normal and variances were homogenous the parameters were analysed with ANOVA Dunnett’s (equal sized groups) or an ANOVA and then a Tukey’s post-hoc test for pair-wise comparisons. If data was not normal or were heterogeneous, they were analysed with a Kruskal Wallis’ ANOVA by ranks followed by a Dunn’s multiple comparison.

Results and Discussion

Results are summarised in the tables below.

Table B.9.1.1-4: Summary of bodyweight of Northern bobwhite quail with CGA329351

Experimental group (mg/kg)	Sex	Body weight (g)		
		Start of test feed	Start of photo stimulation	Adult termination
Control	Male Female	202.7 204.7	214.3 216.4	227.6 263.1
100	Male Female	200.4 204.8	213.5 215.3	229.5 257.4
300	Male Female	202.9 203.4	215.5 214.7	231.5 256.9
900	Male Female	205.0 205.2	222.2 216.3	233.7 255.5

Table B.9.1.1-5: Summary of mean feed consumption (g/cage/week).

Experimental group (mg/kg)	Feed week											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Control	238.2	264.4	224.1	352.6	233.9	205.1	226.0	208.7	231.3	156.1	278.1	284.8
100	226.3	261.6	224.2	340.4	225.0	207.7	221.4	201.4	229.5	161.5	258.3	271.6
300	232.4	273.6	234.9	348.9	234.1	208 3	226.2	208.3	243.6	164.7	286.9	291.1
900	234.5	266.5	224.6	343.8	225.9	206.4	237.6	204.9	236.5	161.5	272.5	277.2
	Feed week											
	13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Control	288.9	276.8	339.5	344.8	353.0	351.3	346.6	361.6	360.5	343.9	336.2	268.1
100	277.2	262.2	316.7	319.5	345.3	343.5	322.2	344.4	337.2	335.7	330.9	264.3
300	283.1	284.1	335.4	347.4	344.2	347.5	344.5	358.8	345.9	346.9	329.0	273.4
900	281.2	283.9	328.8	329.7	344.1	334.7	334.7	347.3	347.6	353.0	329.9	266.8

Table B.9.1.1-6: Summary of reproductive performance from Northern bobwhite quail reproduction with CGA329351

Parameter	Experimental group (mg/kg)

	Control	100	300	900
Adult mortalities	One male and one female. The male was bloated, emaciated black intestine, and the gall bladder was enlarged. The female was from a cage had two females in error.	One male and two females. The male was emaciated, the females had head scalped, head lacerations, bald head and neck, blood on breast area, mass of yellowish fluid in mouth, pale liver.	One male and one female. The male was emaciated, and the female back was scalped.	One male and two females. The male had pecked eyes, whilst the females head pecked, spot on left lung, white foam over heart, and infected feet.
Number of replicates	18	17	18	17
Total eggs laid	1053	935	1045	1061
Eggs cracked	11	2	8	6
Eggs cracked of eggs laid (%)	1	0	1	1
Eggs set	959	854	956	970
Fertile eggs	886	797	882	912
Viable embryos	877	792	858	902
Hatchlings	846	767	829	868
14-day survivors	477	566*	550	614*
Number of eggs laid per day per hen	0.82	0.77	0.82	0.88
Numbers of eggs set per day per hen	0.75	0.71	0.75	0.80
Fertile eggs/eggs incubated (%)	92	93	92	94
Viable embryos/fertile eggs (%)	99	99	97	99
Hatchlings of eggs set (%)	88	90	87	89
Hatchlings/viable embryos (%)	96	97	97	96
14-day old survivors/hatchling (%)	56	74	66	71
14-day old survivors of eggs	50	74	66	71

Parameter	Experimental group (mg/kg)			
	Control	100	300	900
set (%)				
Hatchling/eggs incubated (%)	88	90	87	89
Mean chick body weights at hatching (g)	6.8	6.7	6.6	6.7
Mean chick body weights at 14 days (g)	27.3	26.9	27.7	26.7

total egg laying days = 71
Cage pairs not laying any eggs are not included in the above

Table B.9.1.1-7: Mean body weight of hatchlings and 14-day old survivors

Experimental group (mg/kg)	Hatchling			14-day old survivors		
	no	mean	std dev	No	mean	std dev
Control	846	6.8	0.6	477	27.3	5.4
100	767	6.7	0.7	566	26.9	5.5
300	829	6.6	0.6	550	27.7	5.3
900	868	6.7	0.7	614	26.7	5.4

Table B.9.1.1-8: Mean eggshell thickness (mm)

Experimental group (mg/kg)	No of eggs measured	Shell thickness mean	Std dev
Control	83	0.222	0.019
100	77	0.221	0.02
300	81	0.216	0.021
900	85	0.215	0.017

Diet: replicates of two test concentrations (100 and 900 mg/kg) were analysed for homogeneity, average concentrations found in these samples were 94.05±20.52 mg a.s./kg for the 100 mg/kg level and 934.49±24.88 mg/kg for the 900 mg/kg level. Replicates of the four test concentrations – 0, 100, 300 and 900 mg/kg were analysed for diet verification. No active substance was found in the control sample, average concentrations found in the treated samples were 96.12±14.47 mg/kg for the 100 mg/kg level, 307.35±34.48 mg/kg for the 300 mg/kg level and 946.10±83.99 mg/kg for the 900 mg/kg level.

All birds appeared normal in appearance and behaviour, with a few exceptions indicating cage wear or pair aggression was normal. All surviving adults were subjected to a post-mortem examination and a majority of birds in all groups were noted to have fatty tissue, the males had enlarged testes and the females had eggs present.

Conclusions

The study was considered during the renewal of metalaxyl-M and considered acceptable. No reproduction parameter showed compound related differences which are biologically relevant and hence the NOEC was set at the highest concentration tested, i.e. 900 mg/kg. This was determined to be equivalent to 84 mg a.s./kg bw/day by the RMS based on a mean body weight of 225 g and a mean food consumption of 21 g/bird/day.

HSE Conclusions:

When assessed by the RMS for the renewal and the associated peer review process the study was deemed to be acceptable and the NOEC was stated to be 900 mg metalaxyl-M/kg or 84 mg a.s./kg bw/day. During the peer review process, the following concerns were raised:

> *'It seems that there was an issue with the % of 14 day old survivors of hatchlings in the control group in this study. The normal values for the bobwhite quail according to OECD 206 are between 75 – 90% whereas in the control group only 56% of hatchlings survived. This may question the reliability of the study. Perhaps historical control information could be used to support the reliability of the study.'*

The RMS responded that:

> *Despite the low number of 14-day survivors in the control, no concentration dependent effect is observed and NOEC = 900 mg a.s./kg feed is justified.*

The applicants responded that:

> *14 days survivors in the controls were low. Despite this there was no evidence of a concentration dependent effect. The power of normalised proportions are relatively high compared to the numerical data. Thus, the NOEL of 900 ppm may be considered as robust despite the poor performance in controls.*

Due to the concerns raised, all four reproduction studies were subject to discussion at an Expert meeting and the outcome is reflected in the above conclusion.

This study (including the concerns raised) is considered in further detail below.

[redacted] and [redacted]. (1998b). The Reproductive Toxicity Test of CGA-329351 with the Mallard Duck (*Anas platyrhynchos*).

Guidelines: Series 71-4 of the EPA Registration Guidelines, Pesticide Assessment Guidelines, Subdivision E. Hazard Evaluation: Wildlife and Aquatic Organisms (EPA, 1982), ASTM Standard Practice for Conducting Avian Reproduction Test, Designation: E 1062-86

GLP:	Yes
Test Material	CGA329351
Lot/Batch #:	FL951095 was used for the study, whilst Lot No FL980418 was used for analytical purposes.
Purity:	Lot no FL 951095 had 97.8% purity, whilst Lot no FL980418 had 95.6% purity.
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	13 June 1998
Treatments	
Test rates:	Control, 100 ppm, 300 ppm and 900 ppm
Food:	Purina Game Bird Ration (Layena)
Water:	Water was provided *ad libitum*
Chemical analysis:	Yes, at 100, 300 and 900 ppm via GC-NPD
Test organisms	
Species:	Mallard duck *(Anas platyrhynchos)* 27 weeks and 1 day of age at the start of the study
Source:	[redacted].
Acclimatisation period:	28 days
Treatment for disease:	None
Weight:	857-1346 g
Test design	
Test cage description:	Polycarbonate-coated, galvanised weld-wire cages size was 76 cm long, 83 cm wide and 44 cm minimum height with a slanted floor to facilitate eggs rolling to the collection tray.
Replication:	16
No. of birds/pen:	2
Duration of test:	181 days
Environmental test conditions	
Temperature:	7.8 and 21.7°C
Humidity:	25 to 69% (mean 55% SD 9.7%)
Photoperiod:	7 hours light for 8 weeks, increased over a period of 6 days to 17 hours light for remainder of the treated feed portion of the

study.

Study Design and Methods

Experimental dates: 25 November 1997 – 25 May 1998

Mature Mallard duck received CGA329351 technical at nominal dietary concentrations of 100, 300 and 900 ppm for 20 weeks and 1 day. A control group receiving basal diet was maintained concurrently with the treatment groups.

The test item was provided at a treated diet. Purina game bird ration was used for feed and was provided *ad libitum* during acclimation and the test period.

Birds were kept in pairs one male and one female per cage.

Body weight was measured 4 times during acclimation and the definitive test. Feed consumption was measured weekly for each pair of birds. Birds where observed daily for signs of behavioural abnormality and mortality. Gross pathological examinations were performed on all birds succumbing prior to adult termination and on all birds surviving the test.

Eggs were collected on a daily basis and marked. Eggs were candled prior to being placed in an incubator. Cracked eggs were recorded as cracked and discarded. Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered cages during even numbered weeks and from each of the even numbered changes on odd numbered weeks. The eggs were cleaned of their contents, eggshells were then allowed to air dry measured at 5 points around the equator.

On day 14 of incubation, eggs were candled for fertility. Those that were not fertile were discarded. On day 21 of incubation eggs were candled for viability, those deemed not viable were discarded.

Upon hatch chicks were weighed and banded with a unique wing tag. Chicks were housed in caging units measuring 46 cm long x 141 cm wide x 46 cm high. Chicks were fed Purina Startena game ration *ad libitum*. Chicks were observed daily for behaviour and mortality. Surviving chicks were euthanized on the 14th day and weighed.

Statistical analysis was performed using TOXSTAT (1994). Data sets were tested for normality using a chi-square test and for homogeneity of variance using a Bartlett's test. Proportional data were arcsine transformed. If the data were normal and variances were homogenous the parameters were analysed with ANOVA Dunnett's (equal sized groups) or an ANOVA and then a Tukey's post-hoc test for pair-wise comparisons.

Findings:

Table B.9.1.1-9: Summary of bodyweight of Mallard Duck with CGA329351

Experimental group (mg/kg)	Sex	Body weight (g)		
		Start of test feed	Start of photo stimulation	Adult termination
Control	Male Female	1020 987	1168 1130	1256 1233
100	Male Female	1044 972	1175 1110	1253 1217
300	Male Female	1038 978	1168 1082	1253 1225
900	Male Female	1044 996	1185 1114	1259 1260

Table B.9.1.1-10: Summary of mean feed consumption (g/cage/week).

Experimental group (mg/kg)	Feed week											
	1	2	3	4	5	6	7	8	9	10	11	12
Control	1298.8	1454.4	1470.8	1257.0	1655.5	1535.8	1152.1	2054.4	1548.3	2144.0	2215.2	2253.4
100	1313.5	1476.4	1535.1	1375.8	1793.1	1487.1	1184.3	2008.9	1437.3	2100.7	2392.9	2204.5
300	1287.8	1571.7	1521.9	1281.0	1603.1	1477.4	1163.1	1965.0	1551.8	2156.3	2315.7	2241.9
900	1356.8	1697.1	1546.3	1229.4	1765.2	1570.7	1290.8	2013.0	1513.4	2069.7	2418.9	2340.2
	Feed week											
	13	14	15	16	17	18	19	20	21			
Control	2367.1	2447.0	2693.1	2754.3	2292.0	2424.2	2360.2	2290.5	352.5			
100	2336.0	2585.6	2737.6	2653.9	2172.4	2318.1	2281.7	2171.6	341.5			
300	2341.6	2836.8	2832.3	3081.0	2484.0	2565.8	2406.8	2349.3	366.5			
900	2438.9	2785.1	2856.3	3081.5	2508.4	2519.3	2623.0	2424.2	393.8			

Table B.9.1.1-11: Major effects of metalaxyl-M observed during the reproduction study of Mallard duck

Parameter	Experimental group (mg/kg)			
	Control	100	300	900
Adult mortalities	None	None	None	One male died from a cage injury; post-mortem indicated an

Parameter	Experimental group (mg/kg)			
	Control	100	300	900
				emaciated state (bw = 885.5 g)
Number of replicates	16	16	16	15
Total eggs laid	942	809	848	856
Eggs cracked	28	25	30	25
Eggs cracked of eggs laid (%)	1	0	1	1
Eggs set	837	713	744	760
Fertile eggs	775	677	716	710
Viable embryos	660	594	647	613
Hatchlings	525	453	472	442
14-day survivors	511	427	447	406
Number of eggs laid per day per hen	0.84	0.72	0.76	0.82
Fertile eggs/eggs incubated (%)	0.75	0.64	0.66	0.72
Viable embryos/fertile eggs (%)	85	88	90	86
Hatchlings/viable embryos (%)	80	76	73	72
14-day old survivors/hatchling (%)	97	94	95	92
14-day old survivors of eggs set (%)	61	60	60	53
Hatchling/eggs incubated (%)	63	64	63	58
Mean chick body weights at hatching (g)	37.03	37.94	38.46	38.80
Mean chick body weights at 14 days (g)	139.92	158.57	149.31	168.31

Table B.9.1.1-12: Mean body weight of hatchlings and 14-day old survivors

Experimental group (mg/kg)	Hatchling			14-day old survivors		
	no	mean	std dev	No	mean	std dev

Control	525	37.03	4.09	511	139.92	34.61
100	453	37.94	3.54	427	158.57	38.24
300	472	38.46	3.50	447	149.31	33.33
900	442	38.80	3.23	406	168.31	35.98

Table B.9.1.1-13: Mean eggshell thickness (mm)

Experimental group (mg/kg)	No of eggs measured	Shell thickness mean	Std dev
Control	76	0.370	0.024
100	69	0.377	0.024
300	72	0.368	0.037
900	71	0.363	0.026

Diet: replicates of two test concentrations (100 and 900 mg/kg) were analysed for homogeneity, average concentrations found in these samples were 94.72±52.45 mg a.s./kg for the 100 mg/kg level and 647.33±135.40 mg/kg for the 900 mg/kg level. Replicates of the four test concentrations – 0, 100, 300 and 900 mg/kg were analysed for diet verification. No active substance was found in the control sample, average concentrations found in the treated samples were 85.95±31.31 mg/kg for the 100 mg/kg level, 267.49±51.24 mg/kg for the 300 mg/kg level and 800.62±142.79 mg/kg for the 900 mg/kg level.

All surviving birds were noted to be normal in appearance and behaviour. No overt signs of treatment related toxicity were observed. All surviving birds were subjected to a post-mortem examination, the majority of birds were noted to have fatty tissue, the males had enlarged testes and the females to have eggs present. One control male was noted not to be sexually active and two females had small eggs. In the 100 mg/kg level, one male was noted as not being sexually active and five females as having small eggs. In the 300 mg/kg level, three males were noted as not being sexually active and three females were noted with enlarged gall bladders. In the 900 mg/kg level two males were not sexually active and one female was noted with small eggs and four females had enlarged gall bladders.

The hatch weight in the treatment group of 900 ppm was statistically significantly greater than the controls. This is not considered to be treatment related.

The mean chick body weights of 14-day old survivors in the treatment groups of 900 ppm were statistically significantly greater than the treatment groups of 100 and 300 ppm and the controls. In addition, the 100 ppm treatment group was statistically significantly higher than the control. These differences do not demonstrate a dose response effect and are not considered to be treatment related.

Conclusions:

The study was considered during the renewal of metalaxyl-M and considered acceptable. No reproduction parameter showed compound related differences which are biologically relevant and hence the NOEC was set at the highest concentration tested, i.e. 900 mg/kg. This was determined to be equivalent to 117 mg a.s./kg bw/day by the RMS based on a mean body weight of 1174 g and a mean food consumption of 152 g/bird/day.

HSE Conclusions:

When originally assessed by the RMS and the peer review process the study was deemed to be acceptable and the NOEC was stated to be 900 mg metalaxyl-M/kg or 117 mg a.s./kg bw day. The following concerns were raised during the peer review process:

> *It is noted that there appears to be a reduction in the number of 14-day old survivors in the treatment groups (although it is noted that the number of eggs laid was also less). Was statistical analysis performed on the number of 14-day old survivors per hen? In addition, the statement underneath table B.9.1.3-4 is not understood – it is indicated that there number of 14-day old survivors in the treatment groups were greater than the control, however, this is not consistent with the results in the table.*

Due to the concerns raised, all four studies reproduction studies were subject to discussion at an Expert meeting and the outcome is reflected in the above conclusion.

This study (including the concerns raised) is considered in further detail below.

<u>Discussion of the reproductive endpoint</u>

Consideration of the Mallard duck study by [redacted] (1980b)

Four avian reproductive studies have been carried out, two on metalaxyl and two on metalaxyl-M. All studies were considered appropriate during the latest EU peer review and the EU endpoint was set on the basis of effects seen in the Mallard duck study conducted by [redacted] (1980b). During the peer review process concern was raised regarding this study in that:

> *There was a 11% decrease in the percentage of normal hatchlings of live 3 week embryos in the test group dosed at 300 mg a.s./kg feed. The level of effect was not statistically significant (although a 13% effect at 900 mg a.s./kg feed) but it should be considered whether this level of effect is biologically relevant given the dose response observed.*

In response to this concern, the applicant stated the following during the peer review process:

> *During previous reviews concerns were raised about the quality of the mallard study conducted by [redacted] (1980b) as it was not conducted to GLP nor to any agreed guidance and an additional study was therefore conducted to*

> *address these concerns[12]. The second study [redacted] (1998) CGA329351/1072 was deemed to be acceptable and was included on the EU endpoint list whereas the study was not considered. The risk assessment is therefore based upon the lowest applicable endpoint obtained with the bobwhite quail [redacted] (1998) CGA329351/1071*.

Concerns, as indicated above, regarding amongst other issues, the lack of information on the purity of the test item, the fact that the study was not carried out to a standard guideline were discussed, however the EU review process considered that the Mallard duck study carried out by [redacted] (1980b) was of sufficient reliability to provide an endpoint for use in the risk assessment. The applicant has now provided further argumentation and outlined 'critical concerns' regarding this study (see Appendix 2 for full details). Presented below is a consideration of the applicant's concerns:

Age of birds: The birds used in the first Mallard duck study were 6 months old and the applicant states that the OECD 206 guideline recommends that birds should be between 9 and 12 months. Whilst the latter is correct, it should be noted that the current US EPA guideline[13] states the following:

> *'Age. Adult test birds used are those approaching their first breeding season and are at least 16 weeks old. All test birds should be the same age within one month.'*

As regards whether the birds had reached peak egg-laying during the study, HSE has re-examined the study and there is no information regarding the weekly productivity of birds, hence it is not possible to determine if the birds had reached peak egg-laying.

Egg-collection: Eggs were collected over an 8-week period in [redacted]; however, the OECD guideline indicates that eggs should be collected over a 10-week period. The applicant highlights the following with regard to the shorter collection period, which it is assumed was relevant for both the control and the treatment birds:

> *'is likely to have contributed to the lower number of eggs laid in the control that treatment groups (666 in control vs 715-818 in treatment groups) and supports that the birds may not have reached peak egg-laying during the study as required in OECD guideline 206.'*

It is appreciated that the egg collection period was less than recommended, impacting the number of eggs collected and hence potentially impacting the sensitivity of the study, however it is not considered to negate the study on its own. Due to the lack of information it is not possible to determine whether the birds had reached peak egg production.

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[12] Please note, as stated above, when originally considered by the EU review process, this study was deemed to be acceptable.

[13] Ecological Effects Test Guidelines OCSPP 850.2300: Avian Reproduction Test, EPA 712-C-023.

The applicant goes on to outline how the control data from [redacted] (1980b) compares to the criteria outlined in both the OECD and the US-EPA guideline, this is presented below:

OCSPP 850.2300 Guideline Parameter	OECD 206 Normal Values	OCSPP 850.2300 Validity Criteria	MLX Mallard Control	Source in Report
Assignment to treatments		Random	Random	Text pg. 2
Adult Mortality	≤ 10%	≤ 10%	5.7%	Text pg. 11
Average number of eggs laid / hen	28-38	≥ 29	**26.6**	Table 2
Viable embryos / eggs set	85-98	≥ 80%	92.0%	Table 1A
Live 3-wk embryos / eggs set		≥ 94%	**90.9%**	Table 1A
Normal hatchlings / viable embryos		≥ 52%	69.7%	Table 1A
Normal hatchlings / eggs set	50-90	≥ 44%	**64.1%**	Table 1A
14-d Survivors / normal hatchlings	94-99	≥ 94%	**77.0%**	Table 1A
Egg shell thickness (mm)	≥ 0.34	≥ 0.316	0.365	Table 3B
Cracked eggs / eggs laid	0.6-6	≤ 13%	4.1%	Table 1A

It is appreciated that the average number of eggs laid/hen, is less than that recommended in either the OECD or the US-EPA guideline. It is noted that the reference to OECD guideline, refers to a range of 28 to 38 eggs/hen over a 10-week period; whilst the US EPA guideline refers to 29 to 61 eggs laid per hen over a 10-week period. As highlighted above, this study only collected eggs over an 8-week period, however a total of 666 eggs were laid by 25 hens, giving 26.64 eggs/hen over an 8-week period. Hence, the number of eggs laid/hen is slightly lower than recommended and hence this study may have potential a lower sensitivity. It should be noted that the avian reproduction study is not particularly sensitive (see Valverde-Garcia *et al* (2018) for a consideration of the minimum detectable differences in key parameters).

Whilst, as stated above, the lower number of eggs/hen does reduce the sensitivity of the study, it is unclear if it negates the study. It is noted that this value is less than 10% lower than that recommended by either guideline, it is also noted that this parameter will impact other related parameters. Overall, whilst not ideal, it does not, on its own, negate the study.

Test material: There is lack of information on the purity of the test substance as well as a lack of any chemical analysis to confirm whether the test concentrations were in fact achieved. This is recommended by the current US-EPA guidelines, where it states:

> *'The dietary levels are confirmed by chemical analysis under test conditions.'*

The OECD guideline states:

> *'There must be evidence that the concentration of the substance being tested has been*

satisfactorily maintained in the diet (it should be at least 80 per cent of the nominal concentration) throughout the test period'

And it goes on to state:

'The test substance concentration in the diet must not drop below 80 per cent of the expected concentration after the first week of the test. During the first week of the test, diets containing the highest and lowest concentrations should be analysed immediately after the initial mixing and again within four hours of replacing with freshly mixed diet, unless the stability of the test substance in the diet can be adequately demonstrated. If all analyses are within 80 per cent of expected concentrations, no further analyses are required, and the test diet should be renewed frequently enough to ensure maintaining the concentrations.

If either set of analyses indicates that concentrations of the test substance in the diet are less than 80 per cent of the expected concentration, adjustments must be made to raise initial concentrations or maintain the actual concentrations by more frequent renewal. Additional analyses during the second week of the test should be conducted to ensure that the adjustments have achieved the 80 per cent goal.'

The applicant also raises concerns regarding carriers used.

The lack of chemical verification does seriously undermine the study and in particular the reliability of it, however it is considered important not to reject the study solely on this basis, especially if it shows potentially adverse effects compared to other studies.

Environmental conditions: The applicant cites several environmental conditions where there were deviations from what is recommended in the OECD guideline, see Appendix 2 for details. Several of these are relatively minor and whilst not ideal, would not normally negate or undermine a study. For example, the applicant cites the following:

'The storage temperature deviates from the OECD 206 recommendation, i.e. 13.3°C (test) compared to 14-16°C (OECD).'

Whilst this is correct, it is noted that according to the US-EPA guideline:

'All eggs are collected daily, marked according to the pen from which collected, and stored at 13 to 16 ºC'

Hence this study is in line with the latter but not the former.

Humidity of 87% was outside that recommended by both guidelines. As for the temperature during brood development, this ranged from 37.8°C to 23.9°C. The applicant considered that this was inappropriate as the OECD 206 guideline requires 32-35°C for days 7-14 and 28-32°C for the latter half of the study. The US-EPA study recommends that a temperature gradient in the brooder pen from approximately 35°C to 22°C. Therefore, whilst both the temperature and humidity are not ideal, it is not considered that the above deviations and the durations are sufficient to negate the study findings.

Historical control: In highlighting their concern, the applicant compared the findings of the [redacted] (1980b) study with those presented in Valverde-Garcia *et al* (2018)[14], and the key table is presented below, with more details in Appendix 2. It should be noted that it is unknown whether the studies considered by Valverde-Garcia *et al* were of adequate quality and had in turn been accepted by a regulatory authority. Furthermore, it is not known whether the studies presented here for metalaxyl-M were in fact in the database used by Valverde-Garcia *et al*.[15]

Mallard Historical Control Values (Valverde-Garcia et al. 2018; Table 5)	MLX Mallard Study Control Values (Table 1B)	Control Mean	95% Lower CL	95% Upper CL	95% Lower PL	95% Upper PL	OECD TG206	OSCPP 850.2300
No. eggs laid / hen / d	**0.48** [a,b]	0.58	0.48	0.69	0.44	0.73		
Eggshell thickness (mm)	**0.365**	0.385	0.372	0.398	0.367	0.403	0.35–0.39	0.316–0.372
Eggs cracked / eggs laid (%)	**4**	1.57	0.52	4.66	0.33	7.2	0.6–7	0–4.0
Viable embryos / eggs set (%)	92	89.7	76.97	95.78	69.27	97.11	85–98	
Live 3 week embryos / viable embryos (%	99	98.27	96.26	99.21	94.89	99.43		94–100
Hatchlings / live 3 week embryos (%)	**71**	78.1	60.67	89.18	52.23	92.08		52–100
14-d old survivors / hatchlings (%)	97	97.95	95.64	99.05	94.09	99.31	94–99	94–100
Hatchlings / eggs set (%)	64.1	69.31	46.86	85.26	37.46	89.49		44–92

[a] Based on 8 weeks of exposure (56 d) as described on pg. 2 of the report.

[b] 666 eggs / 25 control females / 56 d

Lower and Upper PL: prediction intervals for the means of the respective reproductive endpoints

The applicant has highlighted in red, those control values from the Mallard duck study that are either outside the 95% confidence interval or differ from the mean value. However, it should be noted that whilst the number of eggs laid/hen/day falls within the 95% confidence limits of the historical control data, this is not a validity criterion of the study. As for eggshell thickness whilst it is outside the confidence limits, it is in line with the validity criteria required of both the OECD and US-EPA guideline. Finally, the hatchling/live 3-week embryos (%) is within that required by the US-EPA study and no criteria for this parameter is defined in the OECD 206 guideline.

Replication: The applicant considers that the replication used in the [redacted] study to be inappropriate, more specifically, they state:

> *Five pens were established for each test treatment and control, with each pen containing 2 drakes and 5 hens compared to the 1 drake to three hens recommended in the guidance, although it is noted that other arrangements may be justified. However, the guidance document does explicitly state that*

[14] This paper has been considered by HSE see Appendix 4

[15] It is noted that Valverde-Garcia *et al* used data from Evans Analytical Group LLC avian toxicoloogy laboratory, which was formerly Wildlife International Ltd. [redacted] (1980a and b) were done at [redacted], whereas the other studies were conducted at [redacted]

when the mallard duck is tested in groups i.e. in ratios of other than 1 drake to 3 hens than at least 8 pens need to be established for each treatment rate and control. Given that only 5 pens were established for each test treatment rate and control then the statistical power of this study is open to question.

The following is stated in the US-EPA guideline:

(i) The experimental unit for this test is the pen. All control and treatment birds should be randomly distributed to pens from the same population. For northern bobwhite and mallard, each of the test substance groups and the control group consist of a minimum of 16 replicate pens. Each pen contains one male and one female. The use of 20 replicate pens in the control group may yield a test with greater statistical power.

(ii) An alternative arrangement of birds may consist of multiple female birds (typically two) and one male bird in each pen. For this arrangement, each pen is considered a replicate. Productivity should be calculated on a per hen basis, with an average given for each pen. Either arrangement is acceptable if productivity reaches the definitive values given in (e)(5)(ii)(A) of this guideline[16].

It is clear that the number of replicates is not in line with the current OECD guideline, as for the US EPA guideline, the study does not meet the criteria in terms of eggs laid per hen (see above). As for the other parameters discussed above, whilst not ideal, it does not necessarily negate the study.

Males per pen: It is noted that in the [redacted] (1980b) study there were 2 male mallards and 5 females per pen. This is contrary to recommendations under both OECD and US EPA guidelines. In contrast there was one male and one female per pen in [redacted] and [redacted] (1998b), i.e. in line with the recommendation for a single male per pen. While the [redacted] (1980b) study predates the current versions of the guidelines, it is apparent that housing 2 males per pen resulted in aggression between males. This will have increased the stress experienced by birds in this study, with mortality occurring in both control and treatment groups. However, the same housing arrangements were used in both control and treatment groups, so this deviation would not be expected to bias results in a particular direction. Additionally, the key parameters impacted by treatment in this study were hatchlings per 3-week embryos, live 3-week of viable embryos and eggshell thickness. It is considered unlikely that the housing of multiple males per pen would have an impact on any of these key parameters.

Overall conclusion on the validity of the [redacted] (1980b) study

As regards the study, whilst clearly not ideal in that there were some deviations from the guideline (e.g. environmental conditions, multiple males per pen) and a few points that could impact the ability to detect an effect, effects were detected that were not detected in the other study and all validity criteria were satisfied. As discussed

[16] The reference relates to the following : Eggs laid. Normal values for both northern bobwhite and mallards are 29 to 61 eggs per hen for a 10 week egg laying period.

above, the number of eggs laid per hen is lower than recommended by either the OECD or US EPA guideline, however the results do not indicate an impact of the test item on this parameter. This parameter would not be expected to impact some of the parameters where effects are seen (eggshell thickness, chick weight, hatchlings per 3-week embryos, live 3-week of viable embryos). As discussed above, the lack of analysis/purity info is a concern but not enough to invalidate in light of the results seen. Therefore, whilst the study is not up to the same standard as modern studies, it is not considered sufficiently unreliable to reject the study on the basis of the above assessment, especially as potentially adverse effects were observed; it is however considered to be more appropriate to consider any effects seen in this study alongside the other three studies submitted.

Robustness of the endpoint from the bobwhite quail study ([redacted] and [redacted] (1998a).

During the EU review concern was raised regarding the percentage of 14-day old survivors/hatchlings. The concern was due to the fact that the number in the control was less than that in the treatments (see Table B.9.1.1.3-5), in addition the percentage was less than that recommended in both the US-EPA and the OECD guideline. The OECD guideline states: the percentage of hatchlings that survive to 14 days should be between 75-90%, whereas the US-EPA guideline states: '14-day-old survivors of eggs hatched. Normal values for northern bobwhite are 77 to 100%'. Presented in the table below are the data for the percentage of 14-day old survivors/hatchling for all the studies considered by the applicant in Appendix 2 and 3.

HSE has compared the control group 14-day old survivors/hatchlings from the study conducted with metalaxyl-M with four other studies conducted in the same laboratory and by the same study director. These are presented in the table below and in Appendices 2 and 3.

Table B.9.1.1-14: Comparison of 14-day old bobwhite quail survivors/hatchlings

	[redacted] and [redacted], 1998	[redacted] and [redacted], 1996	[redacted] and [redacted], 1998b	[redacted] and [redacted], 1996a	Taliaferro et al., 1998	OECD	US-EPA
	CGA329351	CGA277476	CGA293343	CGA215944	CGA24705		
14-day old survivors/hatchling (%)	56	88	57*	72	55	75-90	77-100

* In Appendix 2, the applicant has quoted a figure of 0.48, this is according to the study report the figure for 14-day old survivors of eggs set.

Only one of the studies submitted meets the criteria outlined in both the OECD and US-EPA guidelines, [redacted] and [redacted] (1996). This study, that used oxysulfuron, has been reviewed by the EU review process and it was concluded that it was a reliable study and an endpoint could be derived from it (see EFSA (2017)[17]). The study on CGA215944 (pymetrozine) had a value of 72% and was deemed to be acceptable when reviewed by the EU review process (see EFSA (2014)[18]). The study on CGA293343 (thiamethoxam) was reviewed by the RMS but was not subjected to peer review by MS and EFSA. The RMS considered that the study was reliable and could be used for setting an endpoint. The study on CGA24705 (metolachlor) has been reviewed by the RMS and is currently undergoing review by the EU process.

It is clear that studies with lower than recommended 14-day old survivors of hatchlings have been accepted as appropriate studies and used in regulatory risk assessments. The applicant considers that the metalaxyl-M study is within the historical control of the other studies. Whilst it is clear that the figure from this study is within that of the other controls, this does not mean that the study is acceptable. Similarly, the values of 75-90% quoted in the OECD guideline are recommended and not validity criteria, hence a study not falling within this range is not invalid. What the low value for the control means is that it is unclear whether the control was performing as well as it should have been and hence whether potentially adverse effects may have been missed due to the low productivity of the control.

The applicant cites that there is an inverse relationship between eggs/laid/hen and 14-day old survivors. HSE has checked this assessment and notes that an incorrect value has been stated for one of the studies regarding control group 14-day old survivors/hatchlings. It is unclear as to how this element should be considered in determining the acceptability of the study and, hence deriving the endpoint.

HSE has checked all other values and whilst it cannot replicate all exactly considers them to be correct.

Overall, the study was well conducted and reported; however, there is uncertainty regarding the potential for this study to detect adverse effects due to an underperforming control in terms of hatchling survival and, in addition, the treatments ‘out-performed’ the control for this parameter.

Overall conclusion regarding the reproductive toxicity endpoint for metalaxyl-M

Four avian reproduction studies have been carried out and submitted and these are briefly summarised below in the table below.

Table B.9.1.1-15: Summary table of the reproductive studies conducting using metalaxyl and metalaxyl-M

[17] EFSA (European Food Safety Authority), 2017. Conclusion on the peer review of the pesticide risk assessment of the active substance oxasulfuron, EFSA Journal 2017;15(3):4722, doi:10.2903/j.efsa.2017.4722

[18] EFSA (European Food Safety Authority), 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance pymetrozine. EFSA Journal 2014;12(9):3817, 102 pp. doi:10.2903/j.efsa.2014.3817

Study	Species	Brief summary	Effects seen	Endpoint
[redacted] (1980a)	Bobwhite quail	Not conducted to modern standards: • lack of details regarding purity of test substance, • no analysis of diet carried out • 1 male:2 female** • duration of study was 18 weeks (10 weeks pre egg-laying, 8 weeks egg-laying)	Statistically significant effects of: • decrease at 900 mg/kg feed in the 14-day old survivors of hatchlings reared (%) • lower body weights of chicks at hatching in all concentrations • lower body weight of adults in all treatments	900 mg/kg feed*
[redacted] (1980b)	Mallard duck	Not conducted to modern standards: • lack of details regarding purity of test substance, • no analysis of diet carried out • age of birds • 5 females:2 males** • duration of study was 18 weeks (10 weeks pre egg-laying, 8 weeks egg-laying) • number of replicates per treatment (5)	Effects on: • live 3-week embryos of viable embryos (%) at 900 mg/kg feed • normal hatchlings of live 3-week embryos (%) at 900 mg/kg feed • statistically lower eggshell thickness at 900 mg/kg feed • lower chick body weights at hatching at 300 and 900 mg/kg feed	300 mg/kg feed
[redacted] and [redacted] (1998a)	Bobwhite quail	Modern standards No apparent adverse effects seen, however concern raised regarding the performance of the control	None	900 mg/kg feed
[redacted] and [redacted] (1998b)	Mallard duck	Modern standards No apparent adverse effects	No statistically significant effects observed but there is a reduction in key reproductive parameters in the percentage of	900 mg/kg feed

Study	Species	Brief summary	Effects seen	Endpoint
			hatchlings/viable embryos (%) (across all concentrations tested), 14-day old survivors/hatchling (%) (across all concentrations tested) and hatchling/eggs incubated (%) (across all concentrations tested) were all reduced compared to the control.	

* There was no apparent discussion either by the RMS or the peer review process of the biological relevance of the effects seen.

** OECD 206 states: 'Birds may be kept in pens as pairs or as groups of one male and two (bobwhite quail and Japanese quail) or three (mallard duck) females. Other arrangements are not excluded if justified.' No justification was provided.

From the above, it is clear that Mallard duck study by [redacted] and [redacted] (1998b) is reliable noting the concerns regarding potential (non-statistically significant) effects at the top concentration, hence there is uncertainty in the endpoint of 900 mg/kg feed. As regards the Bobwhite quail study by [redacted] and [redacted] (1998a), whilst conducted to modern standards, there are concerns regarding the performance of the control and hence the overall reliability of the study. The two earlier studies, both by [redacted], were not conducted to modern standards, and in particular there was a lack of information on the test item, whether concentrations were achieved, the stability of the test item and in the case of the Mallard Duck study, the replication used. However, there is information in these studies that indicates potential adverse effects and whilst they are not ideal, they cannot be ignored.

Body weights

Lower body weights of chicks at hatching was observed in both [redacted] studies, in the Bobwhite quail study it was in all concentrations, whereas in the Mallard duck study a reduction was observed in the top two concentrations tested.

Adult body weights were reduced in the Bobwhite quail study ([redacted], 1980a), whilst there was no impact on adult body weight in the Mallard Duck study ([redacted] (1980b).

In the two modern studies, there was no obvious impact on body weight of either adults or chicks, neither was there any impact on food consumption.

There is no clear reason as to why effects on body weight of chicks and to a lesser extent adults should occur in the [redacted] studies and not the more modern studies. One possible explanation is the grouping of the test birds.

Eggshell thickness

In [redacted] (1980b), a statistically significant effect on the thickness of eggshells was noted, this was not observed in any of the other studies. It is unclear as to why this parameter was impacted in the one study at the top concentration tested.

Reproductive parameters

In the [redacted] Mallard duck study (1980b), the following effects were observed:

- live 3-week embryos of viable embryos (%)
- normal hatchlings of live 3-week embryos (%)

It is noted that whilst not statistically significant, there is a reduction, in key reproductive parameters in the [redacted] and [redacted] (1998b) Mallard Duck study, namely, the percentage of hatchlings/viable embryos (%), 14-day old survivors/hatchling (%) and hatchling/eggs incubated (%) were all reduced compared to the control. Therefore, whilst the [redacted] (1980a) study is not up to modern standards and has some potentially significant deficiencies, there is some evidence that similar effects are occurring in the modern study by [redacted] and [redacted] (1998b).

Whilst in the [redacted] Bobwhite Quail study (1980a), the following effect was observed

- decrease at 900 mg/kg feed in the 14-day old survivors of hatchlings reared (%)

The effects seen are concentration related and hence potentially treatment related. No adverse effects were observed in the modern study by [redacted] and [redacted] (1998a), however this is the parameter that was questioned by the EU review process and is discussed above. Therefore, it is not possible to draw a conclusion on this parameter.

When all four studies are examined it is considered that the endpoint selected by the EU review process is appropriate, i.e. **a NOEC of 300 mg/kg feed, equivalent to 24.6 mg/kg bw/day**. This endpoint is from the [redacted] (1980b) Mallard duck study and covers the embryo/hatchling effects seen in that study. It also covers potential adverse effects seen in the newer Mallard duck study. In addition, whilst a firm conclusion cannot be reached on hatchling survival effects in the [redacted] and [redacted] (1998a) bobwhite quail, the proposed endpoint should cover any concerns with this study and [redacted] (1980a). This endpoint will be used in the following risk assessment.

B.9.1.2. Effects on terrestrial vertebrates other than birds

Mammalian toxicity endpoints from the EU review of metalaxyl-M are reported in the following table.

Table B.9.1.2-1: Mammalian endpoints for Metalaxyl-M

Species	Substance	Exposure system	Results	Reference [a]
Rat	Metalaxyl-M	Oral 1 d Acute	LD_{50} = 953 mg/kg bw (male) **LD_{50} = 375 mg/kg bw** (female)	EFSA Journal 2015;13(3):3999; [redacted], 1994; CGA329351/0002
Rat	Metalaxyl-M	Dietary Reproductive toxicity	**NOAEL = 96 mg/kg bw/d**	EFSA Journal 2015;13(3):3999; [redacted] *et al.*, 1980; CGA48988/0597

Acute toxicity:

Mammalian acute oral studies have been carried out with metalaxyl-M and with the EU representative formulation 'Apron XL'. These studies were reviewed by the RMS in the EU renewal review of metalaxyl-M and were summarised in Volume 3 section B.6 of the draft Renewal Assessment Report (RAR). The lowest LD_{50} of 375 mg a.s./kg bw for females from the [redacted] (1994a) study will be used in the acute risk assessment for mammals. This is in line with the EU renewal review of metalaxyl-M.

It is noted that an acute oral toxicity study is also available for mice ([redacted], 1996d). In principle a geometric mean LD_{50} could be derived from the rat and mouse data. However, this is complicated by the fact that a precise LD_{50} was not determined for mice (LD_{50} = 500-1000 mg a.s./kg bw). If it were conservatively assumed that the LD_{50} for mouse was 500 mg a.s./kg, then the geometric mean using the mouse and rat values would be 433 mg a.s./kg bw/d. Therefore, the difference between this geometric mean LD_{50} and the rat-only value is minor. It can be considered whether the use of the geometric mean LD_{50} will have an impact on the outcome of the risk assessment in the higher tier evaluation.

Long-term/reproductive toxicity:

Mammalian short-term dietary and long-term reproduction studies have been carried out with either metalaxyl or metalaxyl-M. These studies were reviewed by the RMS in the EU renewal review of metalaxyl-M and were summarised in Volume 3 section B.6 of the Renewal Assessment Report (RAR). From these data it was concluded for the EU renewal review of metalaxyl-M that the ecotoxicological toxicity to mammals of the enantiomer metalaxyl-M is comparable to that of the racemate metalaxyl. The lowest NOAEL of 96 mg a.s./kg bw/d from the [redacted] et al. (1980) study will be used in the reproductive risk assessment for mammals. This is in line with the EU renewal review of metalaxyl-M.

It is noted that no acute or reproductive toxicity studies are available with the formulated products, however, no such studies are considered necessary and the risk assessment can be based on the active substance toxicity.

B.9.1.3. Higher tier studies – birds

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	IIIA 10.1.7/01 [redacted] (2002) Acceptance of sugar beet pills by birds: field monitoring of birds in the Netherlands. [redacted], Report Number [redacted]. Syngenta File Number N/1158, VV-339361 (owned by Bayer Crop Science, Syngenta access)

Guidelines
None

GLP: Yes

Study Design and Methods

Experimental dates: April to May 2001

Objectives: To determine the numbers of sugar beet pills (pelleted seeds) on the field surface after drilling, the types of birds using the fields and their feeding activity to determine if they were likely to be eating the pills.

Study area: The field monitoring was performed in 5 different areas of the Netherlands: Goirle (Noord Brabant), Alphen (Noord Brabant), Eindewege (Zeeland), Kloetinge (Zeeland), Dinteloord (Noord Brabant) in the spring of 2001.

Test plots:

Location		Test substance	Colour
Field 1*	Goirle	Laetitia Brigitta	Orange orange
Field 2	Alphen	Laetitia	orange
Field 3	Eindewege	Toledo Cyntia	orange
Field 4	Kloetinge	Toledo 514 Lenora	Blue orange
Field 5	Dinteloord	Cyntia	orange

* Goirle had 15 kg of barley and 25 kg of winter wheat applied in the same morning to protect the sol from wind erosion.

Test organisms: Natural bird communities.

Method and parameters:
Fields were drilled with a 50 cm distance between the rows. The distance between the seed in each row ranged from 16 cm to 18 cm. The drilling rates ranged from 0.8 unit/ha to 1.1 unit/ha.

Two areas on each field were chosen to assess the exposure of sugar beet pills after drilling. One midfield area of 50 m x 50 m, and one end row of rectangular shape of different sizes ranging from 500 m^2 to 2000 m^2. Field 3 did not have an end row, as the field was not rectangular, therefore only the midfield area was used for the exposure assessment. The whole field areas were searched for spillages, in case of spillage the position of the spillage and number of seeds per spillage were determined.

Artificial spillages were generated on day 0 in field 2 and field 3. Two areas in each field of 5 m x 5 m had 250 pills left visible on the surface to simulate a worst-case availability. In one area, blue pills were used, and in another area, orange pills were used.

Observations:
On each field number of seeds, which remained on the surface after drilling, were counted. Additionally, on field 1, the number of cereal seeds remaining on the surface were counted on 2 midfield areas.

On two fields (Alphen, Eindewege) artificial spillages were laid out and collected two days later to determine if any had been removed (consumed).

Birds were observed on the day of drilling (during and after drilling) until dusk and on the two following days for the whole daylight period. The field was scanned with binoculars or a spotting scope (scan-sampling method). Bird species were divided into three groups: small seed eating birds, large seed eating birds, and non-seed eating birds. All birds present on the field were recorded and the number of individuals showing a distinct behaviour was estimated. Data sets were normalised into index values based on one observation interval (5 minutes) per hectare for comparison.

Data analysis: Not applicable.

Results and discussion

Exposure assessment:
The mean number of exposed sugar beet pills on the soil surface after drilling the midfield was 0.011 pills/m^2 (range 0.0004 - 0.03 pills/m^2), and after drilling end rows was 0.155 pills/m^2 (range 0.011-0.37 pills/m^2). The percentage per m^2 of seeds on the surface in the midfield areas was 0.111 %, whereas the percentage per m^2 of seeds on the surface in the end row was 1.527 %. This showed that in all fields there was a higher number of seeds remaining in the end row areas than in the midfield areas. The highest number of seeds remaining on the soil surface in the midfield areas were found in Goirle (field 1) and Alphen (field 2) with a range of 0.02 – 0.03 pills/m^2. Both areas had sandy soil and the sowing depth was 1 – 1.2 cm. In the other fields where the sowing depth was deeper (over 2 cm), the number of pills remaining on the surface were < 0.003 pills/m^2.

Expressed as percentage of uncovered pills related to the drilling rate, 0.111 % mean of the pills remained uncovered in the midfield areas.

Table B.9.1.3-1: Percentage of seeds/m² in relation to the drilling rate

Location	Drilling rate		Pills found/m²	Percentage per m²
Midfield	Units/ha	Pills/m²		
Goirle	1.06	10.6	0.03	0.283
Alphen	1.06	10.6	0.02	0.189
Eindewege*	1.04	10.4	0.003	0.029
Kloetinge	0.8	8	0.004	0.050
Dinteloord	0.8	8	0.0004	0.005
		Mean	**0.011**	**0.111**
		SD	**0.013**	**0.120**
End row	Units/ha	Pills/m²	Pills found/m²	Percentage per m²
Goirle	1.06	10.6	0.17	1.604
Alphen	1.06	10.6	0.37	3.491
Kloetinge	0.8	8	0.011	0.138
Dinteloord	0.8	8	0.07	0.875
		Mean	**0.155**	**1.527**
		SD	**0.157**	**1.440**

* Eindewege did not have an end row due to the shape of the field.

Artificial spillages:

In fields 2 and 3, no birds were observed at the artificial spillage. When the sugar beet pills were recollected, 7 were missing in field 2, and 15 in field 3. It is likely that birds or mammals ingested them. It was not apparent that the birds were attracted to either the blue or orange pills.

Table B.9.1.3-2: Artificial Spillages of sugar Beet Pills on two fields

Location	Laying out			Recollection	
	Date	Number	Colour	Date	Number
Alphen	12.04.01	250	Blue	14.04.01	243
		250	Orange		251
Eindewege	02.05.01	250	Blue	04.05.01	235
		250	Orange		250

Bird Observation:

There was a high diversity of birds in all areas ranging from 11 species in Alphen (field 2) to 27 at Eindewege (field 3). The mean number of individuals/ha for each scan over days 0-2 ranged from 0.043 at Alphen to 6.076 at Goirle. The mean for all fields during days 0-2, except Goirle, was 0.460. The highest abundancies were recorded for Goirle (field 1), the availability of cereals on the surface (mean 8.1 seeds/m²) was much higher than that of the sugar beet pills.

Table B.9.1.3-3: Number of species in each location

	Goirle	Alphen	Eindewege	Kloetinge	Dinteloord
Day 0	9	0	16	8	4
Day 1	11	8	24	20	9
Day 2	11	7	19	13	6
Number of	13	11	27	22	10

species all together					

Table B.9.1.3-4: Bird activity (total/interval/hectare)

		Goirle (field 1)	Alphen (field 2)	Eindewege (field 3)	Kloetinge (field 4)	Dinteloord (field 5)	Mean (field 2 – 5)
Day 0	Index of individuals	4.207	0	0.931	0.874	0.048	**0.463**
	Index of foraging individuals	4.152	0	0.577	0.222	0.042	**0.210**
Day 1	Index of individuals	5.147	0.066	0.851	0.949	0.078	**0.486**
	Index of foraging individuals	4.674	0.034	0.584	0.385	0.064	**0.267**
Day 2	Index of individuals	7.221	0.036	0.579	1.060	0.067	**0.436**
	Index of foraging individuals	6.565	0.022	0.314	0.114	0.056	**0.127**
Day 0 - 2	Index of individuals	6.076	0.043	0.737	0.989	0.069	**0.460**
	Index of foraging individuals	5.435	0.023	0.474	0.261	0.057	**0.203**

Table B.9.1.3-5: Guild activity (total/interval/hectare)

		Goirle (field 1)	Alphen (field 2)	Eindewege (field 3)	Kloetinge (field 4)	Dinteloord (field 5)	Mean (field 2 – 5)
Day 0 - 2	Index of individuals (large seed eater)	5.889	0.023	0.168	0.072	0.038	**0.075**
	Index of foraging individuals (LS)	5.394	0.008	0.129	0.064	0.034	**0.059**
Day 0 - 2	Index of individuals (Small seedeater)	0	0.013	0.001	0.001	0	**0.003**
	Index of foraging individuals (SS)	0	0.011	0.001	0.001	0	**0.003**

Day 0 - 2	Index of individuals (non-seedeater)	0.043	0.006	0.568	0.916	0.031	**0.380**
	Index of foraging individuals (NS)	0.041	0.003	0.344	0.197	0.023	**0.197**

Goirle was not included in the calculation of the mean since this field was too dissimilar to the others due to the high availability of wheat/barley seeds.

The number of foraging individuals/ha for each scan over days 0-2 ranged from 0.023 at Alphen to 5.435 at Goirle. The mean for all fields during days 0-2, except Goirle, was 0.203.

Conclusions

Under the study conditions the proportion of pelleted sugar beet seed (pills) remaining on the surface was low due to the technique of precision drilling. Despite the high diversity of species there was no evidence of birds eating sugar beet pills and no observations or measurements of seed being recovered from artificial spills.

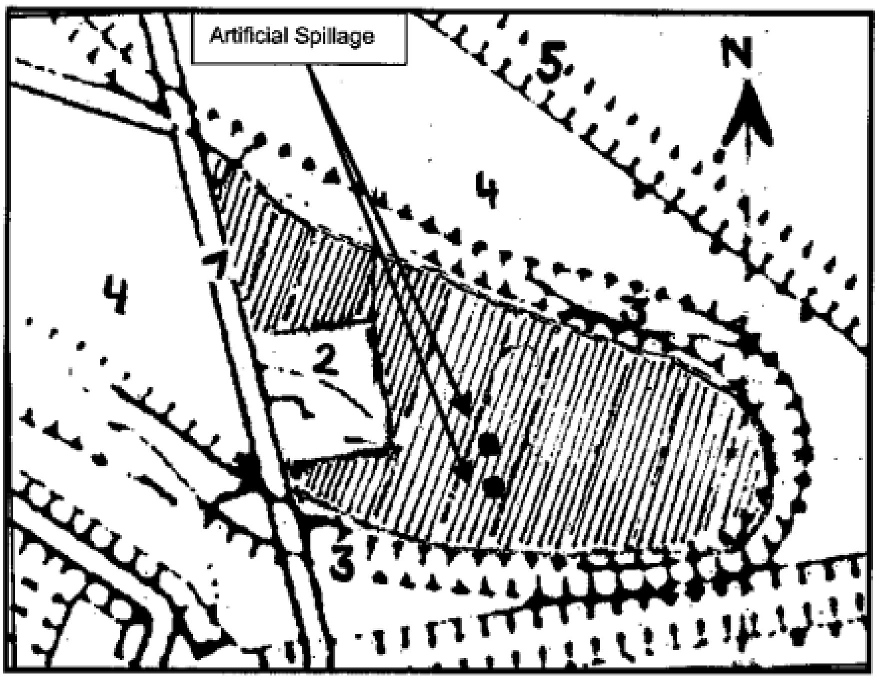
([redacted], 2002)

HSE Comments:

Pelleted sugar beet seeds were applied in five different locations to determine the number of pills left of the surface after drilling. Additionally, the type and activity of birds visiting the drilled areas was observed and noted.

All of the test sites except for Eindewege (field 3) were rectangular in shape and contained a mid-field area of 50 x 50 m and a rectangular end row area ranging from 500 m^2 to 2000 m^2. Eindewege did not contain an end row area. The applicant has stated this is due to the shape of the field not being a rectangle, and therefore no end row area existed as the tractor could drill without turning around.

Figure 7 Test Location Site 3: Eindewege, Detail



1= street, 2= farm,3= hedge of trees with ditch, 4=field (north : partly winter seed, west: not yet sowed), 5= summer dike

Figure 7 above shows the shape of the field in Eindewege. HSE considers the tractor would still have to turn during drilling of this field and therefore an end row should have been included.
Sugar beet pills were drilled into the soil at a rate between 0.8 – 1.1 unit/ha. A unit of seeds corresponds to 100 000 seeds, the proposed drilling rate of 100 000 – 130 000 seeds/ha of the product is comparable to the GAP.

Sugar beet pills of an orange colour were applied in all fields. Sugar beet pills of a blue colour were additionally drilled in Kloetinge (Field 4). While blue pills were only drilled in field 4, artificial spillages were created in field 2 and field 3, using both orange and blue pills. HSE can conclude that this was sufficient to give an indication if the colour of the sugar beet pill contributed to the attractiveness of the pill as a food item to birds.

EFSA, 2009, discusses how the type of soil and constituent composition can affect the extent to which birds may be exposed to test items in grit. Non-granivorous birds take in grit with soil particles whilst granivorous birds will selectively pick up grit particles. The applicant has briefly described the soils as sandy, clay or marsh. HSE considers that more information could have been provided with regards to the composition of the soil. The nature of the soil did affect the drilling depths of the sugar beet pills. The two sandy sites; Goirle and Alphen were drilled to a depth of 1 and 1.2 cm respectively. The other three sites were drilled to a depth between 2 – 2.5 cm. The results showed more pills were left on the surface of Goirle and Alphen, indicating that the shorter drilling depth has an effect on the number of seeds found on the surface.

The fields were observed for bird activity for the whole daylight period after drilling. 90 % of each field was visible for scanning by binocular. HSE considers this to be an acceptable level of observation. The results were calculated into an index value by summarising the data from all observation intervals of day, dividing by the number of 5-minute intervals, and dividing again by the hectare size of the field. HSE agrees with this approach to allow for reliable data comparison between the field sites.
The results indicate that a mean of 0.111 % of the sugar beet pills drilled into the soil were left uncovered in the mid field areas. Higher numbers of sugar beet pills were left uncovered in the end-rows with a mean of 1.527 % of the sugar beet pills drilled into the soil left uncovered.

The artificial spillages were recollected and counted. 4.4 % of the blue sugar beet pills were missing, HSE agrees it is likely that birds or mammals ingested them. Upon recollection there was one additional orange sugar beet pill than drilled at the beginning across the two sites. HSE considers this to be a counting error either during the initial drilling or collection. HSE would suggest these results show that birds and mammals are slightly more attracted to the blue pills than the orange pills. However, as there is no statistical analysis, it cannot be confirmed if this is significant.

Overall HSE considers this study to be acceptable for use in discussion in the risk assessment.

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	IIIA 10.1.7/02 [redacted] (2015), Thiamethoxam: Measured Residues in Sugar Beet Seedlings Emerging from A9765R-Treated Seed, Germany 2015, Report Number S15-01163. Eurofins Agroscience Services Ltd Slade Lane, Wilson, Melbourne, Derbyshire, DE73 8AG, United Kingdom (Syngenta file No. A9765R_10126, VV-414090).

Guidelines
Designed to comply with Regulation (EC) 1107/2009 and Guideline 7029/VI/95.

GLP: Yes

Study Parameters

Parameter	Description
Test materials:	Thiamethoxam (CGA293343) Batch: POR3J05671 Valid until: End of March 2017 Formulation a.s. content: Nominal: 600 g/L; Actual: 618 g/L
Seed loading with	Nominal treatment rate: 0.45 mg a.s./seed

thiamethoxam (CGA293343)	Actual treatment rate: 0.441 mg a.s./seed
Identification of untreated seed	Sugar beet variety: Callas Batch No.: 15904001-03
Identification of treated seed	Sugar beet variety: Callas Batch No.: 15904001-04
Seed appearance	Pelleted seed – blue in colour
Stability of the test item	The test item is assumed to be stable for the period of use in the study.
Reference items	Thiamethoxam: Batch WRS 1239/3; purity: 98.9% CGA322704: Batch BPS 1143/2; purity: 98%
Trial site details	<u>Trial number S15-01163-01 (ZP):</u> Germany; Northrhine-Westphalia; Rhineland, Zuelpich; Drilling date: 22 April 2015 Soil type: Silty-loam (19.3% Clay, 51.0% Silt, 29.7% Sand) Soil pH: 6.5 Pesticide use: none Fertilizer use: none Irrigation: none <u>Trial number S15-01163-04 (ML):</u> Germany; Miehelen; Drilling date: 10 April 2015 Soil type: Very clayey silt (18.4% Clay, 69.5% Silt, 12.1 % Sand) Soil pH: 5.3 Pesticide use: none Fertilizer use: none Irrigation: none <u>Trial number S15-01163-05 (BB):</u> Germany; Rhineland, Bedburg Drilling date: 04 May 2015 Soil type: Medium clayey silt (12.4% Clay, 85.0% Silt, 2.6 % Sand) Soil pH: 6.7 Pesticide use: none Fertilizer use: none Irrigation: none <u>Trial number S15-01163-06 (OH):</u> Germany; Holsteiner Land, Scharbeutz Drilling date: 12 May 2015 Slightly clayey sand (5.7% Clay, 7.4% Silt, 86.9 % Sand) Soil pH: 4.1 Pesticide use: none Fertilizer use: none Irrigation: none
Product application rate	Nominal: 75 mL product/100,000 seeds Actual: 72.8 mL product/100,000 seeds

Drilling rate per meter row	Trial number S15-01163-01 (ZP): Control: 7500 seeds/plot; Approx. 100 rows, row length 10 m Treated: 6500 seeds/plot; Approx. 100 rows, row length 10 m; Trial number S15-01163-04 (ML): Control and treated: 1300 seeds/plot; Approx. 100 rows, row length 10 m; Trial number S15-01163-05 (BB): Control: 5000 seeds/plot; Approx. 100 rows, row length 10 m Treated: 6700 seeds/plot; Approx. 100 rows, row length 10 m; Trial number S15-01163-06 (OH): Control: 1300 seeds/plot; Approx. 100 rows, row length 10 m Treated: 8330 seeds/plot; Approx. 100 rows, row length 10 m;
Drilling equipment	Seeds were drilled by hand, with a hand-driven drilling machine. Plot was covered with netting after drilling in order to prevent wildlife from feeding on the drilled seed and emerging seedlings.
Weather data	Trial number S15-01163-01 (ZP): Control: Air temp: 8.6 °C; Humidity: 73 %; soil temp at 5 cm: 11.1 °C Treated: Air temp: 12.4 °C; Humidity: 68 %; soil temp at 5 cm: 11.8 °C Trial number S15-01163-04 (ML): Control: Air temp: 21.1 °C; Humidity: 40 %; soil temp at 5 cm: 11.5 °C Treated: Air temp: 22.5 °C; Humidity: 42%; soil temp at 5 cm: 12.7 °C Trial number S15-01163-05 (BB): Control: Air temp: 23.0 °C; Humidity: 54 %; soil temp at 5 cm: 14.4 °C; Treated: Air temp: 26.6 °C; Humidity: 38 %; soil temp at 5 cm: 17.7 °C; Trial number S15-01163-06 (OH): Control: Air temp: 14.2 °C; Humidity: 56 %; soil temp at 5 cm: 10.5 °C; Treated: Air temp: 17.1 °C; Humidity: 61 %; soil temp at 5 cm: 19.8 °C;
Sampling details	Control: day 0, 14 and 21 after emergence Treatment: day 0, 1, 2, 3, 5, 7, 10, 14 and 21 after emergence By hand, cutting seedlings just above soil surface with scissors, following a 'W' shape pattern across the plot. Care was taken to avoid getting soil in the sample container.

Description of field methods and environmental monitoring

Four residue field trials on sugar beet were successfully conducted in Germany during 2015. The trials were conducted in four different locations distributed over

Germany: (1) near Zuelpich, Rhineland (ZP), (2) near Bedburg, Rhineland (BB), (both located in Northrhine-Westphalia), (3) near Scharbeutz, Holsteiner Land (OH) and (4) Miehlen (ML) in Palatine.

Two plots of a size of approximately 100 m^2 each were prepared for drilling at each trial site. The control plot (Plot 0) was drilled with untreated sugar beet seeds, and the treated plot (Plot 1) was drilled with sugar beet seeds treated with thiamethoxam (Cruiser 600 FS). The seeds were treated with A9765R, a FS formulation containing 600 g thiamethoxam per litre, prior to this study, at a nominal rate of 0.45 mg a.s./seed. Drilling was conducted by hand (drilling rate of up to 6 times the normal commercial rate of approx. 130,000 seeds/ha) at a depth of 2 to 3 cm, according to Good Agricultural Practice. Each plot was covered with netting to prevent wildlife from feeding on the drilled seeds and emerging seedlings.

Before drilling, a sample of approx. 2000 treated and 2000 untreated seeds were taken directly from the package. These will be stored (≤ −18°C) as reference samples should any analysis be required in the future.

Seedlings emerging from drilled treated seeds were sampled at 0, 1, 2, 3, 5, 7, 10, 14, 21 days after emergence (DAE: BBCH 10), using a systematic ‘W’ shaped sampling pattern within the exposure area. Seedlings emerging from drilled untreated seeds (control plot) were sampled at 0, 14 and 21 DAE.

Sample sizes from the untreated (Plot 0) and treated plot (Plot 1) comprised of a minimum of 40 seedlings with a minimum biomass of 10 g wet weight. The target number and biomass for Plot 1 was reduced to 20 seedlings and 5.0 g in cases of poor emergence. The target sample size for the untreated plot (Plot 0) was increased to 100 seedlings on 0 DAE, in order to ensure sufficient biomass for matrix matched standard preparation, if required.
Air temperature, rainfall and sunshine hours, as well as soil parameters, were recorded at every trial location.

Description of analytical procedures
5 g of crop sample was homogenized with 25 mL of MeOH:H2O (50:50, v/v) for 3-5 minutes and then centrifuged to separate solid particles from the sample extract. An aliquot of the sample extract was removed and mixed with H2O/MeOH (90/10, v/v) + 0.1% acetic acid to yield the sample final fraction. Determination was achieved by LC-MS/MS.

Results and Discussion
The weight of the seedlings was on average 7 times greater 0 days after emergence to 228 times greater on 21 days after emergence. Between 3DAE and 5DAE the average increase reduced from 13.86 to 13.48. This is due to the emerged seedlings being sampled on 3DAE, leaving behind plants which were smaller in size.

Table B.9.1.3-6: Seed and seedling weight in S15-01163-01 (ZP)

Days after emergence	Average single seed weight (g)	Average single seedling weight (g)	Increase in weight

0DAE	0.0306	0.195	6.364
1DAE	0.0306	0.286	9.361
2DAE	0.0306	0.291	9.529
3DAE	0.0306	0.423	13.831
5DAE	0.0306	0.309	10.118
7DAE	0.0306	0.465	15.218
10DAE	0.0306	1.342	43.898
14DAE	0.0306	2.644	86.508
21DAE	0.0306	9.757	319.223

Table B.9.1.3-7: Seed and seedling weight in S15-01163-04 (ML)

Days after emergence	Average single seed weight (g)	Average single seedling weight (g)	Increase in weight
0DAE	0.029	0.197	6.793
1DAE	0.029	0.343	11.841
2DAE	0.029	-	-
3DAE	0.029	0.443	15.298
5DAE	0.029	0.545	18.842
7DAE	0.029	1.033	35.696
10DAE	0.029	-	-
14DAE	0.029	1.511	52.247
21DAE	0.029	4.979	172.126

Table B.9.1.3-8: Seed and seedling weight in S15-01163-05 (BB)

Days after emergence	Average single seed weight (g)	Average single seedling weight (g)	Increase in weight
0DAE	0.029	0.227	7.909
1DAE	0.029	0.377	13.146
2DAE	0.029	0.344	12.011
3DAE	0.029	0.436	15.223
5DAE	0.029	0.372	12.998
7DAE	0.029	0.353	12.317
10DAE	0.029	0.665	23.228
14DAE	0.029	2.153	75.166
21DAE	0.029	7.796	272.194

Table B.9.1.3-9: Seed and seedling weight in S15-01163-06 (OH)

Days after emergence	Average single seed weight (g)	Average single seedling weight (g)	Increase in weight
0DAE	0.029	0.285*	9.887
1DAE	0.029	0.345	11.969
2DAE	0.029	0.290	10.061
3DAE	0.029	0.320	11.101
5DAE	0.029	0.345	11.969
7DAE	0.029	0.330	11.448
10DAE	0.029	0.490	16.999
14DAE	0.029	0.695*	24.111
21DAE	0.029	4.375*	151.778

* HSE has discarded the data from plot 1 as the weight was significantly greater (0DAE weight = 0.945, 14DAE weight = 4.708, 21 DAE weight = 10.92)

Table B.9.1.3-10: Average weight increase across the four test plots from seed to seedling

Days after emergence	Weight increase S15-00163-01 (ZP)	Weight increase S15-00163-04 (ML)	Weight increase S15-00163-05 (BB)	Weight increase S15-00163-06 (OH)	Average weight increase
0DAE	6.364	6.793	7.909	9.887	7.738
1DAE	9.361	11.841	13.146	11.969	11.579
2DAE	9.529	-	12.011	10.061	10.534
3DAE	13.831	15.298	15.223	11.101	13.863
5DAE	10.118	18.842	12.998	11.969	13.482
7DAE	15.218	35.696	12.317	11.448	18.670
10DAE	43.898	-	23.228	16.999	28.042
14DAE	86.508	52.247	75.166	24.111	59.508
21DAE	319.223	172.126	272.194	151.778	228.830

Table B.9.1.3-11: Test Site Weather Data- April, May, June 2015

Location	Average Daily Air Temperature (ºC)	Average Precipitation (mm)	Average Sunshine Hours (hh:mm)
ZP	12.7	0.616	5:20
BB	14.6	1.1	6:1
OH	12.9	1.13	7:1
ML	13.5	1.45	7:1

Table B.9.1.3-12: UK Average Weather Data- April, May, June 1991-2020

Location	Average Daily Air Temperature (ºC)	Average Precipitation (mm)	Average Sunshine Hours (hh:mm)
UK	8.59	73.31	-

Conclusions

The data shows that when the seedlings emerge, the weight is on average a minimum of 7 times larger than the seed weight. Soon after emergence on DAE 0, 1,2 and 3, the seedling weight was 7.738, 11.579, 10.534, and 13.482 times greater than the seed.
The weight increase reduced on 2DAE, as the larger plants had been sampled on 1DAE, leaving smaller samples behind.

([redacted], 2015)

HSE Comments:

The study above has been used to refine the risk assessment for birds and mammals, based on the predicted concentration of the active substance in the seedlings.
As the weight data of the seeds predrilling and the seedlings post emergence is of the most interest to HSE, this has been focused on in the study summary. Four field sites were used, with two plots drilled in each; one control plot with untreated seeds and one plot drilled with treated seeds.
HSE has combined the weight data for both plots in all sites except for Trial number S15-01163-06 (OH). The weight data for the seedlings from plot 0 in this trial were significantly higher than the weight data for the seedlings from plot 1, and HSE considered this would affect the reliability of the mean.
During the course of the study, the weather conditions were reported to be adverse. This resulted in poor emergence on all sites. As a result, a number of the trials were restarted on different plots. S15-01163-04 (ML) could not be restarted, and therefore no samples were taken at 2 DAE, and 10 DAE. HSE considers these adjustments acceptable.
Due to the poor emergence of the seedlings, the weight may be smaller than emerged seedlings from another year. This would produce a more conservative increase in weight from seed to seedling.
HSE has looked at average weather data for the UK to compare with the weather from the study. The average daily temperature range for the UK is lower than the temperature during the test study. Additionally, there was a higher level of rainfall during the three months. It was not possible to compare the sunlight hours. The lower temperature in the UK could potentially have an effect on the weight of the seedlings, producing smaller seedlings, whereas the increased rainfall in the UK could potentially have a positive effect on the size of seedling emerged. However, as the data for the UK is a 30-year average, and weather conditions do change year to year, HSE does not consider the difference in weather to have had an effect on the overall results.

The following study was presented for the renewal of metalaxyl-M. A revised summary is presented below.

Report:	**KIIIA 10.1.8/02 [redacted] (2004) Untreated seeds: seed selection by the house sparrow *(Passer domesticus*). [redacted] Report Number [redacted].** **Syngenta File Number MK936/1043**

Guidelines

No guidelines. Protocol developed specifically to address the objectives.

GLP: No. Conducted at a GLP compliant laboratory

Study Design and Methods

Experimental dates: 05 January to 18 February 2004

Objectives: To identify the attractiveness of different untreated seeds to the house sparrow (*Passer domesticus*) and to determine if they were dehusked.

Study area: Outdoor aviaries at [redacted].

Method and parameters: Wild caught house sparrows (*Passer domesticus*) were acclimatised for at least 14 days to outdoor aviaries and ground feeding conditions in groups (3 birds) to induce feeding conditions which might be expected in the field. This comprised first allowing *ad lib* feeding on a proprietary finch seed mixture together with untreated test seeds, this was followed by deprivation of food for a short period before exposure to only a single untreated test seed type.

Seeds types evaluated were:

sugar beet (*Beta vulgaris*),
tomato (*Lycopersicum esculentum*),
pepper (*Capsicum annuum*),
cucumber (*Cucumis sativa*),
melon (*Cucumis melo*),
carrot (*Daucus carota*),
lettuce (*Lactuca sativa*),
cotton (G*ossypium hirsutum*),
maize (*Zea mays*),
soya (*Glycine max*),
pea (*Pisum sativa*),
oilseed rape (*Brassica napus*) and
wheat (*Triticum aestivum*).

Nine house sparrows were used in this experiment (3 groups of 3 birds) in a design that varied the order in which test birds were presented with seed, to prevent order effects from confounding the results. Birds were exposed to untreated seed treatments for 2 hours after a fasting overnight, noting that birds would not normally feed overnight, and the weight of seed removed/eaten estimated by weight. Background accuracy of the study was measured in controls by collecting and weighing seed without birds. Dehusking was described but not measured quantitatively.

Data analysis: Results were subjected to AVOVA testing and Duncan's multiple testing procedure was used to separate seed types with statistically different levels of consumption.

Results and discussion

There were 3 negative values, two for tomato and one for soya seeds, the study author considered that this may have been due to initial moisture content differences between the control and treatment batches. All analyses were performed for the original data as well as with the negative values replaced with 0. Based on a mean loss of finch seed weight of 0.40 g (dry wt) under the similar environmental conditions in the pilot study it is considered that a mean intake of small seeds (carrot, lettuce, pepper, tomato) of 0.40 g or less (dry wt.) can be considered insignificant.

The level of determination in this study was 0.4 g based on the control seed weight changes. As expected, intake of the proprietary seed mix was highest, followed by wheat, cucumber, pea, melon, oilseed rape, pepper and tomato. Only wheat and cucumber consumptions exceeded 1g. There were wide variations in the acceptability of different seed types and within groups of birds feeding on the same seed type.

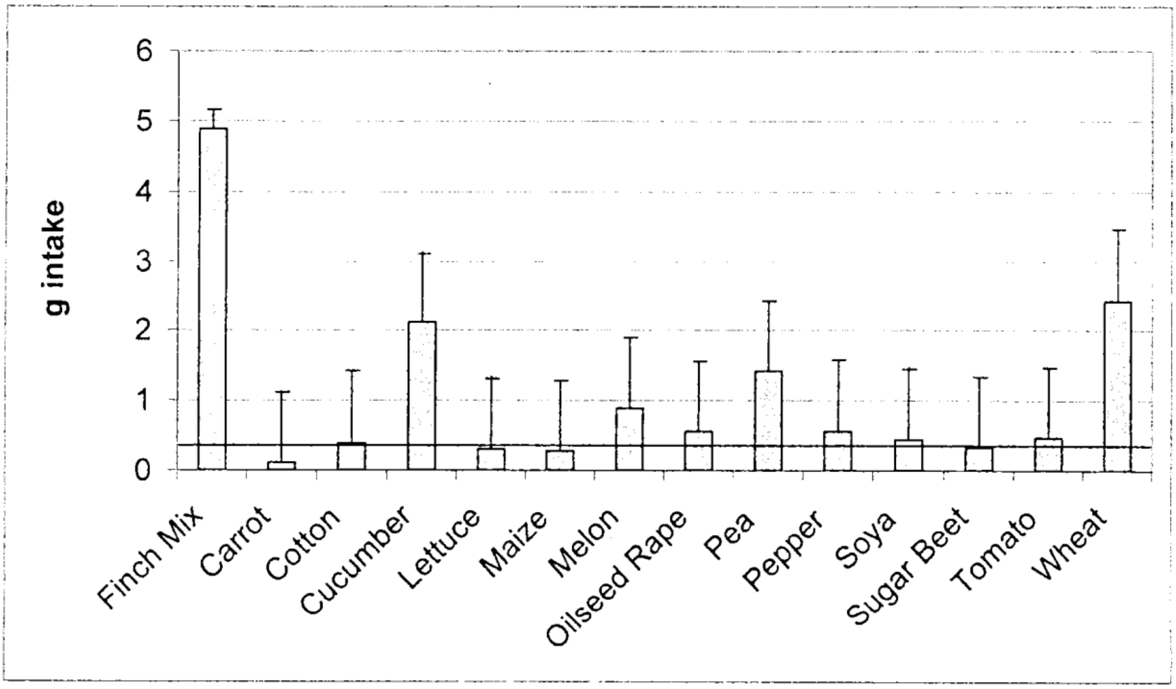
Note in the following table the units are g seed consumed per bird.

Simultaneous confidence intervals (studentized maximum modulus procedure)

	Original data			Negative values replaced with 0		
		Confidence interval			Confidence interval	
Seed	mean	lower limit	upper limit	mean	lower limit	upper limit
Finch Mix[1]	4.89	4.61	5.17	4.89	4.61	5.17
Carrot	0.11	-0.90	1.13	0.11	-0.89	1.12
Cotton	0.40	-0.61	1.41	0.40	-0.61	1.41
Cucumber	2.11	1.09	3.12	2.11	1.10	3.11
Lettuce	0.32	-0.70	1.33	0.32	-0.69	1.32
Maize	0.28	-0.73	1.29	0.28	-0.73	1.29
Melon	0.89	-0.12	1.91	0.89	-0.11	1.90
Oilseed Rape	0.55	-0.47	1.56	0.55	-0.46	1.55
Pea	1.42	0.40	2.43	1.42	0.41	2.42
Pepper	0.57	-0.44	1.59	0.57	-0.43	1.58
Soya	*0.44*	*-0.57*	*1.45*	*0.46*	*-0.55*	*1.46*
Sugar Beet	0.33	-0.68	1.34	0.33	-0.68	1.34
Tomato	*0.37*	*-0.64*	*1.39*	*0.47*	*-0.54*	*1.48*
Wheat	2.44	1.43	3.45	2.44	1.43	3.45

[1]Mean data across all pens and all pre-trial (2hr consumption) data

Figure B.9.1.3-1: Mean consumption of seed types (model derived simultaneous confidence limits are shown by bards and line shows limit of detection for seed consumption of 0.40 g.)



As regards dehusking, the following results were presented in the report:

Seed type	No. aviaries where dehusking/residue observed	Comments	Approx number seeds consumed/pen[1]
Carrot	1	Approx 5 seeds	0[a]
Cotton	2	1 seed per aviary	1-9
Cucumber	3	4-20 seeds	26-92
Lettuce	3	4-30 seeds	0[a]
Maize	1	1 seed cracked	1-3
Melon	3	2-4 seeds	23-50
Oilseed rape	3	2-10 seeds	0[a] –198 seeds
Pea	1	1 seed	2-11
Pepper	2	2-20 seeds	0-123
Soya	2	2 seeds	0-10
Sugar beet	2	5-15 seeds	8-21
Tomato	2	2-12 seeds	0-552
Wheat	2	3-4 seeds	54-76

[1] based on minimum change in weight of 0.40g for small seeds
[a] evidence of dehusking/residue but less than 0.40g consumed

Under the conditions of the study house sparrow (*Passer domesticus*) can be expected to consume amounts of wheat (small grain cereal), peas and cucumber seed if available on the soil surface. All other seeds tested, sugar beet, oilseed rape, pepper, melon, carrot, lettuce, cotton, maize, soya and tomato were consumed to a lesser extent.

([redacted], 2004)

HSE Conclusions:

The study was considered as part of the EU review and the following is presented in EFSA (2015):

> ***Untreated seeds: Seed selection by the house sparrow (*****Passer domesticus*****)***
>
> *Under the conditions of the study house sparrow can be expected to consume significant amounts of wheat (small grain cereal), peas and cucumber seed if available on the soil surface. All other seeds tested, sugar beet, oilseed rape, pepper, melon, carrot, lettuce, cotton, maize, soya and tomato can be considered unattractive or eaten in negligible amounts. These results were consistent with Prosser (1999).*

While this study has not been used by the applicant in their latest risk assessment, it is included here since it contains information on the consumption and dehusking of pea and sugar beet seeds.

The following study was presented for the renewal of metalaxyl-M. A revised summary is presented below.

Report:	**KIIIA 10.1.8/03 [redacted] (2004a) Untreated seeds: seed selection by the grey partridge (*Perdix perdix*). [redacted], Report Number [redacted].** **Syngenta File Number MK936/1044**

Guidelines

No guidelines. Protocol developed specifically to address the objectives.

GLP: No. Conducted at a GLP compliant laboratory.

Study Design and Methods

Experimental dates: 05 January to 18 February 2004

Objectives: To identify the attractiveness of different untreated seeds to the grey partridge (*Perdix perdix*) and to determine if they were dehusked.

Study area: Outdoor aviaries at [redacted]

Method and parameters: Grey partridge were acclimatised to outdoor aviaries and ground feeding conditions in groups (3 birds) to induce feeding conditions which might be expected in the field. This comprised first allowing *ad lib* feeding on a proprietary seed mixture together with untreated test seeds. Food was removed at dusk before the trial day, hence there was overnight deprivation however birds would not normally feed during the night.

Seeds types evaluated were:

sugar beet (*Beta vulgaris*),
tomato (*Lycopersicum esculentum*),
pepper (*Capsicum annuum*),
cucumber (*Cucumis sativa*),
melon (*Cucumis melo*),
carrot (*Daucus carota*),
lettuce (*Lactuca sativa*),
cotton (*Gossypium hirsutum*),
maize (*Zea mays*),
soya (*Glycine max*),
pea (*Pisum sativa*),
oilseed rape (*Brassica napus*) and
wheat (*Triticum aestivum*).

Nine grey partridge were used in this experiment (3 groups of 3 birds) in a design that varied the order in which test birds were presented with seed, to prevent order effects from confounding the results. Birds were exposed to untreated seed treatments for 2 hours after a fasting overnight and the weight of seed removed/eating estimated by weight. Background accuracy of the study was measured in controls by collecting and weighing seed without birds. Dehusking was described but not measured quantitatively.

Data analysis: Results were subjected to AVOVA testing and Duncan's multiple testing procedure was used to separate seed types with statistically different levels of consumption.

Results and discussion

The level of determination in this study was 2.9 g based on the control seed weight changes.

There were four negative consumption values, 3 for pea and 1 for soya which may have been due to initial moisture content differences between the control and treatment batches.

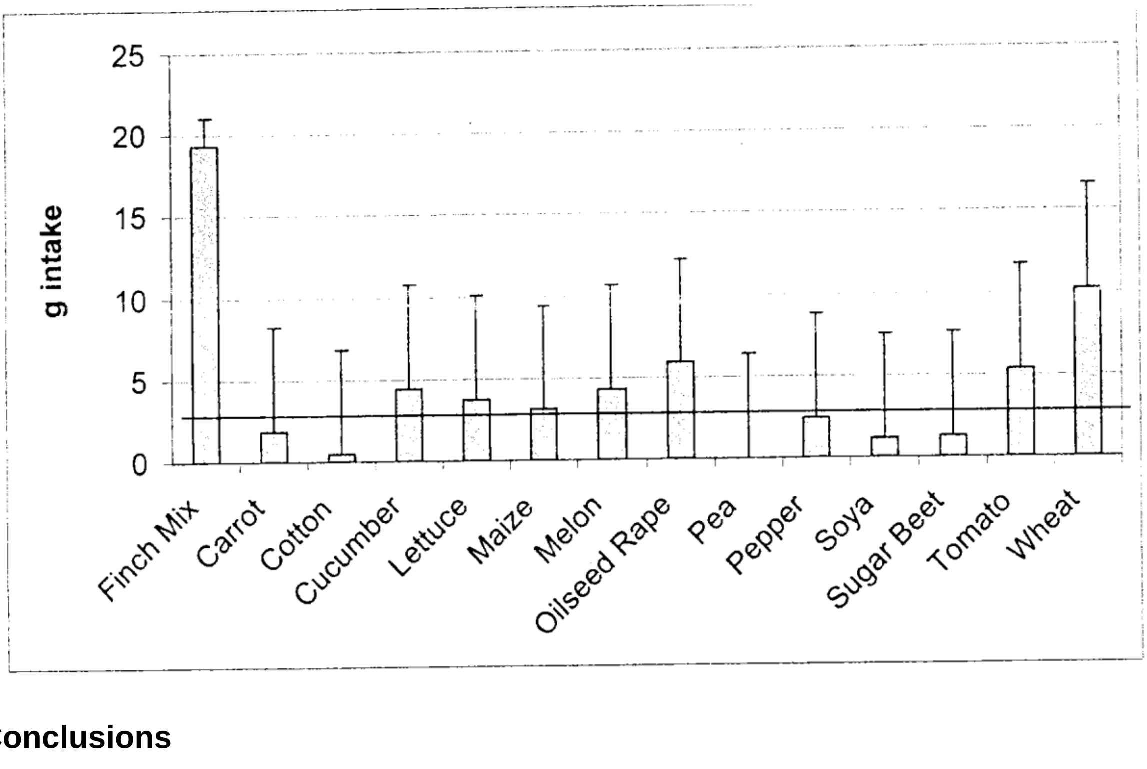
As expected, intake of the proprietary seed mix was highest, followed by wheat, oilseed rape, tomato, cucumber, melon, lettuce and maize. There was negligible measurable consumption or dehusking of the other seeds. Only wheat, oilseed rape and tomato consumption exceeded 5 g. There were wide variations in the acceptability of different seed types and within groups of birds feeding on the same seed type.

The units are g seed consumed per bird.

	Original data			Negative values replaced with 0		
		Confidence interval			Confidence interval	
Seed	mean	lower limit	upper limit	mean	lower limit	upper limit
Finch Mix[1]	19.29	17.53	21.05	19.29	17.53	21.04
Carrot	1.86	-4.48	8.21	1.86	-4.47	8.20
Cotton	0.49	-5.86	6.83	0.49	-5.84	6.82
Cucumber	4.44	-1.90	10.79	4.44	-1.89	10.78
Lettuce	3.72	-2.62	10.07	3.72	-2.61	10.05
Maize	3.07	-3.28	9.41	3.07	-3.27	9.40
Melon	4.28	-2.07	10.62	4.28	-2.06	10.61
Oilseed Rape	5.87	-0.47	12.22	5.87	-0.46	12.20
Pea	*-0.13*	*-6.48*	*6.22*	*0.00*	*-6.33*	*6.33*
Pepper	2.47	-3.88	8.82	2.47	-3.86	8.80
Soya	*0.89*	*-5.46*	*7.23*	*1.17*	*-5.17*	*7.50*
Sugar Beet	1.32	-5.03	7.66	1.32	-5.01	7.65
Tomato	5.36	-0.98	11.71	5.36	-0.97	11.69
Wheat	10.20	3.86	16.55	10.20	3.87	16.53

[1] Mean across all pens and all pre-trial (2hr) data

Figure B.9.1.3-2: Mean consumption of seed types (model derived simultaneous confidence limits are shown by bards and line shows limit of detection for seed consumption (2.9 g).



Conclusions

Under the conditions of the study grey partridge can be expected to consume significant amounts of wheat (small grain cereal), oilseed rape and tomato seed if available on the soil surface. All other seeds tested, sugar beet, pepper, cucumber, melon, carrot, lettuce, cotton, maize, soya and pea were not consumed to the same extent.

([redacted], 2004a)

HSE Conclusions:

The study was considered by the EU review and the following is presented in EFSA (2015):

> ***Untreated seeds: Seed selection by the grey partridge (Perdix perdix)***
>
> *Under the conditions of the study grey partridge can be expected to consume significant amounts of wheat (small grain cereal), oilseed rape and tomato seed if available on the soil surface. All other seeds tested, sugar beet, pepper, cucumber, melon, carrot, lettuce, cotton, maize, soya and pea can be considered unattractive or eaten in negligible amounts. With the exception of peas, these results were consistent with Prosser (1999).*

While this study has not been used by the applicant in their latest risk assessment, it is included here since it contains information on the consumption and dehusking of pea and sugar beet seeds.

The following study summary is reproduced verbatim from Section B.9 of the EU draft Renewal Assessment Report for metalaxyl-M

Report:	**IIIA 10.1.2/03. [redacted]. (2012). Fludioxonil, Thiabendazole, Azoxystrobin and Metalaxyl-M - Dissipation of residues on treated maize seed and shoots.**

Syngenta file: 14918E_10216
Guidelines: No guideline applicable.
GLP: Yes

Previous evaluation: No, submitted for the purpose of renewal of a.s. approval

Executive summary:

Residue dissipation of fludioxonil, thiabendazole, azoxystrobin and metalaxyl-M was measured on treated maize seeds placed on the soil surface to refine exposure estimates for birds and mammals that might consume seeds. In addition, the shoots of seedlings emerging from drilled seed were analysed for thiabendazole and metalaxyl-M because both substances are systemic and may be eaten by bird and mammals.

Residues studies were replicated at 3 sites in the United Kingdom during 2011.

Samples of maize seed from the surface (protected and unprotected) were collected at 0, 1, 2, 4, 7, 10, 14 and 21 days. Protected seeds were sheltered from rain by a polythene film that was later shown to filter out most UV light (300-400nm).

Maize seed was drilled and allowed to emerge before sampling of the above ground plant shoots. Shoots were sampled at 7 time points to be representative of growth stages from emergence up to BBCH 15.

There was significant dissipation of fludioxonil, thiabendazole, azoxystrobin and metalaxyl-M on maize seeds placed on the soil surface.

There was initial uptake followed by dissipation of thiabendazole and metalaxyl-M in shoots of maize seedlings.

Dissipation was different for protected and unprotected seeds. Seed protection resulted from both rainfall and UV light (300-400nm).

Material:

Test material: Maxim Quattro treated maize seed
Content of active substance: 3.32% fludioxonil, 26.50% thiabendazole, 1.33% azoxystrobin, 2.65% metalaxyl-M
Formulation type: treated maize seed

Study design and Methods:

Seeds on soil surface: On each plot 20 batches of approximately 100 seeds were applied directly to the soil surface 1 seed deep to represent unincorporated or spilled seed during sowing (i.e. seeds were not piled on top of each other). The seed was protected from foraging birds and mammals by placing a metal mesh cage over the exposed seed.

Ten batches were left uncovered and open to environmental conditions, e.g. rainfall, sunlight. The other ten batches were covered with a water impermeable membrane.

A single sample was taken from the seed batch used to supply the seed on the soil surface (day 0). At both the Fera and STC trial sites the seed, which was placed on the surface, was taken directly from the opened bag of seed. At the Buttercrambe site, following the drilling of the seed, the remaining seed was collected from the drilling equipment and then used for the seed on the surface phase. Samples were collected from seed placed on the soil surface on days 1, 2, 4, 7, 10, 14 and 21 days after drilling.
Samples were taken by using forceps to collect samples of seeds separately from the covered and uncovered batches. Ten seeds were sampled from each of the 10 uncovered batches on the soil surface and combined into a single sample and ten seeds were sampled from each of the 10 covered batches on the soil surface and combined into a single sample.

Drilled seeds for shoots: The treated seeds were drilled at standard commercial drilling rates (10-30 cm between plants, 20 cm between rows) and depths into each plot (no GLP compliance was claimed for this aspect). The area planted (approx. 100-200 m^2) was sufficient to generate samples of shoots over time for analysis. The drilling was made with calibrated equipment.

Samples were taken at 7 time points to represent the time from planting to growth stage BBCH 15 (5 leaves unfolded) or day 21 after drilling whichever was later.

Samples were taken from representative areas within the plot by dividing the plot into 10 approximately equally sized subplots and taking a sample of at least 10 shoots from each subplot. Shoots were cut at the soil surface, weighed, sealed in a bag and stored deep frozen prior to analysis at -15 to -25^{o}C (the study plan stated the shoots would be dried with silica gel overnight but this proved unworkable and has no impact on the study).

All samples were stored directly in the dark in a freezer at -15 to -25^{o}C or within 1hr in a portable freezer at a maximum of -10^o C and were then transferred to a freezer at -15 to -25^{o}C within 12 hrs where they were stored prior to analysis.

Residue analysis and validation: The samples of maize shoots were prepared by homogenising using ‘mini-blender’ in the presence of ice. For the samples of maize seed no homogenisation was required.
The method V7YG/02 was successfully validated for analysis of azoxystrobin, fludioxonil, metalaxyl-M and thiabendazole in maize seed and shoots.

Results:

Table B.9.1.3-13: Data from the Fera Site – Protected Maize Seeds

Sample No.	Sampling Interval (days)	Fludioxonil		Thiabendazole		Azoxystrobin		Metalaxyl-M	
		mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed
D0 Drilled Seed	0DAD[a]	19.70	6.0	113.3	34.3	7.45	2.3	14.96	4.5
Plot P1/Fera Seed D1/S/P/F	1DAD	14.94	4.5	115.6	35.0	7.39	2.2	11.04	3.3
Plot P1/Fera Seed D2/S/P/F	2DAD	10.50	3.2	105.6	32.0	6.45	2.0	10.61	3.2
Plot P1/Fera Seed D4/S/P/F	4DAD	9.29	2.8	98.2	29.8	5.71	1.7	9.03	2.7
Plot P1/Fera Seed D7/S/P/F	7DAD	8.68	2.6	94.0	28.5	5.77	1.7	8.38	2.5

Plot P1/Fera Seed D10/S/P/F	10DAD	7.34	2.2	86.5	26.2	5.30	1.6	8.36	2.5
Plot P1/Fera Seed D14/S/P/F	14DAD	7.40	2.2	92.2	27.9	5.43	1.6	7.77	2.4
Plot P1/Fera Seed D21/S/P/F	21DAD	4.53	1.4	77.4	23.5	4.43	1.3	5.33	1.6

[a] taken from opened bag on day of drilling. DAD = days after drilling

Table B.9.1.3-14: Data from the Fera Site – Unprotected Maize Seeds

Sample No.	Sampling Interval (days)	Fludioxonil		Thiabendazole		Azoxystrobin		Metalaxyl-M	
		mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed
D0 Drilled Seed	0DAD[a]	19.25	5.8	171.8	52.1	6.70	2.0	14.27	4.3
Plot P1/Fera Seed D1/S/F	1DAD	13.32	4.0	142.2	43.1	6.44	2.0	10.33	3.1
Plot P1/Fera Seed D2/S/F	2DAD	11.93	3.6	130.3	39.5	5.91	1.8	9.18	2.8
Plot P1/Fera Seed D4/S/F	4DAD	8.24	2.5	104.0	31.5	5.00	1.5	6.48	2.0
Plot P1/Fera Seed D7/S/F	7DAD	6.24	1.9	82.8	25.1	4.46	1.4	3.86	1.2
Plot P1/Fera Seed D10/S/F	10DAD	5.05	1.5	66.7	20.2	3.35	1.0	2.23	0.7
Plot P1/Fera Seed D14/S/F	14DAD	3.61	1.1	50.3	15.3	2.07	0.6	0.87	0.3
Plot P1/Fera Seed D21/S/F	21DAD	2.20	0.7	37.8	11.5	1.57	0.5	0.69	0.2

[a] taken from opened bag on day of drilling. DAD = days after drilling

Table B.9.1.3-15: Data from the Fera Site – Maize Shoots

Sample No.	Sampling Point/ Growth Stage	Thiabendazole (mg/kg)	Metalaxyl-M (mg/kg)
P3/Fera BBCH 10/F	BBCH 10	1.20*	0.06
P3/Fera BBCH 11-12/F	BBCH 11-12	2.93*	0.13
P3/Fera BBCH 12-13/F	BBCH 12-13	2.52*	0.07
P3/Fera BBCH 13/F	BBCH 13	2.51*	0.03

P3/Fera BBCH 13-14/F	BBCH 13-14	1.69*	0.02
P3/Fera BBCH 14/F	BBCH 14	0.63*	< 0.01
P3/Fera BBCH 14-15/F	BBCH 14-15	0.08	< 0.01
P3/Fera BBCH 15/F	BBCH 15	0.07	< 0.01

*Original analysis demonstrated residues were outside of the calibration range, therefore a dilution of the extracts was performed and the extracts re-analysed. This data is calculated using results from the analysis of the diluted extracts.

Table B.9.1.3-16: Data from the STC Site – Protected Maize Seeds

Sample No.	Sampling Interval (days)	Fludioxonil		Thiabendazole		Azoxystrobin		Metalaxyl-M	
		mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed
D0 Drilled Seed	0DAD[a]	21.57	6.5	150.0	45.5	7.84	2.4	15.32	4.6
Plot P1/STC Seed D1/S/P/Sc	1DAD	13.02	3.9	128.5	38.9	6.13	1.9	12.63	3.8
Plot P1/STC Seed D2/S/P/Sc	2DAD	11.05	3.4	121.3	36.7	6.06	1.8	12.48	3.8
Plot P1/STC Seed D4/S/P/Sc	4DAD	9.14	2.8	117.7	35.7	5.79	1.8	9.88	3.0
Plot P1/STC Seed D7/S/P/Sc	7DAD	8.57	2.6	114.1	34.6	5.58	1.7	8.86	2.7
Plot P1/STC Seed D10/S/P/Sc	10DAD	8.75	2.7	109.7	33.3	5.60	1.7	9.17	2.8
Plot P1/STC Seed D14/S/P/Sc	14DAD	5.56	1.7	92.5	28.0	4.76	1.4	7.91	2.4
Plot P1/STC Seed D21/S/P/Sc	21DAD	4.70	1.4	89.8	27.2	4.46	1.4	6.93	2.1

[a] taken from opened bag on day of drilling. DAD = days after drilling

Table B.9.1.3-17: Data from the STC Site – Unprotected Maize Seeds

Sample No.	Sampling Interval (days)	Fludioxonil		Thiabendazole		Azoxystrobin		Metalaxyl-M	
		mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed
D0 Drilled Seed	0DAD[a]	17.48	5.3	133.3	40.4	6.92	2.1	12.47	3.8

Plot P1/STC Seed D1/S/Sc	1DAD	10.74	3.3	84.4	25.6	5.17	1.6	6.50	2.0
Plot P1/STC Seed D2/S/Sc	2DAD	9.32	2.8	80.5	24.4	5.07	1.5	5.16	1.6
Plot P1/STC Seed D4/S/Sc	4DAD	7.34	2.2	55.2	16.7	3.60	1.1	2.52	0.8
Plot P1/STC Seed D7/S/Sc	7DAD	5.06	1.5	32.0	9.7	2.10	0.6	1.17	0.4
Plot P1/STC Seed D10/S/Sc	10DAD	3.99	1.2	27.9	8.5	1.84	0.6	0.83	0.3
Plot P1/STC Seed D14/S/Sc	14DAD	3.59	1.1	28.2	8.5	1.60	0.5	0.49	0.2
Plot P1/STC Seed D21/S/Sc	21DAD	3.20	1.0	18.1	5.5	1.15	0.3	0.51	0.2

[a] taken from opened bag on day of drilling. DAD = days after drilling

Table B.9.1.3-18: Data from the STC Site – Maize Shoots

Sample No.	Sampling point	Thiabendazole mg/kg	Metalaxyl-M mg/kg
P3/STC/BBCH 10/Sc	BBCH 10	0.85*	0.05
P3/STC/BBCH 11-12/Sc	BBCH 11-12	2.93*	0.09
P3/STC/BBCH 12-13/Sc	BBCH 12-13	2.49*	0.04
P3/STC/BBCH 13/Sc 6/6	BBCH 13	0.79*	< 0.01
P3/STC/BBCH 13/Sc 7/6	BBCH 13	0.83*	< 0.01
P3/STC/BBCH 13-14/Sc	BBCH 13-14	0.51*	< 0.01
P3/STC/BBCH 14/Sc	BBCH 14	0.37*	< 0.01
P3/STC/BBCH 15/Sc	BBCH 15	0.05	< 0.01

*Original analysis demonstrated residues were outside of the calibration range, therefore a dilution of the extracts was performed and the extracts re-analysed. This data is calculated using results from the analysis of the diluted extracts.

Table B.9.1.3-19: Data from the Buttercrambe Site – Protected Maize Seeds

Sample No.	Sampling Interval (days)	Fludioxonil		Thiabendazole		Azoxystrobin		Metalaxyl-M	
		mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed
D0 Drilled Seed	0DAD[a]	16.77	5.1	161.8	49.0	8.13	2.5	13.17	4.0
Plot P1/Melb Seed D1/S/P/M	1DAD	10.70	3.2	106.8	32.4	6.18	1.9	8.44	2.6
Plot P1/Melb Seed D2/S/P/M	2DAD	9.68	2.9	102.4	31.1	6.15	1.9	8.80	2.7
Plot P1/Melb Seed D4/S/P/M	4DAD	8.43	2.6	94.1	28.5	5.82	1.8	7.54	2.3
Plot P1/Melb Seed D7/S/P/M	7DAD	6.78	2.1	94.9	28.8	5.63	1.7	6.41	1.9
Plot P1/Melb Seed D10/S/P/M	10DAD	6.07	1.8	92.0	27.9	5.15	1.6	6.16	1.9
Plot P1/Melb Seed D14/S/P/M	14DAD	5.63	1.7	84.4	25.6	4.78	1.4	5.84	1.8
Plot P1/Melb Seed D21/S/P/M	21DAD	4.46	1.4	75.1	22.8	3.91	1.2	5.35	1.6

[a] taken from opened bag on day of drilling. DAD = days after drilling

Table B.9.1.3-20: Data from the Buttercrambe Site – Unprotected Maize Seeds

Sample No.	Sampling Interval (days)	Fludioxonil		Thiabendazole		Azoxystrobin		Metalaxyl-M	
		mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed	mg/kg	µg/seed
D0 Drilled Seed	0DAD[a]	19.21	5.8	181.9	55.1	8.68	2.6	17.15	5.2
Plot P1/Melb Seed D1/S/M	1DAD	9.59	2.9	100.5	30.4	4.54	1.4	3.78	1.1
Plot P1/Melb Seed D2/S/M	2DAD	8.01	2.4	81.2	24.6	4.51	1.4	3.64	1.1

Plot P1/Melb Seed D4/S/M	4DAD	6.80	2.1	76.0	23.0	3.12	0.9	1.83	0.6
Plot P1/Melb Seed D7/S/M	7DAD	5.64	1.7	58.4	17.7	2.54	0.8	1.01	0.3
Plot P1/Melb Seed D10/S/M	10DAD	5.32	1.6	55.9	16.9	2.40	0.7	0.90	0.3
Plot P1/Melb Seed D14/S/M	14DAD	6.20	1.9	67.8	20.5	2.77	0.8	1.37	0.4
Plot P1/Melb Seed D21/S/M	21DAD	3.85	1.2	34.0	10.3	1.41	0.4	0.72	0.2

[a] taken from opened bag on day of drilling. DAD = days after drilling

Table B.9.1.3-21: Data from the Buttercrambe Site – Maize Shoots

Sample No.	Sampling point	Thiabendazole mg/kg	Metalaxyl-M mg/kg
P3/Melb/BBCH 10/M	BBCH 10	1.09*	0.07
P3/Melb/BBCH 11/M	BBCH 11	0.65*	0.06
P3/Melb/BBCH 12/M	BBCH 12	1.01*	0.03
P3/Melb/BBCH 12-13/M	BBCH 12-13	1.36*	0.02
P3/Melb/BBCH 13/M	BBCH 13	0.66*	< 0.01
P3/Melb/BBCH 14/M	BBCH 14	0.08	< 0.01
P3/Melb/BBCH 15/M	BBCH 15	0.02	< 0.01

*Original analysis demonstrated residues were outside of the calibration range, therefore a dilution of the extracts was performed and the extracts re-analysed. This data is calculated using results from the analysis of the diluted extracts.

Table B.9.1.3-22: Summary of residues on maize seeds placed on the soil surface

Sampling time	Metalaxyl-M (mg/kg)	Thiabendazole (mg/kg)	Fludioxonil (mg/kg)	Azoxystrobin (mg/kg)
Nominal Seed Concentration[a]	16.5	165	21.5	8.3
Protected Seed 0DAD[b]	13.17 – 15.32	113.3 – 161.8	16.77 – 21.57	7.45 – 8.13
Protected Seed 1DAD	8.44 – 12.63	106.8 – 128.5	10.70 – 14.94	6.13 – 7.39
Protected Seed 21DAD	5.33 – 6.93	75.1 – 89.8	4.46 – 4.70	3.91 – 4.46
Seed unprotected 0DAD	12.47 – 17.15	133.3 – 181.9	17.48 – 19.25	6.70 – 8.68
Seed unprotected 1DAD	3.78 – 10.33	84.4 – 142.2	9.59 – 13.32	4.54 – 6.44
Seed unprotected 21DAD	0.51 – 0.72	18.1 – 37.8	2.20 – 3.85	1.15 – 1.57

[a] nominal seed concentration refers to the calculated nominal concentration present on seeds taken directly from an opened bag. This concentration is comparable to the higher validation level (appropriate to seeds) performed for all analytes.
[b] taken from opened bag on day of drilling. DAD = days after drilling

Table B.9.1.3-23: Summary of residues in seedling shoots after planting

Sampling time	Metalaxyl-M	Thiabendazole
BBCH 10	0.05 – 0.07	0.85 – 1.20
BBCH 15	< LOQ[a]	0.02 – 0.07

[a] LOQ = 0.01 mg/kg

Conclusions:

The study is considered acceptable.

There was significant dissipation of fludioxonil, thiabendazole, azoxystrobin and metalaxyl-M on maize seeds placed on the soil surface.

There was initial uptake followed by dissipation of thiabendazole and metalaxyl-M in shoots of maize seedlings.
Dissipation was different for protected and unprotected seeds. Seed protection resulted from both rainfall and UV light (300-400nm).

([redacted]., 2012)

<u>HSE comments</u>

This study was previously evaluated in the EU renewal review for metalaxyl-M. It is considered further by HSE in the higher tier reproductive risk assessments for birds and mammals (sections 9.1.5 & 9.1.6).

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	IIIA 10.1.7/01 [redacted] (2021a). Metalaxyl-M - Dissipation of Residues on A9642H - Treated Spinach Seed Scattered on the Soil Surface in Poland, Germany and Northern France in 2020. Report Number CEMR-9570. CEM Analytical Services, Oaklands Park, Wokingham, Berkshire RG41 2FD United Kingdom. Task Number: TK0538580 (VV-894877).

Guidelines

The field phase was designed under consideration of recommendations in the current guidance document on risk assessment for birds and mammals (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).

Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document).
OECD Guidance Document on Crop Field Trials, Series on Pesticides No. 66 and Series on Testing and Assessment No. 164, ENV/JM/MONO(2011)50.

OECD Guidance Document on Overview of Residue Chemistry Studies (as revised 2009), Series on Testing and Assessment (No. 64) and Series on Pesticides (No. 32), ENV/JM/MONO(2009)31.

Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.

OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009.
European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000).

OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).

GLP: Yes

Study Parameters

Parameter	Description
Test materials:	Metalaxyl-M Formulation code: A9642H Batch: SMU7LP001 Valid until: 31-Jan-2022 Formulation a.i. content: Metalaxyl-M – nominal: 350 g/L; actual: 355 g/L
Seed loading:	Metalaxyl-M – nominal: 67.8 g a.s./100kg seed
Identification of untreated seed:	Spinach variety: El Lucio batch 15071335
Identification of treated seed:	Spinach variety: El Lucio batch 15071335
Stability of the test item:	The test item is assumed to be stable for the period of use in the study.
Growing system:	Seeds were scattered on soil, each individual square metre was protected by netting. There was no control plot, control seeds were sampled from untreated seeds supplied by the sponsor.
Seed Scattering method:	Seeds scattered by hand in single layer on soil surface, seed rate at least 300 kg seed/ha.
Trial site details:	<u>Trial number SRPL20-046-037FR:</u> Poland; Town/Village: Nowa Wies; State/Province: Mazowieckie; County: Warszawski Scattering date: 15 Sept 2020 Soil type: sandy clay Soil pH: 5.9 Pesticide use: none Fertilizer use: none Irrigation: none Weather:

	At scattering	Between scattering and last sampling	Long term monthly weather (compared to

			average)
Air temperature (ºC)	30.5	7.8 - 24.2	Normal
Humidity (%)	51.1	-	-
Wind speed (m/s)	3	-	-
Precipitation (mm)	none	59.2	Above

Trial number SRPL20-047-037FR:
Poland; Town/Village: Murczyn; State/Province: Kujawsko-pomorskie; County: Bydgosko-torunski
Scattering date: 16 Sept 2020
Soil type: loamy sand
Soil pH: 6.4
Pesticide use: none
Fertilizer use: none
Irrigation: none

Weather:

	At scattering	Between scattering and last sampling	Long term monthly weather (compared to average)
Air temperature (ºC)	25.2	14.6	Normal
Humidity (%)	98.2	-	-
Wind speed (m/s)	2	-	-
Precipitation (mm)	none	71.6	Above

Trial number SRDE20-144-037FR:
Germany; Town/Village: Untergruppenbach; State/Province: Baden-Wurttemberg; County: Heilbronn, Landkreis
Scattering date: 6 Aug 2020
Soil type: silty clay
Soil pH: 6.9
Pesticide use: none
Fertilizer use: none
Irrigation: none

Weather:

	At scattering	Between scattering and last sampling	Long term monthly weather (compared to average)

	<table><tr><td>Air temperature (ºC)</td><td>28.7</td><td>18.9 – 27.9</td><td>Above</td></tr><tr><td>Humidity (%)</td><td>37</td><td>-</td><td>-</td></tr><tr><td>Wind speed (m/s)</td><td>0.0</td><td>-</td><td>-</td></tr><tr><td>Precipitation (mm)</td><td>none</td><td>4.8</td><td>Below</td></tr></table> <u>Trial number SRFR20-057-037FR:</u> France; Town/Village: La Chapelle de Guinchay; State/Province: Bourgogne-Franche-Comte; County: Saone-et-Loire Scattering date:4 Aug 2020 Soil type: loam Soil pH: 6.9 Pesticide use: none Fertilizer use: none Irrigation: none Weather: <table><tr><td></td><td>At scattering</td><td>Between scattering and last sampling</td><td>Long term monthly weather (compared to average)</td></tr><tr><td>Air temperature (ºC)</td><td>29.6</td><td>17.2 – 31.3</td><td>Above</td></tr><tr><td>Humidity (%)</td><td>36</td><td>-</td><td>-</td></tr><tr><td>Wind speed (m/s)</td><td>0.3 – 1.5</td><td>-</td><td>-</td></tr><tr><td>Precipitation (mm)</td><td>none</td><td>4.9</td><td>Below</td></tr></table>
Method of sampling:	Before scattering, seeds were taken from the packet by hand. After scattering, seeds were collected from the soil surface using forceps, avoiding the edge of the plot and contamination with soil.

Selection of samples to be analysed, and shipment

Following scattering of the seed on the soil surface, treated samples were collected before scattering from the treated seed packet and at 0, 1, 2, 3, 4, 5, 7, 10 and 14 days after scattering (DAS). Untreated samples were collected before scattering from untreated seed bag.

Samples were kept deep frozen at or below -18°C during transport and storage, prior to analysis.

Residue analysis

The analytical phase was conducted at the test site CEM Analytical Services LTD, located in the United Kingdom, using method REM 181.13A for the analysis of Metalaxyl-M. Seed samples were homogenized with methanol, before centrifugation.

An aliquot was removed and cleaned using an Oasis^{TM} HLB solid phase extraction procedure. Final determination was made by HPLC with triple quadropole mass spectrometry detection (LC-MS/MS). The limit of quantification (LOQ) was 1.0 mg/kg. The method REM181.13A has been validated according to SANCO/3029/99 (rev. 4, 11/07/2000) in a range of crops.

Results and Discussion

Table B.9.1.3-24: Results of Analysis of Field Trial Samples

Nominal Rate of Seed Treatment g ai/100kg seed)	Nominal Sampling Interval (days after scattering)	Crop Part	Metalaxyl-M (mg/kg)			
			SRPL20-046-037FR	SRPL20-047-037FR	SRDE20-144-037FR	SRFR20-057-037FR
67.8	0 DBS	Seed	512.42	510.18	493.77	496.23
67.8	0 DAS	Seed	396.13	448.17	478.01	494.61
67.8	1 DAS	Seed	272.74	348.95	299.21	376.03
67.8	2 DAS	Seed	305.75	323.54	294.26	331.82
67.8	3 DAS	Seed	351.33	343.86	316.69	290.79
67.8	5 DAS	Seed	240.59	305.29	257.97	283.48
67.8	7 DAS	Seed	286.11	272.73	252.77	279.06
67.8	10 DAS	Seed	260.53	263.54	108.53	219.38
67.8	14 DAS	Seed	268.57	265.17	123.90	179.72
67.8	21 DAS	Seed	29.97	33.93	76.08	49.52
Control	0 DBS	Seed	<1.0	<1.0	<1.0	<1.0

DAS – Days after scattering, DBS - Days before scattering
No correction of results for either control residues or recovery values has been performed

Table B.9.1.3-25: Results of the residue analysis against rainfall in study SRPL20-046-037FR

Nominal sampling interval (days)	SRPL20-046-037FR		SRPL20-047-037FR		SRDE20-144-037FR		SRFR20-057-037FR	
	Precipitation (mm)	**Metalaxyl-M (mg/kg)**	Precipitation (mm)	**Metalaxyl-M (mg/kg)**	Precipitation (mm)	**Metalaxyl-M (mg/kg)**	Precipitation (mm)	**Metalaxyl-M (mg/kg)**
0 DBS	0	512.42	0	510.18	0	493.77	0	496.23
0 DAS	0	396.13	0	448.17	0	478.01	0	494.61
1 DAS	0	272.74	0	348.95	0	299.21	0	376.03
2 DAS	0	305.75	0	323.54	0	294.26	0	331.82
3 DAS	0	351.33	0	343.86	0	316.69	0	290.79
5 DAS	0	240.59	0	305.29	0	257.97	0	283.48
7 DAS	0	286.11	0	272.73	0	252.77	0	279.06
10 DAS	0	260.53	0	263.54	0.4	108.53	0	219.38
14 DAS	2.2	268.57	5.2	265.17	0	123.90	0.5	179.72
21 DAS	1.8	29.97	0.4	33.93	0	76.08	0	49.52

Conclusions

Four residue field trials on treated spinach seeds were successfully conducted in Poland, Germany and northern France during 2020.

Metalaxyl-M was applied to spinach seeds as A9642H, an emulsion for seed treatment (ES) formulation containing 350 g of metalaxyl-M per litre. A seed treatment was made at a nominal rate of 67.8 g a.s./100 kg seed for metalaxyl-M and treated seeds were supplied by the Sponsor. Untreated seeds were also provided by the Sponsor.

At the field sites, provided treated seeds were scattered on the soil surface.
Treated samples were collected before scattering from the treated seed packet and at 0, 1, 2, 3, 4, 5, 7, 10 and 14 days after scattering (DAS). Untreated samples were collected before scattering from untreated seed packet.

Samples were shipped frozen to the analytical facility where they were analysed for metalaxyl-M.

Samples were analysed using method REM 181.13A.

The study design as detailed above was successfully carried out leading to the following conclusions.

Residues of metalaxyl-M are summarised in the table below.

Table B.9.1.3-26: Summary of residue values in spinach seeds

Sampling Interval (days)	Metalaxyl-M Residues (mg/kg)
Treated Plot (P2): seeds treated at a nominal rate of 67.8 g a.s.l9o /100 kg of seed	
Spinach seed	
0 DBS	493.77 – 512.42
0 DAS	396.13 – 494.61
1 DAS	272.74 – 376.03
2 DAS	294.26 – 331.82
3 DAS	290.79 – 351.33
4 DAS	240.59 – 305.29
5 DAS	252.77 – 286.11
7 DAS	108.53 – 263.54
9-10 DAS	123.90 – 268.57
14 DAS	29.97 – 76.08
Control plot (C1) No residues of metalaxyl-M at or above the limit of quantification (LOQ, 1.0 mg/kg) were found in any of the untreated spinach seed samples. This LOQ was assigned due to apparent residues of up to 0.05 mg/kg of metalaxyl-M in the control seed samples. Raising the LOQ to 1.0 mg/kg was agreed with the Study Manager. The justification for this was that the lowest residue found in the treated samples was	

29.97 mg/kg. Procedural recoveries were carried out at 1.0 mg/kg and 1000 mg/kg.

DBS: days before scattering
DAS: days after scattering

([redacted], 2020a)

HSE Comments

Four residue field trials were completed on spinach seeds treated with Metalaxyl-M in Poland, Germany and Northern France. Seeds were treated at a nominal rate of 67.8 g a.s./100 kg seed, and the seeds were scattered on the soil surface.

While the applicant has proposed using this study in the risk assessment for use on vining peas, it is noted that the seeds used in these residue field trials were spinach. Additionally the seed treatment applied was a single active substance formulation containing metalaxyl-M, rather than Wakil XL, which also contains other active substances. It is also noted that the application rate of 67.8 g a.s./100 kg seed used in this study is higher than in the proposed GAP for vining peas, which has an application rate of 33.9 g a.s./100 kg seed. These extrapolations will be considered further in the risk assessment section.

The locations used in the residue field trials in Germany, Poland and Northern France are similar in climate to GB, HSE considers these locations to be acceptable. During the field trials the weather was recorded and compared to the long term monthly average. The two trials performed in Poland (SRPL 20-046-037FR and SRPL 20-047-037FR) had an average temperature in line with the 'normal' long term monthly average in the area. The precipitation during the period of the trial was above the long term monthly average. Trial SRDE20-144-037FR, in Germany, and Trial SRFR20-057-037FR, in Northern France, had temperatures above the long term monthly average, however the precipitation was below. HSE considers the low precipitation could provide a more conservative result for residue decline, as there is less opportunity for the test substance to be washed-off or diluted. HSE has compared the rainfall data to the results of the residue analysis. There was very little precipitation during the study period, particularly early on, and does this not seem to coincide with any enhanced decline in residues.

The proposed time for use of the active on vining peas is from February to mid-April. The date of the application was reported along with the method for scattering the seeds and collecting samples. The date of the studies where in August, this would result in warmer weather conditions and less rainfall then expected during the proposed use. Extrapolation of data from summer to spring furthers adds to the uncertainty and will be considered further in the risk assessment.

The edge of the test plot was avoided when collecting seeds for sampling, as recommended in the EFSA recurring issues document, 2019. HSE considers that the methods used were appropriate. The timings for sampling were 0, 1, 2, 3, 4, 5, 7, 9 – 10, and 14 days after scattering. HSE considers that these time intervals will cover the main period of time for residue decline.

Historic data for pesticide use was provided for each of the sites used in the residue decline trials. No pesticides or fertilisers were used for the duration of the residue decline field trial. The site for trial SRPL20-046-037FR used Polifoska 6 (350 kg/ha), Saletrzak (200kg/ha), and Urea (100 kg/ha) on 25th April 2020. This was five months prior to the scattering date of 15 September 2020, HSE considers this acceptable. The site for trial SRPL20-047-037FR used Polifoska (500 kg/ha), Potassium salt (100 kg/ha), and Magnesium sulfate (100 kg/ha) on 14 April 2020. This was five months prior to the scattering of treated seeds for the residue decline field trail. HSE considers this an adequate time gap. No additional fertilisers or pesticides were used in the 6 months before the residue field trial in SRDE20-144-037FR and SRFR20-057-037FR.

The analytical method for determining Metalaxyl-M residues in the seed was reported as REM 181/13A, using LC-MS/MS. The LOQ was 1.0 mg/kg. No measurements from treated seeds were below the LOQ. The LOD was not reported.

The analytical method used has been considered by a HSE chemistry specialist. The method 'REM 181.13A' is fully validated in accordance with SANCO 3029/99 rev 4. for the determination of metalaxyl-M in various crop groups (RAR, 2014). Acceptable procedural recoveries and chromatograms were provided for spinach seeds; the method is considered sufficiently validated for the purpose of determining residues of metalaxyl-M in spinach seeds.

Overall, HSE considers the above residue field trial acceptable for use in the risk assessment, however, there is uncertainty in using the data for the vining pea risk assessment, due to the use of a different crop and timing to the proposed GAP.

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	IIIA 10.1.7/02 [redacted] (2021). Metalaxyl-M - Dissipation of Residues on A9642H - Treated Spinach Seed Scattered on the Soil Surface in Spain and Portugal in 2020. Report Number CEMR-9570. CEM Analytical Services, Oaklands Park, Wokingham, Berkshire RG41 2FD United Kingdom. Task Number: TK0538579 (VV-901662).

Guidelines

The field phase was designed under consideration of recommendations in the current guidance document on risk assessment for birds and mammals (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).

Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document).

OECD Guidance Document on Crop Field Trials, Series on Pesticides No. 66 and Series on Testing and Assessment No. 164, ENV/JM/MONO(2011)50.

OECD Guidance Document on Overview of Residue Chemistry Studies (as revised 2009), Series on Testing and Assessment (No. 64) and Series on Pesticides (No. 32), ENV/JM/MONO(2009)31.
Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.

OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009.
European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000).

OECD Guidance Document on Pesticide Residue Analytical Methods, ENV/JM/MONO(2007)17 (Unclassified, 13 Aug 2007).

GLP: Yes

Study Parameters

Parameter	Description
Test materials:	Metalaxyl-M Formulation code: A9642H Batch: SMU7LP001 Valid until: 31-Jan-2022 Formulation a.s. content: Metalaxyl-M – nominal: 350 g/L; actual: 355 g/L
Seed loading:	Metalaxyl-M – nominal: 67.8 g a.s./100kg seed
Identification of untreated seed:	Spinach variety: El Lucio batch 15071335
Identification of treated seed:	Spinach variety: El Lucio batch 15071335
Stability of the test item:	The test item is assumed to be stable for the period of use in the study.
Growing system:	Seeds were scattered on the soil in a single layer with no seeds touching each other. The soil was protected with netting to prevent seed consumption from birds and mammals. There was no control plot, control seeds were sampled directly from the untreated seed container supplied by the sponsor.
Seed scattering method:	Seeds were scattered by hand, seed rate of 600 kg seed/ha.
Trial site details:	<u>Trial number SRES20-444-037FR:</u> Spain; Town/Village: Picanya; State/Province: Valencia; County: Valencia Scattering date: 3 Aug 2020 Seed loading: 600 kg seed/ha Soil type: clay loam

	Soil pH: 7.05 Pesticide use: imidacloprid 0.05%- 12.5g/m², alpha-cypermethrin 1% - 30 g/m² Fertilizer use: none Irrigation: none Weather: <table><tr><th></th><th>At scattering</th><th>Between scattering and last sampling</th><th>Long term monthly weather (compared to average)</th></tr><tr><td>Air temperature (ºC)</td><td>29.8</td><td>22.89 – 31.62</td><td>25.79 (Above)</td></tr><tr><td>Humidity (%)</td><td>53</td><td>-</td><td>-</td></tr><tr><td>Wind speed (m/s)</td><td>0.6</td><td>-</td><td>-</td></tr><tr><td>Precipitation (mm)</td><td>none</td><td>0.0</td><td>7.78 (Below)</td></tr></table> Trial number SRES20-445-037FR: Spain; Town/Village: Yecla; State/Province: Murcia; County: Murcia Scattering date: 03 Aug 2020 Seed loading: 600 kg seed/ha Soil type: clay-loam Soil pH: 8.4 Pesticide use: imidacloprid 0.05%- 12.5g/m², alpha-cypermethrin 1% - 30 g/m² Fertilizer use: none Irrigation: none Weather: <table><tr><th></th><th>At scattering</th><th>Between scattering and last sampling</th><th>Long term monthly weather (compared to average)</th></tr><tr><td>Air temperature (ºC)</td><td>31.9</td><td>26.05 – 26.85</td><td>24.63 (Above)</td></tr><tr><td>Humidity (%)</td><td>52</td><td>-</td><td>-</td></tr><tr><td>Wind speed (m/s)</td><td>0.0</td><td>-</td><td>-</td></tr><tr><td>Precipitation (mm)</td><td>none</td><td>2.80</td><td>0.0 (Below)</td></tr></table> Trial number SRPT20-087-037FR: Portugal; Town/Village: Caldas da Rainha; State/Province: Centro; County: Oeste

	Scattering date: 24 Aug 2020 Seed loading: 330 - 370 kg seed/ha Soil type: sandy Soil pH: 6.5 Pesticide use: Fontana Bio 50 g/kg (03 Sept 2020) Fertilizer use: none Irrigation: none Weather:

	At scattering	Between scattering and last sampling	Long term monthly weather (compared to average)
Air temperature (ºC)	29.8	6.8 – 30.5	August: 19.4 (Normal) September: 20.3 (Normal)
Humidity (%)	53	-	-
Wind speed (m/s)	0.6	-	-
Precipitation (mm)	none	1.5	August: 25.48 (Above) Sept: 17.35 (Above)

<u>Trial number SRPT20-088-037FR:</u>
Portugal; Town/Village: Porto de Muge; State/Province: Alentejo; County: Leziria do Tejo
Scattering date: 8 Sep 2020
Seed loading: 330 - 370 kg seed/ha
Soil type: loam
Soil pH: 8.5
Pesticide use: none
Fertilizer use: none
Irrigation: none

Weather:

	At scattering	Between scattering and last sampling	Long term monthly weather (compared to average)
Air temperature (ºC)	29.9	12.6 – 37.7	22.9 (Normal)
Humidity (%)	36	-	-
Wind speed (m/s)	0.0	-	-
Precipitation (mm)	none	15.0	0.00 (Below)

Method of sampling:	Before scattering seeds were taken from the original bags by hand. After scattering, seeds were collected from the soil surface using forceps, avoiding the edges of the plot.

Selection of samples to be analysed, and shipment
Following scattering of the seed on the soil surface, treated samples were collected before scattering from the treated seed packet and at 0, 1, 2, 3, 4, 5, 7, 10 and 14 days after scattering (DAS). Untreated samples were collected before scattering from untreated seed bag.
Samples were kept deep frozen at or below -18°C during transport and storage, prior to analysis.

Residue analysis
The analytical phase was conducted at the test site CEM Analytical Services LTD, located in the United Kingdom, using method REM 181.13A for the analysis of Metalaxyl-M. Seed samples were homogenised with methanol. Final determination was by LC-MS/MS. The limit of quantification (LOQ) was 1.0 mg/kg. The method REM181.13A has been validated according to SANCO/3029/99 (rev. 4, 11/07/2000) in a range of crops.

Results and Discussion

Table B.9.1.3-27: Results of Analysis of Field Trial Samples: Metalaxyl-M

Nominal Rate of Seed Treatment g ai/100kg seed)	Nominal Sampling Interval (days after scattering)	Crop Part	Metalaxyl-M (mg/kg)			
			SRES20-444-037FR	SRES20-445-037FR	SRPT20-087-037FR	SRPT20-088-037FR
67.8	0 DBS	Seed	528.19	549.31	480.78	531.82
67.8	0 DAS	Seed	550.46	557.27	427.00	446.43
67.8	1 DAS	Seed	311.25	329.66	338.63	348.10
67.8	2 DAS	Seed	310.73	280.59	198.50	340.64
67.8	3 DAS	Seed	284.81	283.08	130.38	314.15
67.8	4 DAS	Seed	280.61	268.26	113.22	269.21
67.8	5 DAS	Seed	257.38	265.40	138.49	251.88
67.8	7 DAS	Seed	208.09	253.45	108.77	225.27
67.8	10 DAS	Seed	222.02	267.45	96.20	148.86
67.8	14 DAS	Seed	119.48	117.19	95.28	-
Control	0 DBS	Seed	<1.0	<1.0	<1.0	<1.0

DAS – Days after scattering, DBS - Days before scattering
No correction of results for either control residues or recovery values has been performed

Conclusions
Four residue field trials on treated spinach seeds were successfully conducted in Spain and Portugal during 2020.

Metalaxyl-M was applied to spinach seeds as A9642H, an emulsion for seed treatment (ES) formulation containing 350 g of metalaxyl-M per litre. A seed treatment was made at a nominal rate of 67.8 g a.s./100 kg seed for metalaxyl-M and treated seeds were supplied by the Sponsor. Untreated seeds were also provided by the Sponsor.

At the field sites, provided treated seeds were scattered on the soil surface.
Treated samples were collected before scattering from the treated seed packet and at 0, 1, 2, 3, 4, 5, 7, 10 and 14 days after scattering (DAS). Untreated samples were collected before scattering from untreated seed packet.

Samples were shipped frozen to the analytical facility where they were analysed for metalaxyl-M.

Samples were analysed using method REM 181.13A.

The study design as detailed above was successfully carried out leading to the following conclusions.

Residues of metalaxyl-M are summarised in the table below.

Table B.9.1.3-28: Summary of residue values in spinach seeds

Sampling Interval (days)	Metalaxyl-M Residues (mg/kg)
Treated Plot (P2): seeds treated at a nominal rate of 67.8 g a.s./100 kg of seed	
Spinach seed	
0 DBS	480.78 – 549.31
0 DAS	427.00 – 557.27
1 DAS	311.25 – 348.10
2 DAS	198.50 – 340.64
3 DAS	130.38 – 314.15
4 DAS	113.22 – 280.61
5 DAS	138.49 – 265.40
7 DAS	108.77 – 253.45
9-10 DAS	96.20 – 267.45
14 DAS	95.28 – 117.19
Control plot (C1) No residues of metalaxyl-M at or above the limit of quantification (LOQ, 1.0 mg/kg) were found in any of the untreated spinach seed samples. This LOQ was assigned due to apparent residues of up to 0.05 mg/kg of metalaxyl-M in the control seed samples. Raising the LOQ to 1.0 mg/kg was agreed with the Study Manager. The justification for this was that the lowest residue found in the treated samples was 95.28 mg/kg. Procedural recoveries were carried out at 1.0 mg/kg and 1000 mg/kg.	

DBS: days before scattering
DAS: days after scattering

([redacted], 2020b)

HSE Comments

Four residue field trials were completed on spinach seeds treated with Metalaxyl-M in Spain and Portugal. Seeds were treated at a nominal rate of 67.8 g a.s./100 kg seed, and the seeds were scattered on the soil surface.
While the applicant has proposed using this study in the risk assessment for use on vining peas, it is noted that the seeds used in these residue field trials were spinach. Additionally the seed treatment applied was a single active substance formulation containing metalaxyl-M, rather than Wakil XL, which also contains other active substances. It is also noted that the application rate of 67.8 g a.s./100 kg seed used in this study is higher than in the proposed GAP for vining peas, which has an application rate of 33.9 g a.s./100 kg seed. These extrapolations will be considered further in the risk assessment section.

HSE considers the locations used in the residue field trials in Spain and Northern Portugal to have a different climate to GB. As such, HSE does not consider the sites to be comparable to the proposed use in Great Britain, as the weather conditions will be more favourable than those in GB. It is also noted that the proposed time for use of the active on vining peas is from February to mid-April. The dates of these studies were in August/September, this would result in warmer weather conditions and less rainfall then expected during the proposed use. Extrapolation of data from summer to spring furthers adds to the uncertainty and will be considered in the risk assessment.

During the field trials the weather was recorded and compared to the long-term monthly average. The two trials performed in Spain (SRES 20-444-037FR and SRES 20-445-037FR) had an average temperature above the long-term monthly average in the area. The precipitation during the period of the trial was below the long-term monthly average. Similarly, the temperature during trial SRPT20-088-037FR in Portugal was in line with 'normal' compared to the long-term monthly average and the precipitation was below the long-term monthly average. The temperature for trial SRPT20-087-037FR in Portugal was 'normal' compared to the long-term monthly average, however the precipitation was above the long-term monthly average.

The edge of the test plot was avoided when collecting seeds for sampling, as recommended in the EFSA recurring issues document, 2019. HSE considers that the methods used were appropriate. The timings for sampling were 0, 1, 2, 3, 4, 5, 7, 10, and 14 days after scattering. HSE considers that these time intervals will cover the main period of time for residue decline.

Historic data for pesticide use was provided for each of the sites used in the residue decline trials. The two trials performed in Spain (SRES 20-444-037FR and SRES 20-445-037FR) used maintenance pesticides during the course of the residue decline trial; imidacloprid (0.05 %, 12.5 g/m^2), alpha-cypermethrin (1 %, 30 g/m^2). Trial SRPT20-087-037FR used Fontana Bio (50 g/kg) on 03 September 2020, 10 days after scattering, whilst samples were still being collected for the residue field trial. Imidacloprid, alpha-cypermethrin, and Fontana Bio are all insecticides.

The analytical method for determining Metalaxyl-M residues in the seed was reported as REM 181/13A, using LC-MS/MS. The LOQ was 1.0 mg/kg. No measurements from treated seeds were below the LOQ. The LOD was not reported. There was a deviation to the study during the transport of samples; on 0 DBS and 1 DAS samples did not enter the freezer within four hours. As the test substance is considered to be stable for the duration of the study, HSE does not consider this to have affected the results.

The analytical method used has been considered by a HSE chemistry specialist. The method 'REM 181.13A' is fully validated in accordance with SANCO 3029/99 rev 4. for the determination of metalaxyl-M in various crop groups (RAR, 2014). Acceptable procedural recoveries and chromatograms were provided for spinach seeds; the method is considered sufficiently validated for the purpose of determining residues of metalaxyl-M in spinach seeds.

Overall, HSE considers the above residue field trial can be used for comparison with results from IIIA 10.1.7.01, however, there is uncertainty over how appropriate the results are for use in the risk assessment. This is due to the differing weather conditions in the field trial locations, alongside the use of a different crop type to the GAP and different application timing.

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	IIIA 10.1.7/04 [redacted] (2021). Metalaxyl-M – Calculation of DT_{50} values from residue decline studies following application to treated Spinach seeds. Report Number RAJ1430B. Syngenta Ltd, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, United Kingdom. Task Number: TK0538580.

Guidelines:
EFSA (2009) Risk Assessment for Birds and Mammals. EFSA Journal 2009; 7(12): 1438

EFSA (2019) Outcome of the Pesticide Peer Review Meeting on general recurring issues in ecotoxicology. EFSA Supporting Publication 2019: EN-1673

FOCUS (2006). Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005, version 2.0, 44 pp.

FOCUS (2014). Generic guidance for estimating modelling and degradation kinetics from environmental fate studies on pesticides in EU registration. Version 1.1, 440 pp.

GLP: Not applicable.

The seed decline of residues of Metalaxyl-M has been studies using the data from two studies with treated spinach seeds.

Modelling:
The kinetic modelling following the FOCUS Kinetics (2006, 2014) flowchart for determining modelling endpoints was carried out using CAKE version 3.4 (2020). Each data set has been considered according to the flowchart and the results of the process are describe below. HSE has also run the kinetic modelling using CAKE version 3.6 (2022).
The acceptability of the kinetic fits was judged as follows:

Visually using a three point scale:

Poor	An unacceptable fit, the fitted curve does not represent the trend of the data points and residuals show strong deviations from random distribution.
Acceptable	The fitted curve describes the trend of the data points, residuals may show some deviation from random distribution but it is not significant.
Good	The fitted curve closely follows all the data points, residuals are randomly distributed.

Confidence of rate constants:

The FOCUS Kinetics guidelines state that the confidence that can be assigned to a parameter must be assessed (FOCUS, 2006). Parameter estimates with a significance level greater than 95% are acceptable and, if greater than 90%, may be accepted where the visual fit is acceptable or good. Where significance levels are less than 90%, the fits are not considered acceptable.

For SFO and DFOP fits the assessment was based on the t-test probability value of the estimate of the degradation rate (k).

Fit to the data points (χ^2 error%):

It is recommended that a χ^2 error% of 15% or less indicates acceptable fits, although for data that may include intrinsically variable data higher values can be tolerated if the visual fit is acceptable. The χ^2 error% parameter has been used to determine goodness of fit and where the scattering of data precluded a statistically acceptable fit, expert judgement has been used to determine the overall acceptability.

CAKE Kinetic Evaluation report for IIA 10.1.7 01 – treated spinach seed scattered in Poland, Germany and Northern France 2020.

HSE considered the results from the study conducted on spinach seeds in Poland, Germany, and Northern France to be acceptable for use in modelling, however, HSE notes that spinach is a different crop to the proposed GAP.

Table B.9.1.3-29: Results for four trials performed in Poland, Germany, and Northern France

Sampling	Nominal	Metalaxyl-M Residue (mg/kg)

Interval (days)	Seed Treatment Rate (g a.s./100kg seed)	SRPL20-046-037FR	SRPL20-047-037FR	SRDE20-144-037FR	SRFR20-057-037FR
0 DBS	67.8	512.42	510.18	493.77	496.23
0DAS	67.8	396.13	448.17	478.01	494.61
1 DAS	67.8	272.74	348.95	299.21	376.03
2 DAS	67.8	305.75	323.54	294.26	331.82
3 DAS	67.8	351.33	343.86	316.69	290.79
4 DAS	67.8	240.59	305.29	257.97	283.48
5 DAS	67.8	286.11	272.73	252.77	279.06
7 DAS	67.8	260.53	263.54	108.53	219.38
10 DAS	67.8	268.57	265.17	123.90	179.72
14 DAS	67.8	29.97	33.93	76.08	49.52

SRPL20-046-037FR (Poland):

Study date: 31 January 2023
Report generated: 31 January 2023

Model Setup:
Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05) Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

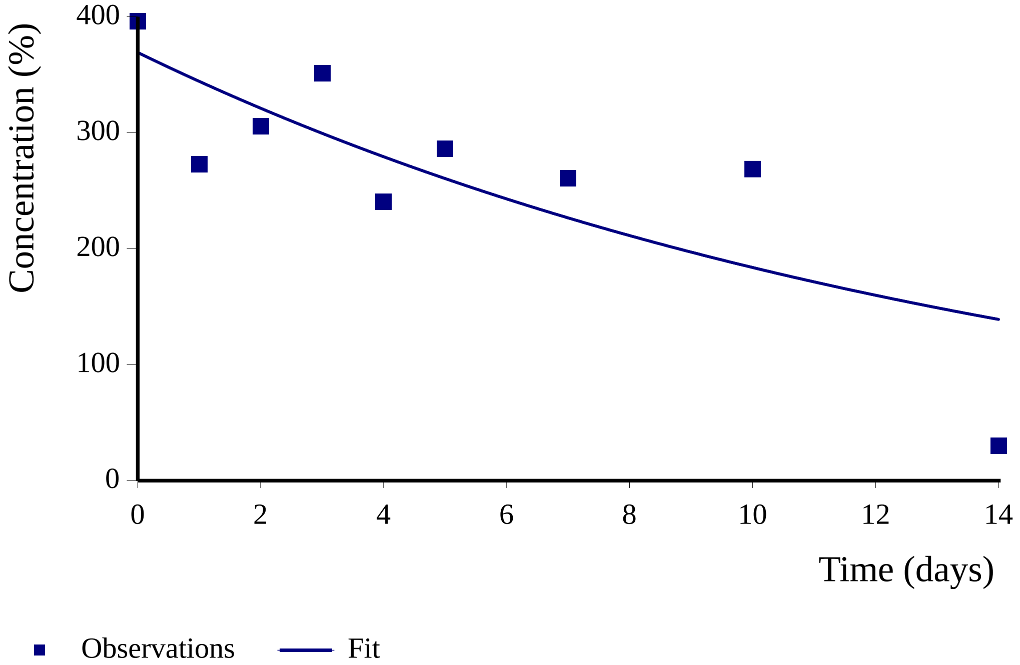
Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final
Used Extra Solver for SFO model fit: No

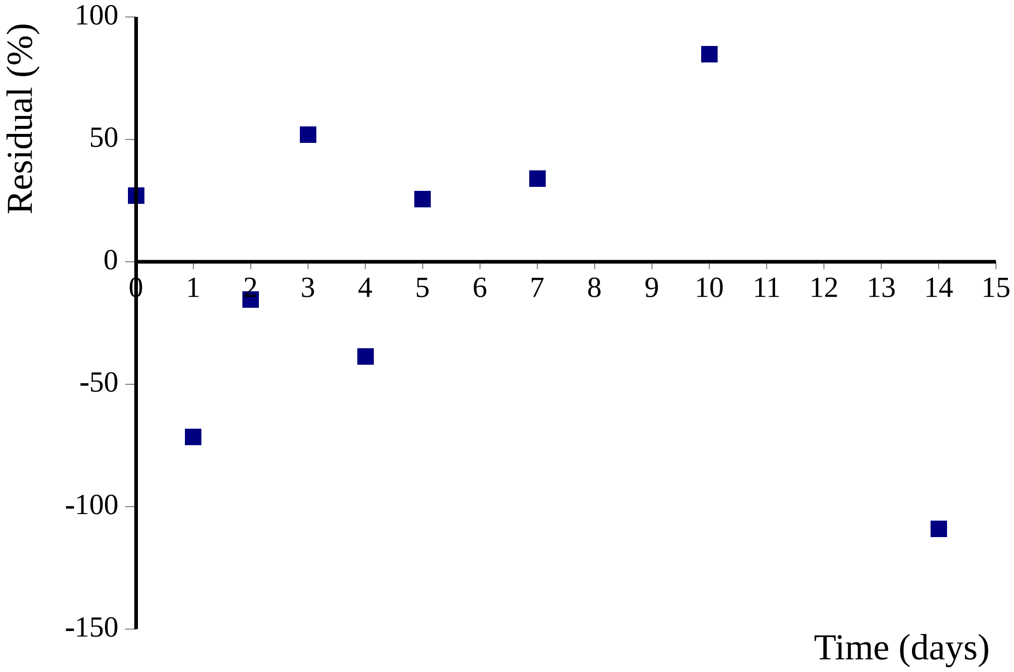
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	369.1	41.8	N/A	289.9	448.3	270.3	468
k_Parent	0.06975	0.02532	0.01415	0.02179	0.1177	0.009886	0.13

Sum of Squared Residuals: 3.116E+04

c²

Parameter	Error %	Degrees of Freedom
All data	17.6	7
Parent	17.6	7

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	9.94	33

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.6252	0.6227
Parent	0.6252	0.6227

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.7088
k_Parent	0.7088	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	396.1	369.1	26.99
1	272.7	344.2	-71.5
2	305.8	321.1	-15.3
3	351.3	299.4	51.91
4	240.6	279.2	-38.66
5	286.1	260.4	25.68
7	260.5	226.5	34.01
10	268.6	183.8	84.82
14	29.97	139	-109

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4515.0

HSE Comments
The residue decline model is acceptable. The fit of the line follows the points except for in day 14, there is no consistent bias above or below the line of fit. On day 14, the predicted decline is less than the actual decline, so this is a conservative model. The residuals shows a mixture of points above and below the line, there is no clear bias.
The prob> t is 0.01415, less than 0.5, chi^2 is 17.6 %, this is greater than the trigger of 15 %, but below 25 %. As the rest of the fit is acceptable, HSE considers this to be acceptable.
Overall HSE accepts this single first order residue decline model.
The DT_{50} for this residue decline study is 9.94.

SRPL20-047-037FR (Poland):

Model Setup:
Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05) Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

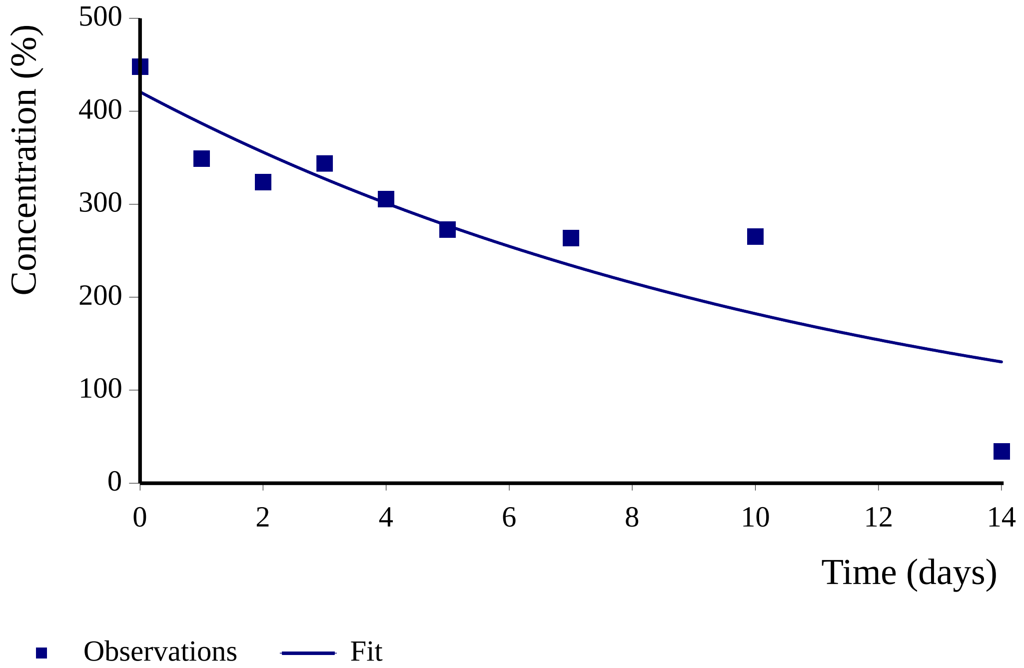
Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final
Used Extra Solver for SFO model fit: No

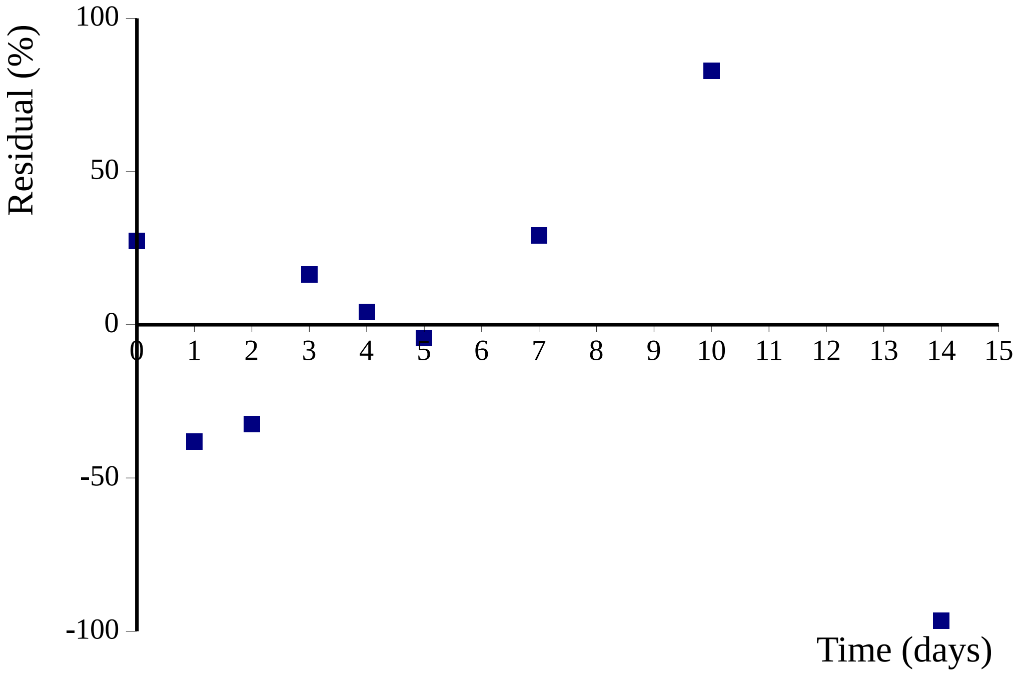
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	420.8	35.1	N/A	354.3	487.3	337.8	503.8
k_Parent	0.08365	0.02011	0.002121	0.04555	0.1217	0.0361	0.131

Sum of Squared Residuals: 2.06E+04

c²

Parameter	Error %	Degrees of Freedom
All data	13.2	7
Parent	13.2	7

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	8.29	27.5

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.7956	0.7939
Parent	0.7956	0.7939

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.7018
k_Parent	0.7018	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	448.2	420.8	27.34
1	349	387.1	-38.11
2	323.5	356	-32.46
3	343.9	327.4	16.43
4	305.3	301.2	4.133
5	272.7	277	-4.261
7	263.5	234.3	29.22
10	265.2	182.3	82.85
14	33.93	130.5	-96.54

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4515.0

HSE comments
The fit of the line is close to the points in the earlier timeframe. Days 7, 10, and 14 are further from the line. There is no consistent in the residual, however, it does not show any clear bias.
Chi^2 is 13.2 %, lower than 15 %, the prob > t is 0.002121, less than 0.5.
HSE accepts this single first order model as acceptable, the DT_{50} 8.29 days.

SRDE20-144-037FR (Germany):
Model Setup:
Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05) Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

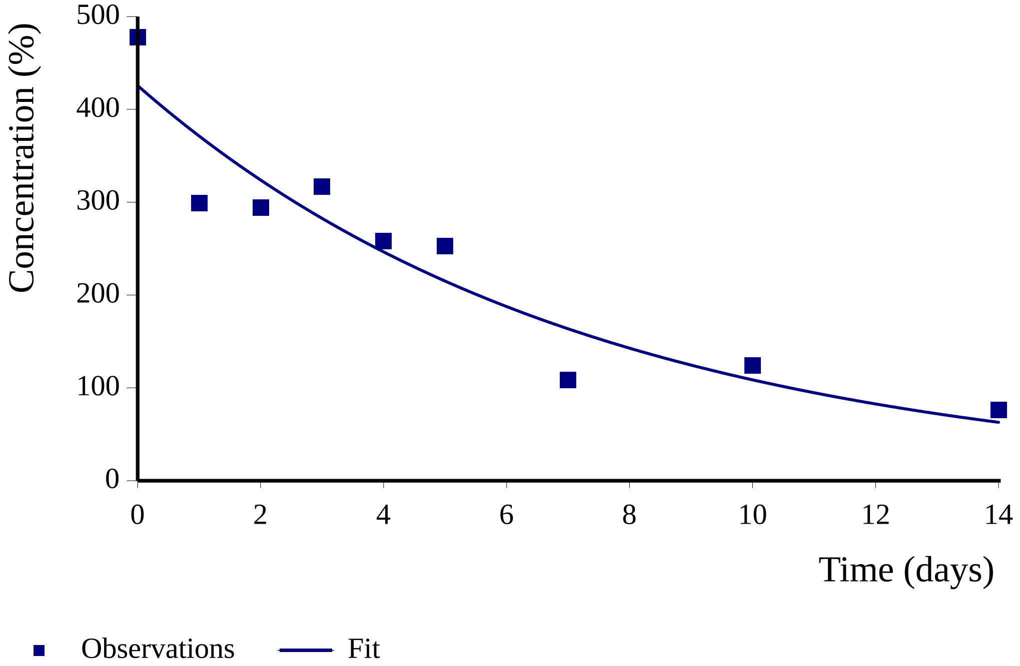
Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final
Used Extra Solver for SFO model fit: No

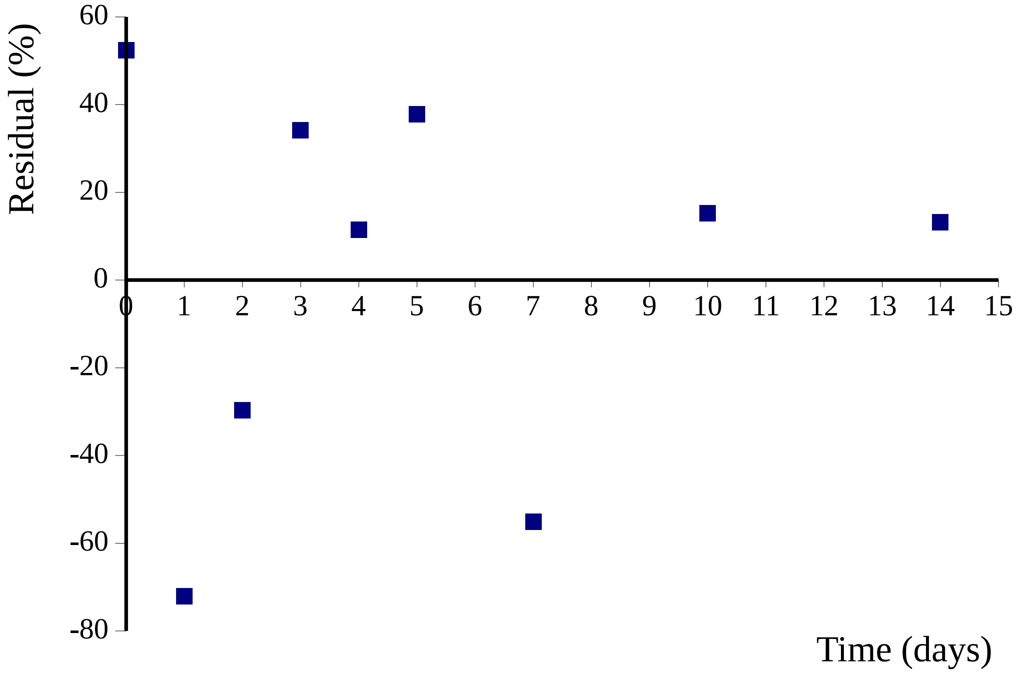
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	425.6	33.28	N/A	362.6	488.7	346.9	504.3
k_Parent	0.1366	0.02447	4.16E-004	0.09022	0.1829	0.07871	0.194

Sum of Squared Residuals: 1.498E+04

c²

Parameter	Error %	Degrees of Freedom
All data	13.3	7
Parent	13.3	7

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	5.08	16.9

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.8821	0.8819
Parent	0.8821	0.8819

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.6812
k_Parent	0.6812	1

Observed v. Predicted:
Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	478	425.6	52.38
1	299.2	371.3	-72.09
2	294.3	323.9	-29.64
3	316.7	282.5	34.14
4	258	246.5	11.49
5	252.8	215	37.76
7	108.5	163.6	-55.09
10	123.9	108.6	15.28
14	76.08	62.9	13.18

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4515.0

HSE comments:
The fit of the line matches the points well, there is no bias in the spread of the points around the line of fit. The residual is also distributed evenly, indicating no bias.
Chi^2 is 13.3 %, below 15 %, prob > t is 4.16 x 10^4, less than 0.5.
HSE accepts this single first order model for residue decline, the DT_{50} is 5.08 days.

SRFR20-057-037FR (France):

Model Setup:
Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05) Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

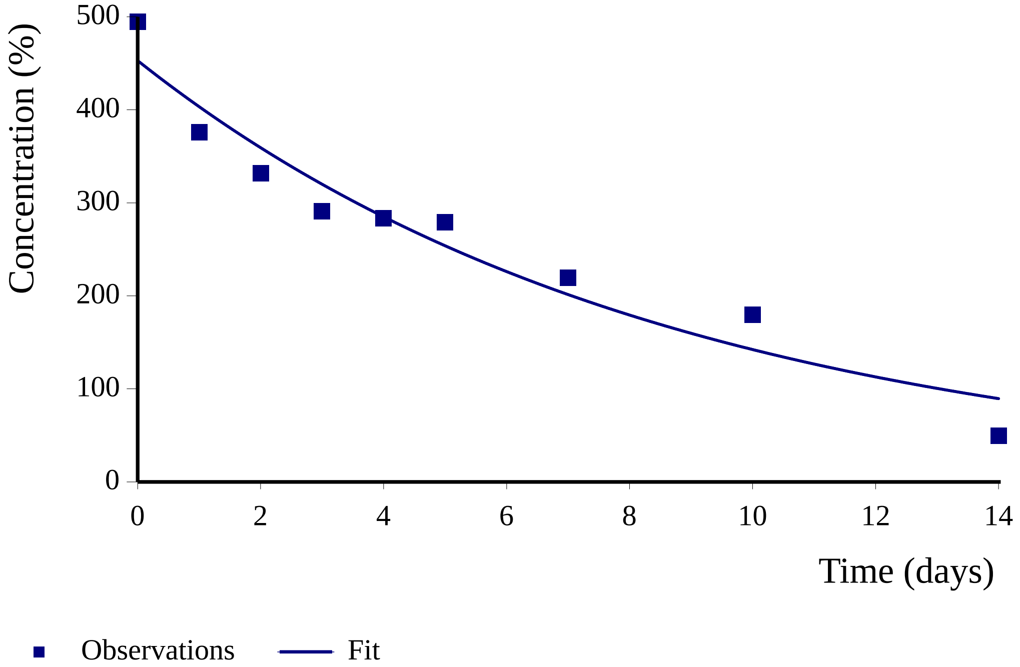
Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final
Used Extra Solver for SFO model fit: No

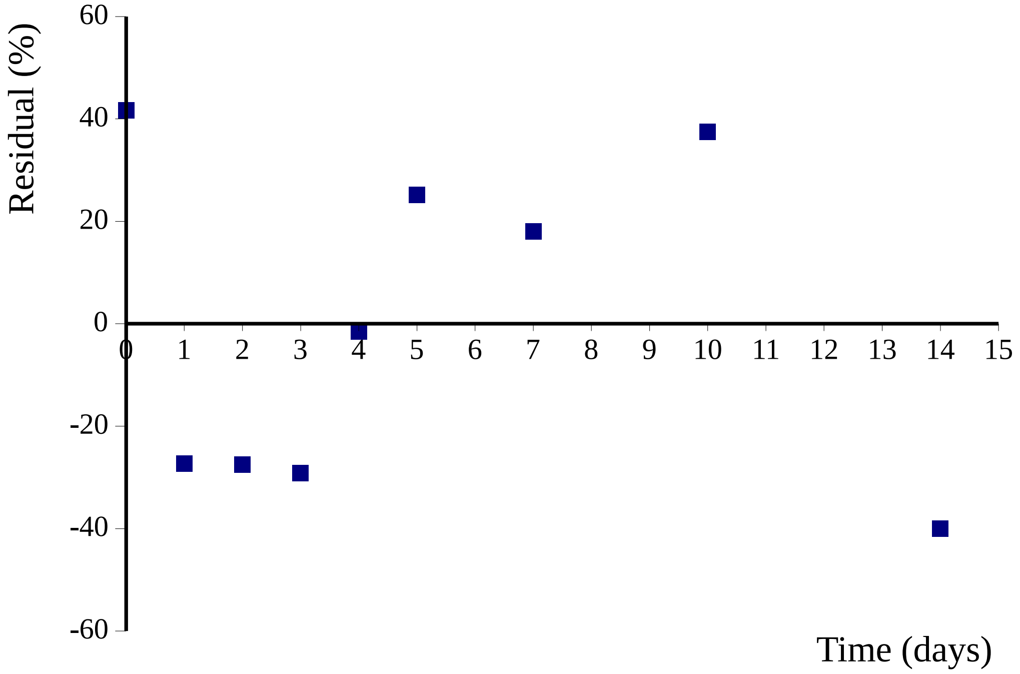
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	452.9	23.49	N/A	408.4	497.4	397.3	508.4
k_Parent	0.1158	0.01473	5.10E-005	0.08788	0.1437	0.08096	0.151

Sum of Squared Residuals: 8060

c²

Parameter	Error %	Degrees of Freedom
All data	8.6	7
Parent	8.6	7

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	5.99	19.9

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9355	0.9355
Parent	0.9355	0.9355

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.6886
k_Parent	0.6886	1

Observed v. Predicted:
Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	494.6	452.9	41.72
1	376	403.4	-27.34
2	331.8	359.3	-27.45
3	290.8	320	-29.2
4	283.5	285	-1.522
5	279.1	253.8	25.22
7	219.4	201.4	18.01
10	179.7	142.3	37.45
14	49.52	89.53	-40.01

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4515.0

HSE comments
The line of fit is good for this model, the fit is close to the points and there is no clear bias. There is a slight fall on day 14, but otherwise the points are all acceptable. The residual shows a mix of points above and below the line.
Chi^2 is 8.6 %, below 15 % and the prob > t is 5.1 x 10^5, below 0.5.
HSE accepts the single first order residue decline model, the DT_{50} is 5.99 days.

CAKE Kinetic Evaluation report for IIA 10.1.7 03 – treated spinach seed scattered in Spain and Portugal, 2020.

HSE has evaluated the study IIA 10.1.7 03, and does not consider the locations used comparable to GB. Additionally HSE notes that spinach is a different crop to the proposed GAP.
HSE has performed modelling on the residue decline results for these studies as a reference to the studies performed in Poland, Germany and Northern France but notes they are not as reliable.

Table IIA 10.1.7 03-1 Results for four trials performed in Northern Spain and Portugal

Sampling Interval (days)	Nominal Seed Treatment Rate (g a.s./100kg seed)	Metalaxyl-M Residue (mg/kg)			
		SRES20-444-037FR	SRES20-445-037FR	SRPT20-087-037FR	SRPT20-088-037FR
0 DBS	67.8	528.19	549.31	480.78	531.82
0DAS	67.8	550.46	557.27	427.00	446.43
1 DAS	67.8	311.25	329.66	338.63	348.10
2 DAS	67.8	310.73	280.59	198.50	340.64
3 DAS	67.8	284.81	283.08	130.38	314.15
4 DAS	67.8	280.61	268.26	113.22	269.21
5 DAS	67.8	257.38	265.40	138.49	251.88
7 DAS	67.8	208.09	253.45	108.77	225.27
10 DAS	67.8	222.02	267.45	96.20	148.86
14 DAS	67.8	119.48	117.19	95.28	-

SRES20-444-037FR (Valencia, Spain):
Model Setup:
Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05) Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

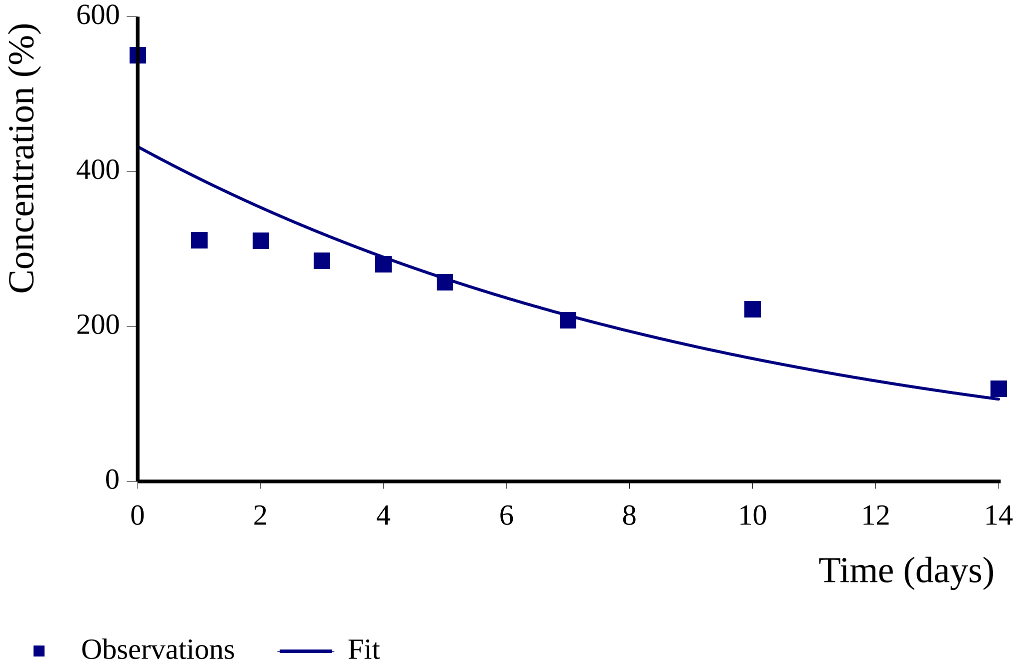
Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final
Used Extra Solver for SFO model fit: No

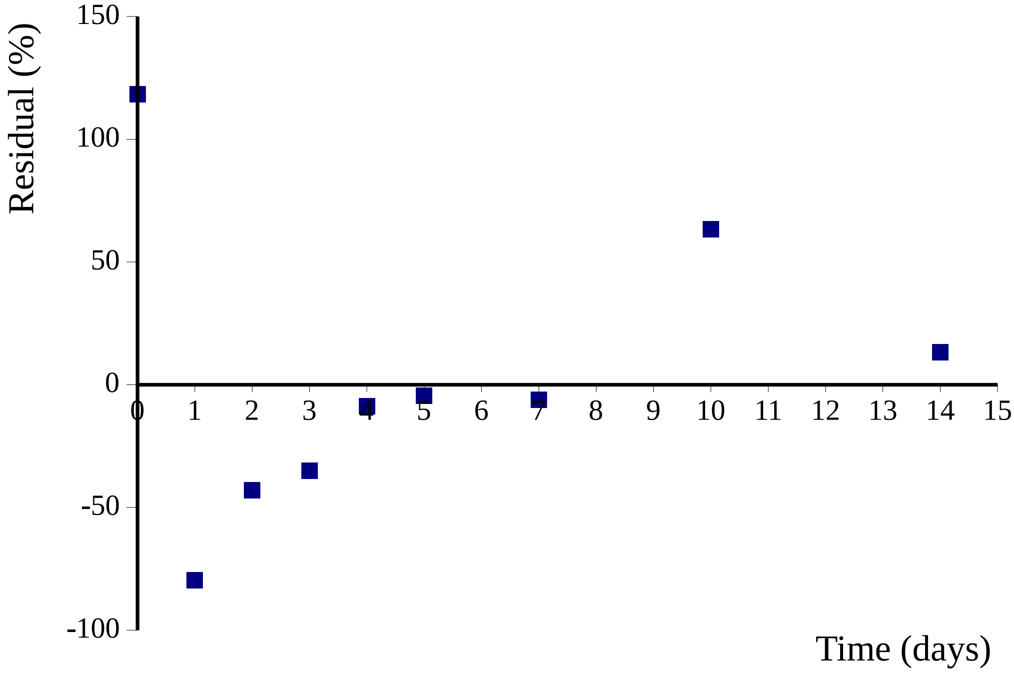
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	432.2	42.25	N/A	352.1	512.2	332.3	532.1
k_Parent	0.1003	0.0257	0.002944	0.05158	0.1489	0.0395	0.161

Sum of Squared Residuals: 2.775E+04

c²

Parameter	Error %	Degrees of Freedom
All data	15.7	7
Parent	15.7	7

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	6.91	23

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.7501	0.7473
Parent	0.7501	0.7473

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.6946
k_Parent	0.6946	1

Observed v. Predicted:
Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	550.5	432.2	118.3
1	311.2	391	-79.71
2	310.7	353.7	-42.93
3	284.8	319.9	-35.11
4	280.6	289.4	-8.789
5	257.4	261.8	-4.41
7	208.1	214.2	-6.133
10	222	158.6	63.45
14	119.5	106.2	13.3

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4515.0

HSE comments
The line of fit is close to the points, however at the beginning of the time period they are majority below the line whereas after day 7, the point are above the line. The residuals show a concentration of the points below the line, up until day 7. This suggests there is some bias in the data.
Chi^2 is 15.7 %, just above 15 %, the prob > t is 0.002944, below 0.5.
HSE does not consider this single first order model to be reliable due to the bias of the fit. A DFOP model may be considered.

SRES20-445-037FR (Mercia, Spain):

Model Setup:

Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05) Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

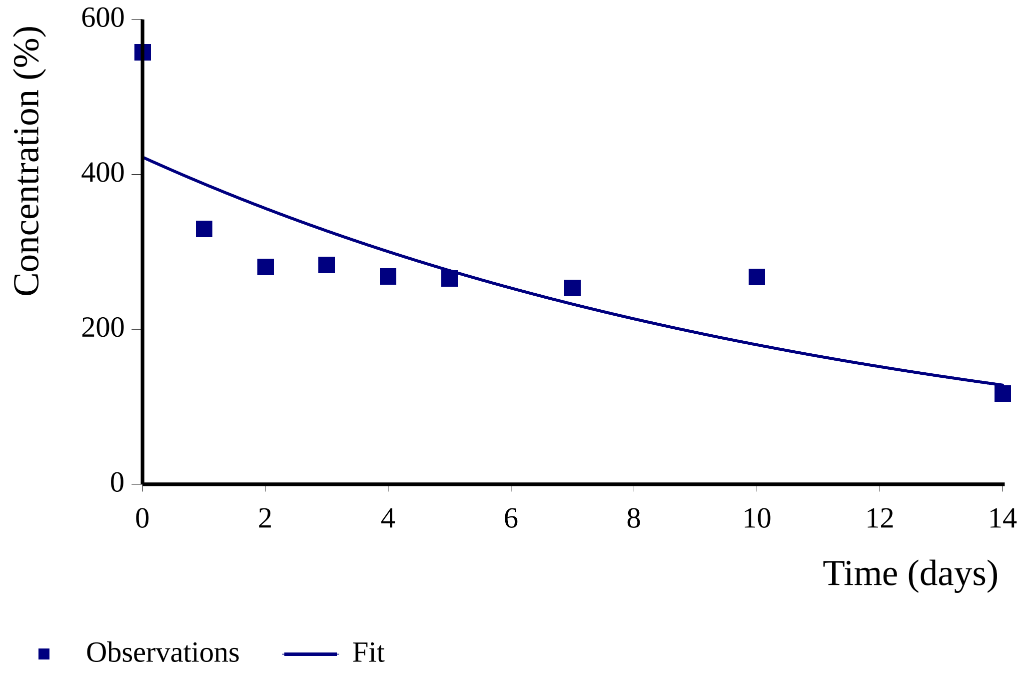
Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final
Used Extra Solver for SFO model fit: No

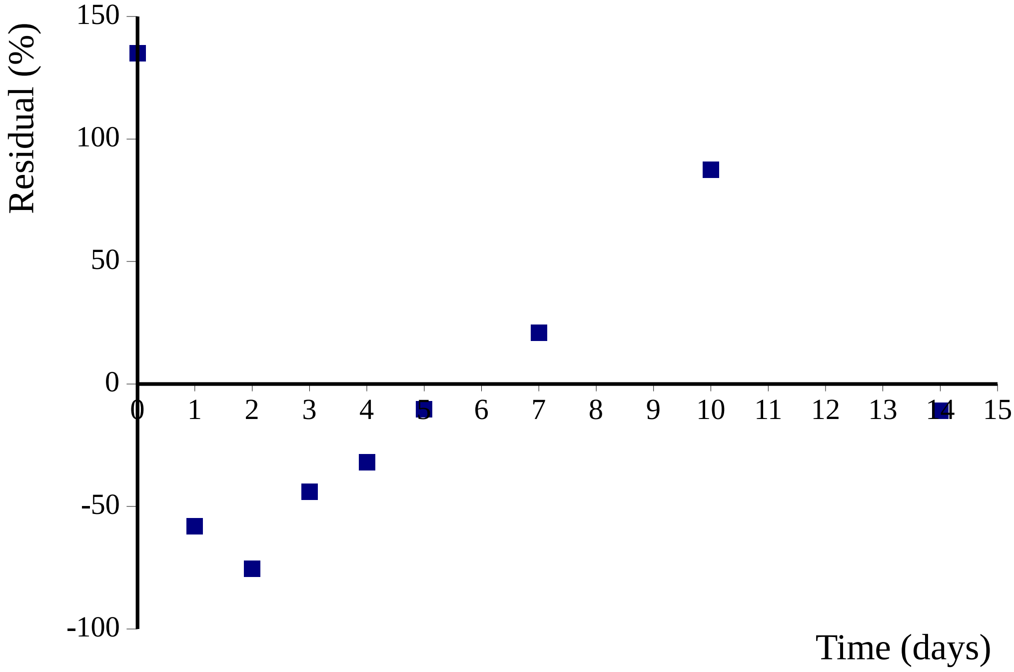
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	422.3	48.2	N/A	331	513.6	308.3	536.2
k_Parent	0.08525	0.02775	0.009009	0.03267	0.1378	0.01963	0.151

Sum of Squared Residuals: 3.855E+04

c²

Parameter	Error %	Degrees of Freedom
All data	18	7
Parent	18	7

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	8.13	27

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.6379	0.636
Parent	0.6379	0.636

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.7011
k_Parent	0.7011	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	557.3	422.3	135
1	329.7	387.8	-58.11
2	280.6	356.1	-75.49
3	283.1	327	-43.9
4	268.3	300.3	-32
5	265.4	275.7	-10.33
7	253.4	232.5	20.94
10	267.4	180	87.41
14	117.2	128	-10.83

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4515.0

HSE comments
The initial fit is close to the line, however, days 10 and 14 do not follow the line of fit. The residuals show there is bias, during the initial time frame the points are below the line, after day 7, the points are above the line except for on day 14.
Chi^2 is 18 %, above 15 %, but below 25 %. Prob > t is 0.009009, below 0.5.
HSE does not consider this single first order model of residue decline to be reliable due to the bias shown in the visual fit. A DFOP model may be considered.

SRPT20-087-037FR (Portugal):
Model Setup:
Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05) Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

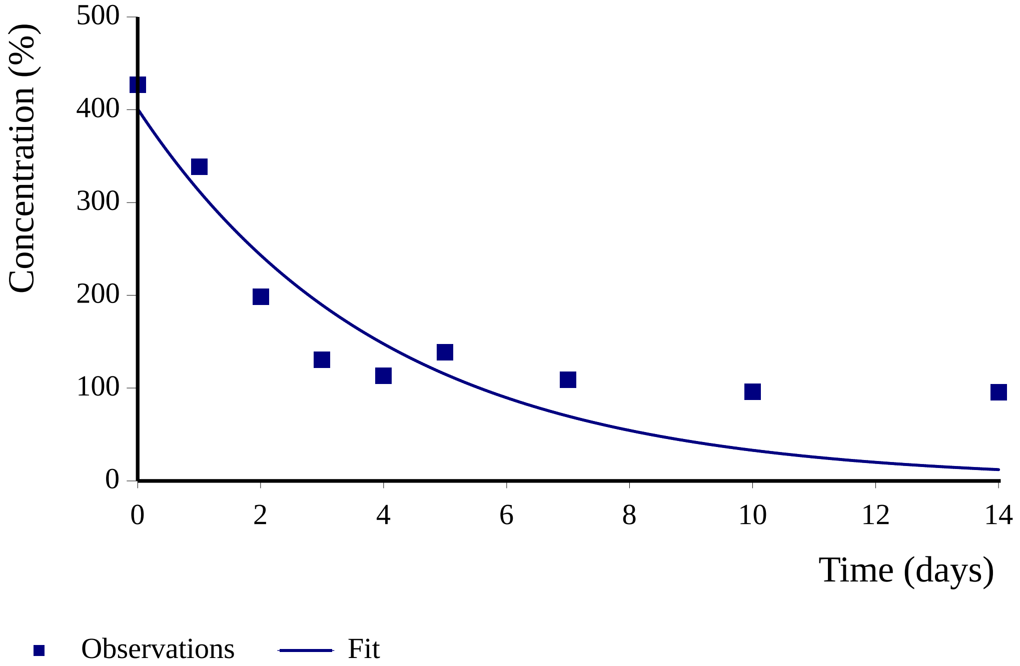
Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final
Used Extra Solver for SFO model fit: No

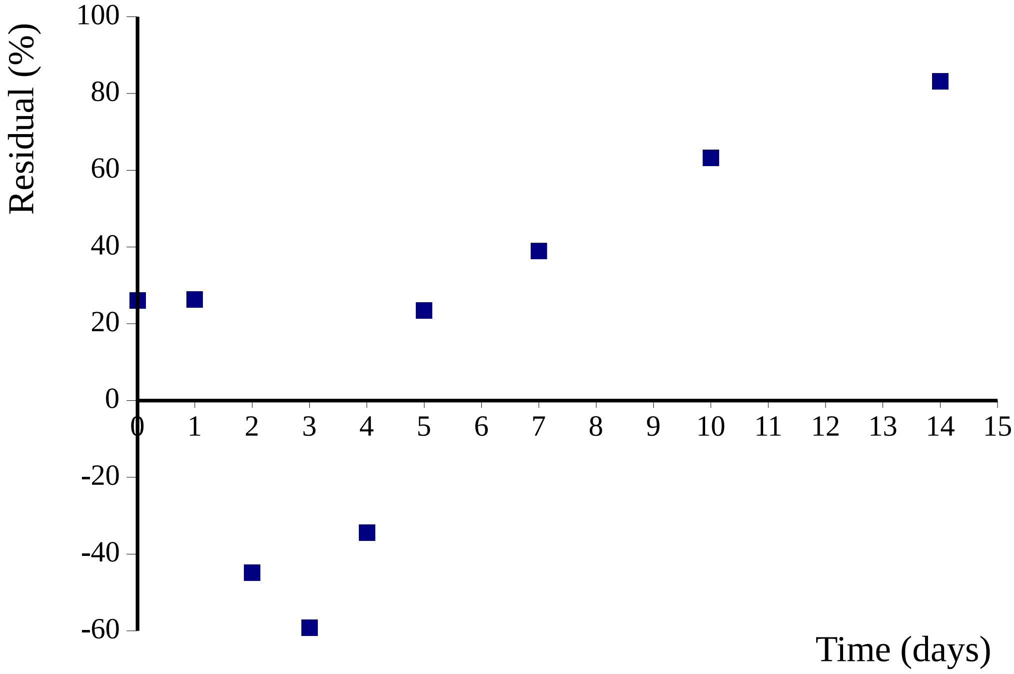
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	400.9	45.41	N/A	314.9	487	293.5	508.3
k_Parent	0.2497	0.05435	0.00125	0.1468	0.3527	0.1212	0.378

Sum of Squared Residuals: 2.104E+04

c²

Parameter	Error %	Degrees of Freedom
All data	21.1	7
Parent	21.1	7

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	2.78	9.22

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.8584	0.816

Parent	0.8584	0.816

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.6374
k_Parent	0.6374	1

Observed v. Predicted:
Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	427	400.9	26.08
1	338.6	312.3	26.31
2	198.5	243.3	-44.8
3	130.4	189.5	-59.15
4	113.2	147.6	-34.42
5	138.5	115	23.47
7	108.8	69.8	38.97
10	96.2	33	63.2
14	95.28	12.15	83.13

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4515.0

HSE comments
The visual fit of the line is good in the initial time period, however, there is a consistent trend above the line during the later time points. The residuals also shows a trend above the line in later time points, indicating bias.
Chi^2 is 21.1 %, above 15 %, but slightly below 25 %. Prob > t is 0.00125, below 0.5.
As there is bias in the visual fit of the data, HSE does not consider this single first order residue decline model reliable, a DFOP model may be considered.

SRPT20-088-037FR (Alentejo, Portugal):

Model Setup:
Topology: Parent only
Optimiser: IRLS (IRLS Its. 10, IRLS Tol. 1E-05, Max. Its. 100, Tol. 1E-05) Extra Solver Option: Use If Required

Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed

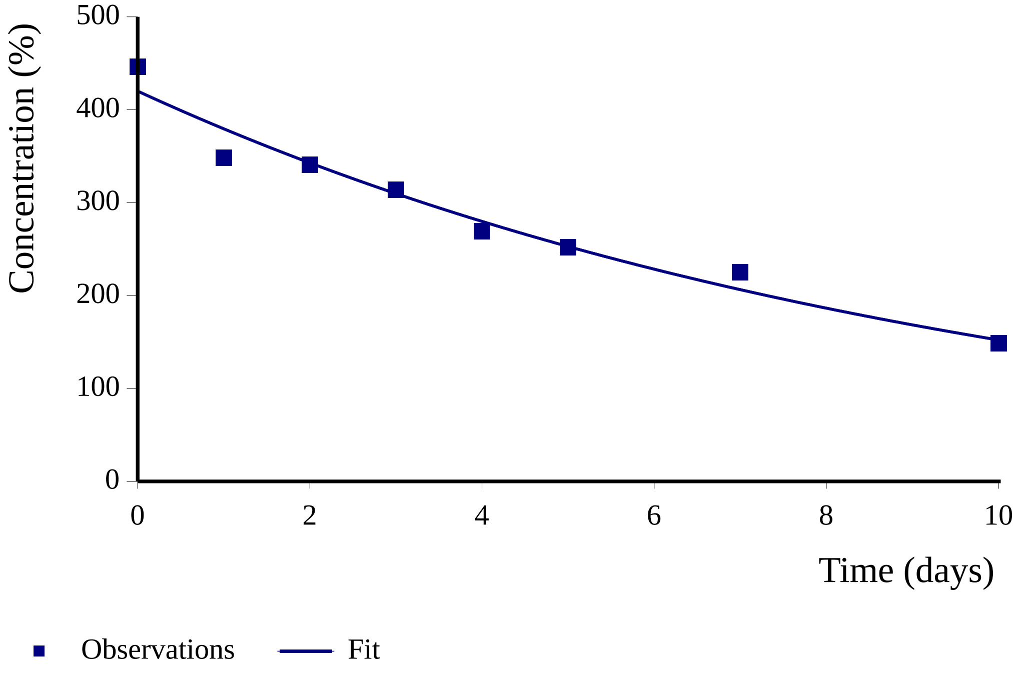
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Fit step: Final
Used Extra Solver for SFO model fit: No

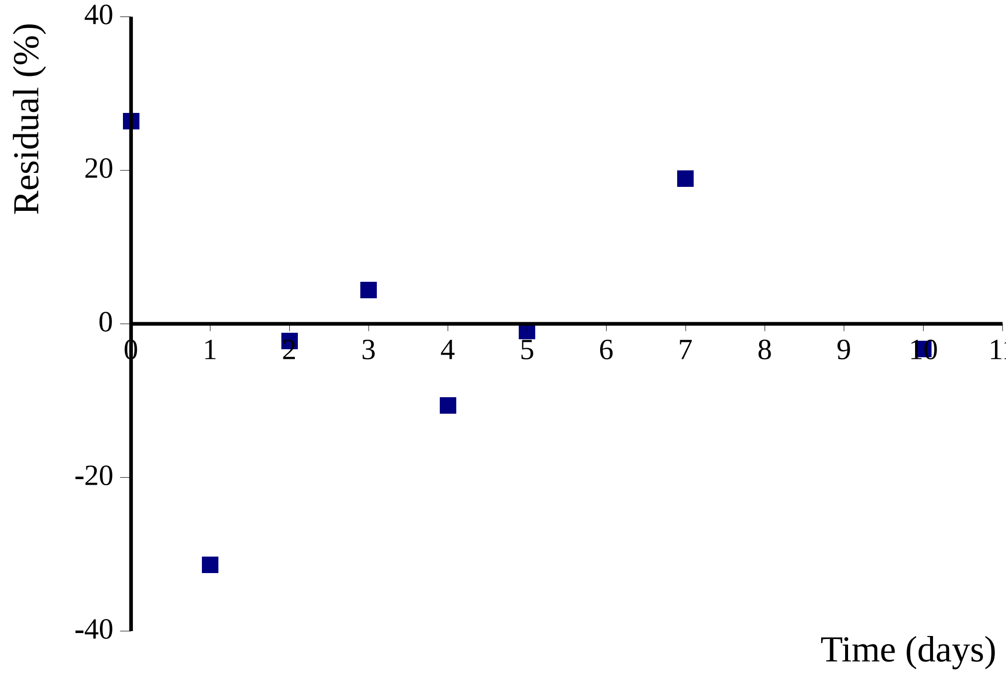
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	420	13.4	N/A	394	446.1	387.3	452.8
k_Parent	0.1015	0.009297	1.75E-005	0.08348	0.1196	0.0788	0.124

Sum of Squared Residuals: 2188

c²

Parameter	Error %	Degrees of Freedom
All data	4.5	6
Parent	4.5	6

Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	6.83	22.7

Additional Statistics:

Parameter	r² (Obs v Pred)	Efficiency

All data	0.9616	0.9616
Parent	0.9616	0.9616

Parameter Correlation:

	Parent_0	k_Parent
Parent_0	1	0.7188
k_Parent	0.7188	1

Observed v. Predicted:
Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	446.4	420	26.39
1	348.1	379.5	-31.38
2	340.6	342.8	-2.194
3	314.1	309.7	4.421
4	269.2	279.8	-10.61
5	251.9	252.8	-0.9206
7	225.3	206.3	18.93
10	148.9	152.1	-3.289

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4515.0

HSE comments
The visual fit is good, the line follows the points closely. The residual shows a good scattering of points, there is no obvious bias.
Chi^2 is 4.5, below 15 % and prob > t is 1.75 x 10^5.
HSE accepts that the single first-order residue decline model is reliable, the DT_{50} is 6.83 days.

<u>DFOP modelling</u>

As the residues in the studies SRES20-444-037FR, and SRES20-445-037FR did not reach DT_{90} during the study period HSE has conducted modelling using a DFOP fit. The SFO model for SRPT20-087-037FR was not considered reliable so a model using a DFOP fit has also been conducted.

SRES20-444-037FR (Valencia, Spain) DFOP:

Initial Values of Sequence Parameters:

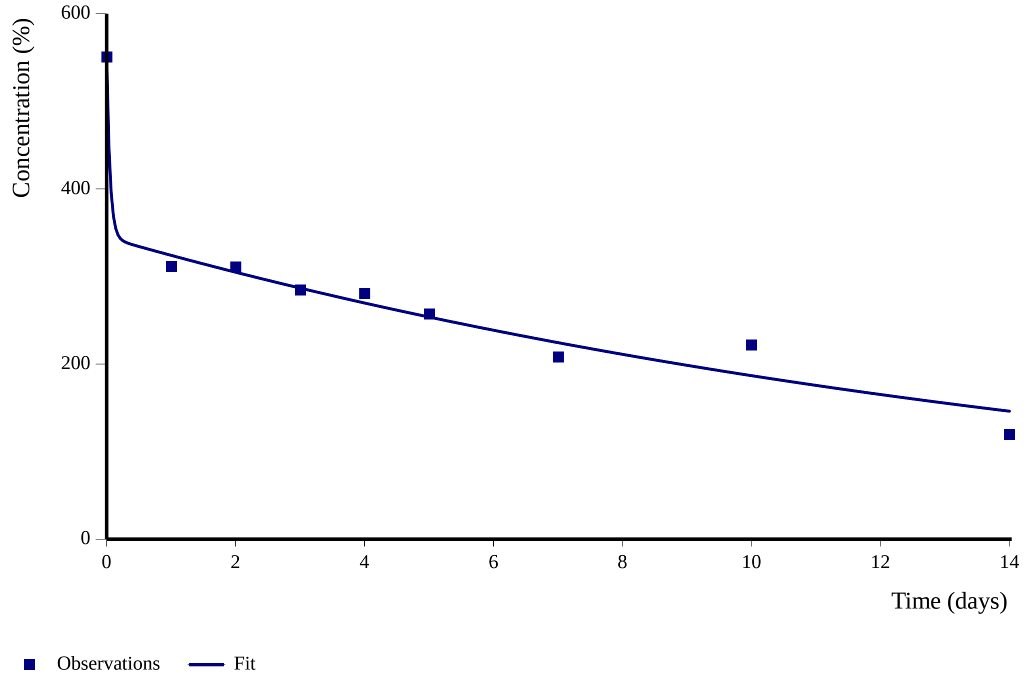
Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Fit step: Final
Used Extra Solver for DFOP model fit: Yes

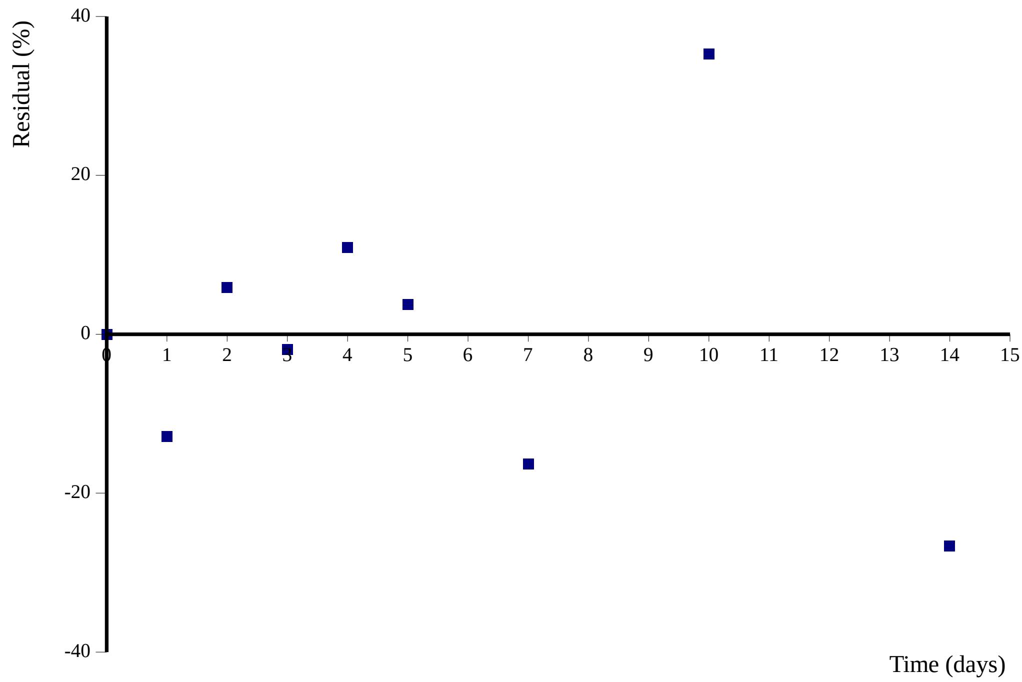
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	550.5	22.62	N/A	504.9	596	492.3	608.6
k1_Parent	19.61	7.88E+003	0.4991	-1.586E+04	1.59E+004	-2.024E+04	2.03E+004
k2_Parent	0.06127	0.00963	7.09E-004	0.04186	0.08067	0.03651	0.086
g_Parent	0.374	0.04071	N/A	0.292	0.456	0.2694	0.479

Sum of Squared Residuals: 2559 c^2

Parameter	Error %	Degrees of Freedom
All data	5.38	5
Parent	5.38	5

Decay Times:

Compartment	DT50 (overall days)	DT90 (overall days)	k1 DT50 (days)	k2 DT50 (days)
Parent	3.67	29.9	0.0353	11.3

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9767	0.9767
Parent	0.9767	0.9767

Parameter Correlation:

	Parent_0	k1_Parent	k2_Parent	g_Parent
Parent_0	1	3.874E-05	2.138E-07	0.632
k1_Parent	3.874E-05	1	8.506E-05	-7.096E-05
k2_Parent	2.138E-07	8.506E-05	1	-0.6079
g_Parent	0.632	-7.096E-05	-0.6079	1

Observed v. Predicted:
Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	550.5	550.5	8.808E-05
1	311.2	324.1	-12.86
2	310.7	304.8	5.886
3	284.8	286.7	-1.917
4	280.6	269.7	10.92
5	257.4	253.7	3.72
7	208.1	224.4	-16.32
10	222	186.7	35.29
14	119.5	146.1	-26.66

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4614.0

HSE comments
The visual fit of the line meets the majority of the points, with the exception of day 10 where the point is slightly above the line. The residual shows a good scatter of the points both above and below the line.
For both the k1 and the k2, the prob > t is below 0.5, and the chi^2 is 5.38 %.
The DT_{50} for k1 is 0.0353 days, and the DT_{50} for k2 is 11.3 days.

SRES20-445-037FR (Murcia, Spain) DFOP:

Initial Values of Sequence Parameters:

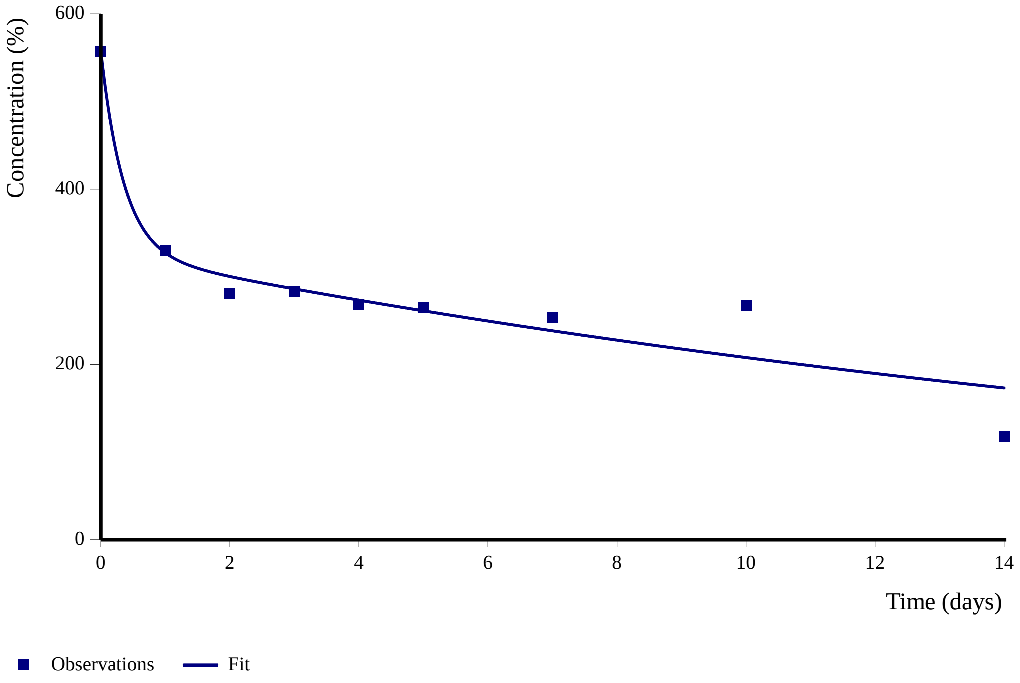
Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Fit step: Final
Used Extra Solver for DFOP model fit: No

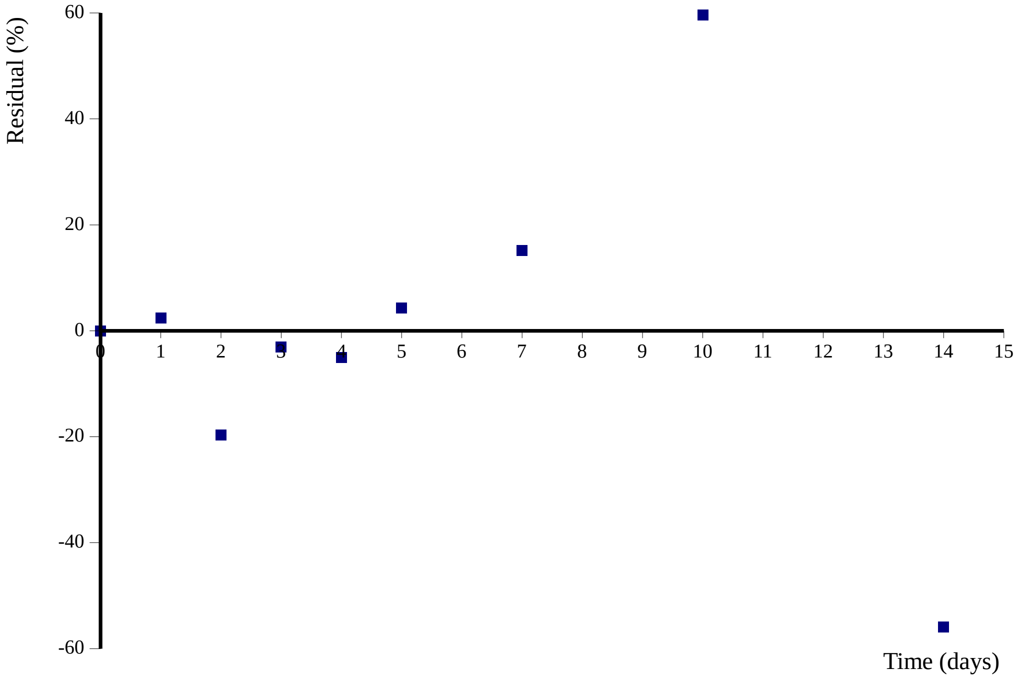
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	557.3	38.37	N/A	480	634.7	458.7	656
k1_Parent	2.811	3.586	0.2343	-4.416	10.04	-6.408	12.03
k2_Parent	0.04567	0.01787	0.02546	0.009662	0.08168	-0.0002656	0.092
g_Parent	0.4113	0.07798	N/A	0.2542	0.5684	0.2108	0.612

Sum of Squared Residuals: 7360

c²

Parameter	Error %	Degrees of Freedom
All data	8.85	5

Parent	8.85	5

Decay Times:

Compartment	DT50 (overall days)	DT90 (overall days)	k1 DT50 (days)	k2 DT50 (days)
Parent	3.58	38.8	0.247	15.2

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9306	0.9305
Parent	0.9306	0.9305

Parameter Correlation:

	Parent_0	k1_Parent	k2_Parent	g_Parent
Parent_0	1	0.04777	0.001573	0.5178
k1_Parent	0.04777	1	0.5005	-0.5157
k2_Parent	0.001573	0.5005	1	-0.7289
g_Parent	0.5178	-0.5157	-0.7289	1

Observed v. Predicted:
Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	557.3	557.3	-0.07277
1	329.7	327.3	2.407
2	280.6	300.3	-19.71
3	283.1	286.1	-3.068
4	268.3	273.3	-5.068
5	265.4	261.1	4.277
7	253.4	238.3	15.12
10	267.4	207.8	59.64
14	117.2	173.1	-55.92

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)

Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4614.0

HSE comments

The line of fit meets the majority of the points, day 10 and day 14 are slightly off the line. The residuals show scattering of the points away from the line, day 10, is significantly above the line, whilst day 14 is significantly below the line.
The prob > t for both k1 and k2 is below 0.5 and chi^2 is 8.85 % below 15 %.
HSE accepts that this model is reliable, the DT_{50} for k1 is 0.247 days and the DT_{50} for k2 is 15.2 days, this is slightly longer than the study period of 14 days.

SRPT20-087-037FR (Cento, Portugal) DFOP:

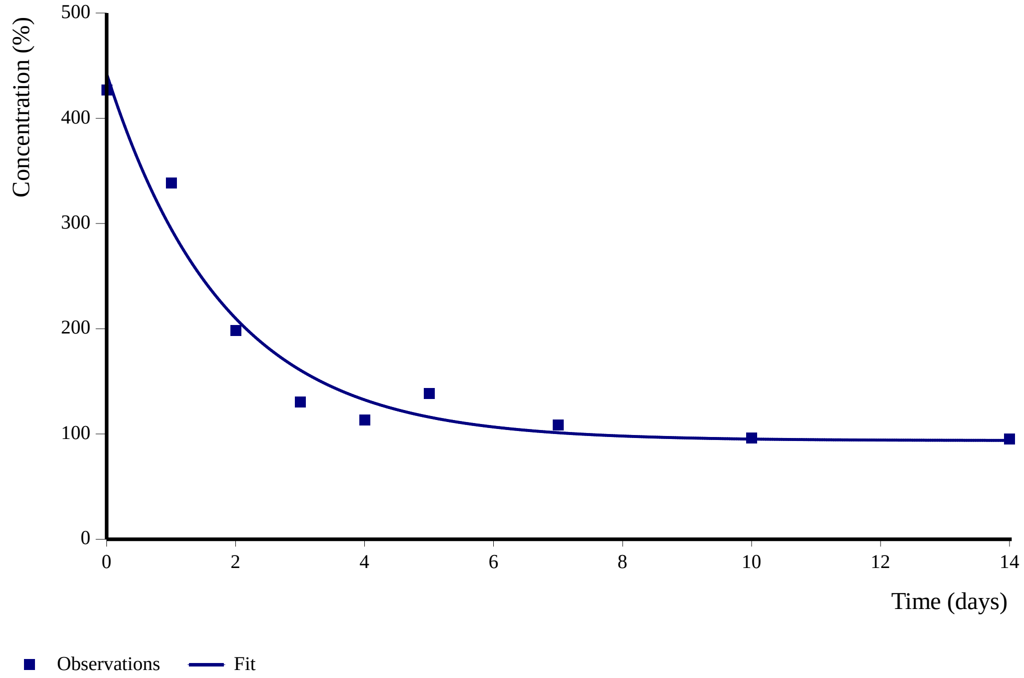
Initial Values of Sequence Parameters:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

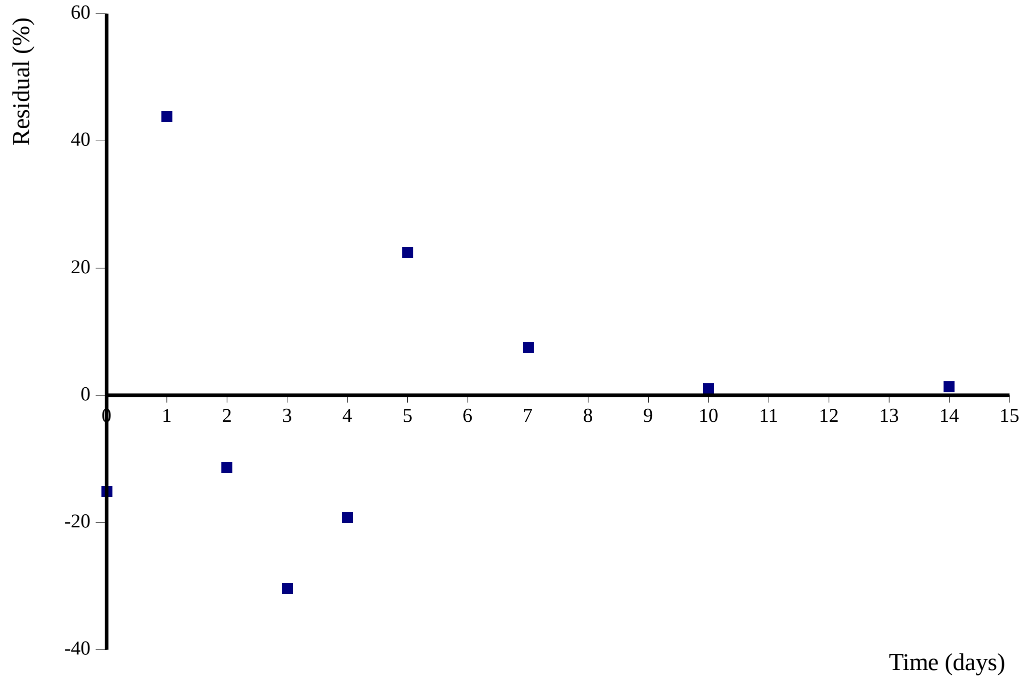
Fit step: Final
Used Extra Solver for DFOP model fit: No
Reference Table:

Compartment	Name
Parent	Parent

Graphical Summary:
Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k1_Parent	0.1	0 to (unbounded)	No
k2_Parent	0.01	0 to (unbounded)	No
g_Parent	0.5	0 to 1	No

Estimated Values:

Parameter	Value	s	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	442.1	27.88	N/A	385.9	498.3	370.5	513.8
k1_Parent	0.5495	0.1886	0.01663	0.1695	0.9296	0.06472	1.034
k2_Parent	9.51E-014	0.0609	0.5	-0.1227	0.1227	-0.1566	0.157
g_Parent	0.7879	0.1384	N/A	0.509	1.067	0.4321	1.144

Sum of Squared Residuals: 4129

c²

Parameter	Error %	Degrees of Freedom
All data	10.6	5
Parent	10.6	5

Decay Times:

Compartment	DT50 (overall days)	DT90 (overall days)	k1 DT50 (days)	k2 DT50 (days)
Parent	1.83	>10,000	1.26	>10,000

Additional Statistics:

Parameter	r^2 (Obs v Pred)	Efficiency
All data	0.9639	0.9639
Parent	0.9639	0.9639

Parameter Correlation:

	Parent_0	k1_Parent	k2_Parent	g_Parent
Parent_0	1	0.4041	0.1757	-0.1118
k1_Parent	0.4041	1	0.8163	-0.8669
k2_Parent	0.1757	0.8163	1	-0.9606
g_Parent	-0.1118	-0.8669	-0.9606	1

Observed v. Predicted:

Compartment Parent

Time (days)	Value (%)	Predicted Value	Residual
0	427	442.1	-15.12
1	338.6	294.9	43.78
2	198.5	209.8	-11.34
3	130.4	160.8	-30.39
4	113.2	132.5	-19.23
5	138.5	116.1	22.39
7	108.8	101.2	7.557
10	96.2	95.21	0.9934
14	95.28	93.94	1.345

Sequence Creation Information:
Fit generated by CAKE version 3.6 (Release)
running on R version 4.1.1 (2021-08-10)
Report Information:
Report generated by CAKE version 3.6 (Release)
CAKE developed by Hybrid Intelligence (formerly Tessella), part of Capgemini Engineering; sponsored by Syngenta
Runtime: .NET Framework 4.8.4614.0

HSE comments

The line of fit follows the majority of the points, there is no clear bias. The residual shows a good scattering of the points above and below the line.
The prob > t is below 0.5 for k1, and 0.5 for k2. Chi^2 is 10.6 %.
HSE accept this model as reliable, the DT_{50} for k1 is 1.25 days and the DT_{50} for k2 is > 10,000 days.

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	KIIIA 10.1.7/06, [redacted], [redacted] and [redacted] (2006). Bird species in pea fields in Brittany (northern France): Field data for the determination of focal species. [redacted], Germany. Unpublished Report No. [redacted]. Syngenta File No. VV-379792

Guidelines

Bibby et al. 1992. Bird census techniques. Academic Press, London

Study Design and Methods

Experimental dates: 26 April to 22 June 2006

Objectives:

The objective of this generic study was to determine the qualitative and quantitative composition of the bird community employing the parameters, frequency of occurrence (FO_{field} and FO_{survey})[19)] and dominance[20)] both as overall and as pea growth stage specific descriptors. Another objective was to then allocate the selected species to defined foraging guilds, diet guilds and size classes.

Study area:

The Brittany region of northern France served as the study area, encompassing 22 pea fields (average transect length 310 ± 21 m; range 168 – 558 m; median 301 m) selected to represent average pea fields, field size and the structure of the landscape. The total area across all transects was 66.0 ha. Many pea fields were characterised by diverse habitat surroundings such as hedgerows, woodlands, agricultural fields, and set aside fields.

Method and parameters:

Field observations covered a period of two months from late April to late June, including the following pea growth stages. The average condition of the majority of crop plants was used to assign growth stages.

Table B.9.1.3-30: Date of surveys and corresponding BBCH growth stages

[19] **FO_{field}**: denotes the number of fields in which a defined species was recorded, given as percentage of the total number of fields, regardless of the number of individuals observed. This approach serves as a measure for the spatial frequency of occurrence. **FO_{survey}**: denotes the number of surveys in which a defined species was recorded, given as percentage of the total number of surveys. This approach gives an approximation for the temporal evenness of occurrence throughout the complete study period.

[20] **Dominance**: denotes the relative occurrence of bird species within the bird community. It is reported as the percentage of individuals of the respective species compared to the total number of individuals of all species (calculated as arithmetic means over all cereal fields).

Survey Period	Growth stage	BBCH code
26.04 – 21.05.2006	Germination/Leaf development	00 – 19
28.04 – 24.05.2006	Stem elongation	30 – 39
24.05 – 22.06.2006	Flowering/Development of fruit	60 – 79

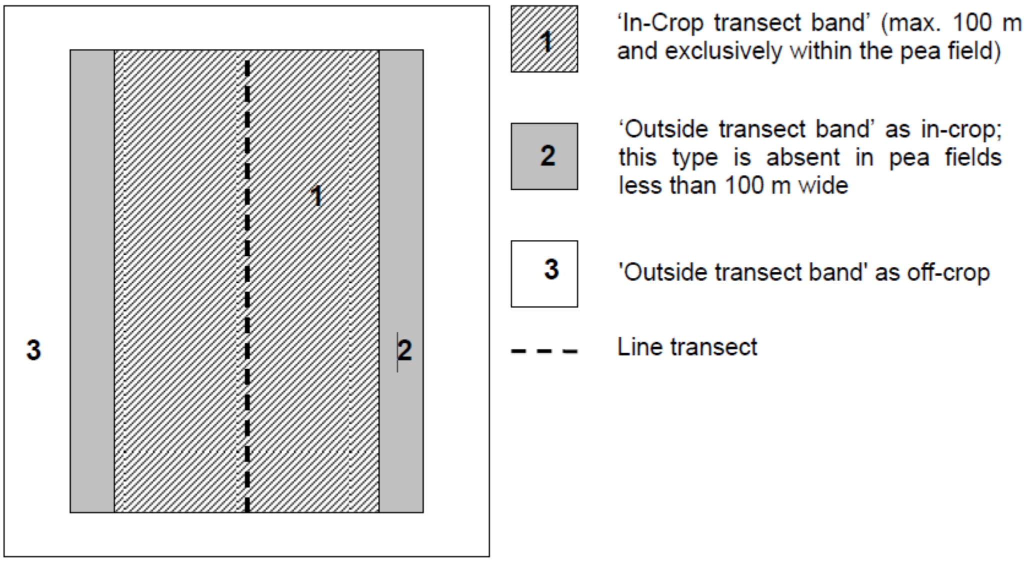
In order to cover different pea growth stages, three line transect surveys were conducted in 2006 for each field in April (germination and leaf development; survey 1), May (stem elongation, survey 2) and June (flowering and development of fruits, survey 3). All field surveys were conducted under comparable weather conditions.

All bird species were recorded in each field by walking slowly along the transect, allowing a clear view between the rows of peas. Each of the individual birds visually or acoustically registered was assigned to one of the following areas:

- ‘In crop transect band’- birds recorded within a 100 m wide band (50 m either side) where the field was at least 100 m wide. For narrower pea fields the band considered was narrowed and contained only the in crop area (i.e. the width of the field).
- ‘Outside transect band’- birds recorded beyond the 100 m central band. Depending on the width of the field the ‘outside transect band’ may include in-crop (for fields > 100 m wide) and off-crop habitat.

Only the birds present in the ‘in crop transect band’ of each field were included for data analysis. Birds flying up to a height of 5 m above crop height were also included in the analysis.

Figure B.9.1.3-2: Graduation of different areas within defined pea fields in Brittany (northern France) as applied in this study.



Data analysis:

The frequency of occurrence (FO_{field}; FO_{survey}) and dominance were considered to be the key parameters for the derivation of focal species in a given period of time.

Frequency of occurrence (FO)

The FO_{field} value describes the likelihood of a defined species occurring in any particular field, given as the percentage of the total number of pea fields regardless of the number of individuals observed. This serves as a measure for the spatial frequency of occurrence.

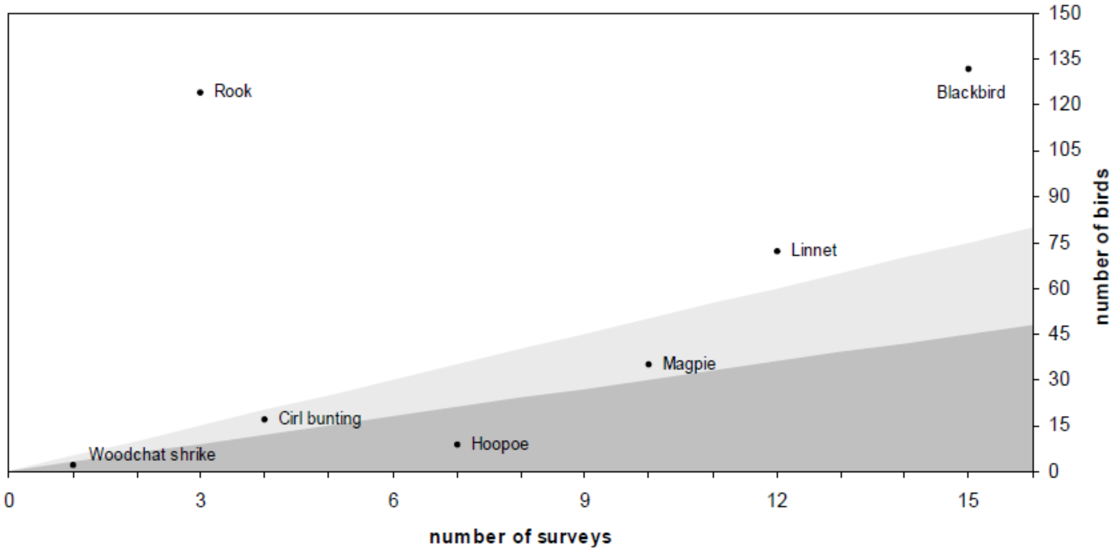
FO_{survey} describes the likelihood of a species occurring in cereal fields at a certain timing. This is given as a percentage of the total number of surveys.

FO_{survey} is more indicative of time-weighted occurrence as opposed to spatial occurrence described by FO_{field}.

Dominance
Dominance is a measure of overall abundance but may be biased by flocking behaviour, so care is needed in interpretation. Dominance denotes the relative occurrence of bird species within the bird community. It is reported as the percentage number of individuals of the respective species, compared to the total number of individuals throughout all species. A species is termed dominant when the dominance value of the respective species was greater than or equal to 5 %.

Flocking behaviour
To distinguish between flocking and non-flocking birds across the different study periods, the number of individual bird contacts were plotted against the number of surveys in which the respective species had been detected. A threshold value of on average more than 5 individual bird contacts per survey was chosen, for bird species occurring only in small groups values between on average 3 – 5 contacts per survey were chosen. Non-flocking species were defined by values on average less than three contacts per survey.

Figure B.9.1.3-3: Example for the number of individual bird contacts versus the number of surveys with positive records during a defined survey period to evaluate numerical occurrence of bird species.



The shaded areas indicate different models of aggregation (dark grey = non-flocking, light grey = species in small groups, white = flocking species)

Abundance
The abundance is defined as the number of individuals per surface area, here referred to as number of birds per hectare (individuals/hectare). The underlying size (ha) for area related calculations was defined by the field length and width of the 'in-crop transect band' (100 m). This allows for fields of different sizes to be compared.

Candidates for focal species
Two different types of criteria were used to generate a list of candidate focal species.

1. Frequency of occurrence (FO) and dominance of birds in the 'in crop transect band' obtained from the field survey.
2. General information on the size and ecology of birds to group the species by guilds based on size, diet, and foraging stratum in accordance with the SANCO guidance document.

Candidate focal bird species were defined primarily as those with an FO_{field} ≥ 20 %, but taking account of FO_{survey} and dominance.

The ranking of species within the list of focal species candidates was then carried out according to the following parameters in decreasing order of importance; FO_{field} > FO_{survey} > dominance. These candidate focal species were then assigned to defined foraging guilds, diet guilds and size classes.

Guilds
The three general criteria for assigning bird species to ecological guilds were the following:

1. Size- small, medium or large as classified in SANCO 2002.
2. Diet- insectivorous, granivorous or herbivorous as in SANCO 2002, most bird species occurring on agricultural land are omnivorous.
3. Foraging stratum- habitat utilisation in terms of foraging strata.

In addition, published data from Perrins, 1998 were employed.

Table B.9.1.3-31: Listed categories of size classes (body weight), diet and foraging stratum

Size classes	Diet	Foraging stratum
Small (< 50 g) Medium (50 – 500 g) Large (> 500 g)	Insectivorous Granivorous Herbivorous Omnivorous	Ground-foraging Foliage foraging Combined stratum user

Once the list of candidate species was available they were grouped as follows; the selected species were grouped by size, then they were grouped according to their predominant guild within the size class, and then by foraging stratum.

Results and discussion

Frequency of occurrence

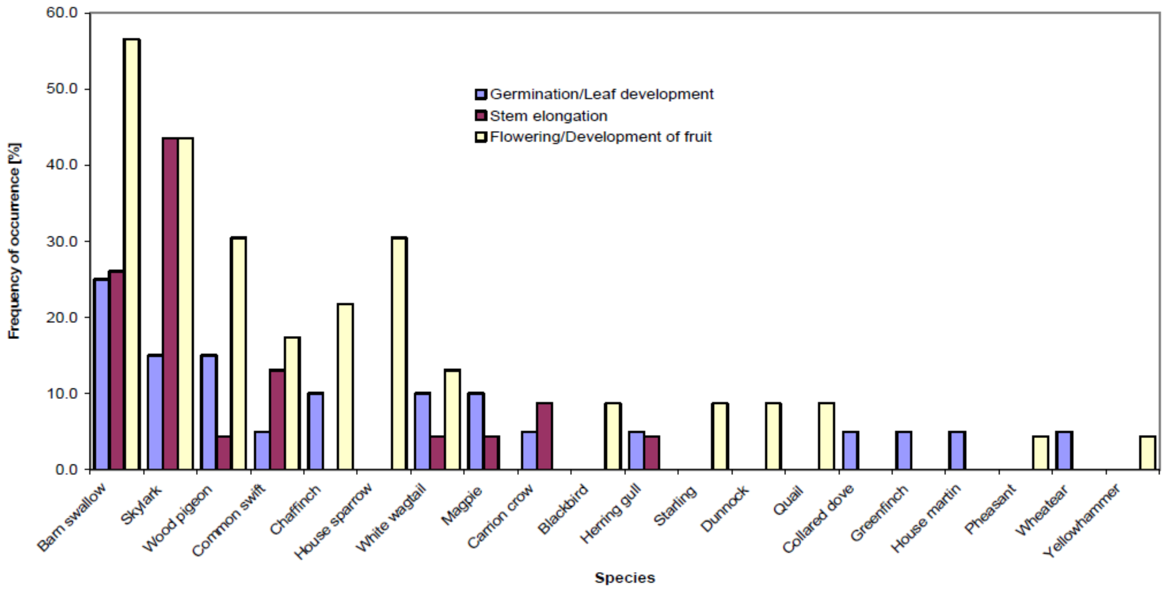
A total of 323 individual bird contacts, comprising 20 different species, was recorded throughout all surveys within the 'in-crop transect bands'. The following seven species listed in the table below were recorded with a frequency of occurrence (FO_{field}) exceeding 20 % and were thus determined as the main candidates for focal bird species. The findings were supported by FO_{survey} and dominance data (see table below).

Table B.9.1.3-32: List of focal bird species candidates grouped by frequency of occurrence in pea fields in Brittany (northern France) ([redacted] et al. 2006)

Species	FO_{field} n = 22 [%]	FO_{survey} n = 66 [%]	Dominance n = 22 [%]
Barn swallow (*Hirundo rustica*)	72.7	36.4	19.8
Skylark (*Alauda arvensis*)	68.2	34.8	15.5
Wood pigeon (*Columba palumbus*)	45.5	16.7	25.7
Common swift (*Apus apus*)	36.4	12.1	7.1
House sparrow (*Passer domesticus*)	31.8	10.6	7.7
Chaffinch (*Fringilla coelebs*)	31.8	10.6	2.8
White wagtail (*Motacilla alba*)	22.7	9.1	3.4

There was variation in the frequency of occurrence for recorded bird species during different growth stages. The relative consistency of occurrence is noted for the barn swallow over all three growth stages while it is more variable for others.

Figure B.9.1.3-4: Frequency of occurrence of bird species utilising pea fields in Brittany (northern France) during different plant growth stages



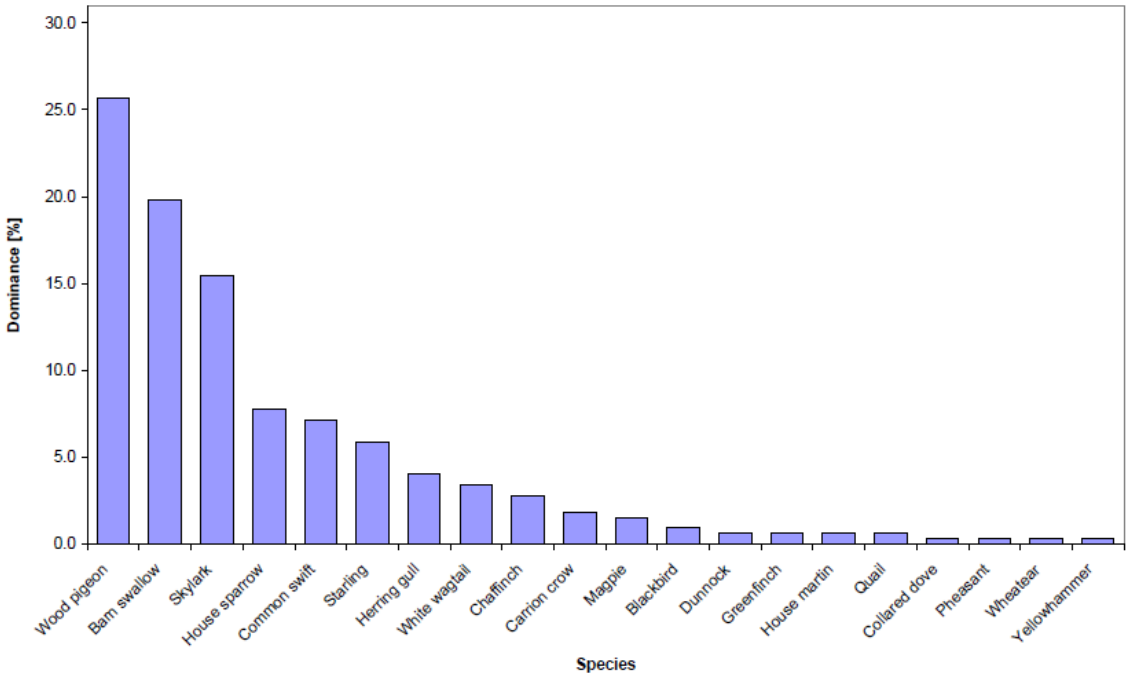
During germination/leaf development 13 species were recorded in the pea fields, of which only one species showed a frequency of occurrence of at least 20 %, the barn swallow (25 %). During stem elongation two out of a total of eight species showed

FO values of at least 20 %, the skylark (43.5 %) and the barn swallow (26.1 %). During flowering/development of fruits five out of a total of 13 recorded species showed a frequency of occurrence exceeding 20 %, the barn swallow (56.5 %), skylark (43.5 %), wood pigeon (30.4 %), house sparrow (30.4 %), and chaffinch (21.7 %). Only five species were present in all three study periods.

When analysing bird frequencies at the three different pea stages the focal species determined showed some variation with regard to their occurrence over time. The barn swallow showed moderate FO values with a peak (56.5%) during the third survey. The skylark showed low FO during the first survey but increased afterwards (43.5% during second and third surveys). The wood pigeon, chaffinch and house sparrow were absent or at least scarce during the first two surveys but displayed higher FO values during the third survey period (30.4%, 21.7% and 30.4%, respectively). Other focal species candidates did not exceed FO values of 20% during different survey periods.

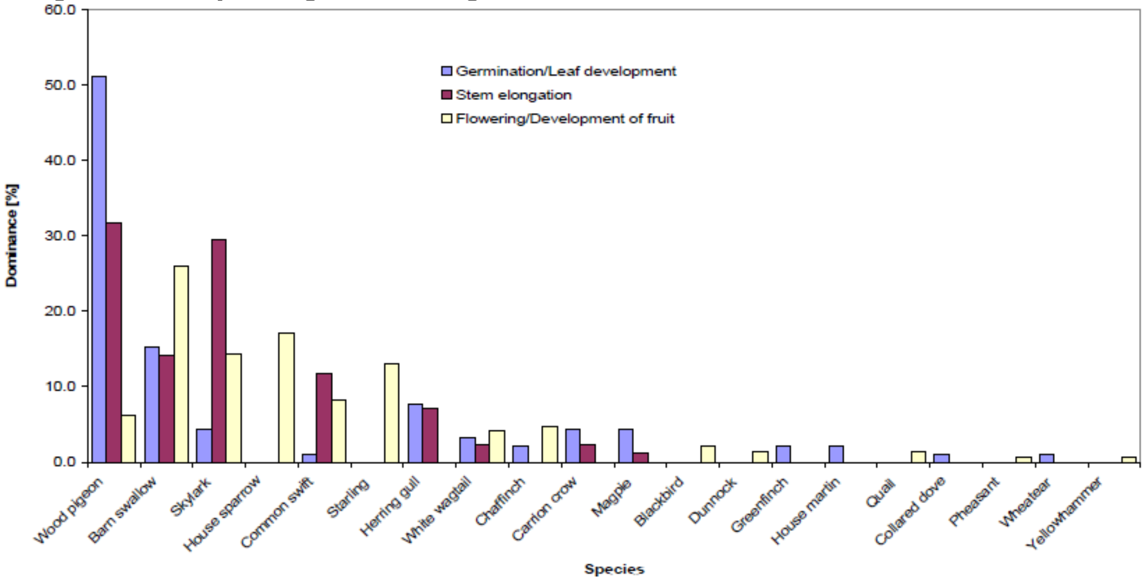
Dominance
The figure below shows the dominance distribution of birds in the pea fields. Only wood pigeon had a dominance higher than 20 % (25.7 %).

Figure B.9.1.3-5: Dominance distribution of birds occuring in pea fields in Brittany



During germination/leaf development 13 species were recorded, of which three species showed dominance exceeding 5 %, wood pigeon (51.1 %), barn swallow (15.2 %), and herring gull (7.6 %). During stem elongation five of a total eight species showed dominance greater than 5 %, wood pigeon (31.8 %), skylark (29.4 %), barn swallow (14.1 %), common swift (11.8 %) and herring gull (7.1 %). During flowering/development of fruit six species out of 13 showed a dominance over 5 %, barn swallow (26 %), house sparrow (17.1 %), skylark (14.4 %), starling (13.0 %), common swift (8.2 %), and wood pigeon (6.2 %).

Figure B.9.1.3-6: dominance of bird species utilising pea fields in Brittany during different plant growth stages



Only five species were present during all three survey periods but with varying respective dominance values. Over the period as a whole, the barn swallow showed the most constant dominance values with a peak during the third survey.

Determination of focal species

The frequency of occurrence (FO_{field}, FO_{survey}) and dominance were considered to be the decisive parameters for the derivation of focal species at a given period of time which could be used to assess the risk of plant protection products to wild species in a refined risk assessment. The major criterion for selection was the of candidate focal species was FO_{field}, the ranking of species based on FO_{survey} showed no differences compared to FO_{field} values.

FO_{field} values were given more significance than FO_{survey} or dominance values. However, FO_{survey} can indicate the importance of the crop for a particular species across several growth stages, and the dominance value will help to identify flocking species compared to rare species.

The species proposed as candidate focal species in pea fields in Brittany (northern France) for at least a certain period are summarised below.

Table B.9.1.3-33: List of focal bird species candidates grouped by size (body weight), dietary guild and foraging stratum according to seasonal aspects in pea fields in Brittany (northern France)[a] ([redacted] et al. 2006)

Species	FO_{field} (%)	FO_{survey} (%)	Dominance (%)	Body weight (g)	Stratum use	Diet guild
Barn Swallow	72.7	36.4	19.8	15.8	Aerial	Insectivorous
Skylark	68.2	34.8	15.5	37.2	Ground	Omnivorous

Wood Pigeon	45.5	16.7	25.7	490.0	Ground	Herbivorous
Common Swift	36.4	12.1	7.1	37.6	Aerial	Insectivorous
House sparrow	31.8	10.6	7.7	27.4	Ground/foliage	Omnivorous
Chaffinch	31.8	10.6	2.8	20.9	Ground/foliage	Omnivorous
White wagtail	22.7	9.1	3.4	21.0	Ground	Insectivorous

The applicant had only considered species candidates with FO values ≥ 20 %, HSE has considered presented all the potential focal species above.

The recorded candidates of focal bird species were assigned to the following guilds in accordance with the SANCO guidance document (ranked by their respective frequency of occurrence and dominance):

- Small insectivore barn swallow > common swift (all aerial) > white wagtail (ground)
- Small omnivore skylark (ground)> house sparrow > chaffinch (all ground/ foliage)
- Medium herbivore wood pigeon (ground)

Conclusions

The barn swallow and skylark were the most characteristic elements of the bird community in pea fields in the Brittany region of northern France across all pea growth stages. Other species, showing peak FO values ≥ 20 % for individual pea growth stages only were the wood pigeon (survey 3), chaffinch (survey 3) and house sparrow (survey 3). All the species listed in the table above can be considered as potential candidates for focal bird species in a refined risk assessment for plant protection products in pea fields in France.

([redacted], [redacted] and [redacted], 2006)

HSE comments

HSE considers the above study to be reliable for the determination of focal species in pea fields in Brittany. Transect counts were performed three times in 22 pea fields to represent average pea fields. The three transects covered the growth stages of peas including BBCH 00 – 19, 30 – 39 and 60 – 79. As the proposed use of ‘Wakil’ is as a seed treatment, BBCH 00 – 19 covers the proposed use.

The study took place in Northern France, which has a similar climate to the UK, however, meteorological data was not recorded during the study. During the transect counts a score was awarded to the weather: 1 = fair, 2 = cloudy, 3 = rainy, 4 = windy. As such, HSE cannot effectively compare the weather for the duration of the study to determine if it is representative of the weather expected during the use of the product.

This study was conducted from April to June, the proposed sowing dates for vining peas in GB using the product 'Wakil' is February to mid-April. The study period does not fully line up with the expected use for GB. However, regarding potential focal species identified in the study, April to early August is the breeding season for skylarks. Skylarks prefer a crop height of 20 – 50 cm for nesting purposes, therefore the study period would potentially attract more skylarks for nesting, resulting in a 'worst case' scenario as more skylarks would be attracted to the shorter vegetation. wood pigeons nest from February to early September, preferring to nest in trees and hedges. The study period does partially cover the nesting season of wood pigeons.

Focal species was determined using the frequency of occurrence as the decisive parameter, however, dominance was also considered. HSE agrees with this approach as it takes into account any flocking birds. Overall HSE accepts that the barn swallow and skylark were the most frequently occurring species in the pea fields in Brittany, followed by the Wood Pigeon. However, barn swallow are insectivorous and are not expected to forage at ground level, therefore they are not considered a relevant focal species for the current risk assessment, where the focus is on birds consuming treated seed or seedlings. When feeding guilds are taken into account, the skylark and the wood pigeon are the most relevant species for the product 'Wakil' as small omnivores and medium herbivores respectively. It is considered that both of these species could have both seed and seedling components to their diets, when foraging in freshly drilled vining pea fields.

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	KIIIA 10.1.7/07, [redacted] & [redacted] (2010) Exposure of birds in maize fields in France - Attractiveness of maize fields, relevant species, diet composition and portion of time. [redacted]. Report Number [redacted]. Syngenta File Number VV-393540

Guidelines
No guideline applicable. The study is consistent with guidance provided in EFSA Journal 2009; 7(12).

GLP: Yes

Study Design and Methods:

Study site

The study was conducted in the South of France in a typical maize growing region south of Toulouse in the departments Haute-Garonne and Ariège.

Methods

The study was conducted in spring 2009 and spring 2010.

Focal species assessment

To confirm the focal species selection, ten study fields were observed at different principle growth stages of maize: immediately after drilling (BBCH 0), after emergence of the seedlings (BBCH 10-11) and in the multi-leaf stage (BBCH 12-16). Observations were made in form of scan sampling lasting for three hours. During these sessions the number of individuals of all bird species occurring on the study fields and their behaviour (foraging or other) was recorded every ten minutes. The abundance, proportion of foraging individuals and frequency of occurrence per scan and per study field was calculated for each species recorded.

PT assessment

Twenty carrion crows and thirteen skylarks were trapped in 2009. Fourteen woodpigeons, 15 starlings and ten crested larks were trapped in 2010.

All skylarks and crested larks monitored during the study were trapped in or in the close vicinity of maize fields (exact distances were not provided in the study report). Woodpigeons were trapped at tree rows, shrubs or forests close to maize fields. Carrion crows and starlings were trapped close to their nesting sites. As stated in the report it was intended to trap preferably at those nests which were adjacent to maize fields however this did not occur for all individuals. The report justified this on the basis of the carrion crows and starlings having ‘home ranges’ that extend over a wide area therefore the chance of them using maize fields was still high, even if the nest or trapping place for some individuals was not adjacent to a maize field. A mixture of methods for trapping birds were used including Larsen and spring, whoosh net, mist nets and inside nest boxes equipped with a trapping device.

All birds were marked individually with one metal and one or more colour rings and equipped with a radio transmitter, allowing the subsequent tracking of the individuals. The birds were tracked continuously over one whole daily activity period (from dawn till dusk), trying to keep the bird under continuous visual observation. To increase the number of tracking sessions, some individuals were tracked twice on different days, which resulted in a total of 24 tracking sessions of carrion crows, 19 tracking sessions of skylarks, 20 tracking sessions of woodpigeons and 11 sessions of crested larks.

For starlings, individuals were only tracked once thus the total number of tracking sessions remained as 15. Some sessions had to be excluded from analysis as the surrounding maize fields were not yet drilled when the tracking was conducted. The proportion of time foraging in maize was estimated for each species using data obtained by radio tracking and visual observation. In addition, proportion of time foraging was also estimated in cereals, sunflower and bare fields pre-drilling. These values were regarded as proportional to diet obtained from the treated area (PT).

Diet selection of the focal species was determined by inspection of faecal samples. For skylarks and crested larks the dry weight proportions of the diet were calculated using correction factors (according to Green 1978) applied to the fragment area of items found in their faeces. Since no correction factors were available to determine the dry weight proportion of food items in the diet of carrion crows, woodpigeons and starlings, uncorrected proportions had to be estimated from faecal samples and are given as proportions of fragment areas of items found in their faeces.

On ten study fields the exposure of maize seeds remaining on the soil surface after drilling was estimated. Counts of maize seeds within a frame of 0.25 m² were conducted every five meters along a 100 m transect in the headland and midfield areas of a study field, respectively.

In order to record any foraging damage to the maize crop potentially caused by birds a sample of maize seedlings was observed once after emergence (BBCH 10-11) and once during the principle growth stages BBCH 12- to 16. Therefore two transects of 100 m were marked on five maize fields. One transect was placed besides the fifth row in from the field border, the other in the centre of the study field (at least 10 m apart from the field order). On both sides of the respective transect two rows of maize seedlings were surveyed for any damage. The total number of maize seedlings inside the monitored rows was counted as well as the damaged ones. The damaged plants were allocated to a damage score (up to 10% missing, between 10% and 50% missing, more than 50% missing, seedling extracted). It was not possible to determine a number of missing plants in one row, as it was not clear if a gap in the row was resulting from a drilling mistake (seed not planted), delayed emergence or from foraging. Plants were regarded as damaged by birds only when all other sources of damage could be ruled out (e.g. damage by insect feeding). The exception was damage by lagomorphs, which could not be distinguished from birds. In this case bird damage recorded represented the worst case.

Statistical calculation of Jacobs index, PT and PD

PT: For PT behaviour was assessed and assigned to one of five categories: potentially foraging (foraging and active), other, reproduction, inactive and unknown (due to lack of signal). The total time a bird is present in all known habitats including maize and the total time the bird is present potentially foraging is recorded during an individual tracking session and the PT is calculated as:

$$\frac{\textit{Potentially foraging time in maize}}{\textit{Potentially foraging time in all known habitats}}$$

As detailed in the later section HSE have calculated the PT values for individuals for all species in emergence (BBCH > 9) in maize for the five species. ‘Consumers only’ PT values were calculated by using PT values for potentially foraging behaviour identified in post emergence maize BBCH >9.

<u>PD:</u> dry weights were measured and correction factors (Green 1978) were applied for all species except the carrion crow, wood pigeon and starling. For these species the fragment area in mm^2 of each food type found in their faeces was measured. For these three species no correction factors are currently available from the literature. In order to quantify their diet selection the fragment area in mm^2 of each food type found in their faeces was measured. For each faeces sample the proportion each food type contribute to the total fragment area was calculated. The specification obtained by this approach solely describe the fragment area proportions of different food types in the faeces and not the dry weight proportions of the diet originally ingested. According to Green (1978) earthworm chaetae were given an arbitrary value of 10% of the total fragment area. For vertebrate remains (e.g. bones, hairs) the same procedure was applied. Diet proportions expressed in 'area' are very similar to 'volume' proportions and much better than 'frequency' proportions that overemphasises smaller food items.

<u>Jacobs index:</u> Analysis of the birds' preference for feeding habitats was done by using the Jacobs' preference index (Jacobs 1974). This index was calculated for each tracking session on the basis of 'minimum convex polygon home range'. The values range from -1 to +1 ('-1': complete avoidance; '+1': exclusive preference; '0': neutral: no avoidance nor preference, i.e. a proportional use of the habitat in relation to its proportion in the home range). The index was calculated using the following equation:

$$[D] = \frac{(r - p)}{(r + p - 2rp)}$$

r is the proportion of time that a bird used a habitat for 'potentially foraging'

p is the spatial proportion of that habitat within the calculated home range

Results

Bird monitoring

Thirty-two bird species were observed during scan sampling sessions on maize fields from drilling through to BBCH principal growth stage 16. The table below gives an overview of the results for all species showing a frequency of occurrence per study field equal or higher than 20.0%. The results were calculated taking all scan sampling sessions into account.

Table B.9.1.3-34: Overview of the scan sampling results for all points in time combined ranked after FO_{field}

Species English name	Scientific name	contacts	A[1] [ind/ha]	F ind[2] [%]	FOscan[3] [%]	FOfield[4] [%]
Carrion crow	*Corvus corone*	81	0.02	98.77	8.25	90.00
Magpie	*Pica*	55	0.01	87.27	5.26	60.00
Wood pigeon	*Columba palumbus*	10	< 0.01	100	1.40	50.00
Starling	*Sturnus vulgaris*	137	0.04	96.35	7.19	50.00
Blackbird	*Turdus merula*	12	< 0.01	75.00	1.93	40.00
Skylark	*Alauda arvensis*	18	< 0.01	22.22	2.63	30.00
Tawny pipit	*Anthus campestris*	3	< 0.01	33.33	0.53	30.00
Crested lark	*Galerida cristata*	58	0.03	51.72	6.67	30.00
Jay	*Garrulus glandarius*	9	< 0.01	100	1.40	30.00
Stonechat	*Saxicola torquata*	17	0.01	41.18	1.75	30.00
Red-legged partridge	*Alectoris rufa*	32	0.01	50.00	2.98	20.00
Common buzzard	*Buteo*	6	< 0.01	33.33	0.70	20.00
Kestrel	*Falco tinnunculus*	4	< 0.01	50.00	0.70	20.00
House sparrow	*Passer domesticus*	15	< 0.01	66.67	0.53	20.00
Pheasant	*Phasianus colchicus*	2	< 0.01	50.00	0.35	20.00
Black redstart	*Phoenicurus ochruros*	8	< 0.01	50.00	1.05	20.00

[1] Abundance
[2] Foraging individuals
[3] Frequency of occurrence per scan
[4] Frequency of occurrence per field

The abundance was also calculated for single dates, when scan sampling was conducted. After drilling, the carrion crow and magpie received the highest mean abundance values. The crested lark, the starling and the carrion crow were on average most abundant after emergence of maize seedlings.

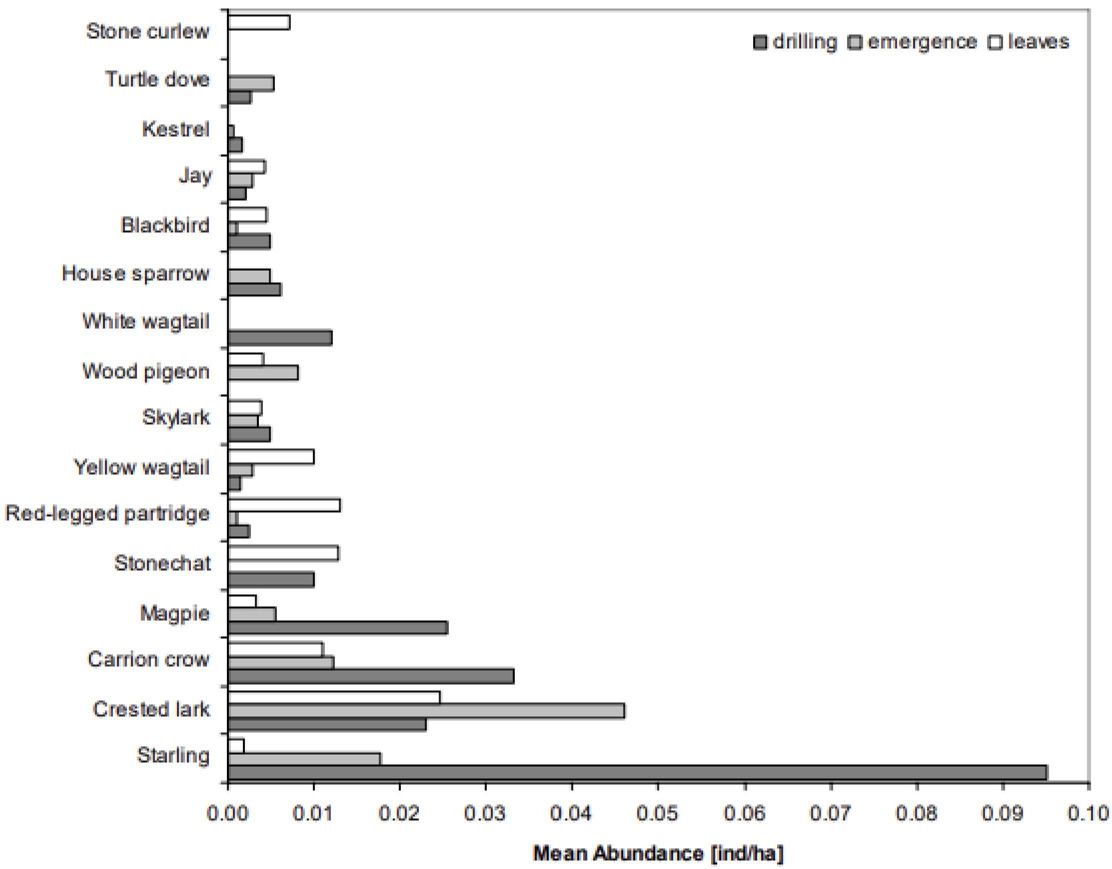


Figure B.9.1.3-7: mean abundance by maize crop stage (at drilling, emergence (BBCH 10-11) and leaves (BBCH 12-16))

PT values and Jacobs' index [D]

Results are presented in the tables below. Radio-tracking combined with visual observations allowed an accurate and representative assessment of potential foraging times in given home ranges in order to calculate reliable PT values.

All tracking sessions were considered in the PT calculation with individuals being potentially consumers on maize fields. The main results section also presents the PT for individuals with maize in their home range and for individuals using maize fields for potentially foraging only. PT was expressed for maize fields in the 'pre-emergence' and 'post-emergence' principal growth stages and for maize fields in total (including all principal growth stages).

Table B.9.1.3-35: Overview of PT values in maize

Proportion of diet obtained in maize fields determined by radio tracking (PT) = 'potentially foraging' time in maize as a proportion of the total 'potentially foraging' time									
Species	**pre-emergence**			**post-emergence**			**total**		
	mean	**50%tile**	**90%tile**	**mean**	**50%tile**	**90%tile**	**mean**	**50%tile**	**90%tile**

Skylark (n[1]=19)	<0.01	0.00	0.00	0.09	0.01	0.17	0.09	0.01	0.17
Woodpigeon (n[1]=20)	0.03	0.00	0.06	0.05	0.00	0.19	0.08	0.00	0.21
Starling (n[1]=15)	0.02	0.00	0.07	0.06	0.01	0.15	0.07	0.06	0.16
Crested lark (n[1]=11)	0.12	0.00	0.28	0.34	0.33	0.65	0.45	0.44	0.75

[1] n = number of sessions

The calculation of the Jacobs' index provided additional information on habitat preferences of focal species (table below). It compares the PT for each bird in maize with the proportion of maize in the home range. A positive Jacobs' index indicates preference for the habitat in question; a negative Jacobs' index represents avoidance of this habitat type.

Table B.9.1.3-36: Overview of Jacobs' indices

Species	Jacobs' index for maize [D]	
	mean	90%ile
Carrion crow	-0.10	0.58
Skylark	-0.66	-0.30
Woodpigeon	-0.50	0.56
Starling	-0.48	-0.09
Crested lark	0.18	0.60

Diet composition

The results of faecal analysis for carrion crows, woodpigeon, starling, crested lark and skylark are given below. Data for skylark are presented for chicks as well as adults. It was noted that appendix 3 on p149 of the final report mentions estimated length rather than dry weights for the crested lark for the PD section. Faeces samples were collected in 2009 and 2010, after the bird was trapped.

Table B.9.1.3-37: Overview of the faeces analysis

Type of food	Carrion crow	Skylark		Woodpigeon	Starling	Crested lark
	Mean content of faeces samples mean area proportion [%] (n=15)	Mean proportion of dry weight in the diet, PD [%]		Mean content of faeces samples mean area proportion [%] (n=6)	Mean content of faeces samples mean area proportion [%] (n=18)	Mean proportion of dry weight in the diet, PD [%] (n=2)
		adults (n=6)	chicks (n=26)			
Animal matter	56.62	75.53	95.55	7.26	75.78	84.69
Seeds (not maize)	12.88	21.11	1.92	26.98	0.29	11.84

Maize seeds	-	-	-	39.22	-	3.47
Leaves	12.68	3.34	2.53	-	7.35	-
Fruits	17.82	-	-	26.44	15.49	-
Other plant matter	-	-	-	0.10	1.09	-

<u>Exposure assessment</u>
The table below shows the results of the conducted counts of maize seeds lying visible on the soil surface.

Table B.9.1.3.38: Overview of the exposure assessment

	Headland	**Midfield**
Quadrat-drops	200	200
Number of quadrats per field	20	20
Area sampled per field [m^2]	5	5
Area sampled in 10 fields [m^2]	50	50
No. of quadrats with seeds	8	3
Total number of seeds found	8	3
Mean density of exposed seeds [seeds/m^2]	**0.16**	**0.06**
SEM[1]	0.07	0.03
Min of all fields [seeds/m^2]	0.00	0.00
Max of all fields [seeds/m^2]	0.60	0.20

[1] Standard error of the mean

Conclusion

Scan samples confirmed the focal species in maize. This study provides measured estimates of PT and diet for use in risk assessments for five species in maize fields after drilling.

([redacted]. and [redacted]., 2010**)**

HSE comments:

This study was evaluated previously for the product ‘Calaris’, and the evaluation can be found in Part B, Section 6 Ecotox, W001591998. HSE has taken into account the original evaluation for consideration of the study’s relevance to this application.

This study was conducted to GLP and was conducted in line with EFSA guidance. For some species the number of birds tracked was relatively low (e.g. crested lark n=6). In addition there were a total of 16 species observed but according to the field scans a number of these species were not frequently observed (10 in total which were 30% and above).

This study was conducted in the Southern zone of the EU, however the proposed GAP is for GB. Furthermore no weather data was provided. The proposed use of

'Wakil XL' and 'Vibrance SB' are as a seed treatment, this GAP is covered by the study which made observations during BBCH 0, BBCH 10 -11, and BBCH 12 – 16.

The crop types proposed in the GAP are vining Peas for 'Wakil XL', and sugar and fodder beet for 'Vibrance SB'. The study does not cover the proposed crop type, therefore there is some uncertainty as to the relevance for determining focal species. This point will need to be considered further in the risk assessment section.

The months for application of 'Wakil XL' would be from February to mid-April whilst the 'Vibrance SB' would likely be drilled March – April. As such, the study period will cover a portion of the time for the prosed use of 'Wakil XL', and most of the proposed use of 'Vibrance SB'.

PT values were determined for the pre-emergence period for the 'consumer' population. Data for wood pigeons are described here. There were only 4 consumer individuals that were tracked, with PT values ranging from 0.02-0.35. Given the small sample size, calculation of a 90th percentile PT value is not considered appropriate.

Session number(s) for individual bird	PT in pre-emergence maize
49	0.35
53	0.19
58	0.02
68	0.04

PT values were also determined for the post-emergence period for the 'consumer' population. Data for wood pigeons and skylarks are described here.

- Wood pigeon – There were PT data for 7 individual consumer population birds, covering 8 tracking sessions. For the bird which was tracked for 2 sessions, a mean PT of 0.19 across the 2 sessions is used. PT values per individual ranged from 0.01 to 0.2. Given the small sample size, calculation of a 90th percentile PT value is not considered appropriate.

Session number(s) for individual bird	PT in post-emergence maize
53	0.07
58	0.01
59	0.20
62, 87	0.19
68	0.05
83	0.20
91	0.13

- Skylark – There were PT data for 9 individual consumer population birds, covering 10 tracking sessions. For the bird which was tracked for 2 sessions, a mean PT of 0.12 across the 2 sessions is used. PT values per individual ranged from 0.01 to 0.91. Given the small sample size, calculation of a 90th percentile PT value is not considered appropriate.

Session number(s) for individual bird	PT in post-emergence maize
22, 36	0.12
26	0.01
27	0.01
29	0.20
32	0.06
34	0.13
38	0.06
40	0.91
41	0.12

For the PD assessments dry weights were measured and correction factors were applied for all species except the carrion crow, wood pigeon and starling. Justification was given as described above for the use of the fragment area but the fact dry weights were not used for these species will require further consideration as dry weight is the recommended procedure.

As shown in the methods section a number of pesticides were applied to the study fields in 2009 including insecticides which could lower the attractiveness of the field for insect-eating birds. Although this should be considered it does not invalidate the study.

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	KCP 10.1.7.11, [redacted] (2005) Generic field monitoring of birds and mammals on maize and beet fields in Austria. [redacted]. Report Number [redacted]. Syngenta File Number N/1155 (Data owner BCS, Syngenta access)

Guideline(s)
The monitoring was especially designed for the purpose of this study.

GLP: Yes

Study Design and Methods

Experimental dates: 19 March to 14 December 2004

Objectives: To identify the significance of maize and sugar beet seeds and seedlings in the diets of skylark and wood mice and confirm their status as focal species.

Study area: The study was conducted in and around 5 maize and 5 sugar beet fields in the 'Tullner Feld' to the west of Vienna in Austria. This region is a typical area of maize and sugar beet cultivation in Europe. The study started some weeks before drilling of maize and sugar beet and was completed when the BBCH-code 14 of maize and 16 of sugar beet was reached.

Method and parameters:
The study was conducted between March 19, 2004 and December 14, 2004.

Birds

Relative abundance of birds
As this was a generic field study, no specific substance was tested. Maize and sugar beet fields were monitored for bird abundance and compared to other crops.

Census counts were carried out along five different transects, representing typical agrarian habitats within the region. The five transects were within and in the vicinities of six monitored fields and were assessed at least once a week. The five transects areas covered 65.4 ha and were monitored 10 times during the whole study period. The abundance of birds was estimated in each crop.

Transects were performed during a slow going walk, only birds within 50 m to both sides of the transect were recorded. To calculate the abundance of birds per ha, the monitored area of each field was measured.

Abundance and feeding behaviour of bird species on beet and maize fields
In addition, scan sampling was done in three maize and in three sugar beet fields. Each field was scanned every 10 minutes for at least three days from dawn till dusk to register all present bird species using the field (51 sessions). The whole visible area of the study field was scanned with a binocular and spotting scope. Species, number of individuals, and behaviour were recorded. The observations were made from inside the car to minimise any disturbance to the wildlife. The proportions (%) of the visible area of the observed study field was recorded.

Before drilling the same fields were 'scan sampled' to monitor the species composition on plain fields. This method offers very detailed information of the bird community using these crops as well as species abundance.

Portion of time Skylarks spent on beet and maize fields
From former studies it was known that Skylarks (*Alauda arvensis*) can be considered to be focal species in sugar beet fields. To quantify the use of sugar beet and maize fields by skylarks over the study period, 18 were trapped, tagged with radio transmitters and 16 were tracked for one to four daylight periods respectively.

The individuals were trapped with mist nets, and the radio tags were attached using the 'Hill Harness' method (Hill et al. 1999). Preferably birds which settle inside or near the prospective maize and sugar beet fields were tagged with a radio transmitter.

During each session a skylark was tracked continuously so that the location, habitat and behaviour could be recorded to get information of the home range, habitat selection and time budget of individuals living in or close to maize and sugar beet cultivation.

To analyse the habitat selection of the skylarks while ‘potentially foraging’ the Jacob’s preference index was calculated based on the minimum convex polygon (MCP) home range (Jacobs 1974). This indicates the relation between the portion of time a given habitat was used for ‘potentially foraging’ by the bird and the spatial portion of this habitat within the birds’ home range:

$$D = (r-p) / (r+p - 2rp)$$

r is the portion the habitat was used by the bird for ‘potentially foraging’
p is the portion of the same habitat within the home range of the bird

The index (D) varies from -1 to 0 for negative selection and form 0 - +1 for positive selection.

Table B.9.1.3-39: Jacobs preference index

-1	Complete avoidance
0	Crop neutral (neither avoidance or preference)
+1	Strong preference

Composition of food of songbirds on and around maize and beet fields
To get information about the diet of skylarks and other bird species, faeces were gathered in maize and sugar beet fields and analysed quantitatively for the composition of animal and plant matter. Additionally, the stomachs of songbirds trapped in hedges which abut the sugar beet and maize fields were flushed. The contents were also analysed quantitively for composition.

<u>Grazing damage</u>
To assess the grazing damage in all maize and sugar beet fields, three lines of 50 m at the field margin and the centre of the field were controlled every fourth day following germination. Every seedling was counted, the damage per seedling was estimated in following categories and the causer (insect, vertebrate, unknown) was recorded.

Table B.9.1.3-40: Grazing damage categories

Category	Damage of the seedling
0	No damage
1	1 – 25 %
2	26 – 50 %
3	51 – 75 %
4	76 – 100 %

Results and Discussion

<u>Birds</u>

Study plots relevant to the monitoring of birds were 3, 5, 6, 7, 8, 9, and 10.

Weather

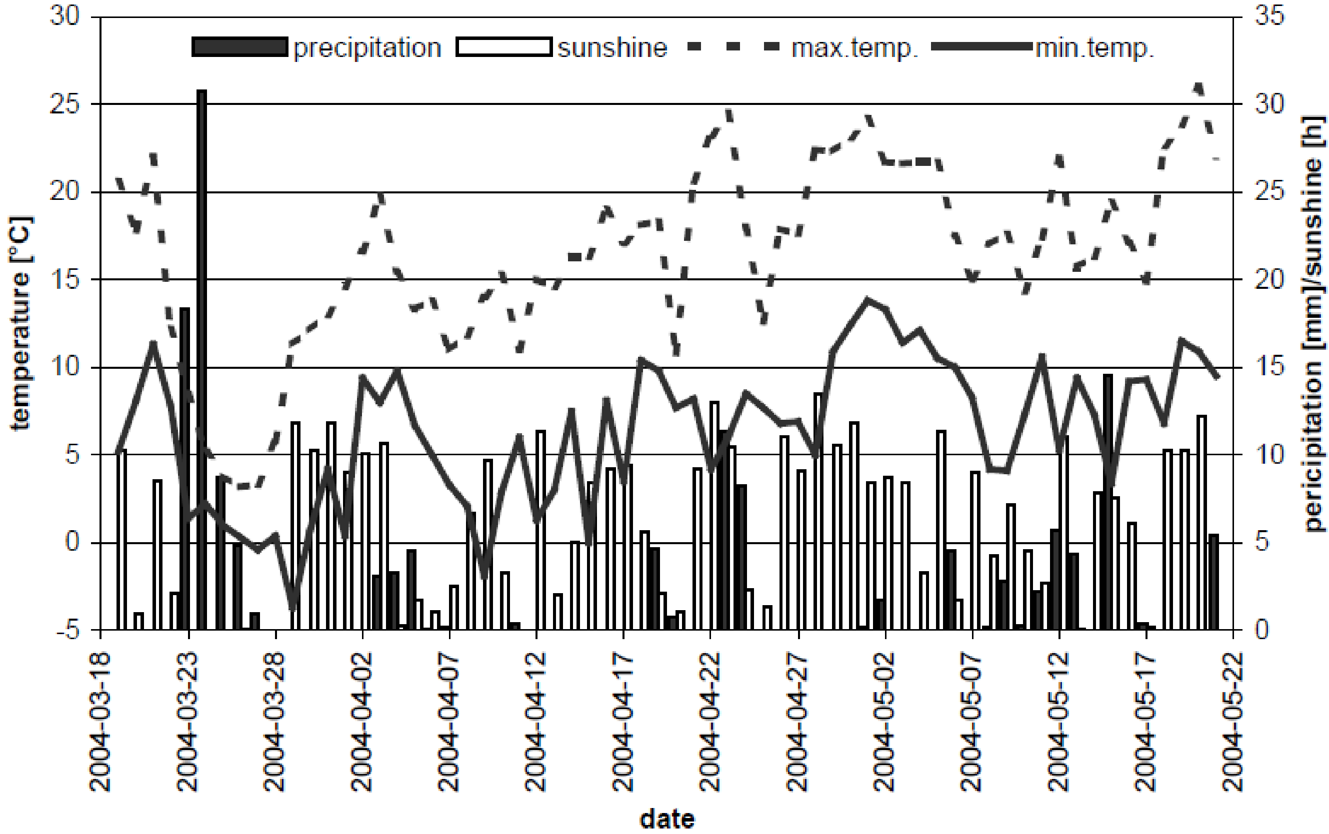


Figure B.9.1.3-8: Climate data provided from the 'Zentralanstalt Meterologie und Geodynamik' Tulln Langebarn.

Relative abundance of bird species in different habitats of the study area
During the whole study period bird transects were performed on five different tracks 10 times respectively. 709 sightings of bird individuals were ascertained during the transect counts. The skylark was the most numerous species with 289 sightings. The Skylark was the most abundant species in plain fields (0.34 ind./ha), drilled maize (0.41 ind./ha), sugar beet fields (0.32 ind./ha), and germinated sugar beet fields (0.31 ind./ha). In germinated maize fields Skylark were the second most abundant species (0.25 ind./ha) with the Carrion Crow being the most abundant (0.29 ind./ha).

Small granivorous birds like Linnets, Greenfinches and Yellow Hammers were found in several crop types. During the transect counts the following species were found only in the surrounding habitats (hedges, trees, path) but never in the fields: Blackbird, Blackcap, Blue tit, chaffinch, Chiffchaff, Great Spotted Woodpecker, Great Tit, Icterine Warbler, Magpie, Nuthatch, Robin, Song Thrush, Stock Pigeon, and Wood Warbler. Barn swallows and House Martins were always flying over, potentially feeding on flying insects connected with the relevant field.

Scan sampling of birds

Plots 3, 5, 6, 8, 9, and 10 were scanned every 10 minutes on nine different days from dusk till dawn. At large 51 sessions and a total of 4319 scans with 3529 bird sightings were made. More than half (28) the sessions took place on bare fields, at least one session was carried out between drilling and germination as well as two after germination on each study plot.

In the bare field stage the scanned fields offered a higher abundance of birds than after drilling or germination of maize, and drilling of sugar beet. Only the fields with germinated sugar beet showed a higher abundance.

In the bare field stage the Skylark was the main species with 38 % of all sightings. After drilling the skylark was sighted 39 % in maize and 56 % in sugar beet. After germination the maize fields were the least attractive habitat with the fewest bird sightings, whereas the sugar beet fields showed even higher numbers of bird sightings after germination.

The high figures of bird sightings in the germinated sugar beet fields were mainly caused by one huge sugar beet field (plot 8) which was adjacent to a rape field holding a large number of breeding Skylarks. After the rape reached above 40 cm, this crop lost its suitability for the Larks and they switched to the adjoining fields. The high numbers of sighting in plot 8 were due to increasing avoidance of the adjacent rape field rather than attraction to the germinated sugar beet itself.

During each scan the observed birds were classified as foraging, labelled as ‘potentially foraging’ if not obviously performing something else. In the majority of cases the birds were regarded as potentially foraging.

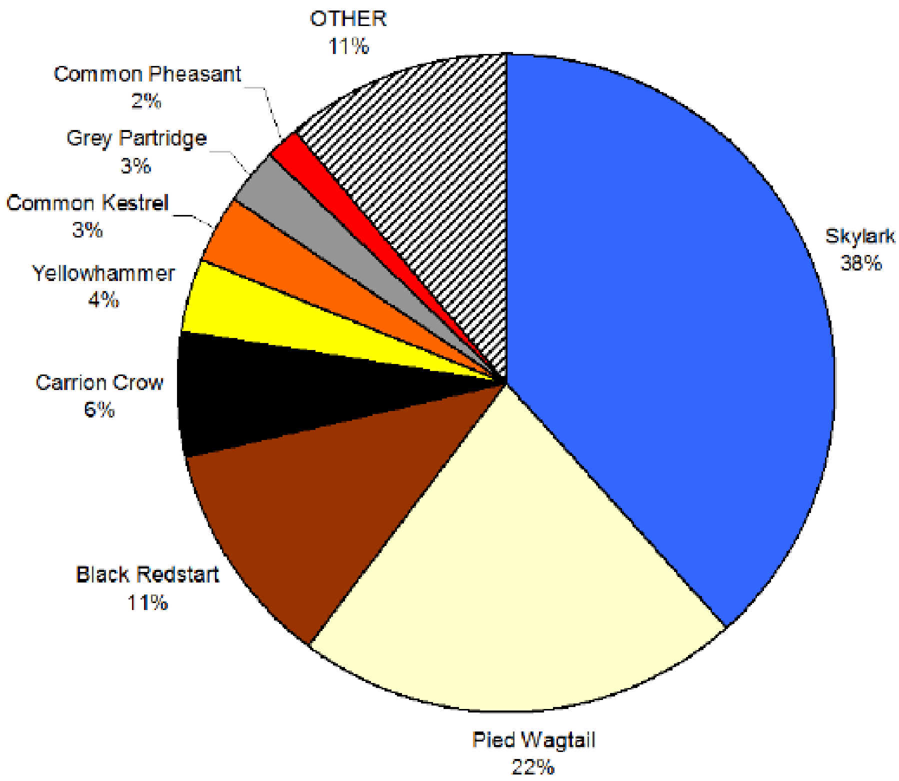


Figure B.1.3-9: Proportion of different bird species in plain fields according to scan sampling

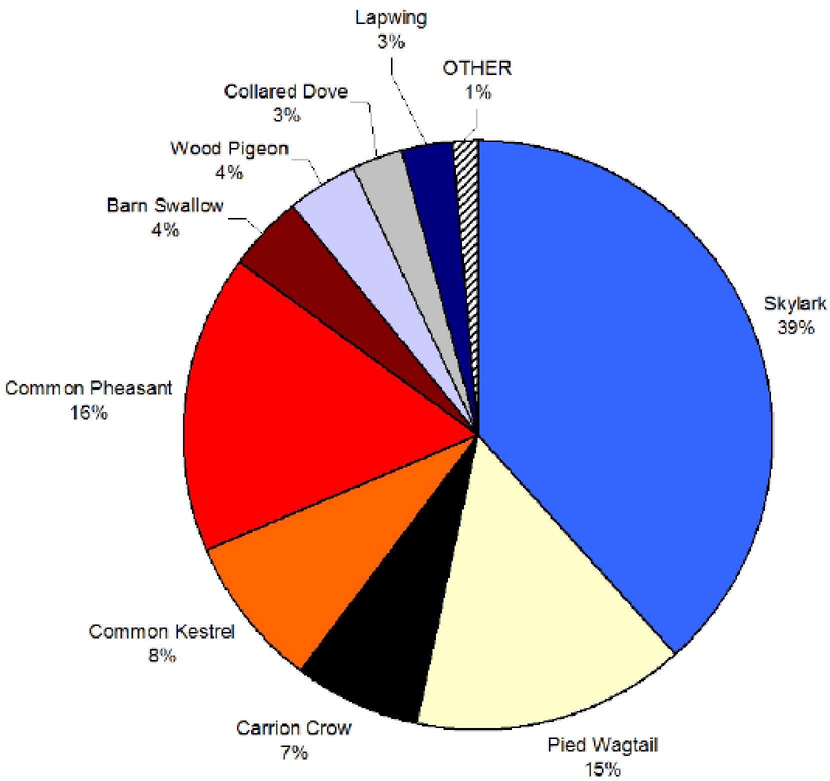


Figure B.9.1.3-10: Portion of bird species in drilled maize fields according to scan sampling

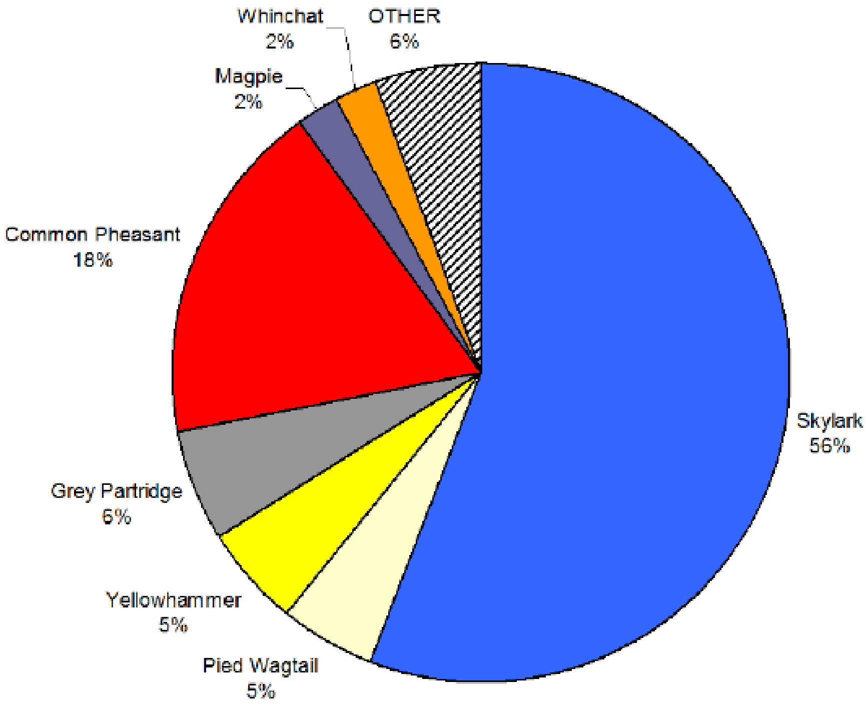


Figure B.9.1.3-11: portion of bird species in drilled sugar beet fields after scan sampling

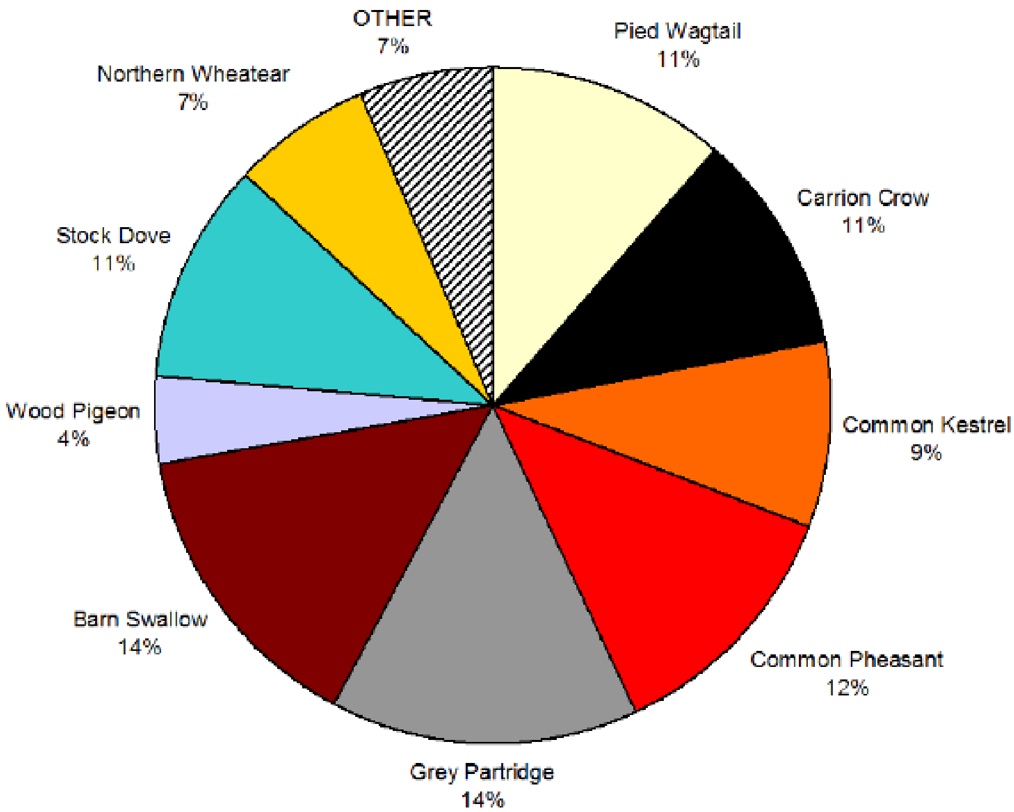


Figure B.9.1.3-12: Portion of bird species in maize fields according to scan sampling

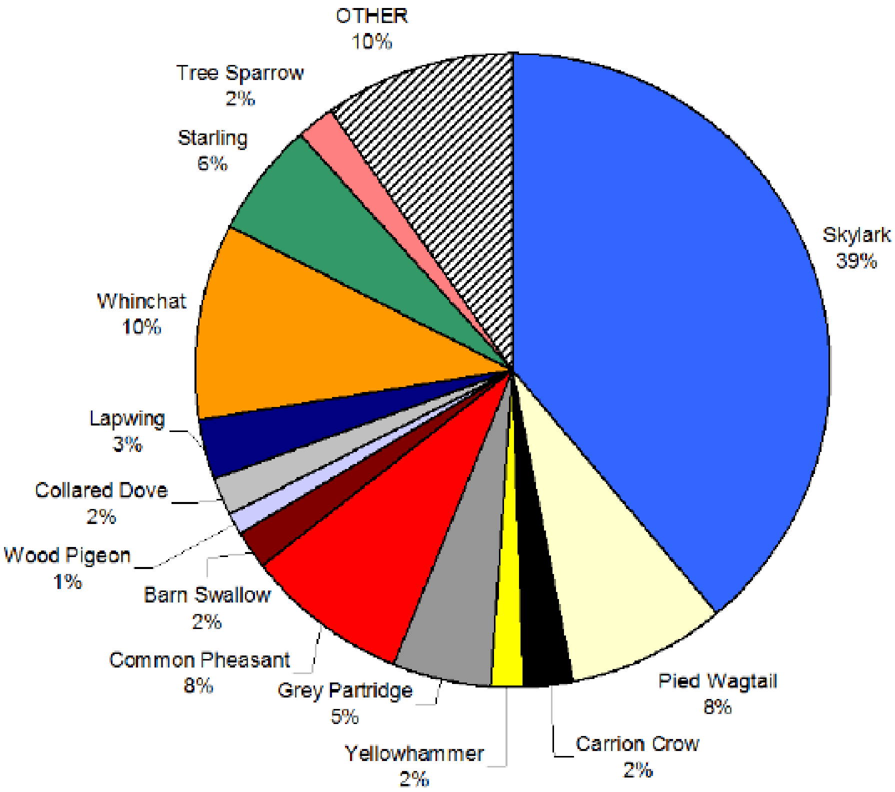


Figure B.9.1.3-13: Portion of bird species in sugar beet fields according to scan sampling

Bird abundance measured by scan-sampling in comparison with transect counts
The abundance of birds offered by the scan sampling method was much lower than the during the transect counts. The main reason for this distinction is due to the different timescales. The transect counts mainly took place during the early morning, the period of highest bird activity.

Radio tracking of Skylarks and tracking data compilation
In total 18 skylarks were trapped and tagged with a radio tag. 13 of the trapped Skylarks were territorial males and 5 were females, at least three of the females were nesting in the immediate vicinity of where trapping took place. One of the birds lost its tag before it could be tracked and another individual left the area after tagging and could not be relocated.

All skylarks were trapped in or close to maize or sugar beet fields. Thus, all Skylarks were offered the opportunity to use these fields as feeding habitats during the course of the study. As such, the selection of birds can be seen as the 'worst case' scenario with regards to exposure to pesticide products.

A total of 30 tracking sessions were carried out on 16 individuals during a period of 43 days. The second excursion of bird 11 produced deficient data and has been excluded from further analysis, thus 29 session of 16 individuals (12 males and 4 females) were used.

For the purpose of this study, the activities ‘foraging’, ‘active and potentially foraging’, and ‘unknown’, have been combined into the classification of ‘potentially foraging’. The potential foraging time of the Skylarks is shown in the table below. The behaviour during the day was heavily dominated by ‘potentially foraging’. Other behaviour was less observed. This could be due to the majority of males within the sample, however, three of the female birds did not differ from the time spent ‘potentially foraging’, and only one female showed a large proportion of ‘reproductive behaviour’.

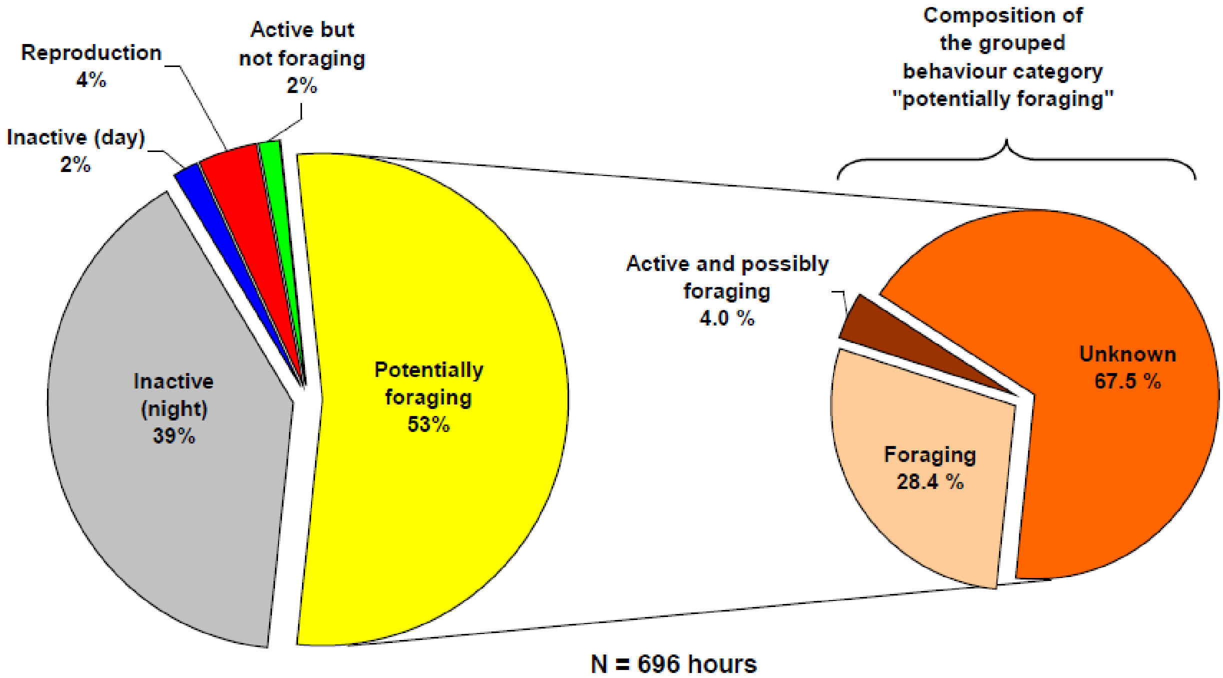


Figure B.9.1.3-14: Summarised proportions of behaviour shown by tracked Skylarks

The habitat selection of the skylarks depended on the availability of habitats within their home ranges. If germinated maize was part of the home range, Skylarks were found there on average for nearly one third (32.4 %) of the tracking time. Sugar beet played a minor role (14.2 %). Tracked Skylarks were found in drilled maize 2.1 % and drilled sugar beet 3.6 %. If winter cereals were a part of the home range Skylarks used fields on average 27 % of the time. The skylarks showed a huge variance in habitat selection during this study.

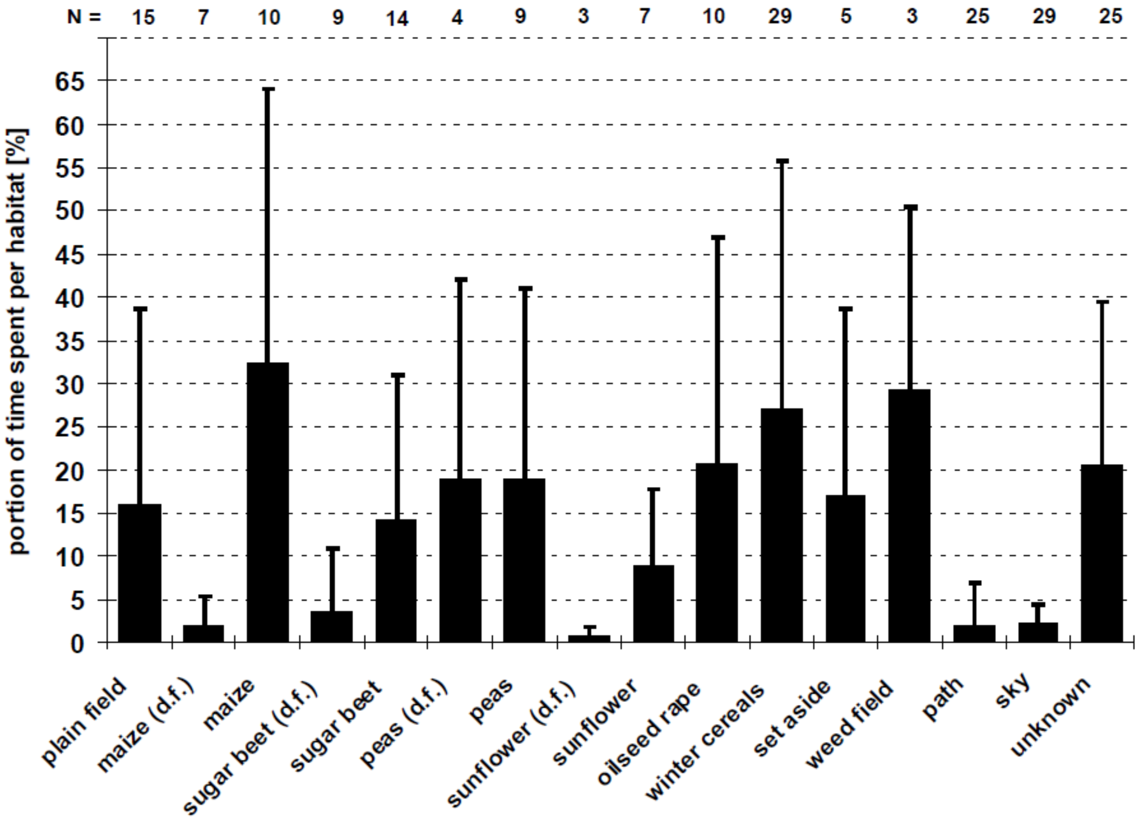


Figure B.9.1.3-15: Mean portion of time individually tracked Skylarks spent per habitat

The mean portion of time Skylarks spent 'potentially foraging' in germinated maize and sugar beet fields was 42.1 ± 39.9 % and 19.7 ± 26.3 % respectively. Within the drilling stage the corresponding values were 4.1 ± 6.4 % for maize and 8.1 ± 19.4 % for sugar beet fields.

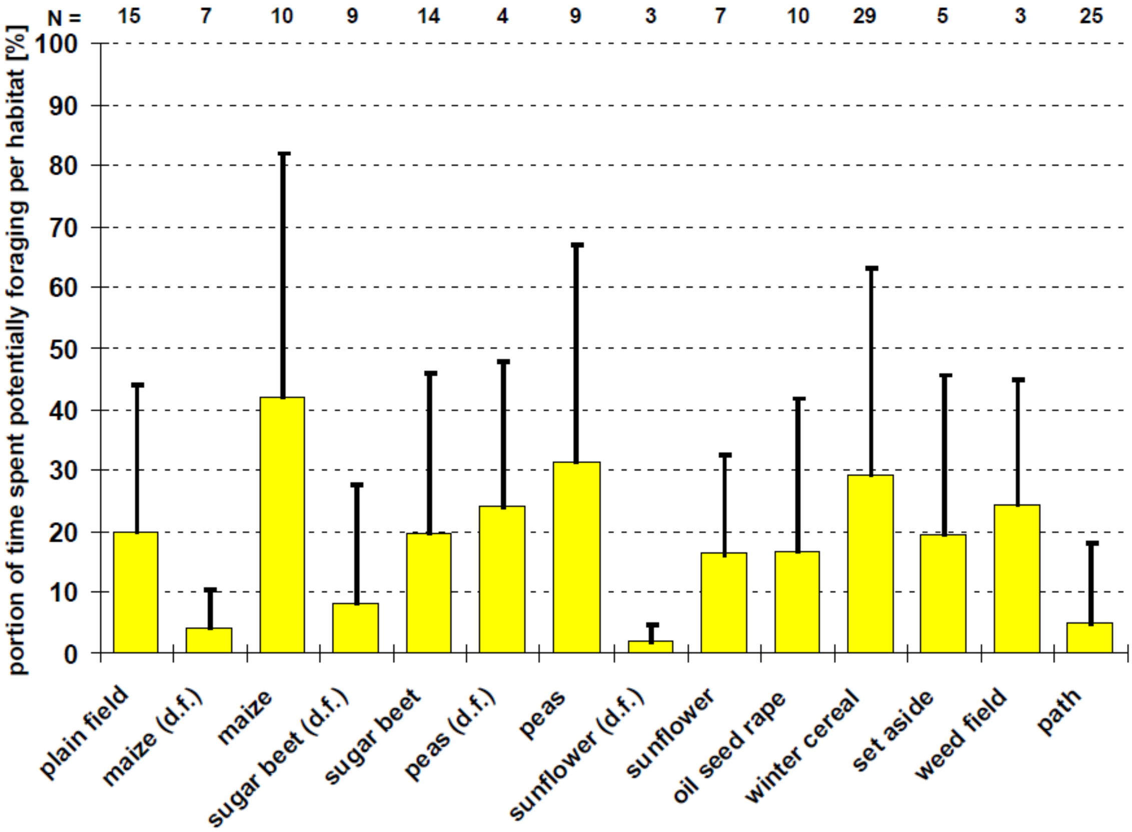


Figure B.9.1.3-16: Mean portion of time Skylarks spent ‘potentially foraging’ (PT) per habitat

Table B.9.1.3-41: Portion of time spent “potentially foraging” per habitat

Portion of time „potentially foraging" per known habitat (%)
each line represents 100%

Bird No. Session No.	Plain field	Maize (drilled field)	Maize	Sugar beet (drilled field)	Sugar beet	Peas (drilled field)	Peas	Sunflower (drilled field)	Sunflower	Oilseed rape	Winter cereals	Set aside	Weed field	Path
1 – 1	72.2					26.8					1.0			0.0
1 – 2	37.1			0.0		61.4					1.4			0.0
2 – 1	3.4					7.9					22.3	66.4		0.0
2 – 2							4.9				64.9	30.1		0.0
3 – 1	19.9									80.1	0.0			
4 – 1	16.0			0.0						23.5	10.3		50.2	0.0
4 – 2			76.6		0.0					0.0	23.4			0.0
5 – 1	70.0			0.0						25.9	0.0		0.0	4.1
7 – 1	6.5			0.0							93.5			0.0
8 – 1	2.3			0.0							34.5			63.2
8 – 2	0.0	13.5									63.8			22.7
8 – 3	21.0	0.0	38.0				0.0				24.2			16.8
8 – 4	0.0	0.0	83.2		0.0		4.8				7.9			4.1
9 – 1				62.0				5.6			32.4			0.0
9 – 2			2.3		82.9				2.5		12.3			0.0
9 – 3					15.5				24.7		56.7			3.1
10 – 1				0.0						0.0	100			
10 – 2	0.0	0.0		0.0						1.1	98.9			0.0
11 – 1	43.3	15.0		11.0						0.3	8.0		22.4	0.0
12 – 1			100		0.0					0.0	0.0			0.0
13 – 1	6.3				55.9		22.7				10.3	0.0		4.8
13 – 2		0.0	2.8		24.9		13.2		49.1		10.0	0.0		0.0
13 – 3					10.3		62.3		23.0		2.3	0.0		2.1
14 – 1		0.0	20.0		0.0		79.2	0.0			0.8			0.0
14 – 2			3.4		0.0		96.6				0.0			0.0
15 – 1					3.6	0.0		0.0	0.0		96.4			0.0
15 – 2			0.0		22.2		0.0		12.5		65.3			0.0
16 – 1	0.0				59.9				2.7	37.4	0.0			
18 – 1			94.9		0.0					0.0	5.1			
N	15	7	10	9	14	4	9	3	7	10	29	5	3	25
50%til	6.5	0.0	29.0	0.0	7.0	17.3	13.2	0.0	12.5	0.7	10.3	0.0	22.4	0.0
90%til	59.3	14.1	95.4	21.2	58.7	51.0	82.6	4.5	34.4	41.6	94.1	51.9	44.7	12.0
mean	19.9	4.1	42.1	8.1	19.7	24.0	31.5	1.9	16.3	16.8	29.2	19.3	24.2	4.8
± sd	24.0	6.4	39.9	19.4	26.3	23.7	35.4	2.7	16.2	24.9	33.9	26.3	20.6	13.1
min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
max	72.2	15.0	100	62.0	82.9	61.4	96.6	5.6	49.1	80.1	100	66.4	50.2	63.2

„Potentially foraging" in unknown habitat is equally distributed over all known habitats noted during the given tracking session

Diet of birds

Direct observations

During scan sampling it was frequently possible to observe a bird ingesting a food item, but only in a few cases was it possible to identify the item. Seedlings could mostly be excluded because the birds were seen feeding between the crop rows. In maize, Skylarks were mostly fed on items which could not be identified. Similarly in sugar beet fields the ingested items could not be identified in most cases.

During radio tracking the Skylarks could be observed feeding on sugar beet seedlings (6 times), on peas (10 times), oilseed rape (once), and invertebrates (3 times). Skylarks were also observed pecking on maize seedlings (4 times) but maize particles were not found in any faeces. As such, it is likely that the birds did not feed on the crop leaves but rather peck food items from the leaves.

Faeces and stomach content analysis
All faeces of Skylarks were gathered in sugar beet and maize fields shortly after germination. The main portion of those faeces gathered in sugar beet comprised animal matter (63 %), the rest was plant matter. Sugar beet leaves and seeds represented only 3.6 % and 0.7 % respectively. The plant matter mainly comprised of *Gramineae* seeds (9.3 % total volume). The faeces gathered in maize fields comprised 81 % of animal matter and 19 % plant matter. Leaves or seeds of maize were definitely not present.

Apart from the Skylark samples, six samples were gathered from other bird species, four faeces and two stomach contents. Two samples came from Greenfinches, two from Yellow Hammers and two from Grey Partridges. Neither maize or sugar beet leaves and seeds were found in any of the faeces.

Table B.9.1.3-42: Results of bird monitoring in sugar beet and maise fields ([redacted] 2005)

<table>
<tr><td colspan="6">PORTION OF TIME potentially foraging (PT) per habitat used by radio tracked Skylarks</td></tr>
<tr><td colspan="2" rowspan="5">potential foraging time [1] 16 skylarks spent per habitat; [mean of sessions], (90%ile, N: no. of tracking sessions considered)</td><td>plain fields</td><td>19.9 %</td><td>(59.3)</td><td>15</td></tr>
<tr><td>drilled maize fields</td><td>4.1 %</td><td>(14.1)</td><td>7</td></tr>
<tr><td>germinated maize fields</td><td>42.1 %</td><td>(95.4)</td><td>10</td></tr>
<tr><td>drilled sugar beet fields</td><td>8.1 %</td><td>(21.2)</td><td>9</td></tr>
<tr><td>germinated sugar beet fields</td><td>19.7 %</td><td>(58.7)</td><td>14</td></tr>
<tr><td colspan="6">HABITAT PREFERENCE of Skylarks according to radio tracking</td></tr>
<tr><td colspan="2" rowspan="5">preference of crop types as a feeding habitat (Jacobs' index [D], Range: –1 to +1; MCP [100%])</td><td>plain fields</td><td colspan="3">-0.47</td></tr>
<tr><td>drilled maize fields</td><td colspan="3">-0.83</td></tr>
<tr><td>germinated maize fields</td><td colspan="3">0.06</td></tr>
<tr><td>drilled sugar beet fields</td><td colspan="3">-0.73</td></tr>
<tr><td>germinated sugar beet fields</td><td colspan="3">-0.29</td></tr>
<tr><td colspan="6">DIET of Skylarks in maize and sugar beet fields</td></tr>
<tr><td rowspan="7">mean portion of diet after the analysis of faeces gathered in sugar beet (63) and maize (6) fields</td><td colspan="2">food item</td><td>PD [%]</td><td colspan="2">Frequency [%] [2]</td></tr>
<tr><td colspan="2">maize seeds</td><td>0</td><td colspan="2">0</td></tr>
<tr><td colspan="2">maize seedlings</td><td>0</td><td colspan="2">0</td></tr>
<tr><td colspan="2">sugar beet seeds[3]</td><td>< 0.7</td><td colspan="2">3.2</td></tr>
<tr><td colspan="2">sugar beet seedlings[3]</td><td>< 3.6</td><td colspan="2">23.8</td></tr>
<tr><td colspan="2">potentially sugar beet seeds[4]</td><td>< 2.1</td><td colspan="2">17.5</td></tr>
<tr><td colspan="2">potentially sugar beet seedlings[4]</td><td>< 10.2</td><td colspan="2">36.5</td></tr>
<tr><td colspan="6">HABITAT of birds according to transect counts (based on population)</td></tr>
<tr><td rowspan="3">abundance of Skylarks and other species</td><td colspan="2">field status</td><td>Skylark</td><td colspan="2">sum of other species</td></tr>
<tr><td colspan="2">plain fields</td><td>0.34</td><td colspan="2">0.41</td></tr>
<tr><td colspan="2">drilled maize</td><td>0.41</td><td colspan="2">0.86</td></tr>
</table>

after 10 transect counts covering 65.4 ha respectively [individuals/ha]	germinated maize	0.25	1.63
	drilled sugar beet	0.32	0.36
	germinated sugar beet	0.31	1.1
	all other fields	0.59	1.1
BIRD ABUNDANCE against crop type and stage according to scan sampling			
Densities of the 3 most abundant bird species in different crop types and stages [ind./ha/scan]	plain field (28 sessions)	Skylark	0.12
		Pied Wagtail	0.07
		Black Redstart	0.03
	drilled maize field (3 sessions)	Skylark	0.07
		Common Pheasant	0.03
		Pied Wagtail	0.03
	germinated maize fields (6 sessions)	Grey Partridge	0.02
		Barn Swallow	0.02
		Common Pheasant	0.02
	drilled sugar beet fields (4 sessions)	Skylark	0.14
		Common Pheasant	0.05
		Grey Partridge	0.01
	germinated sugar beet fields (10 sessions)	Skylark	0.21
		Whinchat	0.05
		Common Pheasant	0.04

[1] Sum of behaviour categories “foraging” + “potentially foraging” + “unknown”
[2] Portion of faeces with remains of the appropriate food item
[3] based on identified food items
[4] Sum of sugar beet and unspecified seeds or rather seedlings, as a conservative worst case approach

Birds and Mammals

Table B.9.1.3-43: Potential grazing damage by vertebrates as % of biomass in sugar beet and maize ([redacted] 2005)

Sum of unknown and vertebrate grazing damage (% of biomass)				
habitat	**BBCH 12**	**BBCH 13**	**BBCH 14**	**BBCH 16**
sugar beet	1.0	not stated	2.8	4.7
maize	0.3	1.5	0.8	not stated

Conclusions

Both the transect count and the scan sampling approach confirmed the choice of the Skylark as the species of concern in maize and sugar beet fields. Accordingly the Skylark was selected species for the radio tracking approach.

Radio tracking of 16 skylarks demonstrated that these fields were used by skylarks but that the seeds and seedlings of sugar beet and maize could mostly be excluded. Skylarks avoided sugar beet fields (negative Jacobs Index). Skylarks neither preferred or avoided germinated maize fields and used them in the same proportion that that they appeared in their home range.

The results of the census counts support the findings of the tracking data that the surrounding fields were generally more attractive to skylarks than the maize and sugar beet fields. The abundance of skylarks in all other habitats was higher than in maize and sugar beet. In maize and sugar beet fields skylark was overall the most abundant species in these crops and prevalent. Potential foraging time for skylarks caught on or close to maize and sugar beet was 4.1% (90^{th} percentile 14.1%) in drilled and 42.1 (90^{th} percentile 95.4%) in germinated maize fields and 8.1% (90^{th} percentile 21.2%) and 19.7% (90^{th} percentile 58.7%) in drilled and germinated sugar beet fields, respectively.

From the analyses of 63 faeces from skylark, gathered in sugar beet fields, it was concluded that the portion of diet (PD) was less than 0.7% for sugar beet seeds and less than 3.6% for sugar beet seedlings. If all unspecified seeds or seedlings actually originated from sugar-beet the portion of diet would have been 2.1% and <10.2% for sugar beet seeds and seedlings' respectively. There was no indication of the ingesting of maize in faeces gathered from maize fields but the numbers of faecal samples analysed was small (n=6).

([redacted] 2005)

HSE comments

This study was conducted to GLP and was conducted in line with EFSA guidance. Maize and sugar beet fields were monitored using transect counts and scan sampling to determine the abundance of different bird species using the habitats. For the purpose of this section, HSE has only considered the bird data provided from this study in this section.

As skylarks have been selected as the focal species in previous studies, these were considered further through radio tagging and tracking of 16 individuals. Additionally, the faeces and stomach content of skylarks in maize and sugar beet fields to ascertain the composition of animal and plant matter.

This study took placed from March to December, this is inclusive of the proposed use of 'Vibrance SB', which will be used from March – April. It also covers the period of drilling for vining pea seeds treated with 'Wakil XL' (February-April). Fields of maize and sugar beet were monitored from pre drilling until growth stage BBCH 16. This is inclusive of the proposed GAP for 'Vibrance SB' in sugar and fodder beet.

The study was performed in Austria. Austria has a very similar climate to the UK, and meteorological data has been provided for the duration of the study. The data showed high rainfall at the beginning of the study, in late March, which reduced for the rest of the study.

The transect count and the scan sampling showed that the skylark was the most abundant species in plain fields. This is relevant for the proposed use of 'Vibrance SB' as it is a seed treatment. The transect counts and scan sampling also showed that skylarks were the most abundant species in drilled and germinated sugar beet fields.

It is noted that the study also contains some information on bird abundance in pea fields from the transect counts, which is relevant for the proposed use of ‘Wakil XL’. In drilled pea fields the only species observed was the skylark, while in germinated fields the skylark, jackdaw, linnet, yellowhammer, carrion crow and woodpigeon were observed, in decreasing order of abundance.

Radio tracking of the 16 skylarks was combined with visual observations. Individual birds were tracked on multiple occasions. A higher number of male birds were tracked than female birds.

For this study, behaviour classified as ‘unknown’, ‘active and possibly foraging’ and ‘foraging’ were placed together in the ‘potentially foraging’ group.

The habitat selection of the tracked individuals showed minor use of sugar beet. Skylarks were found in much higher percentages in maize, winter cereals, weed fields, oilseed rape, ‘unknown’, peas and plain field. Additionally, the mean time spent foraging in sugar beet fields was low compared to the other field types.

Faeces and stomach content analysis showed that 63 % of the composition of faeces collected in sugar beet fields was animal matter. Sugar beet seeds represented 3.6 % and sugar beet seedlings were 0.7 %. No faeces samples were taken from pea fields.

The results of this study show that skylarks can be considered the most abundant species in sugar fields, in part due to the relatively lower populations of other species using the fields. However, radio tracking shows they do not show any preference to sugar beet fields, in fact the mean portion of time skylarks spent in sugar beet fields was much lower than some of the other field types available. Additionally, skylarks are not exclusively consuming the sugar beet material, rather their faeces show over half of their diet is animal matter. The results and use of this study will be considered further in the higher tier risk assessment for birds.

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	KIIIA 10.1.7/08 [redacted], [redacted] (2018), PT of woodpigeons in pre- and post-emergence maize fields in Germany, central zone (2017). Report Number: [redacted]. [redacted]. (Syngenta file no.VV-470595)

Guidelines: Regulation (EC) No 1107/2009 and EFSA Guidance Document Risk Assessment for Birds and Mammals (2009)

GLP: Yes

Materials

Test Material No specific substance was tested.

Test organisms

Species: Woodpigeons (*Columba palumbus*)

Crop: Maize

Drilling date: 15th April – 8th May 2017

Test design Radio tracking to assess use of maize fields as feeding habitat by radio tracking individuals, between drilling (BBCH 00) and BBCH 18, in order to obtain PT values. The study plan was prepared under consideration of recommendations in the current EFSA guidance document on risk assessment of Plant Protection Products (PPP) for birds and mammals (EFSA 2009).

Duration of study: 3 months

Study Design and Methods

Experimental dates: 11th April – 15th June 2017

The purpose of the study was to assess the proportion of diet (PT) that woodpigeons (*Columba palumbus*) obtain in maize fields during the pre-emergence and post-emergence period in spring.

The study was conducted in the area of Bersenbrück, in the districts of Osnabrück and Cloppenburg, in the north-west of Germany (province of Lower Saxony). The area is considered representative for the cultivation of maize by the study authors (though specific information has not been provided to substantiate this point). The size of the fields ranged from 1.4 to 13.3 ha, with an average of 5.5 ha.

Due to the usually large home range of the woodpigeon, single agricultural fields were of lower importance for the study. However, the trapping locations were close ≤ 150 m) to a maize field or to a field soon-to-be-drilled with maize.

Eight trapping locations (A-H) were selected based on the presence and distribution of woodpigeons in the study area. Trapping took place in April and May 2017, following procedures specified in [redacted] (2016). The trapping methods used for the woodpigeons were spring traps, mist net and whoosh net. Captured individuals were fitted with an aluminium ring on the right leg (tarsus), in total, 39 woodpigeons were tagged with radio transmitters. The weight of the respective transmitters did not exceed 3 % of the birds' body weight. Transmitters were fixed using a 'Side-Tube-Backpack-Harness'. The transmitters were tested prior to tagging the birds in order to detect any malfunction.

The respective tag frequency and health status of each bird was recorded, and a bird ID was assigned to each tagged woodpigeon. In order to estimate the location and status (alive or dead) of radio-tagged birds, the birds were checked regularly. After taking the relevant measurements, the check of health status, attachment of radio transmitters, ringing and examination, each trapped bird was released back into the study area.

During a tracking session, one individual was followed non-stop by car or on foot for a daylight activity period, maximum 16 hours. Every change in behaviour and location was recorded. An exchange of observer took place every few hours to ensure full attention of the person tracking the bird. Unidirectional Yagi-antennas were used to locate the tagged individual. In order to describe the behaviour of the tracked bird as accurately as possible and to verify its location, visual contact with optical devices (scope, binoculars) was attempted. To ensure that the observer did not affect the behaviour of the bird, an appropriate safe distance was maintained.

Data collected were divided into sessions A and B. During session A maize fields were pre-dominantly at the pre-emergence stage. During session B maize field were predominantly at growth stages BBCH 10-18. In total 21 individuals were tracked pre-emergence of maize and 23 individual from BBCH 10-18.

General information									
Study design	Generic								
Study period	April - June 2017								
Crop	Maize								
Test item	None								
Focal species	Woodpigeon (*Columba palumbus*)								
Field information									
Trapping location	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**TOTAL**
No. study fields	3	3*	3	3*	2	3	3	3	**22**
Drilling dates	15 April - 08 May 2017								**-**
Focal species information									
No. of radio tracked birds	6	2	6	1	1	7	1	6	**30**
Total radio tracking sessions									
BBCH 00 - 09	4	2	4	1	1	5	-	4	**21**
BBCH 10 - 18	4	1	4	-	1	7	1	5	**23**

* one field (B-2) shared by locations B and D

The availability of freshly drilled and emerged maize fields in the study area was monitored by regular checks (in most cases every 3-4 days) of 22 selected study fields for their status, drilling time and development of the crop (i.e. BBCH growth stages).

Meteorological data (temperature and precipitation) was obtained from a nearby weather recording station (Diepholz, Lower Saxony) for the duration of the field phase.

Three different approaches were used to determine the PT, as defined as the “consumer”-approach whereby PT was estimated only from those individuals having used maize fields as foraging habitat, as shown by radio-tracking. These individuals are thus considered as “consumers”. The “home range”-approach comprises of the individuals which included maize fields in their home range, irrespective of whether these fields were used for potentially foraging during the tracking session. All these individuals together are considered “potential consumers”. For the “all individuals”-approach all tracking sessions were considered to calculate a PT estimate. This approach gives an indication of the risk to the wider farmland populations that occur in the vicinity of maize fields but do not all necessarily visit the fields during the tracking session.

PT is defined as the proportion of an animal´s daily diet obtained in habitat treated with pesticide (EFSA 2009). The proportion of diet obtained in pre- and post-emergence maize fields (PT) was quantified for intensively radio-tracked individual woodpigeons, based on the assumption that the time an animal spends active in a particular habitat is a good estimation of the proportion of diet obtained in this defined area. Bird behaviour was defined as ‘foraging’, ‘active (possibly foraging)’, ‘active (definitely not foraging)’, ‘inactive’, ‘unknown (weak signal)’ or ‘no signal’. The appropriate category was determined based on the signal and/or visual contact. Potential foraging time was determined by adding the time spent engaged in ‘foraging’ and ‘active (possibly foraging)’ behaviours. PT values were then determined by dividing the time spent potential foraging in maize fields by the total time spent potentially foraging.

The PT values (mean, median and 90%-percentile of PT) for pre-emergence and post-emergence were calculated for each individual and session separately. A method for estimating home range sizes is the ‘minimum convex polygon’ method (MCP, Mohr 1947). This home range size measure reveals the area of a convex polygon plotted around all locations (GPS positions) which an animal used during one tracking session. Moreover, the Jacobs’ preference index (D) was calculated for each tracking session. The index provides additional information that helps to quantify the habitat preferences of an individual or species and can help to interpret the PT values for different landscapes. The Jacobs’ index was calculated for the maize fields, for the pre-emergence and post-emergence periods separately.

Results and Discussion

Among the trapping locations, the timing of drilling and emergence was variable. Therefore the radiotracking sessions were conducted according to the status of the reference fields. A combined analysis of the crop development of the reference fields shows the general availability of the pre- and post-emergence maize fields in the study area during the time of the tracking sessions

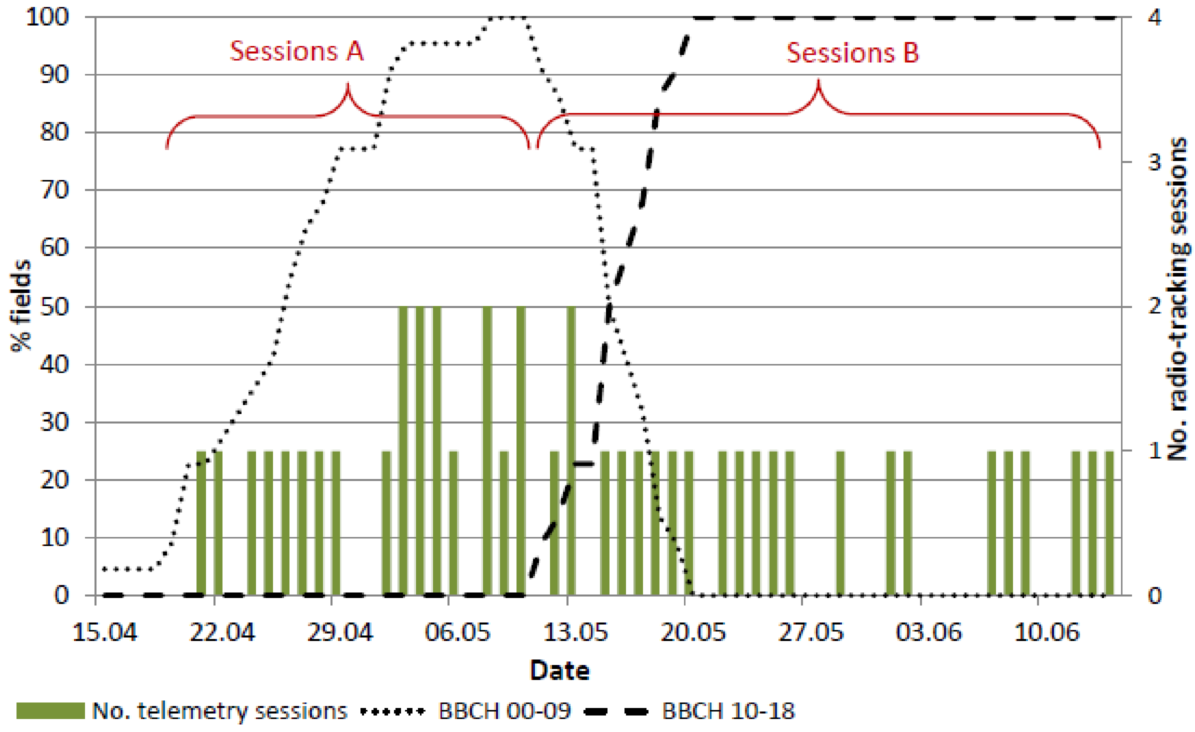


Figure B.9.1.3-17: Percentage of monitored maize fields in pre- and post-emergence stage and number of radio-tracking sessions performed during the study

Mean temperatures per day during the study period varied from 1.4-11.3 °C in April, 8.6-20 °C in May and 9.7-22.8 °C in June. The average mean temperature was 6.8 °C in April, 14.7 °C in May and 16.9 °C in June. Average daily precipitation was 1.2 mm in April, 1.1 mm in May and 1.5 mm in June.

In total 44 radio-tracking sessions were successfully conducted in order to evaluate the attractiveness of the maize fields before and after emergence. Continuous tracking sessions on 21 woodpigeons pre-emergence and 23 woodpigeons post-emergence, allowed a representative assessment of potential foraging times. In total, 76.2% of the individuals entered the pre-emergence maize fields (n = 9) or overflew this habitat during their tracking session (n = 7). The remaining birds (n = 5) with no freshly drilled maize in their home range were individuals with high activity on forests, shrubs and settlements and comparatively small home ranges. During the post-emergence period, 100% of the woodpigeons were either inside maize fields (n = 17) or overflew the habitat (n = 6).

Table B.9.1.3-44: Summary of PT for woodpigeons in pre- and early post-emergence maize

Session	Parameter	"Consumers"-Approach	Home Range Approach	All Individuals Approach
A (BBCH 00 - 09)	Mean	0.04	0.02	0.02
	Median	0.02	0.01	0.00
	Max	0.17	0.17	0.17
	90%ile	0.09	0.06	0.04
B (BBCH 10 - 18)	Mean	0.13	0.10	0.10
	Median	0.06	0.02	0.02
	Max	0.78	0.78	0.78
	90%ile	0.31	0.25	0.25

Mean PT values for consumers were relatively low (0.04 for BBCH 00-09 and 0.13 for BBCH 10-18), especially when compared with the mean proportion of maize fields in the home ranges (17.5% and 31.4% respectively). During the pre-emergence period, the mean home range size for woodpigeons (n = 21 individuals) was 41.96 ha, but home range size varied from 2.60 ha to 325.40 ha. During the post-emergence period, the mean home range size (n = 23 individuals) was 52.50 ha, and home ranges varied from 2.41 ha to 364.0 ha. Individuals which were tracked in both sessions A and B showed high intra-individual differences (SD between ± 1.77 and ± 41.53 ha).

Table B.9.1.3-45: Size of home ranges of radio-tracked woodpigeons in post-emergence period

Minimum Convex Polygon, MCP [ha]	n	Mean	Median	Max.	Min.	SD	90%ile
Session A (BBCH 00 – 09)	21	41.96	23.58	325.40	2.60	68.56	70.48
Session B (BBCH 10 - 18)	23	52.50	17.78	364.00	2.41	88.39	80.84

Pre-emergence maize fields were part of the home ranges of 16 out of the 21 radio-tracked individuals (76.2%), during 21 tracking sessions. Overall, the proportion of agricultural area in the total home ranges of woodpigeons was 61.2% and 34.7% of this area was occupied by drilled maize. The main habitats within the home ranges of the woodpigeons after the fields of drilled maize were meadows and forests. Forest was also the habitat used by almost all individuals (n = 20) during the pre-emergence radio-tracking sessions

Post-emergence maize fields were part of the home range of all 23 individuals (100%) during 23 tracking sessions. The overall proportion of agricultural area in the total home range of woodpigeons was 66.2%, of this agricultural area 42.0% were post-emergence maize fields. The main habitats within the home ranges of the woodpigeons were, after maize fields, meadows, forests and cereal fields. From them, forest was also the only habitat used by all individuals (n = 23) during the post-emergence radio-tracking sessions.

Jacobs' indices [D] for the 9 woodpigeons which used pre-emergence maize fields (as consumers) ranged from -0.95 to -0.40 with a mean value of -0.79. The mean value These overall negative results, together with a relatively low variability among individuals (SD ±0.18), indicate that drilled maize fields were infrequently used as feeding habitat and were generally avoided by woodpigeons.

Jacobs' indices [D] for the 17 woodpigeons which used the post-emergence maize fields (as consumers) ranged from -0.98 to 0.77, with a mean value of -0.60. The mean value indicates a rather negative position of woodpigeons regarding maize fields as feeding habitat, though there is high variability among individuals (SD ±0.48). In 2 sessions woodpigeons showed a preference for the maize fields, whereas 15 sessions indicated an avoidance of the study crop.

Conclusion

This study demonstrated that maize fields, at pre- and post-emergence growth stage, are not especially attractive as feeding habitat for woodpigeons although they were not always avoided.

- In total 30 potentially consumer birds, which were trapped close to the fields, were radio-tracked before and after emergence in order to measure their use of maize fields as feeding habitat.
- During pre-emergence, nine out of 21 individuals entered the maize fields; during postemergence, 17 woodpigeons out of 23 entered emerged maize fields.
- For consumers during the pre-emergence period, the 90%ile of the PT value was 0.09 (mean PT = 0.04) over all 21 sessions. In the post-emergence period, 23 sessions were conducted, and the 90%ile of the PT value was 0.31 (mean PT = 0.13) over all consumers.

In summary, the study outcome gives representative PT values for woodpigeons in pre-emergence and post-emergence maize fields.

([redacted] and [redacted], 2018)

HSE comments:

This study was conducted to GLP and follows sound methodological practices, based around EFSA guidance. The study provides information on the proportion of total foraging time that wood pigeons spend in freshly drilled maize fields and maize fields at BBCH 10-18. Maize is not a crop subject to consideration for this Article 7 application, so the relevance of the data for the higher tier assessment is considered further in the bird risk assessment section.

It is noted that the study was conducted in Germany and not the UK. The focal species tracked is relevant for the GB assessment and it is considered that there were relatively high percentages of maize fields in the study area. The study area is considered broadly representative of bird foraging behaviour around maize fields in GB though, as noted above, there is a degree of uncertainty extrapolating the results to other crops.

The field phase of the study was conducted in May-July, whereas the proposed uses of ‘Vibrance SB’ are in March to April and ‘Wakil XL’ is to be used in February to April. Given the differences in timing, the relevance of the data for the higher tier assessment is considered further in the bird risk assessment section.

Agricultural practices were recorded for each of the study fields. In 15/22 of the study fields the seed coating product Mesurol (containing the active substance methiocarb) was applied to maize seeds. In a further 6 fields no seed coating was used, with Kurit Plus applied to seeds at a single field. It is possible that use of a seed treatment that did not contain metalaxyl-M could impact the attractiveness of treated seed to birds, i.e. if birds avoid seed treated with methiocarb more than seed treated with metalaxyl-M, using radio-tracking data from this study could underestimate the ‘true’ PT value for the metalaxyl-M risk assessment. However, the birds with highest PT values for pre-emergence maize in this study were tracked in locations where maize seeds had been treated with Mesurol. Therefore, the presence of Mesurol does not appear to have led to birds avoiding fields with treated seed. It is also noted that a range of herbicides were applied to maize fields during the study period. These are considered indicative of typical agricultural practice and do not impact the reliability of the study.

During tracking sessions there were periods where it was not possible to determine bird location/behaviour, i.e. categories ‘unknown (weak signal)’ or ‘no signal’. The study authors have only included tracking data from sessions where it was possible to determine bird location/behaviour for at least 80% of the monitored period. The minimum percentage of valid tracked time per session was 85.1%, with most sessions over 95%. Periods where the location/behaviour was unknown were excluded from the PT calculations. This approach is appropriate and the duration for which location/behaviour was unknown is considered relatively small and largely unavoidable.

HSE has summarised the individual session PT data from the study in the tables below, and has checked the summary statistics derived for the consumer population.

Table B.9.1.3-46: PT in maize fields for 21 radio-tracked woodpigeons during 21 sessions in pre-emergence maize fields (BBCH 00 - 09, session A)

Bird ID	Status	PT in pre-emergence fields	Total percentage foraging time
A1	Non-consumer	0.00	59.4
A2	Consumer	0.01	63
A3	Potential consumer*	0.00	48.5
A4	Non-consumer	0.00	73.4
B1	Potential consumer*	0.00	48.1
B2	Potential consumer*	0.00	65.9
C1	Potential consumer*	0.00	52.9
C2	Non-consumer	0.00	55.3
C5	Consumer	0.02	79.2
C6	Potential consumer*	0.00	91.3
D1	Non-consumer	0.00	53.5
E1	Consumer	0.02	57.8
F1	Consumer	0.01	56.6
F2	Potential consumer*	0.00	48.7
F3	Consumer	0.17	70.9
F4	Consumer	0.02	57.6
F5	Consumer	0.03	34.7
H1	Non-consumer	0.00	58.1
H2	Consumer	0.04	60.4
H4	Consumer	0.07	68.8
H5	Potential consumer*	0.00	77.3

*Based on presence of newly drilled maize field in home range

During most sessions birds were potentially foraging for at least 50% of the contact time. Therefore, there the data provide a good indication of bird locations during potential foraging activity. For pre-emergence maize fields there were 9 consumer birds, with PT values ranging from 0.01-0.17. The mean consumer PT was 0.04 and the 90th percentile was 0.09. The appropriate PT value to use in higher tier risk assessment is discussed further in the risk assessment section.

Table B.9.1.3-47: PT in maize fields for 23 radio-tracked woodpigeons during 23 sessions in postemergence (BBCH 10 - 18, session B)

Bird ID	Status	PT in post-emergence fields	Total percentage foraging time

A2	Consumer	0.02	84.3
A3	Consumer	0.02	74.2
A7	Consumer	0.01	58.2
A8	Consumer	0.01	65.0
B2	Consumer	0.06	79.8
C4	Potential consumer*	0.00	89.5
C5	Consumer	0.05	86.0
C6	Consumer	0.01	69.4
C7	Potential consumer*	0.00	58.0
E1	Consumer	0.02	79.6
F1	Potential consumer*	0.00	86.4
F2	Potential consumer*	0.00	44.9
F3	Consumer	0.05	58.0
F4	Consumer	0.07	57.2
F5	Consumer	0.16	69.1
F6	Consumer	0.10	73.5
F7	Potential consumer*	0.00	63.1
G1	Consumer	0.15	52.4
H2	Consumer	0.78	91.9
H4	Consumer	0.28	74.2
H5	Potential consumer*	0.00	37.7
H7	Consumer	0.06	68.9
H9	Consumer	0.37	53.4

*Based on presence of maize field at BBCH 10-18 in home range

During most sessions birds were potentially foraging for at least 50% of the contact time. Therefore, there the data provide a good indication of bird locations during potential foraging activity. For early post-emergence maize fields (BBCH 10-18) there were 17 consumer birds, with PT values ranging from 0.01-0.78. The mean consumer PT was 0.13 and the 90th percentile was 0.32. The appropriate PT value to use in higher tier risk assessment is discussed further in the risk assessment section.

This study is considered suitable for use in risk assessment, though the relevance of the study for the particular risk assessment questions at issue will be considered in the higher tier bird risk assessment section.

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	KIIIA 10.1.7/09 [redacted]. (1999), Migration of Metalaxyl-M in Soil After Seed Treatment with [Phenyl-(U)-14C] CGA 329351, Report Number 98SV04. Novartis Crop Protection AG CH-4002 Basel, Switzerland. (Syngenta File No. CGA329351/1151; VV-312789)

Guidelines:

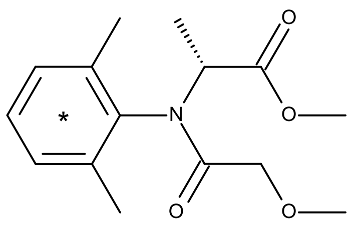
EPA OPPTS 860.1300
Council Directive 91/414/EEC

GLP: Yes

MATERIALS AND METHODS

Materials

Test Materials:

Structure/Label	[Phenyl-(U)-14C] CGA 329351
	 (* = 14C position)
CAS Number	70630-17-0
Batch Number	ILS-181.1
Specific Activity	2.54 MBq/mg 68.65 µCi/mg
Radiochemical Purity	> 98 %

Test System

In sub-study 1, field pea var. Piccolo (40 treated and 10 untreated seeds) were seeded in plastic pots filled each with about 900 ml of soil. Plants were watered regularly and fertilizer was given as required.

In sub-study 2, forty soil samples (each 50 g based on dry weight) were kept in Erlenmayer flasks. Samples were adjusted to approx. 40 % of the max. water holding capacity and kept at this level during the whole study.
Both sub-studies were performed using the same soil under the same climatic conditions

<u>Test Soil</u>

A loam soil was used. The characteristics of the soil were as follows (Agro Lab AG, Ebikon, Switzerland):

ph (KCl)	7.4
CaCo3	7.5%
Carbon (organic)	2.3%
Sand	41.8%
Slit	48.6%
Clay	9.6%
Cation exchange capacity	13.8 meq/100 g dry soil
Maximum water holding capacity (pF 1.0)	75.9%
Microbial biomass	1.098 mg C/g dry soil

STUDY DESIGN

Experimental Conditions

Sub-study 1

Nominal Application Rate	35 g a.s./100 kg of seeds
Number of Applications	1
Target Application Rate	35 g a.s./100 kg of seeds (i.e. 165 g a.s./ha assuming 470 kg seeds/ha)
Achieved Application Rate	11’986’911 dpm/seed i.e.0.079 mg a.s./seed
Formulation type	XL 350 ES
Formulation code	A-9642 C
Formulation concentration and application solution	20.0 mg of [Phenyl-(U)-14C] CGA 329351 dissolved in 43 µL blank formulation 43 µL formulated Test Item dispersed in 300 µL pure water.
Volume rate of application solution	343 µL /40 g seeds
Method of application	Seeds were added to the application solution in an Erlenmayer flask and shaken for 1 minute.
Growth stage at application	Seed application
Environmental conditions	Growth chamber (14 h day length) Temperature = 16 ± 2 °C (day); 12 ± 2 °C (night); Relative humidity = 54-57% (day); 59-69% (night)

a Assuming a seed weight of 225 to 250 g/1000 pea seeds, achieved rate is 31.46 to 34.9 g a.s./100 kg seed (between 90 and 99.9% of target rate)

Sub-study 2

Nominal Application Rate	150 g a.s./ha
Number of Applications	1

Target Application Rate	150 g a.s./ha (i.e. 0.2 mg a.s./kg soil)a
Achieved Application Rate	141 g a.s./ha (i.e. 0.188 mg a.s./kg soil)a
Application solution	0.5 mg [Phenyl-(U)-14C] CGA 329351 dissolved in 0.5 mL acetone followed by dilution with water to a final volume of 10 mL
Volume of application solution/treatment	200 µL/50 g soil (dry weight)
Method of application	Direct treatment of soil with application solution
Environmental conditions	Identical to sub-study 1, in the dark

[a] assuming an incorporation depth of 5 cm and a bulk density of 1.5g/cm3.

Test Samples

Just after treatment on June 8, 1998 (= interval 0), twenty seeds were analysed to determine the dressing efficiency and to define the initial value for the radioactivity on the seeds of sub-study 1. Seeds were washed with methanol: water (1: 1 v: v) and the wash solution analysed by LSC. The washed seeds were analysed by combustion and LSC. Total radioactive residue was calculated as the sum of the radioactivity in the wash solution and the remaining radioactivity on the seed. In sub-study 2, three aliquots of the application solution were analysed directly by LSC.

At intervals 1-7 (1, 2, 4, 7, 14, 28 and 53 DAT) 3 replicates of each sub-study were taken and analysed.
In sub-study 1, each replicate was divided into the seed or aerial plant part, a soil core and the remaining soil. The soil core (Ø2.8 cm) was taken around the seed/plant, including roots, and further separated into 3 layers of 0-4 cm, 4-7 cm and 7-10 cm depth.

Each sample generated from each replicate in sub-study 1 and each replicate in sub-study 2 was analysed separately.

Sample Preparation

In sub-study 1, the layers of the soil cores were directly taken for extraction with homogenization. The seeds were analysed by combustion/LSC. The aerial plant parts were homogenized in the presence of liquid nitrogen using a Cut-O-mat homogenizer. The remaining soil was air dried and homogenized in a disk mill. Homogenates of aerial plant parts and remaining soil were analysed by combustion/LSC.

In sub-study 2, each soil sample was directly taken for extraction with homogenization.

Extraction and Fractionation of Residues

The layers of the soil cores in sub-study 1 and the soil samples in sub-study 2 were directly extracted by means of a Polytron homogenizer with 80 % methanol in water. The extraction procedure was repeated five times or until recovered radioactivity was less than 5 % compared with the radioactivity recovered in the first extraction step .The extracts were combined and used for TLC analysis. The residue resulting from the extraction was radio-assayed by combustion/LSC.

In sub-study 1, samples of the remaining soil containing > 10 ppb of TRR and >10% AR (intervals 4-7) were extracted following the same procedure as described above.

RESULTS AND DISCUSSIONS

Distribution of Radioactivity and Extractability

Table B.9.1.3-48: Distribution of Radioactivity and Residual [Phenyl-(U)-14C] CGA 329351 in Plant and Soil Parts at Various Intervals in Sub-study 1 (A) and in Soil at Various Intervals in Sub-study 2 (B)

A)DAT (interval)	Substrate	TRR (ppb)a	Extractable (%)b	Non – Extractable (%)b	Total (%)b
0 (0)	Seed	-	-	-	100.0
1 (1)	Seed	-	-	-	19.3
	Soil core 00-04 cm	3282	89.9	0.9	90.8
	Soil core 04-07 cm	3	<0.1	<0.1	0.1
	Soil core 07-10 cm	3	<0.1	<0.1	0.1
	Remaining soil	1	0.6	<0.1	0.6
	Aerial part	-	-	-	-
	Total	-	90.6	0.9	110.8
2 (2)	Seed	-	-	-	23.7
	Soil core 00-04 cm	2083	70.7	1.3	72.0
	Soil core 04-07 cm	<1	<0.1	<0.1	<0.1
	Soil core 07-10 cm	2	<0.1	<0.1	<0.1
	Remaining soil	<1	0.3	<0.1	0.3
	Aerial part	-	-	-	-
	Total	-	71.0	1.3	96.1
4 (3)	Seed	-	-	-	12.8
	Soil core 00-04 cm	1962	58.2	2.1	60.3
	Soil core 04-07 cm	26	0.5	<0.1	0.5
	Soil core 07-10 cm	<1	<0.1	<0.1	<0.1
	Remaining soil	7	4.7	<0.1	4.7
	Aerial part	-	-	-	-
	Total	-	63.4	2.1	78.4
7 (4)	Seed	-	-	-	8.4
	Soil core 00-04 cm	1492	47.2	4.4	51.6
	Soil core 04-07 cm	4	<0.1	<0.1	<0.1
	Soil core 07-10 cm	<1	<0.1	<0.1	<0.1
	Remaining soil	28	10.8	9.2	20.0
	Aerial part	-	-	-	-
	Total	-	58	13.6	80.1

[a] in CGA 329351 equivalents,
[b] in %AR

DAT (interval)	Substrate	TRR (ppb)[a]	Extractable (%)[b]	Non – Extractable (%)[b]	Total (%)[b]
14 (5)	Seed	-	-	-	8.7
	Soil core 00-04 cm	748	17.8	6.0	23.8
	Soil core 04-07 cm	19	0.2	0.3	0.5
	Soil core 07-10 cm	<1	<0.1	<0.1	<0.1
	Remaining soil	53	24.7	12.6	37.3
	Aerial part	1089	-	-	0.5
	Total	-	42.8	18.9	70.6
28 (6)	Seed	-	-	-	-
	Soil core 00-04 cm	616	12.2	7.3	19.5
	Soil core 04-07 cm	22	0.2	0.3	0.5
	Soil core 07-10 cm	4	<0.1	<0.1	<0.1
	Remaining soil	70	19.9	33.0	52.9
	Aerial part	165	-	-	0.6
	Total	-	32.3	40.6	73.6
53 (7)	Seed	-	-	-	-
	Soil core 00-04 cm	250	2.2	6.4	8.6
	Soil core 04-07 cm	129	3.1	0.7	3.8
	Soil core 07-10 cm	50	1.0	0.3	1.3
	Remaining soil	50	19.2	20.8	40.0
	Aerial part	118	-	-	2.1
	Total	-	25.5	28.2	55.9

[a] in CGA 329351 equivalents,
[b] in %AR

B)

DAT (interval)	Substrate	TRR (ppb)[a]	Extractable (%)[b]	Non – Extractable (%)[b]	Total (%)[b]
0 (0)	Soil	188	-	-	100.0
1 (1)	Soil	184	94.7	2.8	97.6
2 (2)	Soil	188	94.9	5.1	100.0
3 (4)	Soil	182	82.7	13.9	96.6
7 (4)	Soil	170	61.6	29.0	90.7
14 (5)	Soil	150	26.9	52.8	79.6
28 (6)	Soil	121	5.3	59.9	65.2
53 (7)	Soil	110	2.8	56.5	59.4

[a] in CGA 329351 equivalents,
[b] in %AR

Characterisation of Radioactivity in soil

The extracted radioactivity was analysed by chromatography. The identified components for soil samples in sub-study 1 and sub-study 2 are summarised in Table IIA 6.2.1-2 (A-C) and (D), respectively.

Table B.9.1.3-49: Quantification of CGA 329351, CGA 62826, CGA 108905, Unknowns and Non-Extractables in Soil at Various Intervals of Sub-study 1 and Sub-study 2

A) Sub-study 1: Soil cores 0-4 cm (% AR)

Metabolite fraction	Days after Treatment						
	1	2	4	7	14	28	53
CGA 329351	84.4	64.8	39.6	30.4	7.1	1.2	0.3
CGA 62826	49.9	5.8	15.2	15.9	9.4	3.0	0.8
CGA 108905	n.d.	n.d.	0.5	0.8	0.4	4.6	0.6
Σ Unknowns	n.d.	n.d.	n.d.	0.3	0.6	3.3	0.5
Unresolved	0.4	0.1	0.5	n.d.	0.3	0.1	n.d.
Non-extractables	0.9	1.3	2.2	4.4	6.3	7.7	7.5
Total	90.6	72.0	58.0	51.8	24.1	19.9	9.7

B) Sub-study 1: Remaining soil (% AR)

Metabolite fraction	Days after Treatment						
	1	2	4	7	14	28	53
CGA 329351	n.a.	n.a.	n.a.	3.2	5.1	4.6	0.5
CGA 62826	n.a.	n.a.	n.a.	6.7	16.8	13.3	14.3
CGA 108905	n.a.	n.a.	n.a.	0.5	0.9	1.3	1.7
Σ Unknowns	n.a.	n.a.	n.a.	0.2	1.0	1.4	2.7
Unresolved	n.a.	n.a.	n.a.	0.1	0.8	0.5	0.1
Non-extractables	n.a.	n.a.	n.a.	9.2	12.6	33.0	20.8
Total	n.a.	n.a.	n.a.	19.9	37.2	54.1	40.1

C) Sub-study 1: Total (soil core 0-4 cm and remaining soil; % AR)

Metabolite fraction	Days after Treatment						
	1	2	4	7	14	28	53
CGA 329351	84.4	64.8	39.6	33.6	12.2	5.9	0.8
CGA 62826	4.9	5.8	15.2	22.6	26.2	16.3	15.1
CGA 108905	n.d.	n.d.	0.5	1.3	1.3	5.9	2.3
Σ Unknowns	n.d.	n.d.	n.d.	0.5	1.6	4.7	3.2
Unresolved	0.4	0.1	0.5	0.1	1.1	0.6	0.1
Non-extractables	0.9	1.3	2.2	13.6	18.9	40.7	28.3
Total	90.6	72.0	58.0	71.7	61.3	74.0	49.8

D) Sub-study 2: Soil (% AR)

Metabolite fraction	Days after Treatment						
	1	2	4	7	14	28	53
CGA 329351	77.6	70.6	47.0	29.8	13.0	2.4	1.0
CGA 62826	16.6	24.2	34.6	29.8	12.1	1.4	0.2
CGA 108905	n.d.	n.d.	n.d.	n.d.	0.2	0.1	0.1
Σ Unknowns	n.d.	n.d.	n.d.	n.d.	1.4	1.1	1.2
Unresolved	0.4	0.2	1.1	1.5	0.2	0.3	0.3
Non-extractables	2.8	5.1	13.9	29.0	52.8	59.9	56.5
Total	97.5	100.1	96.7	90.1	79.6	65.2	59.4

<u>Storage Stability</u>

Analysis of all samples was performed less than 4 months after collection. Therefore, no storage stability data were established

Conclusions

An estimate of the distribution of CGA 329351 residues in soil and plant samples over time was provided allowing a determination of movement and dissipation of residues in soil and uptake of residues by the plants following seed application.

Radioactive residues were adequately identified or characterised in accordance with guideline triggers.
The half-lives (DT50) and disappearance times for 90 % (DT90) in soil following seed application (sub-study 1) and in a laboratory soil dissipation study (sub-study 2) were determined for CGA 329351 and it's major soil metabolite CGA 62826.

In sub-study 1, CGA 329351 was applied at a nominal rate of 35 g a.s./100 kg of seeds (i.e. 165 g a.s./ha assuming 470 kg seeds/ha). Seed radioactivity was rapidly released to the surrounding soil (80% AR found in soil core of 0-4 cm depth at 1 DAT). Highest levels of radioactivity remained in the soil core top layer (0-4 cm) for the first 7 days. Afterwards the radioactivity was moved horizontally to the outer soil counting for 40% AR at 53 DAT. Only low amounts of the applied radioactivity were found in the aerial plant part.

In sub-study 2, CGA 329351 was applied at a nominal rate of 0.2 mg/kg dry weight soil, equivalent to a single field application rate of 150 g a.s./ha (assuming an incorporation depth of 5 cm and a bulk density of 1.5g/cm3).
The recovery of the applied radioactivity decreased from 110.8 % (1 DAT) to 55.9 % (53 DAT) and from 97.6 % (1 DAT) to 59.4 % (53 DAT) in sub-study 1 and sub-study 2, respectively.

In both sub-studies, CGA 329351 was metabolized rapidly via cleavage of the methyl ester bond to the major metabolite CGA 62826. In sub-study 1, the concentration of CGA 329351 in the upper soil core decreased from interval 1 (1 DAT) to interval 7(53 DAT) from 84.4 % to 0.3 % AR. The major metabolite CGA 62826 amounted to 4.9% AR (1 DAT), reached its maximum of 15.9 % (7 DAT) and decreased to 0.8 % (53 DAT). Oxidation of one methyl group on the phenyl ring of CGA 329351 forming CGA 108905 accounted for a maximum of 5.9 % AR (28 DAT) in sub-study 1, while only trace amounts were found in sub-study 2.

The dissipation rates of parent compound are very similar in both studies. For the metabolite CGA 62826 the dissipation times are longer in sub-study 1 compared to sub-study 2. This is explained by the fact, that its rate of formation was slower due to the formation of CGA 108905 at the same time.

([redacted] 1999)

HSE comments

This study was conducted to GLP and follows a sound scientific methodology. The study was primarily conducted for the purpose of assessing migration and dissipation of metalaxyl-M (and the major metabolite) in soil from treated seeds. However, the study also included measurement of the update of metalaxyl-M into aerial plant parts, which is potentially relevant for the higher tier bird and mammal risk assessments.

On the day of treatment 100% AR was in the treated seeds. This rapidly declined to 19.3% AR by 1 DAT and 8.4% AR by 7 DAT. From 1 DAT onwards the majority of the AR was found in soil, with a pattern of lateral spreading from the location of the seed over time. Total AR in the system also decreased over time, reaching 55.9 %AR by the end of the first sub-study (53 DAT). These results therefore indicate that metalaxyl-M (or metabolites) rapidly moved from the treated seed into soil and that residues in soil declined over the 53 day monitoring period.

Measurements of radioactivity in aerial plant parts were only determined from 14 DAT onwards. It is assumed at earlier time points insufficient seedlings had emerged. The % AR in aerial plant parts was relatively low at all measured timepoints – 0.5 % AR (14 DAT), 0.6 % AR (28 DAT) and 2.1 % AR (53 DAT). While the % AR in aerial plant parts slightly increased over time, this does not necessarily mean that concentrations in aerial plant parts would be higher over time, given the volume of above ground plant material will increase. In fact the highest TRR in aerial plant parts was at 14 DAT, which was determined to be equivalent to 1089 ppb metalaxyl-M (or 1.089 mg a.s./kg).

It is noted that pea seeds were treated with Apron XL, containing only the active substance metalaxyl-M. 'Wakil XL' contains additional active substances that are not present in Apron XL. Seeds were treated at a rate of 350 mg a.s./kg seeds in this study. The amount of metalaxyl-M applied per seed with 'Wakil XL' is 339.2 mg a.s./kg seed, which is very close to the treatment rate used in the [redacted] (1999) study.

The use of this study will be considered further in the higher tier risk assessments for birds and mammals from consumption of pea seedlings.

The following study summary is reproduced verbatim from Section B.9 of the EU draft Renewal Assessment Report for mancozeb

Reference	Green, R., 1978
Title	Factors Affecting the Diet of Farmland Skylarks, *Alauda arvensis*. Journal of Animal Ecology 47: 913-928.
Guidelines	No guidelines applicable
GLP	No
Justification	Submitted for support of risk assessment – Higher tier risk assessment omnivorous birds cereals and potatoes– PD refinement

I. Aim

The diet of skylarks on three farmland areas determined by faecal analysis and using a correction factor for different food items, are described and compared.

II. Summary

(1) The diets of skylarks on three farmland areas, determined by faecal analysis, are described and compared. Similarities between the feeding ecology of some of the common farmland bird species are pointed out.

(2) The feeding rates and the sizes and metabolizable energy contents of food items are established for skylarks using each of the three main winter foraging methods. This permits the calculation of rates of metabolizable energy intake from foraging.

(3) Birds fed on cereal grain from stubbles and sowings when it was available. These food sources gave rates of energy intake higher than were found for the other foraging methods. However these other food sources were used to some extent even when grain was widely available.

(4) When cereal grain was unavailable, in late winter, skylarks could either search ploughed land for weed seeds or graze the leaves of winter wheat. The rate of

energy intake possible from feeding on seeds varied with seed size and density but that from grazing was relatively constant from field to field. Birds spent more time foraging for seeds on ploughed land than grazing only where seed densities and sizes allowed a higher rate of energy intake from searching for seeds than from grazing. Where seed densities were low, leaves formed the greater part of the food in late winter

III. Methods

The study was conducted on three areas of arable farmland, one in West Suffolk and two in Cambridgeshire (all UK) from November 1974 to June 1977. Two of the sites had both spring and winter cereals (Barrow and Balsham) and the third (Prickwillow) only had spring cereals.

In each area skylarks were counted at least once every 5 weeks. Feeding skylarks were watched and the number of successful and unsuccessful pecks at food items and, where possible, the types of food being taken were recorded. In the course of a bird count 15-30 faecal pellets were collected from which ten were taken at random for analysis.

The relationship between the quantities of different foods eaten by skylarks and the composition of the resulting faeces was established by a series of feeding experiments on three captive birds.

Six foods were chosen to represent the main types of food taken by wild birds (barley (*Hordeum vulgae*) seeds, *Polygonum lapathifolium* seed, cotyledon stage seedling of sugar beet (*Beta vulgaris*), barley seedling, weevils *Sitona lineatus* and flowers of *Poa annua*).

The birds were individually caged for at least 3 months before the experiments began. Before each experiment they were starved until no faeces had been produced for one hour. The cages were cleaned and measured quantities of food were introduced. After about 5 hours the food remaining was measured and the consumption calculated by difference. The birds were starved after the feeding period and their faeces collected periodically until none had appeared for 90 minutes.

Faeces were dispersed in water, poured through a sieve (0.2 mm apertures) and fragments remaining in the sieve were stained with safranine, light green SF and Canada balsam.

Relative area of seed, grain, leaf, cotyledon or bract epidermis and weevil cuticle were estimated using a binocular microscope and following the point quadrat method, counting about fifty hits for each slide. Then for each food item a correction factor was calculated related to barley leaf which was used as reference item.

The faeces of wild skylarks were processed in the same way as those from the experiments except that faecal pellets were analysed individually and each was broken up and examined for earthworm chaetae before sieving. Fragments of insect cuticle, seed testa, plant epidermis, etc. were identified by comparison with a reference collection.

The composition of the diet was estimated from samples of ten faecal pellets using the formula:

$$P_i = \frac{C_i * FFA_i}{\sum C * FFA}$$

Where, P_i is the proportion by dry weight of food 'i' in the diet
C_i is the correction factor of the food item relative to barley leaf
FFA is the faecal fragment area

The correction factors from are shown below:

TABLE 2. Correction factors (see text) from faecal analysis calibration experiments with captive skylarks

Food type	Correction factor relative to barley leaf
Barley grain	5·11 ± 0·63
Polygonum lapathifolium seed	1·90 ± 0·21
Sugar beet cotyledons	3·55 ± 0·40
Poa annua flowers	0·50 ± 0·07
Sitona weevils	3·34 ± 0·35

Mean ± 1 S.E. for five experiments with each food.

IV. Results

Table 9.1.3.50 shows the correction factor of five food items related to barley leaves.

Table 9.1.3.50: Correction factors of five food items related to barley leaves

Food item	Correction factor relative to barley leaf
Barley grain	5.11 ± 0.63
Polygonum lapathifolium seed	1.90 ± 0.21
Sugar beet cotyledons	3.55 ± 0.40
Poa annua flowers	0.50 ± 0.07
Sitona weevils	3.34 ± 0.35

The main food sources are shown below:

Food source	Main foods
Stubble	Grain
Cereal sowing	Exposed grain
Cereal sowing	Germinated grain
Ploughed land	Weed seeds from soil surface
Winter wheat fields (November–April)	Wheat leaves
Leys, harvested beet and vegetable fields (April–August)	Weed seeds from seeding plants
Growing crops of all types (April–August)	Insects, grass flowers, leaves, weed seeds

The figure below shows the seasonal variation in the importance of six main components of the diet. In all three areas cereal grain was the main food in the autumn, when it was taken from stubbles and sowings, and for a short period at the time of spring sowing.

Leaves, particularly those of wheat and barley, predominated in the diet in mid-winter and late spring at Barrow and Balsham, but were much less important at Prickwillow. Invertebrates formed a large part of the diet in summer.

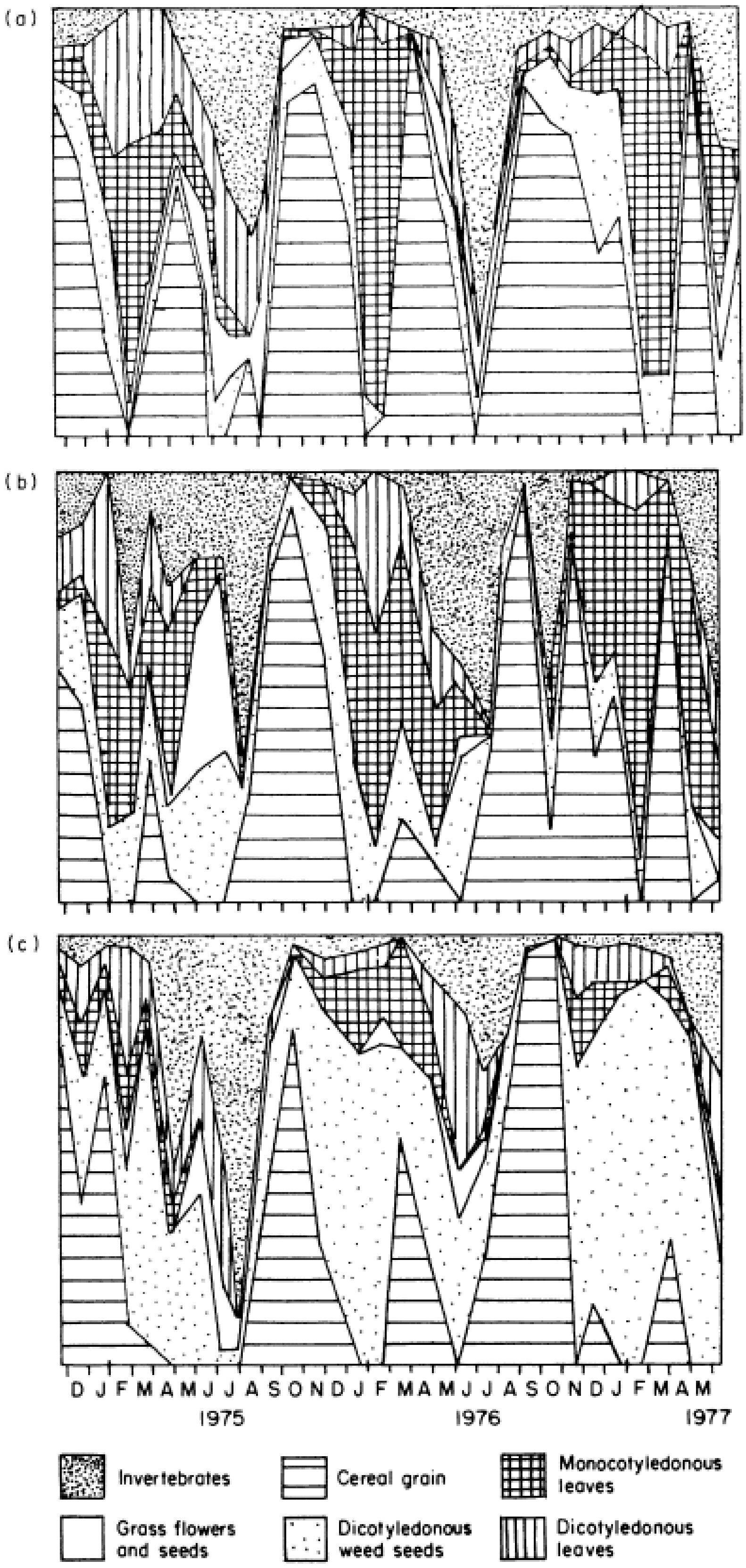


FIG. 3. Seasonal variation in the proportion in the diet of the main foods of skylarks. The vertical extent of the hatched and stippled areas indicates the proportion of the food by dry weight in the diet. (a) Barrow, (b) Balsham, (c) Prickwillow.

Figure B.9.1.3.18: Seasonal variation in the proportion in the diet of the main food of skylarks

Figure B.9.1.3.18 shows the variation in diet (in proportion of dry weight) over the two and a half years of the study at the three sites, but does not give the numerical values. The table below summarises the proportions read from the graphs. There will be some error in deriving these values because it is from a visual examination of the graphs.

Table B.9.1.3.51 summarises the results for each site (dicotyledon weed seeds and grass flowers and seeds have been combined to a single group called weed seeds).

Table B.9.1.1.51: PD data from Green (1978)

Site	Month	Cereal grain	Weed seeds	Mono leaves	Dicot leaves	Invertebrates
A Barrow	D	0.54	0.25	0.13	0.01	0.06
	J	0.23	0.47	0.18	0.10	0.02
	F	0.06	0.10	0.67	0.16	0.01
	M	0.35	0.12	0.36	0.13	0.03
	A	0.67	0.09	0.09	0.08	0.07
	M	0.27	0.19	0.16	0.12	0.26
	J	0.00	0.30	0.13	0.09	0.48
	J	0.21	0.16	0.03	0.10	0.50
	A	0.49	0.06	0.00	0.16	0.29
	S	0.64	0.18	0.00	0.03	0.15
	O	0.75	0.14	0.01	0.04	0.06
	N	0.68	0.20	0.03	0.04	0.05
B Balsham	D	0.35	0.21	0.27	0.13	0.05
	J	0.08	0.15	0.51	0.26	0.00
	F	0.03	0.17	0.53	0.13	0.14
	M	0.43	0.15	0.26	0.11	0.05
	A	0.13	0.10	0.49	0.07	0.22
	M	0.00	0.41	0.18	0.05	0.37
	J	0.10	0.47	0.05	0.01	0.37
	J	0.33	0.05	0.05	0.00	0.57
	A	0.72	0.05	0.00	0.00	0.23
	S	0.63	0.09	0.00	0.00	0.28
	O	0.71	0.09	0.04	0.00	0.16
	N	0.57	0.16	0.05	0.20	0.02
C Prickwillow	D	0.21	0.53	0.13	0.09	0.04
	J	0.22	0.62	0.07	0.06	0.03
	F	0.05	0.71	0.07	0.13	0.03
	M	0.29	0.47	0.16	0.03	0.05
	A	0.08	0.48	0.11	0.06	0.27
	M	0.00	0.45	0.01	0.27	0.26
	J	0.10	0.28	0.00	0.22	0.40
	J	0.25	0.18	0.00	0.00	0.57

	A	0.61	0.16	0.00	0.00	0.23
	S	0.80	0.12	0.00	0.02	0.06
	O	0.89	0.08	0.00	0.01	0.02
	N	0.12	0.63	0.13	0.08	0.04

Cereal grain predominated in the diet in spring and autumn (Fig. 3) suggesting that the birds fed on it whenever it was available from sowings and stubbles and resorted to leaves or weed seeds when it was scarce.

V. Conclusion

Correction factors were calculated for all main food items of skylark in arable areas. These correction factors can be used for the calculation of PD for higher tier risk assessments.

VI. HSE Comments:

This study can be used to calculate the proportions of different food items in the diet of skylarks at different times of year. The study area covered three areas of arable farmland in the UK.

The use of this study will be considered further in the higher tier risk assessment for birds from consumption of pea seedlings.

B.9.1.4. Higher tier studies - mammals

The following study summary is reproduced verbatim from Section B.9 of the EU draft Renewal Assessment Report for metalaxyl-M

Exposure reduction of seed treatments through dehusking behavior of the wood mouse (*Apodemus sylvaticus*).

Carsten Albrecht Brühl, Bernd Guckenmus, Markus Ebeling, Ralf Barfknecht

Environ Sci Pollut Res (2011) 18:31-37

Abstract:

Background, aim and scope:
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 animals, 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.

Materials 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 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 around 60% for cereals to almost 100% for sunflower seeds as a result of the dehusking behaviour.

Discussion:
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.

Conclusions:
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.

Recommendations and perspectives:
Seed treatments are used for most of the cereal crops grown in Europe today. Their advantages usually include a lower application rate and the reduction of drift compared to a conventional spraying regime. However, there is a potential risk especially for granivorous mice, and its assessment is challenging in case of a high residue concentration on the dressed seeds. The concept of a dehusking factor in the risk assessment scheme for seed treatments for granivorous mice is a valid approach to account for the behavioural exposure reduction, and generic data could be easily generated also for other wild mammal species and other seed types, possibly analysing the pigment in commercial seed treatment formulations.

Table B.9.1.4-1: Determination of seed treatment concentration (St_{conc} on fungicide and pigment) on the different treated seed types (mean and SD of five batches of approx. 6 g)

	Seeds	Residue amount in sample of 6 g seed (mg)	Seed treatment concentration St_{conc} (mg/kg seed)
Fungicide	Wheat	0.451 ± 0.013	75.101 ± 2.223
	Barley	0.500 ± 0.017	83.263 ± 2.912
Pigment	Wheat	1.246 ± 0.020	207.643 ± 3.827
	Barley	1.162 ± 0.052	193.633 ± 8.705
	Maize	1.259 ± 0.026	209.753 ± 4.746
	Sunflower	5.544 ± 0.138	923.973 ± 23.536

Table B.9.1.4-2: Residues on seed husks and sand and the resulting calculated exposure reduction through behaviour (mean and SD) for the different seed types and both seed treatments

	Fungicide		Pigment			
	Wheat (N = 13)	Barley (N = 14)	Wheat (N = 12)	Barley (N = 11)	Maize (N = 12)	Sunflower (N = 11)
Consumed seeds S_c (g) measured	1.66 ± 0.76	1.67 ± 0.69	2.672 ± 0.842	2.585 ± 1.025	4.917 ± 1.479	2.390 ± 0.919
Potential exposure E_{pot} (mg) calculated	0.125 ± 0.057	0.139 ± 0.057	0.555 ± 0.175	0.501 ± 0.198	1.031 ± 0.310	2.208 ± 0.849
Residues of seed treatment on husks R_h (mg) measured	0.068 ±0.041	0.102 ± 0.044	0.326 ± 0.144	0.425 ± 0.187	0.587 ± 0.147	2.182 ± 0.846
Residues of seed treatment in sand R_s (mg) measured	0.010 ± 0.005	0.009 ± 0.003	-	-	-	-
Residues of seed treatment on husks and in sand R_{hs} (mg) calculated	0.078 ± 0.043	0.111 ± 0.046	-	-	-	-
Actual exposure E_{actual} (mg) calculated	0.047 ± 0.026	0.028 ± 0.015	0.229 ± 0.088	0.075 ± 0.046	0.444 ± 0.232	0.026 ± 0.045

Reduction through behaviour ER (%) calculated	61.38 ± 15.12	79.47 ± 7.50	58.04 ± 14.55	83.95 ± 9.28	58.97 ± 13.08	98.78 ± 2.03
Dehusking factor DH calculated	0.39	0.21	0.42	0.16	0.41	0.01

N number of mice

(Brühl CA., Guckenmus B., Ebeling M., Barfknecht R., 2011)

HSE comments

This study was previously evaluated in the EU renewal review for metalaxyl-M. It is considered further by HSE in the higher tier reproductive risk assessments for mammals (section 9.1.6).

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	KIIIA 10.3.3/04: [redacted] (2014) Consumption of vegetable seeds by wood mice (*Apodemus sylvaticus*) Report Number [redacted]. [redacted] (Syngenta File No. VV-409633)

Guideline(s): No guidelines were explicitly followed for this study

Deviations: No

GLP: Yes

Acceptability: Yes/ No/ Supplementary

Duplication (if vertebrate study) No

Materials

Test organisms

Species: Wood mouse (*Apodemus sylvaticus*) aged >6 weeks

Source: [redacted]

Acclimatisation period: 14 days

Test design

Test cage description:	30cm wide, 60cm long and 15cm deep
Replication:	10 wood mice
No. of mice/pen:	1

Environmental test conditions

Temperature:	16-20 °C
Photoperiod:	Reversed daylight with night corresponding to red light and day period corresponded to main lights

Study Design and Methods

Experimental dates: 21 June 2012 – 18 September 2012

The test was performed in two parts, a no choice test on day 0 and a choice test for the following three days: On test day 0, ten individual mice per seed type, were given access to seeds (spinach, carrot, onion, oilseed rape or wheat) for six hours. Wheat was used as a reference as it has been shown to be palatable in previous studies. After 6 hours mice were given access to normal maintenance diet and the test seeds removed. On days 1, 2 and 3 mice were offered a choice between the same seeds and an alternative food (normal diet).

The mice were housed individually in mouse/rat colony cages (approx. 30cm wide, 60cm long and 15cm deep) and a 50:50 male to female ratio for each seed type was used.

Reversed daylight was used so that night corresponded to red light on from 0900 to 2100, dusk periods corresponded to low light on from 0830 to 0900 and 2100 to 2130 and the day period corresponded to main lights on from 2130 to 0830. Any food and test seeds provided to the animals were presented at ambient temperature.

Mice welfare was routinely checked on day 0 when the seeds were presented and when the seeds were removed and again daily for the 3-day choice phase. No abnormal behaviour or losses were recorded during this trial. Other data recorded data include: weight of crushed diet, weight of whole seeds and weight of recovered seeds and husks.

The data was analysed using an analysis of covariance (ANCOVA). Two covariates were also considered in this analysis; bodyweights of the mice during the acclimatisation phase and the arithmetic mean of consumption of normal diet during the pre-trial phase. Both covariates for the day 0 ‘no choice trial’ were found to be insignificant at the 5% level, (p=0.40) for mouse bodyweight and (p=0.12) for consumption of normal diet, both covariates were therefore removed from any further analysis. Similarly, in the ‘choice trial’ both covariates were found to be insignificant at the 5% level. Average bodyweights during acclimatisation (p=0.97) and average food consumption (p=0.50) were therefore removed from any further analysis.

Results and Discussion

The seeds presented in this trial varied in attractiveness to wood mice and under no choice conditions ranked from highest to lowest for wheat, spinach, spring oilseed rape (SOSR), onion, winter oilseed rape (WOSR) to carrot. Mice ate less of all seeds under no choice conditions compared to pre-trial normal diet. Under choice conditions mice preferred wheat and spinach to their normal diet and preferred normal diet to summer oilseed rape, onion, winter oilseed rape to carrot. There was relatively little ‘hoarding’ of seed.

Table B.9.1.4-3: Average consumption of normal diet and seeds (mean ± SE)

Seed type	Pre-trial normal diet (g)	Day 0 seed (no choice) (g)	Day 1 seed (g)	Day 1 normal diet (g)	Day 2 seed (g)	Day normal diet (g)	Day 3 seed (g)	Day 3 normal diet (g)
Wheat	1.78 (0.198)	1.41 (0.073)	1.25 (0.143)	0.36 (0.116)	1.49 (0.121)	0.53 (0.136)	1.56 (0.142)	0.45 (0.106)
Spinach	1.55 (0.231)	0.95 (0.083)	1.07 (0.087)	0.52 (0.143)	1.27 (0.178)	0.65 (0.118)	1.59 (0.119)	0.58 (0.112)
SOSR	1.24 (0.121)	0.55 (0.051)	0.57 (0.057)	0.87 (0.061)	0.62 (0.100)	0.88 (0.064)	0.63 (0.098)	0.71 (0.060)
Onion	1.49 (0.158)	0.44 (0.092)	0.09 (0.024)	1.37 (0.149)	0.25 (0.116)	1.67 (0.157)	0.21 (0.045)	1.73 (0.233)
WOSR	1.21 (0.237)	0.29 (0.044)	0.24 (0.032)	1.53 (0.178)	0.22 (0.033)	1.76 (0.205)	0.23 (0.049)	1.31 (0.130)
Carrot	1.76 (0.240)	0.26 (0.088)	0.16 (0.063)	1.51 (0.220)	0.38 (0.111)	1.67 (0.163)	0.36 (0.091)	1.57 (0.186)

In almost all cases, with the exception of two animals presented with wheat, evidence of dehusking was found for all seed types presented on day 0. Mice dehusked all vegetable seed types although the proportion of seeds dehusked could not be quantified explicitly. Some manual dehusking of spinach seeds when compared to wood mice dehusking indicated that on average wood mice dehusk close to 100% spinach seeds, on the basis that following manual dehusking about 50% of the whole seed is represented by husk. Wood mouse dehusking estimates for spinach seeds are however variable ranging from 65 to 163%. The source of this variability is uncertain, possibly due to proportions of husk eaten and seed fragments left by mice during feeding, recovering seed fragments from husk fragments is extremely difficult.

Table B.9.1.4-4: Dehusking behaviour

Seed type	Average wt. Whole seeds eaten (g)	Average weight of husk recovered (g)	% Average husk remaining to weight of seed consumed	Estimate of the population that completely dehusk %
Spinach	1.758	0.9168	94.35	30
Onion	0.6733	0.3628	101.5	60
Wheat	1.5145	0.1624	8.963	0
SOSR	0.786	0.201	36.01	0
WOSR	0.3732	0.1277	43.97	10
Carrot	0.333	0.0432	40.77	10

Conclusions

There was a clear seed preference effect, and this was statistically significant for the ‘no choice’ day 0 phase of the trial, ($p<0.001$). In the ‘choice test’ There appears to be a palatability difference between the two types of oilseed rape seeds tested and consumption of SOSR was higher. There was a significant difference between consumption of the two seeds at the 5% significance level.

Almost all animals displayed dehusking behaviour, ~97%, with the exception of two mice in the wheat seed treatment group. The ratio of seed husk recovered to that of seed consumed does indicate that mice have a predisposition to dehusk.

Overall, there are clear differences in the palatability of the vegetable seeds and the reference wheat seed tested in this trial. Trends in palatability were largely consistent under both choice and no choice conditions.

([redacted] and [redacted], 2014)

HSE comments:

This study was conducted to GLP and investigated seed consumption and dehusking behaviour by wood mice. It is apparent from the study results that while mice ate less of all seed types during the ‘no choice’ phase than they did standard diet during the pre-trial period, wheat and spinach seeds were the two seed types with the highest consumption values in the ‘no choice’ phase. Also, during the ‘choice’ phase of the study it is noted that mice consumed a greater weight of spinach and wheat seeds than their standard diet, with wheat seeds being the most extensively consumed of the six seed types evaluated. Therefore, across the study phases it appears wheat seeds are the most favoured of the seeds tested as a food source for wood mice, with spinach seeds the second most favoured.

The study report states that dehusking was estimated from seed consumed, husk recovered and the relative proportions of husk to seed derived from manual separation. However, it is not clear how the *‘% Average husk remaining to weight of seed consumed’* figures reported in the above table were derived and HSE have been unable to replicate these values from the data in the study report. Further summary dehusking data is provided in the table below, which is reproduced from the study report.

Table B.9.1.4-5: Detailed dehusking data for wood mice

Seed Type	Ave. wt. whole seeds eaten (g)	Min. weight of Husk rec.(g)	Max. weight of Husk rec. (g)	Ave. weight of Husk rec. (g)	Estimate of the population that completely dehusk (%)	Estimate of the population that dehusk to some degree(%)	Percent low to high - levels of dehusking recorded (standard deviation / variance)
Spinach	1.758	0.473	1.565	0.9168	30	70	65 – 163 (31 / 972)
Onion	0.6733	0.033	1.122	0.3628	60	40	3 – 174 (63 / 3974)
Wheat	1.5145	0	0.918	0.1624	0	80	0 – 46 (14 / 204)
SOSR	0.786	0.037	0.71	0.201	0	100	7 – 93 (25 / 628)
WOSR	0.3732	0.025	0.364	0.1277	10	90	11 – 125 (37 / 1392)
Carrot	0.333	0.017	0.077	0.0432	10	90	5 – 114 (36 / 1319)

It is also not clear from the study report how the '*Percent low to high – levels of dehusking recorded*' figures were derived and again HSE have been unable to replicate these values from the data in the study report. Additionally, the basis for considering that individual mice fall within the '*population that completely dehusk*' or whether they fall within the '*population that completely dehusk*' categories is not specified in the study report.

Since seed husks were found in the cages of all individual wood mice presented with vegetable seeds, it is apparent that all individuals dehusked vegetable seeds to some extent. It is noted that this was also the case with other seed types, except wheat where 2 individuals did not dehusk at all and 5 other mice dehusked only to a limited extent (based on the low weight of husk found). Overall the dehusking results provide information on the prevalence of dehusking behaviour in wood mice but the information provided regarding the extent to which wood mice dehusk seeds is more limited and difficult to interpret. The dehusking results from this study are further discussed in the higher tier acute risk assessment for granivorous mammals.

This study was considered in the EU draft Renewal Assessment Report for metalaxyl-M, but no summary of the study was included. Therefore, this study has been summarised and evaluated by HSE below.

Report:	10.2.1, Barber I., Tarrant K.A., Thompson H.M., (2003), Exposure of small mammals, in particular the wood mouse *Apodemus sylvaticus*, to pesticide seed treatments. Environmental Toxicology and Chemistry, 22(5):1134-1139. (Syngenta file no. VV-860234)

Guidelines

None followed

GLP: No

Aim

The aim of this study was to investigate the proportion of seed in the diet of small mammals. Small mammals were trapped for 4 days and 4 nights per week for three weeks, and stomach contents of the rodents were analysed content using microscopy. The proportion of the stomach containing seed husk compared to other food stuffs was quantified.

MATERIALS AND METHODS

Test item:	One field drilled with seed treated with fluquinconazole (750 mg/kg) and one field drilled with seed treated with bitertanol (34.5 mg/kg) and fuberidazole (562.5 mg/kg)
Test species:	Wood Mouse (*Apodemus sylvaticus*) and Voles (*Microtus agrestis* and *Clethrionomys glareolus*)
Crop:	Fields were drilled with treated winter wheat (*Triticum aestivum* variety Savannah) seeds.
Total field size:	2 fields; one termed the ‘drain’ field (4.7 ha) and one termed the ‘wood’ field (5.6 ha). Both fields had edge habitat (thick hedges, woodland) on three sides.
Application:	The study took place in two fields; a drain field was drilled with fluquinconazole-treated seed and a wood field was drilled with bitertanol and fuberidazole-treated seed.

Study design and methods

Experimental dates: October 1999 from drilling of seed to seedling emergence (3-week period).

The study was carried out on study fields near York, United Kingdom. Two fields were used, a drain field (4.7 ha) drilled with fluquinconazole-treated seed and a wood field (5.6 ha) drilled with bitertanol and fuberidazole-treated seed. Both fields were of loamy sand and clay soil types, typical of the area. The fields were separated by at least 50 m of uncultivated arable land and a 50- x 20-m wood, and each field had sufficient edge habitat (thick hedges, woodland etc.) to ensure that small mammals foraging on each were from distinct populations.

Pre-drilling trapping and seed application

A month prior to the study, live trapping was carried out with Longworth traps to confirm whether a sufficient number of small mammals were foraging on both study fields. Before drilling, the fields were disc-harrowed to bury any residual cereal seed from the previous harvest, in an attempt to minimise any additional sources of cereal seed from the field sites and adjacent margins. Each field was plough immediately before drilling and the seed sown at typical agricultural rates and depth. The fields were not harrowed post-drilling due to heavy rainfall following drilling operations.

Grain counts and residue levels

The density of exposed seed on the fields and headlands was measure on the day of drilling and again at 1, 3, 7 and 14 days after drilling. Samples were also taken of fluquinconazole-treated seeds from the drill hopper during drilling and from the drain field on days 1, 3, 7, 14, and 21 (shoots) for residue analysis.

Post-drilling trapping

Immediately after drilling, 200 snap-traps were used on each field for trapping small mammals. The traps were set up for 4 days and 4 nights per week for three weeks. Traps were baited with cheese and set out on a 20- x 10-trap grid, with traps spaced approximately 10 m apart. A row of 20 traps was set in the hedge bottom, with a second row placed 10 m into the field and a third row placed 20 m into the field, and so on. Traps in the field were operated throughout the week until 10 animals had been caught, checked at dawn and dusk. Trapped animals were removed, weighed and sexed.

Stomach contents and residue analysis

Stomachs of the mammals were dissected and examined using a dissecting microscope. The proportion of the stomach containing seed husk versus other solid matter was quantified, and the proportion of the stomach consisting of wheat was then calculated. This was calculated by suspending the stomach contents in ethanol and poured in petri-dish placed over a counting grid, marked in 1-cm squares. The area containing seed husk versus other solid matter was quantified and the proportion of the stomach contents consisting of wheat was then calculated. Residues of fluquinconazole in the stomach, liver and intestine were quantified for the drain field study site (fluquinconazole treated seeds) only. Residues in the stomach were used as a measure of recent consumption of treated seed before capture, whereas analysis of liver and intestine provided an indication of recent exposure in the past day even if no evidence of seed was observed in the stomach.

RESULTS AND DISCUSSION

Grain counts

Overall, mean (± SD) seed densities on both sites were in the range of 1.2 (± 3.2) to 19 (± 32) seeds/m^2 exposed on the field, except for the period following heavy rainfall and high winds on the wood field. In contrast, on the headlands, the number of exposed seeds was higher and more variable than on the field, especially during the first few days after drilling, with mean grain counts between 6.8 (±13) and 60 (± 68) seeds/m^2 over the duration of the study. Authors report that seed density on the soil surface immediately after drilling was similar to that reported in previous studies and that the density of exposed wheat seed is likely to have been typical for autumn drilling scenarios. From this, authors state that the study can be considered representative for autumn drilling of treated seed according to typical agricultural practices conducted in the United Kingdom.

Trapping

Prior to drilling, approximately 80 wood mice were caught on the wood field and 67 on the drain field. Voles were also caught but mainly in the field hedge adjacent to the wood field. After drilling, a total of 19 wood mice (11 female and 8 male) and 8 field/bank voles (4 male and 4 female) were caught on the wood field, and 51 wood mice (27 female and 24 male) were caught on the drain field. All but one of the voles, and 71% of the wood mice were caught in the field hedge. Weights of trapped mice ranged between 9.25 and 23.8 g (n = 70) and included both juveniles and mature adults.

However, regardless of this population of small mammals, authors state that the trapping effort to catch the wood mice was 3.2-fold greater on the field compared to the hedge. It is not clear how they measured this. Nevertheless, voles were trapped almost exclusively in the field hedge adjacent to the crop, as were a high proportion of the wood mouse population.

Stomach contents

The proportion of wheat seed in the stomachs of small mammals trapped on both sites, in the hedge and field, was similar. None of the voles contained any visible evidence of wheat seed in the stomach, and for many of the wood mice (>60%), wheat seed contributed less than 5% of total stomach contents.

Of the 70 individual wood mice caught on the fields (19 in the wood field; 51 on the drain field) , it is stated that 70 to 90%, had some wheat seed in the stomach, with the maximum content of seed in the stomach being 40% of total stomach contents. Over 90% of mice caught in the field contained less than 25% seed in the stomach contents.

Only 22% of mice caught in the hedgerow contained evidence of wheat seed consumption, with the maximum content of seed in the stomach being 28% of total stomach contents. Over 90% of the mice caught adjacent to the field contained less than 5% seed in the stomach contents. The study authors state that seed consumption was lower than reported in previous studies (Pelz, 1989) which estimated that wood mice consumed 30 – 50 % of their diet as cereal grain during the autumn months. Overall, regardless of trap location, 90% of individuals had consumed 20% or less wheat seed, and all had consumed less than 50% wheat seed according to stomach contents.

Residue analysis

Approximately half the trapped mice from the drain field had no measurable exposure to fluquinconazole (limit of quantification (LOQ) was 0.2 µg for liver and intestine samples (~0.1–0.2 mg/kg) and 1 µg for stomach contents solutions (~0.04 µg/ml) immediately before being caught. Mice with detectable residues of the fungicide contained most of the residue in the stomach, although in several cases, significant residues were detected in the intestine and/or liver even though no residue was detected in the stomach. Instances were also observed in which up to 10% of the stomach contents were identified as wheat seed, but fluquinconazole levels were below the level of quantification. Based on the residues determined in the stomach, liver, and intestine of the trapped mice, the maximum estimated exposure concentration of fluquinconazole was 3.93 mg/kg body weight.

De-husking

In the study, authors speculate that the presence of de-husked wheat seed capsules on the drain field (i.e., fluquinconazole-treated site) but not on the wood field showed that some mice removed the seed coat, presumably because they found the fluquinconazole seed-treatment unpalatable. This suggests it is possible for mice to further reduce their exposure to the fungicide, even if they forage on treated seeds by removing the outer seed coat. Residue analysis of the amount of fungicide in the stomach contents for those mice that had consumed seed provided support for this behavioural avoidance of the fungicide, because quantities of fluquinconazole in the stomachs of the mice were lower than expected for the amount of seed consumed.

CONCLUSION

The study shows that exposure of wild mammals to seed treatments can be significantly lower than assumed in the initial, worst-case risk characterisation. If the regulatory goal is to protect small mammals (specifically wood mice) within an arable landscape, it is more realistic to assume that individuals will not feed exclusively on the treated seed. In the present study, seed intake did not typically exceed 20% of the total diet. Furthermore, as illustrated, exposure can additionally be reduced as a result of lower-than-expected pesticide residues on seed and by behavioural avoidance of the seed. The latter may be predictable, at least qualitatively, using relatively simple laboratory palatability studies with appropriate species.

(Barber I., Tarrant KA. and Thompson HM., 2003)

HSE comments

The use of this study in a risk assessment has focussed on the data reported for the wood mouse (*Apodemus sylvaticus*) and voles (*Microtus agrestis* and *Clethrionomys glareolus*) in autumn cereal fields shortly after drilling, in order to gain more realism of the focal species' feeding ecology. The study was not conducted to Good Laboratory Practice (GLP) and did not follow any guidelines. This limits the study's reliability and so it can only be used as supporting information in the risk assessment. No statistical analysis was conducted on the results.

Focal species

The study considers whether the assumption that small mammals extensively forage on freshly drilled cereal crops is appropriate in refined risk assessments. Firstly, trapping data from the study makes it clear that wood mice are an appropriate focal species in comparison to voles due to increased captures in the study fields and adjacent habitat. However, 71 % of the wood mice were caught in the field edge and this suggests that small mammals are more active here in comparison to the interiors of freshly drilled fields. The Authors make it clear that the trapping of wood mice in study fields was increasingly difficult in comparison to the field edges. Although wood mice have been identified as the appropriate small mammal focal species, the low numbers trapped in field interiors questions whether wood mice (and subsequently small mammals) consider the freshly drilled autumn cereal field to be an attractive foraging habitat. However, it is noted that mice were predominantly caught in the hedge rather than the field interior, meaning these individuals reflect the ‘potential consumer’ population rather than the ‘consumer’ population.

Stomach content

There is some variability displayed between individuals’ stomach contents for those caught on the study fields. Authors report 70 – 90 % of mice had some wheat in the stomach, with the maximum amount of seed in the stomach being 40% of total stomach contents. Other stomach contents are not reported and how full the stomach was is also not stated. The raw data is not provided but the visible wheat seed in the stomach plotted against the percentage of mice trapped (%) is displayed in figure 3 (see graph below).

Exposure of small mammals to pesticide seed treatments

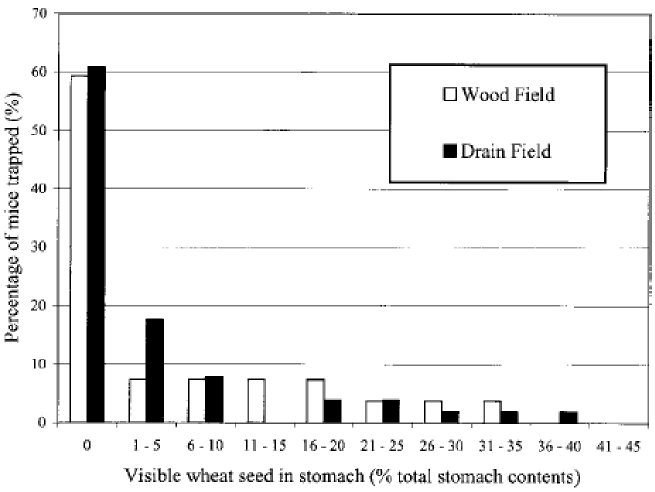


Fig. 3. Percentage of wheat seed in the stomachs of small mammals.

Of the 70 mice caught in the fields it is not clear if all of these individuals were used for stomach content analysis. Irrespective of this, the data indicate that the majority of wood mice trapped (where stomach contents were analysed) are not extensively foraging on the treated seed (~ 60 % for those caught in both study fields), but there is clear variation between individuals. Some mice are clearly foraging on wheat fields to some degree, with up to 40 % of their stomach contents being wheat. Overall, this suggests the first-tier risk assessment assumption that wood mice exclusively forage on freshly drilled autumn cereal fields (i.e. PT = 1) and that all food items obtained from such fields are treated seeds (PD = 1) may be refined at higher tiers, However, it also highlights the need to consider individual variability in foraging behaviour and food selection in the risk assessment. There are limitations with the use of these data to inform on the proportion of food taken by wood mice from freshly drilled autumn cereal fields or the proportion of food items taken from freshly drilled autumn cereal fields that are cereal seeds:

- The proportion of wheat seeds in the stomach of a wood mouse caught on a freshly drilled autumn cereal field does not account for differences in digestibility between food items.

- The study contains no information on food items other than wheat seeds that were found in wood mouse stomachs.

- It is not clear how full the stomachs were of the wood mice analysed.

- Information on surrounding habitats is limited and could influence wood mouse foraging behaviour.

Palatability/dehusking

There is a question regarding treated seed palatability and whether this could act as a potential mitigating factor for reducing overall exposure. Authors report de-husking behaviour of the fluquinconazole-treated seed and that stomach content residues were lower than expected for mice for this study field. However, the authors only explore the residue analysis of fluquinconazole-treated seed study field (drain field) and this dehusking behaviour was not observed at the wood field site. Therefore, conclusions cannot be considered as definitive. The presence of dehusking behaviour on the drain site but not the wood field site could be explained by multiple factors, e.g. an impact of the seed treatment or variability in wood mouse behaviour between locations/populations. It is therefore not clear if the de-husking behaviour observed was a result of the palatability of the treated seed and whether such behaviour can be extrapolated with certainty to other seed treatments, such as those containing metalaxyl-M.

Residues

Authors report that there were instances where up to 10% of the stomach contents were identified as wheat seed, but fluquinconazole levels were below the level of quantification (~0.04 µg/ml). There is no extensive information on the surrounding habitats other than being bordered by hedges and the two sites separated by 50m of uncultivated arable land. There is a question whether mice could potentially be foraging wheat seed from the surrounding areas resulting in low residues of fluquinconazole in the stomach contents. Authors attribute this result to behavioural avoidance of the fungicide, because quantities of fluquinconazole in the stomachs of the mice were lower than expected for the amount of seed consumed. Unfortunately, authors do not explore residue analysis for the wood (bitertanol and fuberidazole treated) field as their focus is on the fluquinconazole fungicide only. However, it would have been useful to explore whether residues for the wood field were more prominent where de-husking was not observed to take place, and subsequently show whether exposure was increased as a result of not displaying this behaviour.

Overall the study provides some support for the wood mouse as an appropriate focal species for freshly drilled autumn cereals and the stomach content results indicate that the PT and PD parameters could be considered further in higher tier risk assessment, though case-by-case consideration will be required. These points are considered further in the risk assessment section.

This study was considered in the EU draft Renewal Assessment Report for metalaxyl-M, but no summary of the study was included. Therefore, this study has been summarised and evaluated by HSE below.

Report:	**Green R., (1979), The ecology of Wood mice (*Apodemus sylvaticus*) on arable farmland. Journal of Zoology, 188:357-377. (Syngenta file no. VV-748945)**

Guidelines

None followed

GLP: No

MATERIALS AND METHODS

Test material:	No test substance was used.
Species:	Wood mice (*Apodemus sylvaticus*)
Test site:	Broom's Barn Farm, Higham, Suffolk. The study area included several fields, but only a short length of hedgerow and was distant from other hedges and woods. Crops grown in the fields included spring barley, spring and winter wheat, field beans, sugar beet and barley under sown with ryegrass.
Trapping site:	Trapping points were laid out in the study area on two 7 x 7 square grids which were 20 m apart. From October 1975, trapping was also carried out at 13 points at 10 m intervals along the northernmost part of the 20 m spaced grids.
Sampling period:	December 1974 to August 1977

Study Design and Methods

Experimental dates: Sampling between December 1974 to August 1977

The study was carried out at Broom's Barn Farm, Higham, Suffolk, an experimental station specialising in sugar beet research. The study area included several fields, but only a short length of hedgerow and was distant from other hedges and woods.

To investigate the ecology of wood mice (*Apodemus sylvaticus*) in arable farmland, trapping points were laid out on two 7 x 7 square grids 20m apart. These grids lay with their central points 240 m apart, within a 5 x 7 rectangular grid with points 80 m apart. Trapping sessions were conducted at approximately 5-week intervals from December 1974 to August 1977. The traps were pre-baited for two nights and then set to catch for a further three nights (if farming operations allowed, otherwise this was reduced to 2 nights).

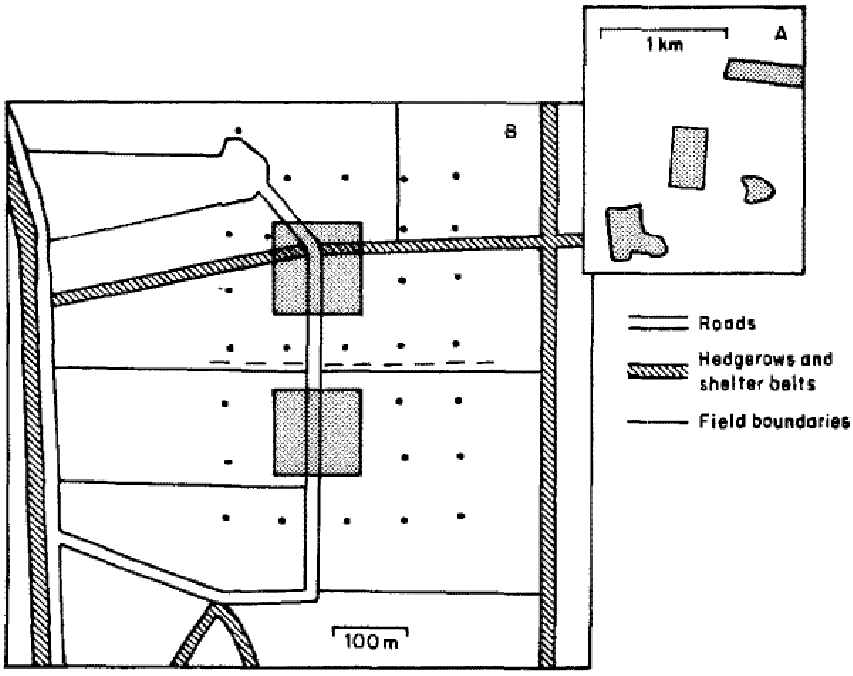


FIG. 1. The study area at Broom's Barn Farm. Map A shows the trapping area (rectangle) in relation to the nearest woods (stippled). Map B shows the trapping area in detail. Trapping points on the 80 m spaced rectangular grid are marked by dots and the two 20 m spaced grids by stippled squares. The dashed line divides the area into the two parts which were trapped separately.

All mice caught were sexed, weighed and individually marked. The point of capture for each mouse was recorded. Sexual characteristics of the mice were also recorded. After examination, the mice were released at the point of capture. The study was divided into breeding and non-breeding periods. Parameters including home range (calculated based on furthest apart trapping locations), trapping efficiency, population density, early juvenile survival, minimum survival rate, proportion of adult mice which had overwintered and habitat preference were assessed.

Samples of stomach contents were taken from mice caught with snap traps baited with cheese on fields adjacent to the live trapping study area. Food types present in the stomach contents were identified by microscopic examination by reference to preparations of animal and plant material collected in the study area. An estimate of the relative area of pieces of different categories of foods was made as an estimate of the composition of the stomach contents by volume.

RESULTS AND DISCUSSION

Wood mice were caught on all of the 29 trapping sessions. The distribution of captures is shown in the table below. There were no differences in the average number of captures per trap per session between the northern part of the grid and the southern part of the grid.

B.9.1.4-7: Distribution of wood mice captures in the study area

Parameter	Northern part of the grid	Southern part of the grid
Number of trap points x number of sessions (A)	1856	1740
Number of captures (B)	977	855
B / A	0.53	0.49

* The figures exclude traps set in the shelter belt

Population dynamics

Estimated wood mice population density showed marked annual density fluctuations, with the highest densities being in early winter and the lowest in late spring-early summer. The lowest density recorded was 0.46 mice/ha and the highest was 17.54 mice/ha. Males and females showed broadly similar changes in density. The pattern of population change varied from year to year, the autumn-early winter peak ranged from 8.44 to 17.54 mice/ha and in late February - early March densities ranged from 1.44 to 8.14 mice/ha.

Onset of breeding and duration of the breeding season

All mice were recorded as non-reproductive for a period in the three winters of the study. The proportion of breeding females which were pregnant or lactating showed no significant trend through the study, but in 1976 this proportion was significantly higher (80.3%) than in 1975 and 1977 (64.2% and 52.3%, respectively).

Production of juveniles

The first young of the season were trapped in May and, from their weights, were estimated to have been born in March. The index of juvenile survival showed considerable variation throughout the breeding season. The index increased to a peak as the breeding season progressed and declined later. There was some tendency for juvenile survival to be inversely correlated with the proportion of overwintered males, but this relationship was not statistically significant. Both male and female density had little effect on juvenile survival.

Table B.9.1.4-8: Correlates of survival index of juvenile wood mice

Variables correlated	Variables excluded	Partial correlation coefficient	Significance level (P)
Juvenile survival index and male density at weaning	Female density and proportion of overwintered males at	- 0.12	> 0.20

	weaning		
Juvenile survival index and female density at weaning	Male density and proportion of overwintered males at weaning	- 0.02	> 0.20
Juvenile survival index and proportion of overwintered males at weaning	Male and female density at weaning	- 0.39	0.20 > P > 0.10

In the 1975 – 1976 winter the mean survival rate was lower than in the two other winters in the period November – February, but mean survival rate declined through this period in all winters. In contrast, the survival rate improved in Spring 1976, but declined in Spring 1975 and 1977.

Mean observed range lengths were greater in summer than in winter for both sexes, with no consistent differences between the sexes in any season. The home range areas of wood mice are shown in the table below.

Table B.9.1.4-9: Home range areas of wood

Season	Year	Mean range area (m^2) (sample size in brackets)	
		Males	Females
Winter	1974 – 1975	2446 (14)	5333 (18)
	1975 – 1976	1874 (10)	3206 (12)
	1976 – 1977	5647 (13)	5342 (3)
	Total (winter)	3416 (37)	4561 (33)
Breeding season	1975	13872 (6)	5342 (6)
	1976	9504 (12)	8028 (6)
	1977	14533 (9)	5885 (9)
	Total (breeding season)	12151 (27)	6337 (21)
Mean range area of known age males in breeding season all years combined			
Known overwintered		13994 (17)	
Known young of year		10803 (8)	

Mean relative densities calculated for the different periods of the year and different crop and habitat types are shown in the table below. There was no preference shown for either ploughed land or winter wheat, the main field types available in winter. However, the relative density of mice in winter wheat increased in the spring and summer, and these fields held greater densities of mice than spring-sown cereals. Sugar beet fields held relatively few mice in spring and summer, but high densities occurred in autumn when the crop provided thick cover and late-germinating weeds seeded.

Table B.9.1.4-10: Relative densities of wood mice in different habitats

Period of year	Habitat	Mean relative density ± 1 standard error	Number of trapping sessions
Oct – March	Ploughed land	0.99 ± 0.08	14
Oct – March	Winter wheat	1.03 ± 0.11	13
April – July	Spring sown cereals	1.02 ± 0.12	16
April – July	Winter wheat	2.30 ± 0.39**	11
April – July	Sugar beet	0.55 ± 0.09***	10
April – July	Field beans	0.79 ± 0.28	10
August – Oct	Sugar beet	1.40 ± 0.19	8
All year	Shelter belt	0.47 ± 0.13***	20

Values significantly different from 1.00 at *p<0.05; **p<0.01; ***p<0.001
Relative densities are densities on the given crop type relative to the overall density on whole area

Food

The information available on the diet of wood mice during the study was divided by time of year and the field type on which the mice were trapped. Three periods were considered:

1) The autumn and early winter (September – December)
2) The late winter (January – March)
3) The spring and early summer (April – June)

No mice were caught for stomach analysis in July or August and the samples for spring and early summer were small. Endosperm from grain and weed seeds formed a large part of the diet of mice caught in autumn and early winter. In winter, wheat sowings and ploughed land which had grown cereals in the previous summer, sown and shed grain was taken in quantity. Based on the stomach contents of the sampled wood mice, wood mice diet in different seasons is summarised in the table below. In winter the main foods were grain, waste sugar beet roots, weed seeds and soil invertebrates and in spring and early summer, the main food was seedling weeds.

Table B.9.1.4-11: Diet of wood mice in the study

	Percentage of stomach contents by volume		
	September -	January – March	April – June

	December		
Winter wheat			
Earthworm	0	16	0
Arthropods	16	16	12
Cereal endosperm	60	55	6
Dicot seed endosperm	24	3	0
Root tissue	0	0	0
Leaf tissue	0	1	1
Other plant tissue	0	7	0
Chickweed seed	0	2	27
Grass flowers and green seeds	0	0	53
Number of stomachs (number of fields sampled in brackets)	8 (1)	30 (5)	15 (2)
Ploughed land, previously cereal			
Earthworm	0	20	-
Arthropods	22	45	-
Cereal endosperm	29	11	-
Dicot seed endosperm	41	12	-
Root tissue	7	8	-
Leaf tissue	2	0	-
Number of stomachs (number of fields sampled in brackets)	18 (6)	10 (2)	-
Ploughed land, previously sugar beet			
Earthworm	0	3	-
Arthropods	4	19	-
Cereal endosperm	18	0	-
Dicot seed endosperm	78	0	-
Root tissue	0	78	-
Number of stomachs (number of fields sampled in brackets)	14 (2)	9 (1)	-

There was a decline in the proportion of endosperm in the diet from early to late winter, but on winter wheat cereals endosperm was still the most important food in late winter. Lepidopteran and coleopteran larvae and earthworms were taken in quantity on all field types. On the ploughed land which had previously grown sugar beet, mice caught in February had been feeding mainly on pieces of sugar beet root left in the soil from previous harvests and caterpillars.

The only samples taken in spring and early summer were from winter wheat fields where the crop was well grown at the time of sampling. Weed seeds formed the bulk of the food found in the mouse stomachs examined.

Mice were found all over the study area, with no tendency for aggregation in or near the shelter belt. Captures were less frequent in the shelter belt that the fields, and since field nest sites were observed at moderate density, it seems that most mice must nest in the field.

Invertebrates were more important as food during the winter. In spring and early summer, the highest densities of mice occurred in winter wheat fields and the lowest on sugar beet fields. The mice living in winter wheat fields in early summer were feeding on mainly seeding weeds which were not available in abundance in spring sown crops due to spring cultivations and herbicide treatments.

CONCLUSION

Wood mice were found on cultivated land away from cover throughout the year and had burrows in the open fields. The average population density, seasonal density changes and timing of breeding seasons were similar to those reported for deciduous woodland. The survival rate of juvenile mice varied greatly within and between breeding seasons. Wood mice showed no marked preference for fields with different crops in winter when the remains of crops, weed seeds and soil invertebrates were the main foods. spring and early summer, the mice preferred winter wheat fields and many of them shifted their home ranges. It is suggested that seeding weeds provide a more abundant food supply in winter wheat fields than in spring sown crops.

(Green R., 1979)

HSE comments:

The population dynamics, ranging behaviour and habitat preferences of wood mice (*Apodemus sylvaticus*) were studied by live trapping methods on arable farmland. The study was not conducted to Good Laboratory Practice (GLP) and did not follow any guidelines. This limits the study’s reliability and so it can only be used as supporting information for the risk assessment. Any statistical analysis conducted was not reported.

For stomach content analysis, a total of 104 stomach contents were analysed between September to June. No mice were caught for stomach analysis in July or August. Between April – June samples were only taken on winter wheat fields. Authors do not go into detail why samples were not obtained for these months.

The percentage volume figures stated in the study do not appear to account for the digestibility of the different food types through correction factors. If the stomach content volumes are not adjusted for the digestibility of individual food items, then the proportion of certain food items that are harder to digest consumed by wood mice may be overestimated relative to easier to digest food items.

The number of stomach content samples varied between study periods, with a change in the number of fields sampled from. For example, (n = number of stomach contents sampled) for winter wheat fields between September – December, n = 8 but only in 1 field; between January – March n= 30 over 5 fields; and between April – June, n= 15 over 2 fields. It is not clear whether the same study fields for the different sampling periods were used (no clear location of where the samples came from is included), and what the surrounding habitat consisted of at that time of year e.g. recently drilled cereal, bare soils etc. There is a question regarding adjacent land uses as favourable/unfavourable nearby habitats may influence a change in stomach contents reported for that fields' land use. From the paper, figure 1. shows the study area (reproduced above). However, this does not provide clarity to what fields were winter wheat, ploughed (previously cereals) and ploughed (previously sugar beet), where mice were captured for that sampling period, and if those captured were close to favourable habitat e.g. hedgerows, or other agricultural practices e.g. nearby more established cereal crops, which could potentially influence stomach content of wood mice throughout the year.

It was not stated why the number of fields sampled from varied over the year (i.e. if any issues were faced, densities decreased for sufficient captures etc) and why no samples were provided for the ploughed areas (previously cereals & sugar beet) between April - June. It was not clear from the study when the winter wheat was drilled and when the ploughed land (previously cereals or sugar beet) was ploughed, which does not allow the associated stomach content to be assessed to see whether these land managements has potential to influence what the mice were foraging on. Additionally, stomach content analysis was averaged between sampling periods between months e.g. an overall value for September – December; January – March; and April – June. Again, it is not clear when individuals were captured during these periods and whether the average stomach contents provided could overlook variations within these study periods.

The use of this study is discussed further in the higher tier risk assessment section.

This study was considered in the EU draft Renewal Assessment Report for metalaxyl-M, but no summary of the study was included. Therefore, this study has been summarised and evaluated by HSE below.

Report:	**Pelz H-J., (1989), Ecological aspects of damage to sugar beet seeds by *Apodemus sylvaticus.* Book chapter in: Mammals as pests (Authors: Pascual J.A., Hart A.D.M., Saunders P.J., McKay H.V., Kilpatrick J., Prosser P.). pp 34-48. (Syngenta file no. VV-419808)**

Guidelines

None followed

GLP: No

Aim

The aim of this paper was to review several factors that possibly influence wood mice (*Apodemus sylvaticus*) damage in sugar beet crops, based on field and laboratory data. The critical time for losses due to wood mice is between sowing and emergence of sugar beet.

MATERIALS AND METHODS

Test item:	No test substance was used.
Test species:	Wood mouse (*Apodemus sylvaticus*)

Test designs

Field diet study

Test sites:	Farmland in the Rhineland, between the cities of Cologne and Aachen
Terrain:	Bushes and trees were completely absent, except for some around the farmhouses, and the nearest wood was approximately 8 km away
Sugar beet sowing times:	Mid-March to mid-May
Dates:	1976 to 1977 and from 1980 to 1986
Number of wood mice:	465 caught in snap traps
Endpoints:	Sex, weight, reproductive state, food components in the stomach

Laboratory palatability study

Test arenas:	Metal test boxes containing eight bowls of food items, hidden below a cardboard layer
Number of wood mice:	6 mice tested per week
Endpoints:	Food choice (gnawing off the cardboard to reach the food) and amount consumed to rank order of preference

Laboratory precipitation study

Test arenas:	Indoor pens containing a light soil
Sugar beet sowing density in the pens:	72 seed pills 1 cm deep in 2 m^2
Number of wood mice per pen:	1
Replicates:	2 per level of precipitation
Precipitation:	Water was evenly sprayed on the dry ground after the sugar beet had been sown (2, 4, 8, 12, 16 or 32 mm/m^2)
Temperature:	25°C

Endpoints:	Number of seeds taken by the wood mouse was determined after 5 hours 30 minutes

Field crop damage survey

Test site:	West Germany
Number of sugar beet fields included in survey:	70/year (average field size 4 to 5 ha)
Dates:	1982 to 1987
Endpoints:	Occurrence of damage, time of sowing and germination, use of pesticides, agricultural practices, precipitation and temperature during the sowing period and soil type
Damage evaluation:	A four point scale of damage severity to sugar beet seeds in fields (very low – damage occurs sporadically without economic importance; low – little but noticeable damage, re-sowing in not necessary; medium – considerable damage to parts of fields, partial re-sowing may be necessary and high – total area is heavily damaged and new sowing is necessary)

Field abundance studies

Traps:	Snap traps
Location:	On the edge of sugar beet fields
Density:	40 m apart
Timing:	Before sowing or immediately after the sugar beet had been sown
Endpoint:	Abundance of wood mice

Study Design and Methods

Experimental dates: Field studies and surveys were conducted in 1976 and 1977 and from 1980 to 1987

Dietary studies

Field studies were conducted in farmland in the Rhineland in 1976 to 1977 and 1980 to 1986. Average field size was 4 – 5 ha; average farm size 30 – 40 ha. Bushes and trees were completely absent (except some near farmhouses), and the nearest wood was present at a distance of 8 km. Farmers practice a three-year crop rotation with sugar beet (mid-March and mid-May) – winter wheat (September/October) – winter barley (mid-September and mid-December ,depending on weather conditions).

During this time 465 wood mice were caught in snap traps, sexed, weighed, reproductive status and stomach contents analysed and recorded.

In laboratory palatability trials, six mice were exposed each week to metal test boxes containing different dietary components available in arable fields. Food choice and amount consumed were recorded to rank order of preference.

Weather conditions

A survey was conducted to determine the effects of rainfall and soil surface temperature on the damage to sugar beet by wood mice between sowing and emergence. Data was collected between 1982 and 1987 from a mean of 70 fields each year in West Germany. In a laboratory precipitation study, the number of pelleted sugar beet seeds found and dug out by a wood mouse in a test plot which had received a set amount of precipitation was determined after 5 hours 30 minutes.

Abundance

Relative abundance of wood mice was calculated on the basis of snap trap catches located on the edge of sugar beet fields set before sowing or immediately after sugar beet sowing.

RESULTS AND DISCUSSION

Dietary studies

Data from field studies investigating the food components in the stomach contents revealed that cereals and invertebrates (of which earthworms were the most consistent element) were the main basic food components and weed seeds and vegetative parts of plants supplemented the diet.

In laboratory palatability studies, cereals, insect larvae and sugar beet seeds were found to be preferred, as shown in the table below. For groups A and B, the results are listed in order of preference.

Table B.9.1.4-12: Dietary preference of wood mice of components available on arable farmland

Group A: High preference	Group B: Low preference	Group C: Not consumed
Helianthus annuus[a]	*Viola arvensis*[a]	*Thlaspi arvense*[a]
Alopecurus myosuroides[b]	*Rumex obtusifolius*[a]	*Matricaria chamomilla*[a]
Hordeum vulgaris[b]	*Lumbricus terrestris*	*Plantago major*[a]
Scotia segetum[d]	*Simapis alba*[a]	-
Beta vulgaris[c]	*Chenopodium album*[a]	-
Triticum sativum[b]	*Capsella bursa-pastoris*[a]	-
Polygonum persicaria[a]	*Secale cereal*[b]	-
Beta vulgaris[e]	-	-
Zea mays[a]	-	-
Avena sativa[b]	-	-

[a] Seeds, [b] caryopses, [c] seeds pills, [d] larvae, [e] root tissues.

Cereals, insect larvae and sugar beets seeds were found to be highly preferred. Earthworms were less often consumed and seem to be of medium preference for wood mice.

Weather conditions

From the survey, there was an inverse relationship between the level of damage and amount of precipitation during the period between sugar beet sowing and emergence, as shown in the Table below.

Table B.9.1.4-13: Average precipitation, wood mouse abundance and damage in March/April

Year	Damage assessment	Relative abundance* of *A. sylvaticus* (%)	Rainfall/24 hours in the period from sowing to emergence (mm)
1982	Medium/high	75	1.2
1983	Very low	203	3.0
1984	High	121	1.0
1985	Low	59	1.7
1986	Low	87	1.5
1987 (preliminary)	Low	55	1.7

*Relative abundance calculated from snap trappings covering field areas of 170 ha on average/year. Average mouse density in 1982 to 1987 was 1.14 individuals/ha.

$$Relative\ abundance = \frac{number\ of\ woodmice\ per\ hectare\ x\ 100\%}{average\ number\ of\ woodmice\ per\ heactare}$$

Damage was also observed at a similar level independent of sowing time. Only in fields sown before 20 March of after 30 April was no damage reported.

In laboratory precipitation studies, there was only a weak relationship between amount of rainfall and ability of wood mice to discover sugar beet seeds. With a precipitation of 32 mm/m^{2,} which is unlikely to occur in one cloudburst in the field, the test animals found more than 60% of the seeds in the soil.

Abundance

Peak densities of wood mice in farmland are observed in sugar beet fields (approximately 40 to 80 per ha). However, population density appeared to have little effect on seed damage, as shown in the table above. In 1982 and 1984 seed loses were high despite low population densities and in 1983 population densities were high but seed losses were low.

CONCLUSION

Data from field studies investigating the food components in the stomach contents of wood mice, over several years, determined that cereals and invertebrates were the main basic food components and weed seeds and vegetative parts of plants supplemented the diet. In laboratory palatability studies with wood mice, cereals, insect larvae and sugar beet seeds were the preferred food choices.

Survey data on the effects of weather conditions (precipitation and soil surface temperature) on the damage to sugar beet crops by wood mice identified an inverse relationship between the level of damage and amount of precipitation and temperature during the period between sowing and emergence (damage also occurred regardless of soil type). In laboratory precipitation studies there was only a weak negative relationship between amount of rainfall and ability of wood mice to discover sugar beet seeds.

Damage to sugar beet seeds appears to occur independently of wood mice population dynamics.

(Pelz HJ., 1989)

HSE comments:

This paper includes field studies, laboratory palatability and precipitation studies and field abundance studies for wood mice (*Apodemus sylvaticu*s). Field crop damage surveys were also conducted. The study was not conducted to Good Laboratory Practice (GLP) and did not follow any guidelines. This limits the study's reliability and so it can only be used as supporting information for the risk assessment. Any statistical analysis conducted was not reported.

Study area

The field study gathered data in a typical sugar beet growing area. Authors report that bushes and trees were absent apart from some around farmhouses with the nearest wood being present 8 km away. Farmers practise a three-year crop rotation with sugar beet (mid-March – mid-May), winter wheat (mid-September) and barley (mid-September and mid-December). A map of the study area in spring 1984 was provided, but it did not show the land uses of each field and how it changed throughout the duration of the study. Therefore, it does not show to what extent drilled, pre-emergence fields were part of the study area during the periods investigated. It does show, however, that the area was extensively arable farmland with little hedges or forests that may be considered more favourable habitats.

Diet composition

The diet composition of wood mice on arable farmland over several years was evaluated by stomach content analysis (n = 346; pooled data of several years). Pelz (1989) reported that cereals and invertebrates are the basic food components, while weed seeds and vegetative parts of plants supplement wood mice diet, according to the season. From figure 3.3, stomach contents for cereal grain and earthworms accounted for > 60% volume of stomach contents in April which decreased to ~ 40 %

in September and continuing to decrease in November. Sugar beet seeds were a small proportion of stomach content volume and seen only between February – April. Dicot seeds were consumed relatively constantly throughout the year, apart from between March – May when consumption on cereals and earthworms was greatest. Vegetative plant tissue was a consistent part of wood mice diet throughout the year.

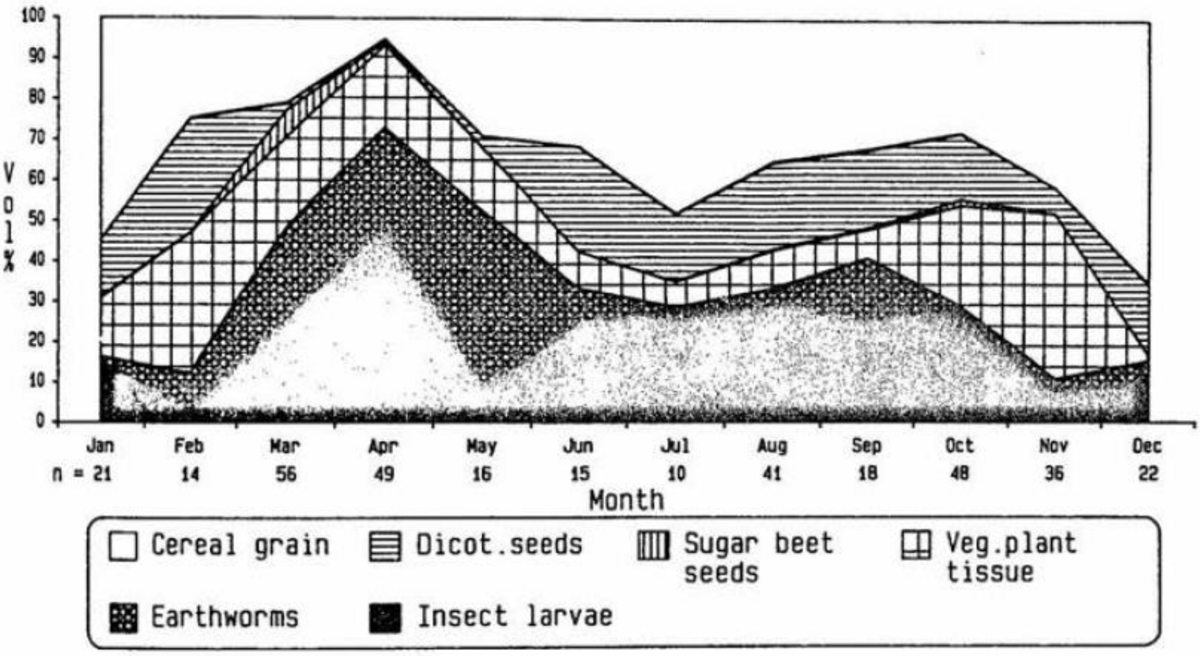


Figure 3.3 Diet of wood mice on arable farmland (analysis of stomach contents, pooled data of several years).

However, it was not clear if the percentage volume figures stated in the study account for the digestibility of the different food types through correction factors. Authors state stomach content analysis was assessed as described by Hansson (1970). If the stomach content volumes are not adjusted for the digestibility of individual food items, then the proportion of certain food items that are harder to digest consumed by wood mice may be overestimated relative to easier to digest food items. It is also not stated if stomach contents were identified by referencing animal and plant material collected in the study area. Additionally, it is not clear from the paper whether arithmetic means or percentile values are used to report the volume of certain food types over the study period. Mean or medians may result in bias, with the diet compositions that are averaged across a long time period having the potential to underestimate the risk to mammals.

The use of this study is discussed further in the higher tier risk assessment section.

This study was considered in the EU draft Renewal Assessment Report for metalaxyl-M, but no summary of the study was included. Therefore, this study has been summarised and evaluated by HSE below.

Report:	**10.2.1, Abt K, F., Brock W., F., (1998), Seasonal variations of diet composition in farmland field mice *Apodemus spp.* and bank voles *Clethrionomys glareolus*. Acta Theriologica, 43(4):379-389. (Syngenta file no. VV-859296)**

Guidelines

None followed

GLP: No

Aim

The aim of this study was to investigate the feeding habits of field mice (*Apodemus flavicollis* and *A. sylvaticus*) and bank voles (*Clethrionomys glareolus*) in a mixed farmland area in Northern Germany. For semi-quantitative analysis of diet composition, faeces were sampled from animals caught during a capture-mark-recapture program in the margins of an arable field surrounded by hedgerows.

MATERIALS AND METHODS

Test item:	No test substance was used.
Test species:	Wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*) and bank vole (*Clethrionomys glareolus*)
Test site:	Mixed farmland area near Kiel in Northern Germany
Sampling site:	Faecal samples were taken along the margins of a field 2.7 ha in size, surrounded by spruce-oak forest, grassland and a maize field with hedgerows bordering on all edges except the eastern edge
Sampling period:	March to December 1992

Study design and methods

Experimental dates: Sampling between March and December 1992

Land management

The field was initially tilled with winter-rye, which was harvested in July. Subsequently, the grass *Lolium perenne* was sown and cut for the first time in October. Large beech -*Fagus sylvatica* and oak-trees -*Quercus robur* within the hedges and on the eastern edge of the field had peak fruit-fall in the autumn of 1992, as had the nearby beech forest.

Sampling

Forty-nine Longworth-traps were set in line around the field in 15 m intervals. From March to December 1992, trapping was carried out twice every month on 2 subsequent days, except where night temperatures were too low to guarantee the survival of trapped animals. Peanuts were used for baiting because their characteristic cellular pattern allowed for easy discrimination from natural food items in faecal analysis.

A total of 254 faecal samples were obtained from 164 faecal specimens summarised in the table below. In April sampling was impossible due to cold night temperatures.

Table B.9.1.4-14: Number of faecal samples/number of specimens by species and month

Species	Mar	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
A. flavicollis	3/3	5/5	6/6	16/14	13/10	10/10	15/13	13/10	3/3	**84/52**
A. sylvaticus	7/7	4/4	11/9	14/12	12/10	14/11	13/13	4/4	4/4	**83/55**
C. glareolus	6/6	7/7	13/10	12/8	15/13	15/14	5/5	11/10	3/3	**87/57**
Total	16/16	16/16	30/25	42/34	40/33	39/35	33/31	28/24	10/10	**254/164**

Diet analysis

Individual samples were cleaned and mounted on slides for microscopic analysis. Food remains were identified under 10 – 100-fold magnification, using histological and morphological criteria from reference collections. Food items were identified to species level, and where possible, assigned to one of the following categories: (0) bait, (1) cereals, (2) tree seeds, (3) other seeds and fruits, (4) flowers, (5) green plant matter, (6) all remaining or non-identified plant matter, and (7) animal matter.

Due to the difficulty in establishing precise volume or weight percentages of food items, a semi-quantitative assessment of food composition was made. The relative proportion of each food item in an individual sample was subjectively estimated on the basis of 5 classes: items making up less than 5% were only noted as present, i.e. given a value of 0. An estimated proportion between 5 and 25% was assigned a value of 12.5%. The three subsequent classes, each covering a range of 25%, were represented by their respective class means, that is 37.5% (25 - 50%), 62.5% (50 - 75%), and 87.5% (75 - 100%). These figures do not necessarily sum up to 100% in an individual sample.

The volume percentage index of each food category was calculated (v-values), and the similarity of diet compositions between two species within one month was also calculated (c-values).

RESULTS AND DISCUSSION

Cereals

Cereals, namely rye, served as the main food for all three species in the period of June – August, and for *A. sylvaticus* up to September. Some rye was still eaten thereafter, presumably originating from caches set up by the animals during the summer. In *A. sylvaticus* and *A. flavicollis,* some cereal matter (maize) was also found in March, suggested to probably have been taken from the remainder of the preceding year's crop in neighbouring fields.

Trees

Tree seeds were of some importance in *Apodemus* diet in spring, but almost disappeared during the summer. In autumn and winter, acorns and/or beechmast became more important.

Other seeds, fruits and caryopses

There was a moderate peak in the proportion of weed seeds and caryopses in *A. sylvaticus* in June. Seeds of Brassicaceae and Chenopodiaceae and caryopses of *Digitaria* spec. were, with one single exception, found only in *Apodemus* samples. These weeds are typical elements of the field flora, and authors attribute this absence in *C. glareolus* samples may indicate the restriction of this species to the immediate vicinity of the hedgerows, whereas *A. sylvaticus* and *A. flavicollis* both range into the field area.

Flowers

Ingested part of both mono- and dicotyledon flowers were mostly anthers. Flowers occurred in small amounts but reached high proportions in May. Authors suggest this is due to beech flowers covering most of the ground and hedgerows as well as in the nearby beech forest. The anthers were found in considerable amount in all samples of May, except for some *C. glareolus.*

Green plant matter

Green plant matter was most prominent in the diet of *C. glareolus*, with a decline in July and August and a significant rise in December, when grass leaves made up the biggest part of the diet. For *A. sylvaticus*, peaks in green plant matter occurred in the diet in July and November.

Animal matter

The proportion of animal matter remained relatively consistent with season. This category was most important for *A. flavicollis*, with v-values ranging between 20 and 40% throughout the year. The animal matter v-values for bank voles were considerably lower, except in May and June. Some insect taxa seemed to be available all year round while earthworms were primarily eaten in the summer. However, authors urge caution with these results as the importance of animal food may be over-estimated due to limitations of the method.

Diet overlap between species

In March, diet overlap between bank voles and the two field mice species was very low, and the overlap between the two field mice species could not be assessed with significant accuracy. In May, diet overlap between bank voles and the two field mice species was higher and overlap between the two field mice species was very high. In the period of June to August, diet overlap between all three species was high due to the high level of rye grain intake.

After August, diet overlap dropped sharply between bank voles and *A. flavicollis.* Overlap between the two field mice species remained above 0.5 throughout the rest of the year, with increased variance. However, the diet overlapping between bank voles and the two field mice species fell below 0.2.

CONCLUSION

The aim of this study was to investigate the feeding habits of field mice (*Apodemus flavicollis* and *A. sylvaticus*) and bank voles (*Clethrionomys glareolus*) in a mixed farmland area in Northern Germany.

Diets of both *Apodemus* species were similar, with *A. flavicollis* tending to consume more animal food and less green plant matter. In general, except in early summer *C. glareolus* consumed less animal matter than both *Apodemus* species, but not consistently more green plant matter than *A. sylvaticus*.

Beech flowers were important food for all species in May, whereas rye grain was the dominant food source until August. In the autumn and winter, field mice consumed beechmast and acorns, while bank voles ate berries and fungi, and in December, large amounts of grass leaves. Dietary overlap was highest from May to August. Overlap between the two field mice species dropped moderately after the summer, while the difference between bank voles and the two field mice species dropped sharply.

(Abt KF. and Brock WF., 1998)

HSE comments:

The use of this study in a risk assessment has focussed on the data reported for the wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*) and bank vole (*Clethrionomys glareolus*) in fields of mixed farmland, in order to gain more realism of the small mammal species' feeding ecology. The study was not conducted to Good Laboratory Practice (GLP) and did not follow any guidelines. This limits the study's reliability and so it can only be used as supporting information in the risk assessment. No statistical analysis was conducted on the results.

Focal species

Authors explore the proportion of diet for 3 rodent species, for the wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*) and bank vole (*Clethrionomys glareolus*) from March to December 1992. Over the study period, the number of individuals and faecal samples obtained for analysis were similar for all 3 species. For wood mice, 83 faecal samples were obtained from 55 individuals, showing that wood mice are present in the area and supports that they are a suitable focal species for the risk assessment.

Proportion of diet over time

The diet consumed by each rodent is given as semi-quantitative proportions of faeces analyses (see figure 1 below). Raw data is not provided in the paper and therefore definitive percentages of diet cannot be concluded and be considered within the risk assessment. Diet proportions can only be considered estimates from the graph due to difficulty in distinguishing between similar colours in the key provided. There were no samples taken in April due to low overnight temperatures.

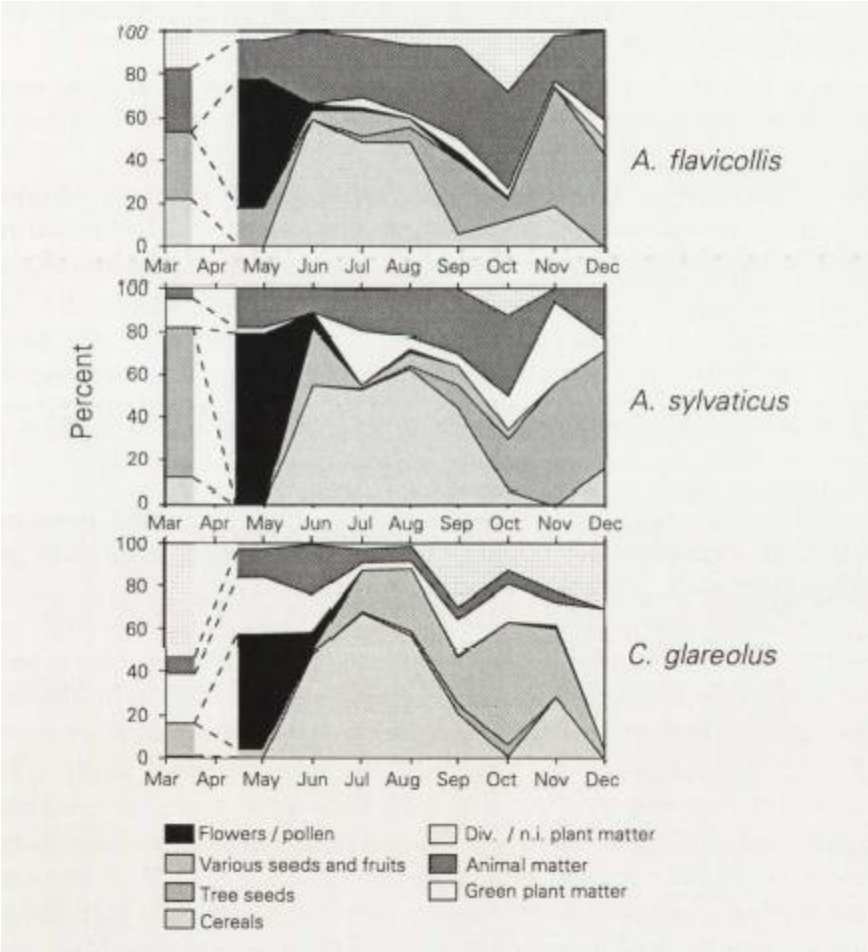


Figure 1. Seasonal variations in the diet composition of rodents

From the graph, there is clear variation of the proportion of the diets between the species throughout the year. Flowers/pollen were important for all species until May, then cereal was prominent until August (for wood mice until September), and this proportion decreases in autumn and towards the winter. From this, dietary overlap of the 3 species was reported to be highest from May to August when cereals form a large proportion of their diet, and variation between species diets increased during autumn and winter. It is clear even when cereals are a high proportion (>50 %) of wood mice diet, mice are not exclusively feeding on cereals. For wood mice, when the proportion of cereal is highest in August (~60%) the remaining diet for wood mice varies and consists of animal matter, green plant matter, various seeds and tree seeds. As mice were only caught on one study site it is not clear whether this is a result of the abundance of favourable alternative foraging habitat.

Study area and surrounding landscape

The field where rodents were trapped along was initially tilled with winter-rye, which was harvested in July. Unfortunately, authors do not report at what point this cereal was drilled and so the overlap between this land management and proportion of diet cannot be considered. Although it is clear that wood mice forage on cereals between May – September, it is not clear whether and to what extent cereals were available prior to May. Flowers/pollen made a higher proportion of diet during May but the lack of information into surrounding habitat and land uses at this specific time does not make it clear whether the flowers were favoured over other available forage, and if the change in foraging behaviour from flowers to cereals after May was a direct result of the drilling of wheat and availability of seeds in the study field, or whether foraging on cereals occurred when the crop was at a more established growth stage.

The study was carried out in a mixed farmland area and surrounded by spruce-oak forest, grassland and a maize field. This maize was found in the diet in *Apodemus* spp. diet in March. Authors suggest this was likely take from the remainder of the preceding year's crop in neighbouring fields. However, the proportions of different cereals within the diet over the year was not clearly reported, and there was no additional information whether this nearby maize field was under management over the study period (i.e. if other freshly drilled crops were present in the immediate area at certain times of year) and whether wood mice were foraging on these crops in addition to the rye. Additionally, the study shows that cereals are still a significant proportion of diet beyond the July harvest for wood mice. Authors suggest that cereals eaten in the autumn were probably stored by animals, but there is a question whether individuals are foraging on nearby cereal fields to account for this continued proportion of their diet. In the autumn and the winter, both field mice species, the decrease in abundance of rye following harvest resulted in a shift towards tree seeds (acorns and beech mast). This was not evident in *C. glareolus* which authors suggest may have been a result from competitive interaction, but this cannot be concluded definitively from the available data.

Diet proportion calculations

Study authors stated that due to different retention times of food in the alimentary tract the true proportion of food items were assumed to be better represented in large faecal samples than in small ones, especially for heterogenous diets. The study calculated diet proportions by volume percentage index V (v-value) for a given food category. This was done using the volume of each component and appropriate weighing factors derived from samples (noting exactly how these weighing factors were derived is not specified in the study). Weighing some measure of a sample mass is considered to eliminate part of the bias associated with faecal samples. The authors specify that this approach can be considered valid as different dietary components have different levels of digestibility and density, meaning that the volume proportion of the components in faecal samples were corrected to represent the proportion of each component of the diet that was ingested. However, they also note that grain-eating rodents tend to discard the outer parts of the caryopses, at least in summer, which probably caused severe underestimation of the proportion of cereals.

Overall it is clear that wood mice, as an omnivorous species, has seasonal variations in diet. Mice feed on green plants, seeds and invertebrates at varying amounts through the seasons. From the study, it is clear that wood mice foraged on cereals between May to September. Wood mice continued to feed on cereal even post-harvest, either as a result of their high mobility to other nearby crops or through seed caching. Authors do not show when the rye field was drilled, meaning this cannot be cross-referenced to the proportion of cereal in the diet at this time-point and the growth stages of the crop were not noted. It is not clear if wood mice were foraging on freshly drilled cereals or more established crops, meaning these conclusions cannot be extrapolated to the risk assessment with certainty.

The use of this study is discussed further in the higher tier risk assessment section.

The following study summary is reproduced verbatim from Section B.9 of the EU draft Renewal Assessment Report for metalaxyl-M

Report: **IIIA 10.3/01. [redacted] (2006). Generic Field Monitoring of Mammals on Cereal Fields in Spring and Summer in Germany.**

Syngenta file n°: N/1153 (data owned by Bayer Crop Science, Syngenta access)
Guidelines: No guideline applicable, the study was specifically designed
GLP: Yes

Previous evaluation: No, submitted for the purpose of renewal of a.s. approval

Executive summary:

The study area comprised winter cereal fields in the region of Thale, Germany. Small mammal use of cereal fields was monitored by means of live-trapping. Three species were caught, wood mouse, common vole and yellow-necked mouse, but the latter species preferred off-field habitats. Individual wood mice and common voles were radio-tracked continuously from dusk till dawn (wood mice) and over 24 hours (common voles). From the telemetry data the portion of time/potential foraging time in cereal fields, the habitat preference (Jacob's index) and individual home ranges were calculated. No common voles and very few wood mice were found in cereal fields prior to BBCH growth stage 30. Radio-tracked wood mice spent most of their time in cereal fields from BBCH 30-90, with mean PT of around 0.88. For common vole, usage of cereal fields increased with growth stage, with a mean PT of 0.64 at BBCH 30-60 and mean PT of 0.94 at BBCH 70-90.

Study design and Methods:

Experimental dates: March 21st to August 31st, 2005

This generic field study has been conducted in and around winter cereal fields in the region of Thale, Sachsen-Anhalt, Germany. This region is a typical area for cereal cultivation in Europe. The observations were initiated when most of the shootings were in BBCH principal growth stage 2 (tillering), with a height of approximately 10-15 cm.

Small mammal species (rodents) were monitored on four study plots within and around winter cereal fields. On each plot a grid of 64 life traps was installed where traps were set up in the cereal field as well as in the adjacent surrounding. The abundance of small mammals was investigated by live-trapping (capture – mark - recapture method). Furthermore, individual wood mice and common voles were radio-tracked continuously from dusk till dawn (wood mice) and over 24 hours (common voles). From the telemetry data the portion of time/potential foraging time in cereal fields, the habitat preference (Jacob's index) and individual home ranges were calculated.

For evaluation and reporting the observations were related to the following three BBCH groups: BBCH group 1 comprised early post-emergence and tillering stages (BBCH principal growth stages 1-2), BBCH group 2 included stem elongation and flowering (BBCH principal growth stages 3-6) and BBCH group 3 combined fruiting and ripening stages until just before harvest (BBCH principal growth stages 7-9).

Results:

Table B.9.1.4-15: Summary of study data

<table>
<tr><td colspan="6">RELEVANT SPECIES in the cereal field habitat (based on live-trapping)</td></tr>
<tr><td rowspan="2">species</td><td colspan="4">mean trapping efficiency (captures/100 trapnights)</td><td rowspan="2">captures in the cereal field (%)</td></tr>
<tr><td colspan="2">cereal field (based on 15168 trapnights)</td><td colspan="2">surrounding (based on 5056 trapnights)</td></tr>
<tr><td>Wood mouse (Apodemus sylvaticus)</td><td colspan="2">2.51</td><td colspan="2">1.17</td><td>68.21</td></tr>
<tr><td>Common vole (Microtus arvalis)</td><td colspan="2">4.61</td><td colspan="2">0.89</td><td>83.82</td></tr>
<tr><td>Yellow-necked mouse (Apodemus flavicollis)</td><td colspan="2">1.39</td><td colspan="2">6.51</td><td>17.59</td></tr>
<tr><td colspan="5">HABITAT USE of wood mice after radio-tracking</td><td></td></tr>
<tr><td rowspan="4">Proportion of habitat types to home range (MCP), based on 15 individuals tracked for one night (whole observed period) [mean], (90%ile)</td><td>winter cereal fields</td><td>93.27</td><td>%</td><td colspan="2">(100.00)</td></tr>
<tr><td>meadow / grass stripe</td><td>4.72</td><td>%</td><td colspan="2">(6.80)</td></tr>
<tr><td>hedgerow / shrub</td><td>1.36</td><td>%</td><td colspan="2">(4.85)</td></tr>
<tr><td>other habitats (track, ditch)</td><td>0.64</td><td>%</td><td colspan="2">(1.86)</td></tr>
<tr><td colspan="6">PORTION OF TIME (PT) in habitat of wood mice after radio-tracking</td></tr>
<tr><td rowspan="4">potential foraging time (surface activity only) spent in winter cereal fields; based on 15 individuals, [mean], (90%tile)</td><td>BBCH group 1 (p.g.s.* 0-2) n = 0</td><td colspan="4">not conducted[a]</td></tr>
<tr><td>BBCH group 2 (p.g.s.* 3-6) n=12</td><td>88.31</td><td>%</td><td colspan="2">(100.00)</td></tr>
<tr><td>BBCH group 3 (p.g.s.* 7-9) n=3</td><td>89.31</td><td>%</td><td colspan="2">(99.43)</td></tr>
<tr><td>total</td><td>88.51</td><td>%</td><td colspan="2">(100.00)</td></tr>
<tr><td colspan="5">HABITAT USE of common voles after radio-tracking</td><td></td></tr>
</table>

Proportion of habitat types to home range (MCP), based on 18 individuals (19 tracking sessions) tracked for 24 h [mean], (90%ile)	winter cereal fields	89.94	%	(100.00)
	meadow /grass stripe	8.58	%	(20.10)
	hedgerow / shrub	1.48	%	(3.90)
PORTION OF TIME (PT) in habitat of common voles after radio-tracking				
potential foraging time (whole observed period) spent in winter cereal fields; based on 19 tracking sessions, [mean], (90%ile)	BBCH group 1 (p.g.s.* 0-2) n=0	not conducted[a]		
	BBCH group 2 (p.g.s.* 3-6) n=8	63.52	%	(100.00)
	BBCH group 3 (p.g.s.* 7-9) n=11	93.78	%	(100.00)
	total	83.86	%	(100.00)

* p.g.s. abbrev. for principle growth stage
[a] no radio-tracking conducted; due to late authorisation, however no common voles and only very few wood mice could be trapped on the field prior to BBCH group 2 (p.g.s. 3-6)

Conclusions:
The study is considered acceptable.

The observations confirmed that wood mice and common voles were the most relevant species and most likely to be captured in cereal fields during spring and summer. Both species were present on all study plots; however, the abundance and appearance differed between the plots. This first appearance of common voles on the field was observed beginning of April (BBCH principle growth stage 3, crop height ≥ 15 cm). The wood mouse was present from the beginning of the investigation period in March (BBCH p.g.s. 2). Apart from one trapping plot (4) wood mice were regularly captured, yet populations showed a slight decrease in summer (BBCH p.g.s. 7-8). Common voles were not trapped on the fields before beginning of April. In more developed growth stages of the crop (BBCH p.g.s 3 and later), radio-tracking of 15 individual wood mice and 18 common voles in winter cereal fields and adjacent habitats showed that this crop was used as a main feeding and nesting habitat by these species.

More than half (60%) of the tracked wood mice spent 100% of their potential foraging time (known active period) in the cereal field habitat. In common voles 10 out of 18 individuals spent 100% of the potential foraging time (24h) in the cereal field, while one individual did not use the field as feeding habitat whilst being radio-tracked. The significance of winter cereal fields as a feeding and nesting habitat for both species is also supported by the live-trapping data of the population of these species within the study area.

For risk assessment purposes the portion of time spend potentially foraging in the cereal fields (PT) was calculated from the radio-tracking data according to the principle crop growth stages defined. Wood mice spent more than 50% of their potential foraging time in the cereal field already at BBCH principal growth stage 3,

whilst typically common voles did not spend significant foraging time on the field before BBCH principal growth stages 4 or 5.
Over the whole observation period, based on the minimum convex polygon, the cereal field habitat accounted on average for approximately 90% of the home ranges of the radio-tracked wood mice and common voles. Both the PT values and the proportions of habitat use indicate that during late spring and early summer the cereal field habitat offered a significant but not exclusive feeding habitat for all tracked wood mice and common voles.

([redacted], 2006)

HSE comments:

This study was previously evaluated in the EU renewal review for metalaxyl-M. It is considered further by HSE in the higher tier reproductive risk assessments for mammals (section B.9.1.6).

Regarding the radio-tracking data for wood mice from this study, no tracking was conducted pre-emergence or for BBCH principal growth stages 1-2 due to late authorisation. However, the study author did note that there were no common voles and few wood mice could be trapped on the field prior to BBCH principal growth stage 2, though how this finding was determined is not clear. Wood mouse radio-tracking sessions were conducted between May 4th and August 1st. Radio-tracking results for wood mice from this study are summarised in the following table, which is reproduced from the study report.

Table 10 Proportion of time wood mice spent "potentially foraging" in known habitats

Proportion of time individual wood mice spent „potentially foraging" in known habitats (time in "unknown" habitat is distributed over all known habitats according to prevailing proportions).

wood mouse no.	portion of habitats used by tracked wood mouse during potential foraging time [%]						habitat type at nest site
	cereal field	hedgerow / shrub	meadow / grass	wood / forest	track	ditch	
1	100.00	0.00	0.00	0.00	0.00	0.00	cereal test field
2	73.50	21.65	4.84	0.00	0.00	0.00	meadow / grass
3	78.85	21.15	0.00	0.00	0.00	0.00	hedgerow / shrub
4	100.00	0.00	0.00	0.00	0.00	0.00	meadow / grass
5	100.00	0.00	0.00	0.00	0.00	0.00	cereal test field
6	100.00	0.00	0.00	0.00	0.00	0.00	cereal test field
7	100.00	0.00	0.00	0.00	0.00	0.00	cereal test field
8	100.00	0.00	0.00	0.00	0.00	0.00	cereal test field
9	10.27	88.47	1.26	0.00	0.00	0.00	hedgerow / shrub
10	100.00	0.00	0.00	0.00	0.00	0.00	cereal test field
11	100.00	0.00	0.00	0.00	0.00	0.00	other cereal field
12	97.13	2.87	0.00	0.00	0.00	0.00	hedgerow / shrub
13	97.17	0.00	2.83	0.00	0.00	0.00	meadow / grass
14	100.00	0.00	0.00	0.00	0.00	0.00	cereal test field
15	70.75	0.00	29.25	0.00	0.00	0.00	meadow / grass
mean	88.51	8.94	2.55	-	-	-	
sd	24.04	23.22	7.52	-	-	-	
50% til	100.00	0.00	0.00	-	-	-	
90% til	100.00	21.45	4.04	-	-	-	
min	10.27	0.00	0.00	0.00	0.00	0.00	
max	100.00	88.47	29.25	0.00	0.00	0.00	

The following study summary is reproduced verbatim from Section B.9 of the EU draft Renewal Assessment Report for metalaxyl-M

Report: IIIA 10.3/02. [redacted] (2008). Generic field monitoring of mammals on freshly drilled summer cereals in Hunsrück, Germany.

Syngenta file n°: NA_11977 (data owned by Bayer Crop Science, Syngenta access)

Guidelines: No guideline applicable. The study was consistent with guidance provided in EFSA Guidance on Risk Assessment for Birds and Mammals (EFSA Journal 2009; 7(12): 1438).

GLP: Yes

Previous evaluation: No, submitted for the purpose of renewal of a.s. approval

Executive summary:

Small mammals were live-trapped in summer cereals (spring sown barley) and surrounding non-cropped habitat in Germany. Wood mouse was identified as the focal species and radio-tracking provided a mean and 90th percentile PT value of 14.37 and 27.5, respectively.

Study design and Methods:

Experimental dates: March 13th, 2006 to April 27th, 2006.

This generic field study was conducted in and around five different freshly sown summer cereal (barley) fields in the region of Sohren, Hunsrück, Germany. This region is a typical area for summer cereal cultivation in Europe. The objective of the study was to identify small mammals that may be exposed to treated summer cereal seeds and quantify their potential for exposure by means of radio-tracking on freshly drilled cereal fields. Small mammals were monitored by live-trapping, before and after drilling, within and around summer barley fields on four study plots. On each study field a grid of 64 live-traps was set up. This grid was divided such that 6 rows were positioned on the cereal field itself and the remaining 2 rows were located within the adjacent woodland. Species diversity and abundance of small mammals were investigated through the implementation of a live-trapping method (capture-mark-recapture).

Wood mice were selected for radio-tracking as the main granivorous species expected to visit open fields and were radio-tracked continuously from dusk till dawn to determine the proportions of time they foraged in cereals and other habit in the landscape. Three wood mice were radio-tracked twice whilst only one radio-tracking night was completed for a fourth wood mouse. During radio-tracking the location, type of habitat and behaviour of the wood mice were recorded following either a change of movement whilst tracking, or if the mouse remained stationary then at 10-15 min intervals. From the radio-tracking data the proportion of time potentially foraging in cereal fields and other habitats, preference and individual home range size were calculated.

Results:

The study was carried out from just before sowing until the first stages of emergence of the summer barley crop. Typical for the early spring season after the winter depression of their populations, a low abundance of small mammals was observed in and around the study fields at the time of summer cereal sowing. Granivorous wood mice and yellow-necked mice, both species of the genus *Apodemus*, were frequently trapped. Despite numerous trappings of yellow-necked mice within the woodland (rows 1 and 2) only a single yellow-necked mouse was caught only once on the open field. However, wood mice were repeatedly trapped in rows 3-8 on the open summer cereal field before and after drilling. Among the herbivorous small mammal species, bank voles were dominant but were exclusively found in the surroundings, as were field voles. Some common voles were captured on the open field but only before seed bed preparation and not after drilling.

Table B.9.1.4-16: Summary of study data

Summary of capture data (before and after drilling combined)

	Rows 1 & 2 (surroundings)	Rows 3-8 Summer cereal fields
Total number of trap nights[a]	1456	4240
Species	Trapping efficiencies[b]	
Wood mouse (*Apodemus sylvaticus*)	2.2	0.3
Yellow-necked mouse (*Apodemus flavicollis*)	2.4	>0.1
Bank vole (*Clethrionomys glareolus*)	5.6	0.0
Common vole (*Microtus arvalis*)	0.3	0.3
Field vole (*Microtus agrestis*)	0.1	0.0
Total	10.5	0.6

[a] trap night is defined as both the activation and checking of traps, for example one trap activated and checked = one trap night.
[b] trapping efficiency is calculated as the number of captures for 100 trap nights

The results from live-trapping confirm the wood mouse as the main granivorous species visiting the open field and therefore its selection as focal species for radio-tracking, in order to quantify the potential for exposure to treated seeds on freshly drilled summer cereal fields in spring. However, even for the wood mouse the surrounding habitats were more attractive since less than 30% of all captures were in the summer cereal field despite the larger number of traps within the fields. The radio-tracking results supported this preference of wood mice for the surroundings. In most cases the freshly drilled summer cereal fields were of minor importance to the wood mice as a potential foraging habitat, thus they spent most of the tracking time in adjacent habitats i.e. wooded areas or hedgerows/woodland edges. No tracked individual used the summer cereal field as a nesting site. For the purpose of risk assessment, the portion of potential foraging time on freshly drilled summer cereal fields was calculated. The freshly drilled summer cereal fields accounted on average for 29.62% of their home ranges, yet the wood mice spent on average only 14.37% (of their potential foraging time within the summer barley fields (PT).

Table B.9.1.4-17: Summary of study data

Habitat use of wood mice based upon radio tracking data					
Track night	Date	Mouse ID	% summer cereals within home range	Jacobs index (D)	% PT in drilled cereal field
1	2006-04-12/13	631152	6.19	-0.052	14.21
2	2006-04-13/14	620651	75.58	0.011	31.38
3	2006-04-	631152	10.07	-1.000	0.00

	14/15				
4	2006-04-17/18	619817	3.01	-0.152	10.94
5	2006-04-18/19	620651	93.97	0.015	25.52
6	2006-04-20/21	619817	6.20	-0.023	8.14
7	2006-04-21/22	175570	12.29	0.003	11.43
	Mean		29.62	-0.171	**14.37**
	90th %ile				**27.5**

The amount of exposed summer barley seeds was counted as part of a line transect on the freshly drilled fields. Mean and ranges for cereals on the surface (seeds/m^2) end rows and mid-field were 25 (8.8-34) and 4.3 (0-10.8), respectively.

Conclusions:
The study is considered acceptable.
The wood mouse was the main granivorous species visiting the open field and was selected as the focal species for radio-tracking. The mean and 90th percentile for the proportion of time (PT) wood mice spent potentially foraging in freshly drilled summer cereals was 14.37% and 27.5%, respectively.

([redacted], 2008)

HSE comments:

This study was previously evaluated in the EU renewal review for metalaxyl-M. It is considered further by HSE in the higher tier reproductive risk assessments for mammals (section 9.1.6).

It is noted that the mean and 90th percentile values for consumers were calculated across 7 tracking sessions, for 4 individual mice. Given that different individuals were tracked for different numbers of sessions, this has the potential to give undue weight to certain individuals in the overall mean and 90th percentile PT calculations. Therefore, mean PT values per individual mouse are presented in the following table.

Table B.9.1.4-18: Radio-tracking data for individual wood mice

Wood mouse ID	% PT in drilled cereal field	Average % PT in drilled cereal field per individual
631152	14.21	7.11
	0	
620651	31.38	28.5
	25.52	
619817	10.94	9.54
	8.14	
175570	11.43	11.43

The following study has been newly submitted and was not evaluated in the EU draft Renewal Assessment Report for metalaxyl-M. This study has therefore been evaluated by HSE and is summarised below.

Report:	KIIIA 10.3.3/01, [redacted]. and [redacted] (2016) Generic Field Study on PT of Wood Mice in freshly drilled Spring Cereal Fields (Germany). Report Number 783266. [redacted], Study owner BASF – Syngenta has access (Syngenta File No. NA_14348).

Guidelines

Regulation (EC) No 1107/2009

EFSA Guidance Document Risk Assessment for Birds and Mammals (2009)

GLP: Yes

Aim

The aim of the study is to quantify PT values for wood mice (*Apodemus sylvaticus)* in spring cereal fields shortly after drilling, in order to gain more realism of the focal species' feeding ecology. Being an omnivorous species, the wood mouse is considered to potentially feed in spring cereal fields and potentially on exposed treated cereal seeds.

Objective

To determine the proportion of diet obtained in cereal fields (PT estimate) by radio tracking individual mice (including visual observations). Observations were carried out using trapping techniques and radio tracking, generating data to calculate the portion of time an animal spent potentially foraging in a specific habitat type (PT), its home range and habitat preferences (Jacobs index), as well as the movements within and between habitats.

MATERIALS AND METHODS

Test item:	No specific substance was tested
Test species:	Wood Mouse (*Apodemus sylvaticus*)
Crop:	6 commercially managed spring cereal fields bordered by off-crop habitats known to be favourable for the presence of wood mice (e.g. hedges or forest).
Total field size:	0.9 ha to 2.8 ha (average 1.68 ha)
Application:	Untreated seeds were used for drilling of the study fields. The fields were drilled between 12th and 22nd of April 2016.

Study design and methods

This generic study was performed to evaluate the extent to which wood mice use spring cereal fields shortly after drilling.

Test locality

The study was conducted on freshly drilled spring cereal fields in Neu-Ulrichstein in Hesse, Germany. The study fields were selected to be representative for commercially managed spring cereal fields and have natural wood mice populations on the fields or in the direct vicinity, later verified with trapping data. Selected study fields were bordered by off-crop habitats known to be favourable for wood mice (e.g. hedges or forest). Therefore, the mice could potentially forage on the freshly drilled spring cereal fields.

Trapping individuals

Live trapping was conducted prior to the drilling of the spring cereal fields from March 17th to April 20th, 2016. At each study site 50-60 'Ugglan' multiple capture live traps were set inside the study field and in addition 40-50 traps were set in suitable off-crop habitat in the close vicinity of the field. At least 50% of the traps were always placed inside the study field. Traps were supplied with bait (e.g. sunflower seeds) which also serves as food for captured animals. At temperatures below 5°C, bedding material (e.g. hay) was added to the traps to provide insulation for captured individuals. Traps were activated for trapping in the evening and checked in the morning, after which they were left open (de-activated) during the day.

Each trapped animal was individually marked with a passive integrated transponder (mini PIT), which was injected subcutaneously. Species, date, location (study field and trap number), first capture or recapture, PIT number (if applied), sex, reproductive state and body weight were recorded as raw data. Afterwards the mice were released at the site of capture.

Radio tagging and tracking

Suitable adult wood mice captured in the live traps were equipped with radio collars ('Pip3-AG379', manufactured by Biotrack Ltd., UK). Animals were regarded as suitable if they had a body weight of 20 g or more (to allow the 0.9 g radio-transmitter to not exceed 5 % of mammals body weight). From April 13th to April 30th, tagged individuals were radio tracked from dusk till dawn continuously for approximately 10 – 12 hours. Authors do not report whether multiple observers were used to track individuals for each session. Every change in behaviour and location (GPS position) were actively recorded to the minute. For each individual, session 1 was conducted within the first three days after drilling (also session 2 of individual 2/4) whereas the following sessions were finished before the cereal plants emerged.

4 study sites (study fields and their vicinity) were used to successfully radio track 11 wood mice in 27-night sessions. The study was conducted on 6 commercially managed spring cereals fields sized from 0.9 ha to 2.8 ha, but only 4 out of the 6 study sites were used in the study for radio-tracking. While not specifically stated in the study report it is assumed that 2 sites were excluded from the radio-tracking phase due to the lack of trapped wood mice at these sites.

Table B.9.1.4-19: Dates of tracking sessions for each individual

Study field	Individual	Session	Start Date	Finish Date
1 (drilled 12.04.2016 with Oats)	1/1	1	13.04.2016	14.04.2016
	1/2	1	14.04.2016	15.04.2016
		2	18.04.2016	19.04.2016
		3	20.04.2016	21.04.2016
	1/3	1	14.04.2016	15.04.2016
	1/4	1	15.04.2016	16.04.2016
		2	18.04.2016	19.04.2016
		3	19.04.2016	20.04.2016
		4	21.04.2016	22.04.2016
	1/5	1	20.04.2016	21.04.2016
		2	21.04.2016	22.04.2016
		3	22.04.2016	23.04.2016
2 (drilled 19.04.2016 with Rye)	2/4	1	19.04.2016	Session aborted
		2	22.04.2016	23.04.2016
		3	23.04.2016	24.04.2016
		4	24.04.2016	25.04.2046
		5	26.04.2016	27.04.2016
4 (drilled 22.04.2016 with Rye)	4/1	1	25.04.2016	Session aborted
	4/3	1	23.04.2016	24.04.2016
		2	26.04.2016	27.04.2016
		3	27.04.2016	28.04.2016
		4	28.04.2016	29.04.2016
	4/7	1	24.04.2016	25.04.2016
		2	27.04.2016	28.04.2016
		3	28.04.2016	29.04.2016
		4	29.04.2016	30.04.2016
	4/8	1	25.04.2016	Session aborted
6 (drilled 14.04.2016 with Barely)	6/1	1	Session not started	
		2	16.04.2016	17.04.2016
	6/2	1	15.04.2016	16.04.2016
	6/3	1	17.04.2016	18.04.2016

During radio tracking sessions the behaviour of the tracked wood mouse was recorded in four activities:

1) **Active**: the animal was active and potentially foraging
2) **Foraging**: the animal was visually observed foraging
3) **Inactive**: the animal was not active and therefore not foraging

4) **Travelling**: the animal was moving very fast leaving no time for the observed to record a position and therefore the animal had no time for foraging.

Authors state that those considered ‘foraging’ were visually observed foraging during sessions. How this was achieved in practice is unclear, as sessions occurred between dusk till dawn (i.e. dark) and wood mice are a small mammal and considered difficult to be visually monitored under these field conditions. Study authors do not go into detail whether any problems were faced or if specialist equipment (i.e. night vision equipment, torches, binoculars etc.) was used for visual observations to confirm the behaviour of individuals.

In order to check whether tagged individuals were still present in the study area between tracking sessions, and whether tags were still functioning, tagged animals were looked for during the daytime. The use of unidirectional ‘Yagi-antennas’ (Biotrack Ltd., United Kingdom) allowed the determination of the direction and a good estimate of the distance to the tagged individual. As the accuracy of GPS devices depends on weather conditions, tree cover etc. and the distance to the animal was estimated, a total accuracy of 3-5 metres could be obtained.

<u>Additional observations</u>

Habitat mapping

Before the radio tracking was started, all habitats within 250 m from each study field border were mapped. Habitat types were taken from satellite photographs (google maps / google earth, 2016) and verified in the field. Habitat mapping was extended if the animal had left the mapped area. Furthermore, growth stages of all arable crops used by the animal were noted, however these were not reported in the study report.

Study site 1 – immediate area consists of ploughed field, field margin, forest, and shrubs/hedges/trees. Wider area consists of meadow, arable crops, orchard, tarmac road, and water & associated vegetation.

Study site 2 – surrounding area consists of ploughed field, field margin, meadow, arable crops, and tarmac road. Wider around consists of fallow land, forest, and water & associated vegetation.

Study site 4 – surrounding area consists of fallow land, shrubs/hedges/trees, arable crops, and field paths. Wider area consists of arable land, field margins, forests, tarmac road, and water & associated vegetation.

Study site 6 – immediate surrounding area consists of field margins, tarmac road, meadows, and forest. The wider area consists of fallow land, arable crops, field paths, and water & associated vegetation.

Climate

The daily precipitation and the temperature data between 19.03.2016 – 20.04.2016 were obtained from the weather station of Neu-Ulrichstein, dependent of the ‘Deutscher Wetterdienst’ (non-GLP). The temperature ranged from -0.5^{o}C – 18.1^{o}C. The total precipitation was 65.5 mm, the average precipitation was 1.99 mm per day.

Agricultural practice

Farmers were responsible for all agricultural practice, which was conducted non-GLP but according to good agricultural practice (GAP). There was no information provided on previous land management for study sites, information regarding the seed bed, whether fields were freshly ploughed, BBCH stages of crops or if any management occurred on study fields during the study. Photographs of study fields show minimal vegetation cover, with exposed bare soil.

Table B.9.1.4-20: Agricultural information for the study fields where radio tracking was conducted

Study field	Area (ha)	Date of drilling	Crop	Variety	Drilling depth (cm)	Drilling density (kg/ha)	Visible seed exposure ?
1	1.6	12.04.2016	Oats	Max	2	140	YES
2	2.2	19.04.2016	Rye	Barwaldo	2	120	YES
4	1.3	22.04.2016	Rye	Barwaldo	2	120	YES
6	2.8	14.04.2016	Barley	Grace	3	160	YES

Analysis of data

PT values were obtained by radio tracking individual wood mice, based on the assumption that the time an animal spends active in a habitat is a reliable measure of the proportion of diet obtained in this defined area.

The proportion of its potential foraging time (PT) an animal spent in a specific habitat type, its home range (through calculating Minimum Convex Polygons (MCP) by GIS software, (Quantum GIS 2.14.6) and its habitat preferences (Jacobs index) were calculated. Movements (speed) within and between habitats by individuals were calculated by measuring the speed of movement between two positions within the same habitat or between habitats. PT values were calculated by adding together the time 'potentially foraging' (time spent 'active' + time spent 'foraging') in a specific habitat and dividing this by the total time observed 'potentially foraging'. Time individuals spent 'travelling' and 'inactive' was not included in PT calculations. Three different approaches to calculate PTs were as follows:

1) 'all individuals' approach – where all successfully tracked individuals with all sessions, regardless of if they used the winter cereal fields or not;
2) 'home range' approach – included only those wood mice that embedded a winter cereal field in their MCP home range. These individuals can be considered 'potential foragers';
3) 'consumers' approach – focussed on individuals that entered a winter cereal field during at least one radio tracking session.

$$\textbf{Potential foraging time (PT)} = \frac{\textit{Time spent foraging in cereal crops 'active' + 'foraging' [hh:mm]}}{\begin{array}{c}\textit{Total time spent potentially foraging}\\ \textit{(in both adjacent and cereal crops 'active' + 'foraging') [hh:mm]}\end{array}}$$

RESULTS AND DISCUSSION

Experimental dates: 17 March 2016 to 01 May 2016.

Trapping

At 6 different locations 5258 trap nights were conducted inside the study fields and 4173 trap nights in the adjacent off crop. During these 9431 trap nights 443 small mammals were captured. 124 of these captures were identified as wood mice. The 124 captures were first and re-captures of 35 individually marked wood mice. 21 out of these 35 wood mice were equipped with radio collars and released. Of the 21 individual wood mice which were tagged with a radio collar, 8 of them disappeared from the study fields and their vicinity before the radio tracking sessions after drilling could be started. Reasons for the disappearance might be individual changes of the used home range, predation by terrestrial or avian predators or technical failures of the radio collars. So, beside a sufficient effort (tagging of 21 wood mice) only 13 could be radio tracked and only 11 out of these 13 gave long enough observation periods of at least 8 hours per night.

The authors report that although EFSA guidance (EFSA 2009, appendix P) indicates individuals caught in the target crop should be preferred for radio tracking, in the present study only 3 tracked individuals (all caught in field 4) out of the 11 radio tracked wood mice were caught inside the study fields. However, authors do not clearly show at what locations individuals used for radio-tracking were trapped. There is no clear information whether captures were in study fields or in the local vicinity for each individual radio-tracked for each session. Although it is reported that 3 tracked individuals were caught inside study fields it is not reported where in the study fields these were caught. For off crop captures there is no information showing where individuals were caught from. This low trapping success in the study fields was achieved even though 5258 trap nights were made in the study field and just 4173 in the adjacent off crop. This questions whether the selected individuals used from the local vicinity were appropriate and subsequently, if the study presents an appropriate scenario for the risk assessment.

Radio tracking individuals

Due to tag failures and/or predation and animals moving far away from the study fields, 13 wood mice were radio tracked for 30 different night sessions. All radio tracking sessions were made after drilling the spring cereal fields but before the emergence of the crop plants. Three of the 30 radio tracking sessions lasted less than 5 hours (0:53, 2:40 and 4:34 hr.). Authors regarded these as possibly biased and were, therefore, excluded from all their analyses. This reduced the number of tracked individuals to 11 and the number of tracking sessions to 27 at 4 different fields.

Table B.9.1.4-21: Overview of captured and tagged wood mice

Study site	Total captures of small	Total captures of wood	Individuals of wood mice	No. of tagged wood mice	No. of successful radio

	mammals	mice	captured		tracked wood mice
1	85	32	8	5	5
2	41	33	11	4	1
3	39	1	1	-	-
4	87	38	9	8	2
5	35	0	-	-	-
6	156	20	6	4	3
TOTAL	**443**	**124**	**35**	**21**	**11**

<u>PT values calculated from 27 radio tracking sessions of 11 different wood mice</u>

During 15 different tracking sessions after drilling six individuals were observed to enter a spring cereal field (see below).

Table B.9.1.4-22: Radio tracking sessions, time foraging and PT values

Individual No.	Radio tracking session	Total time potentially foraging observed	Time potentially foraging in spring cereal fields [study report]	Time potentially foraging in spring fields [values from raw data for verification recalculated by HSE]	PT in spring cereal fields	PT in spring fields [if different from those reported, recalculated by HSE]
1/1	1	5:20	0:00	0:00:00	0.000*	0.000*
1/2	1	8:12	0:02	0:02:00	0.004***	0.004***
	2	7:44	0:00	0:00:00	0.000*	0.000*
	3	6:24	0:00	0:00:00	0.000*	0.000*
1/3	1	7:41	0:00	0:00:00	0.000**	0.000**
1/4	1	7:40	0:01	0:01:00	0.002***	0.002***
	2	7:27	0:02	0:02:00	0.004***	0.004***
	3	7:09	0:00	0:00:00	0.000*	0.000*
	4	4:20	0:00	0:00:00	0.000*	0.000*
1/5	1	6:28	0:03	0:03:00	0.008***	0.008***
	2	8:09	0:00	0:00:00	0.000*	0.000*
	3	8:04	0:02	0:02:00	0.004***	0.004***
2/4	2	7:13	0:04	0:04:00	0.009***	0.009***
	3	7:21	0:00	0:00:00	0.000**	0.000**

	4	7:06	0:00	0:00:00	0.000*	0.000*
	5	9:13	0:09	0:09:00	0.016***	0.016***
4/3	1	6:02	1:03	1:03:00	0.174***	0.174***
	2	7:24:50	1:16:20	**01:15:50**	**0.172*****	**0.171*****
	3	6:13	1:40	01:40:00	0.268***	0.268***
	4	7:29	2:15:30	**02:15:00**	**0.302*****	**0.301*****
4/7	1	7:02	0:36	00:36:00	0.085***	0.085***
	2	7:51	1:38	**01:37:30**	**0.208*****	**0.207*****
	3	6:43:30	0:56:30	**00:56:00**	**0.140*****	**0.139*****
	4	8:04	1:46:30	01:46:30	0.220***	0.220***
6/1	1	8:41	0:00	00:00:00	0.000**	0.000**
6/2	1	5:06	0:00	00:00:00	0.000*	0.000*
6/3	1	5:41	0:00	00:00:00	0.000**	0.000**
'All sessions' approach [n=27 sessions]	Mean				0.60	0.60
	Median				0.004	0.004
	90^{th} percentile				0.213	**0.212**
'Home range' approach [n=19 sessions]	Mean				0.085	0.089
	Median				0.009	0.013
	90^{th} percentile				0.230	**<u>0.234</u>**
'Consumers only' approach [n=15 sessions]	Mean				0.108	0.107
	Median				0.085	0.085
	90^{th} percentile				0.249	0.249

*1st approach: PT value was only used to calculate the 'all sessions' approach
**2nd approach: PT value was used for the calculations of the 'all sessions' and the 'home range' approach
***3rd approach: PT value was used for the calculations of all three approaches

Values that are calculated from the raw data and different to what is reported are in bold

The 1st approach for deriving overall PT values from this dataset included all radio tracked wood mice captured in or next to the spring cereal fields, 'all sessions' approach. The 2nd approach used only tracking sessions when the individual home range calculated for this session embedded the spring cereal field. Due to the method of MCP a field can be part of the home range, even if the animal did not enter (use) this field during tracking sessions (‘home range’ approach) and these individuals could be considered as ‘potential consumers’. The 3rd approach used only sessions for mean, median and percentile calculations if the animal was observed (radio tracked) in the spring cereal field, ‘consumers only’ approach.

It should be noted that some errors were found in authors’ reported values for the total time an individual was observed foraging on spring cereals in a session, typically just by a few seconds. However, to ensure errors were not translated into overall PT values these errors have been highlighted above. It is clear there is a slight change in overall PT values for ‘all sessions’ and ‘home range’ but because these are only slight changes, this is not thought to influence the reliability of the study.

<u>Home range, Jacobs index and speed of wood mice in spring cereal fields</u>

Home range

The home range size of 11 wood mice were calculated as MCP based on radio tracking data. The home range for 9 out of 11 radio tracked wood mice included a spring cereal field, ranging between 1.85 % and 68.74 % of the total home range, with an average home range percentage of spring cereal fields of 18.58 %.

Jacobs preference index

Wood mice in this study showed a preference for three habitat types available within their home ranges. These habitats were shrubs, hedges and solitary trees (SHT), water and associated trees and bushes (WAT) and the forests (FOR). All habitat types under agricultural use, field margins (FM), arable land (ARA), ploughed fields (PLO), meadows (MEA), fallow land (FAL), field paths (F) and among them the freshly drilled spring cereal fields (DRI), were avoided. As summarised in table 10.3.3/01-5, all radio tracked individuals had indices between -1 to 0, showing avoidance of freshly drilled spring cereals habitat.

Table B.9.1.4-23: Summary of proportional home ranged, Jacobs index and the speed of wood mice in spring cereal fields

Study field	Individual No.	No. of tracking sessions	Spring cereal fields		
			% of spring cereal fields in home range	Jacobs Index	Speed (m/h)
1	1/1	1	0	-	-
	1/2	3	1.85	-0.85	-
	1/3	1	39.83	-1.00	-
	1/4	4	2.02	-0.83	-
	1/5	3	1.61	-0.63	-
2	2/4	4	29.37	-0.97	2880.24
4	4/3	4	68.74	-0.76	278.85
	4/7	4	37.35	-0.50	403.73
6	6/1	1	9.83	-1.00	-
	6/2	1	0	-	-
	6/3	1	13.82	-1.00	-

Land management

Study sites were chosen due to being bordered by off-crop habitats known to be favourable for the presence of wood mice, so mice could potentially use the adjacent freshly drilled spring cereal fields for foraging. For study sites 1 and 2, a large proportion of the immediate area was agricultural land (ploughed fields or arable crops) which was avoided by wood mice populations (shown through the Jacobs preference index; ploughed field -0.87; arable field -0.37) and it is seen from radio tracking maps that individuals typically cluster in more favourable habitats on the field edges. Conversely, the individual tracked for site 2 does move into arable land, but it is still reported to have a negative Jacobs index value (-0.97). The unfavourable bordering habitat questions whether the chosen landscape fully supported wood mice populations, and if the study sites were appropriate for the study.

Conversely, areas surrounding study sites 4 and 6 were predominately shrubs, hedges & solitary trees and forests which provide cover (considered favourable to wood mice). Study site 6 shows that individuals spent their time in forest habitats or field margins, with no tracking data within the freshly drilled spring study site. This questions whether the site was appropriate for radio tracking wood mice populations, as the more attractive extensive forest habitat would draw populations away from the study field. The only study site that experienced foraging from wood mice was site 4. Authors report that study field 4 was previously set-aside land and that this area was ploughed just before drilling the spring cereals. It is suggested that even after ploughing the weed seed bank is likely to be much more substantial than in fields under years of arable use. However, although plausible, this speculation by the authors is not supported with additional data. Radio tracking was not conducted in the weeks before drilling, and this notion cannot be confirmed. The report does not explore the previous land uses or management of **all** the study fields before seed drilling which raises the question whether the study sites are comparable in their attractiveness for wood mice populations.

Ultimately there is no information provided on the previous land uses or management of the study areas which may give further context for the suitability of the study sites. It is not clearly identified whether there were any land management practices conducted on or on nearby areas to the study sites during the study period. It is clear that the wood mice population are not extensively foraging on the study fields, but there are questions over whether the previous land uses/management practices and surrounding habitats at the study locations mean that all study fields are representative of locations where wood mice would have a relatively high potential to forage in spring cereal fields (i.e. there is uncertainty whether wood mouse behaviour in the study fields is representative of wood mouse behaviour in spring cereal fields in general).

Speed

Wood mice moved much faster in spring cereal fields (on average 1187.61 m/h = ~ 1.2 km/h) than in all other habitat types. Authors conclude that due to these speeds observed in the spring cereal fields, this clearly suggests that they were travelling rather than foraging, as the speeds moved through the habitat provided no time to forage. However, the mean speed in spring cereal fields was calculated for just three different individuals. Three other wood mice observed in cereal fields left the field instantly after they had entered it, thus allowing no 'speed in habitat' calculation and so the study does not include the time travelling in PT calculations. However, for one individual (4/3) 16 minutes 30 seconds was spent travelling in the study field. This amount of time for an individual to be solely travelling across a relatively small area of land for this amount of time is considered questionable, as the study authors report, wood mice can move quickly. Authors do not give a justification of what behaviour the animal was exhibiting and omits this travelling time from their potential foraging PT calculations. However, as this amount of time 'travelling' can be considered a significant amount of time of the individual in spring cereals during that session, it is proposed that the time should be considered within PT calculations which would result in a PT value of 0.201 for that individual session (excluding travel time = 0.171). Despite this difference, all PT 90th percentile calculations remain the same and therefore this exclusion of the travel time is not considered to have significantly impacted the results. However, for further calculations within this evaluation, the value including the travel time will be used for this individual.

CONCLUSIONS

In the present study freshly drilled spring cereal fields were not considered attractive for wood mice even if they were included in the animals' home ranges, with PT values were found to be low. Wood mice with relatively high PT values spent less time in the freshly drilled spring cereal fields than in their adjacent habitats.

([redacted]. and [redacted], 2016)

HSE comments:

The use of this study in risk assessment has focussed on the data reported for the wood mouse (*Apodemus sylvaticus*) in spring cereal fields shortly after drilling, in order to gain more realism of the focal species' feeding ecology.

It is noted that 443 small mammal captures occurred with only 124 of these being wood mice. However, the study authors do not state what other species were caught by the Ugglan multi-capture traps, and from this if there were other mammals possibly using these habitats more frequently that could potentially be a more appropriate focal species for the risk assessment. Authors report the selection of the fields on the basis that the surrounding area supports the wood mice population. Jacobs' index results indicate that individuals are avoiding freshly drilled spring cereals and surrounding areas are considered more attractive for foraging, though these results are likely a function of the nature of the surrounding habitats and the general applicability of these results is therefore uncertain.

The study states that for the selection of individuals which were radio tracked repeatedly, animals were already known as consumers (i.e. were radio tracked in the spring cereal fields) and these were preferred to ensure a conservative worst-case scenario. The use of radio-tracking from multiple sessions with the same individual is discussed in the higher tier reproductive risk assessment. It is noted that the small number of individuals trapped in the cereal fields (with no clear mapping of where these traps were located) and low number of individuals observed foraging, although considered known foragers, questions whether the area can be considered to be representative in terms of the population of this species. Although, from the study, it is clear that at these study sites during spring most wood mice do not extensively forage in freshly drilled spring cereals, it is questionable whether all of the study fields investigated represent an appropriate scenario for the risk assessment (i.e. whether the results from the study fields represent a reasonable worst-case scenario in terms of wood mouse behaviour).

When deriving PT values, authors report the mean, median and 90th percentile for 'all sessions', 'home range' and 'consumer only' approaches, with values of 0.213, 0.230 and 0.249, respectively. The number of individuals tracked was 11 individuals (over 27 tracking sessions). However, it was noted that some individuals were tracked on separate occasions, sometimes up to 4 separate sessions from 13.04.2016 – 30.04.2016. It is noted that seven individuals were tracked under different sessions (all within 11 days after seed drilling; with sessions carried out on consecutive days for each individual). For individuals with multiple tracking sessions an arithmetic mean PT value has been calculated in the following table.

Table B.9.1.4-24: HSE calculation of PT values per individual wood mouse

Individual No.	Radio tracking session	Total time potentially foraging	Time potentially foraging in	PT in spring cereal	Mean PT values

		observed	spring cereal fields	fields	(consumers)
1/1	1	05:20	0:00:00	0.000	-
1/2	1	08:12	0:02:00	0.004	0.001
	2	07:44	0:00:00	0.000	
	3	06:24	0:00:00	0.000	
1/3	1	07:41	0:00:00	0.000	-
1/4	1	07:40	0:01:00	0.002	0.002
	2	07:27	0:02:00	0.004	
	3	07:09	0:00:00	0.000	
	4	04:20	0:00:00	0.000	
1/5	1	06:28	0:03:00	0.008	0.004
	2	08:09	0:00:00	0.000	
	3	08:04	0:02:00	0.004	
2/4	2	07:13	0:04:00	0.009	0.006
	3	07:21	0:00:00	0.000	
	4	07:06	0:00:00	0.000	
	5	09:13	0:09:00	0.016	
4/3	1	06:02	1:03:00	0.174	**0.236****
	2	07:24:50	**01:15:50**	**<u>0.201*</u>**	
	3	06:13	01:40:00	0.268	
	4	07:29	**02:15:00**	**<u>0.301</u>**	
4/7	1	07:02	00:36:00	0.085	0.163
	2	07:51	**01:37:30**	**<u>0.207</u>**	
	3	06:43:30	**00:56:00**	**<u>0.139</u>**	
	4	08:04	01:46:30	0.220	
6/1	1	08:41	00:00:00	0.000	-
6/2	1	05:06	00:00:00	0.000	-
6/3	1	05:41	00:00:00	0.000	-
90th percentile PT value (only values >0)					0.199

***** PT value for 4/3 radio tracking session 2 as includes the travel time for this session (see *Speed* section for justification)
** Endpoint that can be considered 'worst-case' endpoint from the study.

It is noted that the 90th percentile PT value calculated for the arithmetic means for individuals in spring cereal fields is 0.199 for all consumers, a smaller value than those reported by the study authors ('consumers only' = 0.249). However, the difference of PT values between study sites suggests that there is clear variation in attractiveness, questioning whether using an overall value across all study sites is appropriate to report PT of wood mice in freshly drilled spring cereals from this study. Study site 4 may represent a more 'worst-case' PT value due to more time spent potentially foraging in this area. The available PT datasets for wood mice are considered further in the higher tier risk assessment section.

The following study has been newly submitted and was not evaluated in the EU

draft Renewal Assessment Report for metalaxyl-M. Therefore, this study has been evaluated by HSE and is summarised below.

Report:	KIIIA 10.3.3/02: [redacted]. and [redacted]. (2017), Generic Field Study on PT of Wood Mice in freshly drilled Winter Cereal Fields (Germany). Report Number: [redacted]. (Syngenta file no. VV-467722)

Guidelines

Regulation (EC) No 1107/2009

EFSA Guidance Document Risk Assessment for Birds and Mammals (2009)

GLP: Yes

Aim

The aim of the study is to quantify PT values for wood mice (*Apodemus sylvaticus)* in winter cereal fields shortly after drilling (pre-emergence), in order to gain more realism of the focal species' feeding ecology. Being an omnivorous species, the wood mouse is considered to potentially feed in freshly drilled winter cereal fields and potentially on exposed treated cereal seeds.

Objective

To determine the proportion of diet obtained in cereal fields (PT estimate) by radio tracking individual mice. Observations were carried out using trapping techniques and radio tracking, generating data to calculate the portion of time an animal spent potentially foraging in a specific habitat type (PT), its home range and habitat preferences (Jacobs index), as well as the movements within and between habitats.

MATERIALS AND METHODS

Test item:	No specific substance was tested
Test species:	Wood Mouse (*Apodemus sylvaticus*)
Crop:	11 commercially managed winter cereal fields bordered by off-crop habitats known to be favourable for the presence of wood mice (e.g. hedges or forest).
Total field size:	0.68 ha to 3.02 ha (average 1.69 ha)
Application:	Untreated seeds were used for drilling of the study fields. The fields were drilled between 9th October and 1st November 2016.

Study design and methods

This generic study was performed to evaluate the extent to which wood mice use winter cereal fields shortly after drilling.

<u>Test locality</u>

The study was conducted on freshly drilled winter cereal fields in Neu-Ulrichstein in Hesse, Germany. The study fields were selected to be representative for commercially managed winter cereal fields and have natural wood mice populations on the fields or in the direct vicinity, later verified with trapping data. Selected study fields were bordered by off-crop habitats known to be favourable for wood mice (e.g. hedges or forest). Therefore, the mice could potentially forage on the freshly drilled winter cereal fields.

Trapping individuals

Live trapping was conducted prior to the drilling of the winter cereal fields from September 24th to October 28th, 2016. Trapping was performed prior to drilling to select suitable animals for radio tracking. At each study site 60-80 'Ugglan' multiple capture live traps were set inside the study field and in addition 20-50 traps were set in suitable off-crop habitat in the close vicinity of the field. At least 50% of the traps were always placed inside the study field. Traps were supplied with bait (e.g. sunflower seeds) which also serves as food for captured animals. At temperatures below 5°C, bedding material (e.g. hay) was added to the traps to provide insulation for captured individuals. Traps were activated for trapping in the evening and checked in the morning, after which they were left open (de-activated) during the day.

Each captured wood mouse (excluding animals below 15 g body weight) was individually marked with a passive integrated transponder (mini PIT), which was injected subcutaneously. Species, date, location (study field and trap number), first capture or recapture, PIT number (if applied), sex, reproductive state and body weight were recorded as raw data. Afterwards the mice were released at the site of capture.

Radio tagging and tracking

Suitable adult wood mice captured in the live traps were equipped with radio collars ('Pip3-AG379', manufactured by Biotrack Ltd., UK). Animals were regarded as suitable if they had a body weight of 20 g or more (to allow the 0.9 g radio-transmitter to not exceed 5 % of mammals body weight). From October 10th to November 6th, tagged individuals were radio tracked from dusk till dawn continuously for approximately 12 hours. Authors do not report whether multiple observers were used to track individuals for each session. Every change in behaviour and location (GPS position) were actively recorded to the minute. For each individual, session 1 was conducted within the first three days after drilling whereas the following sessions were finished before the cereal plants emerged.

7 study sites (study fields and their vicinity) were used to successfully radio track 16 wood mice in 38-night sessions. The study was conducted on 11 commercially managed winter cereals, but only 7 out of the 11 study sites were used in the radio-tracking phase of the study. It is not clearly explained why only 7 study sites were used, though it appears that the number of trapped wood mice was low at the other 4 sites.

Table B.9.1.4-25: Information for tracking sessions for each individual

Study site	Date of drilling winter cereal	Individual No.	No. of tracking sessions	Date of tracking sessions
2	28.10.2016	2-1	1	Not specified
		2-3	5	Not specified
		2-4	1	Not specified
3	09.10.2016	3-1	5	Not specified
4	09.10.2016	4-1	5	Not specified
8	17.10.2016	8-1	1	Not specified
		8-2	2	Not specified
		8-3	5	Not specified
		8-4	1	Not specified
9	18.10.2016	9-1	1	Not specified
		9-2	5	Not specified
10	01.11.2016	10-1	2	Not specified
		10.2	1	Not specified
13	01.11.2016	13-3	1	Not specified
		13-5	1	Not specified
		13-6	1	Not specified

Study authors do not provide the date of each tracking session for each individual but report the first session being conducted within 3 days after the winter cereal was drilled.

During radio tracking sessions the behaviour of the tracked wood mouse was recorded in the following activities:

5) **Active**: the animal was active and potentially foraging
6) **Foraging**: the animal was visually observed foraging
7) **Inactive or in the burrow**: active or resting but definitely not foraging outside
8) **Travelling**: moving fast through the habitat without foraging.

Authors state that those considered 'foraging' were visually observed foraging during sessions. How this was achieved in practice is unclear, as sessions occurred between dusk till dawn (i.e. dark) and wood mice are a small mammal and considered difficult to be visually monitored under these field conditions. Study authors do not go into detail whether any problems were faced or if specialist equipment (i.e. night vision equipment, torches, binoculars etc.) was used for visual observations to confirm the behaviour of individuals.

In order to check whether tagged individuals were still present in the study area between tracking sessions, and whether tags were still functioning, tagged animals were looked for during the daytime. The use of unidirectional ‘Yagi-antennas’ (Biotrack Ltd., United Kingdom) allowed the determination of the direction and a good estimate of the distance to the tagged individual. As the accuracy of GPS devices depends on weather conditions, tree cover etc. and the distance to the animal was estimated, a total accuracy of 3-5 metres could be obtained.

Additional observations

Habitat mapping

Before the radio tracking was started, all habitats within 250 m from each study field border were mapped. Habitat types were taken from satellite photographs (google maps / google earth, 2016) and verified in the field. Habitat mapping was extended if the animal had left the mapped area. Furthermore, growth stages of all arable crops used by the animal were noted (BBCH stages), however these were not reported in the study report.

Study site 2 – immediate area consists of shrubs/hedges/trees, field margins, meadows, harvested field and tarmac road. Wider area consists of water & associated vegetation, forest, settlement, other winter cereal fields, and arable fields.

Study site 3 – immediate area consists of forest, meadows and field margin. Wider area consists of harvested field, arable land, field path, other winter cereal fields, and water & associated vegetation.

Study site 4 – immediate area consists of meadows, field margin, field path and forest. Wider area consists of fallow land, other winter cereal fields, tarmac road and arable land.

Study site 8 – immediate area consists of meadow, field margins, forest, ploughed field, and arable crop. Wider area consists of settlement, tarmac road, fallow land and other winter cereal field.

Study site 9 – immediate area consists of shrubs/hedges/trees, meadows, forests, field margins and winter cereal field. Wider area consists of ploughed field, fallow land, settlement and water & associated vegetation.

Study site 10 – immediate area consists of shrubs/hedges/trees, arable crops, field margins and ploughed field. Wider area consists of harvested field, winter cereal fields and tarmac roads.

Study site 13 – immediate area consists of arable crop, winter cereal field, harvested field, field path, forest, meadow and shrubs/hedges/trees. Wider area consists of fallow land, water & associated vegetation and tarmac road.

Climate

The daily precipitation and the temperature data between 29.09.2016 – 04.11.2016 were obtained from the weather station of Neu-Ulrichstein, dependent of the ‘Deutscher Wetterdienst’ (non-GLP). The temperature ranged from -1.0^{o}C – 23.5^{o}C. The total precipitation was 46.7 mm, the average precipitation was 1.42 mm per day.

Agricultural practice

Farmers were responsible for all agricultural practice, which was conducted non-GLP but according to good agricultural practice (GAP). There was no information provided on previous land management for study sites, information regarding the seed bed, whether fields were freshly ploughed, BBCH stages of crops or if any management occurred on study fields during the study.

Photographs of study fields show some vegetation cover for study site 2 and 9; stubbles for study site 10; and minimal vegetation with exposed bare soil for study sites 3, 4, 8 and 13. It is not clear from the report if photographs were taken before or after drilling for all study sites.

Table B.9.1.4-26: Agricultural information for the study fields where radio tracking was conducted

Study field	Area (ha)	Date of drilling	Crop	Drilling depth (cm)	Drilling density	Visible seed exposure?
2	1.6	28.10.2016	Winter cereal (wheat)	5	155 kg/ha	YES
3	1.96	09.10.2016	Winter cereal (wheat)	2-3	470 seed/m^2	YES
4	0.68	09.10.2016	Winter cereal (wheat)	2-3	470 seed/m^2	YES
8	1.7	17.10.2016	Winter cereal	5	160 kg/ha	YES
9	1.82	18.10.2016	Winter cereal	3	160 kg/ha	YES
10	0.92	01.11.2016	Winter cereal (wheat)	2	180 kg/ha	YES
13	0.88	01.11.2016	Winter cereal (wheat)	Not specified	350 kg/ha	YES

Analysis of data

PT values were obtained by radio tracking individual wood mice, based on the assumption that the time an animal spends active in a habitat is a reliable measure of the proportion of diet obtained in this defined area.

The proportion of foraging time (PT) an animal spent in a specific habitat type, its home range (through calculating Minimum Convex Polygons (MCP) by GIS software, (Quantum GIS 2.14.6) and its habitat preferences (Jacobs index) were calculated. Movements (speed) within and between habitats by individuals were calculated by measuring the speed of movement between two positions within the same habitat or between habitats. PT values were calculated by adding together the time ‘potentially foraging’ (time spent ‘active’ + time spent ‘foraging’) in a specific habitat and dividing this by the total time observed ‘potentially foraging’. Time individuals spent ‘travelling’ ‘in the burrow’ and ‘inactive’ was not included in PT calculations. Three different approaches to calculate PTs were as follows:

1. ‘all individuals’ approach – where all successfully tracked individuals with all sessions, regardless of if they used the winter cereal fields or not;

2. ‘home range’ approach – included only those wood mice that embedded a winter cereal field in their MCP home range. These individuals can be considered ‘potential foragers’;
3. ‘consumers’ approach – focussed on individuals that entered a winter cereal field during at least one radio tracking session.

$$\textbf{Proportion of time and animal spend potential foraging time (PT)} = \frac{\textit{Time spent foraging in cereal crops 'active' + 'foraging' [hh:mm]}}{\textit{Total time spent potentially foraging (in both adjacent and cereal crops 'active' + 'foraging') [hh:mm]}}$$

RESULTS AND DISCUSSION

Experimental dates: 24 September 2016 to 06 November 2016.

Trapping

At 11 different locations 8220 trap nights were conducted inside the study fields and 4728 trap nights in the adjacent off crop. During these 12948 trap nights 1606 small mammals were captured. 401 of these captures were identified as wood mice. The 401 captures were first and re-captures of 175 individually marked wood mice. Out of these 175 wood mice 24 were equipped with radio collars and released. It is not clearly stated by authors why these 24 individuals were selected. In total 16 wood mice were successfully radio tracked for 38 different night sessions.

The authors report that although EFSA guidance (EFSA 2009, appendix P) indicates individuals caught in the target crop should be preferred for radio tracking, in the present study only 2 tracked individuals (individuals 10-1 and 10-2, all caught in field 10) out of the 16 radio tracked wood mice would occasionally be caught inside the study fields. However, authors do not clearly show at what locations individuals used for radio-tracking were trapped. There is no clear information whether captures were in study fields or in the local vicinity for each individual radio-tracked for each session. Although it is reported that 2 tracked individuals were *occasionally* caught inside study fields it is not reported where in the study fields these were caught. For off crop captures there is no information showing where individuals were caught from. This low trapping success in the study fields was achieved despite the fact that 8220 trap nights were made in the study fields and just 4728 in the adjacent off crop. This questions whether the selected individuals used from the local vicinity were appropriate and subsequently, if the study presents an appropriate scenario for the risk assessment.

Radio tracking individuals

Authors do not state why only 16 out of the 24 tagged wood mice were successfully tracked. No radio tracking sessions were excluded from analysis. All radio tracking sessions were made after drilling the winter cereal fields but before the emergence of the crop plants. Overall, 16 individuals were successfully tracked over 38-night sessions at 6 different study fields.

Table B.9.1.4-27: Overview of captured and tagged wood mice

Study site	Total captures of small mammals	Total captures of wood mice	Individuals of wood mice captured	No. of tagged wood mice	No. of successful radio tracked wood mice
1	162	7	6	1	-
2	264	35	19	4	3
3	181	22	15	2	1
4	129	66	25	1	1
6	119	12	6	-	-
8	129	63	33	4	4
9	95	62	17	4	2
10	267	46	21	2	2
11	17	2	2	-	-
12	5	3	2	-	-
13	238	83	29	6	3
TOTAL	**1606**	**401**	**175**	**24**	**16**

<u>PT values calculated from 38 radio tracking sessions of 16 different wood mice</u>

During 13 different tracking sessions after drilling 7 individuals were observed to enter a winter cereal field (see table 10.3.3/02-4 below).

Table B.9.1.4-28: Radio tracking sessions, time foraging and PT values

Wood mouse number	Radio tracking session	Status [A = all sessions C = consumers HR = home range]	Time potentially foraging [hh:mm]		PT in drilled winter cereal fields	Individual mean PT in drilled winter cereal fields
			All habitats	Drilled winter cereal fields		
2-1	1	A	08:00:00	00:00:00	0.000	0.000
2-3	1	C	03:29:00	01:04:00	0.306	0.612
	2	C	05:11:30	00:57:30	0.185	
	3	C	00:58:00	00:58:00	1.000	
	4	C	06:10:00	05:05:00	0.824	
	5	C	04:29:00	03:21:00	0.747	
2-4	1	C	09:12:00	00:04:00	0.007	0.007
3-1	1	A	10:11:00	00:00:00	0.000	0.000
	2	HR	09:07:00	00:00:00	0.000	

	3	HR	07:55:00	00:00:00	0.000	
	4	HR	07:00:00	00:00:00	0.000	
	5	A	05:50:00	00:00:00	0.000	
4-1	1	C	09:42:00	00:00:20	0.001	0.000
	2	C	09:19:00	00:00:30	0.001	
	3	A	08:49:00	00:00:00	0.000	
	4	A	06:53:00	00:00:00	0.000	
	5	A	09:01:00	00:00:00	0.000	
8-1	1	A	09:11:00	00:00:00	0.000	0.000
8-2	1	C	09:09:00	00:00:30	0.001	0.001
	2	HR	08:23:00	00:00:00	0.000	
8-3	1	A	08:57:00	00:00:00	0.000	0.001
	2	C	10:59:00	00:02:30	0.004	
	3	A	10:38:00	00:00:00	0.000	
	4	C	09:08:00	00:01:00	0.002	
	5	A	10:01:00	00:00:00	0.000	
8-4	1	A	06:29:00	00:00:00	0.000	0.000
9-1	1	A	07:30:00	00:00:00	0.000	0.000
9-2	1	A	09:36:00	00:00:00	0.000	0.005
	2	C	10:22:00	00:16:00	0.026	
	3	A	09:30:00	00:00:00	0.000	
	4	A	10:49:00	00:00:00	0.000	
	5	A	08:57:00	00:00:00	0.000	
10-1	1	C	08:53:00	00:01:30	0.003	0.001
	2	A	07:46:00	00:00:00	0.000	
10-2	1	A	07:41:00	00:00:00	0.000	0.000
13-3	1	HR	10:12:00	00:00:00	0.000	0.000
13-5	1	A	09:44:00	00:00:00	0.000	0.000
13-6	1	A	09:31:00	00:00:00	0.000	0.000
All individuals approach* (n=16 individuals)				Mean	0.039	
				Median	0.000	
				90th percentile	0.006	
Home range approach ** (n= 9 individuals)				Mean	0.070	
				Median	0.001	
				90th percentile	0.128	
Consumers approach*** (n=7 individuals)				Mean	0.090	
				Median	0.001	
				90th percentile	0.249	

*All individuals [A]: PT value included all tracked individuals
**Home range [HR]: PT value includes those with 'consumers' and the 'home range' status
***Consumers approach [C]: PT value includes only those with 'consumer' status
Mean, median and 90th percentile values are generated from calculated mean PT values.

The 1st approach for deriving overall PT values from this dataset included all radio tracked wood mice captured in or next to the winter cereal fields, 'all individuals approach. The 2nd approach used only tracking sessions when the individual home range calculated for this particular session embedded the winter cereal field. Due to method of MCP a field can be part of the home range, even if the animal did not enter (use) this field during the tracking session (‘home range’ approach) and these individuals could be considered as ‘potential consumers’. The 3rd approach used only sessions if the animal was observed (radio tracked) in the winter cereal field, ‘consumers’ approach.

Home range, Jacobs index and speed of wood mice in winter cereal fields

Home range

The home range for 9 out of 16 radio tracked wood mice included a winter cereal field, ranging between 0.0 % and 91.72 % of the total home range, with an average home range percentage of winter cereal field of 14.65 %.

Jacobs preference index

Wood mice in this study showed a preference for three habitat types available within their home ranges. These habitats were shrubs, hedges and solitary trees (SHT), water and associated trees and bushes (WAT) and the forests (FOR). All habitat types under some kind of agricultural use, field margins (FM), arable land (ARA), meadows (MEA), and field paths (F) and among them the freshly drilled winter cereal fields (WC), were avoided. When accounting for all radio tracking data, authors report a Jacobs index of winter cereal fields of -0.83, showing wood mice displayed strong avoidance of this habitat. Individual 3 for study site 2 (2-3) where the majority of its time during radio tracking sessions were on the winter cereal fields in addition to reportedly having its burrow located inside the study field, displayed avoidance of this habitat (Index value of -0.29). As summarised in the table below, all radio tracked individuals had indices between -1 to 0, showing avoidance of freshly drilled winter cereals habitat.

Table B.9.1.4-29: Summary of proportional home ranged, Jacobs index and the speed of wood mice in winter cereal fields

Study site	Individual No.	No. of tracking sessions	Winter cereal fields		
			% of winter cereal fields in home range	Jacobs Index	Speed* (m/h)
2	2-1	1	0.00	-	-
	2-3	5	70.27	-0.29	52.33
	2-4	1	13.88	-0.91	-
3	3-1	5	11.69	-1	-
4	4-1	5	0.35	-0.83	-
8	8-1	1	0.00	-	-

	8-2	2	11.23	-0.99	-
	8-3	5	25.30	-0.99	-
	8-4	1	0.00	-	-
9	9-1	1	0.00	-	
	9-2	5	91.72	-1*	478.49
10	10-1	2	1.03	-0.75	-
	10.2	1	0.00	-	-
13	13-3	1	8.97	-1	-
	13-5	1	0.00	-	-
	13-6	1	0.00	-	-

* the animal 9-2 had a Jacobs index of -0.996 which was rounded to -1

Land management

Study sites were chosen due to being bordered by off-crop habitats known to be favourable for the presence of wood mice, so mice could potentially use the adjacent freshly drilled winter cereal fields for foraging. The report does not explore the previous land uses or management of all the study fields before seed drilling which raises the question whether the study sites are comparable in their attractiveness for wood mice populations.

Ultimately there is no information provided on the previous land uses or management of the study areas which may give further context for the suitability of the study sites. It is not clearly identified whether there were any land management practices conducted on or on nearby areas to the study sites during the study period. It is clear that the wood mice population are not extensively foraging on the study fields, but there are questions over whether the previous land uses/management practices and surrounding habitats at the study locations mean that all study fields are representative of locations where wood mice would have a relatively high potential to forage in winter cereal fields (i.e. there is uncertainty whether wood mouse behaviour in the study fields is representative of wood mouse behaviour in winter cereal fields in general).

Speed

The average speed of wood mice moving in winter cereal fields (265.41 m/h) was similar to speeds observed in arable fields (269.91 m/h) and when moving from one habitat into another (266.36 m/h). In other habitat types the average movements were much slower from 11.65 m/h in field margins to 94.24 m/h in meadows; 56.04 m/h in forest habitats and 53.34 h/h in shrubs, bushes and trees, habitats known to be favoured by wood mice populations. These speeds therefore indicate that the wood mice in winter cereal fields were rather travelling than foraging. These data, however, were obtained from a few observations as speed inside a habitat could only be calculated when at least two consecutive radio tracking locations fell into the same habitat type. Therefore, average speed in winter cereal fields is based on just two individuals with very different behaviour. The wood mouse 9-2 at field number 9 ran with high speed (478.49 m/h) across the field to reach the forest at the other side whereas individual 2-3 at field 2 made its burrow inside the winter cereal field and moved with a moderate average speed of 52.33 m/h.

CONCLUSIONS

Observations by the means of radio tracking revealed that wood mice occasionally used freshly drilled winter cereal fields for foraging. In the present study an average of ~14% of the home range area were freshly drilled winter cereal fields for all tracked individuals. A single individual obtained PT values up to 1 but all other wood spent less than 3% of their foraging time in winter cereal fields. A second indicator of attractiveness, the Jacobs index, showed that the winter cereal fields were avoided, i.e. they were less frequently used than a random use of all available habitats would suggest. It can be concluded that freshly drilled winter cereal fields were occasionally entered by wood mice, but the majority of individuals avoided them as foraging habitats.

([redacted]. and [redacted], 2017)

HSE comments:

The use of this study in risk assessment has focussed on the data reported for the wood mouse (*Apodemus sylvaticus*) in winter cereal fields shortly after drilling, in order to gain more realism of the focal species' feeding ecology.

It is noted that 1606 small mammal captures occurred with only 401 of these being wood mice. However, the study authors do not state what other species were caught by the Ugglan multi-capture traps, and from this if there were other mammals possibly using these habitats more frequently that could potentially be a more appropriate focal species for the risk assessment. Authors report the selection of the fields on the basis that the surrounding area supports the wood mice population. Jacobs' index results indicate that individuals are avoiding freshly drilled winter cereals and some surrounding areas are considered more attractive for foraging, though these results are likely a function of the nature of the surrounding habitats and the general applicability of these results is therefore uncertain. The study states to overcome this that the selection of individuals which were radio tracked repeatedly, animals were already known as consumers (i.e. were radio tracked in the winter cereal fields) and were preferred to ensure a conservative worst-case scenario. The use of radio-tracking from multiple sessions with the same individual is discussed in the higher tier reproductive risk assessment. It is noted that the small number of individuals trapped in the cereal fields (with no clear mapping of where these traps were located) and low number of individuals observed foraging, although considered known foragers, questions whether the area can be considered to be representative in terms of the population of this species. Although, in the study, it is clear that most wood mice do not forage in freshly drilled winter cereals to a significant extent, it is unclear whether the fields investigated represent an appropriate scenario for the risk assessment (i.e. whether the results from the study fields represent a reasonable worst-case scenario in terms of wood mouse behaviour).

When deriving PT values, authors report the mean, median and 90th percentile for 'all individuals, 'home range' and 'consumers' approaches, with 90th percentile values of 0.006, 0.128 and 0.249, respectively. The number of individuals tracked was 16 individuals (over 38 tracking sessions), 7 individuals were tracked for 1 to 4 additional nights each. It was noted that some individuals were tracked on separate occasions, sometimes up to 5 separate sessions each consisting of 12 hours. Although dates of tracking sessions were not disclosed, authors state 1st sessions were within 3 days of drilled winter cereals, additional sessions before crop emergence. It is noted that seven individuals were tracked under different sessions. Arithmetic mean PT values across sessions have been calculated for individuals tracked for multiple sessions. It is noted that the mean PT values for individual wood mice for winter cereal fields ranged from 0.000 – 0.612. Across study sites, the PT value is typically low, apart from individual 3 for study site 2. This individual had its burrow inside the study field and was active within the winter cereal field during all radio tracking sessions, which resulted in a PT value of 0.612. Conversely, other individuals for this study site had PT values of 0.000 and 0.007, i.e. they were not foraging on the winter cereal study field or foraging to a very limited extent. Unfortunately, there is no trapping data to compare where each individual was captured in relation to the study site, and whether this could potentially have had an impact. Nevertheless, this value was included in the 'consumers' approach, which resulted in a 90th percentile PT value of 0.249.

The available PT datasets for wood mice are considered further in the higher tier risk assessment section.

The following study summary is reproduced verbatim from Section B.9 of the EU draft Renewal Assessment Report for mesotrione

[redacted], [redacted] & von [redacted] (2013). Generic field study on small mammals focal species and wood mouse (*Apodemus sylvaticus*) PT in maize fields in Germany. [redacted]. Oxon unpublished Report No.: [redacted]. Syngenta File Number [redacted] (Data owner: Oxon Italia, S.p.A., Syngenta access). 16 December 2013.

Sponsor	OXON ITALIA S.p.A., Regulatory Affairs Dept., Via Sempione, 195, 20016 Pero (MI), Italy
Test facility	[redacted]
Report No.	[redacted]
Completion date	16 December 2013
Guidelines	None – protocol was designed for purpose
Deviations	N/A
GLP	Yes

Aim

Generic monitoring of small mammals in general and wood mice in particular in maize fields and adjacent natural off-crop habitats in Germany (Central Europe) based on live trapping, scan sampling and radio-tracking (only for wood mice) data.

Material and methods

The occurrence of small mammals in freshly drilled maize fields was assessed by small mammal live trapping, nocturnal scan sampling and radio-tracking. The study was conducted on ***five study fields*** located in southern Germany during spring, 27 April – 17 May 2013 (for structure of study fields please see the figures below). Four fields were used for nocturnal scan sampling and radio-tracking.

Table B.9.1.4-30: Information on study plots

Study field	Emergence of maize [dd.mm.yyyy]	Surrounding habitats
1	07.05.2013	arable fields (partly freshly drilled, partly ploughed), trees, shrub, hedges, ditch, street/path, field margin
2	14.05.2013	arable fields (including maize), trees, shrub, hedges, meadows, street/path, field margin
3	13.05.2013	arable fields (including maize), forest, trees, shrub, hedges, street/path, settlement, wetland, meadows
4	16.05.2013	meadows, riding arena, pond, field margin, wetland/reed, ditch, street/path, trees, shrub, hedges, settlement
5	16.05.2013	arable fields, trees, shrub, hedges, street/path

Figure B.9.1.4-1: Sketch of study field 1 with location of trap lines (dashed lines)

Figure B.9.1.4-2: Sketch of study field 2 with location of trap lines (dashed lines)

Figure B.9.1.4.3: Sketch of study field 3 with location of trap lines (dashed lines)

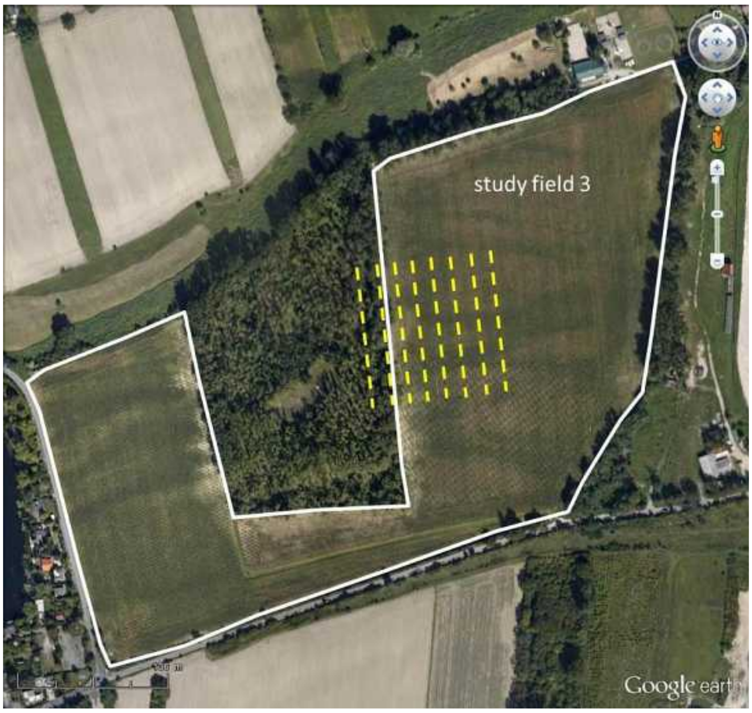


Figure B.9.1.4-4: Sketch of study field 4 with location of trap lines (dashed lines)

Figure B.9.1.4-5: Sketch of study field 5 with location of trap lines (dashed lines)

The ***live trapping*** of small mammals was used to generate a list of small mammal species and their abundance in and around freshly drilled maize fields. Trapping was carried out from 27 April 2013 until 17 May 2013 mostly before BBCH 10 (i.e. after sowing but before emergence; 1560 trapnights in-crop, 520 trapnights off-crop) and additionally until max. BBCH 12 (720 trapnights in-crop, 240 trapnights off-crop) on five different maize fields with a total trapping effort of 3,040 trapnights, with 25% of the traps set up in the adjacent off-crop habitats. At least two trapping sessions of two consecutive nights were conducted applying 80 traps on each field (26 trapping nights before and 12 trapping nights after emergence). The applied Ugglan multiple capture live traps are suitable for all small mammals. Live trapping followed a Capture-Mark-Recapture (CMR) design to allow the identification of individually marked animals upon recapture. Each captured individual >7.0 g was individually marked by subcutaneous injection of a Passive Integrated Transponder (PIT, 0.11 g). Individuals below 7.0 g body weight were marked with individual fur cuts.

Table B.9.1.4-31: Schedule of small mammal live trapping on all study plots

Date [dd.mm.yyyy]	study plot 1	study plot 2	study plot 3	study plot 4	study plot 5
27.04.2013	x				
28.04.2013	x				
29.04.2013	x				
30.04.2013	x				
01.05.2013	x				
02.05.2013	x				
03.05.2013	x				
04.05.2013	x				
05.05.2013					
06.05.2013	x				
07.05.2013	x	x	x		
08.05.2013	x	x	x		
09.05.2013	x	x	x	x	x
10.05.2013		x	x	x	x
11.05.2013				x	
12.05.2013					
13.05.2013					
14.05.2013		x	x	x	x
15.05.2013		x	x	x	x
16.05.2013		x		x	x
17.05.2013		x		x	

Note: The day of emergence is dashed.

Table B.9.1.4-32: Trapping effort as trapnights[21]

Plot No.	BBCH 00-09		BBCH 10-12		Total
	in-crop	off-crop	in-crop	off-crop	
1	540	180	180	60	960
2	240	80	240	80	640
3	240	80	120	40	480
4	300	100	120	40	560
5	240	80	60	20	400
Total	**1560**	**520**	**720**	**240**	**3040**

Note: trapping effort was divided to pre-emergent and post-emergent periods by the RMS following the schedule of small mammal live trapping

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. Observations (186 scan sessions) were conducted in-crop between BBCH growth stages of 10-16. For one entire night the whole

[21] 1 trapnight = 1 trap set for 1 night

selected scan sampling area was searched for five minutes in every 15 minutes. The size (i.e. ≤ 50 g, > 50 g; if possible genus was given) and the behaviour (i.e. foraging/potentially foraging, other) of the spotted individuals were recorded. The total area of each observation was 0.108 ha.

Table B.9.1.4-33: Dates of nocturnal scan sessions

Study field	1st scan session [dd.mm.yyyy]	2nd scan session [dd.mm.yyyy]
1	09.05.2013	11.05.2013
2	17.05.2013	31.05.2013
3	15.05.2013	26.05.2013
4	30.05.2013	04.06.2013

With the purpose to obtain more detailed information about the use of freshly germinated maize fields (period: BBCH 10-16) by wood mice, 20 individual wood mice were provided with transmitters and radio-tracked. Wood mice were individually radio tracked for one entire activity period (i.e. from dusk till dawn). PT (proportion of diet obtained from the treated area) was determined for all complete ***radio-tracking*** sessions (n = 17), for sessions including maize field in the home range of wood mice (n = 13) and for consumers only (n = 9).

Results

Trapping – Small mammal species

The wood mouse was the most abundant species with a total of 121 captures comprising of 33 individuals, followed by the bank vole (*Myodes glareolus*) with 110 captures (26 individuals) and the yellow-necked mouse (*Apodemus flavicollis*) with 25 captures (10 individuals). Bank vole and yellow-necked mouse were not captured within the maize fields. The house mouse (*Mus musculus*) was captured once only and no captures of common voles (*Microtus arvalis*) or shrews (*Soricidae*) were made in the course of this study.

The only one in-crop capture of wood mouse occurred on the 2nd plot where the highest capture of 33 was achieved in the surrounding area.

Nocturnal scan sampling – Monitoring behaviour and activity

During all scans only 19 observations of mammals were made; 16 observations of small mammals (i.e. mammals ≤ 50 g) and 3 observations of medium sized/large mammals (i.e. mammals ≥ 50 g; one rabbit (*Oryctolagus cuniculus*; potentially foraging; corresponding to 0.05 individuals/ha), one marten (*Martes* sp.) and one fox (*Vulpes vulpes*)). The majority of observations were determined as „mouse" (i.e. *Apodemus* or *Mus* species, no voles). One single observation of a presumed vole was made. Mammal observations were made on all study fields, resulting in a mean abundance of 1.0 individuals per scan per ha during all observation periods.

Radio-tracking

The PT values of wood mice in maize fields after emergence (BBCH 10) until BBCH 16 (mean and 90th percentile of „all tracked mice" and „consumer only") are all below 0.1.

The following tables give an overview of the key results.

Table B.9.1.4-34: Trapping success as number of wood mouse individuals in BBCH 00-09 and 10-12

Study plot	BBCH 00-09		BBCH 10-12	
	In-crop	Off-crop	In-crop	Off-crop
1	0	20	0	9
2	0	20	1	33
3	0	13	0	2
4	0	13	0	5
5	0	4	0	1
Total	**0**	**70**	**1**	**50**

Table B.9.1.4-35: Trapping efficiency for wood mouse as captures/100 trapnights in BBCH 00-09 and 10-12*

Study plot	BBCH 00-09		BBCH 10-12	
	In-crop	Off-crop	In-crop	Off-crop
1	0	11.11	0	15.00
2	0	25.00	0.42	41.25
3	0	16.25	0	5.00
4	0	13.00	0	12.50
5	0	5.00	0	5.00

Note: *Calculated by the RMS

Table B.9.1.4-36: Trapping efficiency as captures/100 trapnights by study plots

Study plot	Wood mouse		Yellow-necked mouse		Bank vole	
	In-crop	Off-crop	In-crop	Off-crop	In-crop	Off-crop
1	0.00	12.08	0.00	1.25	0.00	19.17
2	0.21	33.13	0.00	7.50	0.00	13.13
3	0.00	12.50	0.00	4.17	0.00	20.83
4	0.00	12.86	0.00	0.71	-	-
5	0.00	5.00	0.00	4.00	0.00	18.00
Mean	0.04	15.11	0.00	3.53	0.00	17.78

Note: - Trapping without success

Table B.9.1.4-37: Trapping efficiency as captures/100 trapnights by BBCH stages (number of individuals in brackets) *

Study plot	Wood mouse		Yellow-necked mouse		Bank vole	
	In-crop	Off-crop	In-crop	Off-crop	In-crop	Off-crop
BBCH 00-09	0 (0)	13.46 (70)	0 (0)	2.31 (12)	0 (0)	14.62 (76)
BBCH 10-12	0.14 (1)	20.83 (50)	0 (0)	5.42 (13)	0 (0)	14.17 (34)

Note: * Calculated by the RMS

Table B.9.1.4-38: Summary of nocturnal scan sampling data on small mammals (BBCH 10-16)

No. of scans		No. of observations				Foraging individuals [%]		Abundance (Mammals ≤ 50 g) [ind./scan/ha]
		Total No.	Mammals ≤ 50 g		Mammals ≥ 50 g	Mammals ≤ 50 g	Mammals ≥ 50 g	
			Mice	Voles				
Total	186	19	15	1	3	31.3	33.3	0.9
Mean (n = 4)	46.5	4.8	3.8	1.0	1.5	31.6	50.0	1.0

Table B.9.1.4-39: Individual PT values of wood mouse in maize (BBCH 10-16)

Session No.	Indivi-dual No.	Plot No.	Date of radio-tracking [dd.mm.yyyy]	PT in maize: *all sessions* (n = 17)	PT in maize: *home range* (n = 13)	PT in maize: *consumer only* (n = 9) focusing on sessions	PT in maize: *consumer only* (n = 7) focusing on individuals *
1	1	1	09.05.2013	0.00	0.00	-	-
2	2	1	10.05.2013	0.00	0.00	-	-
3	3	1	12.05.2013	0.03	0.03	0.034	0.034
5	5	1	14.05.2013	0.00	-	-	-
6	6	3	20.05.2013	0.00	-	-	-

Session No.	Indivi-dual No.	Plot No.	Date of radio-tracking [dd.mm.yyyy]	PT in maize: *all sessions* (n = 17)	PT in maize: *home range* (n = 13)	PT in maize: *consumer only* (n = 9) focusing on sessions	PT in maize: *consumer only* (n = 7) focusing on individuals *
7	7	2	18.05.2013	0.04	0.04	0.045	0.026
8	8	2	20.05.2013	0.07	0.07	0.068	0.068
9	9	2	20.05.2013	0.14	0.14	0.139	0.139
10	10	2	21.05.2013	0.03	0.03	0.028	0.030
11	11	2	22.05.2013	0.00	0.00	-	-
12	7	2	22.05.2013	0.01	0.01	0.008	-
13	13	2	23.05.2013	0.01	0.01	0.013	0.007
14	14	2	24.05.2013	<0.01	<0.01	0.004	0.004
15	15	4	25.05.2013	0.00	-	-	-
16	16	4	27.05.2013	0.00	-	-	-
17	13	2	29.05.2013	0.00	0.00	-	-
18	10	2	01.06.2013	0.03	0.03	0.031	-

Notes: Session No. 4, 19 and 20 were incomplete and therefore not used for evaluation. The data are based on 17 tracking sessions of 14 individual wood mice. * Including mean values for individuals with 2 tracking sessions (No. 7, 10 and 13).

Table B.9.1.4-40: PT values for each radio-tracked wood mice (BBCH 10-16)

PT parameter	PT calculation				PT in maize: *all sessions* (n = 17)	PT in maize: *home range* (n = 13)	PT in maize: *consumer only* (n = 9) focusing on sessions	PT in maize: *consumer only* (n = 7) focusing on individuals
	Behaviour in all known habitats [%]		Behaviour in maize fields [%]					
	Not foraging	Potentially foraging	Not foraging	Potentially foraging				
50th %ile	9.56	90.44	0.00	0.36	0.00	0.01	0.030	0.030
90th %ile	13.77	95.57	1.93	4.79	0.05	0.06	0.084	0.096
Mean	9.40	90.60	0.65	1.98	0.02	0.03	0.041	0.044
Max	18.20	99.46	5.90	13.05	0.14	0.14	0.139	0.139

Conclusions

Three small mammal species occurred regularly on and in the surrounding of maize fields after emergence until BBCH 16: the wood mouse (*Apodemus sylvaticus*), the bank vole (*Myodes glareolus*) and the yellow-necked mouse (*Apodemus flavicollis*). Only the wood mouse was found inside maize fields and only in small numbers.

During nocturnal scan sessions 16 observations of small mammals (mostly *Apodemus* and *Mus* species, only 1 vole) and 3 medium sized mammals were made (one *European rabbit* (*Oryctolagus cuniculus*), one marten (*Marten* sp.) and one fox (*Vulpes vulpes*).

Radio-tracking data support a low portion of diet obtained from treated area by wood mouse: the worst case PT was 0.139.

([redacted]., [redacted], and [redacted]., 2013)

HSE comments:

This study was conducted to GLP and is considered acceptable for use in regulatory risk assessment.

For further evaluation and comments please see the relevant subsections in the higher tier risk assessment.

B.9.1.5. Risk assessment – birds

B.9.1.5.1. *Acute risk assessment*

The key metalaxyl-M toxicity endpoint for the acute risk assessment is as follows:

- Geometric mean LD_{50} = 1180 mg a.s./kg bw

<u>**First tier – Vibrance SB**</u>

For pelleted seeds, such as sugar and fodder beet, it is assumed that birds do not intentionally consume seeds for food but may consume pelleted seed as/with grit (EFSA, 2009). Therefore exposure of birds ingesting seeds as/with grit has been considered.

Acute exposure – Tier 1 assessment birds ingesting seeds with/as grit

The acute Daily Grit Dose ($DGritD_{acute}$) can be calculated for large pelleted seeds using the following equation:

$$DGritD_{acute} = 2453 \times (G_{density} / (71 + G_{density})) \times G_{loading}$$

Where:

$G_{density}$ Number of seeds on soil surface
$G_{loading}$ Amount of active substance in one seed

In a worst-case conservative assessment, the initial $DGritD_{acute}$ calculation assumes that all seeds are available to birds. Based on a sowing rate of 130000 seeds/ha, this equates to 13 seeds/m^2. It is noted that results from [redacted] (2002) indicate that a much smaller number of sugar beet seeds are likely to be available per m^2. Data from [redacted] (2002) can be factored into a refined exposure assessment, if required.

The $DGritD_{acute}$ is calculated for a large bird on a per bird rather than per kg bw basis. Therefore to convert the $DGritD_{acute}$ into a per kg bw basis, a default body weight of 300 g is assumed for the generic large bird.

Calculation of the DDD for the representative use patterns for A20607B are given in the table below.

Table B.9.1.5-1: Acute exposure estimate for birds following the consumption of seeds with/as grit

Crop	Active substance	Generic focal species	$G_{loading}$ (mg a.s./seed)	$DGritD_{acute}$ (mg a.s./bird)	$DGritD_{acute}$ (mg a.s./kg bw)
Sugar and fodder beet	Metalaxyl-M	Large bird	0.0048	1.82	6.07

Acute exposure – Tier 1 assessment emerged seedlings

The acute Daily Dietary Dose (DDD_{90}) as a result of the consumption of emerged seedlings can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR/5}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

For the Tier I risk assessment a small omnivorous and a large herbivorous bird with food intake rates (FIR/bw) of 0.5 and 0.3 respectively will be used as relevant indicator species.

Table B.9.1.5-2: Acute exposure estimate for birds following the consumption of treated sugar and fodder beet seedlings

Crop	Active substance	Generic focal species	NAR/5 (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Sugar and fodder beet	Metalaxyl-M	Small omnivorous bird	39.96	0.5	19.98
	Metalaxyl-M	Large herbivorous bird	39.96	0.3	11.99

Exposure for birds through drinking water

There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water.

Leaf scenario

The ‘Leaf scenario’ is relevant for birds taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation water sufficiently to attract birds. Since the proposed use of metalaxyl-M is as a seed treatment in sugar and fodder beet the leaf scenario does not apply.

Puddle scenario

This scenario is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all uses of metalaxyl-M and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:

> *"Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg)."*

With a K(f)oc of 78.9, metalaxyl-M belongs to the group of less sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the maximum use rate of 0.62 g a.s./ha in sugar and fodder beet is used. Since the ratio of the application rate to the toxicity endpoint is less than the trigger value, an acceptable acute risk to birds can be concluded for the puddle scenario and no further consideration is required.

Table B.9.1.5-3: Acute risk to birds from drinking water – puddle scenario

Substance	Crop	Application Rate (g/ha)	Endpoint (mg/kg bw)	Ratio AR (g/ha)/endpoint (mg/kg bw)	Trigger value
Metalaxyl-M	Sugar and fodder beet	0.62	LD_{50} = 1180	0.00053	≤ 50

Acute toxicity exposure ratio (TER_A) – Exposure via birds ingesting seeds with/as grit

Acute risk is assessed by comparing the relevant DDD values from above with the appropriate LD50 endpoint to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ \text{(mg/kg bw)}}{\text{DDD}}$$

The resulting TER_A value is given in the table below.

Table B.9.1.5-4: Tier 1 acute risk to birds – consumption of treated seeds with/as grit

Crop	Active substance	Generic focal species	$DGritD_{acute}$ (mg a.s./kg bw)	LD50 (mg a.s./kg bw)	TER_A
Sugar and fodder beet	Metalaxyl-M	Large bird	6.07	1180	194.46

The acute TERs is above the trigger value of 10. An acceptable acute risk to birds from the consumption of treated seed with/as grit had therefore been demonstrated.

Acute toxicity exposure ratio (TER_A) – Exposure via consumption of emerged seedlings

Acute risk is assessed by comparing the relevant DDD values from above with the appropriate LD_{50} endpoint to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ (\text{mg/kg bw})}{\text{DDD}}$$

The resulting TER_A values are given in the table below.

Table B.9.1.5-5: Tier 1 acute risk to birds – consumption of emerged seedlings

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Sugar and fodder beet	Metalaxyl-M	Small omnivorous bird	19.98	1180	59.06
	Metalaxyl-M	Large herbivorous bird	11.99	1180	98.43

The acute TERs for all generic focal species are above the trigger value of 10. Acceptable acute risks to birds from the consumption of emerged seedlings have therefore been demonstrated.

<u>**First tier – Wakil XL**</u>

Acute exposure – Tier 1 assessment treated seeds

The acute Daily Dietary Dose (DDD_{90}) can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

Vining pea seeds are considered as 'large seeds' according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). Consequently, for the Tier I risk assessment a large granivorous bird with a food intake rate (FIR/bw) of 0.1 will be used as relevant indicator species.

Calculation of the DDD for the representative use patterns for A9873C are given in the table below.

Table B.9.1.5-6: Acute exposure estimate for birds following the consumption of treated vining pea seeds

Crop	Active substance	Generic focal species	NAR (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Vining peas	Metalaxyl-M	Large granivorous bird	339.2	0.1	33.92

Acute exposure – Tier 1 assessment emerged seedlings

The acute Daily Dietary Dose (DDD_{90}) as a result of the consumption of emerged seedlings can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR/5}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

For the Tier I risk assessment a small omnivorous and a large herbivorous bird with food intake rates (FIR/bw) of 0.5 and 0.3 respectively will be used as relevant indicator species.

Table B.9.1.5-7: Acute exposure estimate for birds following the consumption of treated vining pea seedlings

Crop	Active substance	Generic focal species	NAR/5 (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Vining peas	Metalaxyl-M	Small omnivorous bird	67.8	0.5	33.92
	Metalaxyl-M	Large herbivorous bird	67.8	0.3	20.35

Exposure for birds through drinking water

There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water.

Leaf scenario

The 'Leaf scenario' is relevant for birds taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation water sufficiently to attract birds. Since the proposed use of metalaxyl-M is as a seed treatment in vining pea the leaf scenario does not apply.

Puddle scenario

This scenario is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all uses of metalaxyl-M and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:

> *"Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg)."*

With a K(f)oc of 78.9, metalaxyl-M belongs to the group of less sorptive substances. The maximum use rate of 76.32 g a.s./ha in vining pea is used. Since the ratio of the application rate to the toxicity endpoint is less than the trigger value, an acceptable acute risk to birds can be concluded for the puddle scenario and no further consideration is required.

Table B.9.1.5-8: Acute risk to birds from drinking water – puddle scenario

Substance	Crop	Application Rate (g/ha)	Endpoint (mg/kg bw)	Ratio AR (g/ha)/endpoint (mg/kg bw)	Trigger value
Metalaxyl-M	Vining pea	76.32	LD_{50} = 1180	0.065	≤ 50

Acute toxicity exposure ratio (TER_A) – Exposure via consumption of treated seeds

Acute risk is assessed by comparing the relevant DDD values from above with the appropriate LD50 endpoint to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ \text{(mg/kg bw)}}{\text{DDD}}$$

The resulting TER_A value is given in the table below.

Table B.9.1.5-9: Tier 1 acute risk to birds – consumption of treated seeds

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Vining pea	Metalaxyl-M	Large granivorous bird	33.92	1180	34.79

The acute TERs is above the trigger value of 10. Acceptable acute risks to birds from the consumption of treated seed have therefore been demonstrated.

Acute toxicity exposure ratio (TER_A) – Exposure via consumption of emerged seedlings

Acute risk is assessed by comparing the relevant DDD values from above with the appropriate LD_{50} endpoint, to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ \text{(mg/kg bw)}}{\text{DDD}}$$

The resulting TER_A values are given in the table below.

Table B.9.1.5-10: Tier 1 acute risk to birds – consumption of emerged seedlings

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Vining pea	Metalaxyl-M	Small omnivorous bird	33.92	1180	34.79
	Metalaxyl-M	Large herbivorous bird	20.35	1180	57.98

The acute TERs for all generic focal species are above the trigger value of 10. Acceptable acute risks to birds from the consumption of emerged seedlings have therefore been demonstrated.

Long-term/reproductive risk assessment

The key metalaxyl-M toxicity endpoint for the long-term/reproductive risk assessment is as follows:

- NOAEL = 24.6 mg a.s./kg bw/d

First tier – Vibrance SB

Long-term/reproductive exposure – Tier 1 assessment birds ingesting seeds with/as grit

The long-term/reproductive Daily Grit Dose ($DGritD_{repro}$) can be calculated for large pelleted seeds using the following equation:

$DGritD_{repro} = 1306 \times (G_{density} / (71 + G_{density})) \times G_{loading}$

Where:

$G_{density}$ Number of seeds on soil surface
$G_{loading}$ Amount of active substance in one seed

In a worst-case conservative assessment, the initial $DGritD_{repro}$ calculation assumes that all seeds are available to birds. Based on a sowing rate of 130000 seeds/ha, this equates to 13 seeds/m^2. It is noted that results from [redacted] (2002) indicate that a much smaller number of sugar beet seeds are likely to be available per m^2. Data from [redacted] (2002) can be factored into a refined exposure assessment, if required.

The $DGritD_{repro}$ is calculated for a large bird on a per bird rather than per kg bw basis. Therefore to convert the $DGritD_{repro}$ into a per kg bw basis, a default body weight of 300 g is assumed for the generic large bird.

Calculation of the DDD for the representative use pattern for A20607B is given in the table below.

Table B.9.1.5-11: Long-term/reproductive exposure estimate for birds following the consumption of seeds with/as grit

Crop	Active substance	Generic focal species	$GI_{loading}$ (mg a.s./seed)	$DGritD_{repro}$ (mg a.s./bird)	$DGritD_{repro}$ (mg a.s./kg bw)
Sugar and fodder beet	Metalaxyl-M	Large granivorous bird	0.0048	0.97	3.23

Long-term/reproductive exposure – Tier 1 assessment emerged seedlings

The long-term/reproductive Daily Dietary Dose (DDD_{50}) as a result of the consumption of emerged seedlings can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR/5}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

For the Tier I risk assessment a small omnivorous and a large herbivorous bird with food intake rates (FIR/bw) of 0.5 and 0.3 respectively will be used as relevant indicator species.

Table B.9.1.5-12: Long-term/reproductive exposure estimate for following the consumption of treated sugar and fodder beet seedlings

Crop	Active substance	Generic focal species	NAR/5 (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Sugar and fodder beet	Metalaxyl-M	Small omnivorous bird	39.96	0.5	19.98
	Metalaxyl-M	Large herbivorous bird	39.96	0.3	11.99

Exposure for birds through drinking water

There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water. As discussed above, the leaf scenario is not relevant for seed treatments, so only the puddle scenario is considered here.

Puddle scenario

This scenario is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all uses of metalaxyl-M and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:

> *"Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg)."*

With a K(f)oc of 78.9, metalaxyl-M belongs to the group of less sorptive substances. The maximum use rate of 0.62 g a.s./ha in sugar and fodder beet is used. Since the ratio of the application rate to the toxicity endpoint is less than the trigger value, an acceptable long-term/reproductive risk to birds can be concluded for the puddle scenario and no further consideration is required.

Table B.9.1.5-13: Long-term/reproductive risk to birds from drinking water – puddle scenario

Substance	Crop	Application Rate (g/ha)	Endpoint (mg/kg bw/day)	Ratio AR (g/ha)/endpoint (mg/kg bw)	Trigger value
Metalaxyl-M	Sugar and fodder beet	0.62	NOAEL = 24.6	0.025	≤ 50

Long-term/reproductive toxicity exposure ration (TERlt) – Exposure via birds ingesting seeds with/as grit

The Tier 1 long-term risk is assessed by comparing the long-term DDD with the NOAEL derived from the toxicity study to give a long-term Toxicity/Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{\text{NOAEL (mg/kg bw/day)}}{\text{DDD (mg/kg bw/day)}}$$

The resulting TER_{LT} value is given in the table below.

Table B.9.1.5-14: Tier I long-term/reproductive risk to birds – consumption of treated seeds with/as grit

Crop	Active substance	Generic focal species	$DGritD_{repro}$ (mg a.s./kg bw/d)	NOAEL (mg a.s./kg bw)	TER_{LT}
Sugar and fodder beet	Metalaxyl-M	Large granivorous bird	3.23	24.6	7.61

The Tier 1 long-term/reproductive TER_{LT} for large birds consuming treated seeds with/as grit is below the trigger of 5. No further consideration of the long-term reproductive risk to birds via consumption of seed with/as grit is required.

Long-term/reproductive toxicity exposure ration (TERlt) – Exposure via consumption of emerged seedlings

The Tier 1 long-term risk is assessed by comparing the long-term DDD with the NOAEL derived from the toxicity study, to give a long-term Toxicity/Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{NOAEL\,(mg/kg\ bw/day)}{DDD\,(mg/kg\ bw/day)}$$

The resulting TER_{LT} values are given in the table below.

Table B.9.1.5-15: Tier 1 long-term/reproductive risk to birds – consumption of emerged seedlings

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	NOAEL (mg a.s./kg bw)	TER_{LT}
Sugar and fodder beet	Metalaxyl-M	Small omnivorous bird	19.98	24.6	**1.23**
	Metalaxyl-M	Large herbivorous bird	11.99	24.6	**2.05**

The Tier 1 long-term/reproductive TER_{LT} for birds consuming treated sugar and fodder beet seedlings is below the trigger of 5 for both generic focal species, indicating unacceptable risks. Further consideration of the long-term/reproductive risk to birds from consumption of treated seedlings is therefore required.

First tier – Wakil XL

Long-term/reproductive exposure – Tier 1 assessment treated seeds

The long-term/reproductive Daily Dietary Dose (DDD_m) can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{FIR}{bw} \text{ X NAR}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

Vining pea seeds are considered as 'large seeds' according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). Consequently, for the

Tier I risk assessment a large granivorous bird with a food intake rate (FIR/bw) of 0.1 will be used as relevant indicator species.

Calculation of the DDD for the representative use patterns for A9873C is given in the table below.

Table B.9.1.5-16: Long-term/reproductive exposure estimate for birds following the consumption of treated vining pea seeds

Crop	Active substance	Generic focal species	NAR (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Vining peas	Metalaxyl-M	Large granivorous bird	339.2	0.1	33.92

Long-term/reproductive exposure – Tier 1 assessment emerged seedlings

The long-term/reproductive Daily Dietary Dose (DDD_{50}) as a result of the consumption of emerged seedlings can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR}/5$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

For the Tier I risk assessment a small omnivorous and a large herbivorous bird with food intake rates (FIR/bw) of 0.5 and 0.3 respectively will be used as relevant indicator species.

Table B.9.1.5-17: Long-term/reproductive exposure estimate for following the consumption of treated vining pea seedlings

Crop	Active substance	Generic focal species	NAR/5 (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Vining peas	Metalaxyl-M	Small omnivorous bird	67.8	0.5	33.92
	Metalaxyl-M	Large herbivorous bird	67.8	0.3	20.35

Exposure for birds through drinking water

There are two scenarios provided in the EFSA Guidance Document for assessing the risk from drinking water. As discussed above, the leaf scenario is not relevant for seed treatments, so only the puddle scenario is considered here.

Puddle scenario

This scenario is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all uses of metalaxyl-M and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:

> *"Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg)."*

With a K(f)oc of 78.9, metalaxyl-M belongs to the group of less sorptive substances. The maximum use rate of 76.32 g a.s./ha in vining pea is used. Since the ratio of the application rate to the toxicity endpoint is less than the trigger value, an acceptable long-term/reproductive risk to birds can be concluded for the puddle scenario and no further consideration is required.

Table B.9.1.5-18: Long-term/reproductive risk to birds from drinking water – puddle scenario

Substance	Crop	Application Rate (g/ha)	Endpoint (mg/kg bw/day)	Ratio AR (g/ha)/endpoint (mg/kg bw)	Trigger value
Metalaxyl-M	Vining pea	76.32	NOAEL = 24.6	3.1	≤ 50

Long-term/reproductive toxicity exposure ration (TERlt) – Exposure via consumption of treated seeds

The Tier 1 long-term risk is assessed by comparing the long-term DDD with the NOAEL derived from the toxicity study, to give a long-term Toxicity/Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{NOAEL\,(mg/kg\ bw/day)}{DDD\,(mg/kg\ bw/day)}$$

The resulting TER_{LT} value is given in the table below.

Table B.9.1.5-19: Tier I long-term/reproductive risk to birds – consumption of treated seeds

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	NOAEL (mg a.s./kg bw)	TER_{LT}
Vining peas	Metalaxyl-M	Large granivorous bird	33.92	24.6	**0.73**

The Tier 1 long-term/reproductive TER_{LT} for large granivorous birds consuming treated vining pea seeds is below the trigger of 5 for metalaxyl-M. Further consideration of the long-term/reproductive risk to birds from consumption of treated seed is therefore required.

Long-term/reproductive toxicity exposure ration (TERlt) – Exposure via consumption of emerged seedlings

The Tier 1 long-term risk is assessed by comparing the long-term DDD with the NOAEL derived from the toxicity study, to give a long-term Toxicity/Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{NOAEL\,(mg/kg\ bw/day)}{DDD\,(mg/kg\ bw/day)}$$

The resulting TER_{LT} value is given in the table below.

Table B.9.1.5-20: Tier 1 long-term/reproductive risk to birds – consumption of emerged seedlings

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	NOAEL (mg a.s./kg bw)	TER_{LT}
Vining peas	Metalaxyl-M	Small omnivorous bird	33.92	24.6	**0.73**
	Metalaxyl-M	Large herbivorous bird	20.35	24.6	**1.21**

The Tier 1 long-term/reproductive TER_{LT} for birds consuming treated vining pea seedlings are below the trigger of 5 for both generic focal species. Further consideration of the long-term/reproductive risk to birds from consumption of treated seedlings is therefore required.

Higher tier risk assessment for the long-term/reproductive risk from treated sugar beet/fodder beet seeds to birds – Vibrance SB

The following areas of the assessment were not demonstrated to result in acceptable long-term/reproductive risks to birds and thus require further consideration:

- Long-term/reproductive risk to birds from exposure to metalaxyl-M via consumption of emerged seedlings;

Long-term/reproductive risk to birds via consumption of emerged seedlings

The first tier assessment of the risk to birds via consumption of seedlings assumes that residues in the seeding are a factor of 5 times lower than residues in the seed (i.e. NAR/5). This is to account for dilution of residues in the growing seedling. The default factor of 5 dilution assumes that the root, seed and seedling are ingested by the animal and that all of the applied substance remains available. This default factor of 5 is assumed for all crops and can be refined in higher tier risk assessment, using crop-specific data or measured residues in seedlings developed from treated seeds.

The applicant has referred to a study by [redacted] **(2015)**, which measured residues of thiamethoxam in sugar beet seedlings in the field. Four trials were conducted and seedlings were sampled by hand, cutting seedlings just above soil surface. While residues of thiamethoxam are not relevant for the current risk assessment, the fresh weight of the sugar beet seedlings were recorded at various times after emergence. By comparing this information with seed weights, dilution factors for sugar beet seedlings can be determined.

It is noted that the default NAR/5 assumes the entire plant is consumed (including root and seed), while the [redacted] (2015) study only weighed the above ground plant parts. Therefore, dilution factors calculated from the [redacted] (2015) data potentially underestimate the dilution in sugar beet plants.

Fresh weight data for seedlings from each site in [redacted] (2015) are summarised in the following table.

Table B.9.1.5-21: Sugar beet single seedling fresh weight data (g) from [redacted] (2015)

Days after emergence	S15-01163-01 (ZP)	S15-01163-04 (ML)	S15-01163-05 (BB)	S15-01163-06 (OH)
0	0.195	0.197	0.227	0.285
1	0.286	0.343	0.377	0.345
2	0.291	-	0.344	0.290
3	0.423	0.443	0.436	0.320
5	0.309	0.545	0.372	0.345
7	0.465	1.033	0.353	0.330
10	1.342	-	0.665	0.490
14	2.644	1.511	2.153	0.695
21	9.757	4.979	7.796	4.375
Geometric mean across days 0-21*	1.299	1.175	1.067	0.655

*Calculated according to Annex 2 of OECD 23

The standard averaging period used for determining exposure in the bird long-term reproductive risk assessment is 21 days. Therefore, determining an average seedling fresh weight over a 21-day period following emergence and comparing this to the seed weight, to calculate a dilution factor, is considered appropriate, and is summarised in the following table.

Table B.9.1.5-22: Calculation of growth dilution factors for sugar beet from [redacted] (2015)

	S15-01163-01 (ZP)	S15-01163-04 (ML)	S15-01163-05 (BB)	S15-01163-06 (OH)
Seed weight	0.0306	0.029	0.029	0.029
Average 0-21 DAE seedling weight	1.299	1.175	1.067	0.655
Dilution factor (seedling weight/see weight)	42.5	40.5	36.8	22.6

Sugar beet single seedling weights were greater than single seed weights by a factor of 22.6-42.5 (mean = 35.6).

The long-term/reproductive risk assessment for birds eating crop seedlings can be updated using the revised dilution factor for sugar beet from [redacted] (2015). Therefore, instead of using the NAR/5, the NAR/35.6 will be used to estimate the residue in the crop seedling over a 21-day period following emergence. As for the Tier I risk assessment a small omnivorous and a large herbivorous bird with food intake rates (FIR/bw) of 0.5 and 0.3 respectively will be used as relevant indicator species.

Table B.9.1.5-23: Refined long-term/reproductive exposure estimate for following the consumption of treated sugar and fodder beet seedlings

Crop	Active substance	Generic focal species	NAR/35.6 (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Sugar and fodder beet	Metalaxyl-M	Small omnivorous bird	5.61	0.5	2.81
	Metalaxyl-M	Large herbivorous bird	5.61	0.3	1.68

Table B.9.1.5-24: Refined long-term/reproductive risk to birds – consumption of emerged seedlings

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	NOAEL (mg a.s./kg bw)	TER_{LT}
Sugar and fodder beet	Metalaxyl-M	Small omnivorous bird	2.81	24.6	8.75
	Metalaxyl-M	Large herbivorous bird	1.68	24.6	14.64

The resulting TER values are all above the trigger value of 5. Acceptable long-term/reproductive risks to birds from consumption of metalaxyl-M via crop seedlings are therefore indicated.

Higher tier risk assessment for the long-term/reproductive risk from treated vining pea seeds to birds – Wakil XL

The following areas of the assessment were not demonstrated to result in acceptable long-term/reproductive risks to birds and thus require further consideration:

- Long-term/reproductive risk to birds from exposure to metalaxyl-M via consumption of seeds as food;
- Long-term/reproductive risk to birds from exposure to metalaxyl-M via consumption of emerged seedlings;

Long-term/reproductive risk to birds from exposure to metalaxyl-M via consumption of seeds as food

The applicant has proposed the following refinements to the risk assessment for granivorous birds:

1. Higher tier chronic avian toxicity endpoint for metalaxyl-M
2. Seed residue dissipation data
3. Selection of relevant focal species based on field study in freshly drilled pea fields
4. Fraction of diet obtained from the treated area (PT)

Higher tier chronic avian toxicity endpoint - metalaxyl-M

The applicant proposed to raise the reproductive endpoint to 84 mg a.s./kg bw/day. The four reproductive studies and the derivation of the toxicity endpoint have been discussed in detail above and as a result, the proposal to use 84 mg a.s./kg bw/day has been rejected. It is considered that the previously agreed NOEC of 24.6 mg a.s./kg bw/d should be retained in the GB risk assessment.

Residue dissipation on treated seeds – metalaxyl-M

i. [redacted] (2012)

In the first tier risk assessment it was assumed that residues of metalaxyl-M on treated seeds remain at initial levels throughout the exposure period for the reproductive risk assessment (i.e. the time-weighted average factor, or TWA, = 1). In order to refine this risk assessment, the rate of dissipation of metalaxyl-M residues on treated seeds can be taken into account, where suitable data are available. In order to consider this further the applicant has proposed using a revised time weighted average factor (TWA) based on the DT50 for residue dissipation of metalaxyl-M on treated seeds from a study conducted by [redacted] **(2012)**. This study investigated the dissipation of residues from A14918E treated maize seeds. A14918E (Maxim Quattro) is a four-way seed treatment that contains 2.65% metalaxyl-M (this formulation also contains the active substances fludioxonil, thiabendazole, azoxystrobin).

The [redacted] (2012) study has been previously considered in the EU renewal review of metalaxyl-M. This study was conducted at 3 sites in the UK, with treated maize seeds placed on the soil surface at a one seed depth (i.e. seeds were placed on the soil surface but were not piled). This was done to be representative of unincorporated or spilled seed. Dissipation of residues of metalaxyl-M (and the other active substances) was investigated over a period of 21 days. Some seeds were left uncovered while others were protected from rain/UV light by a polythene sheet. Metalaxyl-M concentrations on seeds were determined using an analytical method that was validated for the EU renewal review. The results for metalaxyl-M residues on treated maize seeds from this study are summarised in the table, which is reproduced from section B.9.1.9.2 of the EU renewal review RAR for metalaxyl-M.

Table B.9.1.5-25: Dissipation of Metalaxyl-M residues from treated maize seeds under protected or unprotected conditions ([redacted], 2012)

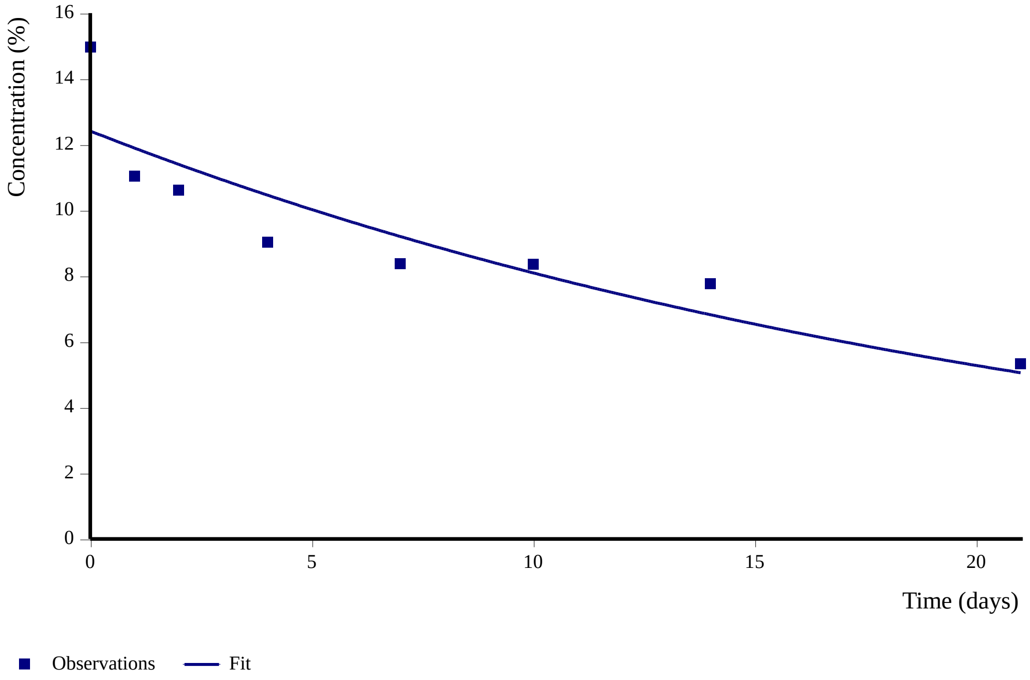
Sampling Interval (days)	Test Site					
	FERA		STC		Buttercrambe	
	Residues mg/kg seed		Residues mg/kg seed		Residues mg/kg seed	
	Protected	Unprotected	Protected	Unprotected	Protected	Unprotected
0DAD[a]	14.96	14.27	15.32	12.47	13.17	17.15
1DAD	11.04	10.33	12.63	6.50	8.44	3.78
2DAD	10.61	9.18	12.48	5.16	8.80	3.64
4DAD	9.03	6.48	9.88	2.52	7.54	1.83
7DAD	8.38	3.86	8.86	1.17	6.41	1.01
10DAD	8.36	2.23	9.17	0.83	6.16	0.90
14DAD	7.77	0.87	7.91	0.49	5.84	1.37
21DAD	5.33	0.69	6.93	0.51	5.35	0.72
	Calculated DT_{50} values (days)					
	16.2	3.7	17.4	1.6	15.6	0.6

[a] taken from opened bag on day of drilling. DAD = days after drilling

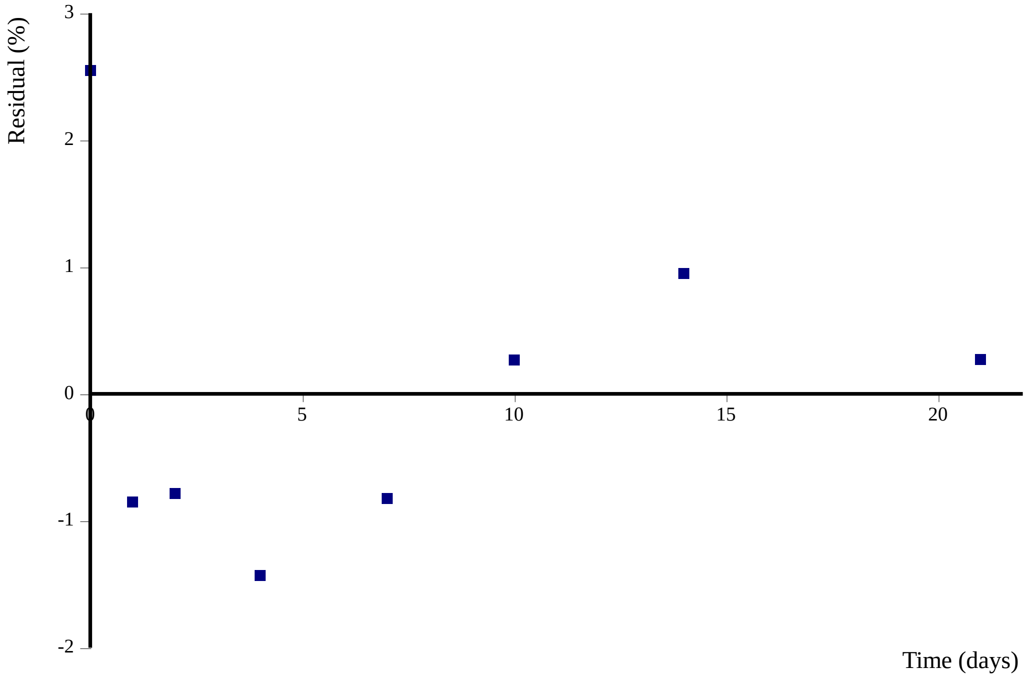
The summary of this study or the risk assessment discussion in the dRAR do not provide details regarding how the DT50 values for metalaxyl-M were derived. Therefore, the kinetics were rerun by HSE assuming first-order dissipation and the DT50 values have been checked using CAKE version 3.2.

ii. Kinetic fitting - FERA protected

Observations and Fitted Model:



Residuals:



Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI

Parent_0	12.41	0.8257	N/A	10.81	14.02	10.39	14.43
k_Parent	0.04275	0.009998	0.002616	0.02332	0.06217	0.01828	0.067

$\square^2$

Parameter	Error %	Degrees of Freedom
All data	10.2	6
Parent	10.2	6

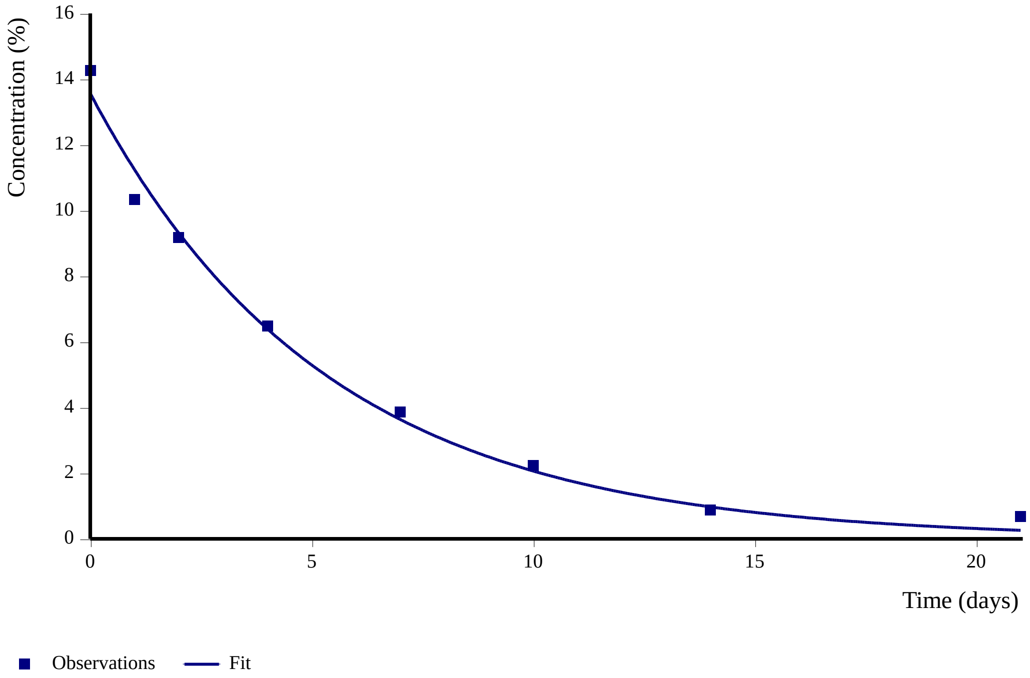
Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	16.2	53.9

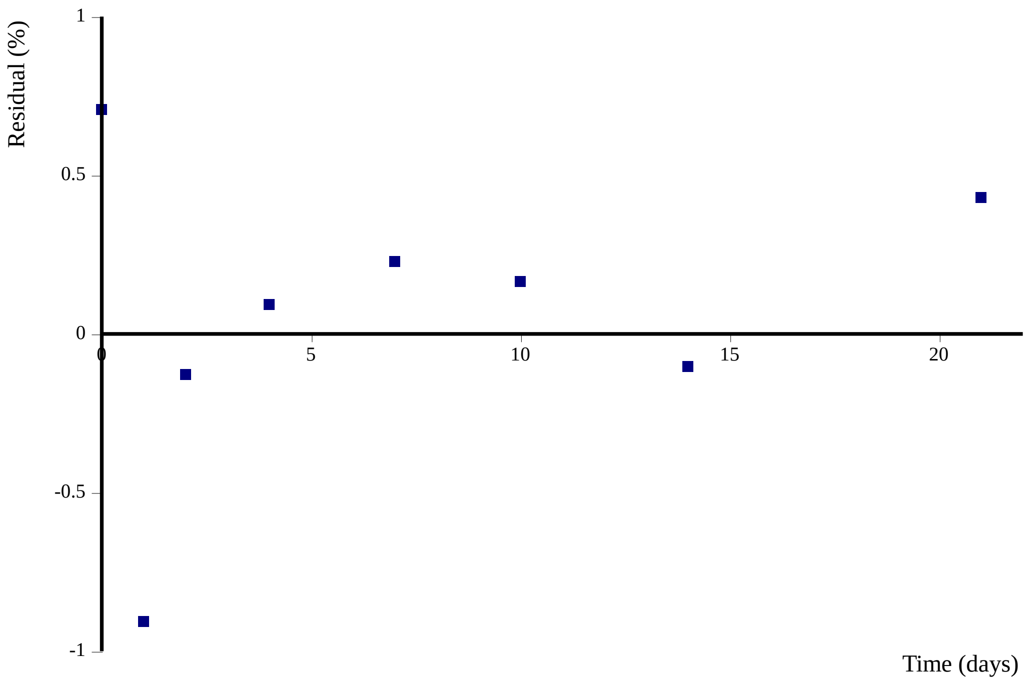
The resulting DT50 of 16.2 days is as reported in the RAR for metalaxyl-M. The Chi-square value is less than 15%, the p (probability) value < 0.05 and the overall fit of the relationship to the underlying data is considered to be reasonable.

iii. Kinetic fitting - FERA unprotected

Observations and Fitted Model:



Residuals:



Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	13.56	0.4064	N/A	12.77	14.35	12.57	14.56
k_Parent	0.1883	0.01303	3.44E-006	0.1629	0.2136	0.1564	0.22

□²

Parameter	Error %	Degrees of Freedom
All data	5.99	6
Parent	5.99	6

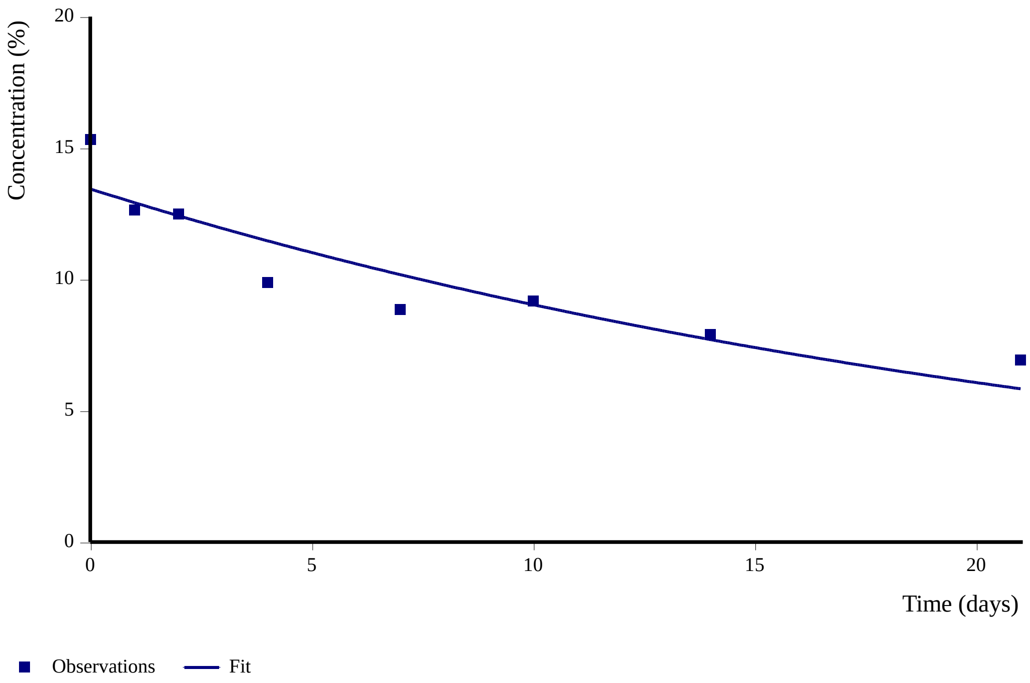
Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	3.68	12.2

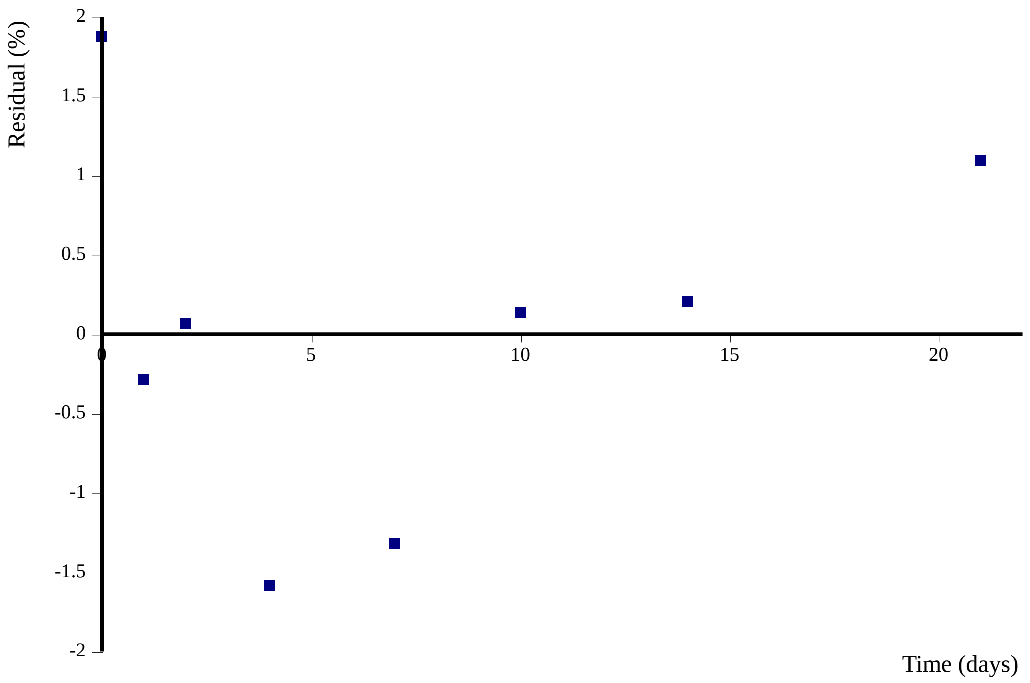
The resulting DT50 of 3.68 days is as reported in the RAR for metalaxyl-M. The Chi-square value is less than 15%, the p (probability) value < 0.05 and the overall fit of the relationship to the underlying data is considered to be good.

iv. Kinetic fitting - STC protected

Observations and Fitted Model:



Residuals:



Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI

Parent_0	13.44	0.7263	N/A	12.03	14.85	11.66	15.22
k_Parent	0.03974	0.007889	0.001182	0.02441	0.05507	0.02043	0.059

□^{2}

Parameter	Error %	Degrees of Freedom
All data	8.19	6
Parent	8.19	6

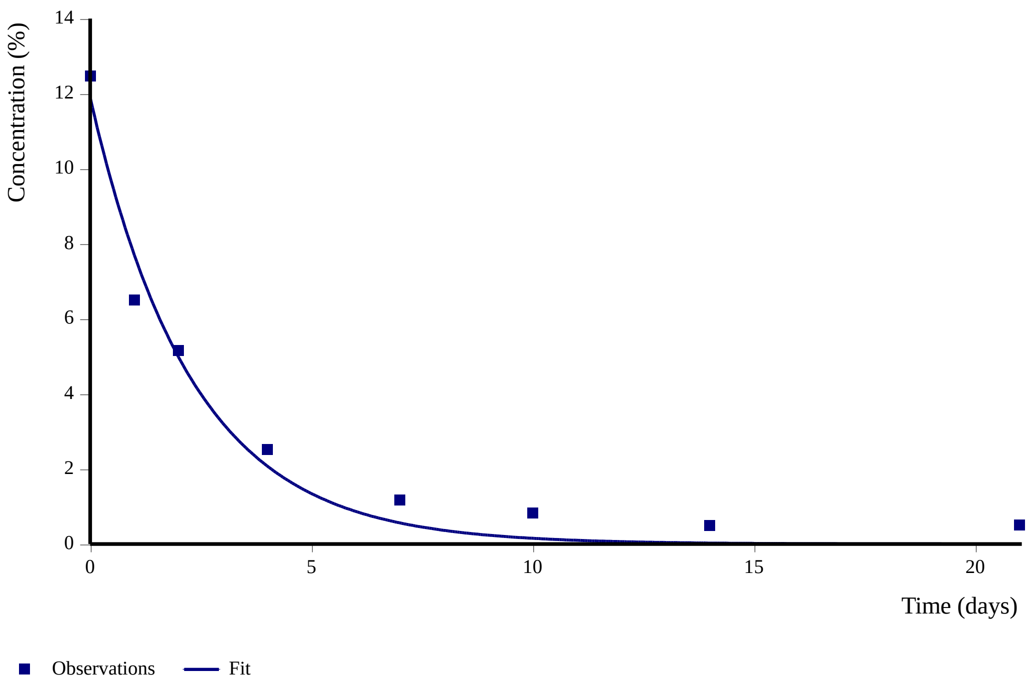
Decay Times:

Compartment	DT50 (days)	DT90 (days)
Parent	17.4	58

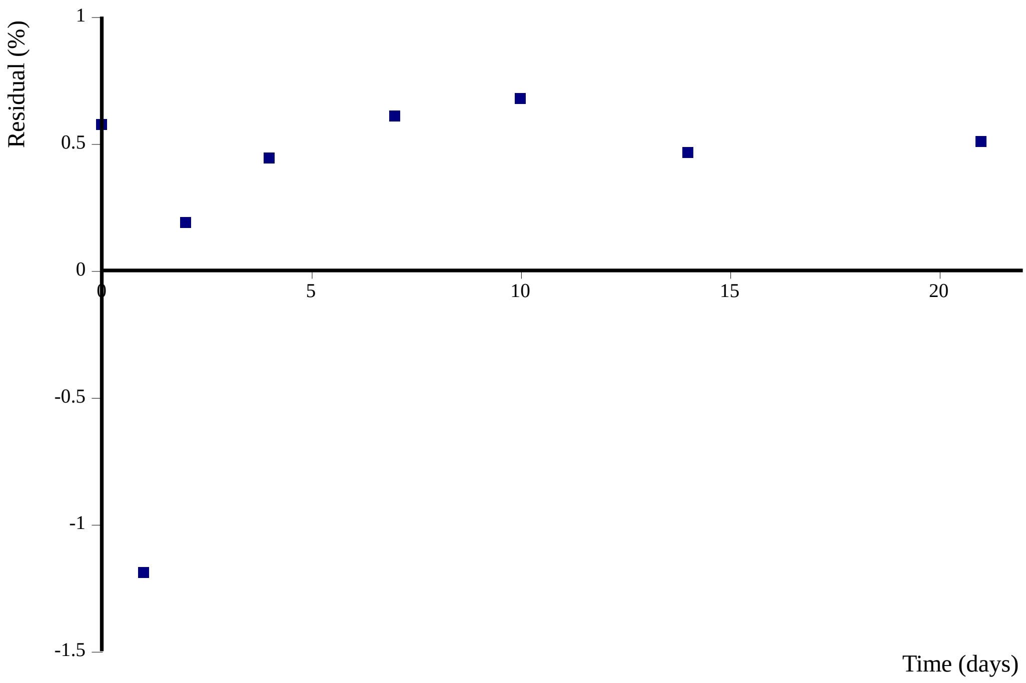
The resulting DT50 of 17.4 days is as reported in the RAR for metalaxyl-M. The Chi-square value is less than 15%, the p (probability) value < 0.05 and the overall of fit of the relationship to the underlying data is considered to be reasonable.

v. Kinetic fitting - STC unprotected

Observations and Fitted Model:



Residuals:



Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	11.89	0.6894	N/A	10.56	13.23	10.21	13.58
k_Parent	0.4362	0.05616	1.20E-004	0.327	0.5453	0.2988	0.574

□^{2}

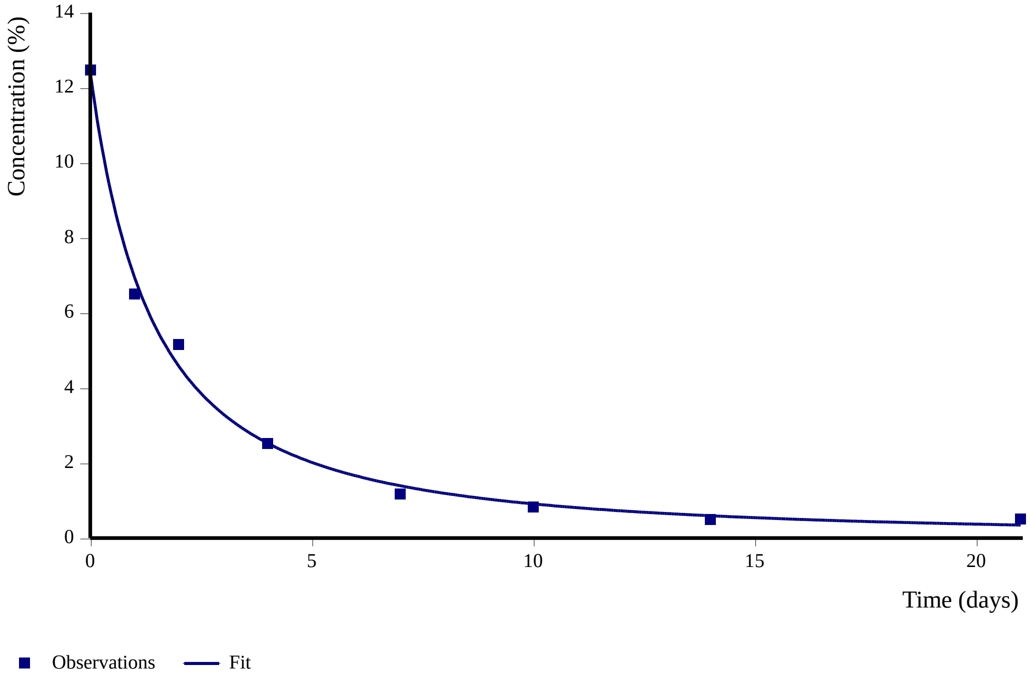
Parameter	Error %	Degrees of Freedom
All data	13.8	6
Parent	13.8	6

Decay Times:

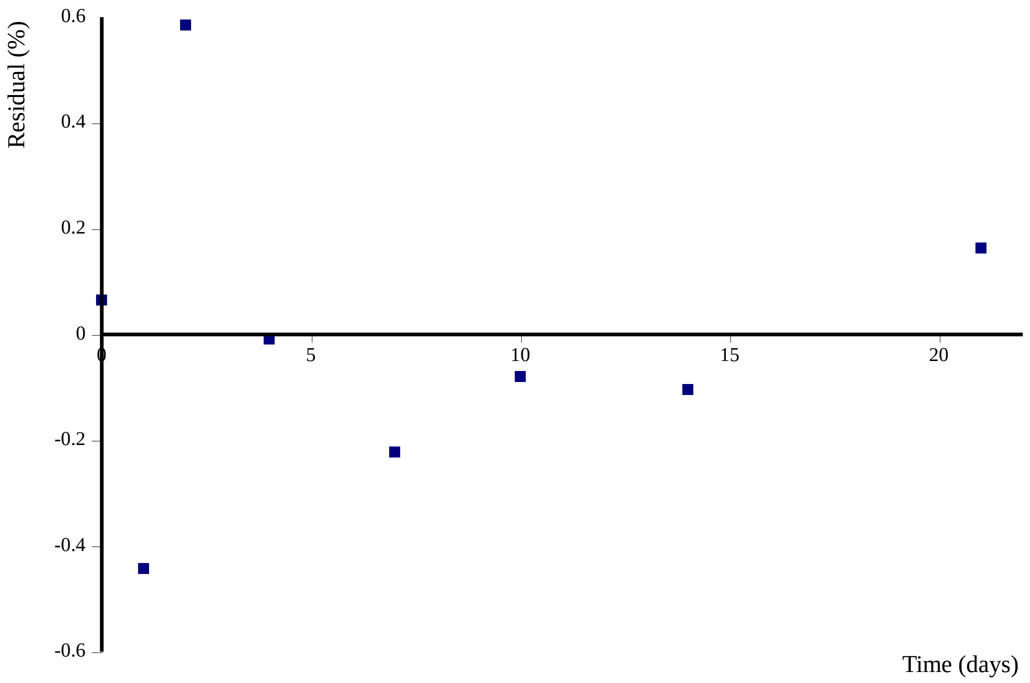
Compartment	DT50 (days)	DT90 (days)
Parent	1.59	5.28

The resulting DT50 of 1.59 days is as reported in the RAR for metalaxyl-M. The Chi-square value is slightly less than 15% and the p (probability) value < 0.05. However, the overall fit of the relationship to the underlying data is considered to be poor with the fitted curve consistently below the data values at most time points. Therefore, this dataset has also been analysed with a FOMC model fitted to the data.

Observations and Fitted Model:



Residuals:



Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	12.41	0.3541	N/A	11.69	13.12	11.49	13.32

alpha	1.494	0.3245	N/A	0.8402	2.148	0.6599	2.328
beta	2.106	0.701	N/A	0.6937	3.519	0.3042	3.908

$\square^{2}$

Parameter	Error %	Degrees of Freedom
All data	6.46	5
Parent	6.46	5

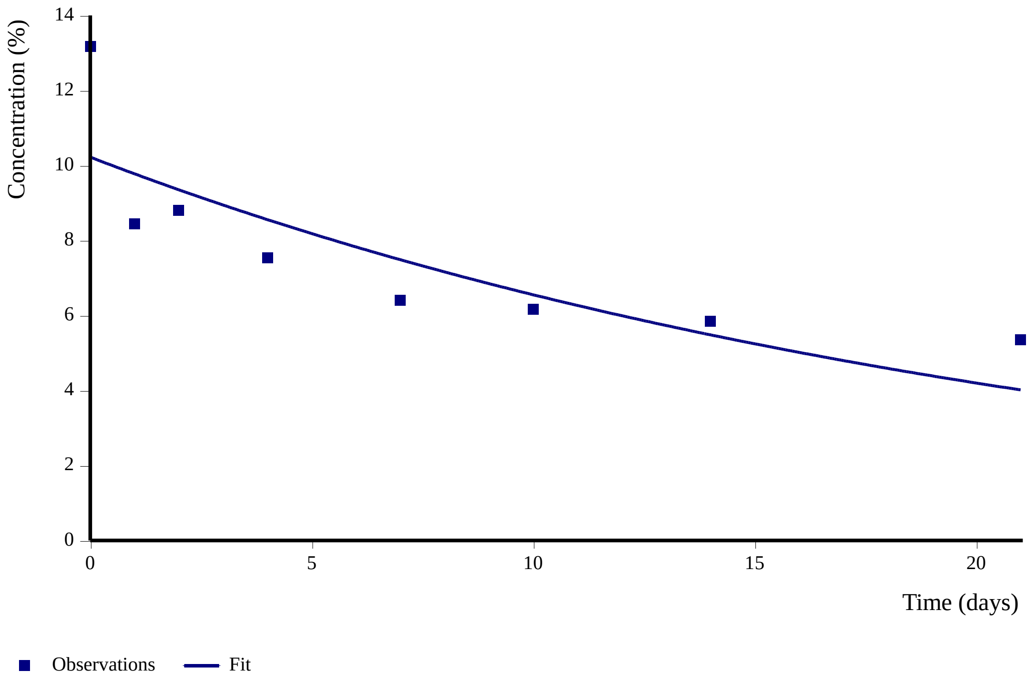
Decay Times:

Compartment	DT50 (days)	DT90 (days)	DT90 / 3.32 (days)
Parent	1.24	7.73	2.33

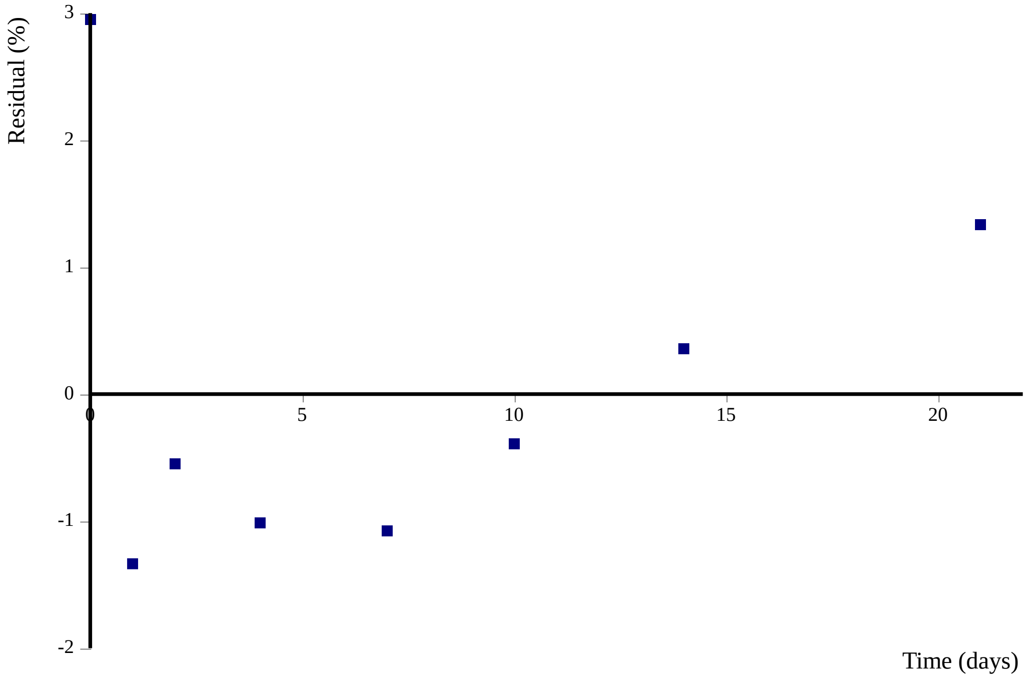
With the FOMC model the Chi-square value is reduced relative to the SFO model and the visual fit is improved, with residuals above and below the line. The error is less than the estimated value for both alpha and beta. Therefore, the FOMC fit is considered to better match the unprotected data from this site. To determine an appropriate DT50 for use in the risk assessment a work around is used, where the DT90 from the FOMC fit (7.73) is divided by a factor of 3.32. The resulting equivalent DT50 of 2.33 d is considered appropriate for this site, noting that this value is higher than that used in the RAR for metalaxyl-M.

vi. Kinetic fitting - Buttercrambe protected

Observations and Fitted Model:



Residuals:



Initial Values for this Step:

Parameter	Initial Value	Bounds	Fixed
Parent_0	100	0 to (unbounded)	No
k_Parent	0.1	0 to (unbounded)	No

Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	10.22	0.9443	N/A	8.385	12.05	7.909	12.53
k_Parent	0.04448	0.01412	0.009906	0.01704	0.07192	0.009929	0.079

□²

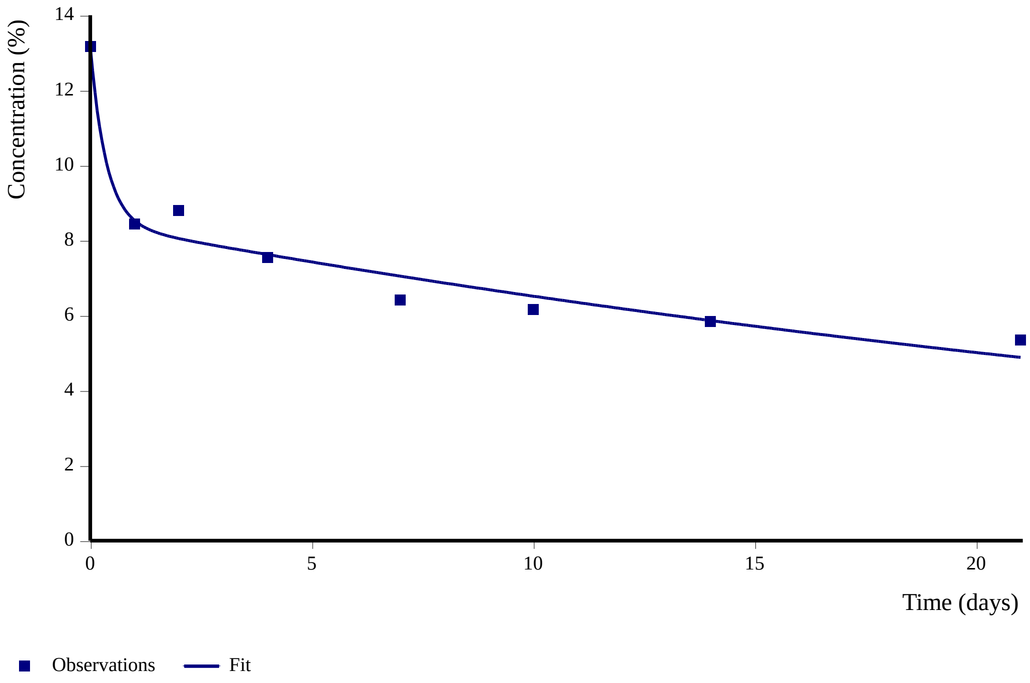
Parameter	Error %	Degrees of Freedom
All data	14.2	6
Parent	14.2	6

Decay Times:

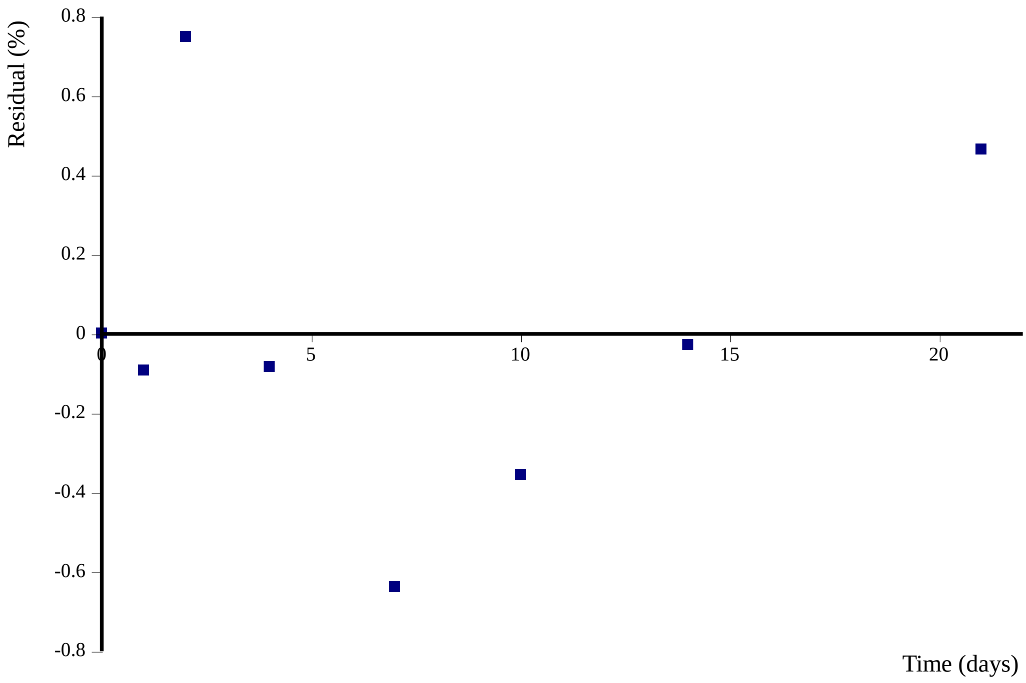
Compartment	DT50 (days)	DT90 (days)
Parent	15.6	51.8

The resulting DT50 of 15.6 days is as reported in the RAR for metalaxyl-M. The p (probability) value < 0.05 and the Chi-square value is slightly less than 15%, but the overall fit of the relationship to the underlying data is considered to be rather poor. Since residues did not decline to 10% of initial values over the study duration a reliable FOMC relationship cannot be fitted to the data. Instead a double first order in parallel (DFOP) model has been considered.

Observations and Fitted Model:



Residuals:



Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	13.17	0.5759	N/A	11.94	14.4	11.57	14.77
k1_Parent	2.803	2.645	0.1745	-2.836	8.443	-4.541	10.15
k2_Parent	0.02619	0.006398	0.007463	0.01255	0.03983	0.00843	0.044
g_Parent	0.3571	0.04933	N/A	0.252	0.4623	0.22	0.4941

Note: Some DFOP parameters have been transformed to enforce convention of k1 as fast rate.

□²

Parameter	Error %	Degrees of Freedom
All data	4.85	4
Parent	4.85	4

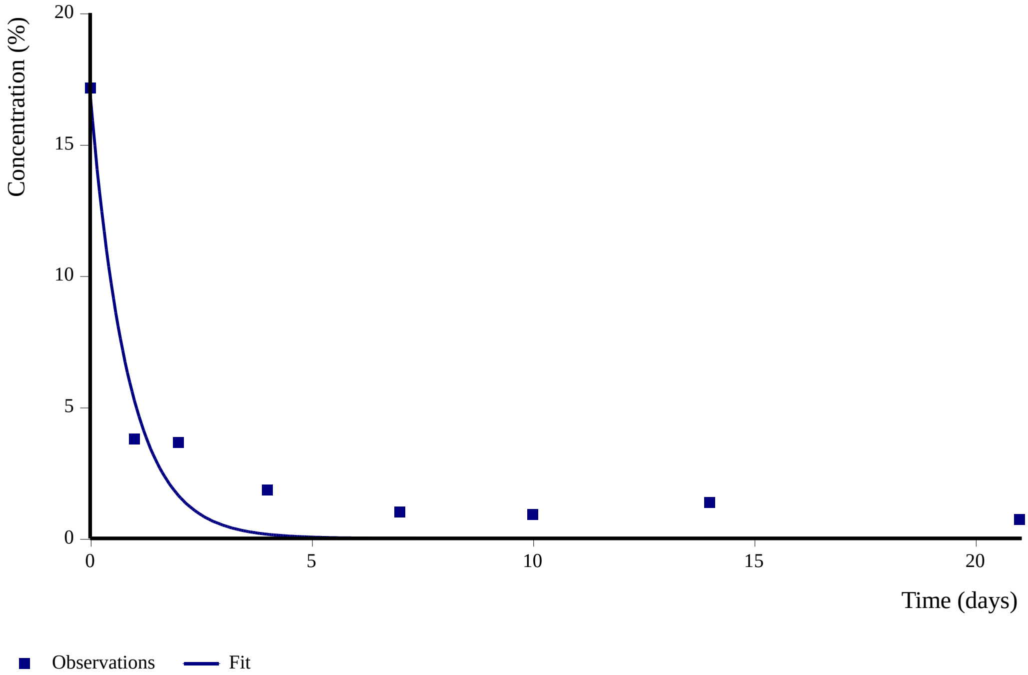
Decay Times:

Compartment	DT50 (overall days)	DT90 (overall days)	k1 DT50 (days)	k2 DT50 (days)
Parent	9.6	71	0.247	26.5

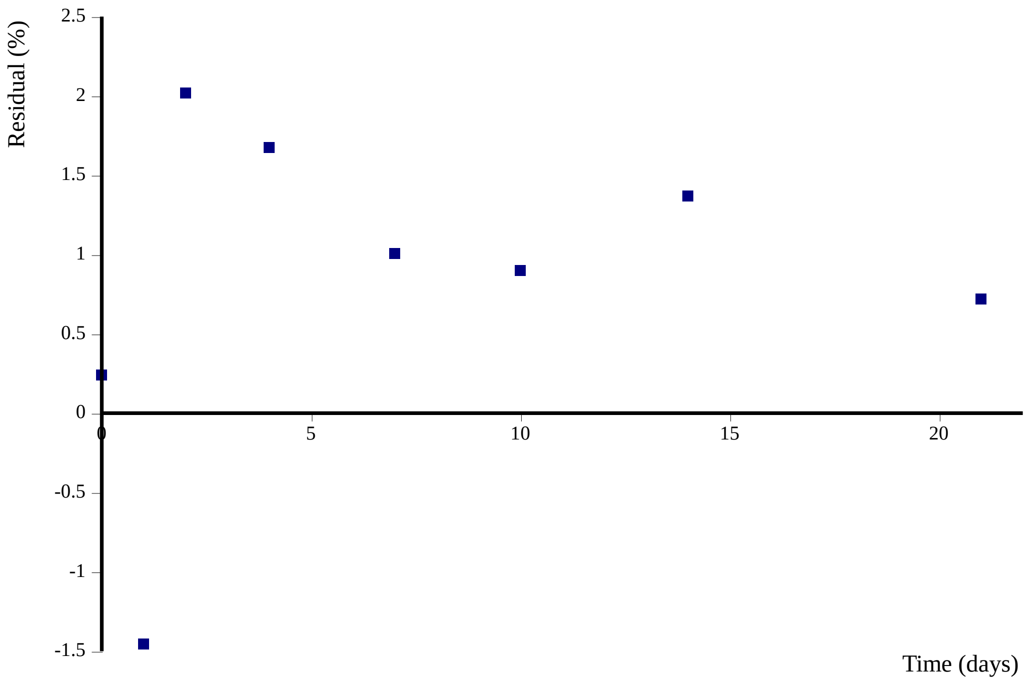
It is noted that the k1 parameter fails the t-test (p >0.05), which is likely to due to the low number of data points for the fast phase. The k2 parameter passes the t-test and the Chi-square is markedly lower than with the SFO fit. The visual fit with the DFOP model to the data is improved relative to the SFO model and residuals are distributed above and below the line. Therefore, the DT50 of 26.5 d from the slow phase of the DFOP model is considered appropriate for risk assessment purposes, noting that this value is higher than that used in the metalaxyl-M RAR.

vii. Kinetic fitting - Buttercrambe unprotected

Observations and Fitted Model:



Residuals:



Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	16.91	1.482	N/A	14.03	19.79	13.28	20.54
k_Parent	1.172	0.2517	0.001738	0.6832	1.661	0.5564	1.788

□²

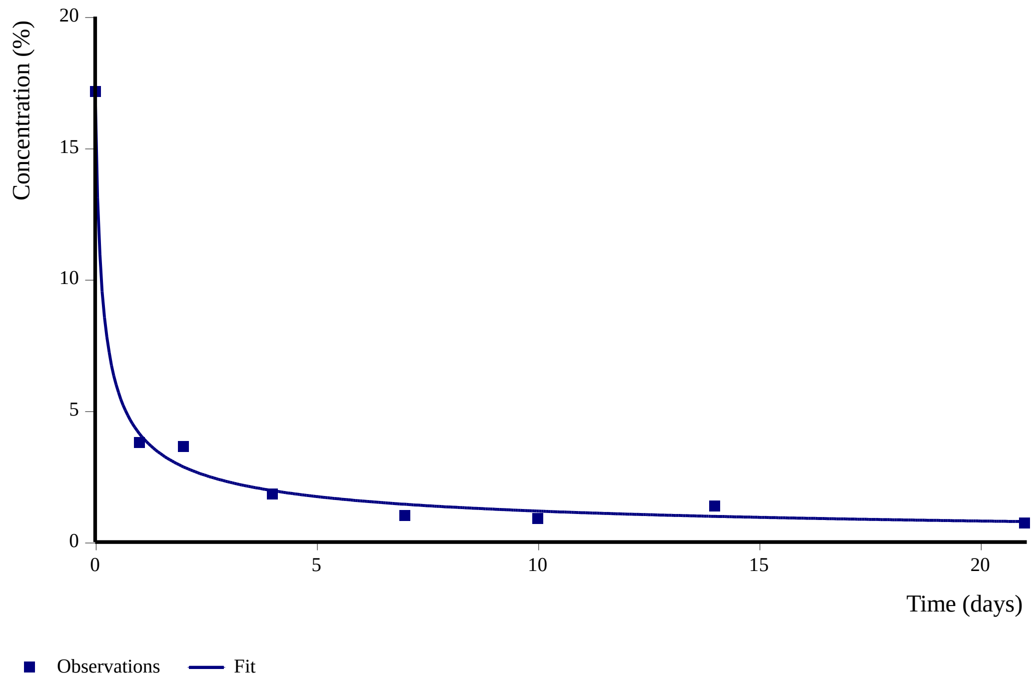
Parameter	Error %	Degrees of Freedom
All data	27	6
Parent	27	6

Decay Times:

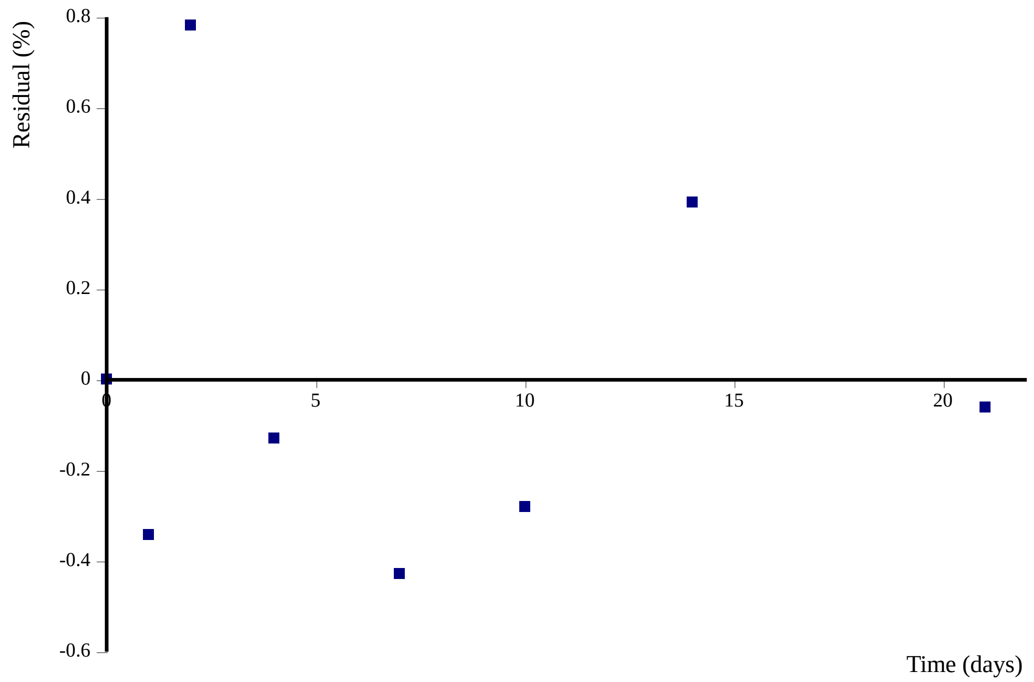
Compartment	DT50 (days)	DT90 (days)
Parent	0.591	1.96

The resulting DT50 of 0.59 days is as reported in the RAR for metalaxyl-M. While the p (probability) value is < 0.05 the Chi-square exceeds 15% and the visual fit of the relationship to the data points is poor, with residuals consistently above the line. Therefore, this dataset has also been analysed with a FOMC model fitted to the data.

Observations and Fitted Model:



Residuals:



Estimated Values:

Parameter	Value	□	Prob. > t	Lower (90%) CI	Upper (90%) CI	Lower (95%) CI	Upper (95%) CI
Parent_0	17.15	0.4827	N/A	16.18	18.12	15.91	18.39

alpha	0.5614	0.1149	N/A	0.33	0.7929	0.2662	0.857
beta	0.0857	0.06085	N/A	-0.0369	0.2083	-0.0707	0.242

$\square^{2}$

Parameter	Error %	Degrees of Freedom
All data	8.54	5
Parent	8.54	5

Decay Times:

Compartment	DT50 (days)	DT90 (days)	DT90 / 3.32 (days)
Parent	0.209	5.09	1.53

With the FOMC model the Chi-square value is reduced relative to the SFO model and below 15%. The visual fit is also improved, with residuals above and below the line. The error is less than the estimated value for both alpha and beta. Therefore, the FOMC fit is considered to better match the unprotected data from this site. To determine an appropriate DT50 for use in the risk assessment a work around is used, where the DT90 from the FOMC fit (5.09) is divided by a factor of 3.32. The resulting equivalent DT50 of 1.53 d is considered appropriate, noting that this value is higher than that used in the dRAR for metalaxyl-M.

Subsequent to the above kinetic evaluation being conducted, the applicant provided their own kinetic evaluation of the data ([redacted], 2021a). It is noted that this report includes analysis of the [redacted] (2012) data using CAKE version 3.4. The outputs from the CAKE modelling by [redacted] (2021a) are identical to the analysis conducted above and the same conclusions were reached regarding goodness of fit and the appropriate fit to consider for each site. Therefore, the [redacted] (2021a) report does not require further consideration.

viii. Residue dissipation DT50s from [redacted] (2012)

The metalaxyl-M residue dissipation DT_{50} values for treated maize seeds derived from the [redacted] (2012) study that are considered reliable for use in risk assessment by HSE are summarised in the following table.

Table B.9.1.5-26: Summary of metalaxyl-M residue dissipation DT50 values on maize seeds ([redacted], 2012)

Site	Covered?	DT_{50} (d)	Model
FERA	Protected	16.2	SFO
	Unprotected	3.68	SFO
STC	Protected	17.4	SFO
	Unprotected	2.33	FOMC (work

			around)
Buttercrambe	Protected	26.5	DFOP (slow phase)
	Unprotected	1.53	FOMC (work around)

The applicant has proposed using only the unprotected data when deriving a TWA for use in the risk assessment, on the basis that these data are more representative of the field exposure situation than the protected data. There is a clear difference in the DT_{50} values obtained between protected (16.2-26.5 d) and unprotected seeds (1.53-3.68 d). Seeds that were protected from rainfall were placed under a membrane (a polythene sheet), which the applicant hypothesises prevented significant transmission of UV light and hence the rate of dissipation via photolysis would be significantly reduced compared to what would be expected for those seeds remaining on the soil surface following drilling of metalaxyl-M treated seeds. In this study the permeability of the covering membrane was determined using a PAR Quantum Sensor. It was determined that 87 % of light within the spectrum 400-700 nm passed through the membrane. However, the applicant proposes that the membrane acted as a UV filter preventing transmission of a significant amount of UV light which had a major impact on photolysis. The study report states that the membrane was further tested by the sponsor using a wider transmission range (a range considered to be more typical of UV radiation). This test concluded that the membrane significantly attenuates the incident radiation in the important 300 - 400 nm region and could therefore not be considered to be UV permeable.

The [redacted] (2012) study report contains detailed information on the weather conditions at the study sites. These data can be analysed to determine the impact of any rainfall events on the DT_{50} values obtained. In this way it can be checked whether the difference between the protected and unprotected study results can be explained by rainfall patterns rather than photolysis. The weather data are considered for each of the three trial sites in the paragraphs below.

At the Fera site there was a total of 23.4 mm rain during the 21-day period over which residues on seeds were evaluated. Rainfall and temperature measurements were taken on site. No rainfall occurred on Day 0, with the first recorded rainfall being on Day 3, though this was small in magnitude (0.6 mm). From the unprotected data there appears to be no sudden fall in residues that coincides with rainfall events when compared with the pattern of decline seen across the whole 21-day monitoring period. Air temperatures during the seed residues evaluation period ranged from 3.67-23.83 °C and soil temperatures ranged from 12.57-17.43 °C.

At the STC site there was a total of 29.8 mm rain during the 21-day period over which residues on seeds were evaluated. Rainfall and temperature measurements were taken on site. There was some rainfall on the day of application, though this was small in magnitude (0.6 mm). From the unprotected data there appears to be no sudden fall in residues that coincides with rainfall events when compared with the pattern of decline seen across the whole 21-day monitoring period. Air temperatures during the seed residues evaluation period ranged from 4.76-23.59 °C and soil temperatures ranged from 10.83-25.69 °C.

Weather data for the Buttercrambe site was taken from the nearby Fera site (within 5 km) for the start of the period over which seed residues were measured, with onsite data available for only the last 6 days of this period. Over the 21-day seed residue evaluation period there was 16.2 mm rainfall, which was less than the other sites, though significant rainfall occurred earlier in the study – rain occurred on Days 1-5, with 4.8 mm on Day 1. At this site there was a rapid decline in residue values for unprotected seeds between Days 0 and 1, which rainfall could have contributed towards. Air temperatures during the seed residues evaluation period ranged from 2.38-23.83 °C and soil temperatures ranged from 12.57-21.59 °C.

Air and soil temperatures across the three sites were in similar ranges. Considering the data on rainfall, while this may have impacted the unprotected DT_{50} for the Buttercrambe site, it does not appear to have had a significant direct influence on residues on seeds at the Fera or STC sites and a direct effect does not explain the differences between the protected and unprotected results. However, rainfall may also contribute to soil moisture and increased soil moisture may lead to increased dissipation of residues from treated seeds. Therefore, while a significant wash off effect of rainfall on seed residues is not apparent at two of the three study sites, rainfall may still have indirectly contributed to increased residue dissipation for unprotected seeds through increasing soil moisture. Such an indirect effect may be expected to be more continuous than the direct wash off effect during a rainfall event and thus would be harder to detect from the pattern of residue decline data. Soil moisture levels in the soil surrounding the placed seeds were not recorded throughout the study, with the only data on soil moisture being available for each site in general and at a single unknown time point (Fera = 5.25% wet basis, STC = 6.58% wet basis, Buttercrambe = 16.12% wet basis).

The hypothesis of the applicant that the membrane used in this study acted as a UV filter, inhibiting photolysis and thus residue dissipation, does not appear to be supported by the RMS assessment of the photolysis of metalaxyl-M in section B.8.1.1.1.2 of the RAR for the EU renewal review of metalaxyl-M. In the Sparrow (1995) soil photodegradation study with artificial light (≥290 nm), half-life times for metalaxyl-M in irradiated and dark samples were comparable, indicating metalaxyl-M is not photodegraded on soil films. Results from additional studies using natural and artificial light ([redacted], 1988a&b) produced contradictory results but ultimately the RMS considered that soil photolysis is not expected to play an important role for the dissipation of the compound. It was however noted that heterogeneous photolytic reactions in the presence of photo sensitizers such as humic acids might occur to some extent under field conditions.

Overall HSE considers that the information available does not adequately support the hypothesis that the large differences between the protected and unprotected seed residue DT_{50}s from the [redacted] (2012) study can be attributed to the protective membrane filtering UV light and thus inhibiting photodegradation of metalaxyl-M. While the unprotected data may be generally more reflective of the expected conditions of use, the appropriate DT_{50} to use in the higher tier risk assessment is considered further below, in light of additional data.

ix. Further consideration of residues using the area under the curve

The applicant has also calculated time-weighted average values using TREC. TREC is a Microsoft Excel©-based tool to employ these kinetic fit parameters to any new exposure scenario including multiple applications, and generates MAF and TWA residues. The Excel worksheet is found online as supplementary information in Ebeling and Hammel (2020[22]). Using TREC the 21-day TWA values for each of the eight trials in [redacted] (2012) were calculated and are presented in the following table.

Table B.9.1.5-27: Time weighted average calculations using residue dissipation trials on maize seeds ([redacted], 2012)

Site	Covered?	21-d TWA
FERA STC	Protected	0.66
	Unprotected	0.68
	Protected	0.77
Buttercrambe	Unprotected	0.25
	Protected	0.14
	Unprotected	0.09
Geometric mean	Protected/unprotected	0.32

x. Additional residues decline data – [redacted] (2021a&b)

In addition to the metalaxyl-M residue decline data with maize seeds, the applicant has also referred to studies investigating dissipation of metalaxyl-M from spinach seeds ([redacted] 2021a&b). Kinetic fitting to determine DT_{50}s from the [redacted] (2021a&b) data is described in [redacted] (2021).

These studies investigated residue decline from treated spinach seeds at four locations in the central zone and 4 in the southern zone. The test item used in these studies was a formulation containing the single active substance metalaxyl-M, rather than 'Wakil XL'. Experimental phases of these studies were conducted in August and September.

While the [redacted] (2021a&b) studies are considered suitable for use in risk assessment and the kinetic fitting in [redacted] (2021) has been checked by HSE, there is uncertainty extrapolating the data to the risk assessment for 'Wakil XL'. This is primarily for the following reasons:

- Spinach seeds were treated in the studies, whereas the proposed use of 'Wakil XL' is on vining peas.
- The application rate of 67.8 g a.s./100 kg seed is higher than in the proposed GAP, which has an application rate of 33.9 g a.s./100 kg seed.
- The formulation tested was not 'Wakil XL' but a formulation containing the single active substance metalaxyl-M.

[22] Ebeling, M., Hammel, K. Evaluating plant residue decline data with KinGUII and TREC: results from case studies involving also non-SFO kinetic models. Environ Sci Eur 32, 116 (2020). https://doi.org/10.1186/s12302-020-00386-7

- Studies were conducted in August-September, whereas the proposed time for use of 'Wakil XL' is from February to mid-April.
- Study sites in [redacted] (2021b) were in Spain and Portugal, where weather conditions differed from that expected in GB.

The above factors could lead to the 'true' rate of residue dissipation from vining pea seeds treated with 'Wakil XL' being either underestimated or overestimated. Acknowledging these uncertainties, the DT_{50} values derived for each site are summarised in the table below.

Table B.9.1.5-28: Summary of residue dissipation data on spinach seeds ([redacted], 2021a&b; [redacted], 2021)

Site	SFO fit acceptable	SFO DT_{50} (d)	Alternative model fit	Alternative model DT_{50}(d)
Poland (SRPL20-046-037FR)	Yes	9.94	NR	NR
Poland (SRPL20-047-037FR)	Yes	8.29	NR	NR
Germany (SRDE20-144-037FR)	Yes	5.08	NR	NR
France (SRFR20-057-037FR)	Yes	5.99	NR	NR
Valencia, Spain (SRES20-444-037FR)	No	NR	DFOP	11.3 (slow phase)
Murcia, Spain (SRES20-445-037FR)	No	NR	DFOP	15.2 (slow phase)
Cento, Portugal (SRPT20-087-037FR)	No	NR	DFOP	>10000 (slow phase)
Alentejo, Portugal (SRPT20-088-037FR)	Yes	6.83	NR	NR

The applicant has also calculated time-weighted average values using TREC. Using TREC the 21-day TWA values for each of the eight trials in [redacted] (2021a&b) were calculated and are presented in the following table.

Table B.9.1.5-29: Time weighted average calculations using residue dissipation trials on spinach seeds ([redacted], 2021)

Site	21-d TWA
Poland (SRPL20-046-037FR)	0.524
Poland (SRPL20-047-037FR)	0.471
Germany (SRDE20-144-037FR)	0.329
France (SRFR20-057-037FR)	0.375*
Valencia, Spain (SRES20-444-037FR)	0.444
Murcia, Spain (SRES20-445-037FR)	0.478
Cento, Portugal (SRPT20-087-037FR)	0.311
Alentejo, Portugal (SRPT20-088-037FR)	0.414
Geometric mean	**0.411**

*Value corrected by HSE

xi. Use of residue dissipation data from [redacted] (2012) and [redacted] (2021a&b) in higher tier risk assessment

In order to consider if and how data from the available residue decline studies can be used in higher tier reproductive risk assessments for birds and mammals, there are a number of factors to take into account. These are briefly discussed below.

- The formulation used in the [redacted] (2012) study was not Wakil XL or the previous representative formulation for metalaxyl-M (Apron XL). The tested formulation was Maxim Quattro, a seed treatment containing metalaxyl-M and the additional active substances fludioxonil, thiabendazole and azoxystrobin. Fludioxonil is also present in Wakil XL but thiabendazole and azoxystrobin are not. In the [redacted] (2021a&b) studies the formulation used was Apron XL (containing only metalaxyl-M) and not Wakil XL. Whether the use of a different formulation or a formulation containing other active substances will impact the rate of dissipation of metalaxyl-M from treated seeds is unknown.
- In the [redacted] (2012) study the amount of metalaxyl-M applied per seed or per kg seed is not stated. In the reporting table for the EU renewal review of metalaxyl-M the applicant states that metalaxyl-M was applied at 1.97 g/100 kg seeds (19.7 mg a.s./kg seed) and initial measured residues in [redacted] (2012) at Day 0 ranged from 12.47-17.15 mg a.s./kg seed. The nominal application rates for Wakil XL on peas seeds is 339.2 mg a.s./kg seed. Therefore, the application rate in the [redacted] (2012) study is much lower than that proposed for the new representative use of metalaxyl-M on peas. In the [redacted] (2021a&b) studies the nominal application rate was 678 mg a.s./kg seed, i.e. above the nominal application rate for vining peas. Whether the magnitude of the initial residue will impact the rate of dissipation observed is uncertain but it is noted that data are available with both higher and lower seed loadings than the proposed GAP.
- [redacted] (2012) assessed residue dissipation on treated maize seeds. The representative use of metalaxyl-M considered here is as a vining pea seed treatment. The applicant has stated that '*although data is not directly available for peas, they are also considered large seeds in the Birds and Mammals guidance and therefore data for maize seeds could be considered relevant for peas*'. It is correct that both pea and maize seeds are considered as being 'large' in the EFSA guidance. However, as well as size of the seed, residue

dissipation could be influenced by the shape and texture of seeds. No information has been provided to consider this point further. In the [redacted] (2021a&b) studies spinach seeds were treated, which are considered small seeds in the EFSA guidance, with no case provided by the applicant to justify read across of data from spinach to pea seeds. Therefore, extrapolating data on residue dissipation from maize and spinach seeds to pea seeds is uncertain and has not been fully justified.

- The period during which dissipation of metalaxyl-M from treated seeds was assessed in [redacted] (2012) was from the 18th April to the 16th June. A HSE efficacy specialist has indicated that vining pea seeds can be drilled from February to mid-April. Therefore, the spring-summer time period assessed in [redacted] (2012) does not correspond to the expected period when vining pea seeds would be drilled in GB. Higher temperatures, longer daylight hours and stronger radiation in summer months could lead to faster dissipation of residues than in colder winter months. Therefore, there is uncertainty in extrapolating the residue decline data from spring-summer to winter-early spring. In the [redacted] (2021a&b) studies data was generated in August and September, therefore there is further uncertainty extrapolating this dataset to the winter-spring period.
- Treated seeds were placed on the surface in the [redacted] (2012) and [redacted] (2021a&b) studies, intended to be representative of unincorporated or spilled seed. The potential for birds and mammals to come into contact with treated seed is considered to be highest when seed is available on the soil surface, either due to being unincorporated or spilled. Therefore, considering residue dissipation from seed on the soil surface is representative of a key exposure scenario.
- Residue dissipation data from treated seed is available in [redacted] (2012) from 3 sites. HSE considers that it would be preferable to have data from 4 or more sites when deriving a DT50 value for use in higher tier risk assessment. It is also noted that 3 sites were geographically close together and that in terms of being representative of GB field conditions in general, it would have been preferable had sites been further apart. Additional data are available from the [redacted] (2021a&b) studies, with each study including 4 sites, though it is noted that none of the sites were in GB and the relevance of the data from southern Europe in [redacted] (2021b) for GB is questionable.
- The number and spacing of the time points when residues of metalaxyl-M on seeds were assessed is considered sufficient and appropriate for deriving robust DT50 estimates in the [redacted] (2012) and [redacted] (2021a&b) studies.
- The [redacted] (2012) study included both treated seed that was protected from rainfall etc. by a polythene sheet and treated seed that was not. There is a clear difference in the DT50 values obtained between protected (16.2-26.5 d) and unprotected seeds (1.53-3.68 d). As discussed above, it remains unclear to what extent this difference in DT50 values is due to exposure of unprotected seeds to rainfall/increased local soil moisture and to what extent it is due to inhibition of photolysis for covered seeds. It can be argued that the unprotected data are more representative of the field situation, where seeds will be exposed to the elements. However, rainfall did occur early in the period over which residues were assessed at two of the study sites and it may be that

the DT50 values obtained from these sites would be lower than DT50 values for field sites where rainfall does not occur soon after sowing.

Taking into account the above points there is considerable uncertainty regarding the extrapolation of seed residue dissipation data from the [redacted] (2012) or [redacted] (2021a&b) studies to the representative use of metalaxyl-M on vining peas.

In the EU renewal review of metalaxyl-M a mean DT_{50} of 5 days was derived from the [redacted] (2012) study, considering both the unprotected and unprotected data, but given the uncertainties with the use of this study MS experts agreed that these data could only be used in a qualitative manner to support a default DT_{50} of 10 days, taken from the EFSA guidance on bird and mammal risk assessment (noting this default value is for spray applications). In light of the revised DT_{50} values considered appropriate by HSE for some sites, the revised geometric mean DT_{50} value using the unprotected and protected values from the [redacted] (2012) study would be 6.79 days, with an arithmetic mean of 11.3 days. However, given the large differences between the protected and unprotected DT50 values, HSE does not support merging these disparate datasets.

The [redacted] (2021a&b) studies expand the data set available on the dissipation of metalaxyl-M for treated seeds, though many of the same uncertainties as highlighted for [redacted] (2012) apply when extrapolating the dataset to vining peas. The sites in Poland, Germany and France are considered more representative of UK conditions, with DT_{50} values ranging from 5.08 to 9.94 days. At southern European sites the data were not observed to follow single first order decline, with slow phase DFOP fit DT50s of 11.3 and 15.2 days. At the Cento, Portugal site residues plateaued in the latter part of the study, meaning a slow phase DT50 could not be determined.

The applicant has acknowledged many of the above issues with the available residue decline dataset in their submission and has proposed that the seed dissipation studies in maize and spinach indicate that the default DT_{50} of 10 days from the birds and mammal guidance is appropriate for the metalaxyl-M risk assessment in seeds as a refinement, albeit a potentially conservative one. Taking into account a 21-day averaging period, the applicant has proposed use of a TWA of 0.53 in the risk assessment, based on a 10 day DT_{50}. This TWA can be compared to 21-day TWA values calculated using the experimental data and TREC tool. From the [redacted] (2012) data 21-day TWA values ranged from 0.09-0.77, with the proposed 0.53 value being towards the upper end of this range, above the mean of 0.32. The 21-day TWA values derived from the [redacted] (2021a&b) data were all below 0.53. Therefore, the proposed 0.53 value does appear broadly representative of a reasonable worst-case 21-d TWA for the available datasets.

In light of the above consideration, HSE considers that a precise metalaxyl-M DT_{50} cannot be defined for vining peas but agrees with the applicant's proposal to assume a DT_{50} value of 10 days in the higher tier risk assessment for treated pea seeds.

Appropriate averaging period

In risk assessments for spray application products, the EFSA guidance document specifies use of a standard 21-day averaging period in the long-term/reproductive risk assessment (assuming long-term effects are not a result of short-term exposure). It is less clear what the appropriate averaging period is for seed treatments. The applicant has assumed a 21-day averaging period in their assessment for metalaxyl-M. The following points are noted:

- Taking a 21-day time window assumes that any reproductive effects are the result of long-term exposure and hence this could underestimate the risk if the effect is a result of short-term exposure.
- There is no information regarding the time to effects in the avian toxicity data.
- The seed is unlikely to be available for 21 days as it may germinate within this time, hence taking a 21 day time window could overestimate the exposure. However, it is feasible that the a.s. may also be found in the seedling and that both, i.e. the seed and the seedling, could be consumed together by the bird (or mammal).
- The field may increase in attractiveness during the 21-day time window due to increased crop cover, conversely, the field may decrease in attractiveness due to the removal, via consumption or germination of seeds.

It is noted that in the EU renewal review of metalaxyl-M a 21-day averaging window was used to determine the TWA factor. This resulted in a TWA of 0.53 (using a DT_{50} of 10 days). This approach is retained by HSE for the current risk assessment but the appropriateness of this averaging period will be considered further when discussing the uncertainties in the risk assessment.

<u>Selection of relevant focal species</u>

The applicant has proposed the wood pigeon as an appropriate focal species for the consumption of treated seed scenario, based on data from the [redacted] **et al. (2006)** study. In this study bird communities were observed on 22 pea fields in Northern France. While Northern France generally has a similar climate to GB, detailed weather conditions were not recorded in the study, so a full consideration of the representativeness of the weather conditions in the study is not possible.

This study was conducted from April to June, while the expected sowing dates for vining peas in GB are February to mid-April. Therefore, only the initial phase of the study overlaps with the expected drilling period.

Data on the presence and abundance of bird species was generated for three time periods, corresponding to growth stages BBCH 00-19, 30-39 and 60-79. Therefore, for the consumption of treated seed scenario (i.e. pre-emergence), only the first of these periods is relevant and even then for some of this period seeds will have germinated and become seedlings.

The [redacted] et al. (2006) study was well-conducted and analysed potential focal species on the basis of frequency of occurrence and abundance, amongst other parameters. During the relevant BBCH 00-19 period, the only bird species observed with a frequency of occurrence per survey above 10% were the barn swallow, skylark and woodpigeon. Of these species barn swallow are not expected to forage at ground level and are insectivorous, hence the barn swallow is not a relevant focal species. The skylark and woodpigeon are both known to consume some seeds in their diets, and as a result, are considered potential focal species. It is noted that the chaffinch was observed in 10% of surveys and is an omnivorous bird that can consume seeds.

During the relevant BBCH 00-19 period, the most dominant species observed was the woodpigeon (51.1%). The next most dominant species were the barn swallow (15.2%) and herring gull (7.6%), though neither of these species are expected to feed on seed to a significant extent. Of the other species observed that do eat seeds, skylark was the most dominant (4.3%).

Based on the results from the [redacted] et al. (2006) study, HSE agrees that the wood pigeon is an appropriate focal species for the higher tier risk assessment for birds consuming treated seed. The woodpigeon was the most frequently observed species and the most dominant during the relevant period. Woodpigeon are herbivorous and could be eating pea seeds and/or pea seedlings during the observation period. Therefore, the wood pigeon is considered a suitable focal species for both the consumption of treated seeds and consumption of seedlings scenarios.

Wood pigeons are known to breed in GB from February to early September[23]. It is expected that vining pea seeds would be drilled in GB from February to mid-April. Therefore, there is some overlap of breeding periods for this species with drilling dates, and it cannot be excluded that woodpigeons would be exposed to metalaxyl-M via consumption of treated seed or seedlings during breeding phases.

While woodpigeon was the most frequently observed and dominant bird species in [redacted] et al. (2006), given the relatively large body size of this species, it needs to be considered whether a smaller bird species that can consume seeds should also be considered in the higher tier risk assessment. Of such species, skylark had the highest frequency of occurrence and dominance in pea fields in the study. Chaffinch was also observed in 10% of surveys during the BBCH 00-19 period.

Skylark are known to breed in GB from April to early August and chaffinch from April to July. It is expected that vining pea seeds would be drilled in GB from February to mid-April. Therefore, there is limited direct overlap between the drilling period and breeding phases for these species, though it is noted that treated seed could remain on the soil surface for a short time after drilling. It is also noted that vining pea seeds are in the size range 190-240 mg/seed (based on HSE efficacy advice) and HSE consider that skylarks would be very unlikely to consume seeds of this size, since their preference is for smaller seeds. It is therefore probable that skylark observed at BBCH 00-19 in the [redacted] et al. (2006) study were foraging on crop seedlings or food items other than treated pea seeds. Taking these points into account, HSE considers that an additional, smaller focal species is not required for the consumption

[23] Literature review of bird and mammal breeding phenologies and the factors affecting them (PS2364). https://randd.defra.gov.uk/ProjectDetails?ProjectId=17615

of treated seeds scenario. However, for the consumption of seedlings scenario, the skylark is a relevant focal species.

- Consumption of treated seed focal species – Woodpigeon
- Consumption of crop seedlings focal species – Skylark and woodpigeon

Refinement of fraction of diet obtained in treated area (PT)

No radio tracking data are available for wood pigeon in freshly drilled vining pea fields. The applicant has instead proposed extrapolating from data derived for freshly drilled maize fields, from studies by [redacted] & [redacted] (2018) and [redacted] (2010). The relevance of each study for the higher tier risk assessment is considered below.

[redacted] & [redacted] (2018):

This study was conducted in north-west Germany, rather than GB. However, the study locations selected are considered broadly representative of GB maize growing areas. The applicant has proposed that as maize is also considered a large seed in EFSA/2009/1438, the use of data obtained in freshly drilled maize fields is also appropriate for vining peas. Additionally, they have proposed that this extrapolation is further supported by the fact that drilling rate and general habitat structure are similar for freshly drilled maize and pea fields. While there is still an element of uncertainty in extrapolating data between crops, HSE agrees with the proposed extrapolation.

It is noted that radio tracking took place between May and July in this study, whereas the drilling period for the proposed use on vining peas is from February to mid-April. The differences in timing will lead to differences in weather conditions between the study and proposed use, with the study conducted during a period of time where typically the temperature is warmer and with less rainfall. While the habitat structure within drilled fields is not expected to significantly differ between crops pre-emergence, the nature of surrounding habitats could differ between timings. The growth stage and available food items in surrounding habitats are likely to impact the proportion of time spent foraging in freshly drilled pea fields. This extrapolation in timing therefore introduces further uncertainty in the use of this study data in the higher tier risk assessment, though it cannot be determined from the available information in which direction this uncertainty would act (i.e. whether it would make it more likely the risk is underestimated or overestimated). It is possible that the PT values derived in this study could underestimate the ‘true’ PT value for the period of use, since in late winter/early spring the availability of alternative food is expected to be lower.

[redacted] (2010):

This study was conducted in southern France in pre-emergence and early post-emergence (BBCH 10-16) maize fields. Sufficient weather data are not available to compare conditions in the study to expected GB conditions during the anticipated drilling period for vining peas. In this study radio-tracking activities were conducted between April and June, i.e. later than the expected drilling time for vining peas. As with the [redacted] & [redacted] (2018) study, there is uncertainty extrapolating radio tracking results from this study to the risk assessment scenario, given that drilled fields were of maize and not peas, and given differences in the timing of observation and timing of drilling for vining peas. Additionally, there is further uncertainty associated with extrapolation of data from southern France to GB.

Study results:

In both the [redacted] & [redacted] (2018) and [redacted] (2010) studies the number of individual consumer birds tracked in pre-emergence maize fields was relatively low. To address this point the applicant has proposed merging the two datasets together and then deriving a 90th percentile PT for the consumer population (0.186). HSE has reservations over this approach, given that one study was conducted in southern Europe and the other in central Europe. Due to the study location, the [redacted] & [redacted] (2018) studies is considered the more relevant for this GB risk assessment. The worst-case PT from this study for consumer population wood pigeon in pre-emergence maize fields was 0.17 (n = 9). The [redacted] (2010) study tracked fewer consumer wood pigeons (n =4), with individual PT values ranging from 0.02-0.35. Overall, HSE considers it appropriate to use the worst-case consumer individual PT for wood pigeon of 0.17 from the [redacted] & [redacted] (2018) study in the higher tier risk assessment for wood pigeon for the consumption of treated seed scenario. However, the uncertainties associated with the relatively small dataset and extrapolation between crops and times of year will need to be factored into the risk assessment.

- Wood pigeon PT in freshly drilled fields = 0.17 (consumer population)

<u>Food intake rate relative to bodyweight (FIR/bw)</u>

The default Tier 1 FIR/bw for the generic focal species can be refined for the focal species woodpigeon. [redacted] et al. (1998) reports a bodyweight of 490 g (range 284-614 g) for wood pigeon.

The daily energy requirement has been determined using the equations presented in Appendix G of the guidance document (EFSA, 2009) and the bodyweight of the woodpigeon (490 g). The relationship between body weight (bw in g) and daily energy expenditure (DEE, in kJ) can be described by the equation: logDEE = log a + b × log bw, using the relevant constants for the species group (non-passerine birds) from Appendix G of EFSA (2009). The energy expenditure of the woodpigeon with a 490 g bw, results in a DEE of 435.3 kJ/day. The amount of vining pea seeds that the woodpigeon needs to consume to meets its daily energy demands and hence the FIR/bw can then be determined.

According to Appendix G (EFSA, 2009), the food intake rate (FIR) is calculated with following equation:

$$FIR = \frac{DEE}{FE\ x\left(1-\frac{MC}{100}\right)x\left(\frac{AE}{100}\right)}$$ (g fresh weight/day)

Where:

DEE Daily Energy Expenditure of the indicator species (kJ/day)
FE Food energy (kJ/dry g)
MC Moisture content (%)
AE Assimilation efficiency (%)

In order to calculate the FIR/bw it is necessary to know the food energy content, food moisture content and the assimilation efficiency of the food item by mammals. Specific values for these parameters are not given for vining seeds in Appendix G of EFSA (2009). Standard values are however stated for weed seeds and cereal seeds, with extrapolation from these values to vining peas considered potentially relevant. The food energy content, food moisture content and assimilation efficiency values for cereal and weed seeds from Appendix G of EFSA (2009) are summarised in the following table.

Table B.9.1.5-30: Summary of food energy parameters

Parameter	Cereal seed (EFSA, 2009)	Weed seed (EFSA, 2009)
Energy content (kJ/g dry)	18.4	21.7
Moisture content (%)	14.7	9.9
Assimilation efficiency	0.76	0.76

Using the default cereal seed inputs and an assumed 100% seed diet, the resulting FIR/bw for the woodpigeon is 0.074. Using the default weed seed inputs and 100% seed diet, the resulting FIR/bw is 0.06. In the absence of specific energy content or moisture content data for vining pea seeds, the more worst-case FIR/bw value of **0.074** will be used in the higher tier risk assessment.

<u>Revised TER calculation for long-term/reproductive risk to birds from consumption of seed treated with metalaxyl-M</u>

The long-term/reproductive TER has been recalculated below using the agreed refinements to focal species (woodpigeon), residue decline (TWA = 0.53), dietary intake (FIR/bw = 0.074) and fraction of diet obtained from the treated area (PT = 0.17), as discussed above.

Table B.9.1.5-31: Refined risk assessment for woodpigeon from consumption of seed treated with metalaxyl-M

Intended use	Focal species	FIR/bw	NAR (mg a.s./kg	TWA	PT	DDD (mg a.s./kg	NOAEL (mg a.s./kg	TER_{LT}

			)			**bw/day)**	**bw/day)**	
Vining pea	Woodpigeon	0.074	339.2	0.53	0.17	2.26	24.6	10.9

The resulting TER value is above the first tier trigger value of 5 by approximately a factor of 2. This potentially indicates that there will be no unacceptable long-term/reproductive risk to birds from consumption of treated seed, however, it is necessary to consider the uncertainties in the risk assessment.

Consideration of uncertainties in the risk assessment

In order to determine whether it has been clearly established that there will be no unacceptable long-term/reproductive risk on birds from the use of metalaxyl-M in the representative product ‘Wakil XL’ on vining pea seeds, it is necessary to consider the main sources of uncertainty in the risk assessment. This is summarised in the table below.

The uncertainties have been considered in the context of the ‘surrogate’ protection goal of ‘making any reproductive effects unlikely’, as specified in section 3 of EFSA (2009).

The +/- symbols indicate whether each source of uncertainty has the potential to make the true risk experienced by a realistic worst case individual higher (+) or lower (-) than the indicated outcome. The number of symbols provides a subjective relative evaluation of the magnitude of the effect.

Table B.9.1.5-32: Evaluation of the uncertainties in the refined long-term/reproductive risk assessment for birds from consumption of vining pea seeds treated with metalaxyl-M

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
Toxicity endpoint – 24.6 mg a.s./kg bw/d	-	Four reproduction studies submitted; lowest endpoint used in the risk assessment. Uncertainty regarding robustness and sensitivity of the studies submitted, 'real' endpoint could be higher, hence risk lower.	+	Four reproduction studies submitted; lowest endpoint used in the risk assessment. Uncertainty regarding robustness and sensitivity of the studies submitted, 'real' endpoint could be lower, hence risk higher.
Choice of focal species (effect on exposure) – wood pigeon	0	Selection is based on relevant and reliable data from pea fields during the freshly drilled/early post-emergence period. Considerably higher frequency of occurrence and dominance than other birds support the wood pigeon selection.	0	Selection of wood pigeon as an appropriate focal species is based on relevant and reliable data from pea fields during the freshly drilled/early post-emergence period. No smaller granivore is included but such species were present very infrequently in the relevant study. Pea seeds are also likely to be too large for many smaller granivores to consume.
Bodyweight – 490 g	0/-	[redacted] et al. (1998) reports a bodyweight of 490 g (range 284-614 g). Therefore some individuals will have greater bodyweights and could experience lesser exposure relative to bodyweight. However, the magnitude of impact this would have on the exposure estimate is relatively small.	0/+	[redacted] et al. (1998) reports a bodyweight of 490 g (range 284-614 g). Therefore some individuals will have smaller bodyweights and could experience more exposure relative to bodyweight. However, the magnitude of impact this would have on the exposure estimate is relatively small.

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
Food intake rate calculation – energy and moisture content	-/0	Specific energy and moisture contents for vining pea seeds have not been used. Extrapolated values have been used for cereal seeds. It is uncertain whether these values represent worst case values or truly realistic values, i.e. the ‘real’ values for vining peas could be higher or lower than those used, hence this could increase or decrease the risk.	+/0	Specific energy and moisture contents for vining pea seeds have not been used. Extrapolated values have been used for cereal seeds. It is uncertain whether these values represent worst case values or truly realistic values, i.e. the ‘real’ values for vining peas could be higher or lower than those used, hence this could increase or decrease the risk.
Loading rate on seed – 339.2 mg a.s./kg	-/0	Nominal loading rate used. The actual rate per seed could be variable.	+/0	Nominal loading rate used. The actual rate per seed could be variable.
Dissipation and degradation of active substance from seeds – DT50 of 10 days	-/0	The DT50 value is representative of metalaxyl-M residue decline data averaged across unprotected and protected sites from [redacted] (2012) and is also representative of data from [redacted] (2021a&b). There is uncertainty due to extrapolating between seeds, products and timings. It is feasible that this value will vary depending on soil and climatic conditions. The ‘real’ DT50 and the associated risk could be smaller.	+/0	The DT50 value is representative of metalaxyl-M residue decline data averaged across unprotected and protected sites from [redacted] (2012) and is also representative of data from [redacted] (2021a&b). DT50s obtained at some individual sites were higher than 10 days, though this was where the data did not follow first order kinetics. There is uncertainty due to extrapolating between seeds, products and timings. It is feasible that this value will vary depending on soil and climatic conditions. The ‘real’ DT50

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
				and the associated risk could be greater.
Averaging period for exposure calculation – 21 days	-/0	Period of exposure responsible for effects seen in the toxicity studies is unknown. Seeds may germinate in less than 21 days. Therefore the ‘real’ risk could be overestimated as seeds may not be available for the full period.	+/0	Period of exposure responsible for effects seen in the toxicity studies is unknown. Seeds may germinate in less than 21 days. However, if the focal species also consumes seedlings then the ‘real’ risk could be higher.
Proportion of diet obtained from the treated area (PT) – 0.17	-/0	Value used is for the worst-case individual from the consumer population. Many individuals will forage within the treated area to a lesser extent. There is uncertainty in extrapolation of the radio-tracking dataset from freshly drilled maize fields to vining pea fields, particularly due to timing differences. Freshly drilled vining pea fields could be less attractive as a foraging habitat than freshly drilled maize fields. It is noted that the PT value used is relatively low, so there is limited scope for the ‘true’ PT value of a foraging bird to be significantly lower.	0/++	Worst-case individual value used but dataset does not come from the crop of concern or the relevant time period. There is high uncertainty in extrapolation of the radio-tracking dataset from freshly drilled maize fields to vining pea fields, particularly due to the timing differences. Freshly drilled vining pea fields could be more attractive as a foraging habitat than freshly drilled maize fields. Freshly drilled crop fields in late winter/early spring may be more attractive than in late spring/summer due to alternative food sources being limited.
Proportion of food types obtained from the treated field (PD) - 1	-/--	PD has not been refined and has remained at 1, as in the first tier. It is likely that individuals will obtain other food items than crop seeds from crop	0	True risk cannot be higher.

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
		fields.		
Reduction in residues consumed due to de-husking – De-husking factor = 1	-	De-husking has not been considered quantitatively, risk may be reduced though de-husking is likely to be variable between and within a species (see [redacted] 2004 and 2004a). Pressure of feeding may also reduce the degree of de-husking. There is a lack of information to indicate whether it would be sufficiently protective.	0	True risk cannot be higher.
Avoidance of treated seed - None	-/--	Avoidance not considered quantitatively, some evidence indicates that treated seed may be avoided, however the relevance of the data to the species of concern and field conditions not known. In addition, relevance for the long-term/reproductive risk assessment uncertain.	0	True risk cannot be higher.
Attractiveness of field drilled with treated seed	-/--	Attractiveness of freshly drilled fields may be low and hence not actively sought out by birds; however, it is not possible to factor this into risk assessment due to the relevance of the data and the potential to extrapolate it to other sites.	0	True risk cannot be higher.
Variation of toxicity between species	---	Focal species could be up to 2 orders of magnitude less sensitive than standard species.	+++	Focal species could be up to 2 orders of magnitude more sensitive than standard species.

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
Uncertainty factor - 5	---	TER is compared with trigger value of 5.	0	Uncertainty factor cannot increase risk.
Overall assessment	**The refined exposure assessment replaces the first-tier generic focal species with an appropriate focal species (woodpigeon) and associated body weight and FIR/bw. Other parameters in the refined TER equation are a DT_{50} relating to the dissipation of the a.s. from the surface of the treated seed, and a PT value for the relevant focal species (noting the uncertainties associated with this value). Other parameters in the refined TER equation are unchanged from the first tier. The refined assessment is considered representative of the risk to a realistic worst-case individual in an exposed population. Certain factors may reduce the risk, for example de-husking or avoidance, however it is not possible to factor these into the risk assessment due to the uncertainties regarding the interpretation of the supporting studies.**			

Overall conclusion regarding the long-term/reproductive risk to birds from the use on vining pea seeds – consumption of seeds scenario

Regulation 1107/2009 requires that for authorisation of a plant protection product to occur it must be clearly established that use of this product will have no unacceptable impact on birds and mammals under field conditions. To this end the EFSA guidance document on bird and mammal risk assessment (EFSA) specifies two protection goals:

- An “actual” protection goal of:
  - “*clearly establishing that there will be no visible mortality and no long-term repercussions for abundance and diversity*.”
- A “surrogate” protection goal of:
  - “*making any mortality or reproductive effects unlikely*”.

The first-tier risk assessment is designed to satisfy the surrogate protection goal but at higher tiers either protection goal can be considered. In the case of metalaxyl-M the data and risk assessment provided do not allow for a consideration of the actual protection goal and therefore both the first and higher tier risk assessments are intended to satisfy the surrogate protection goal, i.e. to demonstrate that reproductive effects are unlikely following the use of the representative formulation.

The first-tier risk assessment for metalaxyl-M indicates an unacceptable reproductive risk to birds consuming treated vining pea seeds (TER = 0.73 compared to the acceptability criterion of ≥ 5).

The higher tier refined exposure assessment for metalaxyl-M takes into account an appropriate focal species, realistic feeding rate, proportion of food obtained from the treated area (PT) and residue dissipation rate. The higher tier refined TER value is above the trigger value of 5 by a factor of around 2 (TER = 10.9, compared to a trigger value of 5). The refined exposure assessment is considered by HSE to be representative of the risk experienced by a realistic worst-case individual in an exposed population. Therefore, this assessment allows for consideration of whether the surrogate protection is addressed, i.e. whether reproductive effects are unlikely. Given the refined TER values is above the trigger value, it is considered that the refined exposure assessment demonstrates that reproductive effects are unlikely.

Additionally, the applicant has proposed that the following risk mitigation labelling statements are appropriate and should be taken into account.

- **STGD-S9**: To protect birds/wild mammals the seeds must be entirely incorporated in the soil; ensure that the seeds are also fully incorporated at the end of rows (except for seeds sown in greenhouses).
- **STGD-S10**: To protect birds/wild mammals remove spillage (except for seeds sown in greenhouses).
- **STGD-S11**: To protect birds/wild animals, treated seeds must be sown (i.e. covered by soil) by using seed drill equipment leaving less than x,xx % of the sown treated seeds on the soil surface (except for seeds sown in greenhouses).

These measures are considered likely to reduce the exposure and hence the long-term/reproductive risk to birds, though the extent to which the risk would be reduced has not been demonstrated.

HSE therefore concludes that it has been clearly established that use of metalaxyl-M as a vining pea seed treatment will have no unacceptable long-term/reproductive impact on birds via consumption of treated seed.

Long-term/reproductive risk to birds from exposure to metalaxyl-M via consumption of emerged seedlings

The applicant has proposed the following refinements to the risk assessment for herbivorous birds:

1. Higher tier chronic avian toxicity endpoint for metalaxyl-M
2. Seedling residue data
3. Selection of relevant focal species based on field study in freshly drilled pea fields
4. Fraction of diet obtained from the treated area (PT)

Higher tier chronic avian toxicity endpoint - metalaxyl-M

The applicant proposed to raise the reproductive endpoint to 84 mg a.s./kg bw/day. The four reproductive studies and the derivation of the toxicity endpoint have been discussed in detail above and as a result, the proposal to use 84 mg a.s./kg bw/day has been rejected. It is considered that the previously agreed NOEC of 24.6 mg a.s./kg bw/d should be retained in the GB risk assessment.

Seedling residue data – metalaxyl-M

The applicant has proposed using a refined residue value in pea seedlings based on data from [redacted] **(1999)**. This study investigated movement of metalaxyl-M residues from seeds to soil and aerial plant parts. The crop used in this study is appropriate for the risk assessment and the application rate per seed was very similar (350 mg a.s./kg seed in the study compared to the GAP of 339.2 mg a.s./kg seed). While the formulation tested was not ‘Wakil XL’, given that metalaxyl-M is the subject of the risk assessment and the tested formulation contained metalaxyl-M, this is considered a minor point. Therefore, the study data are considered relevant for the risk assessment situation.

In the [redacted] (1999) study concentrations of metalaxyl-M in aerial plant parts were not analytically determined, however, they were estimated based on the %TRR. The results indicated that metalaxyl-M rapidly moved to the soil, with the highest %AR in aerial plant parts only 2.1% at the end of the measurement period (53 DAT). The highest TRR in aerial plant parts was on 14 DAT and was determined to be equivalent to 1089 ppb metalaxyl-M (i.e. 1.089 mg a.s./kg).

The applicant has proposed replacing the default first tier assumption that residues in seedlings will be equivalent to 20% of the seed NAR. Instead they have proposed that for pea seedlings it can be assumed that residues will be a maximum of 2.1% of the NAR. Using a NAR of 339.2 mg a.s./kg seed this equates to 7.12 mg a.s./kg seedling. It is noted that this is higher than the metalaxyl-M equivalent concentration for the highest TRR in plants found in the study (1.089 mg a.s./kg). In the absence of a direct measurement of metalaxyl-M specifically in above ground plant parts, the HSE higher tier risk assessment will consider both of these alternative values.

It is acknowledged that radioactivity in above ground plant parts was only determined from 14 DAT in [redacted] (1999) and it is possible that seedlings may emerge in the field earlier and hence potentially may have higher residues when they could be consumed by birds. However, from a practical perspective there needs to be sufficient plant material present for the analysis to be conducted. This is an area of uncertainty that can be further considered when characterising the outcome of the risk assessment.

The following refined residue values will be used in the higher tier risk assessment for birds consuming pea seedlings:

- Residue in seedling = 1.089 mg a.s./kg (based on maximum TRR in above ground plant parts)
- Residue in seedling = 7.12 mg a.s./kg (based on 2.1% AR in above ground plant parts)

Focal species

Data on bird abundance in freshly drilled and early growth stage pea fields is available in [redacted] et al. (2006). The use of this study and relevant focal species are discussed above in the consideration for birds consuming treated seed. HSE considers the appropriate focal species for the consumption of vining pea seedlings scenario to be the **skylark** and **wood pigeon**.

It is noted that that there is some further observational information on bird species on pea fields from transect counts in Austria in [redacted] (2005). The most abundant bird species observed on germinated pea fields in this study was the skylark, supporting the identification of this focal species.

Refinement of fraction of diet obtained from treated area (PT)

Skylark:

The applicant has proposed using a refined PT value of 0.826 for skylark, from a study by [redacted] (2005). This study included radiotracking and monitoring of skylarks on 5 maize and 5 sugar beet fields in Austria. A total of 30 tracking sessions were carried out on 16 individuals during a period of 43 days. Skylark tracking sessions took place in April and May. In total there were over 433 hours of tracking time for skylarks in this study.

While the focus of [redacted] (2005) was maize and sugar beet fields rather than pea fields, there were pea fields in the home ranges of some birds and these fields were used by some of the skylarks. Considering both drilled and germinated pea fields together, 5 birds were recorded actively foraging within pea fields (i.e. were consumers). Across these birds, PT values in peas > 0 were found during 10 sessions. The relevant data are summarised below.

Table B.9.1.5-33: Summary of skylark radiotracking data from [redacted] (2005) for consumer individuals in pea fields

Bird number	Session number	Proportion of potentially foraging time per habitat (%)			Mean PT per individual
		Drilled peas	Germinated peas	All peas	
1	1	26.8	-	26.8	44.1
1	2	61.4	-	61.4	
2	1	7.9	-	7.9	6.4
2	2	-	4.9	4.9	
8	4	-	4.8	4.8	4.8
13	1	-	22.7	22.7	32.7
13	2	-	13.2	13.2	
13	3	-	62.3	62.3	
14	1	-	79.2	79.2	87.9
14	2	-	96.6	96.6	

Since different numbers of tracking sessions are available for individual birds a mean PT value per individual has been calculated for each individual bird. While it is acknowledged that this dataset is very small, i.e. only 5 consumer birds, it does give an indication of the variability between individuals for the PT parameter. Given the high variability and small sample size, use of the maximum individual PT (0.879) is considered appropriate for this dataset.

Radio-tracking data for skylarks in post-emergence maize fields is available in the study by [redacted] (2010). There were 9 consumer individuals, with PT values varying from 0.01 to 0.91. While the crop was different and the study was conducted in southern Europe, it does provide some support to the wide variability found in PT values for skylark and the fact that for some individuals PT can be high, even close to 1.

The following PT value will be used in the higher tier risk assessment:

- Skylark PT in post-emergence pea fields = 0.879

Woodpigeon:

Radio-tracking data are not available for wood pigeons in post-emergence pea fields in the studies provided. Data are available for post-emergence maize fields, which can be considered further.

In [redacted] & [redacted] (2018) wood pigeons were tracked in maize fields at early post-emergence growth stages (BBCH 10-18) in Germany. There were 17 consumer birds tracked, with PT values varying from 0.01-0.78. It is noted though that the maximum 0.78 value was for a single individual, with most birds having PT values close to zero. The 90th percentile PT value for the consumer population was determined to be 0.32. Wood pigeons were also tracked in post-emergence maize fields in the [redacted] (2010), in southern France. Also in this study PT values in post-emergence maize were found to be relatively low, ranging from 0.01-0.2 (n = 7). Therefore, the [redacted] (2010) is generally supportive of the [redacted] & [redacted] (2018) dataset.

Extrapolation of PT between crops is a source of uncertainty in higher tier risk assessment. At early crop growth stages the habitat structure and food availability in maize and vining pea fields is not expected to be substantially different. However, as discussed above, there are differences in timing between when radio-tracking was conducted in the [redacted] (2010) and [redacted] & [redacted] (2018) studies compared to the period when vining pea seedlings would be present in GB. This could impact the attractiveness of surrounding areas as potential foraging locations, thus influencing PT in pea fields. It is unknown in which direction this uncertainty would act, i.e. whether using a PT value from maize would lead to underestimating or overestimating exposure. It is possible that the PT values derived in this study could underestimate the ‘true’ PT value for the period of use, since in April/May when pea seedlings have emerged, the availability of alternative food may be lower than in summer, noting the tracking sessions were conducted from May until late June.

Noting the uncertainty associated with the extrapolation of data, the following PT value is considered suitable for use in the higher tier risk assessment.

- Wood pigeon PT in post-emergence pea fields = 0.32

<u>Food intake rate relative to bodyweight (FIR/bw)</u>

The default Tier 1 FIR/bw for the generic focal species can be refined for the focal species. [redacted] et al. (1998) reports a bodyweight of 37.2 g for skylark and 490 g for woodpigeon.

The daily energy requirement has been determined using the equations presented in Appendix G of the guidance document (EFSA, 2009) and the bodyweight of the woodpigeon (490 g). The relationship between body weight (bw in g) and daily energy expenditure (DEE, in kJ) can be described by the equation: logDEE = log a + b × log bw, using the relevant constants for the species group (passerine birds for skylark, non-passerine birds for woodpigeon) from Appendix G of EFSA (2009). The energy expenditure (DEE) is 124.1 kJ/day for the skylark and 435.3 kJ/day for the woodpigeon.

No data are available directly measuring the food items consumed by birds when foraging in vining pea fields at early growth stages. At first tier a diet of 100% crop seedlings is assumed for the large herbivorous bird generic focal species. This represents an absolute worst-case diet. The same diet will be assumed for the woodpigeon focal species in the higher tier assessment. For the small omnivorous bird generic focal species the first tier FIR/bw assumes a diet of 25% crop seedling, 25% weed seed and 50% ground arthropods. The same diet will be considered for the skylark focal species in the higher tier risk assessment. However, in the absence of specific data on skylark diets it is unclear whether this particular dietary mix is appropriate for the skylark and protective of the risk to other small herbivorous/omnivorous species. If skylark consume a higher proportion of seedlings than 25% when foraging in crop fields, then the ‘true’ risk could be underestimated. Therefore, an additional scenario will also be included for skylark, assuming 50% crop seedlings in the diet, along with 25% weed seed and 25% ground arthropods. While this additional diet is not supported by specific data for pea fields, a study by Green (1978) in general farmland habitat consistently found that skylarks consume mixed diets throughout the year (i.e. an assumption of 100% crop seedlings would be unrealistic). The maximum proporion of dicot plant matter in skylark diets in any month at any site was 27%. Therefore, the Green (1978) data is considered to support the use of a 50% figure as a sufficiently conservative assumption, in the absence of specific data.

The amount of food items that the skylark and woodpigeon need to consume to meet their daily energy demands and hence the FIR/bw is determined using the following approach.

According to Appendix G (EFSA, 2009), the food intake rate (FIR) is calculated with following equation:

$$\text{FIR} = \frac{DEE}{FE\ x \left(1 - \frac{MC}{100}\right) x \left(\frac{AE}{100}\right)} \text{ (g fresh weight/day)}$$

Where:

DEE Daily Energy Expenditure of the indicator species (kJ/day)

FE Food energy (kJ/dry g)
MC Moisture content (%)
AE Assimilation efficiency (%)

In order to calculate the FIR/bw it is necessary to know the food energy content, food moisture content and the assimilation efficiency of the food item by birds. Specific values for these parameters are not given for vining seeds in Appendix G of EFSA (2009), however, default values for non-grass herbs can be used as a surrogate. The relevant food energy content, food moisture content and assimilation efficiency values from Appendix G of EFSA (2009) are summarised in the following table.

Table B.9.1.5-34: Summary of food energy parameters for skylark and woodpigeon

Parameter	Non-grass herbs	Weed seeds	Arthropods
Energy content (kJ/g dry)	17.8	21.7	22.7
Moisture content (%)	88.1	9.9	68.8
Assimilation efficiency (skylark)	0.76	0.8	0.76
Assimilation efficiency (woodpigeon)	0.53	NR	NR

Using the default non-grass herbs inputs and an assumed 100% seedling diet, the resulting FIR/bw for the woodpigeon is 0.79. For skylark a mixed diet is expected but specific data on the proportion of different food items taken in pea fields is unavailable. Assuming a diet of 25% seedlings, 25% weed seeds and 50% arthropods, the resulting FIR/bw for skylark is calculated to be 0.48. Assuming a diet of 50% seedlings, 25% weed seeds and 25% arthropods, the resulting FIR/bw for skylark is calculated to be 0.55.

<u>Revised TER calculation for long-term/reproductive risk to birds from consumption of emerged seedlings containing metalaxyl-M residues</u>

The long-term/reproductive TER has been recalculated using the agreed refinements to focal species (skylark and woodpigeon), seedling residue (1.09 or 7.12 mg/kg), dietary intake (FIR/bw = 0.79 and 0.48/0.55) and fraction of diet obtained from the treated area (PT = 0.879 and 0.32), as discussed above. No data are available on decline of residues from emerged crop seedlings, so a default 10 day DT_{50} and TWA of 0.53 are assumed.

Table B.9.1.5-35: Refined risk assessment for birds from consumption of crop seedlings containing metalaxyl-M

Intended use	Focal species	FIR/bw	PD	Residue (mg a.s./kg)	TWA	PT	DDD (mg a.s./kg bw/day)	NOAEL (mg a.s./kg bw/day)	TER_{LT}

Vining pea	Skylark (mixed diet)	0.48	0.25	7.12	0.53	0.879	0.398	24.6	61.8
		0.48	0.25	1.09	0.53	0.879	0.061	24.6	404
	Skylark (100% seedlings)	0.55	0.5	7.12	0.53	0.879	0.912	24.6	27
		0.55	0.5	1.09	0.53	0.879	0.14	24.6	176
	Woodpigeon	0.79	1	7.12	0.53	0.32	0.954	24.6	25.8
		0.79	1	1.09	0.53	0.32	0.146	24.6	168

The resulting TER values are above the first tier trigger value of 5 by at least a factor of 5. This potentially indicates that there will be no unacceptable long-term/reproductive risk to birds from consumption of vining pea seedlings, however, it is necessary to consider the uncertainties in the risk assessment.

Consideration of uncertainties in the risk assessment

In order to determine whether it has been clearly established that there will be no unacceptable long-term/reproductive risk on birds from the use of metalaxyl-M in the representative product ‘Wakil XL’ on vining pea seeds, it is necessary to consider the main sources of uncertainty in the risk assessment. This is summarised in the table below.

The uncertainties have been considered in the context of the ‘surrogate’ protection goal of ‘making any reproductive effects unlikely’, as specified in section 3 of EFSA (2009).

The +/- symbols indicate whether each source of uncertainty has the potential to make the true risk experienced by a realistic worst case individual higher (+) or lower (-) than the indicated outcome. The number of symbols provides a subjective relative evaluation of the magnitude of the effect.

Table B.9.1.5-36: Evaluation of the uncertainties in the refined long-term/reproductive risk assessment for birds from consumption of vining pea seedlings from seeds treated with metalaxyl-M

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
Toxicity endpoint – 24.6 mg a.s./kg bw/d	-	Four reproduction studies submitted; lowest endpoint used in the risk assessment. Uncertainty regarding robustness and sensitivity of the studies submitted, 'real' endpoint could be higher, hence risk lower.	+	Four reproduction studies submitted; lowest endpoint used in the risk assessment. Uncertainty regarding robustness and sensitivity of the studies submitted, 'real' endpoint could be lower, hence risk higher.
Choice of focal species (effect on exposure) – skylark and wood pigeon	0	Selection is based on relevant and reliable data from pea fields during the early post-emergence period ([redacted] et al., 2006). The species selected were the most frequently observed and the most dominant.	0	Selection is based on relevant and reliable data from pea fields during the early post-emergence period ([redacted] et al., 2006). The species selected were the most frequently observed and the most dominant.
Bodyweight – 490 g (woodpigeon), 37.2 g (skylark)	0/-	[redacted] et al. (1998) reports a woodpigeon bodyweight of 490 g (range 284-614 g) and skylark bodyweight of 37.2 g (range 29-51 g). Therefore some individuals will have larger bodyweights and could experience less exposure relative to bodyweight. However, the magnitude of impact this would have on the exposure estimate is relatively small.	0/+	[redacted] et al. (1998) reports a woodpigeon bodyweight of 490 g (range 284-614 g) and skylark bodyweight of 37.2 g (range 29-51 g). Therefore some individuals will have smaller bodyweights and could experience more exposure relative to bodyweight. However, the magnitude of impact this would have on the exposure estimate is relatively small.
Food intake rate calculation – energy	-/0	Specific energy and moisture contents for vining pea seedlings have not been	+/0	Specific energy and moisture contents for vining pea seedlings have not been

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
and moisture content		used. Extrapolated values have been used for non-grass herbs. It is uncertain whether these values represent worst case values or truly realistic values, i.e. the 'real' values for vining peas could be higher or lower than those used, hence this could increase or decrease the risk.		used. Extrapolated values have been used for non-grass herbs. It is uncertain whether these values represent worst case values or truly realistic values, i.e. the 'real' values for vining peas could be higher or lower than those used, hence this could increase or decrease the risk.
Residue in seedlings – 1.089 or 7.12 mg a.s./kg	-/0	Data derived for pea seedlings from [redacted] (1999). Metalaxyl-M was applied to seeds at a representative nominal application rate. Only a single soil was tested and this parameter could vary between soils. The highest residue from the study in above ground plant material was used, so in many cases residues would be expected to be lower. However, data are only available from 14 DAT and residues could be higher at an earlier time point.	+/0	Data derived for pea seedlings from [redacted] (1999). Metalaxyl-M was applied to seeds at a representative nominal application rate. Only a single soil was tested and this parameter could vary between soils. The highest residue from the study in above ground plant material was used, however, data are only available from 14 DAT and residues could be higher at an earlier time point.
Dissipation and degradation of active substance from seedlings – DT50 of 10 days	-/0	A default DT50 value for above ground plant material has been assumed. This default is based on data from spray applications and the relevance of this for the consumption of seedlings (from treated seed) is unknown. The 'true' DT50 could lower, with residue	0/+	A default DT50 value for above ground plant material has been assumed. This default is based on data from spray applications and the relevance of this for the consumption of seedlings (from treated seed) is unknown. The 'true' DT50 could be higher but residue

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
		dissipation data with seeds indicate that the persistence of the active substance in plant material is not high.		dissipation data with seeds indicate that the persistence of the active substance in plant material is not high.
Averaging period for exposure calculation – 21 days	-/0	Period of exposure responsible for effects seen in the toxicity studies is unknown. Therefore the 'real' risk could be overestimated, due to an averaging period being used that is too short.	+/0	Period of exposure responsible for effects seen in the toxicity studies is unknown. Therefore the 'real' risk could be underestimated, due to an averaging period being used that is too.
Proportion of diet obtained from the treated area (PT) – 0.879 (skylark), 0.32 (woodpigeon)	-/0	For skylark tracking data comes pea fields ([redacted] (2005). The PT value used is for the worst-case individual from the consumer population. Many individuals will forage within the treated area to a lesser extent. For woodpigeon tracking data comes from maize fields ([redacted] & [redacted], 2018). The PT value used is a 90th percentile for the consumer population. Many individuals would be expected to have lower PT values than this. There is uncertainty in extrapolation of the radio-tracking dataset from freshly drilled maize fields to vining pea fields, particularly due to timing differences. Freshly drilled vining pea fields could be less attractive as a foraging habitat	0/+	Worst-case individual value used for skylark, so the 'true' value would not be expected be higher, though noting the small sample size. There is uncertainty in extrapolation of the radio-tracking dataset for woodpigeon from freshly drilled maize fields to vining pea fields, particularly due to timing differences. Freshly drilled vining pea fields could be more attractive as a foraging habitat than freshly drilled maize fields.

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
		than freshly drilled maize fields.		
Proportion of food types obtained from the treated field (PD) - 1	-/--	PD has not been refined from first tier for woodpigeon - a diet of 100% seedlings is assumed. It is likely that individuals will obtain other food items than seedlings when foraging in crop fields. For skylark, diets assuming 25% and 50% pea seedlings have been considered. Field data for skylark indicates that many individual birds are likely to consume a smaller proportion of dicotyledenous material in their diets than 50%.	0/+	For woodpigeon the true risk cannot be higher. For skylark both a tier 1 diet and diet with increased seedling content (50%) have been considered. It cannot be excluded that some individuals will obtain higher proportions of crop seedlings from crop fields.
Attractiveness of field at early post-emergence growth stage	-	Attractiveness of early post-emergence fields may be low and hence not actively sought out by birds; however, it is not possible to factor this into risk assessment due to the relevance of the data and the potential to extrapolate it to other sites.	0	True risk cannot be higher.
Variation of toxicity between species	---	Focal species could be up to 2 orders of magnitude less sensitive than standard species.	+++	Focal species could be up to 2 orders of magnitude more sensitive than standard species.
Uncertainty factor - 5	---	TER is compared with trigger value of 5.	0	Uncertainty factor cannot increase risk.
Overall assessment	**The refined exposure assessment replaces the first-tier generic focal species with appropriate focal species (woodpigeon and skylark) and associated body weight and FIR/bw. Other parameters**			

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
	in the refined TER equation are a default DT_{50} relating to the dissipation of the a.s. from seedlings, specific data on the maximum residue of metalaxyl-M in pea seedlings, and PT values for the relevant focal species in pea fields. Other parameters in the refined TER equation are unchanged from the first tier. The refined assessment is considered representative of the risk to a realistic worst-case individual in an exposed population.			

Overall conclusion regarding the long-term/reproductive risk to birds from the use on vining pea seeds – consumption of seedlings scenario

Regulation 1107/2009 requires that for authorisation of a plant protection product to occur it must be clearly established that use of this product will have no unacceptable impact on birds and mammals under field conditions. To this end the EFSA guidance document on bird and mammal risk assessment (EFSA) specifies two protection goals:

- An “actual” protection goal of:
    - “*clearly establishing that there will be no visible mortality and no long-term repercussions for abundance and diversity*.”
- A “surrogate” protection goal of:
    - “*making any mortality or reproductive effects unlikely*”.

The first-tier risk assessment is designed to satisfy the surrogate protection goal but at higher tiers either protection goal can be considered. In the case of metalaxyl-M the data and risk assessment provided do not allow for a consideration of the actual protection goal and therefore both the first and higher tier risk assessments are intended to satisfy the surrogate protection goal, i.e. to demonstrate that reproductive effects are unlikely following the use of the representative formulation.

The first-tier risk assessment for metalaxyl-M indicates an unacceptable reproductive risk to birds consuming treated vining pea seedlings (TER = 0.73 and 1.21 compared to the acceptability criterion of ≥ 5).

The higher tier refined exposure assessment for metalaxyl-M takes into account appropriate focal species, realistic feeding rates, proportions of food obtained from the treated area (PT), initial residues in pea seedlings and residue dissipation rate. The higher tier refined TER values are above the trigger value of 5 by a factor of at least 5 (lowest TER = 25.8, compared to a trigger value of 5). The refined exposure assessment is considered by HSE to be representative of the risk experienced by a realistic worst-case individual in an exposed population. Therefore, this assessment allows for consideration of whether the surrogate protection is addressed, i.e. whether reproductive effects are unlikely. Given the refined TER values are well above the trigger value, it is considered that the refined exposure assessment demonstrates that reproductive effects are unlikely.

<u>HSE therefore concludes that it has been clearly established that use of metalaxyl-M as a vining pea seed treatment will have no unacceptable long-term/reproductive impact on birds via consumption of crop seedlings.</u>

B.9.1.6. Risk assessment – mammals

B.9.1.6.1. *First tier acute risk assessment for mammals*

The key metalaxyl-M toxicity endpoint for the acute risk assessment is as follows:

- LD_{50} = 375 mg a.s./kg bw

Vibrance SB – sugar beet

For pelleted seeds (which includes sugar and fodder beet), consideration of the risk to mammals from consumption of treated seeds is not required. This is specified in section 5.2.1 of EFSA (2009), which states:

'Pelleted seeds may be consumed by wood mice (e.g. Pelz, 1989) but the Joint Working Group considered that the risk in these cases may be reduced due to animals cracking and discarding the pellet with most of the residue before ingesting the seed.'

Consideration is required of the risk to mammals from consumption of treated crop seedlings.

Acute exposure – Tier 1 assessment emerged seedlings

The acute Daily Dietary Dose (DDD_{90}) as a result of the consumption of emerged seedlings can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR/5}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

For the Tier I risk assessment a small omnivorous and a large herbivorous mammal with food intake rates (FIR/bw) of 0.24 and 0.4 respectively will be used as relevant indicator species.

Table B.9.1.6-1: Acute exposure estimate for mammals following the consumption of treated sugar and fodder beet seedlings

Crop	Active substance	Generic focal species	NAR (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Sugar and fodder beet	Metalaxyl-M	Small omnivorous mammal	199.8	0.24	9.59
	Metalaxyl-M	Large herbivorous mammal	199.8	0.4	15.98

Exposure for mammals through drinking water

The EFSA Guidance Document for assessing the risk from drinking water specifies that consideration for mammals is required for the puddle scenario only.

Puddle scenario

This scenario is relevant for mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all uses of metalaxyl-M and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:

> *"Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg)."*

With a K(f)oc of 78.9, metalaxyl-M belongs to the group of less sorptive substances. The maximum use rate of 0.62 g a.s./ha in sugar and fodder beet is used. Since the ratio of the application rate to the toxicity endpoint is less than the trigger value, an acceptable acute risk to mammals can be concluded for the puddle scenario and no further consideration is required.

Table B.9.1.6-2: Acute risk to mammals from drinking water – puddle scenario

Substance	Crop	Application Rate (g/ha)	Endpoint (mg/kg bw)	Ratio AR (g/ha)/endpoint (mg/kg bw)	Trigger value
Metalaxyl-M	Sugar and fodder beet	0.62	LD_{50} = 375	0.0017	≤ 50

Acute toxicity exposure ratio (TER_A) – Exposure via consumption of emerged seedlings

Acute risk is assessed by comparing the relevant DDD values from above with the appropriate LD_{50} endpoint to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\text{ (mg/kg bw)}}{\text{DDD}}$$

The resulting TER_A values are given in the table below.

Table B.9.1.6-3: Tier 1 acute risk to mammals – consumption of emerged seedlings

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Sugar and fodder beet	Metalaxyl-M	Small omnivorous mammal	9.59	375	39.1
	Metalaxyl-M	Large herbivorous mammal	15.98	375	23.46

The acute TERs for all generic focal species are above the trigger value of 10. Acceptable acute risks to mammals from the consumption of emerged seedlings have therefore been demonstrated.

<u>Wakil XL – vining pea</u>

Acute exposure – Tier 1 assessment treated seeds

The acute Daily Dietary Dose (DDD_{90}) can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

Vining pea seeds are considered as ‘large seeds’ according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). Consequently, for the

Tier I risk assessment a small omnivorous mammal with a food intake rate (FIR/bw) of 0.24 will be used as relevant indicator species.

Calculation of the DDD for the representative use patterns for A9873C is provided in the table below.

Table B.9.1.6-4: Acute exposure estimate for mammals following the consumption of treated vining pea seeds

Crop	Active substance	Generic focal species	NAR (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Vining pea	Metalaxyl-M	Small omnivorous mammal	339.2	0.24	81.41

Acute exposure – Tier 1 assessment emerged seedlings

The acute Daily Dietary Dose (DDD_{90}) as a result of the consumption of emerged seedlings can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR/5}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

For the Tier I risk assessment a small omnivorous and a large herbivorous mammal with food intake rates (FIR/bw) of 0.24 and 0.4 respectively will be used as relevant indicator species.

Table B.9.1.6-5: Acute exposure estimate for mammals following the consumption of treated vining pea seedlings

Crop	Active substance	Generic focal species	NAR/5 (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Vining peas	Metalaxyl-M	Small omnivorous mammal	67.8	0.24	16.28
	Metalaxyl-M	Large herbivorous mammal	67.8	0.4	27.14

Exposure for mammals through drinking water

The EFSA Guidance Document for assessing the risk from drinking water specifies that consideration for mammals is required for the puddle scenario only.

Puddle scenario

This scenario is relevant for mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all uses of metalaxyl-M and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:

> *“Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg).”*

With a K(f)oc of 78.9, metalaxyl-M belongs to the group of less sorptive substances. The maximum use rate of 76.32 g a.s./ha in vining pea is used. Since the ratio of the application rate to the toxicity endpoint is less than the trigger value, an acceptable acute risk to mammals can be concluded for the puddle scenario and no further consideration is required.

Table B.9.1.6-6: Acute risk to mammals from drinking water – puddle scenario

Substance	Crop	Application Rate (g/ha)	Endpoint (mg/kg bw)	Ratio AR (g/ha)/endpoint (mg/kg bw)	Trigger value
Metalaxyl-M	Vining pea	76.32	LD_{50} = 375	0.20	≤ 50

Acute toxicity exposure ratio (TER_A) – Exposure via consumption of treated seeds

Acute risk is assessed by comparing the relevant DDD values from above with the appropriate LD50 endpoint to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ (\text{mg/kg bw})}{\text{DDD}}$$

The resulting TER_A value is given in the table below.

Table B.9.1.6-7: Tier 1 acute risk to mammals – consumption of treated seeds

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Vining pea	Metalaxyl-M	Small omnivorous mammal	81.41	375	**4.61**

The acute TER for metalaxyl-M is below the trigger value of 10. Further consideration is required for the acute risk to mammals from the consumption of treated seed.

Acute toxicity exposure ratio (TER_A) – Exposure via consumption of emerged seedlings

Acute risk is assessed by comparing the relevant DDD values from above with the appropriate LD_{50} endpoint, to give an acute Toxicity: Exposure Ratio (TER_A):

$$TER_A = \frac{LD_{50}\ (\text{mg/kg bw})}{\text{DDD}}$$

The resulting TER_A values are given in the table below.

Table B.9.1.6-8: Tier 1 acute risk to mammals – consumption of emerged seedlings

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	LD_{50} (mg a.s./kg bw)	TER_A
Vining peas	Metalaxyl-M	Small omnivorous mammal	16.28	375	23.03
	Metalaxyl-M	Large herbivorous mammal	27.14	375	13.82

The acute TERs for all generic focal species are above the trigger value of 10. Acceptable acute risks to mammals from the consumption of emerged seedlings have therefore been demonstrated.

B.9.1.6.2. *First tier long-term/reproductive risk assessment for mammals*

The key metalaxyl-M toxicity endpoint for the long-term/reproductive risk assessment is as follows:

- NOAEL = 96 mg a.s./kg bw

Vibrance SB – sugar beet

Long-term/reproductive exposure – Tier 1 assessment emerged seedlings

The long-term/reproductive Daily Dietary Dose (DDD_{50}) as a result of the consumption of emerged seedlings can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR/5}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

For the Tier I risk assessment a small omnivorous and a large herbivorous mouse with food intake rates (FIR/bw) of 0.24 and 0.4 respectively will be used as relevant indicator species.

Table B.9.1.6-9: Long-term/reproductive exposure estimate for following the consumption of treated sugar and fodder beet seedlings

Crop	Active substance	Generic focal species	NAR (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Sugar and fodder beet	Metalaxyl-M	Small omnivorous mammal	199.8	0.24	9.59
	Metalaxyl-M	Large herbivorous mammal	199.8	0.4	15.98

Exposure for mammals through drinking water

The EFSA Guidance Document for assessing the risk from drinking water specifies that consideration for mammals is required for the puddle scenario only.

Puddle scenario

This scenario is relevant for mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all uses of metalaxyl-M and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:

> *“Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg).”*

With a K(f)oc of 78.9, metalaxyl-M belongs to the group of less sorptive substances. The maximum use rate of 0.62 g a.s./ha in sugar and fodder beet is used. Since the ratio of the application rate to the toxicity endpoint is less than the trigger value, an acceptable long-term/reproductive risk to mammals can be concluded for the puddle scenario and no further consideration is required.

Table B.9.1.6-10: Long-term/reproductive risk to mammals from drinking water – puddle scenario

Substance	Crop	Application Rate (g/ha)	Endpoint (mg/kg bw/day)	Ratio AR (g/ha)/endpoint (mg/kg bw)	Trigger value
Metalaxyl-M	Sugar and fodder beet	0.62	NOAEL = 96	0.0065	≤ 50

Long-term/reproductive toxicity exposure ration (TERlt) – Exposure via consumption of emerged seedlings

The Tier 1 long-term risk is assessed by comparing the long-term DDD with the NOAEL derived from the toxicity study, to give a long-term Toxicity/Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{NOAEL\,(mg/kg\ bw/day)}{DDD\,(mg/kg\ bw/day)}$$

The resulting TER_{LT} values are given in the table below.

Table B.9.1.6-11: Tier 1 long-term/reproductive risk to mammals – consumption of emerged seedlings

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	NOAEL (mg a.s./kg bw)	TER_{LT}
Sugar and fodder beet	Metalaxyl-M	Small omnivorous mammal	9.59	96	10.01
	Metalaxyl-M	Large herbivorous mammal	15.98	96	6.01

The Tier 1 long-term/reproductive TER_{LT} for mammals consuming treated sugar and fodder beet seedlings are above the trigger of 5 for all generic focal species. Acceptable long-term/reproductive risks to mammals consuming treated seeds can be concluded.

Wakil XL – vining peas

Long-term/reproductive exposure – Tier 1 assessment treated seeds

The long-term/reproductive Daily Dietary Dose (DDD_m) can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{FIR}{bw} \text{ X NAR}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

Vining pea seeds are considered as 'large seeds' according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). Consequently, for the Tier I risk assessment a small omnivorous mammal with a food intake rate (FIR/bw) of 0.24 will be used as relevant indicator species.

Calculation of the DDD for the representative use patterns for A9873C is given in the table below.

Table B.9.1.6-12: Long-term/reproductive exposure estimate for mammals following the consumption of treated vining pea seeds

Crop	Active substance	Generic focal species	NAR (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Vining pea	Metalaxyl-M	Small omnivorous mammal	339.2	0.24	81.41

Long-term/reproductive exposure – Tier 1 assessment emerged seedlings

The long-term/reproductive Daily Dietary Dose (DDD_{50}) as a result of the consumption of emerged seedlings can be calculated using the following equation:

$$\text{DDD (mg a.s./kg bw/day)} = \frac{\text{FIR}}{\text{bw}} \times \text{NAR/5}$$

Where:

FIR Food intake rate of indicator species (g fresh weight/day)
bw Bodyweight (g)
NAR Nominal loading/ application rate of active substance (mg/kg seed)

For the Tier I risk assessment a small omnivorous and a large herbivorous mouse with food intake rates (FIR/bw) of 0.24 and 0.4 respectively will be used as relevant indicator species.

Table B.9.1.6-13: Long-term/reproductive exposure estimate for following the consumption of treated vining pea seedlings

Crop	Active substance	Generic focal species	NAR/5 (mg a.s. /kg seed)	FIR/bw	Daily Dietary Dose (mg/kg bw)
Vining peas	Metalaxyl-M	Small omnivorous mammal	67.8	0.24	16.28
	Metalaxyl-M	Large herbivorous mammal	67.8	0.4	27.14

Exposure for mammals through drinking water

The EFSA Guidance Document for assessing the risk from drinking water specifies that consideration for mammals is required for the puddle scenario only.

Puddle scenario

This scenario is relevant for mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all uses of metalaxyl-M and should therefore be assessed. The EFSA Guidance Document (ref. 5.5, Step 2b) states the following:

> *"Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary since the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg)."*

With a K(f)oc of 78.9, metalaxyl-M belongs to the group of less sorptive substances. The maximum use rate of 76.32 g a.s./ha in vining pea is used. Since the ratio of the application rate to the toxicity endpoint is less than the trigger value, an acceptable long-term/reproductive risk to mammals can be concluded for the puddle scenario and no further consideration is required.

Table B.9.1.6-14: Long-term/reproductive risk to mammals from drinking water – puddle scenario

Substance	Crop	Application Rate (g/ha)	Endpoint (mg/kg bw/day)	Ratio AR (g/ha)/endpoint (mg/kg bw)	Trigger value
Metalaxyl-M	Vining pea	76.32	NOAEL = 96	0.8	≤ 50

Long-term/reproductive toxicity exposure ration (TERlt) – Exposure via consumption of treated seeds

The Tier 1 long-term risk is assessed by comparing the long-term DDD with the NOAEL derived from the toxicity study, to give a long-term Toxicity/Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{NOAEL\,(mg/kg\ bw/day)}{DDD\,(mg/kg\ bw/day)}$$

The resulting TER_{LT} value is given in the table below.

Table B.9.1.6-15: Tier I long-term/reproductive risk to mammals – consumption of treated seeds

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	NOAEL (mg a.s./kg bw)	TER_{LT}
Vining pea	Metalaxyl-M	Small omnivorous mammal	81.41	96	**1.18**

The Tier 1 long-term/reproductive TER_{LT} for small omnivorous mammals consuming treated vining pea seeds is below the trigger of 5 for metalaxyl-M. Further consideration of the long-term/reproductive risk to mammals from consumption of treated seed is therefore required.

Long-term/reproductive toxicity exposure ration (TERlt) – Exposure via consumption of emerged seedlings

The Tier 1 long-term risk is assessed by comparing the long-term DDD with the NOAEL derived from the toxicity study, to give a long-term Toxicity/Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{\text{NOAEL}\,(\text{mg/kg bw/day})}{\text{DDD}\,(\text{mg/kg bw/day})}$$

The resulting TER_{LT} values are given in the table below.

Table B.9.1.6-16: Tier 1 long-term/reproductive risk to mammals – consumption of emerged seedlings

Crop	Active substance	Generic focal species	DDD (mg/kg bw/day)	NOAEL (mg a.s./kg bw)	TER_{LT}
Vining peas	Metalaxyl-M	Small omnivorous mammal	16.28	96	5.90
	Metalaxyl-M	Large herbivorous mammal	27.14	96	**3.54**

The Tier 1 long-term/reproductive TER_{LT} for mammals consuming treated vining pea seedlings are above the trigger of 5 for the small omnivore but below the trigger value for the large herbivore. Further consideration of the long-term/reproductive risk to large herbivores via consumption of pea seedlings is therefore required.

Higher tier acute risk assessment for mammals

Higher tier risk assessment for the acute risk from treated vining pea seeds to mammals – Wakil XL

The following areas of the assessment were not demonstrated to result in acceptable acute risks to mammals and thus require further consideration:

- Acute risk to mammals from exposure to metalaxyl-M via consumption of seeds as food;

Acute risk to mammals from exposure to metalaxyl-M via consumption of seeds as food

The applicant has proposed for the following refinements to the risk assessment for granivorous mammals:

1. Focal species – wood mouse
2. PD (proportion of food type in the diet) refinement wood mouse
3. Additional weight of evidence:
    a. Dehusking behaviour of wood mouse
    b. Lower formulation toxicity compared to predicted toxicity

These proposed refinements are considered in turn below.

<u>Focal species</u>

The applicant has proposed refining the risk to small omnivorous mammals, using the wood mouse as a focal species. This is justified on the basis that the wood mouse is among the most frequent mammals in freshly drilled fields and therefore is considered as the relevant focal species. The applicant has not referred to specific data to support this selection, so HSE have briefly considered the available field study data submitted.

In the EU renewal review of metalaxyl-M the wood mouse was concluded to be an appropriate focal species for the use as seed treatments on sunflower and spinach. However, given the difference in crop to that considered under this evaluation (vining pea) and since higher tier studies have been submitted by the applicant which investigate the presence of small mammal species in/around pre-emergence crop fields, some further consideration of this point is warranted.

[redacted] (2008) trapped small mammals in/around pre-emergence spring cereal fields. Wood mouse was the most frequently trapped species within the open field, with yellow-necked mice, bank voles and field voles almost exclusively trapped in the surroundings. Common voles were also trapped on open fields, but this was before drilling and not after drilling. Species trapped and locations caught are not transparently presented in the [redacted] & [redacted] (2016) or [redacted] & [redacted] (2017) studies, so these studies provide limited information on focal species – only to confirm that wood mice were trapped in and around pre-emergence winter and spring cereal fields. In the non-GLP Barber et al. (2003) literature study wood mice and bank voles were trapped in and around cereal fields, with the number of wood mice trapped in the cereal fields substantially higher than for bank voles.

It is noted that none of these studies were specifically designed to identify focal species in freshly drilled vining pea fields. The array of small mammal species present in freshly drilled fields of different crops at the same time of year may be expected to be similar, given the degree of ground cover and the type of food items available would be expected to be similar. However, potential differences in the attractiveness of crop seeds as a food source could impact the extent to which a small mammal species forages within a freshly drilled field. Therefore, this point has been considered further. In a study by [redacted] et al. (2004), wild caught wood mice were provided with different crop seeds for 6 hours during their peak activity period. No alternative food was provided during this period, with 5 males and 5 females used per seed type. The amount of seeds consumed per individual was investigated by measuring food bowl weight change (accounting for spillage). The mean weight of pea seeds consumed was 0.58 g, compared to 1.27 g for wheat seeds. The individual maximum seed consumption was 1.3 g for pea and 2.6 for wheat. Therefore, there is no indication of pea seeds being a more attractive food source than wheat seeds for wood mice, based on the findings of the [redacted] (2004) study. As such, it is agreed that the wood mouse is an appropriate focal species for addressing the risk to small mammals from consumption of treated vining pea seeds.

<u>PD (composition of diet obtained from treated area)</u>

At first tier the risk assessment for mammals consuming treated seeds assumes that the diet of the generic focal species consists of 100% treated seeds, which is a worst-case assumption. The applicant has proposed refining the acute risk assessment for mammals consuming treated seeds by considering data on wood mouse diets. In their quantitative risk assessment, the applicant has used dietary data from 3 public literature studies: Abt & Bock (1998), Green (1979) and Pelz (1989).

It is noted that these same studies were referred to in the dRAR for metalaxyl-M by the RMS and were used to support a refined diet for the focal species wood mouse in post-sowing, pre-emergence sunflower fields. However, this PD refinement was not accepted following the peer review process and does not appear in the EFSA conclusion for metalaxyl-M (EFSA, 2015). Additionally, HSE does not consider refinement of the PD parameter to be appropriate for the acute risk assessment since sufficient exposure to result in mortality could come from consumption of a relatively small number of food items obtained during a short time period. Therefore, including a mixed diet composition that is averaged across a longer time period has the potential to underestimate the acute risk to mammals. A diet of 100% treated seed will therefore be retained in the risk assessment.

FIR/bw

The default Tier 1 FIR/bw for the generic focal species small omnivorous mammal can be refined for the focal species wood mouse. The applicant has used a bodyweight of 21.7 g for the wood mouse in the FIR/bw calculations. Gurney *et al*. (1998)[24] reports a mean wood mouse bodyweight of 18 g, with a range of 13-27 g. While the mean bodyweight from Gurney *et al*. (1998) is lower than the value used by the applicant, the difference is slight, and it is noted that a bodyweight of 21.7 g was used for wood mice in the renewal assessment report for metalaxyl-M. Therefore, a bodyweight of 21.7 g for wood mice is used in the following FIR/bw calculation.

The daily energy requirement has been determined using the equations presented in Appendix G of the guidance document (EFSA, 2009) and the bodyweight of the wood mouse (21.7 g). The relationship between body weight (bw in g) and daily energy expenditure (DEE, in kJ) can be described by the equation: logDEE = log a + b × log bw, using the relevant constants for the species group (mammals) from Appendix G of EFSA (2009). The energy expenditure of the wood mouse with a 21.7 g bw, results in a DEE of 58.8 kJ/day. The amount of vining pea seeds that the wood mouse needs to consume to meets its daily energy demands and hence the FIR/bw can then be determined.

According to Appendix G (EFSA, 2009), the food intake rate (FIR) is calculated with following equation:

$$\text{FIR} = \frac{DEE}{FE\ x\left(1-\frac{MC}{100}\right)x\left(\frac{AE}{100}\right)}\ \text{(g fresh weight/day)}$$

Where:

DEE Daily Energy Expenditure of the indicator species (kJ/day)
FE Food energy (kJ/dry g)
MC Moisture content (%)
AE Assimilation efficiency (%)

In order to calculate the FIR/bw it is necessary to know the food energy content, food moisture content and the assimilation efficiency of the food item by mammals. Specific values for these parameters are not given for vining seeds in Appendix G of EFSA (2009). Standard values are however stated for weed seeds and cereal seeds, with extrapolation from these values to vining peas considered potentially relevant. The food energy content, food moisture content and assimilation efficiency values for cereal and weed seeds from Appendix G of EFSA (2009) are summarised in the following table.

[24] Gurney J.E., Perrett J., Crocker D.R & Pascual, J.A. (1998). CONTRACT PN0910/PN0919 MILESTONE REPORT Mammals and farming: information for risk assessment. CSL Project No. M37.

Table B.9.1.6-17: Summary of food energy parameters

Parameter	Cereal seed (EFSA, 2009)	Weed seed (EFSA, 2009)
Energy content (kJ/g dry)	18.4	21.7
Moisture content (%)	14.7	9.9
Assimilation efficiency	0.84	0.84

Using the default cereal seed inputs and an assumed 100% seed diet, the resulting FIR/bw for the wood mouse is 0.21. Using the default weed seed inputs and 100% seed diet, the resulting FIR/bw is 0.16. In the absence of specific energy content or moisture content data for vining pea seeds, the more worst-case FIR/bw value of 0.21 will be used from the cereal seed and weed seed FIR/bw values. This approach is in line with the EU renewal review assessment for metalaxyl-M in the dRAR and EFSA conclusion.

<u>Dehusking behaviour</u>

Residues on treated seeds will be mainly located on the outside of the seeds (husk, testa, pericarp), whereas concentrations in the inner parts of the seeds (endosperm, embryo) will be significantly lower. Thus, exposure of granivorous mammals may be markedly reduced if they dehusk seeds before consumption. Regarding consideration of dehusking in higher tier risk assessment, Section 6.1.7 the EFSA guidance on bird and mammal risk assessment (EFSA, 2009) states the following:

> '*Due to the lack of reliable data and the known uncertainties, dehusking should only be considered in higher-tier assessments with case-specific justifications. Evidence must be provided that dehusking may actually play a role under field conditions for the relevant focal species. If this is the case, the available information should be checked for the conditions under which dehusking occurs and the extent to which it has been observed for this species. Specific care should be taken for seed treatments with a high toxicity per single seed. If the LD50 is already reached with one or few seeds/particles, consideration of dehusking in the risk assessment might not be justified.*
>
> *To obtain an estimate on the actual efficiency of dehusking, studies with the relevant focal species, the relevant seed type and the relevant product are preferable, since extrapolation is always connected with increasing uncertainty.'*

The applicant has proposed using data on dehusking of seeds by wood mice as part of a weight of evidence approach to the higher tier risk assessment for metalaxyl-M. As part of this argumentation, the applicant has referred to results from the **Brühl *et al*. (2011)** and **DEFRA (2010)** studies. These studies have been summarised in section B.9.1.4 are discussed in detail below.

In addition to these specific data, the applicant has referred to a number of other published studies to provide supporting evidence of dehusking behaviour in wood mice. They have stated that:

> '*In several studies de-husking by wood mice has been reported, e.g. Barber* et al*. (2003) for wheat seeds, Westerman* et al*. (2003)*[25] *for weed seeds, Tew* et al*. (2000*[26]*) on sterile brome (Bromus sterilis) and common wild oat (Avena fatua) seeds, Pelz (1989; 1979*[27]*) and Gersdorf (1971*[28]*) for sugar beet seeds.*'

Further details or discussion of the results and methodology from these additional published studies have not been submitted by the applicant. However, it is apparent that no specific information regarding dehusking of vining pea seeds by wood mice has been referred to and that these studies did not involve seeds treated with metalaxyl-M or Wakil XL. Therefore, these studies have not been considered further. While it is accepted that dehusking of seeds can be part of the typical behaviour of granivorous mammals, case-specific consideration is needed, addressing the points highlighted in Section 6.1.7 of EFSA (2009).

For the studies investigating dehusking behaviour where the applicant has provided detailed consideration (Brühl et al., 2011; DEFRA, 2010), these are discussed below in relation to whether they provide case-specific information, addressing the points highlighted in Section 6.1.7 of EFSA (2009). In addition, further DEFRA research investigating dehusking behaviour in wood mice (DEFRA, 2015) and an additional published study investigating dehusking behaviour (Morris & Dennis, 2014) are also considered.

Brühl *et al*. (2011):

Brühl *et al.* (2011) conducted a laboratory study with wood mice to measure the reduction in residue uptake through dehusking behaviour on wheat, barley, maize and sunflower. This is a published study and was not conducted to GLP. In the EU renewal review of metalaxyl-M Brühl *et al.* (2011) was considered a key study with respect to dehusking behaviour by wood mice.

This study adopted two methods. In the first of these methods a fungicide seed treatment was applied to wheat and barley seeds (Prothioconazole 100 FS at 100 mL/kg seed). Twenty wild-caught wood mice were then provided with 6 g of these seeds for 24 h, after which time husks and surrounding sand were collected and analysed for prothioconazole. The percentage reduction in exposure due to dehusking behaviour has been calculated. This calculation takes into account the

[25] Westerman, P., Hofman, A., Vet, L. and Van der Werf, W. (2003). Relative importance of vertebrates and invertebrates in epigeaic weed seed predation in organic cereal fields. Agriculture Ecosystems and Environment, 95, 417-425.
[26] Tew, T.E., Todd, I.A. and Macdonald, D.W. (2000). Arable habitat use by wood mice (Apodemus sylvaticus). 2. Microhabitat. Journal of Zoology (London), 250, 305-311.
[27] Pelz, H.J. (1979). Die Waldmaus, Apodemus sylvaticus L., auf Ackerflächen: Populationsdynamik, Saatschäden und Abwehrmöglichkeiten. Zeitschrift für angewandte Zoologie, 66, 261-280.
[28] Gersdorf, E. (1971). Waldmäuse und Vögel zerstören pillierte Rübensaat. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, 23, 151-152.

potential exposure from the consumed seeds had the whole seed been consumed and the amount of active substance remaining on the husks/sand.

In the second method no active substance was used and instead a blank seed treatment containing a pigment was applied to wheat, barley, maize and sunflower seeds. A similar methodology to the first method was then adopted, where 20 wild-caught wood mice were given 6 g of treated seeds and the percentage reduction in exposure due to dehusking was calculated through analysing residues of the pigment on seed husks. The second method differed from the first in that it included a 16 h starvation period prior to exposure.

The results from the second study method showed that dehusking behaviour reduced the residue uptake most in sunflower seeds (mean reduction of 98.78% with a standard deviation ± 2.03%), followed by barley seeds (83.95 ± 9.28%) and wheat and maize seeds (58.04 ± 14.55% and 58.97 ± 13.08% respectively). Similar results were obtained for wheat and barley seeds using the first method, supporting the use of the pigment method. Data for individual wood mice is not presented, so the full range of dehusking values is not apparent, though standard deviation values have been quoted in addition to the mean.

The Brühl *et al*. (2011) study satisfies some of the criteria indicated in the EFSA guidance (EFSA, 2009) regarding the use of dehusking data in higher tier risk assessment. Data are available on the relevant focal species for metalaxyl-M (wood mouse). However, the study was not conducted under field conditions and there are a number of respects in which the motivation of test animals to feed may underestimate the field situation, which could then lead to overestimating the frequency or extent to which wood mice dehusk seeds under field conditions (on the assumption that where the motivation to feed is higher, dehusking is less likely to occur/will be less effective in reducing residues). In this study mice were housed individually (i.e. there was no competition for food), laboratory temperatures were warm (21.1-29.8 °C) and mice did not have to forage for food (both factors which may reduce their energy requirement). The starvation period in the second method may have artificially increased the motivation of mice to feed to some extent but it is noted that the majority of the starvation period occurred during the ‘light’ phase of the study and wood mice would be expected to be more active and predominantly consume food during the ‘dark’ phase. Importantly, it is also noted that none of the seeds used in this study were treated with metalaxyl-M and vining pea seeds were not included.

Defra (2010):

The applicant has provided detailed consideration of results from an investigation of dehusking of seeds by small mammals from work by **Defra (2010) PS2349**[29]. In this work dehusking behaviour was assessed for 10 captive bred wood mice for each of seven different seed types (wheat, maize, barley, pelleted sugar beet, peas, oilseed

[29] DEFRA PS2349 (2010): (http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=16264)

rape and beans). The purpose of this work was mainly to establish a suitable testing methodology for investigating dehusking behaviour, rather than to establish specific dehusking factors for risk assessment. Mice were housed individually in cages, with an acclimation period of at least one month (12 :12 reversed light /dark cycle and temperature approximately 18 °C). Each animal received seeds dressed with 319009 Eurogran Brilliant Blue FCF dye at the start of the 'dark' phase, with food withheld for the preceding 16 h. After 3 h seed has removed, and faecal samples were collected over a period of 3 days. The amount of dye recovered in faecal samples was used to estimate the amount ingested and the amount of dye remaining on seed husks was used to estimate the amount dehusked. The percentage of dye dehusked was estimated from the amount remaining on the husk and the total recovery of dye from the husk and faeces. These dehusking results are summarised in the following table.

Table B.9.1.6-18: Wood mouse dehusking data from DEFRA (2010) using food dye method

Crop	Mean percentage of dye dehusked	Range of dehusking percentages across individuals
Peas	89.4	74.2-97
Oilseed rape	41.7	0-81
Beans	66.7	36.1-88.9
Wheat	60.2	28.8-100
Sugar Beet	98.6	95.1-99.6
Barley	55.4	13.9-89
Maize	62.2	0-89.4

The mean percentage of dye dehusked varied significantly between crops and for some crops there was also high variability between individuals, with some individuals dehusking seeds to a great extent and others not at all.

As part of the work by Defra (2010) dehusking behaviour was also assessed for bank voles, using comparable methodology to that used for wood mice, though only wheat and barley seeds were tested. The mean percentage of dye dehusked for bank voles with barley sees was 46.7% (range 0-84.1%) and the mean percentage of dye dehusked for bank voles with wheat seeds was 27.6% (range 0-61.6%). Therefore, the bank vole results were similar to the wood mouse results for barley seeds but for wheat seeds dehusking behaviour reduced dye ingestion less for bank voles than it did for wood mice.

The Defra work involved seeds from seven different crops, including peas but it did not include seeds treated with metalaxyl-M or any other seed treatment (noting that the main aim of the study was to establish a suitable testing methodology rather than specific dehusking factors). The study was also not conducted under field conditions. Given the temperature, confined area to forage in and individual housing of test animals, conditions may underestimate the motivation of animals in the field to feed and hence potentially overestimate the prevalence and/or effectiveness of dehusking behaviour in reducing exposure. However, it is noted that a 16 h food deprivation period was observed prior to exposure and that in preliminary trials where the length of the food deprivation period was varied, this did not have a clear effect on dehusking behaviour.

Defra (2015):

Further work evaluating dehusking of treated seeds by wood mice is available in Defra (2015) PS2369[30]. Dehusking of barley and maize seed by wood mice (*Apodemus sylvaticus*) was assessed for five different seed treatments (five fungicides for barley and four fungicides and one insecticide for maize). The aim of the project was to generate a generic dehusking factor independent of the seed treatment for use in risk assessments. The study methodology adopted was similar to and based on that followed in [redacted] *et al*. (2011).

Captive bred adult wood mice were housed individually in cages, with 6 mice used for each crop/seed treatment combination (3 males, 3 females). Mice were housed under normal maintenance conditions with a mean temperature of 18°C under reverse daylight conditions; dark regime 09:00 – 21:00. There was 3-week acclimation period prior to study conditions and the seed type before the trial began. During this 3-week pre-trial period mice were presented on 3 occasions with the relevant seed in a terracotta dish (3 g for maize, 6 g for barley) for a 4 h exposure period, following a 16 h starvation period (to ensure mice were motivated to feed). This starvation period incorporated the 12-hour daylight period plus an additional four hours dark at the end of the night cycle. Each of these training periods in the pre-trial phase was separated by 7 days. The same methodology was used for the trial phase, though this time the seed was treated with the relevant plant protection product and the single exposure phase was 6 h in duration. At the end of the 6-hour exposure period, seed husks, whole and partially eaten seeds were recovered from the test cage and weighed together. For barley, residue analysis was performed on whole and partially eaten seeds, husk and seed debris recovered from the cage. With maize, residue analysis was performed on partially eaten seed, husk and seed debris; no whole seeds were analysed.

Under conditions of hunger and no choice, all wood mice ate barley and maize seeds treated with commercially available seed treatments with no mortalities or observed ill effects. The amount of presented food consumed ranged from 18-94% for maize seeds and 17.7-48.5% for barley seeds. For barley seeds mean dehusking factors saw a decrease in actual exposure compared with the nominal exposure to pesticide, giving dehusking factors below 1 (these ranged from below zero to 0.975, with a mean of 0.29). For maize seeds 2/5 seed treatments showed a decrease in actual exposure compared with the nominal exposure to the active substance, however, for 3/5 seed treatments dehusking factors were above 1 (i.e. there was an increase in actual exposure compared to nominal). Mean dehusking factors ranged from 0.703 to 1.669 for maize seeds, with a mean of 1.3. A dehusking factor of 1 indicates that the amount of residue consumed by the animal is equal to the nominal amount available

[30] DEFRA PS2369 (2015): http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=2&ProjectID=18266

on the food consumed. Dehusking factors above 1 can occur where the animal eats only part of the seed and the part eaten contains higher residues than the part remaining (e.g. the husk is preferentially consumed).

In this study it was not possible to tell whether the between-experiment variation was due to random experimental variation or caused by real differences in dehusking behaviour associated with different seed treatments. It was determined that the 90th percentile dehusking factor for 'a treatment' for barley seeds could lie between 0.71 and 1.30, depending on how much of the observed between-experiment variation is caused by different treatments. For maize seeds it was determined that the 90th percentile dehusking factor for 'a treatment' could lie between 1.57 and 1.9. It is noted that the dehusking factors calculated assume that any unrecovered residue has been consumed by the mouse. The authors concluded that due to the levels of uncertainty in dehusking factors for both barley and maize, it would be inappropriate to use a generic dehusking factor for risk assessment purposes.

[redacted] & [redacted] (2014):

This study was conducted to GLP and investigated seed consumption and dehusking behaviour by wood mice. Ten captive-bred wood mice were individually housed under laboratory conditions and presented with bare seeds (i.e. no seed treatment was applied). Seed consumption was assessed over a 3-day period and dehusking data were collected on the first day of exposure. Six seed types were evaluated in this study, though this did not include vining peas. Therefore, while the study contains information for the relevant focal species, it does not include the relevant seed type and did not involve treating seeds with Wakil XL or another metalaxyl-M formulation.

The primary focus of the study appears to be investigating seed consumption, with the data on dehusking more difficult to interpret. Based on the fact that vegetable seeds were consumed, and seed husks were found in the cages of all individual wood mice, it is apparent that all study mice dehusked vegetable seeds to some extent. There is also data on the weight of seed husks recovered from each cage, but this information is difficult to interpret in isolation. From the weight of seed husks recovered relative to the weight of seeds consumed, it is evident that mice dehusked seeds to a significant extent, but an exact quantitative proportion or range cannot be determined. Additionally, and more fundamentally, the data on dehusking in the [redacted] & [redacted] (2014) study only informs on the proportion of a seed that may be consumed by a wood mouse and the proportion that may be discarded. It does not provide information on the proportion of a seed treatment that is applied to the seed that a wood mouse would be exposed to. Mice may be exposed to a seed treatment during handling activities, so exposure to the seed treatment can occur even if the mouse discards the husk and consumes only the kernel. Therefore, without applying a treatment to the seeds used in this study (a pesticide product or proxy), the study only provides limited information regarding dehusking.

As with the above discussions of the Brühl *et al*. (2011) and Defra (2010 & 2015) studies, dehusking behaviour was assessed under laboratory conditions in [redacted] &

[redacted] (2014) and therefore the relevance of these data for field conditions is uncertain. Like the above studies mice were housed individually in confined areas, under relatively warm conditions. Additionally, there was no starvation period prior to exposure. Therefore, the [redacted] & [redacted] (2014) study may underestimate the motivation of wood mice to feed under field conditions and hence potentially overestimate the prevalence/extent of dehusking behaviour.

Barber *et al.* (2003):

This non-GLP public literature study investigated foraging behaviour and food consumption in wood mice and voles. Animals were trapped on arable fields drilled with winter wheat. Wheat seed was present in the stomach contents of 70-90% of individual wood mice sampled. Dehusked wheat seed capsules were found on the field where seed was treated with fluquinconazole (drain field) but no such capsules were found on the other study field (wood field), which was treated with bitertanol and fuberidazole. Residue analysis of the amount of fungicide in the stomach contents for those mice that had consumed seed from the drain field site provided support for this behavioural avoidance of the fungicide, since quantities of fluquinconazole in the stomachs of the mice were lower than expected based on the amount of seed consumed. Therefore, the study results provide evidence that dehusking behaviour does occur under field conditions but the prevalence of such behaviour among the local wood mouse population and the extent to which this reduces exposure to a seed treatment is not clear. The absence of dehusked wheat seed capsules on the wood field may indicate that the extent of dehusking behaviour is influenced by the plant protection product applied to the seed.

Use of dehusking data in the higher tier risk assessment for Wakil XL:

In the EU renewal review for metalaxyl-M the use of data on dehusking behaviour of wood mice in higher tier risk assessment for granivorous mammals was considered. The RMS originally accepted this as a quantitative refinement for higher tier TER calculations. The RMS used a dehusking factor of 0.01 for sunflower and 0.42 for spinach seeds, with these values being the sunflower seed and overall worst-case seed dehusking factors from the [redacted] *et al*. (2011) study respectively (i.e. the proportion of the residue on the seed consumed when accounting for dehusking behaviour). The use of this refinement was commented on by other MS and EFSA during the peer review stage of the EU review and the dehusking data were therefore discussed at an ecotoxicology expert meeting (Pesticides Peer Review Meeting 119). Ultimately the experts at this meeting concluded that the dehusking data did not support the use of a dehusking factor in the quantitative higher tier risk assessment but that the dehusking data could be used in the risk assessment in a qualitative manner as part of a weight of evidence approach.

The dehusking data now provided by the applicant is the same as that considered in the EU renewal review of metalaxyl-M. HSE has also considered updated work from Defra (2015). The same overall conclusion regarding the use of the dehusking data in higher tier risk assessment as was reached in the EU renewal review of metalaxyl-M is considered to apply here. Therefore, dehusking can be considered in a qualitative way as part of a weight of evidence approach but not as a quantitative factor in higher tier risk assessment. This is in line with the approach proposed by the applicant.

Regarding the qualitative use of the available data on dehusking behaviour by wood mice in the higher tier risk assessment for mammals consuming vining pea seeds, the following points are noted.

- The only data that directly considered dehusking behaviour of wood mice with pea seeds comes from the DEFRA (2010) study. Under the conditions of the study mice dehusked 89.4% of dye from pea seeds. The extent of dehusking of pea seeds between individuals varied between 74.2-97%. Therefore this study indicates that dehusking of pea seeds can greatly reduce wood mouse exposure to seed treatments but the study is subject to limitations (as discussed above).
- The Brühl *et al.* (2011), DEFRA (2015), [redacted] & [redacted] (2014) studies and other sources contain additional data on the dehusking behaviour of wood mice for other seed types but extrapolation of these data to pea seeds is uncertain. Use of such data in the risk assessment could lead to the real risk to small granivorous mammals from consumption of vining pea seeds being underestimated or overestimated.
- Data on dehusking behaviour are available for the relevant focal species the wood mouse. However, it must be considered whether a risk assessment for the focal species wood mouse that takes into account dehusking behaviour will also be protective of the risk to other small granivorous mammals that may consume pea seeds (e.g. bank voles), given these species may not dehusk or may dehusk to a more limited extent. Defra (2010) contains dehusking information for the bank vole, with the dehusking results for wood mice and bank voles being similar for barley seeds. However, for wheat seeds the reduction in exposure due to dehusking behaviour was greater for wood mice than for bank voles, indicating a wood mouse risk assessment for this crop that includes dehusking may not be protective of bank voles. Without specific information on dehusking of pea seeds by other small granivorous mammals it is unknown whether a wood mouse risk assessment that uses dehusking as a refinement is protective of other species. Therefore, extrapolation of dehusking results for pea seeds from wood mice to other small granivorous mammal species is uncertain. This uncertainty could lead to the real risk to these species being underestimated or overestimated.
- While information is available on dehusking behaviour from studies conducted under laboratory conditions, there is limited available data concerning dehusking behaviour under field conditions. Laboratory conditions may underestimate the motivation of small mammals to feed under field conditions and hence this could potentially overestimate the prevalence of dehusking behaviour and/or the extent to which such behaviour reduces exposure. Therefore, extrapolation of laboratory dehusking results to field conditions is uncertain and use of such data in risk assessment could underestimate the risk to small mammals consuming treated seed under field conditions.

- None of the studies investigating dehusking behaviour used seeds treated with the representative formulation Wakil XL or metalaxyl-M (or any of the other active substances in Wakil XL). Therefore, there is uncertainty in extrapolating results where a different active substance/product or pigment/dye proxy has been used to a risk assessment for metalaxyl-M. The Defra (2015) study found high variability in wood mouse dehusking factors between barley and maize seeds treated with different seed treatments and concluded that no dehusking factor for a generic ‘seed treatment’ could be identified for use in risk assessment. The dehusking results from the [redacted] *et al.* (2003) study also indicate that the plant protection product used to treat seeds may have a significant impact on whether and to what extent dehusking behaviour occurs. Therefore, extrapolating dehusking data between seed treatments could lead to the real risk being underestimated or overestimated.
- It should be noted that there is not an established, uniform method for calculating dehusking factors and there are differences in the calculation approaches used in the available studies. Therefore, comparing dehusking factors across studies where different calculation methods were used may not be appropriate.
- The EFSA guidance (Section 6.1.7) indicates that consideration of dehusking as a refinement may not be justified for situations where the LD50 is already reached with one or few seeds/particles. Assuming a bodyweight of 21.7 g for the wood mouse and using the EU agreed acute toxicity LD50 of 375 mg a.s./kg bw results in a revised LD50 for wood mouse of 8.14 mg a.s./mouse. Taking into account the standard assessment factor of 10 (to cover a range of uncertainties), this results in a regulatory acceptable dose per wood mouse (LD50/10) of 0.814 mg a.s./mouse. Considering the amount of active substance applied per seed, the number of seeds that would need to be consumed to exceed the LD50/10 has been calculated in the table below. From these results is apparent that the proposed seed treatment uses of metalaxyl-M on vining pea do not constitute situations where the LD50 or LD50/10 for the relevant focal species is reached with one or few seeds, though the number required to reach the LD50/10 is not very high.

Table B.9.1.6-19: Number of vining pea seeds required to exceed the LD50/10 for wood mouse

Crop	Nominal application rate (mg a.s./kg seed)	Seed weight (mg)	Loading per seed (mg a.s./seed)	Seeds required to exceed LD50/10
Metalaxyl-M	339.2	225	0.0763	10.7

Considering the above, there is evidence to indicate that dehusking behaviour can reduce exposure of wood mice to a seed treatment applied to pea seeds under controlled conditions. However, there are a number of factors that result in a high degree of uncertainty when extrapolating these dehusking results to the metalaxyl-M risk assessment. The above points will be taken into account when concluding on the acute risk to wood mice from consumption of treated vining pea seeds.

Lower formulation toxicity compared to predicted toxicity

The applicant has referred to a study of the acute toxicity of the formulation 'Wakil XL' (A9873C) to rats. A LD_{50} > 2000 mg/kg bw is reported from the formulation study ([redacted], 1998). The applicant has proposed that this result indicates that the formulation is less toxic than the predicted LD_{50} for the formulation, based on the active substance data and the assumption of additive toxicity. However, for this Article 7 evaluation only the risk from the metalaxyl-M component of the formulation is under consideration. The risk from the other active substances and the combined formulated product will need to be assessed at the product evaluation stage. It is noted that if the formulation study endpoint is expressed in terms of metalaxyl-M only (a potentially very conservative approach as it assumes metalaxyl-M is responsible for 100% of the toxicity seen in the formulation study), then the LD_{50} > 339.2 mg a.s./kg bw is potentially similar to the EU agreed endpoint for metalaxyl-M (LD_{50} = 375 mg a.s./kg bw).

Refined acute TER calculation

Refinements to the focal species and FIR/bw are accepted and used in revised higher tier calculations of the acute risk to mammals from consumption of treated seed in the table below. The proposed refinements to the composition of diet obtained from treated area are not accepted for use in the acute risk assessment and it is considered that no dehusking factor should be used in the quantitative risk assessment.

Table B.9.1.6-20: Acute risk to wood mice exposed to residues of metalaxyl-M via the consumption of treated seeds of vining peas

Focal species	FIR/bw	Nominal application rate (mg a.s./kg seed)	Daily dietary dose (mg a.s./kg bw/day)	LD_{50} (mg a.s./kg bw)	TER
Wood mouse	0.21	339.2	71.23	375	**5.26**

The resulting higher tier TER remains below the first tier trigger value of 10, indicating that it has not been demonstrated that there is no unacceptable risk. If the geometric mean LD_{50} from the rat and mouse data was used in the above calculation (LD_{50} = 433 mg a.s./kg), the resulting TER would be 6.08, i.e. still below the trigger value.

In table B.9.1.6-19 it has been calculated how many treated seeds a wood mouse would need to consume to exceed the acute regulatory dose (i.e. LD_{50}/10). This number is 10.7 seeds. This is considered a relatively small number of seeds that could conceivably be consumed by a wood mouse in a single feeding bout. No information is available on vining pea seed availability on the soil surface following drilling. Therefore, it is not possible to determine the area that would need to be foraged by a wood mouse to encounter 10.7 seeds on the soil surface. If, in the absence of data, it were assumed that all treated seed were available on the surface, then an area 0.11 m^2 would provide sufficient seeds (noting an assumption of 100% seed availability is unrealistic).

<u>Consideration of uncertainties in the risk assessment</u>

In order to determine whether it has been clearly established that there will be no unacceptable impact on mammals from the use of metalaxyl-M in the representative product Wakil XL, it is necessary to consider the main sources of uncertainty in the risk assessment. This is summarised in the tables below. The uncertainties have been considered in the context of the ‘surrogate’ protection goal of ‘*making any mortality unlikely*’, as specified in section 3 of the EFSA bird and mammal guidance document (EFSA, 2009). In these tables the +/- symbols indicate whether each source of uncertainty has the potential to make the true risk experienced by a realistic worst case individual higher (+) or lower (-) than the indicated outcome. The number of symbols provides a subjective relative evaluation of the magnitude of the effect.

Table B.9.1.6-21: Evaluation of the uncertainties in the refined acute risk assessment for mammals from consumption of treated vining pea seeds

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
Choice of focal species (effect on exposure) – wood mouse	0	Wood mouse is the most commonly recorded small mammal species foraging on post-drilling, pre-emergence crop field interiors across the field studies submitted. Data are not available for vining pea specifically but identification of a different focal species for post-drilling, pre-emergence vining pea fields is considered unlikely.	0	Wood mouse is the most commonly recorded small mammal species foraging on post-drilling, pre-emergence crop field interiors across the field studies submitted. Data are not available for vining pea specifically but identification of a different focal species for post-drilling, pre-emergence vining pea fields is considered unlikely.
Bodyweight – 21.7 g	0	Default tier 1 value from EFSA (2009). Similar to the mean wood mouse bodyweight of 18 g from Gurney *et al*. (1998).	+	Gurney *et al*. (1998) reports a mean wood mouse bodyweight of 18 g, with a range of 13-27 g, so actual body weight may be slightly lower, which would increase the FIR/bw and hence the risk.
Food intake rate calculation – energy and moisture content	-/0	Specific energy and moisture contents for vining pea seeds have not been used. Extrapolated values have been used for cereal seeds. It is uncertain whether these values represent worst case values or truly realistic values, i.e. the 'real' values for vining peas could be higher or lower than those used, hence	+/0	Specific energy and moisture contents for vining pea seeds have not been used. Extrapolated values have been used for cereal seeds. It is uncertain whether these values represent worst case values or truly realistic values, i.e. the 'real' values for vining peas could be higher or lower than those used,

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
		this could increase or decrease the risk.		hence this could increase or decrease the risk.
Loading rate on seed – 339.2 mg a.s./kg seed	-/0	Nominal loading rate, actual rate per seed could be variable.	+/0	Nominal loading rate, actual rate per seed could be variable.
Diet – 100% seeds	-	Literature studies indicate wood mice have mixed diets; therefore, the true risk could be lower, but these studies do not contain specific information on the food items taken from post-drilling, pre-emergence fields.	0	True risk cannot be higher.
Availability of untreated seeds – 0%	-	Actual feeding, even for the worst-case individual, may be a mix of treated seeds and other non-treated seeds from the natural seed-bank.	0	True risk cannot be higher.
Proportion of diet obtained from the treated area - 1	-/0	Radio-tracking data on post-drilling, pre-emergence arable fields with wood mice found daily PT values <1 for all individuals tracked but higher PT values than these would be expected for shorter durations. No data are available for vining peas specifically.	0	True risk cannot be higher.
Reduction in residues consumed due to	-/--	Dehusking has not been considered quantitatively. The risk may be reduced through de-husking, though it is likely to	+	Dehusking values above 1 were found for some active substances in Defra (2015).

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
dehusking – Dehusking factor = 1		be variable between and within a species. Feeding pressure may also reduce the degree of dehusking. Evidence that wood mice can dehusk a range seed types but the prevalence of this behaviour and the impact on exposure is unknown. Data are not available with Wakil XL treated seeds or under field conditions.		
Avoidance of treated seed - None	-/---	No data are available for mammals with metalaxyl-M treated seed. True contributions vary between species and pesticides. Could be negligible or could prevent mortality.	0	True risk cannot be higher.
Variation of toxicity between species	--	In general focal species could be up to 2 orders of magnitude less sensitive than standard species. In this case rats and mice were of similar sensitivity (rat LD_{50} = 375 mg a.s./kg bw, mouse LD_{50} = 500-1000 mg/kg bw), therefore less uncertainty extrapolating to wood mice is likely.	++	In general focal species could be up to 2 orders of magnitude more sensitive than standard species. In this case rats and mice were of similar sensitivity (rat LD_{50} = 375 mg a.s./kg bw, mouse LD_{50} = 500-1000 mg/kg bw), therefore less uncertainty extrapolating to wood mice is likely.
Variation of toxicity between	0	LD50 value used so representative of the central tendency of the sampled population.	+/+++	Most sensitive individuals could be two to ten times more sensitive than reported LD50.

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
individuals				
Uncertainty factor - 10	---	TER is compared with trigger value of 10.	0	Uncertainty factor cannot increase risk.
Overall assessment	**The refined exposure assessment replaces the first tier generic focal species with an appropriate focal species (wood mouse), an associated FIR/bw value. Other parameters in the refined TER equation are unchanged from the first tier. The refined assessment is considered to be representative of the risk to a realistic worst-case individual in an exposed population. Certain factors may reduce the risk, for example de-husking, however it is not possible to factor these into the risk assessment due to the uncertainties regarding the interpretation of the supporting studies.**			

Conclusion on acute risk to mammals consuming treated seed – Wakil XL

Regulation 1107/2009 requires that for authorisation of a plant protection product to occur it must be clearly established that use of this product will have no unacceptable impact on birds and mammals under field conditions. To this end the EFSA guidance document on bird and mammal risk assessment (EFSA) specifies two protection goals:

- An “actual” protection goal of “*clearly establishing that there will be no visible mortality and no long-term repercussions for abundance and diversity*.”
- A “surrogate” protection goal” of “*making any mortality or reproductive effects unlikely*”.

The first tier risk assessment is designed to satisfy the surrogate protection goal but at higher tiers either protection goal can be considered. In the case of metalaxyl-M the data and risk assessment provided do not allow for a consideration of the actual protection goal and therefore both the first and higher tier risk assessments are intended to satisfy the surrogate protection goal, i.e. to demonstrate that any mortality is unlikely following use of the representative formulation.

The first tier risk assessment for metalaxyl-M indicates an unacceptable acute risk to mammals consuming treated vining pea seeds (TER = 4.61 compared to the acceptability criterion of ≥ 10). Table 14 in Appendix C of EFSA (2009) specifies the following regarding the first tier acute risk assessment scheme for birds and mammals for seed treatments:

> ‘*Although conservative, the scheme appropriately represents the risk to the worst case individuals, therefore it may often be necessary to refine the assessment in order to assess the product against the ultimate protection goals of visible mortality and long term repercussions for abundance and diversity’*.

For the higher quantitative risk assessment the applicant proposed refining the PD parameter for the wood mouse focal species, to reflect a mixed diet. However, HSE does not consider refinement of the PD parameter to be appropriate for the acute risk assessment since sufficient exposure to result in mortality could come from consumption of a relatively small number of food items obtained during a short time period. It is also noted that the PD refinement was not accepted following the EU peer review process and does not appear in the EFSA conclusion for metalaxyl-M (EFSA, 2015). A higher tier acute TER has been calculated for the relevant focal species (wood mouse), assuming a diet of 100% peas seeds, with the resulting TER of 5.26 still below the first tier trigger value of 10.

Based on the data available, this TER is considered by HSE to be representative of the risk experienced by a realistic worst-case individual in an exposed population. Therefore, this assessment allows for consideration of whether the surrogate protection is addressed, i.e. whether it is clearly established that any mortality is unlikely. Given the TER value is below the trigger value of 10, it is considered that it does not demonstrate that any mortality is unlikely.

The applicant has also proposed using the available dehusking data with wood mice in the risk assessment as part of an overall weight of evidence approach. However, in the absence of specific data on dehusking of treated vining pea seeds by wood mice under field conditions, it cannot be clearly established that such behaviour would be effective in preventing mortality occurring. Therefore, there is a high degree of uncertainty associated with this line of evidence.

Additionally, the applicant has proposed that the following risk mitigation labelling statements are appropriate and should be taken into account.

- **STGD-S9**: To protect birds/wild mammals the seeds must be entirely incorporated in the soil; ensure that the seeds are also fully incorporated at the end of rows (except for seeds sown in greenhouses).
- **STGD-S10**: To protect birds/wild mammals remove spillage (except for seeds sown in greenhouses).
- **STGD-S11**: To protect birds/wild animals, treated seeds must be sown (i.e. covered by soil) by using seed drill equipment leaving less than x,xx % of the sown treated seeds on the soil surface (except for seeds sown in greenhouses).

The effectiveness of these measures in reducing the exposure and hence the risk to small mammals to an acceptable level has not been demonstrated. **HSE therefore concludes that it has not been clearly established that use of metalaxyl-M as a vining pea seed treatment will have no unacceptable impact on small mammals exposed to treated seed.**

B.9.1.6.3. Higher tier reproductive risk assessment for mammals

Higher tier risk assessment for the long-term/reproductive risk from treated vining pea seeds to mammals – Wakil XL

The following areas of the assessment were not demonstrated to result in acceptable long-term/reproductive risks to mammals and thus require further consideration:

- Long-term/reproductive risk to mammals from exposure to metalaxyl-M via consumption of seeds as food;
- Long-term/reproductive risk to mammals from exposure to metalaxyl-M via consumption of emerged seedlings.

Long-term/reproductive risk to mammals from exposure to metalaxyl-M via consumption of seeds as food

The applicant has proposed the following refinements to the risk assessment for granivorous mammals:

The refined risk assessment presented below is based on the following refinements:

1. Focal species
2. Seed residue dissipation data
3. PD refinement based on published literature data
4. PT refinement based on field studies

These proposed refinement are considered in turn below.

<u>Focal species</u>

As discussed for the acute assessment, the wood mouse is considered to be an appropriate focal species for exposure of mammals via consumption of treated vining pea seeds, though it is noted that it has not been fully justified for the proposed crop.

<u>Seed residue dissipation data</u>

The available data on dissipation of residues from seeds treated with metalaxyl-M are discussed above in the bird risk assessment section. It is concluded that a DT50 of 10 days and associated 21-day TWA of 0.53 can be used in the higher tier risk assessment.

<u>PD (composition of diet obtained from treated area)</u>

Applicant's proposal:

At first tier the risk assessment for mammals consuming treated seeds assumes that the diet of the generic focal species consists of 100% treated seeds, which is a worst-case assumption. The applicant has proposed refining the reproductive risk assessment for mammals consuming treated seeds by considering data on wood mouse diets. In their quantitative risk assessment, the applicant has used dietary data from 3 public literature studies: **Abt & Bock (1998)**, **Green (1979)** and **Pelz (1989)**.

In Green (1979) the diet composition of 53 wood mice (determined by stomach content analysis) caught on winter wheat fields between September and June in the UK was analysed. The study area contained arable fields (cereals, sugar beet, beans), with short length of hedgerow and was distant from other hedges and woods. The applicant has proposed using dietary information from this study for the spring and early summer period (April to June), which is presented in the table below. The number of mice for which stomach contents were sampled during this period was 15. A single percentage volume value for each dietary component is presented for each time period. Therefore, there is no information on the extent to which diets varied between individual wood mice. It is also noted that during the spring and early summertime period wood mice were caught on well grown winter wheat fields. Higher proportions of seed/endosperm in wood mouse stomach contents were reported for September to December (84%) and January to March (60%).

Table B.9.1.6-22: Wood mice diet in winter wheat fields given as percentage of stomach contents by volume (Green, 1979)

Time of the year (month)	Diet fractions				
	Arthropods	Leaf tissue	Cereal seeds	Grass flowers & green seeds	Weed seed
April to June	12	1	6	53	27

Another, multi-annual study was conducted on farmland in Germany (Pelz, 1989). The diet composition of wood mice in the study area over several years was evaluated by stomach content analysis (n = 346). The study area was extensively arable farmland (sugar beet, winter wheat, barley) with few hedges or wooded areas. The study report does not contain information on crop growth stages during the period over which dietary compositions were assessed. The dietary results from this study are presented in terms of the volume composition of stomach contents. The applicant has proposed using dietary information from this study for the March to May period (presented in the table below). It is noted that the proportion of cereal and weed seed in the diet was higher at other times in the study, e.g. June to August. Mean values are presented, with no information in the study report on variability in stomach contents between individuals.

Table B.9.1.6-23: Wood mice diet on arable farmland given as percentage of stomach volume, pooled data (Pelz, 1989)

Time of the year (month)	Diet fractions			
	Animal matter	Green plant tissue	Cereal seeds	Weed seeds
Mean March to May	58.9	20	17.2	4.1

Dietary data are also available from a study on mixed farmland in northern Germany by Abt & Bock (1998). The diet composition of wood mice living at field margins was determined between March and December (semi quantitative faeces analysis). The test field where trapping took place was initially tilled with winter-rye, which was harvested in July. Subsequently, the grass *Lolium perenne* was sown and cut for the first time in October. The date when drilling on winter rye took place is not specified and no information is available on the growth stage of the crop during each month. Field surroundings were forest, grassland and a maize field, with hedges on all boundaries except one. A total of 83 faecal samples were obtained from 55 wood mice. The applicant has proposed using dietary fractions averaged across the March-May period in the risk assessment. It is noted that the proportion of cereal seed in the diet was highest in June-September. No individual level data are available, so variability in wood mouse diets is unknown.

Table B.9.1.6-24: Wood mice diet on arable farmland given as semi quantitative proportions of faeces analyses (Abt & Bock, 1998)

Time of the year (month)	Diet fractions			
	Animal matter	Veg. Pant tissue	Other seeds	Cereal seeds
Mean March to May	12.2	47.6	34.7	6.1

Table B.9.1.6-25: Average food of wood mice for seed treatments (mean March to May, based on appropriate literature data)

Reverence	Animal matter [%]	Green plant matter [%]	Cereal seeds [%]	Weed seeds [%]
Green, 1979*	12	27.5	19.3	40.3
Pelz, 1989	58.9	20	17.2	4.1
Abt & Bock, 1998	12.2	47.6	6.1	34.7
Mean	**27.7**	**31.7**	**14.2**	**26.4**

*Grass flowers & green seeds data has been divided equally between green plant material and seed categories

Results from the **Barber et al. (2003)** study were also considered to support the above dietary results. In this study wood mice were caught in and around freshly drilled autumn cereal fields and their stomach contents were analysed. The maximum proportion of wood mouse stomach contents that constituted wheat seeds was 40%, with most individuals having <20% wheat seeds in their stomach contents. However, the other food items found in wood mice stomachs were not reported and the impact of differences in digestibility between different food items was not taken into account.

The applicant has used the three PD datasets described above from the Green (1979), Pelz (1989) and Abt & Bock (1998) studies to determine the daily food consumption for the individual dietary components. To do this they have assumed that an equivalent proportion of vining pea seeds are consumed as cereal seeds in the study data. The green plant material has been set as dicot leaves and animal material is taken to represent arthropods.

Table B.9.1.6-26: Calculation of daily dietary consumption for the wood mouse (21.7 g bodyweight) feeding on peas as part of a mixed diet

Food type	Energetic content of food [a)]	Assimilation efficiency [b)]	Energetic content of food, weighted by assimilation efficiency	Proportion of different food items in diet mix	Energy uptake per gram of diet mix [c)]	DEE	Daily food consumption [d)]
	(kJ/g wet wt)	(%)	(kJ/g wet wt)	(% of diet wet weight)	(kJ/g wet wt)	(kJ)	(g wet wt/day)
Peas	15.70	84	13.18	14.2	1.87		0.99
Weed seeds	19.55	84	16.42	26.4	4.33		1.84
Non-grass herbs	2.12	76	1.61	31.7	0.51		2.21
Arthropods	7.08	87	6.16	27.7	1.71		1.93
Total					8.42	58.83	

a) Taken from Appendix L, Table 19 of the Guidance Document on Risk Assessment for Birds and Mammals

b) Taken from Appendix L, Table 2 of the Guidance Document on Risk Assessment for Birds and Mammals

c) Calculated as Energetic content of food, weighted by assimilation efficiency x proportion of different food items in diet mix/100

d) Calculated as (DEE ÷ Total energy uptake per gram of diet) x Proportion of different food items in diet mix

Use of dietary data in the higher tier risk assessment for Wakil XL:

Regarding treatment of vining pea seeds with metalaxyl-M, based on advice from a HSE efficacy expert, it is anticipated that in GB vining pea seeds would generally be drilled from early February to mid-April. Therefore, while there is overlap with the time period considered by the applicant using data from the Green (1979), Pelz (1989) and Abt & Bock (1998) studies, the assessed period does not fully cover the timespan when treated vining pea seeds may be available to granivorous/omnivorous mammals. Therefore, there is uncertainty as to whether the data proposed by the applicant are fully representative of the expected period when exposure could occur.

It is noted that the Green (1979), Pelz (1989) and Abt & Bock (1998) studies were previously referred to in the dRAR for metalaxyl-M by the RMS and were used to support a refined diet for the focal species wood mouse in post-sowing, pre-emergence sunflower/spinach fields (noting vining peas were not considered). However, this PD refinement was not accepted following the peer review process and does not appear in the EFSA conclusion for metalaxyl-M (EFSA, 2015). The expert meeting report where the higher tier mammal risk assessment for metalaxyl-M was discussed (Pesticides Peer review Meeting 119) concluded that the majority of experts agreed that no PD refinement was possible, considering the available data, and that therefore the wood mouse diet used in the reproductive risk assessment should consist of 100% contaminated seeds.

Section 5.2.3 of the guidance document on bird and mammal risk assessment (EFSA, 2009) specifies that additional care is needed when refining ecological parameters for the risk assessment from treated seed. It goes on to state that:

> '*Simple dietary assessments assume that food obtained on treated fields follows the same dietary composition as measured for the general population in all habitats. This will probably underestimate the intake of crop seed for animals feeding on newly drilled fields. Therefore, the conservative assumption of taking only treated seed should be retained unless there is specific data on the foods taken on relevant fields.*'

The Green (1979), Pelz (1989) and Abt & Bock (1998) studies contain information on wood mice diets in arable landscapes. However, none of these studies include data on food items taken from post-drilling, pre-emergence fields specifically. Therefore, the studies referenced by the applicant provide information on the dietary composition of the general population of wood mice but do not contain specific information on the food items taken in fields sown with vining pea seeds or other post-drilling, pre-emergence arable fields. Additionally, in all three studies only average dietary composition data are presented, so the degree of variability in diets between individuals remains unknown. Further data are available in Barber et al. (2003) from freshly drilled cereal fields but the information available in this study does not provide a complete picture of wood mouse diets in such fields. Finally, it is noted that different food items will vary in terms of how easily they are digested by wood mice, which can impact dietary intake estimates if not appropriately adjusted for. This issue has been considered to varying extents in the Green (1979), Pelz (1989), Abt & Bock (1998) and Barber et al. (2003) studies but uncertainty remains regarding this point in all studies. In light of these points and the guidance document recommendations, a diet of 100% treated seeds will be used in the higher tier reproductive risk assessment. However, the likelihood that the food items obtained by wood mice from drilled vining pea fields constitute <100% treated seeds will be considered when discussing the uncertainties and level of conservatism in the risk assessment.

FIR/bw

As discussed for the acute risk assessment, a refined FIR/bw of 0.21 can be used in the higher tier risk assessment for exposure via the consumption of treated vining pea seeds. This value is for the wood mouse focal species and assumes a diet of 100% treated seed.

PT (proportion of diet obtained from the treated area)

Available datasets:

The first tier risk assessment assumes that PT equals 1.0, i.e. the mammal obtains all its food from the treated crop. However, it is likely that the focal species will not feed exclusively in vining pea fields – part of its diet being obtained in other crops/habitats (i.e. PT < 1.0). Current EFSA guidance refers to the possibility of conducting mammal behaviour field studies in which the use of radio-tracking, together with observational information, is used to estimate active time spent foraging in different crops – which can then be directly extrapolated to the proportion of diet obtained in these crops (enabling an estimate of PT to be derived).

The applicant has proposed refining the proportion of diet wood mice obtain from the treated area (PT) using data from the study by [redacted] **et al. (2013)**. This study involved live trapping, scan sampling and radio-tracking of small mammals in and around maize fields. The study was conducted in a typical maize growing region in south-western Germany in the federal state of Rhineland-Palatinate. The applicant has proposed that since maize and pea seeds are both considered as 'large' seeds in the EFSA bird and mammal guidance document (EFSA, 2009), data from freshly drilled maize fields are representative for pulses as well.

The PT data from [redacted] et al. (2013) is briefly summarised in the table below. In total 17 tracking sessions of 14 individuals of wood mouse were conducted in freshly germinated maize fields (on days 2-18 after emergence; BBCH 10-16). 13 sessions contained wood mice with maize in their home range. The 9 tracking sessions of the "consumer only" group comprised 7 individuals (8 of their sessions were conducted on the same plot No. 2).

Table B.9.1.6-27: PT values for wood mouse by individuals (BBCH 10-16)

Study	Wood mouse individual No.	PT by session [%]	PT by individual [%]
[redacted] *et al.*,	3	3.4	3.4

2013	7	4.5	2.6
	7	0.8	
	8	6.8	6.8
	9	13.9	13.9
	10	2.8	3.0
	10	3.1	
	13	1.3	0.7
	13	0.0	
	14	0.4	0.4

The PT dataset from [redacted] et al. (2013) is relatively small, including only 7 consumers. It is questionable whether this dataset is of sufficient size to refine the PT parameter, given the variability in this value expected between individuals and locations. While a case could be made to use the maximum individual PT of 13.9% in the higher tier risk assessment, the relevance of this study for the metalaxyl-M risk assessment must first be considered.

The following key differences are noted between the proposed representative uses of Wakil XL and the [redacted] et al. (2013) study:

- Wakil XL is used to treat vining pea seeds, whereas the [redacted] et al. (2013) study investigated foraging behaviour in maize fields.
- Wood mice may be exposed to metalaxyl-M immediately following drilling of crop seeds (i.e., pre-emergence of the crop), whereas the [redacted] et al. (2013) study investigated foraging behaviour in early post-emergence crop fields.
- The expected GB drilling period for vining pea seeds is from early February to mid-April, whereas radio-tracking sessions in [redacted] et al. (2013) took place almost exclusively in May (there was a single session in June).

In addition to the [redacted] et al. (2013) study, the applicant has access to other radio-tracking studies that can be considered. Unfortunately there are no studies where wood mice were tracked on pre-emergence vining pea fields, though there are a number of studies where wood mice were tracked on cereal fields. These are discussed below.

In the EU renewal review of metalaxyl-M, the RMS evaluated two field studies, which investigated the behaviour of wood mice in cereal fields – [redacted] **(2006)** and [redacted] **(2008)**. The summaries of these studies from the EU review are reproduced in section 9.1.4. Use of these studies in the reproductive risk assessment for wood mice via consumption of treated seeds was discussed at the Pesticides Peer Review Meeting 119. These studies were conducted in pre-emergence and post-emergence cereal fields in Germany in spring and summer. While it was questioned whether extrapolation of these data to the representative seed treatment uses of metalaxyl-M was appropriate, it was ultimately agreed at this meeting that given the growth stage in [redacted] (2008) was bare soil, that a PT value from this study could be derived and used in the reproductive risk assessments for uses on sunflower and spinach seeds. Based on the information available and given the small number of consumer individuals tracked, the experts agreed to use a 90th percentile consumer PT value of 0.51 in the higher tier reproductive risk assessment. However, it is noted that this value did not come from either the [redacted] (2006) or [redacted] (2008) studies but from UK DEFRA radio-tracking data PS2328 (**DEFRA, 2009**). The 0.51 figure comes from a wood mouse radio-tracking dataset for pre-emergence winter cereal fields. This 0.51 figure is the 90th percentile PT values for the consumer population (n = 12).

Two additional studies investigating the behaviour of wood mice in freshly-drilled cereal fields have previously been submitted by the applicant – [redacted] **&** [redacted] **(2016)** and [redacted] **&** [redacted] **(2017)**. These studies were not considered in the EU renewal review of metalaxyl-M and are therefore reviewed by HSE. Therefore, in total 5 radio-tracking datasets for wood mice in pre-or post-emergence cereal fields are available. These are summarised in the table below, alongside the [redacted] et al. (2013) study.

Table B.9.1.6-28: Summary of the wood mouse radio-tracking datasets

Source	Crop	Timing of tracking sessions	Multiple tracking sessions for individuals?	Number of consumers	Consumer PT values
DEFRA (2009)	Winter cereals, pre-emergence	Autumn*	No	12	0.00-0.77 90th percentile = 0.51
[redacted] (2006)	Winter cereals, post-emergence (BBCH 30-90)	May 4th to August 1st	No	15	0.1-1 Mean = 0.885 90th percentile = 1
[redacted] (2008)	Spring cereals, pre-emergence	April 12th-21st	Yes	4	0.071-0.285
[redacted] & [redacted] (2016)	Spring cereals, pre-emergence	April 13-30th	Yes	6	0.001-0.236
[redacted] & [redacted] (2017)	Winter cereals, pre-emergence	October 10th to November 6th	Yes	7	0.001-0.612
[redacted] et al. (2013)	Maize, post-emergence (BBCH 10-	May 9th to June 1st	Yes	7	0.004-0.139

	16)				

*Insufficient information in report to be precise - data predominantly come from autumn but it may include some spring data too

In addition to the above radio-tracking data, information on wood mouse diets from the [redacted] **et al. (2003)** may also be relevant for the consideration of the proportion of diet obtained from the treated area. This is a published literature paper that is not GLP compliant and can therefore only be used as supporting information. [redacted] *et al*. (2003) was conducted on freshly drilled winter cereal fields in October and among other factors, the stomach contents of wood mice trapped in the study area were investigated. Of wood mice caught on wheat fields, 70-90% had some wheat seed in their stomachs, though for 90% of wood mice trapped on these fields wheat seeds constituted <25% of the stomach contents (by area). The maximum recorded amount of wheat seeds as a percentage of total stomach contents for any individual wood mouse was 40%. However, as discussed in the summary of this study, there is uncertainty extrapolating data on the wheat seed content of stomachs to the proportion of diet obtained from freshly drilled fields.

Extrapolation of datasets to the risk assessment scenario:

Of the 6 available radio-tracking datasets, 4 of these were conducted in pre-emergence cereal fields, 1 was conducted in post-emergence cereal fields (BBCH 30-90) and one in early post-emergence maize (BBCH 10-16). Given the scenario under consideration for the higher tier risk assessment is wood mice consuming treated seed, the pre-emergence data closer matches this scenario. Due to the relatively late growth stage of the crop during the study (BBCH 30-90), the [redacted] (2006) study is considered of limited relevance for the risk assessment scenario and may overestimate exposure of wood mice. Therefore the [redacted] (2006) study is not considered further.

Of the pre-emergence cereal studies, two of the remaining radio-tracking datasets were conducted in freshly drilled spring cereal fields ([redacted], 2008; [redacted] & [redacted], 2016) and two were conducted in freshly drilled winter cereal fields (Defra, 2009; [redacted] & [redacted], 2017). In both spring and winter cereal fields wood mouse PT values were generally low, even amongst the consumer subset of the population. However, a small number of individual wood mice had higher PT values. The highest individual PT from the studies with winter cereals were higher than the equivalent values from the studies with spring cereals. Therefore, potentially freshly drilled winter cereal fields in autumn are a more attractive foraging habitat for wood mice than freshly drilled spring cereal fields in spring, noting that population densities in the spring may be lower and hence be reflected in lower numbers occurring. However, given the size and variability of the datasets, no definitive conclusion can be reached on this point. While using a different methodology, the stomach content data from Barber et al. (2003) is broadly consistent with the PT values from the pre-emergence winter cereal radio-tracking datasets (though extrapolating data on the proportion of wheat seeds in wood mouse stomachs to inform on the proportion of diet obtained from treated fields is uncertain).

It is noted that while all of the available pre-emergence PT data is for cereal fields, the proposed use of metalaxyl-M being considered is the treatment of vining pea seeds. Therefore, it must be considered whether it is appropriate to extrapolate PT data from pre-emergence cereal fields to pre-emergence vining pea fields. This extrapolation can be considered in terms of vegetation cover, timing of drilling, and the food items available.

1. Vegetation cover – Minimal differences would be expected between freshly drilled cereal fields and freshly drilled vining pea fields.
2. Time of drilling – A HSE efficacy specialist has indicated that in GB vining pea seeds are generally drilled from early February to mid-April. The available studies overlap with the later period of drilling but do not include February or March. It is difficult to know how significant these differences in the timing of the tracking period could be without additional data. Changes in timing could result in differences in food availability in areas surrounding drilled fields, which in turn could impact wood mouse foraging behaviour (e.g. if less food is available outside the field, more time may be spent foraging within the field). Alternatively, wood mouse populations would be expected to be lower in abundance in late winter than in spring, hence during late winter there may be less competition for optimal territory and hence a lower likelihood of mice foraging within freshly drilled fields.
3. Food items available and their attractiveness – The amount of green plant material available would be expected to be very low in fields freshly drilled with cereal or vining pea seeds. There is no basis to indicate that there would be significant differences in arthropod communities or weed seed availability in freshly drilled cereal fields compared to freshly drilled pea fields. However, the attractiveness of the crop seed itself as a food source for wood mice could vary between seed types. This point therefore requires further consideration. In a study by [redacted] et al. (2004), wild caught wood mice were provided with different crop seeds for 6 hours during their peak activity period. No alternative food was provided during this period, with 5 males and 5 females used per seed type. The amount of seeds consumed per individual was investigated by measuring food bowl weight change (accounting for spillage). The mean weight of pea seeds consumed was 0.58 g, compared to 1.27 g for wheat seeds. The individual maximum seed consumption was 1.3 g for pea and 2.6 for wheat. Therefore, there is no indication of pea seeds being a more attractive food source than wheat seeds for wood mice, based on the findings of the [redacted] (2004) study.

In light of the above points, it is considered reasonable to extrapolate wood mouse radio-tracking data from freshly drilled cereal fields to freshly drilled vining pea fields. It is acknowledged that there is some uncertainty in this approach, primarily relating to differences in the time of drilling for pea seeds sown early in the year, however, the available radio-tracking datasets for freshly-drilled cereals do cover a range of times of year. It is noted that in the EU renewal of metalaxyl-M, extrapolation of wood mouse radio-tracking data from freshly drilled cereal fields to freshly drilled sunflower and spinach fields was also accepted.

Use of radio-tracking datasets to refine the PT parameter:

The Defra (2009), [redacted] (2008), [redacted] & [redacted] (2016) and [redacted] & [redacted] (2017) wood mouse radio-tracking datasets are considered are potentially relevant for deriving a PT value for use in the higher tier risk assessment for wood mice consuming vining pea seeds treated with metalaxyl-M. In order to conclude on how to use these datasets in the refined risk assessment, a number of factors require consideration.

- Scope for refinement of the default PT value of 1 – In the available studies, no tracked individuals spent 100% of their potential foraging time in pre-emergence cereal fields, and the majority of individuals spent a low proportion of time foraging in such habitats. These results are also supported by the trapping and dietary findings of Barber *et al*. (2013). It is therefore apparent that freshly drilled cereal fields are not extensively foraged by the majority of wood mice in local populations and that the assumption that such fields are extensively foraged could overestimate exposure within the risk assessment. Therefore, taking into account more realistic foraging behaviour from studies conducted under field conditions in the higher tier risk assessment is appropriate.
- Relevant population to consider – The higher tier risk assessment can consider the protection goal of determining whether any reproductive effects are unlikely following use of the plant protection product. To do this it is considered appropriate for the higher tier risk assessment to address the risk to individuals that are potentially exposed to metalaxyl-M. The 'consumer' population can be exposed to metalaxyl-M as these individuals are known to forage within freshly drilled crop fields.
- Multiple tracking sessions for some individuals - In the [redacted] (2008), [redacted] & [redacted] (2016) and [redacted] & [redacted] (2017) studies some individual wood mice were tracked across multiple sessions, with the number of tracking sessions varying between individuals. If all tracking sessions were to be included unadjusted when determining an appropriate PT value for use in the risk assessment, then this would potentially over-represent certain individuals in the PT dataset (i.e. those individuals tracked for the most sessions). To account for this point HSE has used an arithmetic mean PT value across tracking sessions for each individual with multiple tracking sessions. However, it is acknowledged that the dependence/independence of these data points is unclear and there is uncertainty over whether this is an appropriate method for taking account of variability in PT for the same individual over time.
- Variability between individuals – While PT values for consumer wood mice are generally low in all of the available radio-tracking studies, there is high variability in this parameter across individuals in each study. In [redacted] (2008) consumer PT values ranged from 0.071-0.285, in [redacted] & [redacted] (2016) they ranged from 0.001-0.263, in [redacted] & [redacted] (2017) they ranged from 0.001-0.612 and in Defra (2009) they range from 0.00-0.77. Therefore, if a mean PT value were determined from each study across

consumer individuals it would potentially underestimate exposure for a significant section of the local consumer population at that site.

- Study methodologies and locations – The [redacted] (2008), [redacted] & [redacted] (2016) and [redacted] & [redacted] (2017) studies adopted broadly comparable methodologies, with wood mice tracked continuously from dusk until dawn in each tracking session. The Defra (2009) study methodology differed, with data from multiple tracking sessions used to determine a dawn to dusk PT value for each individual. The [redacted] (2008), [redacted] & [redacted] (2016) and [redacted] & [redacted] (2017) studies were conducted in Germany, while the Defra (2009) study was conducted in the UK.
- Size of the datasets – The size of the sampled consumer population varied between studies – 12 in Defra (2009), 7 in [redacted] & [redacted] (2017), 6 in [redacted] & [redacted] (2016) and 4 in [redacted] (2008). HSE considers that a mean or 90th percentile PT value cannot be reliably estimated for a population from a sampled dataset of <10 consumer individuals, given the variability in this parameter.
- Potential to combine multiple datasets – As discussed above, there is some indication that pre-emergence winter cereal fields in autumn may be a more attractive foraging habitat than pre-emergence spring cereal fields in spring for wood mice, but a definitive conclusion can’t be reached on this point. Therefore, it is not clear whether it is appropriate to merge the spring and winter cereal datasets. Given the difference in study design it is also not clear if it is appropriate to merge winter cereal datasets from the [redacted] & [redacted] (2017) and Defra (2010) studies. Additionally, since the available radio-tracking datasets have different numbers of consumer individuals sampled, any approach in which multiple datasets are merged risks giving undue weight to the datasets with the higher number of consumers.

In the EU renewal review of metalaxyl-M it was agreed by MS to use the 90th percentile PT value of 0.51 from the Defra (2009) dataset on pre-emergence winter cereal fields in the higher tier risk assessments for wood mice consuming treated spinach and sunflower seeds. This is the 90th percentile value for the consumer population, from a study conducted at a time of year when PT values were highest. While the crop under consideration here is different, vining peas, it is considered reasonable to extrapolate radio tracking data from pre-emergence cereal fields to pre-emergence pea fields (noting there is uncertainty associated with this extrapolation). Additional datasets are now available, but these contain small sample sizes for consumer individuals, and it has not been sufficiently established that it is appropriate to merge the datasets from these studies. The results from the [redacted] & [redacted] (2017) study are in line with those from Defra (2009) and the datasets from pre-emergence spring cereal fields ([redacted], 2008; [redacted] & [redacted], 2016) indicate lower PT values than Defra (2009). Therefore, it is considered appropriate to use a PT value of **0.51** in the higher tier reproductive risk assessment for wood mice consuming treated vining pea seeds.

Dehusking

The available evidence regarding dehusking behaviour in wood mice is discussed in the higher tier acute risk assessment section for mammals consuming treated seed (see above). It is concluded that there is evidence to indicate that dehusking behaviour can reduce exposure of wood mice to a seed treatment applied to pea seeds. However, there are a number of factors that result in a high degree of uncertainty when extrapolating these dehusking results to the metalaxyl-M risk assessment. The points raised in the discussion in the acute section are also relevant for the reproductive risk to wood mice from consumption of treated vining pea seeds. As for the acute risk, it is considered that dehusking behaviour can be considered as part of a weight-of-evidence, but should not be used to refine the quantitative risk assessment.

Refined reproductive TER calculation

Refinements to the focal species, FIR/bw, PT and TWA are accepted and used in revised higher tier calculation of the reproductive risk to mammals from consumption of treated seed in the table below. The proposed refinements to the composition of diet obtained from treated area are not accepted for use in the reproductive risk assessment and it is considered that no dehusking factor should be used in the quantitative risk assessment.

Table B.9.1.6-29: Reproductive risk to wood mice exposed to residues of metalaxyl-M via the consumption of treated seeds of vining peas

Focal species	FIR/bw	Nominal application rate (mg a.s./kg seed)	PT	TWA	Daily dietary dose (mg a.s./kg bw/day)	NOAEL (mg a.s./kg bw)	TER
Wood mouse	0.21	339.2	0.51	0.53	19.25	96	**4.99**

The resulting higher tier TER value for the vining pea use is almost exactly equal to the standard first tier trigger value of 5.

Consideration of uncertainties in the risk assessment

In order to determine whether it has been clearly established that there will be no unacceptable impact on mammals from the use of metalaxyl-M in the representative product Wakil XL, it is necessary to consider the main sources of uncertainty in the risk assessment. This is summarised in the tables below. The uncertainties have been considered in the context of the 'surrogate' protection goal of '*making any reproductive effects unlikely*', as specified in section 3 of the EFSA bird and mammal guidance document (EFSA, 2009). In these tables the +/- symbols indicate whether each source of uncertainty has the potential to make the true risk experienced by a realistic worst case individual higher (+) or lower (-) than the indicated outcome. The number of symbols provides a subjective relative evaluation of the magnitude of the effect.

Table B.9.1.6-30: Evaluation of the uncertainties in the refined reproductive risk assessment for mammals from consumption of treated vining pea seeds

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
Choice of focal species (effect on exposure) – wood mouse	0	Wood mouse is the most commonly recorded small mammal species foraging on post-drilling, pre-emergence crop field interiors across the field studies submitted. Data are not available for vining pea specifically but identification of a different focal species for post-drilling, pre-emergence vining pea fields is considered unlikely.	0	Wood mouse is the most commonly recorded small mammal species foraging on post-drilling, pre-emergence crop field interiors across the field studies submitted. Data are not available for vining pea specifically but identification of a different focal species for post-drilling, pre-emergence vining pea fields is considered unlikely.
Bodyweight – 21.7 g	0	Default tier 1 value from EFSA (2009). Similar to the mean wood mouse bodyweight of 18 g from Gurney *et al*. (1998).	+	Gurney *et al*. (1998) reports a mean wood mouse bodyweight of 18 g, with a range of 13-27 g, so actual body weight may be slightly lower, which would increase the FIR/bw and hence the risk.
Food intake rate calculation – energy and moisture content	-/0	Specific energy and moisture contents for vining pea seeds have not been used. Extrapolated values have been used for cereal seeds. It is uncertain whether these values represent worst case values or truly realistic values, i.e. the ‘real’ values for vining peas could be higher or lower than those used, hence	+/0	Specific energy and moisture contents for vining pea seeds have not been used. Extrapolated values have been used for cereal seeds. It is uncertain whether these values represent worst case values or truly realistic values, i.e. the ‘real’ values for vining peas could be higher or lower than those used, hence

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
		this could increase or decrease the risk.		this could increase or decrease the risk.
Loading rate on seed – 170, 339 and 678 mg a.s./kg seed	-/0	Nominal loading rate, actual rate per seed could be variable.	+/0	Nominal loading rate, actual rate per seed could be variable.
Dissipation and degradation of active substance of seeds – DT50 = 10 days	-/0	The DT50 value is representative of metalaxyl-M residue decline data averaged across unprotected and protected sites from [redacted] (2012) and is also representative of data from [redacted] (2021a&b). There is uncertainty due to extrapolating between seeds, products and timings. It is feasible that this value will vary depending on soil and climatic conditions. The 'real' DT50 and the associated risk could be smaller.	+/0	The DT50 value is representative of metalaxyl-M residue decline data averaged across unprotected and protected sites from [redacted] (2012) and is also representative of data from [redacted] (2021a&b). DT50s obtained at some individual sites were higher than 10 days, though this was where the data did not follow first order kinetics. There is uncertainty due to extrapolating between seeds, products and timings. It is feasible that this value will vary depending on soil and climatic conditions. The 'real' DT50 and the associated risk could be greater.
Averaging period for exposure calculation – 21 days	-/0	Period of exposure responsible for effects seen in the toxicity studies is unknown. Seeds may germinate in less than 21 days. Therefore the 'real' risk could be overestimated as seeds may	+/0	Period of exposure responsible for effects seen in the toxicity studies is unknown. Seeds may germinate in less than 21 days. However, if the focal species also consumes seedlings then

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
		not be available for the full period.		the ‘real’ risk could be higher.
Diet – 100% seeds	-	Literature studies indicate wood mice have mixed diets; therefore, the true risk could be lower, but these studies do not contain specific information on the food items taken from post-drilling, pre-emergence fields.	0	True risk cannot be higher.
Availability of untreated seeds – 0%	-	Actual feeding, even for the worst-case individual, may be a mix of treated seeds and other non-treated seeds from the natural seed-bank.	0	True risk cannot be higher.
Proportion of diet obtained from the treated area – 0.51	-/0	Value used is a 90th percentile for the consumer population. Many individuals will forage within the treated area to a lesser extent.	+/0	Value used is a 90th percentile for the consumer population. Some individuals may forage in the treated area to a greater extent.
Reduction in residues consumed due to dehusking – Dehusking factor = 1	-/--	Dehusking has not been considered quantitatively. The risk may be reduced through de-husking, though it is likely to be variable between and within a species. Feeding pressure may also reduce the degree of dehusking. Evidence that wood mice can dehusk a range seed types but the prevalence of this behaviour and the impact on exposure is unknown. Data are not	+	Dehusking values above 1 were found for some active substances in Defra (2015).

Parameter, assumption or omission	Potential for true risk to be lower	Explanation	Potential for true risk to be higher	Explanation
		available with Wakil XL treated seeds or under field conditions.		
Avoidance of treated seed - None	-/---	No data are available for mammals with metalaxyl-M treated seed. True contributions vary between species and pesticides. Could be negligible or could prevent mortality.	0	True risk cannot be higher.
Variation of toxicity between species	---	Focal species could be up to 2 orders of magnitude less sensitive than standard species.	+++	Focal species could be up to 2 orders of magnitude more sensitive than standard species.
Variation of toxicity between individuals	-/0	Lowest NOAEL used from the available reproductive and developmental toxicity studies. NOAEL is influenced by dose spacing. The 'real' endpoint could be higher, hence risk lower.	+/++	Sensitive individuals could experience effects at exposure levels below the reported NOAEL since this is an average value.
Uncertainty factor - 5	---	TER is compared with trigger value of 5.	0	Uncertainty factor cannot increase risk.
Overall assessment	**The refined exposure assessment replaces the first tier generic focal species with an appropriate focal species (wood mouse), an associated FIR/bw vale, a refined DT50 value and a refined fraction of diet obtained from the treated area (PT). Other parameters in the refined TER equation are unchanged from the first tier. The refined assessment is considered to be representative of the risk to a realistic worst-case individual in an exposed population. Certain factors may reduce the risk, for example de-husking, however it is not possible to factor these into the risk assessment due to the uncertainties regarding the interpretation of the supporting studies.**			

Conclusion on reproductive risk to mammals consuming treated seed

Regulation 1107/2009 requires that for authorisation of a plant protection product to occur it must be clearly established that use of this product will have no unacceptable impact on birds and mammals under field conditions. To this end the EFSA guidance document on bird and mammal risk assessment (EFSA) specifies two protection goals:

- An “actual” protection goal of “*clearly establishing that there will be no visible mortality and no long-term repercussions for abundance and diversity*.”
- A “surrogate” protection goal” of “*making any mortality or reproductive effects unlikely*”.

The first tier risk assessment is designed to satisfy the surrogate protection goal but at higher tiers either protection goal can be considered. In the case of metalaxyl-M the data and risk assessment provided do not allow for a consideration of the actual protection goal and therefore both the first and higher tier risk assessments are intended to satisfy the surrogate protection goal, i.e. to demonstrate that any reproductive effects are unlikely following use of the representative formulation.

The first tier risk assessment for metalaxyl-M indicates an unacceptable reproductive risk to mammals consuming treated sunflower seeds (TER = 1.18 compared to the acceptability criterion of ≥ 5).

The higher tier refined exposure assessment for metalaxyl-M takes into account an appropriate focal species (wood mouse), realistic feeding rates for this species, the proportion of food obtained from the treated area and the dissipation of residues from treated seeds. The higher tier refined TER value is equal to the trigger value of 5 (TER = 4.99). The refined exposure assessment is considered by HSE to be representative of the risk experienced by a realistic worst-case individual in an exposed population. Therefore, this assessment allows for consideration of whether the surrogate protection is addressed, i.e. whether it is clearly established that any reproductive effects are unlikely. Given the refined TER value is essentially equal to the trigger value and in light of the consideration of uncertainties indicating that in the quantitative risk assessment there are more factors that would lead to the ‘true’ risk being overestimated rather than underestimated, it is considered that the refined exposure assessment demonstrates that any reproductive effects are unlikely.

Additionally, the applicant has proposed that the following risk mitigation labelling statements are appropriate and should be taken into account.

- **STGD-S9**: To protect birds/wild mammals the seeds must be entirely incorporated in the soil; ensure that the seeds are also fully incorporated at the end of rows (except for seeds sown in greenhouses).
- **STGD-S10**: To protect birds/wild mammals remove spillage (except for seeds sown in greenhouses).
- **STGD-S11**: To protect birds/wild animals, treated seeds must be sown (i.e. covered by soil) by using seed drill equipment leaving less than x,xx % of the

sown treated seeds on the soil surface (except for seeds sown in greenhouses).

While it is agreed that these measures would help to limit exposure of small mammals via consumption of treated seed, the effectiveness of these measures in reducing the exposure and hence the risk to small mammals has not been demonstrated.

HSE therefore concludes that it has been clearly established that use of metalaxyl-M as a vining pea seed treatment will have no unacceptable impact on small mammals exposed via consumption of treated seed.

Long-term/reproductive risk to mammals from exposure to metalaxyl-M via consumption of emerged crop seedlings

The applicant has proposed refining the growth dilution factor used in estimating the metalaxyl-M residue in emerged vining pea seedlings. To support this refinement data from Volllmin (1999) have been used and are considered the higher tier risk assessment section for birds. The following refined residue values will be used in the higher tier risk assessment for mammals consuming pea seedlings:

- Residue in seedling = 1.089 mg a.s./kg (based on maximum TRR in above ground plant parts)
- Residue in seedling = 7.12 mg a.s./kg (based on 2.1% AR in above ground plant parts)

At first tier the only generic focal species requiring further consideration was the ‘large herbivorous mammal’ (TER = 3.54). Revised TER calculations are summarised below, using the residue data from [redacted] (1999). All other parameters remain unchanged from tier 1.

Table B.9.1.5-31: Higher tier long-term/reproductive risk to mammals – consumption of emerged seedlings

Crop	Generic focal species	Residue (mg a.s. /kg seed)	FIR/bw	DDD (mg/kg bw/day)	NOAEL (mg a.s./kg bw)	TER_{LT}
Vining peas	Large herbivorous mammal	1.089	0.4	0.436	96	220
		7.12	0.4	2.85	96	33.7

The resulting TER value is above the trigger value of 5, regardless of which of the residue values is used. This refined assessment is considered by HSE to be representative of the risk experienced by a realistic worst-case individual in an exposed population. Given the margin of safety in the refined risk assessment and the fact that only a single parameter has been refined compared to the first tier assessment, it is considered that the higher tier risk assessment is sufficient basis to conclude that the surrogate protection goal is met, i.e. it is clearly established that any reproductive effects are unlikely from exposure via consumption of vining pea seedlings.

B.9.1.7. Overall conclusion for terrestrial vertebrates

The overall outcomes of the risk assessments for birds and other terrestrial vertebrates are summarised in the following tables. For the proposed use of 'Vibrance SB' as a sugar beet seed treatment it has been demonstrated that there will be no unacceptable impacts on terrestrial vertebrates for all exposure scenarios. For the proposed use of 'Wakil XL' as a vining pea seed treatment, while for most scenarios it is concluded that there will be no unacceptable impacts on terrestrial vertebrates, it has not been demonstrated that there will be no unacceptable impact to mammals from acute exposure via consumption of treated seed. It is noted that in light of risk assessment for the consumption of seedlings scenario conducted in this amendment evaluation, no time interval is necessary before seedlings grown under full and permanent protection can be planted outside.

Table B.9.1.7-1: Summary of risk assessment conclusions for use of Vibrance SB on sugar beet and fodder beet

Exposure route	Birds		Mammals	
	Acute	Reproductive	Acute	Reproductive
Ingestion of treated seed	No unacceptable impact demonstrated at first tier	No unacceptable impact demonstrated at first tier	No assessment required	No assessment required
Ingestion of germinated seedlings	No unacceptable impact demonstrated at first tier	No unacceptable impact concluded at higher tier	No unacceptable impact demonstrated at first tier	No unacceptable impact demonstrated at first tier
Ingestion of drinking water	No unacceptable impact demonstrated at screening step	No unacceptable impact demonstrated at screening step	No unacceptable impact demonstrated at screening step	No unacceptable impact demonstrated at screening step

Table B.9.1.7-2: Summary of risk assessment conclusions for use of Wakil XL on vining pea

Exposure route	Birds		Mammals	
	Acute	Reproductive	Acute	Reproductive

Exposure route	Birds		Mammals	
	Acute	Reproductive	Acute	Reproductive
Ingestion of treated seed	No unacceptable impact demonstrated at first tier	No unacceptable impact concluded at higher tier	Unacceptable impact cannot be excluded	No unacceptable impact concluded at higher tier
Ingestion of germinated seedlings	No unacceptable impact demonstrated at first tier	No unacceptable impact concluded at higher tier	No unacceptable impact demonstrated at first tier	No unacceptable impact concluded at higher tier
Ingestion of drinking water	No unacceptable impact demonstrated at screening step	No unacceptable impact demonstrated at screening step	No unacceptable impact demonstrated at screening step	No unacceptable impact demonstrated at screening step

The following risk mitigation labelling is required for the use of Vibrance SB on sugar beet and fodder beet:

'To protect birds and wild mammals, treated seed should not be left on the soil surface. Bury or remove spillages.

Treated seed should not be broadcast.'

B.9.2. APPENDICES

B.9.2.1. Appendix 1 Literature Review

A literature search was conducted for Metalaxyl-M and its relevant metabolites according to the requirements of Article 8(5) of Regulation No 1107/2009. The literature search was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009".

The objective of the reviews was to determine if any scientific peer-reviewed open literature, before the submission date of the dossier, was relevant for consideration during the risk assessment process.

Literature searches related to the active substance and its metabolites have been submitted by the applicant. For the literature search in total 8 studies have been identified during the literature review of relevance to ecotoxicology, 3 for metalaxyl; 4 for rac-metalaxyl, R-metalaxyl or S-metalaxyl; and 1 study for metalaxyl-M (Riomil Gold Bravo ®). There were no records found for relevance and reliability for terrestrial vertebrates.

The conduct of the literature search method in relation to ecotoxicological studies has been evaluated; the conclusions of which are presented here. For this amendment evaluation only the risks to terrestrial vertebrates are within the scope of the HSE assessment, and so HSE have only considered the relevance and reliability of any literature studies identified in the context of this area of the risk assessment.

Databases searched

Table B.9.2.1-1 summarises the databases searched by the applicant as part of the respective literature searches.

Table B.9.2.1-1: Databases searched

	Applicant
	Syngenta
Total number of databases searched	13

List of databases used in the literature review	MEDLINE EMBASE Exerpta Medica ESBIOBASE AGRICOLA BIOSIS CABA CAPLUS Chemical Abstracts Plus FSTA GEOREF TOXCENTER PQSCITECH SCISEARCH Science Citation Index ANABSTR – Analytical abstracts
Time window of search	01 August 2010 – 16 June 2020
Date span of the search	9 years, 10.5 months

Search Parameters

The search used search terms and synonyms for metalaxyl-M, metalaxyl, Apron XL, and its metabolites. The results, returned from this "single concept search" for each database, were then checked for duplicates which were removed. The search was then filtered using the search terms specific to a technical aspect.

Search parameters/queries used during the literature search are presented in Table B.9.2.1-2. Those highlighted in grey are relevant for terrestrial vertebrates.

Metalaxyl-M was used as a case study in an EFSA supporting publication by Berger *et al.,* (2013)[31] for guidance detailing how to conduct literature reviews for the approval of pesticide active substances under Regulation (EC) No 1107/2009. The search terms the applicant has provided follow what is detailed in the EFSA case study.

Table B.9.2.1-2: <u>Search strategy used</u>

Input parameters	**STN Query – ecotoxicology search filters**
	L1 QUE ((RIPARIAN? OR REPTILE? OR SNAKE? OR LIZARD?) OR (TORTOISE? OR TURTLE? OR TERRAPIN? OR CROCODIL?) OR (ALLIGATOR? OR CAIMAN? OR GHARIAL? OR HOVERFLIES) OR ((MEADOW#(W)VOLE#) OR PSEUDOKIRSCHNERIELLA)) L2 QUE ((RHAPHIDOCELIS OR NITZSCHIA OR CYCLOTELLA OR MICROCYSTIS) OR (OSCILLATORIA OR

[31] Berger, E., Čoja, T., Dellantonio, A., Hrdina-Zödl, B., Hutzenlaub, N., Jölli, D., Müller, M., Prohaska, C. (2013). Case studies for the application of the Guidance of EFSA on Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, using substances for which dossiers are submitted under Regulation (EU) No 1141/2010. EFSA supporting publication 2013:EN-511.

	APHANIZOMENON OR ANKISTRODESMUS) OR (TEILINGRIA OR MONORAPHIDIUM OR RADIOCOCCACAE OR TETRASPORALES)) L3 QUE ((TETRAEDRON OR TREUBARIA OR WILLEA OR COSMOCLADIUM) OR (HYPOASPIS OR (SOIL(3A)MICROORGAN?) OR ECHINOCHLOA OR SPARTINA) OR (SALVINIA OR NAJAS OR CALLITRICHE OR MYOSOTIS OR STRATIOTES)) L4 QUE ((HIPPURUS OR PERSICARIA OR CLOEON? OR CORBICULA?) OR (NEOCARIDINIA? OR NEOCARIDINA? OR MYSID? OR CICHLIDAE) OR (CICHLID# OR LEPOMIS? OR SERRANIDAE OR PERCIFORMES)) L5 QUE ((ICTALURUS? OR POECILIA? OR ORYZIAS? OR GASTEROSTEUS?) OR (GASTEROSTEIDAE OR SALVELINUS OR BRACHYDANIO? OR CARASSIUS?) OR (MISGUMUS? OR CYPRINODON? OR FUNDULUS? OR MISGURNUS?)) L6 QUE ((BREAM OR ROTIFER# OR GAMMARUS OR GAMMARID? OR MAYFLY?) OR (BIVALVE# OR MUSSEL# OR MOLLUSK# OR MOLLUSC# OR BUFO) OR (NEWT# OR SCALLOP# OR CLAM# OR GAMBUSIA OR OREOCHROMIS)) L7 QUE ((OSTRAC? OR TUBIFEX? OR TURBELLARIA OR COPEPODA) OR (PREDACE? OR PREDACI? OR PARASITOID? OR APIS OR APIDAE) OR (BOMBUS OR BOMBINAE OR WORM# OR LUMBRICIDAE OR LUMBRICUS)) L8 QUE ((ALLOBOPHORA? OR DENDROBAENA? OR APORRECTODEA? OR DENDRODRILUS?) OR (EISENIA? OR OCTOLASION? OR (LACE(W)WING#) OR NEUROPTER?) OR (CARABID? OR CARBUS OR STAPHYLINID? OR COCCINEL? OR ADALIA?)) L9 QUE ((STETHORUS? OR SCYMNUS? OR WASP# OR VESPIDAE OR SPHECOIDEA) OR (SPHECIDAE OR STIZIDAE OR OPIUS OR (ICHNEUMON(W)FL?)) OR (ICHNEUMONID? OR BRACONID? OR CHALCID? OR CYNIP? OR APHIDI?)) L10 QUE ((EUCOILID? OR IBALIID? OR FIGITID? OR EURYTOM? OR TORYM?) OR (ORYM? OR EUCHARIT? OR PERILAMP? OR PTEROMAL? OR CHRYSOLAMP?) OR (EUPELM? OR ENCYRT? OR SIGNIPHOR? OR APHELIN? OR ELASMID?)) L11 QUE ((ELASMUS OR TETRACAMP? OR MYMAR? OR HELOR? OR PROCTOTRUP?) OR (DIAPRI? OR SCELION? OR PLATYGASTR? OR PLATYGASTER?) OR (CERAPHRON? OR MEGASPIL? OR ARANE? OR OPILION? OR PHALANG?)) L12 QUE ((ARACHNID? OR HARVESTM? OR DADDYLONGLEG? OR (DADDY(W)LONG(W)LEG?)) OR ((DADDY(W)LONGLEG?) OR COLLEMB? OR (SPRING(W)TAIL?)

	OR CYDNODROMUS?) OR (PARDOSA? OR ORIUS? OR TYPHLODROM? OR PHYTOSEIULUS? OR SYRPHID?)) L13 QUE ((METASYRPHUS? OR SYRPHUS? OR EUPEODES? OR EPISYRPHUS? OR SYRPHIAN?) OR (EPISTROPHE? OR AMBLYSEIUS? OR POECILUS? OR TRECHUS? OR BEMBIDION?) OR (NEBRIA? OR PTEROSTICHUS? OR CALOSOMA? OR TACHYPORUS? OR NABIDAE?)) L14 QUE ((GEOCORIS? OR HYMENOPT? OR HAEMATOLOECHA? OR CHRYSOPID? OR SYMPHYTA?) OR (OULEMA? OR APHYTIS? OR BATHYPLECTES? OR LINPHIIDAE? OR LYNPHIIDAE?) OR (LINYPHIIDAE? OR ERIGONE? OR BATHYPHANTES? OR MEIONETA? OR OEDOTHORAX?)) L15 QUE ((LEPTHYPHANTES? OR LYCOSID? OR LYCOSA? OR CHRYSOPA? OR DACNUSA?) OR (CYRTORHINUS? OR CRYPTOLAEMUS? OR ZETZELLIA? OR LEPTOMASTIX?) OR (TRICHOGRAMMA? OR ENCARSIA? OR MACROLOPHUS? OR CHRYSOPERLA?)) L16 QUE ((ALEOCHARA? OR CHRYSOPID# OR CHRYSOPIDAE OR DIABROTICA) OR (PALEXORISTA? OR MAMMAL## OR ANIMAL? OR RABBIT? OR RODENT#)) L17 QUE ((BLACKBIRD# OR (BLACK(W)BIRD#) OR ((TURDUS OR T)(W)MERULA)) OR (CHAFFINCH? OR ((FRINGILLA OR F)(W)COELEBS) OR GREENFINCH?) OR (((CARDUELIS OR C)(W)CHLORIS) OR SONGTHRUSH?) OR (SONG(W)THRUSH?)) L18 QUE (((TURDUS OR T)(W)PHILOMELOS) OR WREN#) OR ((TROGLODYTES OR T)(W)TROGLODYTES) OR (WILLOW(W)WARBLER#) OR (((PHYLLOSCOPUS OR P)(W)TROCHILUS) OR (GREAT(W)TIT#)) OR (((PARUS OR P)(W)MAJOR) OR ROBIN# OR GOLDFINCH?) L19 QUE (((ERITHACUS OR E)(W)RUBECULA) OR DUNNOCK#) OR (((CARDUELIS OR C)(W)CARDUELIS) OR LINNET#) OR (((PRUNELLA OR P)(W)MODULARIS) OR SKYLARK# OR (SKY(W)LARK#)) L20 QUE ((HEDGE(W)(SPARROW# OR ACCENTOR#))) OR (((CARDUELIS OR C)(W)CANNABINA) OR ((ALAUDA OR A)(W)ARVENSIS)) OR ((RED(W)LEGGED(W)PARTRIDGE#) OR ((ALECTORIS OR A)(W)RUFA)) L21 QUE ((MEADOW(W)PIPIT#) OR MEADOWPIPIT# OR ((ANTHUS OR A)(W)PRATENSIS)) OR (LAPWING# OR ((VANELLUS OR V)(W)VANELLUS) OR PEEWIT#) OR (STARLING# OR ((STURNUS OR S)(W)VULGARIS)) L22 QUE ((TURTLE(W)DOVE#) OR ((STREPTOPELIA OR S)(W)TURTUR)) OR (YELLOWHAMMER# OR (YELLOW(W)HAMMER#) OR (YELLOW(W)WAGTAIL#)) OR (((EMBERIZA OR E)(W)CITRINELLA) OR (YELLOW(W)WAG(W)TAIL#))

	L23 QUE (((MOTACILLA OR M)(W)FLAVA) OR (FAN(W)TAILED(W)WARBLER#)) OR ((GREY(W)LAG(W)G!!SE) OR ((ANSER OR A)(W)ANSER)) OR (REEDBUNTING# OR (REED(W)BUNTING#) OR ((EMBERIZA OR E)(W) SCHOENICLUS)) L24 QUE (CHAFFINCH? OR BLUETIT? OR (BLUE(W)TIT?)) OR (((PARUS OR P)(W)CAERULEUS) OR (SYLVIA(W)COMMUNIS)) OR (((GALERIDA OR G)(W)CRISTATA) OR (TREE(W)SPARROW#)) L25 QUE (((COTURNIX OR C)(W)COTURNIX) OR (GREY(W)PARTRIDGE#)) OR (((PERDIX OR P)(W)PERDIX) OR ((PHASIANUS OR P)(W)COLCHICUS)) OR (((MILIARIA OR M)(W)CALANDRA?) OR GREYLAGG!!SE) L26 QUE ((GREYLAG(W)G!!SE) OR ((COLUMBA OR C)(W)PALUMBUS?)) OR (((STREPTOPELIA OR S)(W)(ORIENTALIS? OR RISORIA?))) OR (((MOTACILLA OR M)(W)ALBA?) OR (CRESTED(W)LARK#)) L27 QUE ((WHITE(W)WAGTAIL#) OR (WOOD(W)PIGEON#) OR (BIRD(W)LIFE)) OR ((SONG(W)BIRD#) OR VANELLUS? OR (PEE(W)WIT#)) OR (AVIFAUNA? OR (AVI(W)FAUNA?) OR SONGBIRD?) L28 QUE (ORNITHOLOG? OR PASSERINE? OR WOODPIGEON#) OR (((PASSER OR P)(W)MONTANUS) OR QUAIL# OR (CALANDRA(W)LARK#)) OR (CISTICOLA? OR (Z(W)CISTICOLA?) OR BIRDLIFE) L29 QUE (GEESE OR GOOSE OR SPARROWS OR PIGEONS OR LARK#) OR (WARBLER# OR PARTRIDGE# OR BUNTING# OR WAGTAIL#) OR (WHITETHROAT# OR PIED# OR (WHITE(W)THROAT#) OR MALLARD OR DUCK OR BOBWHITE OR ANAS? OR COLINUS?) L30 QUE ((FORAGING OR FARMLAND OR GRASSLAND)(3A)BIRD#) OR (BLUEBIRD# OR (ROCK(W)PTARMIGAN#) OR (BLACK(W)REDSTART#)) OR ((PREDATOR? OR NONTARGET? OR (NON(W)TARGET))(3A)BIRD#) OR ((CORN(W)BUNTING#) OR SERINS OR SERINUS) L31 QUE (L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30) L32 QUE (L31 NOT (JAPANESE? OR JAPONICA?)) L33 QUE (((SMALL OR WILD)(3A)MAMMAL#) OR (WILD(3A)ANIMAL?)) OR (VOLE# OR GLIS OR DORMOUSE OR DORMICE OR ELIOMY#) OR (LEROT# OR LAGOMORPH# OR LEPORID? OR LEPUS OR ORYCTOLAGUS? OR VERTEBRAT? OR RAT) L34 QUE (HARE# OR SORICIDAE? OR SOREX? OR NEOMY# OR CROCIDURA?) OR (SHREW# OR WOOD MOUSE OR WOODMICE OR APODEMUS? OR MICROTUS?) OR (CLETHRIONOMYS? OR CRICETIDAE? OR MICROTIN?)

	L35 QUE (RAPTOR# OR MARMOSET# OR GOPHER# OR GRASSCUTTER#) OR ((PREDATOR? OR NONTARGET? OR (NON(W)TARGET?))(3A)MAMMAL#) OR ((WOOD(W)(MOUSE OR MICE)) OR ARVICOLA?) OR (MEADOW#(W)VOLE#) L36 QUE (L33 OR L34 OR L35) L37 QUE (ECOTOX? OR LC50 OR ((LC OR EC OR LR)(W)50) OR EC50 OR LR50) L38 QUE ((ECO OR ECOL OR ECOLOG? OR ENV OR ENVIRONM? OR AQUATIC?) (5A)(TOX? OR RISK? OR IMPACT? OR EFFECT?)) L39 QUE (AQUATIC? OR FRESHWATER? OR (FRESH(W)WATER?)) L40 QUE (FLORA OR FAUNA OR BIOTA OR ORGANISM? OR INSECT?) OR (ENVIRONM? OR LIFE OR INVERTEB? OR CRUSTA? OR SPECIES) OR (ENTOMOFAUNA OR (ENTOMO(W)FAUNA)) L41 QUE (L39(5A)L40) L42 QUE (MAGNA? OR (D(W)MAGNA?) OR CHIRON? OR BRACHIONUS?) OR (LIMNEA? OR CRASSOSTREA? OR ALG? OR FISH OR FISHES) OR (ONCORHYNCHUS? OR SALMONIDAE? OR CYPRINUS? OR CYPRINID?) L43 QUE (PIMEPHALES? OR PISCES OR TROUT OR SUNFISH? OR CARP) OR (MINNOW? OR (F(W)MINNOW?) OR CATFISH? OR ZEBRAFISH?) OR (GOLDFISH? OR (ZEBRA(W)DANIO#) OR GUPPY OR GUPPIES) L44 QUE (KILLFISH? OR FATHEAD? OR BLUEGILL? OR SALMON#) OR (THUNDERFISH? OR (WATER(W)(FLY OR FLEA?)) OR WATERFLEA?) OR (FROG# OR AMPHIB? OR SHRIMP# OR PRAWN# OR CRAB# OR TOAD#) L45 QUE (TADPOLE# OR CRAYFISH? OR SHELLFISH? OR LOBSTER#) OR (OYSTER# OR SNAIL# OR RANA OR RANIDAE? OR PLANKTON? OR crusta? OR ESTUARINE OR GASTROPOD?) L46 QUE (L37 OR L38) L47 QUE ((NONTARGET? OR (NON(W)TARGET?))(5A)(PLANT? OR FLORA?)) OR ((AQUATIC(3A)(PLANT? OR (PHYTO(W)TOX?) OR PHYTOTOX?))) OR (SEDIMENT? OR HYDROSOIL? OR DUCKWEED? OR PONDWEED?) L48 QUE (((DUCK OR POND)(W)WEED#) OR MACROPHYT? OR PERIPHYTON?) OR (POTAMOGETON? OR CHAROPHYTA? OR ELODEA? OR HYDROCHARITA?) OR (CERATOPHYL? OR CHLAMYDOMON? OR SELENASTRUM? OR CHLORELLA?) L49 QUE (SCENEDESMUS? OR SKELETONEMA? OR NAVICULA? OR ANABAENA?) OR (MYRIOPHYLLUM? OR GLYCERIA?) L50 QUE (NONTARGET? OR (NON(W)TARGET?) OR BENEFICIAL?)

	L51 QUE (EFFECT? OR INVERTEB? OR ORGANISM? OR ARTHROPOD? OR INSECT?) OR (FAUNA OR SPECIES OR (ENTOMO(W)FAUNA?) OR ENTOMOFAUNA?) L52 QUE (L50 (5A)L51) L53 QUE (PREDAT? OR (NATURAL(W)ENEM?) OR BEE OR BEES OR API? OR HONEYBEE#) OR (BUMBLEBEE# OR ((HONEY OR BUMBLE)(W)BEE#) OR EARTHWORM?) OR ((EARTH(W)WORM?) OR LADYBUG# OR LADYBEETLE# OR LADYBIRD#) L54 QUE ((LADY(W)(BUG# OR BEETLE# OR BIRD#)) OR HOVERFLY) OR (HOOVERFLIES OR SAWFLY OR SAWFLIES OR DRONEFLY) OR (DRONEFLIES OR FLOWERFLY OR FLOWERFLIES OR LACEWING?) L55 QUE (((HOVER OR DRONE OR FLOWER OR SAW)(W)(FLY OR FLIES))) OR (SPIDER# OR SPRINGTAIL? OR (ROOT(W)WORM#) OR ROOTWORM# OR MACRO(W)ORGANISM) L56 QUE (L52 OR L53 OR L54 OR L55) L57 QUE (BIRD? OR AVES OR AVIAN? OR (AVI(W)FAUNA?) OR AVIFAUNA?) OR (SONGBIRD? OR (SONG(W)BIRD?) OR ORNITHOLOG?) L58 QUE ((WILD(3A)(LIFE OR ANIMAL#)) OR WILDLIFE OR SQUIRREL?) OR (VOLE# OR SCIURUS OR GLIRID? OR GLIS OR DORMOUSE) OR (DORMICE OR ELIOMYS OR LEROT# OR MUSTELID? OR MINK#) L59 QUE (MUSTELINE# OR WEASEL? OR STOAT? OR MUSTEL? OR BADGER?) OR (MELES OR MELINAE OR OTTER# OR LUTRA OR LUTRINAE) OR (LAGOMORPH# OR LEPORID? OR LEPUS OR ORYCTOLAGUS OR HARE#) L60 QUE (TALPA OR MOLE OR MOLES OR HEDGEHOG? OR (HEDGE(W)HOG?)) OR (CROCIDURA? OR SHREW# OR WOOD MOUSE OR WOODMICE OR APODEMUS) OR (MICROTUS OR ARVICOLA OR CLETHRIONOMYS? OR CRICETIDAE?) OR (ERINACEUS OR ERINACEIDAE? OR SORICIDAE? OR SOREX) L61 QUE ((ENDOCRIN? OR HORMON?)(5A)(DISRUPT? OR MIMIC? OR MODULAT? OR DISORDER? OR DISEASE?)) L62 QUE (DAPHNI? OR CERIODAPHNI? OR HYALELLA? OR ASSELLUS) L63 QUE (L41 OR L42 OR L43 OR L44 OR L45 OR L62) L64 QUE (PHYTOPLANKTON? OR AUFWUCH# OR LEMNA? OR ARALES OR CHARA OR SEDIMENT(W)DWELL OR HAZARD OR ADVERSE OR BIOACCUMULAT? OR BIOMAGNIFICAT? OR BIOCONCENTRAT? OR POISON OR SEWAGE OR ACTIVATED(W)SLUDGE) L65 QUE (L47 OR L48 OR L49 OR L64) L66 QUE (NEOMYS OR MICROTINAE? OR MICROBIAL OR VEGETATIVE(W)VIGO? OR SEEDLING OR

	GERMINAT? OR MONOCOT? OR DICOT?) L67 QUE (L58 OR L59 OR L60 OR L66) L68 QUE (LOACH? OR STICKLEBACK? OR MUMMICHOG# OR TILAPIA? OR ASELLUS) L69 QUE (L63 OR L68) L70 QUE (L46 OR L69 OR L65 OR L56 OR L57 OR L67 OR L61) L71 QUE (L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16) L72 QUE (L70 OR L71 OR L32 OR L36) SAVE L72 ECOTOX/Q
Substances searched for	For the literature search on the active substance metalaxyl-M, also information on the active substance metalaxyl was included. Metalaxyl-M is the biologically active isomer in metalaxyl, which is a racemate of R-(metalaxyl-M) and S-isomers. The following were used to search for metalaxyl-M and its metabolites: IUPAC name, CAS number, other names given to the substance/trade names. It was not clear if codes and abbreviations, molecular structure, molecular formula, molar mass and other names/codes were used. Patent literature was not considered to be relevant and so the search was filtered to remove patent document types. Substance name: Metalaxyl-M; IUPAC name: 2R)-2-(N-(2-methoxy-1-oxoethyl)-2,6-dimethylanilino)propanoic acid methyl ester; (2S)-2-(N-(2-methoxy-1-oxoethyl)-2,6-dimethylanilino)propanoic acid methyl ester; 2-(N-(2-methoxy-1-oxoethyl)-2,6-dimethylanilino)propanoic acid methyl ester; methyl (2R)-2-[(2,6-dimethylphenyl)-(2-methoxyethanoyl)amino]propanoate; methyl (2S)-2-[(2,6 dimethylphenyl)-(2-methoxyethanoyl)amino]propanoate; methyl 2-[(2,6-dimethylphenyl)-(2-methoxyethanoyl)amino]propanoate. Other names given to the substance/trade name: BION MX, BION MX 44 WG, RIDOMILGOLD R, RIDOMIL GOLD R WG, FLARE GOLD R WG, MEXIL ORO R WG, RIDOMIL GOLD, RIDOMIL GOLD SL, FONGANIL GOLD, SANTHAL GOLD, SUBDUE, SUBDUE GOLD, SANTHAL JARDIN, FLARE GOLD, APRON XL, APRON XL 350 ES, MAXIM QUATTRO, INFLUX QUATTRO, CELEST QUATTRO, WAKIL XL, WAKIL, WAKIL 325, WAKIL XL 32.5 WG,MAXIM XL, MAXIM XL 350 FS, MAXIM XL 035 FS, MAXIM XL 034.7 FS, CELEST XL, INFLUX XL, VIBRANCE SB, VIBRANCE XL, VIBRANCE CORN, RIDOMIL GOLD MZ, RIDOMIL GOLD MZ, RIDOMIL GOLD MZ 68 WG, RIDOMIL GOLD MZ PEPITE, FUBOL GOLD, FUBOL GOLD WG, CROCODIL MZ 67.8 WG, RIDOMIL GOLD MZ PEPITE 67.8 WG, EPERON JARDIN, EPERON PEPITE,

	EPERON PRO, ROSTER 68WG, MANTOX 68WG, FLARE GOLD MZ PEPITE, MEXIL ORO MZ WG, EPERON MZ PEPITE, PLAY MZ PEPITE, SIMPLIA MZ PEPITE, MILDISAN MZ, RIDOMIL GOLD 67.9 PEPITE, VIBRANCE OSR, FOLIO GOLD, FOLIO GOLD, FOLIO GOLD SC, FOLIO GOLD 537.5 SC, RIDOMIL GOLD, COMBI PEPITE 45 WG, RIDOMIL GOLD COMBI WG, RIDOMIL GOLD COMBI PEPITE, RIDOMIL GOLD COMBI, RIDOMIL GOLD COMBI 45 WG, EPERON COMBI PEPITE, PLAY COMBI PEPITE, SIMPLIA COMBI PEPITE, EPERON GOLD PEPITE, ACYLON GOLD COMBI 45 WG, RIDGOLD F, FLARE GOLD COMBI PEPITE, CRUISER OSR 322 FS, CERTICOR 50 FS CAS numbers: 70630-17-0; 6951634-3; 57837-19-1

The search terms were used to establish databases of relevant literature. These were further used with regard to ecotoxicologically specific terms. The results were considered further based on the criteria for relevance (see below), for inclusion/exclusion from the studies relevant to the ecotoxicology risk assessment. The applicant used a range of input parameters for terrestrial vertebrates, with 2204 results (ecotox only, excluding duplicates) retrieved from the search to be screened for relevance. Based on the number of summary records retrieved, the key words for the selected bibliographic databases were considered reasonable.

Evaluation of studies for inclusion/exclusion based on relevance

Initial rapid assessment – Titles were first scanned to identify whether the studies were relevant or not. Summary abstracts were then requested for the remaining titles and a further rapid assessment was conducted, where again any clearly irrelevant studies were removed.
The following rapid assessment criteria for **irrelevance** were used:

- Analytical
- **Bio**
- Biochemistry
- Climatic Zone
- Consumer Safety
- **Endocrine Disruptor**
- Efficacy
- EFSA review
- Enzyme activity
- **Exposure modelling**
- Human exposure modelling
- Human Health
- Metabolism
- Multi-stressors
- Modelling

- **Monitoring**
- Not chemical related
- QSAR modelling
- Results given in abstract EC50> 10mg/L
- Results given in abstract LC50> 10mg/L
- **Results given in abstract LD50> 500 mg/kg**
- **Use as a bird repellent – no effect**
- Other sections not related to Ecotox.

The criteria used for irrelevance highlighted in bold text above are either not considered transparent or not considered appropriate (excluding 22 studies in total). More specifically, the term 'Bio' is vague and there is no reasoning of what is meant by this term. 'Endocrine Disruptor' is relevant for ecotoxicology. Similarly, 'Exposure modelling' and 'monitoring' are very broad terms and exposure modelling for non-targets or effect modelling would be of interest, so it is unclear why these are considered irrelevant when approaching the literature review. It is not clear why the 'Results given in abstract LD50> 500 mg/kg' is used as irrelevance criteria, and this needs to be noted as the acute bird LD50 endpoint for metalaxyl-M is 981 mg a.s./kg bw. Finally, the 'Use as a bird repellent – no effect' criteria could result in relevant information with potential supporting information being excluded unnecessarily.

It is not clear why the applicant would not consider such aspects as relevant to ecotoxicology and without further justification HSE do not agree with all criteria for exclusion of records (though it is noted that a relatively small number of studies were excluded based on these criteria). The other criteria can be considered acceptable with regard to ecotoxicology. In total 2153 articles were excluded at the rapid assessment phase.

Criteria for relevance – The following detailed assessment criteria were used:

Laboratory Studies

1. Well defined test material (including purity/content)
2. Number of organisms per group sufficient to establish a statistical significance
3. Applicable test species
4. Test organisms are not previously exposed to the test material or other contaminants
5. Several dose levels tested, at least 3, including a negative control, to establish a dose-response, unless the study design is specifically a limit test. Control must be run concurrently with treatments and mortality to be within test validity criteria.
6. Exposure route is clearly defined, is environmentally relevant and, if appropriate, suitably quantified.
7. If conducted, analytical confirmation of dosing or sufficient information provided to determine concentrations were within acceptable range (e.g. 80-120%) of nominal targets.

8. Effects are related to single test item, and a quantitative relationship exists between the reported endpoint and risk assessment endpoints of growth, mortality, behaviour and/or reproduction.
9. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust.
10. **Study conditions should not differ significantly from recommended protocols.**
11. Study conditions should not interfere with the interpretation of the study results.

Field Studies

12. Appropriate and relevant geoclimatic conditions (setting), appropriate application method and rates (exposure) and observation data (biological relevance) to derive endpoints.
13. Well defined test material (including purity/content)
14. Applicable test species
15. Exposure route is clearly defined, is environmentally relevant and, if appropriate, suitably quantified.
16. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust (e.g. pre-treatment details, characterisation of physico-chemical parameters, replication, statistical methods, appropriate sampling regime).
17. Study conditions should not differ significantly from recommended protocols, if available for field study.
18. Study conditions should not interfere with the interpretation of the study results

Criteria highlighted in bold exhibit some issue(s) that mean they are ambiguous or unsuitable for use as a relevance criterion (explained further below) as they are more appropriately considered at the reliability assessment stage. In addition, for the list of criteria for relevance it is not clear how the defined criteria were applied. Did all have to be true for a study to be considered relevant or was there a more flexible system for assessing relevance based on the number of applicable criteria that were true?

<u>Regarding the criteria not considered transparent the following open questions/issues have been identified:</u>

- **Well defined test material (including purity/content) -** Whilst it should be possible to relate effects in a study to a specific test item, it would not be necessary for the purity or batch number of a test item to have been identified and exclusion of studies on this basis is not considered appropriate.

- **Number of organisms per group sufficient to establish a statistical significance -** Any issues related to the suitability of study methods or statistical analysis are not relevance criteria but instead should be addressed in the reliability assessment.

- **Applicable test species –** How was this criterion applied with regard to ecotoxicological studies? What test species are considered applicable? How are applicable test species defined? It would not be appropriate to exclude species based on their geographic distribution (e.g. non-European native species) as they will still inform about the range of sensitivity of organisms to a substance. Were the species defined as relevant only those that belonged to groups that should be considered in the risk assessment, such as birds, mammals, aquatic plants, amphibians/reptiles?

- **Several dose levels tested, at least 3, including a negative control, to establish a dose-response, unless the study design is specifically a limit test. Control must be run concurrently with treatments and mortality to be within test validity criteria -** Any issues related to the suitability of study methods or statistical analysis are not relevance criteria but instead should be addressed in the reliability assessment.

- **If conducted, analytical confirmation of dosing or sufficient information provided to determine concentrations were within acceptable range (e.g. 80-120%) of nominal targets -** Any issues related to the suitability of study methods or statistical analysis are not relevance criteria but instead should be addressed in the reliability assessment.

- **Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust -** Any issues related to the suitability of study methods or statistical analysis are not relevance criteria but instead should be addressed in the reliability assessment.

- **Study conditions should not differ significantly from recommended protocols -** Any issues related to the suitability of study methods or statistical analysis are not relevance criteria but instead should be addressed in the reliability assessment.

- **Appropriate and relevant geoclimatic conditions (setting), appropriate application method and rates (exposure) and observation data (biological relevance) to derive endpoints –** as a result of this relevance criteria, 1 terrestrial vertebrate study (Bro *et al.,* 2015[32]) was excluded for evaluation on the basis that is was a field study conducted in South Korea (inappropriate geoclimatic conditions). It is unclear why non-European field studies are dismissed based on location without some consideration of whether it is representative or not of European conditions in detail. From this, it remains unclear whether it should have been considered non-relevant at the stage, but instead considered further within detailed assessment for relevance. The study report and summary have not been provided and so it cannot be considered further. However, from the title it is suggested that the study considers only exposure and so this would not be considered to impact the risk assessment conclusions.

[32] Bro, E., Millot, F., Decors, A., & Devillers, J. (2015). Quantification of potential exposure of gray partridge (Perdix perdix) to pesticide active substances in farmlands. *Science of the Total Environment*, *521*, 315-325.

From the issues highlighted above this may have led to inappropriate exclusion of relevant/potentially relevant studies at the relevance assessment stage. From the applicant's literature review there are studies related to ecotoxicology that have been excluded based on criteria which would be considered to assess reliability instead of relevance (criteria highlighted in bold above). However, as this literature review is to assess publications for terrestrial vertebrates this is not considered to have an impact, as only one studies for terrestrial vertebrates was excluded under these criteria **and it is thought that this exclusion would not impact the risk assessment conclusions.**

Search result

The result of the search method is summarised below in Table B.9.2.1-3. Table B.9.2.1-4 summarises the number of full text articles not excluded for relevance and summarised by the applicant as full text documents of relevance to ecotoxicology. In total 8 studies have been identified during the literature review of relevance to ecotoxicology, 3 for metalaxyl; 4 for rac-metalaxyl, R-metalaxyl or S-metalaxyl; and 1 study for metalxyl-M (Riomil Gold Bravo ®). Test species consisted of 2 freshwater mussel species (Unionidae), cyanobacterial species, earthworm *Eisenia foetida*, (*Lens esculentus*)-specific *Rhizobium sp.* Strain MRL3, mycorrhizal fungi, *Tenebrio molitor* larvae, and Zebrafish. Although the search did not identify any literature directly relevant for risks to terrestrial vertebrates, studies KIIA 8.2.6 and KIIA 8.2.6 explore bioaccumulation in *Tenebrio molitor* larvae and earthworms, respectively. These can be considered potentially relevant for the bird and mammal secondary poisoning risk assessment. However, secondary poisoning is outside of the scope of this amendment evaluation, so these studies don't need to be considered further at this time.

Table B.9.2.1-3: Summary of literature search results (all subject areas)

Summary of review	Number (search: June 2020)
Total number of summary records retrieved from search	4615 (ecotox only)
Total number of summary records retrieved after removing duplicates from all database searches	2204 (ecotox only)
Number of summary records excluded after rapid assessment for relevance (by title/abstract) *	2153
Number of studies excluded from risk assessment after detailed assessment of full-text documents (i.e. not relevant)	43
Number of studies not excluded for relevance after detailed assessment (i.e. reliable studies and studies of unclear reliability)	8

* aligned with ESA Journal 2011; 9(20:2092: rapid assessment means exclusion of *"obviously irrelevant records"* based on titles, and titles plus abstracts.

Table B.9.2.1-4: Summary of full text articles not excluded for relevance and related to ecotoxicology

Data requirement (indicated by the corresponding CA and CP data point number)	Title
KIIA 8.4 (CA 8.2.6)	Differential responses of two cyanobacterial species to R-metalaxyl toxicity: Growth, photosynthesis and antioxidant analyses.
KIIA 8.16.1 (CA 8.7)	Ecotoxicological assessment of pesticides towards the plant growth promoting activities of Lentil (*Lens esculentus*)-specific *Rhizobium sp*. Strain MRL3.
KIIA 8.2.6 (CA 8.2.2.3)	Enantiomerization and enantioselective bioaccumulation of metalaxyl in *Tenebrio molitor* larvae.
KIIA 8.2.6 (CA 8.2.2.3)	Enantioselective bioaccumulation and toxic effects of metalaxyl in earthworm *Eisenia foetida.*
KIIA 8.2 (CA 8.2)	Enantioselectivity in Developmental Toxicity of rac-metalaxyl and R-metalaxyl in Zebrafish (*Danio rerio*) Embryo.
KIIA 8.2 (C.A 8.2)	Exposure to Metalaxyl Disturbs the Skeletal Development of Zebrafish Embryos.
KIIA 8.16.1 (CA 8.7)	Placing arbuscular mycorrhizal fungi on the risk assessment test battery of plant protection products (PPPs).
KIIA 8.3.1.4 (CA 8.2.4)	Sensitivity of multiple life stages of 2 freshwater mussel species (Unionidae) to various pesticides detected in Ontario (Canada) surface waters.

HSE comments

The number and types of databases searched appears appropriate to address ecotoxicological risks. The search terms used are considered appropriate to capture relevant studies for the risk to terrestrial vertebrates from the ecotoxicological risks from the active substance, representative formulation and metabolites. The date span of the literature search is from 01 August 2010 – 16 June 2020 (9 years, 10.5 months). Literature searches should explore relevant scientific peer-reviewed open literature published in the last 10 years before the submission date of the dossier. Therefore, it is not clear why the applicant did not search for literature up to 10 years prior to submission date, even if it was only 1.5 months under this specific period. However, the EFSA case study[33] explores publications between 2003 – 2013 and did not report any relevant studies for terrestrial vertebrates between this time period and so this is not considered to have an impact.

[33] Berger, E., Čoja, T., Dellantonio, A., Hrdina-Zödl, B., Hutzenlaub, N., Jölli, D., Müller, M., Prohaska, C. (2013). Case studies for the application of the Guidance of EFSA on Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, using substances for which dossiers are submitted under Regulation (EU) No 1141/2010. EFSA supporting publication 2013:EN-511.

Some of the rapid relevance criteria were considered ambiguous or unsuitable for use as a relevance criterion. It is unclear without further clarification as to why the applicant would not consider such criteria ‘Bio’, ‘Endocrine disruptor’, ‘Exposure monitoring’ ‘monitoring’, ‘Results given in abstract LD50> 500 mg/kg’, and ‘Use as a bird repellent – no effect’ as relevant to ecotoxicology.

For studies assessed under detailed relevance criteria these include aspects of both relevance and reliability assessment. This could potentially result in studies being incorrectly excluded at the relevance assessment stage on the grounds of criteria relating to study reliability, which instead should have been transparently considered at the reliability evaluation stage. It is at this stage the applicants’ reasoning for exclusion would have been clearly assessed. For example, one field monitoring study for terrestrial vertebrates was assessed for relevance, but this was excluded at this stage as it was considered geographically irrelevant i.e. a field study conducted in South Korea. Although this is a non-European field study, the exclusion of this study on the basis of location without some consideration of whether it is representative or not of European conditions is considered inappropriate, and it remains unclear whether it should have been considered as non-relevant at this stage. As the study report and a summary have not been provided this cannot be considered further. However, from the study title it suggests that the study only considers exposure of pesticide active substances in farmlands to grey partridge and will not impact the risk assessment conclusions. For this amendment evaluation only the risks to terrestrial vertebrates are within the scope of the HSE assessment. The literature review found no other studies for terrestrial vertebrates which were excluded under unsuitable relevance criteria.

Subsequently, of all the records considered relevant and reliable by the applicant, none of these explored the risk of metalaxyl-M, metalaxyl, Apron XL, and its metabolites to terrestrial vertebrates. Overall, the literature review can be considered to survey the open literature, though there are deficiencies within the respective search strategies as highlighted above. The outcome of the literature review submitted by the applicant closely follows the outcome to the EFSA case study for metalaxyl-M[3]. Similarly, Berger *et al.,* (2013) did not conclude any relevant studies for terrestrial vertebrates between 2003 – 2013. This gives further evidence that while there are aspects of the relevance assessment provided by the applicant that are considered deficient, the literature review does not identify any data that require further consideration in the amendment evaluation regarding risks to terrestrial vertebrates.

B.9.2.2. Appendix 2 applicant's case to amend both the avian acute and the long-term/reproductive endpoints

Presented below the applicant's argument for amending the both the agreed acute and long-term/reproductive endpoints.

Justification for new endpoints

Bird Acute endpoint

The EFSA conclusion (2015) for metalaxyl-M proposes a geometric mean of 1180 mg/kg bw/day as endpoint for the bobwhite quail (*Colinus virginianus).* The EFSA Guidance for birds and mammals *(*2009 - point 2.4.1 and 2.4.2)[34]proposes that when multiple studies are available an overall geometric mean is determined. In situations when more than one study is available with a single species (as is the situation for the bobwhite quail) a geometric mean is calculated for that species and used as a single input in determining an overall geometric mean for all species tested.

In addition to studies with the bobwhite quail a study was conducted with the mallard duck (*Anas platyrhynchos*) in which a LD_{50} of 1466 mg/kg bw/day was determined. Using these two endpoints (i.e. LD_{50} of 1180 mg/kg bw/day and LD_{50} of 1466 mg/kg bw/day), and in accordance with the EFSA guidance (2009), an overall geometric mean of **1315 mg/kg bw/day** should be used in the acute risk assessment.

Consideration of reproductive endpoints for metalaxyl-M used in the risk assessment

According to the EFSA Guidance (2009), an estimated reproductive endpoint should be obtained by using the acute oral LD_{50} (from a single species or geometric mean) and divided by 10 to obtain an LD_{50}/10. This LD_{50}/10 is used as an endpoint in the reproductive assessment to take account of the possibility of reproductive impairment due to sub-lethal effects on pair formation and breeding site selection, incubation, parental care of nestlings, and survival of fledgling birds (in accordance with **Appendix J** of the EFSA Guidance). If the LD_{50}/10 is lower than the lowest reproductive endpoint, then this should be used as the reproductive endpoint.

For metalaxyl-M the LD_{50} used in the acute risk assessment is 1315 mg/kg bw, generating an LD_{50}/10 value of 131.5 mg/kg bw.

EFSA in 2015 concluded a value for NOEL of 24.6 mg / kg bw /day based on the mallard duck reproductive toxicity study with the racemic mixture metalaxyl ([redacted]***, 1980a; CGA48988/0152***), while, the previous EU evaluation (2002) and the current applicant position support a value for NOEL of 84.0 mg /kg bw/day based on the bobwhite quail study with Metalaxyl-M ([redacted] ***and*** [redacted]***, 1998; CGA329351/1071***). Both NOEL values are lower than the LD_{50}/10 value and thus the NOEL values will be used in the risk assessment.

[34]European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [139 pp.]

Consideration of reproductive endpoints for metalaxyl-M used in the risk assessment

During the original EU review (2002) it was concluded that the bird reproduction study conducted by [redacted] ***(1980a; CGA48988/0152)*** with the racemic mixture metalaxyl was not suitable for use in a regulatory risk assessment due to a number of critical concerns over the conduct of the study, details of which are discussed below. Therefore, the applicant conducted two additional studies looking at the effect of metalaxyl-M on the bobwhite quail ([redacted] ***and*** [redacted]***, 1998; CGA329351/1071***) and the mallard duck ([redacted] ***and*** [redacted]***, 1998a; CGA329351/1072***), which were included in the Supplementary Dossier for renewal (2012).

The applicant maintains the position that the NOEL of 84 mg a.s./kg bw/day obtained from the bobwhite quail study ***([redacted] and [redacted] 1998a; CGA329351/1071)*** should be the appropriate endpoint to use in the regulatory risk assessment as it is a robust study and guidance compliant (OECD 206). This position was already evaluated and supported by the RMS (Belgium) during the evaluation for the renewal (peer review expert meeting in November 2014).

During the EU peer review expert meeting (2014) for the renewal of metalaxyl-M further consideration as to which endpoint was most appropriate for use in the regulatory risk assessment took place and further clarification on historical control data for hatchling survivorship in bobwhite quail was requested by some MSs experts. However, the EU process at that stage of the evaluation did not allow the applicant to provide additional information.

In this application are reported the new information which supports the use of the NOEL of 84 mg a.s./kg bw/day obtained with the bobwhite quail study ***([redacted] and [redacted] 1998; CGA329351/1071)***.

<u>Robustness of the endpoint from the bobwhite quail study ([redacted] ***and*** [redacted] ***- 1998; CGA329351/1071)***:</u>

During the EU peer review (2014), it was noted that the hatchling survivor rate at 14 days was low (0.56) in the bobwhite quail study ([redacted] ***and*** [redacted] ***- 1998; CGA329351/1071)***. The studies in the HCD dataset were conducted with bobwhite quail according to GLP and to OECD 206 guideline by the same authors in the same facility and in the same time frame as the above study were sourced. The hatchling survivorship from each study was collated to provide historical control data (HCD). These data showed hatchling survivor rates of 0.48-0.94 (Table IIIA **10.1-**02).

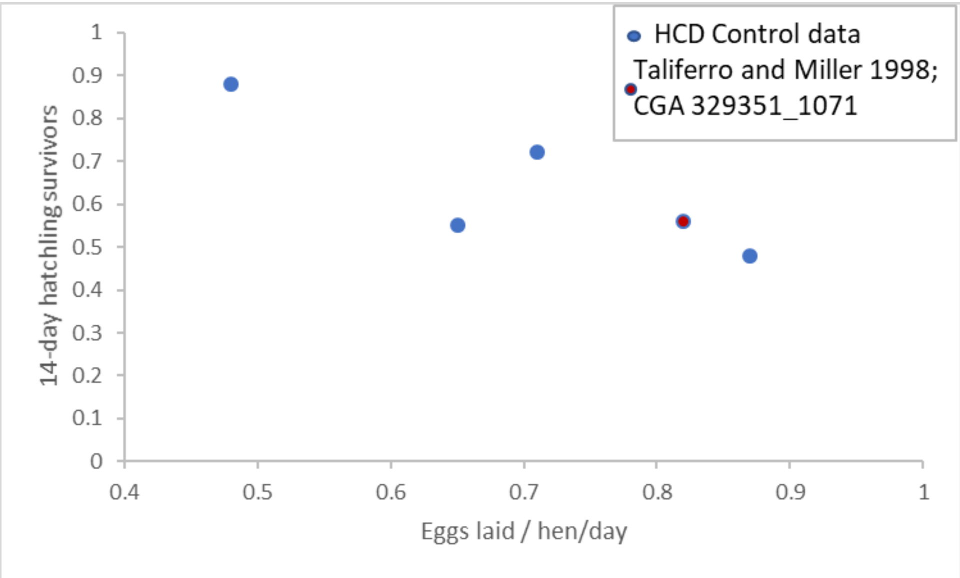
The data from the facility shows an inverse relationship between the number of eggs laid by control birds and 14-day old survivors / hatchlings (Figure IIIA 10.1-01). This position on the representativeness of the historical control data for the facility is further supported by the 14-day survivor values obtained in the test treatments within the study which were 0.74 (100 ppm), 0.66 (300 ppm) and 0.71 (900 ppm, the proposed endpoint). This confirms that 14-day survivors/hatchling for all the treatments were within the range of the facility HCD. Furthermore, it should be noted that there are no treatment related effects observed within the study (e.g. no dose response relationships). Therefore, it can be concluded that the NOEL of 84 mg a.s./kg bw/day obtained in the study is considered to be robust and suitable for use in a regulatory risk assessment.

Table IIIA 10.1-06: Summary of HCD from the bobwhite quail studies observed in the controls of several reproductive toxicity tests from [redacted]

	Control group eggs laid per female	Control group 14-day old survivors / hatchlings	Study
14-day old survivors / hatchlings	0.82	0.56	[redacted] *and* [redacted], *1998 (CGA329351/1071)* [A]
	0.48	0.88	[redacted] *and* [redacted], *1996 (CGA277476/0292)*
	0.87	0.48	[redacted] *and* [redacted], *1998b (CGA293343/0653)*
	0.71	0.72	[redacted] *and* [redacted], *1996a* CGA215944/0344.
	0.65	0.55	[redacted] *et al., 1998 (CGA24705/2591)*

[A] Study with MFX-M and has been fully discussed

Figure IIIA 10.1-07: Relationship between eggs laid per female per day and 14-day old survivors in control groups of several toxicity tests from [redacted]



With regards to the historical control data (HCD) the remaining endpoints are in line with both OECD 206 and published historical control data (***Valverde-Garcia et al. 2018***[35]). It should be noted though that the number of eggs laid per hen per day is significantly higher compared the published HCD and the OECD 206 guideline (Table IIIA 10.1-03).

This is in fact the case for 4 out of the 5 studies conducted by [redacted] which are summarised above. It is likely that the low percentage of 14-day old survivors is related to the high number of eggs laid per hen. In terms of number of 14-day old survivors per hen the control group has actually performed above average. The average number of 14-day old survivors per hen in the control group is well above 12 (26.5 respectively) which is the validity criteria for this endpoint according to the OECD 206 Test Guideline. Table 3 of the OECD 206 Test Guideline states that for the bobwhite quail the normal value of the number of 14-day hatchlings per hen is 14-25. So, in the study conducted by [redacted] ***and*** [redacted] ***(1998; CGA329351/1071)*** the number of 14-day old survivors per hen is actually slightly above the normal values according to OECD 206.

Based on the reasoning above the applicant is of the opinion that the NOEL of 84.0 mg a.s./kg bw/day from the study by [redacted] ***and*** [redacted] ***(1998; CGA329351/1071***) is robust and fit for purpose for long-term avian risk assessment.

Table IIIA 10.1-08: Comparison of control data from [redacted] *and* [redacted]*, 1998 (CGA329351/1071)* with published historical control data and OECD guideline 206

[35] Valverde-Garcia P., T. Springer, V. Kramer, M. Foudoulakis, J.R. Wheeler. 2018. An avian reproduction study historical control database: A tool for data interpretation. Regulatory Toxicology and Pharmacology 92:295-302.

Endpoint	[redacted] *and* [redacted]*, 1998 (CGA329351/1071)*	*Valverde-Garcia et al. 2018*			OECD 206
		Mean	95% lower CL	95% Upper CL	
no.eggs laid/hen/day	0.82	0.52	0.44	0.61	0.4-0.54
Eggshell thickness (mm)	0.222	0.223	0.215	0.231	0.19-0.24
Eggs cracked/eggs laid %	1	2.84	0.93	8.33	0.6-2.0
Viable embryos/eggs set %	92	90.84	79.37	96.24	75-90
Live 3 week embryos/viable embryos	99	98.97	97.45	99.59	
Hatchlings/live 3 week embryos %	96	93.32	86.64	96.78	
14-day old survivors/hatchlings %	56	91.17	82.31	95.82	75-90
Hatchlings/eggs set %	88	84.02	67.58	92.98	50-90

Critical concerns over the mallard duck study ([redacted]***, 1980a; CGA48988/0152***):

The applicant maintains the position that the study conducted with the mallard duck and metalaxyl ([redacted]***, 1980a; CGA48988/0152***) is not acceptable due to fundamental technical issues with the conduct of the study (e.g. age of birds, egg collection, etc..), which were identified in a previous EU review (2002), further than, it was not conducted according to GLP and some information (e.g. on purity) was missing. The above mentioned critical concerns have also been highlighted by the USA EPA. For this reason a more robust study on bobwhite quail [redacted] ***and*** [redacted] ***(1998; CGA329351/1071)*** was conducted.

The fundamental issues raised by the applicant are supported by the criteria set in the OECD 206 (1984) guideline published after the [redacted] ***(1980a; CGA48988/0152)*** study was conducted. According to the OECD 206 guideline criteria, several technical deficiencies with the study design and environmental conditions for the birds during testing were highlighted, and these confounding variables likely invalidated the test results which were raised in the previous European regulatory review (SANCO/3037/99-final) resulting in using the study on bobwhite quale ([redacted] ***and*** [redacted] ***- 1998; CGA329351/1071)*** to establish the EU endpoint.

More details on the critical concerns of the [redacted] ***(1980a; CGA48988/0152)*** study are provided hereafter:

Age of the birds: The study was conducted on ducks that were only 6 months old at the initiation of the study; the OECD 206 recommended age is 9-12 months. This, together with the shortened egg collection period (8 vs 10 weeks) results in it being unclear that the birds effectively reached peak egg-laying during the study. If the birds were not in the peak egg-laying phase of their life cycle this may have impacted on the either the number of quality of eggs produced.

Egg collection: In the study the eggs were only collected over an eight-week period rather than the ten-week egg collection period recommended under OECD 206. This is likely to have contributed to the lower number of eggs laid in the control that treatment groups (666 in control vs 715-818 in treatment groups) and supports that the birds may not have reached peak egg-laying during the study as required in OECD guideline 206.

These data were compared to OECD Normal Values and OCSPP Validity Criteria (Table IIIA 10.1-04). Those values in red do not meet the criteria or normal values. The value in blue is low compared to the OECD normal value range:

Table IIIA 10.1-09: Comparison of control data in [redacted] *(1980a; CGA48988/0152)* study with OECD Normal Values and OCSPP Validity Criteria

OCSPP 850.2300 Guideline Parameter	OECD 206 Normal Values	OCSPP 850.2300 Validity Criteria	MLX Mallard Control	Source in Report
Assignment to treatments		Random	Random	Text pg. 2
Adult Mortality	≤ 10%	≤ 10%	5.7%	Text pg. 11
Average number of eggs laid / hen	28-38	≥ 29	**26.6**	Table 2
Viable embryos / eggs set	85-98	≥ 80%	92.0%	Table 1A
Live 3-wk embryos / eggs set		≥ 94%	**90.9%**	Table 1A
Normal hatchlings / viable embryos		≥ 52%	69.7%	Table 1A
Normal hatchlings / eggs set	50-90	≥ 44%	**64.1%**	Table 1A
14-d Survivors / normal hatchlings	94-99	≥ 94%	**77.0%**	Table 1A
Egg shell thickness (mm)	≥ 0.34	≥ 0.316	0.365	Table 3B
Cracked eggs / eggs laid	0.6-6	≤ 13%	4.1%	Table 1A

Test material dosing: It was noted in the expert meeting (2014) that there was no information on the purity of the test substance given. However, the lack of any chemical analysis of the amount of test material in the diet is of far greater concern. The OECD 206 requires test substance concentrations in diet to be confirmed during at least the first week of the test. In addition, there is no information on the amount of corn oil used as an inert carrier and there is no indication whether the same amount of corn oil was added to the control diet; OECD 206 requires no more than 2% of the weight of the basal diet and clearly the same level of carrier should be added to both treatment groups and control.

Environmental conditions: There are certain environmental conditions that are required to ensure an optimum test conduct as per OECD 206 (1984), however many of these conditions were not met. Again, this calls into question the validity of the test as the hatchlings and birds may have been of sub-optimal health resulting in the lower endpoint than identified in the [redacted] ***and*** [redacted] ***(1998a; CGA329351/1072)*** study conducted in the mallard in compliance with the OECD 206 guideline.

"*The temperature and humidity in the research facility <u>were allowed to fluctuate with ambient temperature</u>. Temperatures below 35.0ºF and above 90.0ºF (1.67-32.2ºC) were prevented through the use of cooling ventilators, exhaust fans and ceiling insulation."*

Examples of these deviations include:

- The storage temperature deviated from the OECD 206 recommendation 13.3°C (test) compared to 14-16°C (OECD).
- Humidity was deviated outside the acceptable range at 87 % compared to 60-85 % (OECD).
- During brood development the temperatures were 37.8°C for days 0-7 compared with the OECD 206 requirement of a 32-35°C and 23.8°C, for days 7-14 compared with the OECD guideline requirement of 28-32°C.

The potential impact of these deviations from guidelines can be assessed by a comparison to background control data for mallard reproduction studies (***Valverde-Garcia et al. 2018***) (Table IIIA 10.1-05). Several control values from the metalaxyl mallard study are either outside the 95% confidence interval or differ from the mean value (shown in red).

Table IIIA 10.1-10: Comparison of control data in [redacted] *(1980a; CGA48988/0152)* study with Valverde-Garcia et al. 2018 background control data

Mallard Historical Control Values (Valverde-Garcia et al. 2018; Table 5)	MLX Mallard Study Control Values (Table 1B)	Control Mean	95% Lower CL	95% Upper CL	95% Lower PL	95% Upper PL	OECD TG206	OSCPP 850.2300
No. eggs laid / hen / d	**0.48** [a,b]	0.58	0.48	0.69	0.44	0.73		
Eggshell thickness (mm)	**0.365**	0.385	0.372	0.398	0.367	0.403	0.35–0.39	0.316–0.372
Eggs cracked / eggs laid (%)	**4**	1.57	0.52	4.66	0.33	7.2	0.6–7	0–4.0
Viable embryos / eggs set (%)	92	89.7	76.97	95.78	69.27	97.11	85–98	
Live 3 week embryos / viable embryos (%	99	98.27	96.26	99.21	94.89	99.43		94–100
Hatchlings / live 3 week embryos (%)	**71**	78.1	60.67	89.18	52.23	92.08		52–100
14-d old survivors / hatchlings (%)	97	97.95	95.64	99.05	94.09	99.31	94–99	94–100
Hatchlings / eggs set (%)	64.1	69.31	46.86	85.26	37.46	89.49		44–92

[a] Based on 8 weeks of exposure (56 d) as described on pg. 2 of the report.

[b] 666 eggs / 25 control females / 56 d

This comparison adds further evidence that the mallards used in the metalaxyl study may have had underlying issues that affected reproductive performance throughout the study. Thus, there is convincing evidence that NOECs generated from this study cannot be attributed solely to metalaxyl.

Replication: Five pens were established for each test treatment and control, with each pen containing 2 drakes and 5 hens compared to the 1 drake to three hens recommended in the guidance, although it is noted that other arrangements may be justified. However, the guidance document does explicitly state that when the mallard duck is tested in groups i.e. in ratios of other than 1 drake to 3 hens than at least 8 pens need to be established for each treatment rate and control. Given that only 5 pens were established for each test treatment rate and control then the statistical power of this study is open to question.

Conclusions

Based on the new information provided for the historical control data of the bobwhite quail study ([redacted] ***and*** [redacted] ***- 1998; CGA329351/1071)*** and the critical concerns identified in the study of mallard duck ([redacted] ***- 1980a; CGA48988/0152)***, the applicant maintains the position that the **NOEL of 84 mg a.s./kg bw/day** is the appropriate value to be used in the regulatory risk assessment.

The value of the NOEC of 300 ppm agreed during the EU peer review (2014) and used in the risk assessment (EFSA Conclusion 2015) was based on a weight of evidence and considering all the available studies at the time. However, the additional HCD provided in this application were not available at the time of the EU peer review evaluation, for this reason the applicant considers that the endpoint need further consideration during this evaluation.

The conclusion on the use of the **NOEL of 84 mg a.s./kg bw/day** in the regulatory risk assessment is further supported by another regulatory review for metalaxyl and mefenoxam (metalaxyl-M) conducted by the USA EPA[36] in 1996. This reviewed that the [redacted] ***(1980a; CGA48988/0152)*** study was only suitable as supplemental data based on the short duration of the egg collection period, uncontrolled environmental conditions, lack individual adult body weight data and a lack of treated diet analysis. Furthermore, a number of parameters in the control groups were lower than the test groups, e.g. total eggs laid resulted in lower numbers of eggs set and viable egg numbers for controls with only 27% of eggs laid surviving to 14 days.

In the USA EPA review the endpoints used for both active substances were those determined in the studies by [redacted] ***and*** [redacted] ***(1998; CGA329351/1071; CGA329351/1072)*** with metalaxyl-M and the earlier studies conducted on metalaxyl ([redacted]***, 1980; (CGA48988/0151; CGA48988/0152)*** were not considered in the dossier having been previously deemed as not being suitable for use in a regulatory risk assessment due to the issues described above.

[36] Anonymous (2016). Metalaxyl and Mefenoxam: Preliminary Ecological Risk Assessment for Registration Review of Metalaxyl and Mefenoxam (Metalaxyl-M) and Proposed Crop Group Conversion for Oilseed Group 20.
PC Code: 113501, 113502. DP Barcode: 433073
https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/113501/113501-171.pdf;
https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/113501/113501-172.pdf

In the USA EPA review the endpoints used for both active substances were those determined in the studies by [redacted] ***and*** [redacted] ***(1998; CGA329351/1071; CGA329351/1072)*** with metalaxyl-M and the earlier studies conducted on metalaxyl ([redacted]***, 1980; (CGA48988/0151; CGA48988/0152)*** were not considered in the dossier having been previously deemed as not being suitable for use in a regulatory risk assessment due to the issues described above. Furthermore, resubmission Art. 7 amendment of approval conditions (EU process) the new endpoint was supported by all member states who commented, including Austria, France, Germany, Poland, Slovenia, and the Netherlands. It is therefore clear than in recent technical reviews by technically robust member states that have a similar agronomic framework to Great Britain that the position of the applicant to base the assessment on the [redacted] ***and*** [redacted] ***(1998)*** studies and hence the endpoint of 84 mg/kg bw should be used in the risk assessment.

For completeness, in this document, both the endpoint from the racemic mixture of 24.6 mg/kg bw/day and the 84 mg a.s./kg bw/day NOEL will be used in the assessment when a higher tier is required, as per justification provided above.

B.9.2.3. Appendix 3: Summary of studies used in the consideration of Historical Control Data (HCD)

Report:	Appendix 1/01: [redacted] (1996) The Reproductive Toxicity Test of CGA-215944 Technical in Northern Bobwhite, *Colinus Virginianus.* Report Number 029502. [redacted] (Syngenta File No. CGA215944/0344)

Guidelines

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms*, subsection 71-4, Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C.

Organization for Economic Cooperation and Development. 1984. *Avian Reproduction Test*. OECD Guideline for Testing of Chemicals. Guideline 206. Paris.

GLP: Yes

Materials

Test Material	CGA-215944 technical
Lot/Batch #:	P102002
Purity:	98.5%
Description:	Yellowish powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	8 July 1996
Density:	Not applicable

Treatments

Test rates:	30, 100 and 300 ppm CGA 215944, alongside an untreated control
Food:	Basal diet (minimum 20% protein and 2.5% fat; maximum 7 % fibre for adults and minimum 30% protein and 2.5 % fat; maximum 6.5 % fibre for the offspring)
Water:	From deep well located on the [redacted].
Analysis of test concentrations	Homogeneity tested day 0 of week 1; stability tested on days 0, 7 and 14. Additional samples collected from each treatment group each time the feed was mixed to

	measure/verify test concentrations. Samples taken from control and treated diets on each occasion.
Test organisms	
Species:	Northern bobwhite (*Colinus virginianus*)
Source:	Birds from the same hatch, obtained from [redacted].
Acclimatisation period:	3.5 weeks
Treatment for disease:	None
Weight:	219.5 – 228.0 g at test initiation
Test design	
Replication:	19
No. of birds/pen and incubation and hatching:	2 (1 male and 1 female) Pens were 50.7 cm x 26 cm x 25 cm and were constructed of epoxy-coated wire mesh. Hatchlings were placed in batteries of brooding pens, each pen was 71 cm x 91 cm x 27 cm, the temperature was maintained at 39°C until the chicks were 14 days old.
Duration of test:	Study phases: Acclimation – approximately 3.5 weeks Pre-photostimulation – approximately 8 weeks Pre-egg laying (with photostimulation) – approximately 2 weeks Treated Feed – approximately 20 weeks Egg laying – approximately 10 weeks Post-adult termination (final incubation, hatching, and 14-day offspring rearing period) – approximately 5 weeks
Parameters monitored:	Adult male and female body weight, adult feed consumption, number eggs laid per day, number eggs set into incubator, number eggs cracked/number eggs laid, number fertile eggs/number eggs set, number viable embryos/number fertile eggs, number of hatchlings/number of fertile eggs, number 14-day survivors/number hatchlings, hatchling weights, 14-day survivor weights, and eggshell thickness.
Environmental test conditions	

Temperature:	18.0 – 30.0°C (Mean = 23.8 °C)
Humidity:	40 – 84 % RH (Mean = 62.1 % (RH))
Photoperiod:	Adult birds: 7 hours light from test initiation to Week 8 17 hours light from Week 8 to adult birds were euthanized (photoperiod was increased over a 3-day period of time to 17 hours of light per day) Fluorescent light, approximately 13.1 foot candles. Hatchlings: 17 hours light per day, however, the brooder heat lamps did provide intermittent infra-red light throughout the 24-hour period.

Study Design and Methods

Experimental dates: 18 April 1995 – 12 October 1995

Adult northern bobwhite (76 males and 76 females) in good health and approaching first breeding season, were randomly assigned into one control group and three treatment groups. The test substance doses were prepared using a premix of appropriate volumes of CGA-215944, such that, once incorporated into the final diet, treatment levels of 30, 100 and 300 ppm CGA-215944 were achieved. The premix for the control groups consisted of ration only. The birds were housed in male/female pairs in batteries of pens measuring approximately 50.7 x 26 x 25 cm high and had slanted floors to facilitate eggs rolling to the front collection tray. The pens were constructed of epoxy-coated wire mesh and steel sheeting and were equipped with food and automatic watering system.

During the test all birds were given feed and water *ad libitum*. Adult birds were observed daily for signs of toxicity or abnormal behaviour. Offspring were observed daily from hatching until 14 days old. Adult body weights were measured at test initiation, end of week 10 and at adult termination. Body weights were not measured during egg-laying. Feed consumption per pen was measured weekly. Spillage was measured from each cage by use of a catch pan under the feeding trays. At the conclusion of the exposure period all adult birds were euthanized with CO_2 and subjected to a gross necropsy.

Eggs were collected daily, marked with the pen number, and stored in weekly interval lots in a cold room until incubation (~10°C). At the end of each weekly interval eggs were counted, indiscriminately selected for measurement of egg shell thickness[37], and candled to detect cracked or abnormal eggs which were discarded. All eggs not discarded or used for egg shell thickness measurements were placed in an incubator. Embryo viability and survival were determined during incubation by candling on days 10-12 and day 20, respectively.

On Day 20 of incubation, eggs were transferred to an incubator configured for hatching, and all hatchlings, unhatched eggs, and egg shells were removed on Day

[37] One egg, when available, collected from odd numbered pens during odd numbered weeks, and even numbered pens during even numbered weeks

22 - 24 of incubation. Surviving hatchlings were leg-banded for identification and their body weights recorded. They were transferred to batteries of brooding pens and fed untreated diet until 14 days old, when the body weights of survivors were recorded.

Data sets were tested for normality using a Chi-Square test and for homogeneity of variance using Bartlett's test or Levene's test. Proportional data was Arcsine transformed and any data set departing from the normal distribution was transformed if the transformation results in a normal distribution. Statistical analyses used were ANOVA with Tukey's post-hoc test for paired comparisons if the data exhibited homogeneity of variance and a normal distribution. If data were heterogeneous or non-normal, they were analysed with a Kruskal-Wallis test followed by Dunn's multiple pair comparisons test. Sample units were individual pens within experimental groups, apart from adult body weight where the sample unit was the individual bird.

Results and Discussion

Samples collected during the test to verify test substance concentrations for the 30, 100 and 300 mg a.s./kg diet CGA-2159444 diets were found to be 27.68 ± 3.56, 100.99 ± 12.22 and 293.35 ± 20.41 mg a.s./kg diet CGA-215944, respectively.

Adult mortality, growth and feed consumption are summarised in the table below.

Table IIIA Appendix 1-01: Summary of effects of CGA-215944 on mortality, growth and feed consumption on adult northern bobwhite (*Colinus virginianus*) following dietary exposure

Nominal dose (ppm CGA-215944)	Mortality after 20 weeks (%)	Mean body weight [g]		Mean Feed Consumption (g/bird/day)	
		Week 10 (Pre-Egg Production)	Week 20 (Overall)	Week 10	Week 20
Control	0[1]	Male: 232.4 Female; 245.7	Male: 246.0 Female; 261.0	19.1	23.3
30	0	Male: 235.8 Female; 250.7	Male: 239.9 Female; 263.0	19.3	23.5
100	0	Male: 227.1 Female; 250.9	Male: 236.3 Female; 261.2	19.1	23.2
300	0[2]	Male: 239.6 Female; 253.6	Male: 248.7 Female; 263.9	19.4	26.9

[1] 3 mortalities found in the Control group in Test week 14, and 1 in test week 17. Post-mortem examinations were performed on all of the mortalities. There were no treatment-related findings.

[2] 1 mortality found in 300 ppm CGA-215944 group in test week 13. A post-mortem examination was performed. There were no treatment-related findings.

There were no treatment related mortalities in the control group or any of the treatment groups, and all birds in all groups were normal in appearance and behaviour for the duration of the test. There were no statistically significant differences in adult body weight or feed consumption between the treatment groups and the control group. No significant pathological changes were observed in any of the surviving birds examined at termination.

Clinical observations: there were no overt signs of toxicity observed in any of the treatment groups.

Mean adult weight data are presented below:

Mean Adult Body Weight (g) from a Northern Bobwhite Quail Study with CGA-215944
EBA Study Number 029502

Experimental Group (ppm a.i.)	Sex	Start of Aclimat.	Change	Start of Test Feed	Change	Start of Egg lay	Change	Study Finish	Total Change
Control	Male	213.8	6.9	220.7	11.7	232.4	13.6	246.0	32.2
	Female	213.3	7.1	220.4	25.3	245.7	15.3	261.0	47.7
30	Male	221.5	3.1	224.6	11.2	235.8	4.1	239.9	18.4
	Female	216.1	3.3	219.4	31.3	250.7	12.3	263.0	46.9
100	Male	216.7	2.8	219.5	7.6	227.1	9.2	236.3	19.6
	Female	214.5	7.8	222.3	28.6	250.9	10.3	261.2	46.7
300	Male	224.3	0.5	224.8	14.8	239.6	9.1	248.7	24.4
	Female	225.5	2.5	228.0	25.6	253.6	10.3	263.9	38.4

Differences between the control and treatment groups were not statistically significant.

Feed consumption data are presented below:

Mean Feed Consumption (g / bird / day)
from a Northern Bobwhite Quail Study with CGA-215944
EBA Study Number 029502

Experimental Group (ppm a.i.)	Feed Period 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Control	11.8	11.5	14.3	12.3	14.3	13.9	11.2	13.3	15.6	19.1	17.8	18.5	20.4	20.8	20.5	22.7	22.4	23.3	23.2	23.3
30	11.3	11.7	12.9	12.7	13.5	13.2	11.1	13.1	17.2	19.3	17.2	19.1	21.0	20.4	21.3	24.1	23.6	23.6	23.8	23.5
100	11.6	11.4	13.3	12.2	13.2	11.9	11.1	13.1	16.7	19.1	17.2	19.0	20.3	20.1	20.7	22.4	22.3	22.6	23.3	23.2
300	12.2	11.1	13.4	12.5	13.5	12.6	11.3	13.3	16.7	19.4	17.3	18.6	20.5	20.9	21.5	23.8	23.1	22.5	22.9	26.9

Differences between the control and each treatment group were not statistically significant. ($p > 0.05$).

Reproductive effects are summarised in the table below.

Table B.9.2.3-1: Summary of effects of CGA-215944 on reproductive parameters for northern bobwhite (*Colinus virginianus*) following dietary exposure

Nominal dose (ppm CGA-215944) [1]	No of reps	Total eggs laid	Fertile eggs/Eggs incubated (normalized as %)[2]	Viable embryos / fertile eggs (normalized as %)[2]	Hatchlings / viable embryos (normalized as %)[2]	14-Day old survivors / hatchlings (normalized as %)[2]	Hatchlings / Eggs Incubated (normalized as %)[2]	14-day old survivors / Eggs incubated (normalized as %)[2]
Control	16	797	0.96	0.99	0.97	0.72	0.93	0.67
30	19	932	0.92	0.98	0.95	0.83	0.86	0.71
100	19	976	0.96	0.99	0.98	0.72	0.93	0.67
300	18	886	0.87	0.96	0.98	0.66	0.82	0.54

[1] number of replicates = 16, 19, 19 and 18 for the control, 30, 100 and 300 ppm groups, respectively
[2]Mean value for all pens in treatment group
Differences between the control and treatment groups were not statistically significant.

There were no treatment-related effects on egg shell thickness, or on any of the reproductive performance parameters tested.

Table B.9.2.3-2: Summary of effects of CGA-215944 on Northern Bobwhite (*Colinus virginianus*) hatchling growth following adult dietary exposure

Nominal dose (ppm CGA-215944)	Hatchlings		14-day old survivors	
	Number	Mean (± SD) body weight of (g)	Number	Mean (± SD) body weight of (g)
Control	663	7.7 (± 0.643)	479	24.4 (± 7.981)
30	722	7.6 (± 0.660)	599	23.4 (± 7.251)
100	823	7.4 (± 0.639)	596	20.8 (± 6.651)
300	648	7.5 (± 0.625)	428	24.6 (± 7.611)

Differences between the control and treatment groups were not statistically significant.

Data on eggshell thickness are presented below:

Mean Egg Shell Thickness Measurements from a Northern Bobwhite Reproduction Study with CGA-215944
EBA Study Number 029502

Experimental Group (ppm a.i.)	No. of Eggs Measured	Shell Thickness Mean	Standard Deviation
Control	79	0.220	0.022
30	81	0.224	0.026
100	92	0.218	0.021
300	84	0.219	0.019

Differences between the control and treatment groups were not statistically significant.

Conclusions
There were no treatment-related mortalities or treatment-related effects observed in any of the parameters tested. The NOEC for dietary exposure to CGA-215944 was determined to be 300 ppm CGA-215944, the highest concentration tested.

Considered in the original assessment and in the renewal; the renewal states:

> *The study was considered valid in the original pymetrozine monograph from 1998. No revision of this evaluation is necessary.*
>
> *Recalculation of concentration in food to daily dose was based on a mean daily food consumption of 17.7 g and a mean body weight of 243.1 g over the 20-week exposure in the NOEC treatment group receiving a diet with 300 mg as/kg. The resulting NOEL amounts to 21.8 mg as/kg bw/d.*

EFSA conclusion of 2014[38] states that the same endpoint, i.e. 300 mg/kg diet equivalent to 21.8 mg/kg bw.

([redacted], [redacted], 1996)

Report:	Appendix 1/02, [redacted], [redacted], [redacted] (1996), The Reproductive Toxicity Test of CGA-24705 In Northern Bobwhite (*Colinus virginianus*), Report Number 029508. [redacted]. (Syngenta File No. CGA24705/2591)

Guidelines

[38] EFSA (European Food Safety Authority), 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance pymetrozine. EFSA Journal 2014;12(9):3817, 102 pp. doi:10.2903/j.efsa.2014.3817

OECD (1984) OECD Guideline 206, Guideline for Testing of Chemicals, Avian Reproductive Test
U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, subsection 71-4

GLP: Yes

Materials

Test Material	Metolachlor Technical CGA-24705 FL 930326
Lot/Batch #:	P.111072
Purity	97.3 %
Description:	Brown liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	01 March 1997
Density:	Not reported

Treatments

Test rates:	50 ppm, 200 ppm and 800 ppm CGA 24705 (adjusted for purity), alongside an untreated control
Food:	Basal diet for the adult birds and their offspring was Purina Layena (minimum 20 % protein, minimum 2.5 % fat and maximum 7 % fibre) and Purina Startena (minimum 30 % protein, minimum 2.5 % fat and maximum 6.5 % fibre), respectively
Water:	Well water from a deep well located on the test facility site
Analysis of test concentrations:	Homogeneity was tested in four samples each from the 50 ppm and 800 ppm diet mix at the beginning of the study. Additional samples collected from the freshly prepared test diet batches to verify test substance concentrations. Samples for analysis of stability were analysed on day 0, 7 and 14 and at 4 months for ambient samples and on days 0, 14, 28 and at 4 months for frozen samples.

Test organisms

Species:	Northern bobwhite (*Colinus virginianus*), 15 weeks old
Source:	Laboratory stock hatched and raised at test facility (Colony 022). Eggs from [redacted]

Acclimatisation period:	2 weeks
Treatment for disease:	None reported
Weight:	162.0 to 229.3 g at start of acclimation period
Test design	
Replication:	18
No. of birds/pen:	Two (1 male and 1 female)
Duration of test:	24 weeks
Parameters monitored:	Adult male bodyweight, adult female body weight, adult feed consumption, number eggs laid, number eggs set into incubator, number eggs cracked / number eggs laid, number fertile eggs / number eggs set, number viable embryos/ number fertile eggs, number hatchlings / number viable embryos, number 14-day survivors / number hatchlings, hatchling weights, 14-day survivor weights, eggshell thickness
Environmental test conditions	
Temperature:	Adult birds (study room range): 16.1 to 29.4 ºC Egg storage prior to incubation: 10 ºC Incubation and hatching (brooding pen): 38.9 ºC
Humidity:	Adult birds, average relative humidity: 62.1 % (range 40 to 84 %)
Photoperiod:	Adult birds: 7 hours of light per day during acclimation and the first 8 weeks, increasing over 13-day period to 17 hours of light per day from week 9 until the adult birds were euthanised. Mean light intensity was approximately 11.1 footcandles. Incubation and hatching: 17 hours of light and 7 hours of dark, intermittent infra-red light was provided by the brooder heat lamps throughout the 24-hour period

Study Design and Methods

Experimental dates: 22 August 1995 to 28 March 1996

Adult northern bobwhite quail (72 males and 72 females), 15 weeks old at experiment start, 27 weeks old at start of egg-laying, and in good health, approaching first breeding season, were randomly assigned to one control group and three treatment groups. A pre-mix of suitable strength was prepared on an as-needed basis within the stability result parameters, by mixing the required quantities of test substance with the basal diet. The test substance doses were prepared by mixing appropriate quantities of pre-mix and basal ration, to achieve treatment levels of 50, 200 and 800 ppm CGA 24705. The mix for the control groups consisted of basal ration only.

Adult mating pairs were housed in epoxy-coated wire mesh battery cages measuring 50.7 x 26 x 25 cm. During the test birds were given food and water *ad libitum*. For the first eight weeks of treated feed, the test birds were held under a photoperiod of 7 hours of light per day. At the end of week 8, the photoperiod was increased to 17 hours of light per day to induce egg laying. Adult continued on a photoperiod of 17 hours light per day until termination. The first eggs were set for incubation at the end of week 14.

Adult birds were observed daily for mortality, signs of toxicity or abnormal behaviour. Offspring were observed daily from hatching until 14 days old. Adult body weights were measured at the beginning of acclimatisation, at exposure initiation, at the end of week 10 and test termination. Feed consumption per pen was recorded weekly throughout the treatment period. At the conclusion of the experiment, all adult birds and hatchlings were examined following euthanasia by carbon dioxide asphyxiation.

Eggs were collected daily and stored in a cold room until incubation. Following each seven-day period, eggs were removed from the cold rooms prior to incubation and candling. The total number of eggs collected, and the number of un-cracked and unblemished eggs were recorded. Non-defective eggs were placed in an incubator. On approximately day 21 of incubation, the eggs were placed in a hatcher and allowed to hatch.

Weekly during the egg-laying period, one egg laid per treatment group was examined for shell thickness. Embryo viability and survival, unhatched eggs, hatchlings, and weight and viability of chicks, were recorded for each hatch. Chicks were not examined *post mortem* upon study completion.
Data sets were tested for normality using a Chi-Square test and for homogeneity of variance using Bartlett's test or Levene's test. Proportional data was arcsine transformed and any data set departing from the normal distribution was transformed if the transformation resulted in a normal distribution. Data parameters were analysed by ANOVA with Tukey's post-hoc test for paired comparisons if the data exhibited homogeneity of variance and a normal distribution. If data were heterogeneous or non-normal, they were analysed with Kruskal-Wallis test followed by Dunn's multiple pair comparisons test.

Results and Discussion

The diet was analysed for homogeneity and to verify the concentration, it was also subjected to an assessment over a 4-month period to ascertain stability. The results are presented below:

Homogeneity: Diet samples collected from the 50 and 800 ppm a.i. (EBA, Inc. Sample Numbers 1800-1883 and 1892-1895, respectively) analyzed to evaluate homogeneity of the test substance in the diet resulted in a mean and Standard Deviation as follows:

Nominal Treatment Level	Verified Mean Level	Standard Dev.
50 ppm	55.50 ppm	8.04
800 ppm	996.26 ppm	117.69

Verification: Diet samples collected from the 50, 200, and 800 ppm a.i. analyzed to verify the ppm of the diet mix resulted in a mean and Standard Deviation as follows:

Nominal Treatment Level	Verified Mean Level	Standard Dev.
50 ppm	51.68 ppm	7.0
200 ppm	185.24 ppm	44.15
800 ppm	902.10 ppm	136.39

Stabilities: Samples for analysis of stability at ambient room temperatures were collected on Day 0 and analyzed on Day 7 and Day 14 and at a 4-month interval. The following values are averages of the samples collected:

Level	Day 7*	Day 14*	4 - Month*
50	91%	77%	85%
800	110%	108%	105%

* % of Day 0

Freezer Stability Tests made on Day 0, 14 and 28 and at a 4 month interval averaged as follows:

Level	Day 14*	Day 28*	4 - Month*
50	111%	108%	128%
800	114%	105%	108%

* % of Day 0

Mortalities: There were 12 mortalities and three birds requiring euthanasia during the feed portion of the study – this involved 4 males and 11 females. Post-mortem examinations were performed on all of these mortalities. The results are presented below:

List of Mortalities

Bird No. and Sex	Cage	Test Week	Treatment Level ppm	Weight at post-mortem (grams)	Comments
52286-M	38	12	0	98.3	Emaciated
52122-F	67	14	0	123.6	Scalped
52256-M	34	15	0	75.0	Emaciated
52217-F	23	15	0	211.1	Dehydrated
52207-F	21	18	0	115.9	Emaciated, feet pecked
52189-F	54	12	50	198.1	Scalped
52288-M	31	22	50	145.0	Emaciated, feet pecked and scraped
52183-F	69	23	50	135.9	Emaciated, feet pecked and dehydrated
52107-F	52	14	200	177.0	Infection, throat and mouth
52127-F	57	17	200	236.3	Scalped
52104-F	16	24	200	125.0	Feet and head pecked pair mate emaciated (weight - 138.3 g)
52236-F	44	14	800	194.6	Scalped - euthanized
52258-M	15	15	800	144.8	Emaciated, eye and feet pecked - euthanized
52220-F	50	18	800	139.3	Pecked, growth behind beak and on head
52129-F	49	23	800	163.8	Pecked head and feet - euthanized

Clinical observations: No overt signs of toxicity were observed in any of the treatment groups during the course of the test.

Body weight data are presented below:

Mean Adult Body Weight (g) from a Northern Bobwhite Quail Study with CGA-24705
EBA Study 029508

Experimental Group (ppm a.i.)	Sex	Start of Acclimat.	Change	Start of Test Feed	Change	Pre- Egg Laying	Change	Study Finish	Total Change*
Control	Male	193.0	0.8	193.8	10.2	204.0	3.7	207.7	14.7
	Female	192.4	1.1	193.5	10.5	204	28.4	232.4	40.0
50	Male	189.0	-2.2	186.8	12.6	199.4	0.3	199.7	10.7
	Female	188.1	1.8	189.9	14.9	204.8	28.1	232.9	44.8
200	Male	193.6	1.8	195.4	5.1	200.5	13.4	213.9	20.3
	Female	191.4	-4.3	187.1	10.3	197.4	9.6	207.0	15.6
800	Male	194.7	-3.8	190.9	13.2	204.1	2.3	206.4	11.7
	Female	194.1	-0.6	193.5	7.7	201.2	22.9	224.1	30.0

* Denotes difference in average weight between start of acclimation and study finish.

No significant differance between Control group and Treatment groups. ($p > 0.05$)

Feed consumption is presented below

Mean Feed Consumption (g / bird / day)
from a Northern Bobwhite Quail Study with CGA-24705
EBA Study 029508

Experimental	Feed Period																							
Group (ppm a.i.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Control	23.9	18.3	15.6	18.5	15.0	16.5	13.7	15.6	14.7	16.0	17.8	17.8	16.7	18.1	17.3	20.5	24.6	24.5	22.1	21.4	23.6	21.9	20.7	19.7
50	21.2	20.5	14.4	16.8	14.6	15.9	14.3	14.9	14.0	15.4	16.5	16.3	15.7	16.7	17.3	20.5	22.2	23.0	21.2	19.5	20.6	19.0	20.5	19.2
200	22.8	19.8	15.2	18.4	14.7	15.4	13.0	15.1	14.5	15.2	16.6	18.0	15.9	17.3	17.4	20.9	21.8	23.2	21.2	19.8	20.2	18.5	19.8	17.7
800	20.9	20.2	15.1	16.3	15.4	16.3	14.7	13.5	13.8	15.8	16.9	17.7	15.4	15.9	16.4	19	20.6	22	21.7	21.6	21.7	19.8	20.2	18.1

No significant difference between Control group and Treatment groups. ($p > 0.05$)

Fecundity is summarised in the table below.

Table IIIA Appendix 1-04: Summary of effects of CGA 24705 on fecundity of northern bobwhite (*Colinus virginianus*) following oral exposure

Parameter	Control	50 ppm CGA 24705	200 ppm CGA 24705	800 ppm CGA 24705
Number of replicates	13	15	14*	14
Total eggs laid	668	587	433	597
Number of eggs laid/hen	0.65	0.5	0.37	0.54
Eggs cracked	24	33	16	29
Eggs for eggshell thickness	62	66	57	56
Eggs set	582	488	360	512
Fertile eggs	540	417	320	477
Viable embryos	533	398	304	455
Hatchlings	497	354	262	391
14-day old survivors	274	170	130	216
Number of eggs laid**	0.65	0.50	0.37***	0.54
Number of eggs set into incubator**	0.57	0.41	0.33***	0.46
Eggs cracked / Eggs laid	0.04	0.06	0.04	0.05
Fertile eggs / Eggs set	0.93	0.85	0.89	0.93
Viable embryos / Fertile eggs	0.99	0.95	0.95	0.95
Hatchlings / Viable embryos	0.93	0.89	0.86	0.86
14-day old survivors / Hatchlings	0.55	0.48	0.50	0.55

* Cage number 19 in the 200 ppm group produced 0 eggs. As a result, it was included only as a statistical replicated for the comparison of eggs laid and not for any of the subsequent reproductive parameters, because no real data values exist for those parameters. Therefore, the number of statistical replicates for eggs laid in the 200 ppm group is 15 and for the remaining comparisons it is 14.
** These parameters computed as number per day per hen. All others computed as total / total (for example: total eggs cracked / total eggs laid)
*** Significantly different from the control group ($p < 0.05$). The effects observed do not reflect the expected dose response, and therefore are probably not treatment related.

There were no treatment-related mortalities or signs of toxicity during the experimental period or in the *post mortem* examination. There were no apparent treatment related effects upon adult body weight or feed consumption at any of the concentrations tested.

There were no apparent treatment-related effects upon reproductive performance, egg shell thickness or offspring body weights at any of the concentrations tested. Birds in the 200 ppm CGA 24705 test concentration showed decrease in number of eggs laid that was statistically significant ($p < 0.05$); however, there were no indications of concentration-dependant effects, it was not considered to be treatment related.

Validity criteria

No validity criteria were reported.

Conclusions

No treatment-related effects were observed for northern bobwhites exposed to CGA 24705 for adult mortality, signs of toxicity, adult body weight or feed consumption, reproductive performance, egg shell thickness or offspring body weight. The no observed effect level was considered to be 800 ppm CGA 24705, which was determined by the RMS to be equivalent to a NOEC 71 mg/kg bw/day.

This active substance is currently undergoing renewal within the EU and the above endpoint has yet to be confirmed.

([redacted], [redacted], [redacted], 1996)

Report:	Appendix 1/04, [redacted] & [redacted] (1998a), The reproductive toxicity test of CGA293343 Technical with the Northern bobwhite (Colinus virginianus), Report Number 029518. [redacted] (Syngenta File No. CGA293343/0653, VV-376393)

Guideline(s): EPA Guideline No.: 71-4

Deviations: No

GLP: Yes

Acceptability:

Duplication (if vertebrate study) No

Materials

Test Material CGA293343

Lot/Batch #: FL961679

Purity: 99.2%

Stability of test Stable under standard conditions.

compound:	
Expiration date:	31 July 1997
Density:	n/a
Treatments	
Test rates:	100 ppm, 300 ppm and 900 ppm
Test organisms	
Species:	Northern bobwhite *(Colinus virginianus)*
Source:	[redacted]
Acclimatisation period:	28 days
Treatment for disease:	None
Weight:	168.7– 237.1 g
Test design	
Replication:	18
No. of birds/pen:	2 (one male, one female)
Duration of test:	204 days
Environmental test conditions	
Temperature:	14.4 to 24.4°C
Humidity:	24 to 82% (mean 47.8% SD 12.1%)
Photoperiod:	7 hours light for 8 weeks, increased over a period of 5 days to 17 hours light for remainder (14.8 footcandles)

Study Design and Methods

Experimental dates: 5 September 1996 to 28 March 1997

Mature Northern bobwhite received CGA293343 technical at nominal dietary concentrations of 100, 300 and 900 ppm for 23 weeks and 5 days. A control group receiving basal diet was maintained concurrently with the treatment groups.

The test item was provided at a treated diet. Purina game bird ration was used for feed and was provided *ad libitum* during acclimation and the test period.

Birds were kept in pairs one male and one female per cage. The cage dimensions were 51 cm deep, 25cm wide and 20.5-25 cm in height, with a floor slope so eggs roll forward and out of the cage on to a collection tray. The study room was maintained between 14.4 and 24.4°C and had relative humidity of between 24-82% with an average of 47.8%. Light exposure was at an average of 14.8 foot candles.

Body weight was measured 4 times during acclimation and the definitive test. Feed consumption was measured weekly for each pair of birds. Birds where observed daily for signs of behavioural abnormality and mortality. Gross pathological examinations were performed on all birds succumbing prior to adult termination and on all birds surviving the test.

Eggs were collected on a daily basis and marked. Eggs were candled prior to being placed in an incubator. Cracked eggs were recorded as cracked and discarded. Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered cages during even numbered weeks and from each of the even numbered changes on odd numbered weeks. The eggs were cleaned of their contents, eggshells were then allowed to air dry measured at 5 points around the equator.

On day 14 of incubation, eggs were candled for fertility. Those that were not fertile were discarded. On day 21 of incubation eggs were candled for viability, those deemed not viable were discarded.

Upon hatch chicks were weighed and banded with a unique wing tag. Chicks were housed in caging units measuring 21 cm long x 21 cm wide x 27 cm high, chicks from no more than two parental pairs were housed in one unit. Chicks were fed Purina Startena game ration *ad libitum*. Chicks were observed daily for behaviour and mortality. Surviving chicks were euthanized on the 14th day and weighed.

Birds were observed once daily during acclimation and during the treatment period. Mortality of test animals was monitored. Gross pathological examinations were performed on all birds succumbing prior to adult termination and on all birds surviving the test.

The following data were collected:

- Number of eggs laid
- Number of eggs cracked
- Number of eggs taken for eggshell thickness
- Number of fertile eggs
- Number of viable embryos
- Number of chicks hatched

The above were used to determine the following parameters:

- Number of eggs laid
- Number of eggs set
- Number of eggs cracked/number of eggs laid
- Number of eggs fertile/number of eggs set
- Number of viable embryos/number of eggs fertile
- Number of eggs hatched/number of viable embryos
- Number of 14-day survivors/number of eggs hatched
- Hatchling weights
- 14-day old survivor weights
- Eggshell thickness

Statistical analysis was performed using TOXSTAT (1994). Data sets were tested for normality using a chi-square test and for homogeneity of variance using a Bartlett's test or Levene's test. Proportional data were arcsine transformed. If the data were normal and variances were homogenous the parameters were analysed with ANOVA Dunnett's post hoc test or an ANOVA and then a Tukey's post-hoc test for pair-wise comparisons. If data was not normal or were heterogeneous, they were analysed with a Steel's Many One Rank Test (equal size groups) or a Kruskal Wallis' ANOVA by ranks followed by a Dunn's multiple comparison (unequal size groups).

Results and Discussion

Results are summarised below.

Average concentrations found in the definitive diet samples were: 95.08±13.33 mg/kg diet for the 100 mg/kg diet level, 309.89±28.47 mg/kg diet for the 300 mg/kg diet level and 946.42±129.58 mg/kg diet for the 900 mg/kg diet level.

There were six adult mortalities during the treated feed portion of the study – two in the control group, three in the 300 mg/kg diet and one in the 900 mg/kg diet. None were deemed to be treatment related.

As regards surviving adults, post-mortem examination indicated that the majority of birds had fatty tissue, the males had enlarged testes and the females had eggs present. Other post-mortem findings were:

> Control: two females were noted to have gaseous intestines
> 100 mg/kg diet: Six females were noted to have gaseous intestines, two males and three females had enlarged livers
> 300 mg/kg diet: Three females were noted to have gaseous intestines. One male was noted to have dark grey-coloured testes. Two females were noted to have very small eggs.
> 900 mg/kg diet: Six females were noted to have gaseous intestines. One male was noted to have dark grey-coloured testes. Two females were noted to have an enlarged liver. One female was noted to have an enlarge intestine. Two females were not sexually active.

The above were not considered to be treatment related.

All birds were normal in appearance and behaviour. No overt signs of treatment-related toxicity were observed.

Table B.9.2.3-2: Summary of bodyweight of Northern bobwhite quail with CGA293343

Experimental group (ppm)	Sex	Body weight (g)		
		Start of test feed	Start of photostimulation	Adult termination
Control	Male Female	200.1 198.4	213.9 212.2	219.8 250.2
100	Male Female	197.7 197.4	215.2 215.4	228.5 254.9
300	Male Female	197.8 196.7	212.8 213.3	219.4 238.4
900	Male Female	197.0 195.3	210.8 208.8	214.0 233.7

No significant difference between the control group and treatment groups ($p<0.05$)

There were no significant differences detected between any of the treatment groups and the controls for male birds. As for females, there were no significant differences detected between any of the treatment groups and the control. There were no significant differences detected between any of the treatment groups.

Table B.9.2.3-3: Summary of reproductive performance from Northern bobwhite quail reproduction with CGA293343

Reproductive parameters	Control	100 mg / kg feed	300 mg / kg feed	900 mg / kg feed
Number of replicates	16	18	15	17
Total eggs laid in each group	990	1139	918	876
Eggs laid / per hen	61.8	63.3	61.2	51.5
Eggs laid / per day per hen	0.87	0.89	0.86	0.73
Eggs cracked	9	10	9	8
Eggs cracked / eggs laid	0.009	0.009	0.010	0.009
Mean egg shell thickness (mm)	0.209	0.207	0.208	0.210
Eggs set	901	1040	835	786
Number of eggs set into incubator	0.79	0.81	0.78	0.65
Fertile eggs	815	986	787	706
Fertile eggs/ eggs set	0.90	0.95	0.94	0.90
Viable embryos	794	972	777	690
Viable embryos / fertile eggs	0.97	0.99	0.99	0.99
Hatchlings	755	933	745	650
Hatchlings / viable embryos	0.95	0.96	0.96	0.94
14-Day old survivors	429	501	497	412
14-Day old survivors / of hatchlings	0.57	0.54	0.67	0.63
Hatchlings / eggs set	0.84	0.90	0.89	0.83
14-Day old survivors / eggs set	0.48	0.48	0.60	0.52
14-Day old survivors/hen	26.8	27.8	33.13	24.2

Mean chick body weight at hatching (g)	6.22	6.39	6.21	6.16
Mean chick body weight at 14 days (g)	22.07	20.94	22.84	22.62
NOEL	**900 mg/kg feed**			

Table B.9.2.3-4: Mean body weight (g) of hatchlings and 14-day old survivors

Mean Body Weight (g) of Hatchlings and 14 Day Old Survivors from a Northern Bobwhite Reproduction Study with CGA-293343
EBA Study 029518

Experimental Group (ppm)	Hatchlings No.	Hatchlings Mean*	Hatchlings Std Dev*	14 Day Old Survivors No.	14 Day Old Survivors Mean*	14 Day Old Survivors Std Dev*
Control	755	6.22	0.57	429	22.07	5.53
100 ppm	933	6.39	0.58	501	20.94	5.09
300 ppm	745	6.21	0.58	497	22.84	5.88
900 ppm	650	6.16	0.66	412	22.62	6.76

*Means and standard deviations are calculated from individual weights, by hatchling and are slightly different from statistical means due to rounding.
No significant different between any treatment group and the corresponding control group, (P > .05).

Table B.9.2.3-5: Mean eggshell thickness measurements (mm)

Mean Eggshell Thickness Measurements (mm) from a Northern Bobwhite Reproduction Study with CGA-293343
EBA Study 029518

Experimental Group (ppm)	No. of Eggs Measured	Shell Thickness Mean	Standard Deviation
Control	80	0.209	0.015
100 ppm	89	0.207	0.015
300 ppm	69	0.208	0.011
900 ppm	82	0.210	0.016

No significant differences between control group and treatment groups. (p > 0.05)
The means and standard deviations are calculated from individual eggshell measurements

Conclusions

According to the study authors and the first review of thiamethoxam the endpoint was a NOEC of 900 mg a.s./kg diet. However, when DE reviewed the study as part of the renewal process, they stated the following:

This study is still considered acceptable and reliable for the risk assessment. When comparing the 900 ppm treatment group to the corresponding control group for the number of Eggs laid / per day per hen, 0.73 and 0.87, respectively, there is a difference of around 16.1% that could be considered biologically relevant. Moreover, for this parameter, there was significant dose responses (p(F) ≤ 0.05), however, due to the low goodness of fit the ECx could not be reliably calculated. RMS considered that even if EC10 could not be reliably calculated, the NOEC should be revised and set to 300 ppm instead of 900 ppm in original monograph. Overall NOEL = 300 ppm.

The above was not subjected to peer review by MS or EFSA.

([redacted] and [redacted], 1998)

Report:	**KCA 8.1.3/01; [redacted] and [redacted], 1996a**
Title:	**The Reproductive Toxicity Test of CGA 277,476 Technical in Northern Bobwhite (*Colinus virgianicus*)**
Report No:	**029401**
Document No:	**Novartis file N°277476/292**
Guidelines:	**US EPA-FIFRA Guidelines No. 71-4(a), OECD Guideline No. 206**
GLP/GEP:	**yes**

Test System: Oxasulfuron technical, Batch No. FL-930038; Purity: 95.4% oxasulfuron content. Four groups of adult Bobwhite quails (*Colinus virginianus*), approaching their first breeding season (average weight 213 g at study initiation), received the test substance at concentrations of 0, 10, 35 and 100 mg a.s./kg feed *ad libitum* during a period of 22 weeks. Each treatment and the control group contained 16 pairs of birds with one male and one female per cage. After 11 weeks of treatment, the first eggs were set for incubation and observed until 14 days post-hatch. Exposure of the parental generation continued for an additional 11 weeks of egg production. Adults were observed daily for mortality, abnormal behaviour and other signs of toxicity (body weight, feed consumption). Reproductive parameters such as number of eggs laid, number of eggs cracked, egg shell thickness, embryonic viability and chick survival were assessed.

<u>Findings:</u>

Table 9.1: Mortalities

Bird I.D. and Sex	Cage	Test Week	Treatment Level	Cause of Mortality
F	31	15	10 ppm	Leg injury
F	36	17	10 ppm	Caught between feed tray and egg ramp
F	71	17	100 ppm	Leg injury
F	25	21	10 ppm	Found dead in cage
F	65	21	Control	Rupture of infundibulum

Table 9.2: Mean Adult Body Weight

Experimental Group (ppm a.i.)	Sex	Start of Aclimat.	Change	Start of Test Feed	Change	Start of Egg lay	Change	Study Finish	Total Change
Control	Male	209.5	4.8	214.3	3.8	218.1	13.4	231.5	22.0
	Female	212.3	2.0	214.3	16.2	230.5	21.8	252.3	40.0
10	Male	210.1	1.7	211.8	4.7	216.5	8.9	225.4	15.3
	Female	209.5	0.9	210.4	20.5	230.9	23.1	254.0	44.5
35	Male	208.4	3.3	211.7	5.1	216.8	12.3	229.1	20.7
	Female	206.4	-3.8	202.6	19.6	222.2	21.4	243.6	37.2
100	Male	220.1	-2.3	217.8	5.2	223.0	19.4	242.4	22.3
	Female	215.2	6.2	221.4	17.1	238.5	25.2	263.7	48.5

No significant differance between Control group and Treatment groups. ($p > 0.05$)

Table 9.3: Mean Food Consumption

Experimental Group	Feed Period 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Control	11.6	11.4	9.9	11.6	10.4	10.9	11.5	10.9	13.2	14.9	16.9	16.8	16.7	17.3	19.2	20.4	20.7	21.7	22.5	23.4	20.9	16.6
10 ppm	11.3	11.5	9.5	11.9	10.6	11.2	11.5	10.6	12.9	14.2	17.1	16.4	15.4	16.4	18.1	20.4	19.9	21.8	21.4	22.5	20.6	15.5
35 ppm	11.0	11.3	9.6	11.7	10.3	11.1	11.6	10.7	12.7	14.9	17.0	15.2	16.7	17.1	18.9	20.8	20.3	21.8	22.5	23.1	22.8	17.2
100 ppm	11.3	10.9	9.6	11.8	10.3	11.3	11.8	10.9	13.1	14.7	17.1	15.5	17.3	18.3	19.9	20.7	19.7	21.6	22.8	22.9	22.3	16.4

No significant differance between the control group and the treatment groups. ($p > 0.05$)

Table 9.4: Reproductive toxicity of technical oxasulfuron to the Bobwhite quail

Reproductive parameters	Control	10 mg /kg feed	35 mg / kg feed	100 mg /kg feed
Number of replicates	15	13	16	15
Total eggs laid	552	561	727	649
Eggs laid per female per day*	0.48	0.56	0.59	0.56
Eggs laid/Hen	36.80	43.15	45.44	43.27
Eggs laid/Hen/Day	0.48	0.56	0.59	0.56

Eggs cracked	7	6	20	9
Eggs cracked of eggs laid*	0.01	0.01	0.03	0.01
Eggs set	486	500	639	577
Fertile eggs	477	475	595	533
Fertile eggs of eggs set*	0.98	0.95	0.93	0.93
Viable embryos	469	469	571	523
Viable embryos of fertile eggs*	0.98	0.99	0.95	0.98
Hatchlings	447	457	546	510
Hatchlings of fertile eggs*	0.94	0.96	0.91	0.96
14-day old survivors	404	414	497	452
14-day old survivors/Hen	26.93	31.85	31.06	30.13
14-day old survivors of normal hatch*	0.88	0.91	0.89	0.88
Chick body weights at hatching (g)	7.4.0 ± 0.6	7.5 ± 0.6	7.4 ± 0.5	7.5 ± 0.6
Chick body weights at 14 days (g)	30.4 ± 6.2	31.3 ± 6	31.1 ± 6	30.8 ± 5.8
Mean egg shell thickness (mm)	0.183 ± 0.019	0.176 ± 0.023	0.175 ± 0.019	0.180 ± 0.020
NOEC	≥ 100 mg/kg feed			

*normalised as proportions

Table 9.5: eggshell thickness (mm)

Experimental Group	No. of Eggs Measured	Shell Thickness Mean (mm)	Standard Deviation
Control	60	0.183	0.019
10 ppm	55	0.176	0.023
35 ppm	68	0.175	0.019
100 ppm	66	0.180	0.020

No significant differences between Control group and Treatment groups. ($p > 0.05$).

Observations: There were no treatment-related adult mortalities or overt signs of toxicity in adult Bobwhite at any of the concentrations tested compared to the control group (p>0.05). There were also no apparent treatment-related effects on the reproductive parameters at any treatment levels tested. Therefore, the no observable effect concentration (NOEC) was ≥ 100 mg a.s./kg feed, the highest concentration tested.

Gross necropsy: all surviving adults were subjected to a *post-mortem* investigation. There were no apparent treatment related effects observed in the study.

Comments (DAR 2000): study and conclusions are acceptable.

RMS Comment from 2015 assessment – note that the following comment is applicable to the two avian reproduction studies submitted for the renewal of oxysulfuron.

The reported studies are GLP compliant. They are conducted according to the US EPA Test Guideline 71-4 from 1982; there were no major deviations from this guideline or the more recent US EPA TG OPPTS 850.2300 from 2012 or with OECD 206 from 1984. The test results are in compliance with the guideline's validity criteria (mortality in the control ≤10%; average number of 14-day-old survivors per hen in the control ≥ 14 for mallard and 12 for bobwhite), except for eggshell thickness in bobwhite quail study that resulted slightly below the recommended value (0.183 vs. 0.19); the 14-day-old survivors per hen in the control were well above the recommended value (26.93 vs. 12), therefore the deviation was considered to not substantially affect the results. The studies are acceptable for regulatory use; endpoints from both studies are identical (no effects at the highest tested dose) and used in TER calculation.

During the commenting phase RMS was required to convert long term endpoints from mg a.s./kg food to mg a.s./kg bw/d taking into account the overall mean value for food consumption and body weight.
For mallard duck, the mean body weight resulted 1130.1 g, and the mean food consumption 143.15 g. The resulting NOEL is 12.67 mg a.s./kg bw/d.

For bobwhite quail, the mean body weight resulted 230.26 g, and the mean food consumption 15.92 g. The resulting NOEL is 6.91 mg a.s./kg bw/d.

B.9.2.4. Appendix 4 HSE's Consideration of Valvedre-Garcia *et al* (2018)

In EFSA (2009) guidance was provided regarding how to use historical control data (HCD). Concerns and questions have been raised regarding the appropriateness of this guidance during both the peer review process and the public consultation. These concerns can be briefly summarised as follows:

- How should HCD be evaluated and, if considered appropriate, be incorporated into a hazard assessment and then the risk assessment.
- Need for the consideration of the biological relevance of the HCD.
- Consistency with mammalian toxicology, for example the timeframe needed to be considered.
- Relevance of HCD compared to the concurrent control, for example should one take precedence over the other

There is reference to HCD in paragraph 3 of Section 5 (Toxicology and Metabolism studies), Section 5.5 and Section 5.6 of Commission Regulation (EU) No 283/2013; the following is stated in the latter two sections:

> *Where submitted, historical control data shall be from the same species and strain, maintained under similar conditions in the same laboratory and shall be from contemporaneous studies. Additional historical control data from other laboratories may be reported separately as supplementary information.*
>
> *The information on historical control data provided shall include:*
>
> a) *identification of species and strain, name of the supplier, and specific colony identification, if the supplier has more than one geographical location;*
> b) *name of the laboratory and the dates when the study was performed;*
> c) *description of the general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed;*
> d) *approximate age, in days, and weight of the control animals at the beginning of the study and at the time of killing or death;*
> e) *description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (such as diseases, infections);*
> f) *name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study;*
> g) *a statement of the nature of the tumours that may have been combined to produce any of the incidence data.*[39]

[39] This point is only relevant to Section 5.5 Long-term toxicity and carcinogenicity

The historical control data shall be presented on a study by study basis giving absolute values plus percentage and relative or transformed values where these are helpful in the evaluation. If combined or summary data are submitted, these shall contain information on the range of values, the mean, median and, if applicable, standard deviation.

Despite the above requirements, there is currently no mammalian toxicology guidance regarding how to interpret and hence use such information.

Valverde-Garcia *et al* (2018)[40] outline a methodology regarding how to interpret and use HCD. In trying to use HCD, Valverde-Garcia *et al* determined confidence and prediction intervals around the mean of the control data in the average study and then compares this to the treatment mean(s) in the study under consideration. Whilst the approach seems logical, ultimately this approach means potential effects could be excluded as they may not be within the range of the HCD (see Box 1 of Valverde-Garcia *et al*). It is unclear whether this approach means that a potential effect relative to the concurrent control and hence a potential effect can be ignored. It is further noted that if this approach was to be followed then it would be logical that the historical control data should be used for interpretation of all observed differences and not just to exclude the significance of observed differences. What this work does indicate is the relevance of the effect when compared to the overall sensitivity of the study.

Box 2 of Valverde-Garcia *et al* illustrates an approach where a control has performed well in so much as it has out-performed the prediction limits. This second example does raise concerns regarding the cohort of birds tested, for example, if they are from a population that performs better than the HCD, then should not they be compared to that control and not the HCD? It could be argued that if the treatment birds had not been exposed to the treatment they would have performed to the same level.

Whilst Valverde-Garcia *et al* provides a useful starting point, it is does not provide a way forward to interpret and hence use HCD.

[40] Valverde-Garcia P, Springer T, Kramer V, Foudoulakis M and Wheeler J R (2018) An avian reproduction study historical control database: A tool for data interpretation. Regulatory Toxicology and Pharmacology 92 (2018) 295–302

B.9.3. References relied on

The applicant has conducted a literature search in order to determine whether there are publicly available data that should be taken into account in the review of metalaxyl-M. The literature search was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009". This literature review is evaluated by HSE in Appendix 1. While there are aspects of the relevance assessment provided by the applicant that are considered deficient, the literature review does not identify any data that require further consideration in this amendment evaluation regarding risks to terrestrial vertebrates.

List of references relied on by HSE – Vibrance SB

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1 / 01	[redacted]	1980 a	One-generation reproduction study - Mallard Duck, CGA-48988 Technical, Report No. 48988 [redacted] Not GLP Unpublished Syngenta File No. CGA48988/0152, VV-341718	Y	N	Expired on 10/1/2012	SYN	Y DAR July 1999 IIA 8.1.3

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1 / 02	Valverde-Garcia P., T. Springer, V. Kramer, M. Foudoulakis, J.R.	2018	An avian reproduction study historical control database: A tool for data interpretation. Regulatory Toxicology and Pharmacology 92 (2018) 295–302 Not GLP Published Syngenta File Number VV-244144	N	N/A	N/A	Published	N
IIIA 10.1.7 /01	[redacted]	2002	Acceptance of sugar beet pills by birds: field monitoring of birds in the Netherlands Report Number BAR/FS008 Bayer AG Crop Protection, Germany GLP Not published Syngenta File Number N/1158, VV-339631	N	N	-	BASF Syngenta access	Y KIIIA1 10.1.7

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1.7 /02	[redacted]	2015	Thiamethoxam: Measured Residues in Sugar Beet Seedlings Emerging from A9765R-Treated Seed, Germany 2015, Report Number S15-01163. Eurofins Agroscience Services Ltd Slade Lane, Wilson, Melbourne, Derbyshire, DE73 8AG, United Kingdom Syngenta file No. A9765R_10126, VV-414090	N	N	New study never submitted before to this country	SYN	N
Appendix 1/01	[redacted]	1996	The Reproductive Toxicity Test of CGA-215944 Technical in Northern Bobwhite, *Colinus Virginianus.* Report Number 029502. [redacted] Syngenta File No. CGA215944/0344, VV-369024	Y	N	New study never submitted before to this country	SYN	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
Appendix 1/02	[redacted]	1996	The reproductive toxicity test of CGA 24705 in Northern Bobwhite (Colinus virginianus) Report No. 029508 Document No. VV-371001 , CGA24705/2591 Test Facility [redacted] GLP Unpublished	Y	N	New study never submitted before to this country	SYN	N
Appendix 1/03	[redacted]	1998	The Reproductive Toxicity Test of CGA-329351 Technical with the Northern Bobwhite (Colinus virginianus). [redacted] GLP, not published [redacted] File No 573-97 Syngenta File No CGA329351/1071, VV-312458	Y	N	Expired on 10/1/2012	SYN	Y DAR addendum May 2000 IIA 8.1.3

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
Appendix 1/04	[redacted]	1998 a	The reproductive toxicity test of CGA 293343 technical with the northern bobwhite (Colinus virginianus) Report No. 029518 Document No. VV-376393 , CGA293343/0653 Test Facility [redacted] GLP Unpublished	Y	N	New study never submitted before to this country	SYN	N
Appendix 1/05	[redacted]	1996 a	The reproductive toxicity test of CGA 277476 technical in northern bobwhite, colinus virginianus Report No. 029401 Document No. VV-352227 , CGA277476/0292 Test Facility [redacted] GLP Unpublished	Y	N	New study never submitted before to this country	SYN	N

List of references relied on by HSE – Wakil XL

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1 / 01	[redacted]	1980 a	One-generation reproduction study - Mallard duck, CGA 48988 technical, [redacted], Rep.Nr. 108-176, 27.10.1980 Owned by Ciba-Geigy Ltd. Basle Switzerland Submitted by Ciba-Geigy Ltd. Basle Not GLP, unpublished Ciba file N° 48988/152 Syngenta File No CGA48988/0152 (VV-341718)	Y	N	Expired on 10/1/2012	SYN	Y DAR July 1999 IIA 8.1.3
IIIA 10.1 / 02	Valverde-Garcia P., T. Springer, V. Kramer, M. Foudoulakis, J.R.	2018	An avian reproduction study historical control database: A tool for data interpretation. Regulatory Toxicology and Pharmacology 92:295-302. Not GLP Published Syngenta File Number VV-244144	N	N/A	N/A	published	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1.7/01	[redacted]	2021a	Metalaxyl-M - Dissipation of Residues on A9642H - Treated Spinach Seed Scattered on the Soil Surface in Poland, Germany and Northern France in 2020. Report Number CEMR-9570. CEM Analytical Services, Oaklands Park, Wokingham, Beerkshire RG41 2FD United Kingdom. GLP unpublished Syngenta File Number VV-894877	N	Y	New study never submitted before to this country	SYN	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1. 7/02	[redacted]	2021 b	Metalaxyl-M - Dissipation of Residues on A9642H - Treated Spinach Seed Scattered on the Soil Surface in Spain and Portugal in 2020. Report Number CEMR-9570. CEM Analytical Services, Oaklands Park, Wokingham, Berkshire RG41 2FD United Kingdom. GLP unpublished Syngenta File Number VV-901662	N	Y	New study never submitted before to this country	SYN	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1. 7/03	[redacted]	2021 a	Metalaxyl-M – Calculation of DT50 Values from a Residue Decline Study Following Application to Treated Maize Seeds. Syngenta Ltd Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY, United Kingdom. Report Number: RAJ1428B. Not GLP unpublished Syngenta File Number VV-903111	N	N	-	SYN	N
IIIA 10.1. 7/04	[redacted]	2021 b	Metalaxyl-M – Calculation DT50 Values Residue Decline Studies Application - Treated Spinach Seeds. Syngenta Ltd Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY, United Kingdom. Report Number: RAJ1430B. Not GLP unpublished Syngenta File Number VV-903094	N	N	-	SYN	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1. 7/05	[redacted]	2012	Fludioxonil, Thiabendazole, Azoxystrobin and Metalaxyl-M - Dissipation of residues on treated maize seed and shoots Syngenta The Food and Environment Research Agency (Fera), Sand Hutton, UK, V7YG 1000 GLP not published Syngenta File No A14918E_10216, VV-402718	N	Y	New study never submitted before to this country	SYN	N
IIIA 10.1. 7/06	[redacted]	2006	Bird species in pea fields in Brittany (Northern France) - Field data for determination of focal species Report No. [redacted] T001019-06 Document No. VV-379792 , N/1085 Test Facility [redacted] Not GLP Unpublished	N	N	-	SYN	Y KIIIA 10.1

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1.7/07	[redacted]	2010	Exposure to birds in maize fields in France – Attractiveness of maize fields, relevant species, diet composition and portion to time. Report No. [redacted] Document No. VV-393540 , NA_11990 Test Facility [redacted] GLP Unpublished	Y	Y	New study never submitted before to this country	SYN	N
IIIA 10.1.7/08	[redacted]	2018	PT of woodpigeons in pre- and post-emergence maize fields in Germany, central zone (2017) Report No. [redacted] Document No. VV-470595 , NA_14734 Test Facility [redacted] GLP Unpublished	Y	N	Syngenta reached agreement with the data owner to access the study. Data owner to provide further details directly if required	BASF (SYN access)	Y KIIIA 10.1

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.1.7/09	[redacted]	1999	Migration of Metalaxyl-M in Soil after Seed Treatment with [Phenyl-(U)-14C] CGA 329351 Report No. 98SV04 Document No. VV-312789 , CGA329351/1151 Test Facility Novartis Crop Protection AG GLP Unpublished	N	Y	New study never submitted before to this country	SYN	N
IIIA 10.1.7/11	[redacted]	2005	Generic field monitoring of birds and mammals on maize and beet fields in Austria Report No. [redacted] Document No. VV-380792 , N/1155 Test Facility [redacted] GLP Unpublished	Y	N	Syngenta reached agreement with the data owner to access the study. Data owner to provide further details directly if required	BCS (SYN access)	Y KIIIA 10.1

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.3. 1.1 / 02	Abt, K.F. and Bock, W.F.	1998	Seasonal variations of diet composition in farmland field mice Apodemus spp. and bank voles Clethrionomys glareolus. Acta Theriologica, 43, 379-389. Not GLP Published Syngenta File No. VV-859296	N	N/A	N/A	Published	N
IIIA 10.3. 1.1 / 04	Green, R.	1979	Green, R. 1979. The ecology of wood mice (Apodemus sylvaticus) on arable farmland. Journal of Zoology (London), 188, 357-377 Not GLP Published Syngenta File No. VV-748945	N	N/A	N/A	Published	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.3. 1.1 / 05	Pelz, H. J.	1989	Ecological aspects of damage to sugar beet seeds by Apodemus sylvaticus. Mammals as pests. R. J. Putman. London, Chapman and Hall: 34-48 Not GLP Published Syngenta File No. VV-845692	N	N/A	N/A	Published	N
IIIA 10.3. 1.1 / 09	Barber, I., Tarrant, K.A. and Thompson, H. M	2003	Exposure of small mammals, in particular the wood mouse Apodemus sylvaticus, to pesticide seed treatments. Environmental Toxicology and Chemistry, 22:1134-1139 Not GLP Published Syngenta File No. VV-860234	N	N/A	N/A	Published	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.3. 1.1 / 14	Brühl, C.A., Guckenmus, B., Ebeling, M. and Barfknecht, R.	2011	Exposure reduction of seed treatments through dehusking behaviour of the wood mouse (Apodemus sylvaticus). Environmental Science and Pollution Research International, 18, 31-37. Not GLP Published Syngenta File No. VV-243922	N	N/A	N/A	Published	N
IIIA 10.3. 1.1 / 15	DEFRA	2010	Dehusking of seed by small mammals - default values for use in risk assessment. Pp. 1-29, DEFRA Not GLP Published Syngenta File No. VV-859262	N	N/A	N/A	Published	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
IIIA 10.3.3/01	[redacted]	2013	Generic field study on small mammals - focal species and wood mouse (Apodemus sylvaticus) PT in maize fields in Germany Report No. [redacted] Document No. VV-406482 , [redacted] Test Facility [redacted] GLP Unpublished	Y	N	Syngenta reached agreement with the data owner to access the study. Data owner to provide further details directly if required	OXON (SYN access)	Y KIIIA 10.1
Appendix 1/01	[redacted]	1996	The reproductive toxicity test of CGA 215944 technical in northern bobwhite, Colinus virginianus Report No. 029502 Document No. VV-369024 , CGA215944/0344 Test Facility [redacted] GLP Unpublished	Y	Y	New study never submitted before to this country	SYN	N

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
Appendix 1/02	[redacted]	1996	The reproductive toxicity test of CGA 24705 in Northern Bobwhite (Colinus virginianus) Report No. 029508 Document No. VV-371001 , CGA24705/2591 Test Facility [redacted] GLP Unpublished	Y	Y	New study never submitted before to this country	SYN	N
Appendix 1/03	[redacted]	1998	The Reproductive Toxicity Test of CGA-329351 Technical with the Northern Bobwhite (Colinus virginianus). [redacted] GLP, not published [redacted] File No 573-97 Syngenta File No CGA329351/1071, VV-312458	Y	N	Expired on 10/1/2012	SYN	Y DAR addendum May 2000 IIA 8.1.3

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previously used Y/N If yes, for which data point?
Appendix 1/04	[redacted]	1998 a	The reproductive toxicity test of CGA 293343 technical with the northern bobwhite (Colinus virginianus) Report No. 029518 Document No. VV-376393 , CGA293343/0653 Test Facility [redacted] GLP Unpublished	Y	Y	New study never submitted before to this country	SYN	N
Appendix 1/05	[redacted]	1996 a	The reproductive toxicity test of CGA 277476 technical in northern bobwhite, colinus virginianus Report No. 029401 Document No. VV-352227 , CGA277476/0292 Test Facility [redacted] GLP Unpublished	Y	Y	New study never submitted before to this country	SYN	N

Additional references from the EU renewal review of metalaxyl-M referred to in the HSE higher tier risk assessments

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner (SYN = Syngenta)	Previous evaluation
KIIIA 1 10.3.2 / 01	[redacted]	2006	Generic Field Monitoring of Mammals in Cereal Fields in Spring and Summer in Germany [redacted] Not GLP, not published Syngenta File No N/1153	Y	-	-	Bayer (SYN access)	Metalaxyl-M EU renewal review assessment
KIIIA 1 10.3.2 / 02	[redacted]	2008	Generic field monitoring of mammals on freshly drilled summer cereals in Hunsruck, Germany [redacted] GLP, not published Syngenta File No NA_11977	Y	-	-	Bayer (SYN access)	Metalaxyl-M EU renewal review assessment

B.9.4. GUIDANCE DOCUMENTS USED IN THIS ASSESSMENT

European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. [139 pp.]. doi:10.2903/j.efsa.2009.1438.

European Food Safety Authority; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011.2092.

B.9.5. Reference List

[redacted], [redacted], and [redacted] (1998) (PDF)- Portable Document Format. Birds and farming: information for risk assessment. 1998 Update Contract PN0919 Milestone Report FERA Project No M37

COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

Ecological Effects Test Guidelines OCSPP 850.2300: Avian Reproduction Test, EPA 712-C-023

EFSA (European Food Safety Authority), 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance metalaxyl-M. EFSA Journal 2015;13(3):3999, 105 pp. doi:10.2903/j.efsa.2015.3999

EFSA (European Food Safety Authority), 2017. Conclusion on the peer review of the pesticide risk assessment of the active substance oxasulfuron, EFSA Journal 2017;15(3):4722, doi:10.2903/j.efsa.2017.4722

EFSA (European Food Safety Authority), 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance pymetrozine. EFSA Journal 2014;12(9):3817, 102 pp. doi:10.2903/j.efsa.2014.3817

DEFRA (2009). Estimating wildlife exposure to pesticides in crops: additional scenarios and data - PS2328.

DEFRA (2015). Evaluating de-husking of treated seeds by wood mice - PS2369.

Gurney J.E., Perrett J., Crocker D.R & Pascual, J.A (1998). CONTRACT PN0910/PN0919 MILESTONE REPORT Mammals and farming: information for risk assessment. CSL Project No. M37.

SANCO/3037/99-final 18 September 2002 COMMISSION WORKING DOCUMENT - DOES NOT NECESSARILY REPRESENT THE VIEWS OF THE COMMISSION SERVICES Review report for the active substance Metalaxyl-M Finalised in the Standing Committee for the Food Chain and Animal Health at its meeting on 19 April 2002 in view of the inclusion of Metalaxyl-M in Annex I of Directive 91/414/EEC.

SANCO/10476/2010 – rev.1 12 March 2010 FINAL Review report for the active substance metalaxyl Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 12 March 2010 in view of the inclusion of metalaxyl in Annex I of Directive 91/414/EEC

SANTE/11112/2019 Rev 5 24 March 2020 Final Review report for the active substance metalaxyl-M finalised by the Standing Committee on Plants, Animals,

Food and Feed on 24 March 2020 in view of the renewal of the approval of metalaxyl-M as an active substance in accordance with Regulation (EC) No 1107/20091