Study Plan:

A large-scale field experiment to quantify the impacts of neonicotinoid (NNI) seed dressings on honeybees in the UK, Germany and Hungary

Version 4.3 (Following full team meeting 12-13 Jan 2015)

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Contents

2 Study Objective

The aim of the study is to quantify whether there is an impact on honeybees of two neonicotinoids (NNI) seed treatments applied to commercially grown oilseed rape (OSR) crops ('Clothianidin' Bayer CropScience and 'Thiamethoxam' Syngenta). Specifically we will test the null hypothesis that there is no impact of neonicotinoid seed treatments on population growth, mortality or overwintering survival of honeybees foraging on oilseed rape treated with this pesticide. Note, that this represents a null hypothesis only and that we make no a priori assumption of the impact of this pesticide on honeybees.

3 Experimental Design

In order to achieve the study aim a large-scale field experiment has been established on commercial farms in the United Kingdom, Germany and Hungary in autumn 2014 for monitoring and harvesting in 2015. This will take full advantage of the European moratorium on NNI use by improving the chances of NNI-free control treatments. A schematic representation of the experimental design is given in Figures 1 & 2 below. Note we include a glossary in the appendix of this document that defines specific terms relating to the experimental design (Appendix 1).

3.1 Definition of terms used in the experimental design

Appendix 1 includes definitions of terms used in this study. However, for clarity the following key terms are described here:

- **Site (36 in total) = a field or group of adjacent fields where a single treatment is applied**
- **Treatment (3 in total) = the experimental intervention applied to a site :(a) control or (b) Clothianidin or (c) Thiamethoxam**
- **Unit of replication (11 in total) = block of each of three treatments (UK = 4; Germany = 3; Hungary = 4)**

See Figure 1 below.

3.2 Treatments

On each site a large area ranging in size from 45-70ha (depending on field size) was sown with oilseed rape in the summer of 2014. Each site was allocated to one of three experimental treatments at random, namely:

a. without Neonicotinoid seed treatment (CONTROL);
b. Clothianidin (Bayer Crop Science Modesto ^{Tm} or Elado ^{Tm}) seed treatment; and
c. Thiamethoxam (Syngenta Cruiser OSR ^{tm}) seed treatment.

In Hungary there are four replicates of the three treatments (12 sites), in Germany there are three replicates (due to the constraints of the experimental licence; 9 sites) and in the UK there are four replicates of the three treatments. **In the UK there will also be an additional three untreated control sites as residual but detectable levels of neonicotinoids were identified within some of the soils of the original control sites. Ultimately exposure of bees to neonicotinoids will be quantified directly in 2015 by chemical detection of the active ingredients within pollen and nectar of the oilseed rape crops. The three additional control sites are intended to reduce this potential risk by providing reserve sites should neonicotinoids be expressed in the oilseed rape crops of the control sites. This will give a total of 12 + 3 = 15 sites for the UK.** The supplementary control sites used in the UK will only be considered if neonicotinoids are expressed in the oilseed rape crop (pollen and nectar) of the original control sites.

3.3 Spatial separation

Each replicate of the three treatments (a, b, c) has been chosen to be in the same general landscape and on the same soil type. Each replicate is separated by at least 10km and each site within a replicate block is separated from its neighbour by a minimum of 3.2 km. This was intended to minimize the possibility of bees from different sites within a block foraging on oilseed rape established as part of the treatments applied to another site within that same block. Note, with the exception of sites B6 and B12 of the [redacted] block in Hungary all other replicate blocks have sites separated by at least 4 km. Within each of the 36 sites the area of treated crop (defined below) will be sufficiently large to ensure foraging honeybees receive a high although realistic exposure to the pesticide, i.e. there will be fewer opportunities to forage on neighboring habitats and possibly untreated oilseed rape (a criticism of some previous field trials). Spatial data sets on land cover and proportion of major crops grown were used to ensure that variation in landscape structure between countries is minimized.

Figure 1. Schematic representation of the experimental design

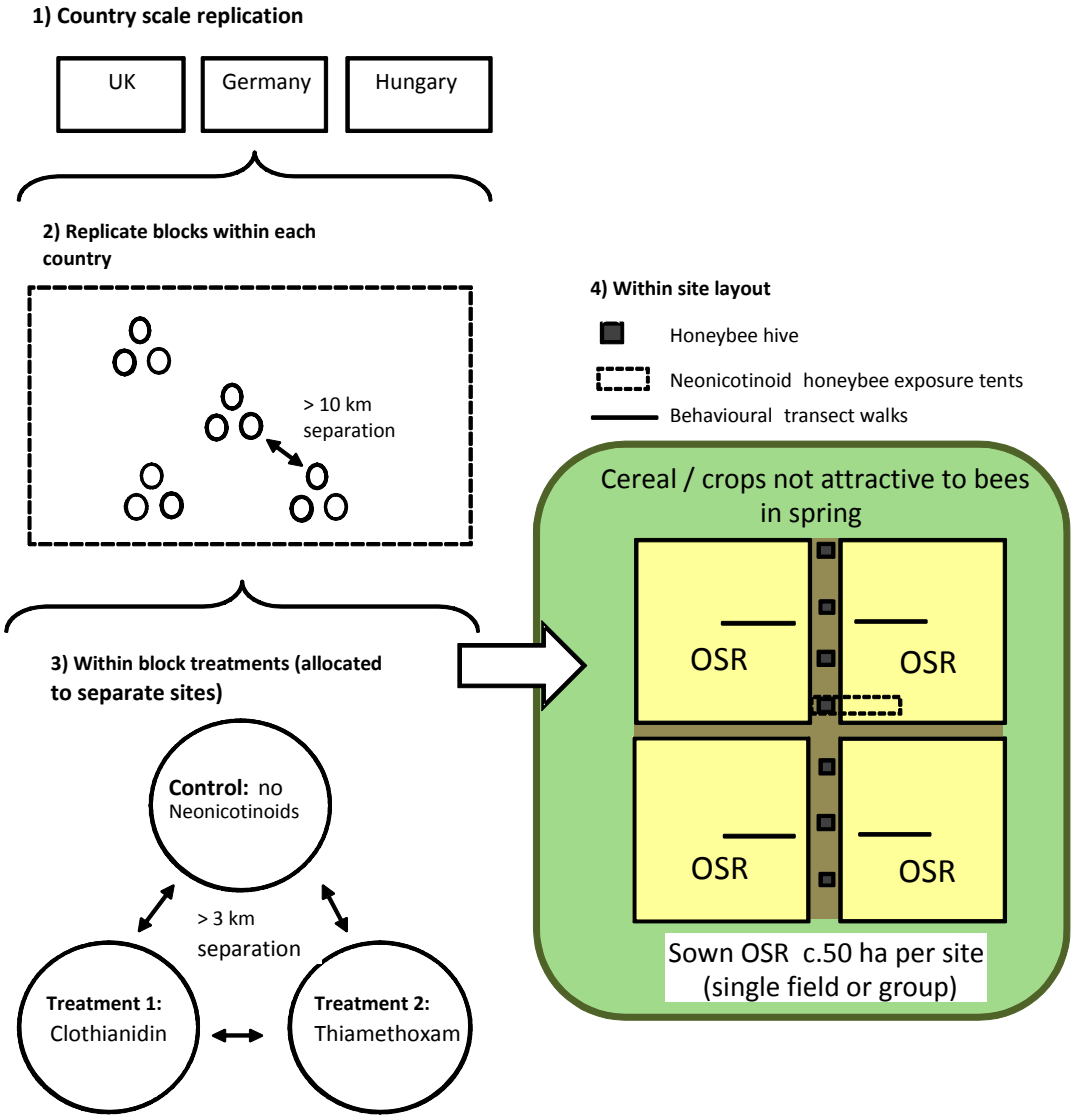
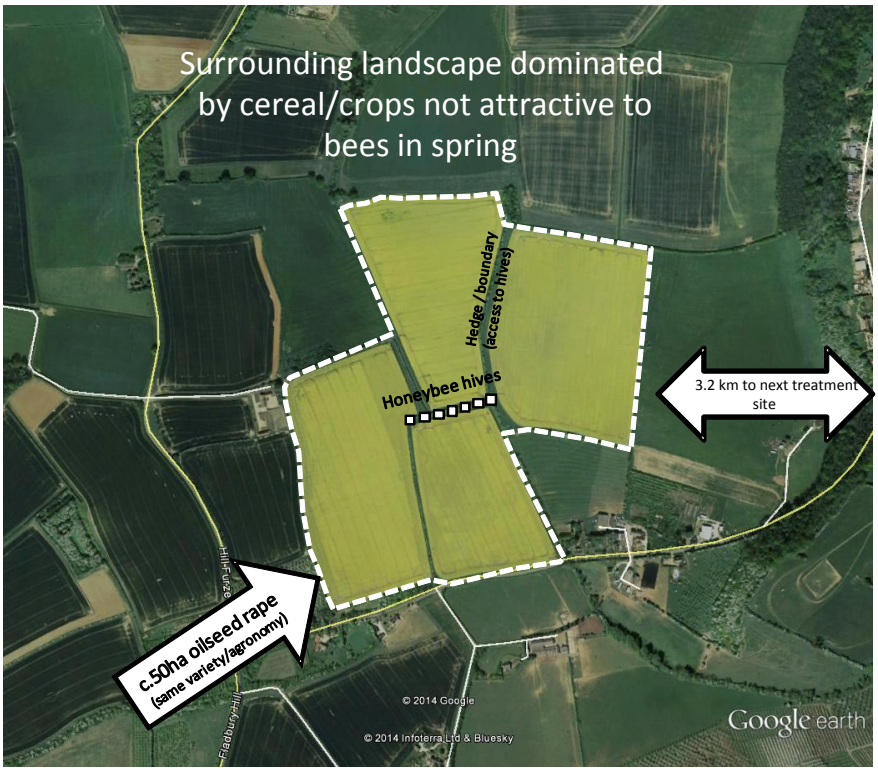


Figure 2. An example of a typical site



3.4 Crop agronomy

The treated and untreated oilseed rape is the same hybrid variety in each country: UK (var. Harper - Bayer); Germany (var. Flyer - Bayer); and Hungary (var. Hybrirock - KWS). The selected varieties were chosen to reflect widely grown varieties for a given country and will be established and grown according to Good Agricultural Practice.

A simple verification of crop plant density will be undertaken at each site in the spring of 2015. This will be achieved by counting and recording plant density per square meter at 20 random locations across each site in representative areas of the crop (i.e. avoiding tramlines or other areas of poor crop establishment). Notes will be made on evidence of damage together with photographs. These counts will be made during a routine site visit in spring 2015. These assessments are intended to provide an overview of the crop state in the immediate proximity of the hives. Given the sometimes very large size of fields it will not be possible to accurately assess crop damage over the whole of these fields.

It is critically important that measures to control pollen beetle infestation at yellow bud and early flower stage do not harm bees and in so doing mask any NNI effects. Therefore measures to control pollen beetle in spring 2015 will be coordinated so as to be consistent in timing across blocks (replicates). Co-ordination of this will be undertaken by Eurofins in Hungary and Germany, and by Wildlife Farming Co. in the UK. At the country level the choice of pesticide products applied to the

oilseed rape will be consistent (and compliant with local regulations on pesticide use). The chosen pesticide products will be selected to pose the minimum possible risk to bees. All application will be applied in the fields of a block within three days.

Proposed approach:

1) UK sites: Position 1 = do not spray for pollen beetle (the case for the last 4 years in the study region); Position 2 = if threshold exceeded at yellow bud stage then all farmers in a replicate block spray with Tau-fluvalinate (trade name Mavrik® ADAMA; the most bee friendly insecticide allowed based on label toxicity). This is to be coordinated by the Wildlife Farming co.. All applications will be applied in the fields of a block within three days.
2) German and Hungarian sites: spraying for pollen beetle or other pests will almost certainly be necessary. In Hungary Maverick will be applied once before flowering if there is considerable pressure and once towards the end of flowering. For the [redacted] Block in Germany the pyrethroid Maverik will be applied before flowering, although it is expected that no pyrethroids will need to be applied during the flowering stage. For the remaining two German blocks either the B4 (no harm to bees) pyrethroid Karate (a.i. Lambda-cyhalothrin), or the B2 (applied after bee flight until 23:00 h) pyrethroids Bulldock (a.i. beta cyfluthrin) and Fury (a.i. zeta-cypermethrin) will be used. All application will be applied in the fields of a block within three days. This will be coordinated by Eurofins.
3) Organophosphate stem weevil treatments will be applied during BBCH55 bud stage (mid to late March) in Hungary (a.i. Chlorpyriphos) and in Germany (Avaunt (a.i. Indoxacarb) and Trebon (a.i. Etofenprox)). All application will be applied in the fields of a block within three days. This will be coordinated by Eurofins.

All agronomic inputs and timings will be recorded for each site, together with estimates of crop yield.

3.5 Measurements

The following measurements will be made to assess exposure and effects of NNIs on honeybees. These are summarized below with full detailed provided in the preceding text:

- Honeybee colony metrics, including size and weight (to the nearest 50 g), growth rate, foraging behavior, reproductive success, incidence of pathogens and parasites, and overwinter survival;
- NNI residues expressed from the crop (nectar and pollen), in stored hive products (honey, pollen and wax), in the soil and plant tissue. Note, soil chemistry measures were taken prior to sowing of oilseed rape in 2014 to identify if control plots were established on fields with residual levels of neonicotinoids. In each case 10 soil cores to a depth of 20 cm were

collected along a transect (separated by 20 m) in individual fields. These cores were homogenized and tested for Neonicotinoid residues.

- Other measures: composition of honeybee pollen loads to measure exposure to oilseed rape and other flower resources;
- Agronomic measures: pesticide and fertiliser input, and yield of the crop. Note that we will collect information on yield, although this will be used to identify if the crop is both fit and healthy. Comparisons of both crop yield and quality in response to the seed treatments will be confounded by management intended to ensure high exposure to neonicotinoids of the bees. For example, in the case in the control plots we aim to ensure good crop health in spite of the absence of the neonicotinoid seed treatment.

Measurements will be taken of the honeybee colonies shortly before the set-up of the hives at the fields (pre-exposure), at several points during crop flowering (the exposure phase), and before and after winter (post-exposure).

4 Materials & Methods

4.1 Test Item and Control

Verification of Sowing and pesticide application rates

Subsamples of the treated and untreated seed in each country will be sent for analysis at the testing facilities at Bayer Crop Science and Syngenta respectively to accurately determine the product dose rate applied. Samples of the treated and untreated seed will be retained by CEH for further analysis if required. Samples are to be sent to [redacted], Centre for Ecology & Hydrology, Crowmarsh Gifford, Wallingford OX10 8BB. UK. We require representative seed.

The information about the test item and control are given in Tables 1-3 below.

Table 1. Details of the test item#1 - clothianidin

	Oilseed rape (*Brassica napus*) seed treated with commercial neonicotinoid **(clothianidin)** and fungicide seed treatment
Name of seed treatment formulation	Elado [tm] / Modesto [tm] 400 g/L (32.5% w/w) clothianidin and 80 g/L (6.51% w/w) beta-cyfluthrin **UK:** Modesto @ 12.5 l/t + Hypro Duet @ 9.0 l/t Modesto = 12.5 ml product /kg seed* Seed drilled @ 50 seeds/m2 **Germany:** Elado (CTL): 25 ml product/kg seed* Seed drilled @ 50 seeds/m2 **Hungary:** Elado (CTL): 25 ml product/kg seed* Seed drilled @ 50 seeds/m2 *Maximum registered application rate for country
TOX-Number	
Batch-ID	#
GAB-code	#
Seed variety	UK – var. Harper Germany – var. Flyer Hungary – var. Hybrirock
1000-grain-weight	5.1 g
Active ingredient(s)	Clothianidin Beta-cyfluthrin
CAS-No.	
Content(s) of a.i. nominal	Modesto contains 400 g/L clothianidin and 80 g/L beta-cyfluthrin applied at 1.25 Litres product per 100 kg seed mg a.s./seed item ai/ha to be confirmed by Bayer
Content(s) of a.i. analyzed	ai/kg to be confirmed by Bayer
Date of certificate	#
Expiry date	#
Appearance	dressed seed
Density [g/mL]	not applicable
Storage	+2 ºC to + 30 ºC
Safety symbol(s)	none
Intended Use (Target(s))	Insecticide-dressed seed

will be given in final report

Table 2. Details of the test item#2 – thiamethoxam

	Oilseed rape (*Brassica napus*) seed treated with commercial neonicotinoid (**thiamethoxam**) and fungicide seed treatment
Name of seed treatment formulation	Cruiser OSR^{tm} 280 g/litre thiamethoxam, 8 g/litre fludioxonil and 32.3 g/litre metalaxyl-M **UK:** Cruiser OSR @ 15.0 l/t 15ml product /kg seed* Seed drilled @ 50 seeds/m^2 20.15 g / 1,000,000 seeds **Germany:** Cruiser OSR @ 15.0 l/t 15ml product /kg seed* Seed drilled @ 50 seeds/m^2 21.22 g / 1,000,000 seeds **Hungary:** Cruiser OSR @ 15.0 l/t 15ml product /kg seed* Seed drilled @ 50 seeds/m^2 22.28 g/ 1,000,000 seeds *Maximum registered application rate for country
TOX-Number	
Batch-ID	#
GAB-code	#
Seed variety	UK – var. Harper Germany – var. Flyer Hungary – var. Hybrirock
1000-grain-weight	**UK** – var. Harper: 5.1 g **Germany** – var. Flyer: 4.8 g **Hungary** – var. Hybrirock: 5.5 g
Active ingredient(s)	Thiamethoxam Fludioxonil Metalaxyl-M
CAS-No.	
Content(s) of a.i. nominal	Cruiser contains 280 g / l TMX
Content(s) of a.i. analysed	**UK** – var. Harper: 3.95 g TMX / Kg seed **Germany** – var. Flyer: 4.42 g TMX / Kg seed

	Hungary – var. Hybrirock: 4.05 g TMX / Kg seed
Date of certificate	#
Expiry date	#
Appearance	dressed seed
Density [g/mL]	not applicable
Storage	+2 ºC to + 30 ºC
Safety symbol(s)	none
Intended Use (Target(s))	Insecticide-dressed seed

will be given in final report

Table 3. Details of the untreated control

	Oilseed rape (*Brassica napus*) seed treated with commercial fungicide seed treatment
Name of seed treatment formulation	**UK:** Control without insecticide; Hypro Duet only @ 9.0 l/t - Seed drilled @ 50 seeds/m2 **Germany:** Control: without insecticide; only TMTD Fungicide (a.i. Thiram) 700 5,71 ml/kg + DMM (a.i. Dimethomorph)10 g/kg Seed drilled @ 50 seeds/m2 **Hungary:** Control: without insecticide; only TMTD Fungicide (a.i. Thiram) 700 5,71 ml/kg + DMM (a.i. Dimethomorph)10 g/kg Seed drilled @ 50 seeds/m2
Batch-ID	#
GAB-code	#
Seed variety	UK – var. Harper Germany – var. Flyer Hungary – var. Hybrirock
1000-grain-weight	To be confirmed by Bayer & Syngenta
Active ingredient	Fungicide ai be confirmed by Bayer & Syngenta
Appearance	native seed
Storage	+2 ºC to + 30 ºC
Safety symbol(s)	none

will be given in final report

Further details of the treated and untreated seed will be summarized in the final report.

4.2 Drilling and crop maintenance details

The oilseed rape treated with neonicotinoid seed dressing and the untreated (control) was sown in each country between July and August of 2014. In each replicate the treated sites were matched with a similar sized untreated control sites. In both cases, sown areas ranged from ca.45-70ha depending on field size. Treatment and control are separated by a minimum of 3.2 km in order to minimise the chance of the bees from one treatment visiting the field plot of the other treatment. The target seeding rate for all three oilseed rape varieties (treated and untreated) is 50 seeds m^{-2} reflecting currently product labeling practice for maximum application rate. The seed used in the control sites were of the same batch and variety as the seed used in the test item treated sites. The control seed was treated with the same fungicide as the test seed (Table 3). Equipment used for drilling treated seeds was calibrated prior to use to ensure accurate application of seed. Details on

the type of equipment used, including sowing date and the actual seeding rate will be documented in the raw data and given in the final report. As this is a non-GLP study drilling equipment was calibrated according to good agronomic practice only.

Each crop will be grown according to Good Agricultural Practice (GAP) guidelines and the timing and nature of management interventions will be recorded. The pesticide use history of all field sites selected for this study will be documented for at least the two previous cropping seasons before the start of the study.

Use of any pesticide products containing neonicotinoids (e.g. Biscaya (Thiacloprid); InSyst (Acetamiprid)) as maintenance treatments is forbidden. Choice of products to be used for maintenance treatments will be discussed and agreed with the Study Director (in consultation with the project team) prior to use. Pollen beetle control measures in spring 2015 will be coordinated within each country to ensure (as far as possible) consistent timing and use of pesticide products in order to minimize negative impacts on honeybees whilst ensuring good pest control. This will be coordinated in the UK by Wildlife Farming Co. and in Hungary and Germany by Eurofins.

It is critically important that measures to control pollen beetle infestation at yellow bud and early flower stage do not harm bees and in so doing mask any NNI effects. Therefore measures to control pollen beetle in spring 2015 will be coordinated so as to be consistent in timing and composition across blocks (replicates) (see Section 3.4). In all countries pesticide products and timing of application will be selected to pose the minimum possible risk to bees. It is accepted that the timing of applications will be selected by the land owning farmers and the coordination role is to reduce variation as much as possible while documenting differences. This will be coordinated in the UK by Wildlife Farming Co. and in Hungary and Germany by Eurofins.

4.3 Test Organism

The test organism for this experiment is the honeybee, *Apis mellifera* L. (Hymenoptera, Apidae).

4.3.1 Attributes of the test honeybee colonies

Bee colonies will be **free of any clinical signs of disease** and of a **consistently high quality** with the following attributes at the start of exposure:

- Young queen / colony (1-2 years old);
- Colonies will be as similar to one another as possible (based on e.g. number of worker bees, age-structure and extent of brood);
- Minimum number of worker bees per colony will be approximately 4000;
- Pending an assessment of colony size in spring, hives to be distributed randomly (or as a stratified random distribution) **within blocks** based on size-class distribution among each country-scale sample. Option to cull from very large colonies is not desirable. Back-up hives will be available to optimize size-class distributions used (see Table 3). The biggest

and smallest of the honey bee hives at a site (out of 8) will be allocated to be either the back-up hive or the hive used in the tent experiment (see section 6.1).

- Race: UK = Buckfast; Germany, Hungary = Carnica;
- Hives with two boxes (lower box = brood chamber = 1; upper box = honeycomb box = 2) including 10 combs each, although this number will depend on hive design (e.g. in the UK 11 combs are present in each hive);
- Additional bodies can be added to the top of the hive as the colony develops;
- Facility for separation of a bee hive in a brood chamber and in a honeycomb box with a queen excluder. This is part of good apicultural practice and is conducted by most bee-keepers to obtain honeycombs which have never been incubated.

4.4 Description of the Test Method

4.4.1 Test Location

Maps showing the exact location of all 36 study sites in Germany (9), Hungary (12) and the UK (15), and the random allocation of treatments have been provided by CEH. The sites are summarized as follows:

Country	Block	Treatment
Germany	[redacted]	Control
Germany	[redacted]	Clothianidin
Germany	[redacted]	Thiamethoxam
Germany	[redacted]	Control
Germany	[redacted]	Clothianidin
Germany	[redacted]	Thiamethoxam
Germany	[redacted]	Control
Germany	[redacted]	Clothianidin
Germany	[redacted]	Thiamethoxam
Hungary	[redacted]	Control
Hungary	[redacted]	Clothianidin
Hungary	[redacted]	Thiamethoxam
Hungary	[redacted]	Control
Hungary	[redacted]	Clothianidin
Hungary	[redacted]	Thiamethoxam
Hungary	[redacted]	Control
Hungary	[redacted]	Clothianidin
Hungary	[redacted]	Thiamethoxam
Hungary	[redacted]	Control
Hungary	[redacted]	Clothianidin
Hungary	[redacted]	Thiamethoxam
UK	[redacted]	Control

UK	[redacted]	Clothianidin
UK	[redacted]	Thiamethoxam
UK	[redacted]	Additional control*
UK	[redacted]	Control
UK	[redacted]	Clothianidin
UK	[redacted]	Thiamethoxam
UK	[redacted]	Control
UK	[redacted]	Clothianidin
UK	[redacted]	Thiamethoxam
UK	[redacted]	Additional control*
UK	[redacted]	Control
UK	[redacted]	Clothianidin
UK	[redacted]	Thiamethoxam
UK	[redacted]	Additional control*

4.4.2 Design and Lay-out of the Test

The field study will be carried out with the treatments and numbers of colonies given in Table 4.

Treatment group	Replicates	No. of hives for exposure study per site	No. of hives for crop residue sampling	Reserve hives	Total hives
Control (C)	11 + 3 additional control sites in the UK = 14	6	1	1	112
Clothianidin (T1)	11	6	1	1	88
Thiamethoxam (T2)	11	6	1	1	88
GRAND TOTAL					**288**

Table 4. Treatment design showing number of hives

4.4.2.1 Hive identifier number

Each honeybee hive will have a unique number (8 hives × 36 sites = 288 unique numbers). The unique number will be attached to the base of each hive in a visible place. This unique number will be noted (together with the unique site number) on the recording form for subsequent data collection relating to the hives.

Site codes for hives will conform to the following system (described in detail in Table 5).

Country code (1 letter)
This is a unique letter for each country

U = UK
H= Hungary
G =Germany

Site identification number (2 digits)
This is a unique two digit code for each of the 36 individual fields. This will always be a two digit number, i.e. '02' not '2'. Individual site identification numbers are continuous and so not replicated in each country (i.e. there will not be a site 02 in Hungary and a field 02 in Germany, rather site will have a unique number)

For Germany these will be 01, 02, 03, 04, 05, 06, 07, 08, 09
Hungary: 10, 11 21
UK: 22, 23.....36

Individual hive code ('H' and 3 digits)

Hive code numbers will always be prefixed by 'H'. This number is a unique three digit code for each of the hives. This will always be a three digit number, i.e. 'H002' not 'H2'. Individual hive identification numbers are continuous and so not replicated in each country (i.e. there will not be a H002 hive in Hungary and a field 002 in Germany, rather site will have a unique number)

For the Germany Blumberg block [redacted] field the hive numbers will be H065, H066, H067, H068, H069, H070, H071, H072

Example unique code descriptors will be: G01-H065, H10-H081 and U24-H201

Table 5. Unique numbering system for all hives in Germany (G), Hungary (H) and the UK (U). This includes sites in the UK that may become rejected from the study following testing of soil neonicotinoid residues.

Country code (1 letter)	Site ident. number (2 digits)	Hive1 code ('H' and 3 digits)	Hive2	Hive3	Hive4	Hive5	Hive6	Hive7 (RESERVE HIVE)	Hive8 (TENT HIVE)	Block name	Block ID	Field name
G	01	065	066	067	068	069	070	071	072	[redacted]	1	[redacted]
G	02	049	050	051	052	053	054	055	056	[redacted]	1	[redacted]
G	03	057	058	059	060	061	062	063	064	[redacted]	1	[redacted]
G	04	001	002	003	004	005	006	007	008	[redacted]	2	[redacted]
G	05	009	010	011	012	013	014	015	016	[redacted]	2	[redacted]
G	06	017	018	019	020	021	022	023	024	[redacted]	2	[redacted]
G	07	033	034	035	036	037	038	039	040	[redacted]	3	[redacted]
G	08	025	026	027	028	029	030	031	032	[redacted]	3	[redacted]
G	09	041	042	043	044	045	046	047	048	[redacted]	3	[redacted]
H	10	081	082	083	084	085	086	087	088	[redacted]	1	[redacted]
H	11	073	074	075	076	077	078	079	080	[redacted]	1	[redacted]
H	12	097	098	099	100	101	102	103	104	[redacted]	1	[redacted]
H	13	129	130	131	132	133	134	135	136	[redacted]	2	[redacted]
H	14	145	146	147	148	149	150	151	152	[redacted]	2	[redacted]
H	15	153	154	155	156	157	158	159	160	[redacted]	2	[redacted]
H	16	161	162	163	164	165	166	167	168	[redacted]	3	[redacted]
H	17	121	122	123	124	125	126	127	128	[redacted]	3	[redacted]

H	18	137	138	139	140	141	142	143	144		3	
H	19	089	090	091	092	093	094	095	096		4	
H	20	105	106	107	108	109	110	111	112		4	
H	21	113	114	115	116	117	118	119	120		4	
U	22	233	234	235	236	237	238	239	240		1	
U	23	225	226	227	228	229	230	231	232		1	
U	24	201	202	203	204	205	206	207	208		1	
U	25	257	258	259	260	261	262	263	264		2	
U	26	289	290	291	292	293	294	295	296		2	
U	27	265	266	267	268	269	270	271	272		2	
U	28	249	250	251	252	253	254	255	256		3	
U	29	273	274	275	276	277	278	279	280		3	
U	30	241	242	243	244	245	246	247	248		3	
U	31	185	186	187	188	189	190	191	192		4	
U	32	177	178	179	180	181	182	183	184		4	)
U	33	169	170	171	172	173	174	175	176		4	
U	34	193	194	195	196	197	198	199	200		1	
U	35	281	282	283	284	285	286	287	288		3	
U	36	209	210	211	212	213	214	215	216		4	
U	37	217	218	219	220	221	222	223	224		3	

4.4.2.2 Hive design

It is noted there are differences in the design of hives between Germany, the UK and Hungary, although we use the same hive design in Germany and UK. The designs are sufficiently compatible to make meaningful comparison between the data and allow consistent management of the hives. Vertical expansion of the hives is possible for both hive designs. For each country photographs and a generalised description of the hive design will be provided to CEH.

4.4.2.3 Field set-up

The bee hives will be placed as close to the centre of the treatment field(s) as practical (Figure 3). CEH have supplied detailed maps showing the centroid of each of the 39 sites. In Germany and Hungary the large field size means it will be necessary to EITHER cut or spray with herbicide an area of crop in the centre of field (together with a suitable access track if required).

In the UK the smaller field size means it will often be possible to site the hives on a suitable field margin or headland in the centre of site. CEH have worked with the participating farmers to locate suitable locations on the UK sites. These will be verified by Eurofins on the January field visit.

It is critically important that the study areas are sufficiently large and well marked with tall canes and flags to protect the hives from pesticide spray drift.

As part of an independently funded add-on to this project wild pollinators (*Bombus spp.*) will be monitored on the 36 sites. This component of the study lies outside of the remit of this protocol, however, their inclusion in the study will have some ramifications for the experimental protocol and therefore they must be considered briefly here. Specifically, the co-location of honeybees and *Bombus* colonies is not desirable due to risks of honeybee robbing from *Bombus*. Therefore TWO patches will be created at each site separated by at least 100m; one patch for honeybees, one for *Bombus*. In addition, *Bombus* colony entrances will be kept closed for first 3-4 days following placement in order to allow honeybees to commence foraging and to minimise risks of invasion/ robbing between colonies. Following opening of the *Bombus* colonies the sugar syrup reservoir will be shut off. *Osmia* cocoons and trap nests will be located on a suitable field edge to encourage return nesting.

Agreed approach: in large fields two experimental patches (One patch will contain the honeybees, the other *Bombus* colonies) will be located either side of the centroid of the field, cut or sprayed off with herbicide (approx. 20×30m minimum; allow for half boom-width at least). There will be a minimum of a 10m buffer between the hives and the edge of the patch to minimize the risk of pesticide spray drift. The two patches will be separated by 100m.

For sites comprising multiple small fields then two central headlands or field margins will be selected (separated by 100m). Again these will be well marked to prevent spray drift.

Figure 3. Suggested field set-up in field centre for sites comprising a single large field

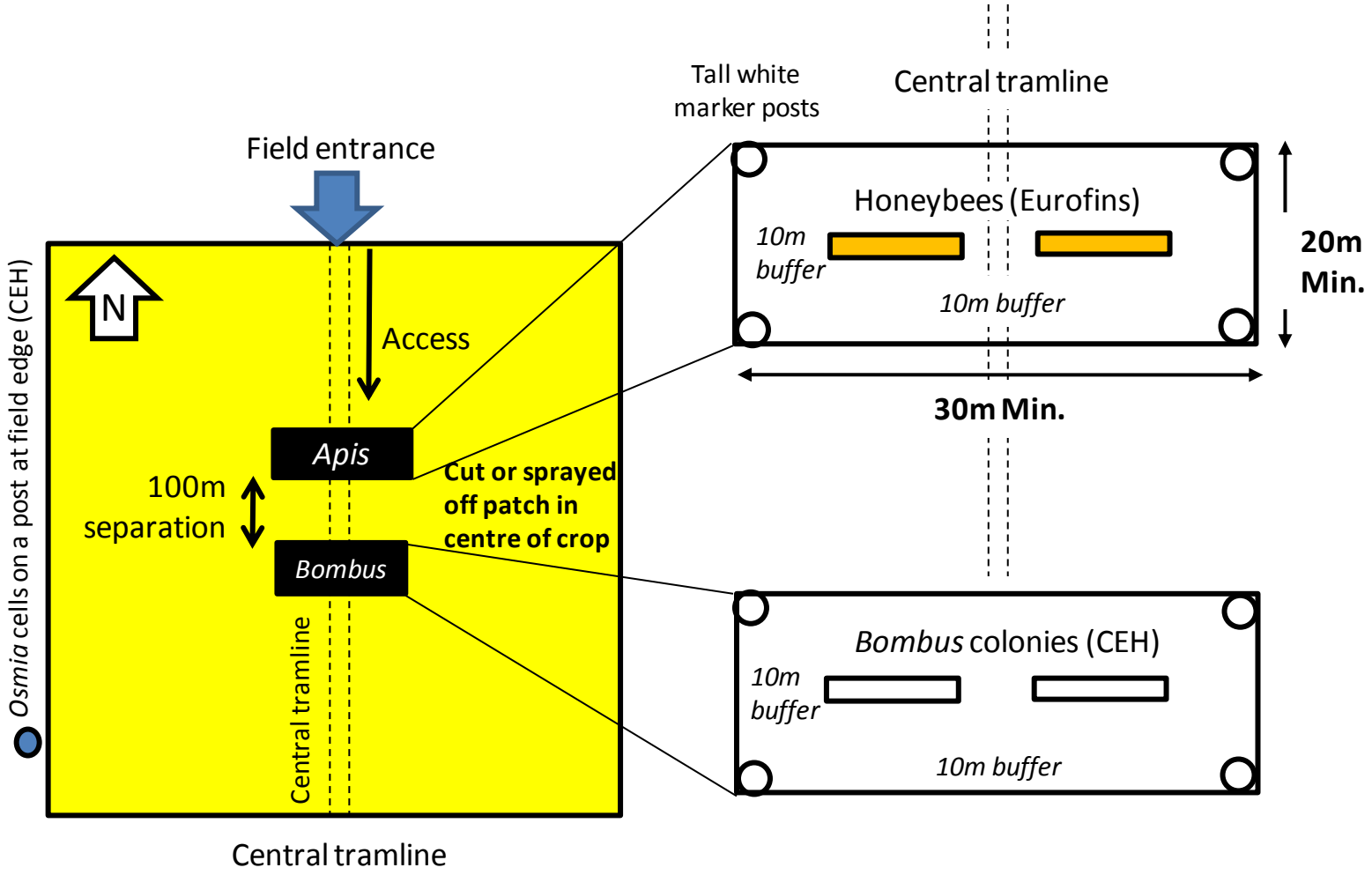
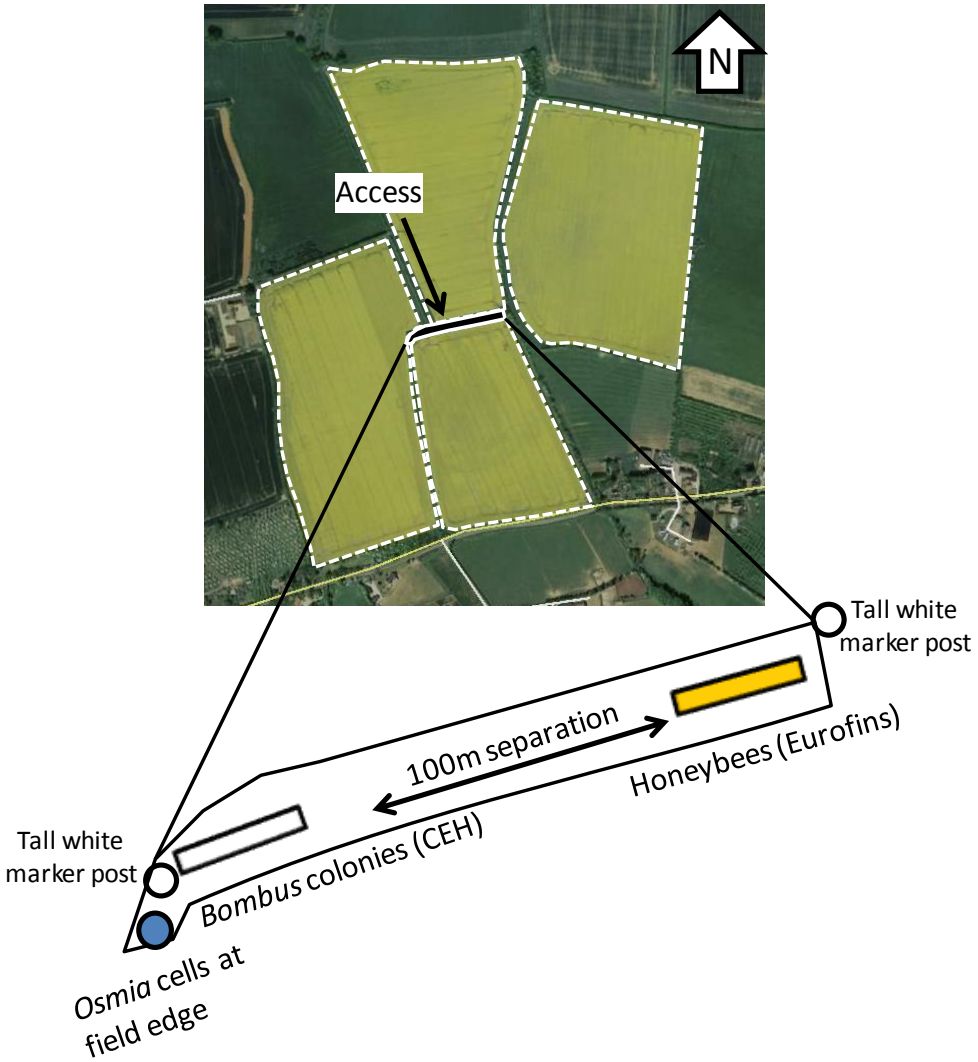


Figure 4. Suggested field set-up on central field margin/headland for sites comprising multiple fields



4.4.2.4 Hive location

Bee hives not part of the current experiment (i.e. hives independently owned by beekeepers not associated with the study) but located within 500m of patches on which the experimental bee hives are located will be moved to reduce the risk of robbing and spread of disease. While we do not expect this to be a significant problem, it is acknowledged that the extent to which this is possible will depend on the goodwill of these beekeepers.

The location of the center of each patch on which the hives are located at each site will be recorded using a hand-held GPS accurate to ±2m. The raw data will be given in the final report.

The colonies will be set up no later than 3 days after the BBCH 61 developmental stage (first flower open on main stem) is reported in the last field of oilseed rape for each site within a block (i.e. there will be a foraging resource for honeybees within each block). At a simple level once a yellow haze of flowers is seen in at least one of the fields of a site within a block (so that resources are present for the honey bees at the site) they can be deployed within all sites in that block. Once this occurs hives will be placed out within a block simultaneously. Hives will remain at the location for the duration of the flowering which is expected to last at least four weeks.

The two 'spare' hives (one back-up and one for cage assessments) will be located with the other six hives at each site so they are exposed to the same conditions if they are required as replacements.

4.4.2.5 Honey management

The aim of the honey management is to maximize exposure of bees to neonicotinoid residues (the 'worst case scenario') rather than representing typical beekeeper practice which would remove oilseed rape honey soon after collection.

Post-exposure, all frames will be left ***in situ*** within hives, accepting any potential crystallisation of OSR honey. At the final beekeeper check before winter any unused frames will be removed and disposed of and the bees fed overwinter. Winter feeding shall be standardized within a country, and will reflect national good beekeeping practice. Details of these practices will be recorded.

4.4.2.6 Control of pests (*Varroa*)

All 288 hives used for the experiment will be managed to a consistently high standard to control pests and disease. Specifically there will be a consistent treatment of *Varroa* comprising two applications of formic acid and one of oxalic acid post-exposure. There will be no treatment <4 weeks prior to exposure (in line with OECD). Timing of treatment will be consistent within a country.

4.4.2.7 Location of hives post-exposure (overwintering)

At the end of the oilseed rape flowering period all the hives will be taken to the same site (e.g. a farm location common to a particular country) that will provide foraging resources sufficient to allow the colonies to overwinter. Within that location the hives from a particular block will be clustered together (e.g. 24 hives from three sites in the same immediate area). Each cluster will be separated

from the next cluster of 24 hives by c. 200 m (see Fig. 5). Depending on the country this will mean for each overwintering site there will be between 3 and 4 clusters. To help reduce robbing the size of the entrances to the hives will be reduced as a mechanical bee management measure to reduce this problem. These overwintering approaches will: a) ensure all hives are exposed to approximately the same climatic conditions and so prevent confounding effects of this factor on overwintering survival; b) limit between hive robbing from hives originating from different sites.

Important points for overwintering

1) Each country will have a single overwintering location
2) Within that location the 24 hives from an experimental block will be clustered together.
3) These clusters will be separated by c. 200 m to minimize robbing.
4) Beekeeper will implement additional measures to minimize between hive robbing. Specifically reducing the size of hive entrances – according to good beekeeping practice.

They will remain here over the winter of 2015/16. During the post-exposure period the hives will be checked frequently (see 4.4.2.9.below). Note although it was originally intended to remove hives away from areas of agriculture, this is unrealistic in some countries (i.e. UK) where the location of such landscapes would require significant movement of hives introducing an additional potential cause of hive mortality.

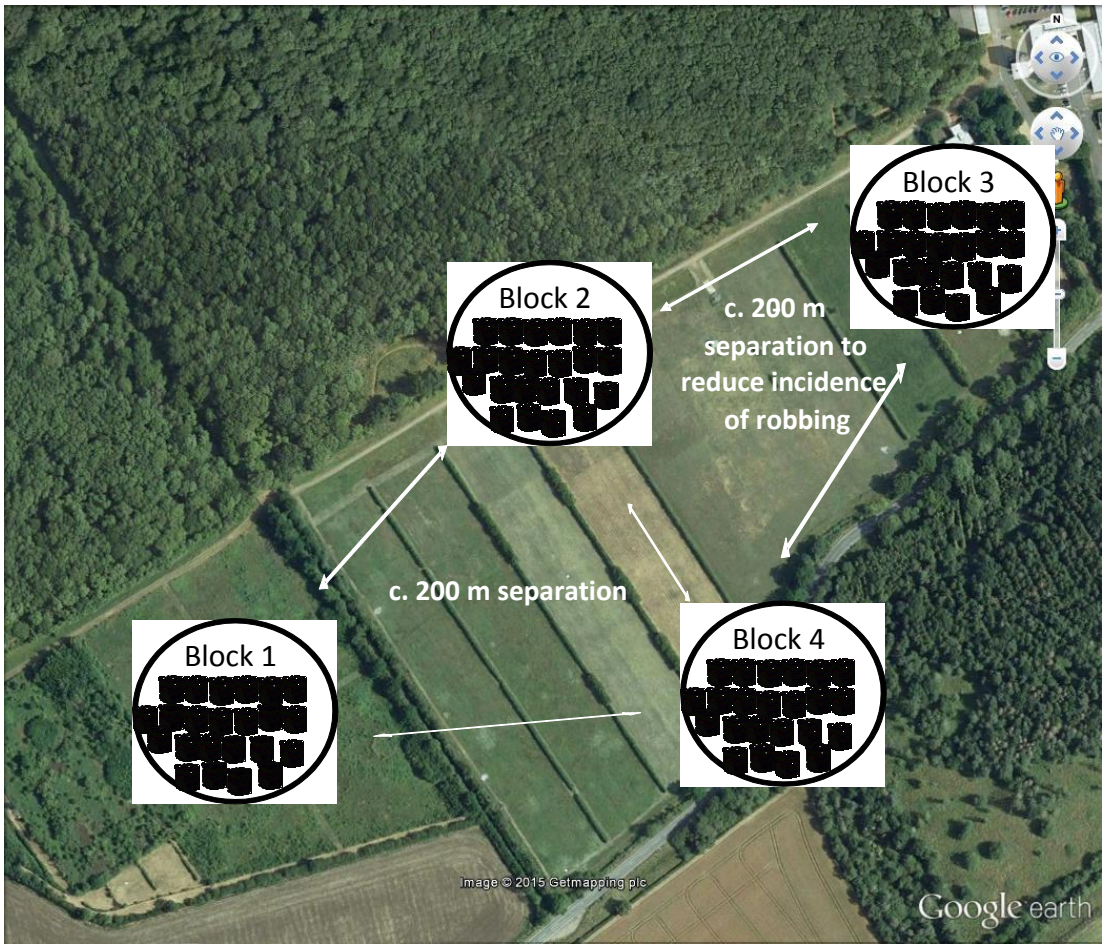
Overwintering sites are:

[redacted]

4.4.2.8 Overwinter management of the hives (non monitoring phase)

Overwinter management will, as far as practicable, be consistent for all hives in all three countries. Critical factors are the management of the honey collected during the exposure phase ('OSR honey', which will be removed prior to winter feeding) and feeding through the winter. The agreed approach is to remove any unused 'Oilseed rape honey' from all hives at the last beekeeper check BEFORE overwintering. Bees will be fed overwinter using a consistent approach at the country level. In the UK this will mean feeding fondant. In Germany and Hungary this will mean feeding sugar solution. Within a country all hives will be fed with the same amount of food (e.g. fondant or sugar solution) on the basis of local best practice. The important component here is consistency within countries and treating all hives the same (within a country).

Figure 5. Suggested field set-up for overwintering honeybee hives. All hives from a country will be overwintered on the same location. The 24 hives from a block (comprised of the hives located in the three sites of a block) will be clustered together, with these separated from the nearest cluster by about 200m. Apertures to hives will be reduced during the overwintering phase to reduce between hive robbing.



4.4.2.9 Beekeeper checking

The beekeepers will check each hive at all experimental locations every 7 days from first exposure through to July, and the final check before winter. Handling of all hives will be done with caution by a professional beekeeper so as to avoid possible disruption, and damage to queen cells in particular.

On each visit the following husbandry and checks will be made:

- Check for queen cells (involves removal of all frames) - remove any found to reduce risk of swarming;

  Swarming will be recorded during assessments of the whole hive during the weekly 'beekeeper checks', but no further action will be taken and monitoring of the hive will continue where possible;

- Record any incidence of queen death assess the possible cause;

- In the event of a queen death the hive will be allowed to regenerate naturally from new eggs and monitoring will continue as per the protocol [analyses can then be undertaken with and without these hives, which may or may not remain queenless];

- Rare incidences of known accidental damage/ death of a queen caused by the beekeeper error (e.g. accidentally killing a queen while checking hives) will be dealt with by removal of the dead queen and searches to confirm presence of new eggs within the hive during the same assessment visit. If new eggs are not apparent within the hive then replacement eggs will be taken from a brood frame from within the (back-up) colony at the same site;

- Check the food store and feed if needed;

- Check for and record the incidence of other disease (e.g. sacbrood, chalkbrood, foulbrood, varroosis etc).

Treatment of disease:

- Treatment for *Varroa* using agreed local practice at the country level. Germany and Hungary typically use formic acid which is considered the favored approach for ALL countries. UK hives may have oxalic acid in addition depending on humidity and temperature levels. In the event of atypical weather conditions, additional approaches can be considered and applied to all hives at the country level (e.g. Thymol).

5 Assessment Methods

5.1 Condition of the colony and brood development

The aim of this assessment is to quantify the effects of high but real world exposure to neonicotinoid pesticides on honeybee populations using standard assessments of colony strength and condition. The condition of each colony (six per site) will be formally recorded on the following occasions (see Table 4):

- Once shortly before set-up of the hives in the field (within 2 weeks pre-exposure);
- At weekly intervals during the exposure phase* (likely to be five times). N.B – in the event of an extended flowering season an additional assessment will be considered if within budget. Eurofins will inform CEH of the likelihood of this during the season;
- Once before the overwintering period (post-exposure – September- to be standardised within 2 week period for all hives within a block);
- Once after overwintering (March).

***Exposure phase is defined according to crop growth stages at the block level. Last field to start (at 10% flowering) and first field to stop flowering (10% flowers remaining) will define minimum period per block of fields.**

On each occasion (Table 6) the following parameters will be assessed:

I. Colony strength using the 'Liebefeld method' (number of bees, estimation adapted to IMDORF & GERIG, 1999, and IMDORF et al., 1987). Visual estimation will be conducted in steps of 5% working through the hive. All field recorders will be experienced and will be trained by Eurofins to maximise consistency and accuracy of recording.
II. Presence of a healthy queen (e.g. presence of eggs)
III. Pollen storage area and area with nectar or honey (estimation adapted to IMDORF & GERIG, 1999, and IMDORF et al., 1987)
IV. Area containing cells with eggs, larvae and capped cells (estimation adapted to IMDORF & GERIG, 1999, and IMDORF et al., 1987)
V. Colony weight measured to ±50 g accuracy by placing the hive on a digital balance.

At each assessment the comb area containing bees and cells with nectar, pollen, eggs, larvae, and capped cells will be estimated per comb side and the total number of bees and cells containing the brood stages, pollen and nectar on the comb will be calculated. This will be done for all combs per hive. For each recording date the raw data for each comb in each hive will be provided for analysis.

Table 6. Assessment of the condition of the colonies

Assessment dates	
1st brood assessment: before set-up of the hives at the field sites;	
	Subsequent brood assessments: weekly intervals (7 ±1 days) during exposure phase. Note this interval will depend to some extent on local weather conditions.
	Brood assessment during monitoring (post-exposure) phase: at set-up of hives at monitoring location and subsequently in September and in March after overwintering at the monitoring sites

5.2 Forager mortality assessment

The aim of this assessment is to quantify the effects of neonicotinoid exposure on honeybee mortality. Adult bee mortality will be assessed for each hive (six per site) once after set-up and then DAILY during the exposure period (crop flowering) using dead bee traps and linen sheets placed in front of each hive (see Table 7).

Water-permeable linen sheets of uniform size (3m × 1m) will be spread out on the ground in front of the hives and left for a consistent period (approx. / min. 24 hours) on each assessment date. Dead bees found on the linen sheets will be counted, recorded and removed. At the same time, dead bee traps, with gauze on the bottom and on the top, will be attached to the entrance of each hive to count and collect dead bees carried out of the hives. The traps will be emptied and counted every day during the exposure phase to measure if mortality is changing.

If more than 50 dead bees are discovered in the dead bee trap from an individual hive (i.e. indicating a spike in mortality) all bees will be collected from each of the six hives for that site and transferred to a deep freezer (≤ -18 °C) within 10 hours of collection. Samples will be kept separate at the hive level (groups of 10 bees) in labeled tubes and archived for the duration of the experiment. Inthis case dead bees on the linen sheets need not be collected. Clinical examination of the dead bee samples will be considered only if a strong, unexplained increase in mortality are detected (see Sections 5.5 and 6).

Table 7: Evaluation of adult bee mortality

Time of the test	**DBE / DAE**	**Evaluations of mortality***
Before setup at exposure site	At least DBE 3 until DBE 1	Once a day at about the same time of day. Preferably in the early morning before beginning of flight activity.
At exposure site	Every day during the exposure phase (crop flowering)	On four separate occasions during exposure (crop flowering)

*Remark: At each evaluation date the dead bees on the linen sheets will be counted and removed. The dead bees in the trap will be counted and sampled, and the sample will be archived.
DBE = Days before exposure to the treated (or control) crop.
DAA = Days after exposure to the treated crop.

5.3 Flight Activity and foraging behavior assessments

The aim of this assessment is to quantify the effects of neonicotinoid exposure on honeybee foraging activity and behavior. Bee activity and foraging behavior will be assessed at each hive (six per site) on four separate occasions during the exposure period (crop flowering). On each assessment day three observers will work in parallel across the three treatment sites within a replicate block to reduce the effects of variation in weather conditions on bee counts. The flight activity at the hive entrance will be documented at the start and end of each fixed observation period (Table 8). Subject to weather conditions meeting set parameters of temperature and wind speed, the number of bees will be counted as follows:

- 5 minutes observation of the number of bees entering the hive (with or without pollen loads)
- A FURTHER five minutes observation of the number of bees leaving the hive

Counts will be made three times per assessment day, spread throughout the day with a minimum of 60 minutes between counts per hive, to allow for variable weather patterns but to capture variation in daily foraging activity. Bee counts will only be made between 10.00 and 16.00 h following the standard limits for weather conditions given by Pollard and Yates (1993). Specifically, temperatures should be above 13°C with a cloud cover of less than 50 % for temperatures between 13-17°C. Above 18°C higher cloud cover is acceptable. Wind speed should be less than 5 on the Beaufort scale which is when branches of a moderate size move and small trees in leaf begin to sway. It should not be raining.

In addition, on the same assessment day, bee density and foraging behavior will be recorded in the flowering crop for a fixed time interval in four separate quadrats of 1m × 1m situated in front of the hives. The first observation quadrat will be situated just off the first tramline (5-10m in front of the hives), with further quadrats situated approximately 20m further into the crop (Figure 6). Bees will

be observed for a total of five minutes in each quadrat. The behavior of each bee at the first point of observation will be recorded and tallied against the following categories: i) nectar collection; ii) pollen collection; iii) all other behavior including abnormal; iv) motionless (Table 9):

Table 8: Evaluation of flight activity and foraging behavior

Time of the test	**DAE**	**Evaluation of flight activity**
Days after setup	Set-up until end of exposure	Four separate occasions during crop flowering

DAE = Days after exposure to the OSR crop.

Figure 6. Suggested set-up for bee observation quadrats in the crop

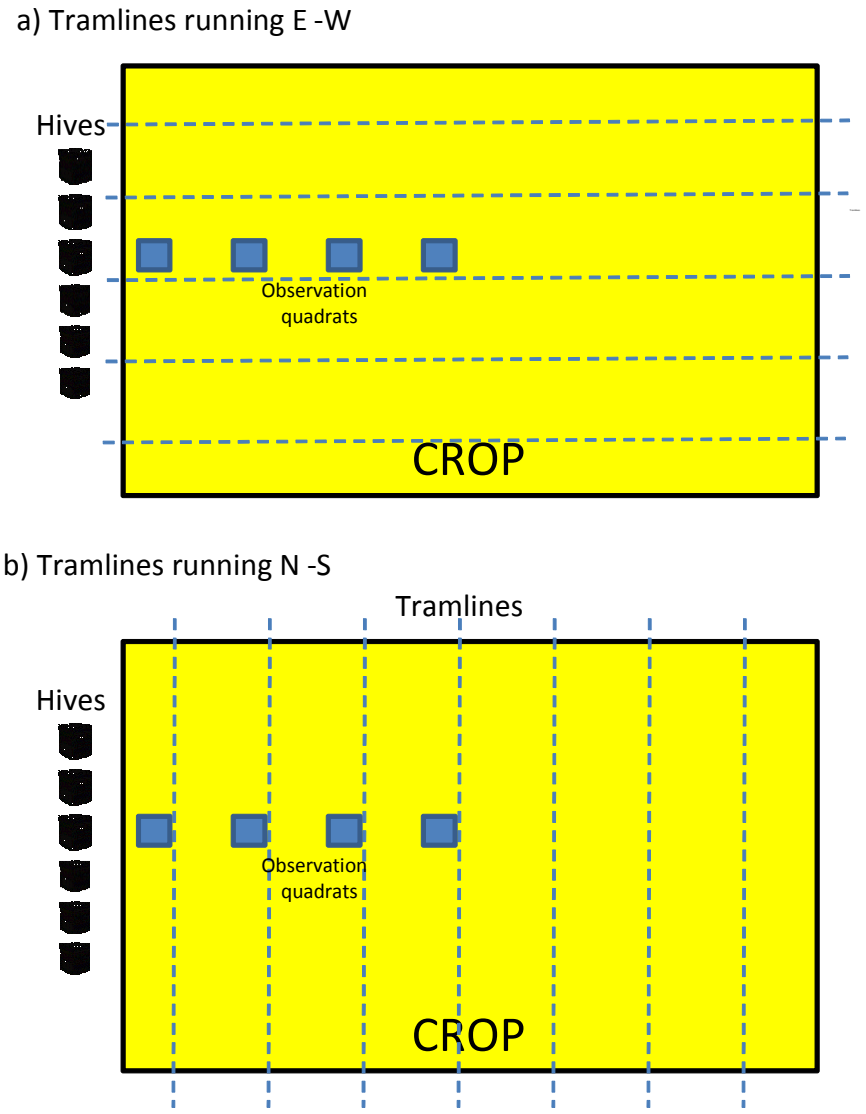


Table 9: Bee behavioral categories

Observed in 5 mins:	**i) Collecting nectar**	**ii)Collecting pollen**	**iii) Other behavior (including abnormal)**	**v)Motionless**
Tally number of bees on first observation in these categories				

The weather conditions at the time of bee flight activity and behavioral assessments will be recorded: shade (ambient) temperature, percentage sunshine and wind speed using the Beaufort scale.

5.4 Survey of surrounding land cover and flowering resources

The aim of this survey is to rapidly record the abundance and diversity of crop and non-crop flower resources likely to be utilized by pollinators during the exposure period. The type, growth stage, approximate area and location of crops and other broad land cover types will be recorded within a 1.5 km radius of the centre of each treatment field on a standard base map once during the study period (Apr-May). CEH will supply standard, large-scale base maps for each site for notation together with colour aerial photographs. In addition, any areas of abundant flowering weeds will be mapped.

For each field / land parcel within a 1.5 km radius of the study site the following information will be marked on a base map or aerial photograph of the site using standard land cover descriptors (Table 9; Figure 7):

- Land cover type: e.g. crops (including crop type), grassland, woodland etc (see Table 10)
- Growth stage (vegetative, flowering): flowering is used here to distinguish between land patches that contain no flowering resources for bees at the time of sampling (e.g. a field of wheat) and those that are likely to be important sources of flowers for bees (defined as flowering).
- Fields or other areas >1ha containing significant areas of non-crop flower resources (e.g. fallows, waste ground, species-rich grasslands)
- Linear features with important flowering resources (e.g. hedgerows, shelter belts, headlands).

For each country we have produced a list of key trees, herbs and shrubs that will be flowering at the same time as the oilseed rape (see below). Where a land parcel contains a large number of flowering plants (described as flowering under growth stage) the dominant species of these will be listed. We are not interested in counts of flowers, simply a list of the main flowering species of trees, shrubs of forbs.

Table 10: Standard land use categories with examples of abundant flowers attractive to bees

Category	Land cover	Growth stage (vegetative, flowering)	Abundant flowers known to be attractive to bees (see lists below)
1) Arable crops			
(e.g. Wheat, barley, legumes, sunflower, oilseed rape, fallow)	Example....Oilseed rape	Flowering	*e.g. Oilseed rape*
2) Horticultural crops			
(e.g. Apples, plums, strawberries, tomatoes, vines, melons)	Example....apple orchard	Flowering	*e.g. Apple*
3) Grassland			
Improved (spp-poor)	Example.....Unimproved grassland	Flowering	e.g. Trifolium repens
Semi-improved			
Unimproved (spp-rich)			
4) Woodland			
Deciduous	Example....shelter belt	Flowering	*e.g. Acer campestre*
Conifer			
Mixed			
Scrub transition			
5) Urban/suburban			
Gardens, parks, waste ground etc			
6) Water			
Lakes, ponds, rivers, ditches			
Wetlands			
Fen, swamp, bog, marsh			
7) Shrubland			
Heather			
8) Linear features			
Hedgerows	Hedgerow	Flowering	
Headlands			
Field margins			
9) Other			

UK key trees/shrubs/herbs

Trees/shrubs

- *Aceraceae - Acer campestre, A. pseudoplatanus* and, near habitation, *A. platanoides*
- *Aquifoliaceae - Ilex aquifolium* coming into flower at the end of the period
- *Caprifoliaceae - Viburnum lantana* mainly associated with the [redacted], but also elsewhere
- *Fabaceae - Ulex europaeus*
- *Rosaceae - Crataegus* species, especially *C. laevigata* on the clay sites [*C. monogyna* will overlap less]; *Malus pumila* and *M. sylvestris; Prunus* species, *P. spinosa* and *P. cerasifera* will be gradually going over, *P. avium* at its best
- *Salicaceae - Salix cinerea, S. purpurea, S. triandra, S. alba, S. x fragilis*

Forbs

- *Amaryllidaceae – Allium ursinum*
- *Asparagaceae – Hyacinthoides non-scripta*
- *Asteraceae – Taraxacum agg*
- *Boraginaceae – Pulmonaria officinalis; Symphytum spp* [Largely garden species of both genera]
- *Brassicaceae* – other wild/naturalised/cultivated *Brassica* species; *Cardamine pratensis*
- *Caryophyllaceae – Silene dioica, Stellaria holostea*
- *Geraniaceae - Geranium robertianum*
- *Lamiaceae – Ajuga reptans, Glechoma hederacea, Lamium album, Lamiastrum galeobdolon*
- *Plantaginaceae – Veronica chamaedrys*
- *Primulaceae – Primula veris, P. vulgaris; Hottonia palustris* (mainly after rape flowering time)
- *Ranunculaceae – Caltha, Anemone nemorosa, Ranunculus bulbosus, Ficaria verna*
- *Rosaceae – Fragaria vesca, Geum urbanum*
- *Rubiaceae – Galium odoratum* (mainly after rape flowering time)
- *Violaceae – Viola hirta, V. odorata, V. reichenbachiana, V. riviniana etc*

German key trees/shrubs/herbs

Trees/shrubs

- *Aceraceae - Acer campestre, A. pseudoplatanus* and *A. platanoides*
- *Caprifoliaceae - Viburnum lantana* sparse
- *Fabaceae - Ulex europaeus* rare
- *Rosaceae - Crataegus* species; *Malus pumila* and *M. sylvestris; Prunus avium*
- *Salicaceae - Salix cinerea, S. purpurea, S. triandra, S. alba, S. x fragilis*

Forbs

- *Amaryllidaceae – Allium ursinum;*
- *Asteraceae – Taraxacum spp*
- *Boraginaceae – Pulmonaria officinalis; Symphytum spp*
- *Brassicaceae – Cardamine amara, C. bulbifera*
- *Convallariaceae – Convallaria majalis, Polygonatum multiflorum* (all mainly after rape flowering time)
- *Lamiaceae – Ajuga reptans, Glechoma hederacea, Lamiastrum galeobdolon; Lamium album;*
- *Liliaceae – Gagea spp*
- *Orchidaceae* – no species really abundant but several contributing to spring nectar source (*Ophrys* spp and various *Anacamptis/Neotinea/Orchis* spp)
- *Papaveraceae – Corydalis bulbosa*
- *Primulaceae – Primula veris, P. vulgaris, Hottonia palustris* (mainly after rape flowering time)
- *Ranunculaceae –Adonis vernalis; Anemone nemorosa, A. ranunculoides; Caltha palustris; Ficaria verna; Pulsatilla/Anemone spp* (like orchids, unlikely to be common)
- *Rosaceae – Fragaria vesca; Geum urbanum*
- *Rubiaceae – Galium odoratum*
- *Violaceae – Viola mirabilis, V. reichenbachiana, etc*

Hungarian key trees/shrubs/herbs

Trees/shrubs

- *Aceraceae - Acer campestre, A. pseudoplatanus, A. tataricum* and *A. platanoides*
- *Caprifoliaceae - Viburnum lantana*
- *Cornaceae – Cornus mas* (may be over by rape flowering time)
- *Fabaceae - Robinia pseudacacia* (abundant locally though this usually flowers from mid/late May onward and the overlap with rape will be small); *Ulex europaeus* rare
- *Oleaceae - Fraxinus ornus*
- *Rosaceae - Crataegus* species (*C. laevigata & C. monogyna*); *Malus pumila*, *M. dasyphylla* and *M. sylvestris*; *Prunus avium, P. spinosa, P. fruticosa* (mainly after rape flowering time); *P. tenella*; *Pyrus pyraster, P. nivalis*

Forbs

- *Amaryllidaceae – Allium ursinum; Leucojum aestivum*
- *Apocynaceae – Vinca spp* (*V. herbacea*)
- *Asparagaceae – Ornithogalum spp* (*O. refractum etc*)
- *Asteraceae – Taraxacum spp*
- *Boraginaceae – Lithospermum purpurocaeruleum; Pulmonaria spp; Symphytum spp*
- *Brassicaceae – Cardamine amara*
- *Caryophyllaceae – Cerastium sylvaticum; Dianthus pontederae*

- *Convallariaceae – Convallaria majalis, Polygonatum latifolium, P. multiflorum* (all mainly after rape flowering time)
- *Fabaceae – Lathyrus vernus*
- *Lamiaceae – Ajuga reptans, Glechoma hederacea, Lamiastrum galeobdolon; Lamium album, L. maculatum*
- *Liliaceae – Gagea spp*
- *Orchidaceae – Anacamptis coriophora*
- *Papaveraceae – Corydalis bulbosa*
- *Primulaceae – Primula veris, P. vulgaris, Hottonia palustris* (mainly after rape flowering time)
- *Ranunculaceae – Adonis vernalis; Anemone nemorosa, A. ranunculoides; Caltha palustris; Ficaria verna*
- *Rosaceae – Fragaria vesca, F. viridis; Geum urbanum*
- *Rubiaceae – Galium odoratum*
- *Violaceae – Viola elatior, V. mirabilis, V. reichenbachiana, V. suavis etc*

Figure 7: Example of a landscape land cover and flower resource assessment

5.5 Disease analysis

The aim of honeybee disease measurements is to provide i) a useful covariate measure of disease loading per hive before exposure and ii) to see how this changes following exposure to NNIs. The quantitative analysis of honeybee disease will complement the frequent beekeeper health checks (4.4.2.9). It is important to note that given the paucity of data on metrics of honeybee diseases there has been no scope for a power analysis to infer appropriate levels of replication. It is impossible therefore to say if we have sufficient replication in this study to identify null effects of neonicotinoids on the susceptibility of bees to different diseases. However, given current evidence of interactions between exposure to pesticides and honeybee susceptibility to disease this component of the methodology will be undertaken although the caveat of unknown required sample size for the detection of significant effects is acknowledged.

5.5.1 Selections of pathogens to study

There are a great many honeybee pathogens that could be studied. However, for practical and economic reasons we consider the following the priority:

- **Gut pathogens**, particularly the enteroviruses such as DWV (deformed wing virus), as even in "normal" colonies these infect the midgut so there could be interactions between these and ingested NNIs;
- ***Nosema*** sp. as there are publications from lab studies indicating sublethal interactions/synergisms with NNIs increasing mortality;
- **Viruses** that act on the nervous system (same site of action as NNIs), including the APV clade (Acute paralysis viruses), KBV (Kashmir bee virus), CPV (chronic paralysis virus). (Note: KBV is not common in Germany but APV is);
- ***Varroa*** as this is a major cause of major cause of increases in DWV as varroa is a vector of DWV. A quantitative assessment of *Varroa* will be used to compliment the beekeeper assessment.

5.5.2 Sampling

Sampling for honeybee disease will be carried out at the following times:

- **Pre-exposure** to get base line data
- **Post-exposure** (post flowering) to determine influence of NNIs on disease loading.
- **Pre-overwintering** is important as most of the virus infection happens between May and September. This may be a key factor explaining overwinter survival.

  Previous experience suggests that post-winter disease loading does not often differ from that of pre-winter. It is therefore recommended that this four sampling point is dropped in favour of wider screening for disease.

At each occasion two separate samples of *c.*100 WORKER bees will be collected per hive (with the aim of assessing diseased in only 60 bees per sample, there remainder being intended to ensure this minimum threshold of bees are collected). For the post-exposure and pre-exposure samples only flying bees (workers) will be sampled. However, for the pre-exposure samples it will be

necessary to collect bees directly from hives due to low levels of flight activity at this time of the year.

The pooled samples (2 separate samples per hive) will be placed in separate labeled tubes and placed immediately into sealed container with dry ice. This is important as most bee viruses are RNA viruses and so storage in dry ice is necessary to prevent catalysis. One of the two samples from each hive will be used for the assessment of viruses / other diseases, the second sample will be used in the assessment of *Varroa* infections. Following their return from the field where they have been temporarily stored in dry ice longer term storage will be in freezers maintaining a temperature of -20 °C. Once all samples for a country have been collected they will be shipped to [redacted] (see address below). Shipped samples will again be stored in dry ice to ensure temperatures remain below -20 °C. To ensure that shipped samples have remained frozen during their transit a data logger will be included in each sample to track temperatures over this period. Samples should be sent on a Monday or Tuesday to ensure they reach their destination before the weekend. Finally, before sending samples [redacted] requests e-mail confirmation of their delivery [redacted] a week before to ensure that sufficient space is available in their laboratory freezers for storage.

[redacted]

The pooled samples of ca.100 bees will be analyzed for the above diseases (5.1) using either microscopy techniques (*Varroa*) or molecular screening (*Nosema*). Incidence of foulbrood and foulbrood will be recorded based on the individual hive checks undertaken by beekeepers only (see section 4.4.2.9)

3 countries, 12 sites in each (± neonics) with 6 + 2 hives on each site and 3 samples (each of *ca.*100 bees) = 3 x 12 x 8 x 3 = 864 samples
A further sample will be taken for *Varroa* counts

This will give a grand total of 1728 samples.

6 Residue sampling

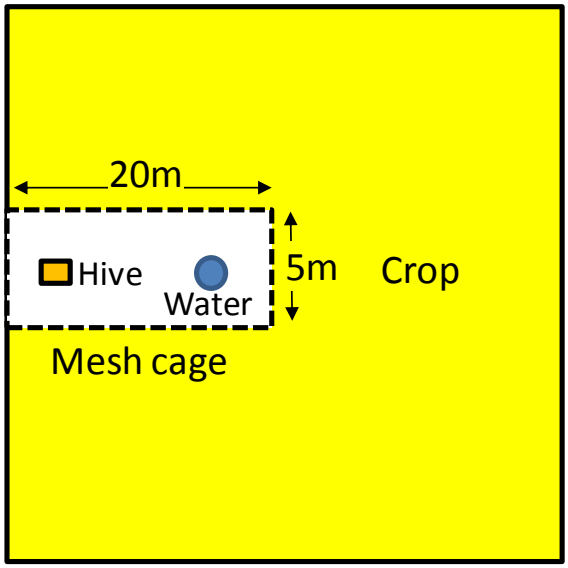
Sampling of neonicotinoid residues from the experiment can be summarised as follows. Full detailed of each approach are given in **Table 11 below**:

Activity	Sample type	Number of hives sampled per site	Samples per site	Sampling frequency	Indicative date of collection	Total samples from 36 sites FOR RESIDUE ANALYSIS
6.1 Crop residue sampling from caged bees (tunnel assessments)	**Nectar**	**1**	**1**	**2**	**Beginning of flowering (early Apr); one week later**	**72**
	Pollen	**1**	**1**	**2**	**Beginning of flowering (early Apr); one week later**	**72**
6.2.1 Residue sampling from collected nectar	**Nectar**	**6**	**1**	**2**	**Three times during crop flowering (end May)**	**72**
6.2.2 Residue sampling from collected pollen	**Pollen**	**6 SEPARATE sample per hive from trap**	**6**	**2**	**Three times during crop flowering (end May) CEH WILL HOMOGENISE SAMPLE**	**72**
6.4 Residue sampling from stored hive products	**Nectar/honey**	**6**	Jun – separate samples per hive; Jul pooled sample, Aug (pooled)	3	**Jun (immediately post-exposure), Jul, Aug**	**Post-exposure (Jun)– separate sample per hive 216 Jul – pooled sample (36) Aug– pooled sample (36) TOTAL = 288**
	Pollen	**6**	1	**3**		**Pre-exposure – pooled sample (36) Post-exposure – pooled sample (36) Pre-winter - – pooled sample (36) TOTAL = 108**
	Wax	**6**	**1**	**3**		**Pre-exposure – 20 random samples Post-exposure – pooled sample (36) Pre-winter – pooled sample (36) TOTAL = 92**
		SEPARATE Samples per hive			**CEH WILL HOMOGENISE SAMPLE**	
TOTAL						**776**

6.1 Residue sampling from caged bees

The aim of this measurement is to quantify the concentration of NNI pesticide expressed in commercial field crops of oilseed rape. This will be achieved by placing a mesh cage over the crop at each of the 36 sites and placing one of the eight honeybee hives in the cage. The bees from this hive will only forage on the oilseed rape crop (treated or untreated) (Figure 8). This will provide an estimate of the 'worst case' exposure.

Figure 8. Layout of the cage in the oilseed rape crop



Each cage will comprise a tunnel of approximately 100m^2 (Table 12) covered with light plastic gauze (mesh size: approximately 1.5 mm) to enclose the bees and ensure they only forage on the treated or untreated crop. Crop at the edge of the cage will be cut or sprayed off and the ground smoothed to ensure the cage is well sealed and the bees cannot escape. Two containers filled with water will be placed into each tunnel as water supply for the bees. The surface of the water will be covered with floatable material to prevent the bees from drowning.

Nectar and pollen will be collected from the caged bees on **two separate occasions** during crop flowering (beginning of flowering and one week later. See Table 11 for detailed activity schedule). On each occasion 400 worker bees will be collected and nectar samples taken and bulked for residue analysis – the primary sample (see Table 13 for methodology). A further sample of 400 bees will be collected at the same time and will be archived as a back-up sample in case insufficient nectar is extracted from the primary sample. It is estimated that a full honey stomach of each bee will yield approximately 6 mg of collected nectar, giving a total sample volume for analysis of around 2.4g. Passive pollen traps will be attached to the front of the hives on two occasions and used to collect pollen loads from returning bees for residue analysis (see Section 6.2.2 for more detail).

This will give a total of 36 nectar samples from two time periods (total 72 samples) and 36 pollen samples from two time periods (total 72 samples) (Table 11). These will be placed in labeled tubes and sent to CEH for pesticide residue analysis.

Table 12: Dimension of the residue tunnel

Tunnel size	Approx. 100 m²
Tunnel length	Approx. 20 m
Tunnel width	Approx. 5 m
Tunnel height	Approx. 3.5 m at the centre
Special requirements	None

Table 13: Detailed activity schedule for tunnel assessment of crop residues

Activity	Timing	Description	**Responsibility**
Set up of exposure tent	Day 0 (evening after bee flight)	Set-up of the colonies in the tunnels	Eurofins
S1	Day 2 (±1)	Sampling of forager bees for nectar and pollen	
S2	Day 9 (±2)	Sampling of forager bees for nectar, pollen and flowers (for residues)	

6.2 Nectar and pollen sampling from free-flying bees

The aim of this measurement is to quantify NNI concentrations in nectar and pollen loads collected from honeybees flying freely in the landscape containing the treated or untreated crops. This will provide an estimate of 'real world' exposure. This will also indicate whether bees are switching foraging choices during the exposure period.

Foraging bees returning to each hive will be sampled on three occasions during crop flowering. Ideally two of these occasions will be on the same day as tunnel assessments, or within one day to allow for comparisons between the 'real world' and 'worst case' exposure (6.1, to avoid major temperature differences between sampling sessions).

6.2.1 Nectar sampling

On each occasion approximately 400 bees will be collected from each SITE ensuring all hives are sampled equally (approx. 60-70 bees per hive) – the primary sample. In addition, a further 400 bees will be collected and retained as a secondary (back-up) sample in case insufficient nectar is extracted

from the primary sample. These bees will be dissected and nectar from the honey stomachs will be pooled into a single sample per site for residue analysis. Dissection of bees will continue until 1 g of nectar is obtained.

This will give a total of 2 occasions × 36 pooled site samples = 72 nectar samples (Table 14). These will be placed in labeled tubes and sent to CEH for pesticide residue analysis.

Table 14: Detailed activity schedule for collecting nectar samples from foraging bees

Specimen description	Forager bees for the preparation of nectar from honey stomachs for residue analysis. At each sampling, the hive entrances will be sealed before the sampling and the forager bees will be subsequently collected as they return to the hive by collecting them into a box containing dry ice, either by brushing or using modified hoovers ("bee vac"), or using tweezers if only few bees are returning. After sampling the hives will be re-opened. On each sampling day a primary sample (400 bees collected approximately evenly from each of the six hives) and a reserve sample (using the same procedure) will be collected at each site.
Retained specimens	Retain specimens should be taken. These will only be analysed if the original specimens are destroyed / damaged prior to analysis / a fault occurs during analysis. They will be stored deep frozen. The retained specimens will be sent to CEH for storage.
Storage conditions	The samples will be chilled directly after sampling and during transport to the freezer (stored on blue ice or dry ice) and will subsequently be stored deep frozen at ≤ -18 °C within 12 h after sampling.
Special requirements	Forager bee samples will be taken from each hive and will be pooled afterwards, resulting in one single sample. Record growth stage of the crop (BBCH) at each day of sampling. Record in raw data conditions on site (air temperature and relative humidity) at each day of sampling. Record in raw data, that forager bee sampling was done from each hive.

6.2.2 Pollen sampling

Pollen traps will be used to collect pollen from foraging bees on four occasions (once pre-exposure and three during crop flowering, to coincide with nectar sampling dates). On each occasion a pollen trap will be attached to the front of each hive. Traps will be set for a consistent time period, with consistent start and end times for each site within a block (Table 15).

Pollen samples from all six hives at each site will be pooled and stored in separate labeled tubes on each occasion. This will give 2 occasions × 36 sites = samples for residue analysis at CEH.

THE POLLEN FROM EACH HIVE AT EACH SITE WILL BE PLACED IN SEPARATE LABELLED TUBES. The pollen samples from each hive will be homogenised and sub-sampled for pollen load analysis by CEH. Pooled samples for each site will be produced for residue analysis.

This will give a total of 2 occasions × 6 hives × 36 samples = 432 pollen samples. These will be sub-sampled for pollen load analysis. Pooled samples will be produced for each site (total 72) and sent to CEH for pesticide residue analysis.

Table 15: Detailed activity schedule for sampling of pollen using pollen traps

Specimen description	Every hive will be equipped with a pollen trap. Bees will strip off the pollen when passing a grid. This pollen grid will only be inserted on sampling days. After collection of the pollen the grid will be removed. After the sampling period the content of the pollen trap will be mixed and divided into three approximately equal portions. One sample will be sent for residue analysis at CEH, one will be sent for pollen source analysis, and one will be retained for archiving.
Retain specimens	Retain specimens should be taken. These will only be analysed if the original specimens are destroyed / damaged prior to analysis / a fault occurs during analysis. They will be stored deep frozen. They can be disposed of 4 weeks after the draft study report has been issued, with the approval of the Study Director, unless the Sponsor requests further storage.
Storage conditions	The samples will be chilled directly after sampling and during transport to the freezer (stored on blue ice / dry ice) and will subsequently be stored deep frozen at ≤ -18 °C within 12 h after sampling.
Special requirements	Pollen samples will be taken from each hive and retained as separate samples. A subsample will be taken from each sample for pollen load analysis. A bulked / pooled sample will be made up from all six hives at a site for residue analysis. Record growth stage of the crop (BBCH) at each day of sampling

	Record in raw data conditions on site (air temperature and relative humidity) at each day of sampling. Record in raw data, that pollen sampling was done from each hive.

6.3 Residue sampling from stored hive products

Individual samples for NNI analysis will require a minimum of 1 g of any given product, e.g. stored products like wax. The total mass of any stored product must be in excess of 1g, but for practical reasons less than 25 g.

The aim of this measurement is to quantify residue concentrations in stored hive products and to determine how these concentrations change over time. Honey and pollen are considered to be the most important products indicating exposure risk as it will be directly ingested. Wax considered less important as an indicator as neonicotinoids are not lypophillic, but needs to be sampled in order to account for exposure via contact (of larvae within cells) as opposed to ingestion, plus need to account for possible contamination of wax used from external sources when constructing frames.

6.3.1 Honey and pollen

Samples of stored hive product (honey, pollen) will be collected from each hive during the routine beekeeper checks (to minimise disruption of the hive) **on three occasions, one immediately post-exposure (June) and again in July and August.**

On each occasion 12 random samples of honey will be taken across all locations and frames in each hive, including capped and un-capped brood cells containing nectar/honey. Eurofins will train field workers using a standard protocol to ensure consistency of sampling.

Stored pollen/bee bread samples will be collected at the same time using a similar consistent approach. A minimum of 1 g if stored

The samples of honey and pollen will be retained in **separate** labeled tubes for each product (honey, pollen) and for each hive on each occasion. The following samples will be analyzed for neonicotinoid residues by CEH:

Honey
June – 6 hives × 36 sites = 216 separate samples analyzed separately
July - 6 hives × 36 sites = 216 separate samples pooled into 36 samples (one per site) for residue analysis
August - 6 hives × 36 sites = 216 separate samples pooled into 36 samples (one per site) for residue analysis
TOTAL = 288 samples for residue analysis

Pollen/bee bread

June - 6 hives × 36 sites = 216 separate samples pooled into 36 samples (one per site) for residue July - 6 hives × 36 sites = 216 separate samples pooled into 36 samples (one per site) for residue analysis
August - 6 hives × 36 sites = 216 separate samples pooled into 36 samples (one per site) for residue analysis
TOTAL = 108 samples for residue analysis

6.3.2 Wax

Samples of wax will be collected pre-exposure from the new brood comb to quantify any contamination with neonicotinoid residues resulting from the mass production of brood comb. In addition, wax will be sampled immediately post- exposure (June) and again in August. The latter two sampling occasions will be coincident with the sampling of honey and pollen from the hives.

On each occasion 12 random samples of wax will be taken across all locations and frames in each hive). The total mass of wax must be in excess of 1g, but for practical reasons less than 25 g. Eurofins will train field workers using a standard protocol to ensure consistency of sampling.

The samples of wax will be retained in **separate** labeled tubes for each hive on each occasion. The following samples will be analyzed for neonicotinoid residues by CEH:

Wax
Pre-exposure - 6 hives × 36 sites = 216 separate samples pooled into 36 samples (one per site); random sub-sample of 20 for residue analysis
June - 6 hives × 36 sites = 216 separate samples pooled into 36 samples (one per site) for residue analysis
August - 6 hives × 36 sites = 216 separate samples pooled into 36 samples (one per site) for residue analysis
TOTAL = 92 samples for residue analysis

6.4 Residue sampling from oilseed rape flowers and leaf material

The aim of this measurement is to provide further proof of exposure in plants, even though lower LOQ will be identified than for nectar and pollen. It is proposed to collect and archive this material, but not undertake any residue analysis unless no residues are identified in nectar and pollen from the caged bees (6.1).

Sampling of oilseed rape flowers and leaf material will be carried out ONCE during peak flowering.

The minimum tissue mass required is 50 g collected from 30 different plants widely spaced along a tramline. From each plant a single healthy leaf will be cut and placed into a labeled sample bag (one bag containing 50g of leaves from a site). From the same plants 10 fully open flowers will also be collected and placed in a separate labeled bag or tube (a total of 300 flowers). Clean (new) scissors will be used at each site to prevent cross-contamination. The samples will be chilled directly after sampling and during transport to the freezer (stored on blue ice / dry ice) and will subsequently be stored deep frozen at ≤ -18 °C within 12 h after sampling.

Sampling of Flowers and Plant Tissue

Specimen description	Winter oilseed rape flowers will be collected (a total of 300 flowers from each site). Therefore single blossoms will be sampled by cutting the blossoms with scissors from the flower heads into a container. The method finally used will be described in the final report.
Retain specimens	Retained specimens will be archived by CEH. These will only be analysed if the original specimens are destroyed / damaged prior to analysis / a fault occurs during analysis. They will be stored deep frozen. They can be disposed of 4 weeks after the draft study report has been issued, with the approval of the Study Director, unless the Sponsor requests further storage.
Storage conditions	The samples will be chilled directly after sampling and during transport to the freezer (stored on blue ice / dry ice) and will subsequently be stored deep frozen at ≤ -18 °C within 12 h after sampling.
Special requirements	The first sampling will be done before the first application and will be used as control specimen. One sample will be taken from plot Ta and two samples will be taken from plot Tb (one in front, the other from the back of the tunnel) from at least 12 different plants, each. They will be pooled afterwards, resulting in one single sample. Record growth stage of the crop (BBCH) at each day of sampling

	Record in raw data conditions on site (air temperature and relative humidity) at each day of sampling.

6.5 Residue Analysis

Samples of nectar, wax and pollen will be sent deep frozen (≤ -18°C) for residue analysis at CEH laboratories. Shipment conditions will be recorded by use of a data logger or a min/max thermometer and documented in raw data.

[redacted]

CEH analytical laboratories have UKAS accreditation for a number of analyses and conducts all analyses according to procedures required for UKAS accreditation. The laboratory will be applying for UKAS accreditation for its methodology during the course of the study which will demonstrate that analysis meet requirement of UKAS accreditation. Full details of QA/QC and LOD will be given in a separate amendment.

7 Other measurements

Data on a number of other variables will be collected to provide background information for the study.

7.1 Recording of the meteorological data

The following climatic data will be recorded at one site in each replicate block (11 in total) to provide a record of local climatic conditions:

- temperature (min/max)
- relative humidity
- rainfall
- sunshine hours

CEH will locate a single weather station at one site within each replicate (11 total)

While colonies are located at their monitoring location, weather data (temperature, humidity and rainfall) will be collected from a nearby official government weather station (non-GLP records).

7.2 Photographical documentation

In addition to the documentation of findings and observations in the raw data pictures will be taken at each site to document:

- Local site conditions and experimental set-up
- Local landscape
- Crop condition
- Foraging activity of the bees

These images will be clearly named with the site code. Care will be taken to ensure the date and time settings on all cameras will be correct.

8 Data management

Data management for the project will follow the Data Management Plan developed by CEH. See separate document. Key points are as follows:

- The experiment will be sampled and analyzed BLIND (i.e. the field workers collecting the data will not know the identity of the treatments at any given sites). This is to minimize the risk of any sampling bias and ensure independence. The only exception to this will be for the chemical analysis where it is good protocol in order to minimize contamination to analyze samples likely to have low concentrations of the neonicotinoids (e.g. control sites) first.

- A consistent labelling convention will be agreed and adhered to for the project. This will be managed by the CEH Data Manager. This will include a single sample labelling matrix based on unique identifier numbers for sites, treatments, samples, measured variables etc.

- Data will be collected in the field in electronic and paper formats. Electronic data input templates based on Microsoft Excel will include macro functions (e.g. drop downs) to minimise and trap errors, and maximise data input consistency in the field. Paper recording sheets will be designed to minimise errors and inconsistencies.

- The original paper data sheets will be checked for accuracy by Eurofins and scanned at 300dpi resolution. The original paper records and a copy of the scanned files will be sent to CEH by recorded delivery/courier.

- CEH will input the paper records using a bespoke database with facility to trap errors and check for data anomalies. Additional queries will be run on the data post-input to check for errors.

- A sample of 10% of the field data sheets will be checked for accuracy.

- All data from the project will be stored on an ORACLE database which is back-up each night with copies stored on servers off-site.

8.1 Data sets

The following raw data sets will be provided by Eurofins to the sponsor in the following formats. Spreadsheet templates will be sent to the CEH Data Manager [redacted] prior to the start of the study to ensure compatibility and compliance with CEH data policy. Copies of the raw data sent by Eurofins will be verified as correct and the final version. A detailed data management plan will be produced by the CEH Data Manager for this project:

Activity			
3.2 Drilling and crop maintenance details			
Crop inputs, rates and dates	Paper	Spreadsheet	
Crop yield and quality	Paper	Spreadsheet	
3.3 Hive details (identifier, origin, size, age, race, hive design)		Spreadsheet	
3.4.2.4 Hive location (GPS)		Spreadsheet	
3.4.2.5 Honey management records	Paper	Spreadsheet	
3.4.2.6 Varroa control records	Paper	Spreadsheet	
3.4.2.7 Beekeeper colony health check records	Paper	Spreadsheet	
3.5.1 Condition of the colony and brood development			
Colony strength (Liebfeld)	Paper	Spreadsheet	
Presence of healthy queen	Paper	Spreadsheet	
Pollen storage area and area with nectar or honey	Paper	Spreadsheet	
Area containing cells with eggs, larvae and capped cells	Paper	Spreadsheet	
Colony weight measured	Paper	Spreadsheet	
3.5.2 Forager mortality assessment			
Dead bee trap counts	Paper	Spreadsheet	
Dead bee sheet counts	Paper	Spreadsheet	
3.5.3 Flight activity and foraging behaviour			
Bee activity counts	Paper	Spreadsheet	
Foraging behaviour tallies	Paper	Spreadsheet	
3.5.4 Survey of surrounding land cover and flowering resources			
Annotated maps of each site showing:	Paper		
Land cover			
Growth stage			
Dominant flowers attractive to bees			
3.5.6 Disease analysis			
Records of quantity, and date of sample collection & shipping		Spreadsheet	

4. Residue sampling			
4.1 Crop residue sampling from caged bees			
Records of quantity, and date of sample collection & shipping	Paper	Spreadsheet	
4.2 Residue sampling from foraging bees			
Records of quantity, and date of sample collection & shipping	Paper	Spreadsheet	
4.3 Residue sampling from collected pollen			
Records of quantity, and date of sample collection & shipping	Paper	Spreadsheet	
4.4 Residue sampling from stored hive products			
Records of quantity, and date of sample collection & shipping	Paper	Spreadsheet	
4.4.2 Weather variables		Spreadsheet	
Temperature (min/max); relative humidity; rainfall			
4.4.3 Photographic documentation			
Local site conditions, crop development, landscape, foraging activity			Digital images

9 Timing of project activities

	Pre-			Exposure					Post-exposure				Over-winter						
	Mar	Apr				May				Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Set-up & management																				
4.4.2.3 Set-up field sites																				
4.4.2.8 Over-winter hives																				
4.4.2.9 Beekeeper checks																				
Measurements																				
5.1 Condition of the colony and brood development																				
5.2 Forager mortality assessment																				
5.3 Flight Activity & foraging behaviour assessments																				
5.4 Survey of surrounding land cover																				
5.5 Disease analysis																				
Sampling																				
Analysis																				
Residue analysis																				
6.1 Residue sampling from caged bees																				
6.2 Nectar sampling from free-flying bees																				
6.3 Pollen sampling from free-flying bees																				
6.4 Residue sampling from stored hive products																				
Wax																				
Honey																				
Pollen																				
6.5 Residue sampling from flowers and leaf material																				
6.6 Residue analysis																				
7.0 Other measurements																				
8.0 Data management																				
Project management																				
QA																				
Data analysis																				
Meetings (including field visits)																				
Reporting																				

10 Archiving

Any material remaining at CEH after sample residue analysis will be retained and archived for a minimum period of 6 months following publication of the data. Raw data in paper format and project documentation will be archived at CEH for a minimum period of 10 years following publication for the final report and peer-reviewed papers.

11 Distribution

11.1 Study Plan

Original:
Testing facility (1 x) (Eurofins)
Sponsor (1 x) (NERC Centre for Ecology and Hydrology)

Copy:
Each Principal Investigator (1 x)
Each Test Site QA (1 x)
Bayer Crop Science(1 x)
Syngenta (1x)

11.2 Raw Data

All copies of the raw data (electronic and paper) sent to the study sponsor (NERC Centre for Ecology and Hydrology). See notes above

12 Responsibilities

12.1 Responsibility

This large-scale field experiment will be undertaken jointly by staff from Eurofins and CEH. Eurofins will have responsibility for the management and monitoring of the honeybee test colonies (in all countries) and the trial crops (in Germany and Hungary only). CEH will have responsibility for pesticide residue analysis (in all countries) and the management of the trial crops in the UK. See first section of report for contact details.

This section explains the responsibilities of persons/functions listed in the study plan.

Study Director and Testing Facility Responsible for Study Management

The study director has the overall responsibility for the conduct and management of this study according to good scientific principles.

Principal Investigators

The field, analytical or processing phase or parts of it are either executed by eurofins-GAB GmbH or by a principal investigator of a co-operator. The Principal Investigator will follow the study plan and amendments including specific arrangements, according to contractual agreement with the testing facility, ensuring GLP-compliance. The Principal investigator will document deviations from study plan and amendments and will inform SD in a timely manner.

The Principal Investigator executing the analytical phase will add the analytical method, if not already described in detail in the study plan, by a study plan amendment that must be approved by the Study Director.

12.2 Deviations from and Amendments to the Study Plan and SOP´s

The PI will inform the Study Director of any necessary amendments to the study plan. A statement will be signed by the persons signing the study plan. Any deviation from the plan with reasons for the deviation and the likely consequences of the deviation will be documented in the raw data and signed by the technician or Principal Investigator. It will be reported immediately to the Study Director, so that corrective actions can be initiated and documented, if necessary.

12.3 Confidentiality

No confidentiality of findings is claimed.

13 References

IMDORF, A. & GERIG, L. (1999): Lehrgang zur Erfassung der Volksstärke, Schweizerisches Zentrum für Bienenforschung.

IMDORF, A.; BUEHLMANN, G.; GERIG, L.; KILCHMANN, V. AND WILLE, H. (1987): Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern, Apidologie 18 (2), 137 – 146.

Pollard E. & Yates T.J. (1993). Monitoring Butterflies for Ecology and Conservation. Chapman and Hall, London.

Appendix 1. Glossary of terminology.

Site: This is the fundamental unit of replication of the study, and represents an individual farm on which either the control, Thiamethoxam or Clothianidin treated oilseed rape will be established. There are 36 sites in the study clustered into replicate blocks of three sites each separated by at least 3.2 km.

Replicate block: Clusters of three sites separated by 3.2 km on which oilseed rape has been established either with no applied neonicotinoids (control), Thiamethoxam or Clothianidin treated crop.

Treatment: This refers to the single treatment of the study, whether or not oilseed rape has been treated with neonicotinoid seed treatments. There are three levels to this treatment. 1) oilseed rape treated with Clothianidin; 2) oilseed rape treated with Thiamethoxam; 3) control oilseed rape not receiving no neonicotinoids.

Country: this is the largest scale of spatial variation. There are between three (Germany) and four (Hungary and UK) replicate blocks within each country.

OSR: Oilseed rape.

Field: The individual land units within which farmers will manage crops. Sites (which represent our smallest experimental unit) may contain multiple field of treated oilseed rape crop.

GLP study: Good Laboratory Practice study.

Quadrat: 1 × 1 m area within which the density of bees is recorded.

Patch: This is the location within each site where bee hives are positioned and is central to the overall area of treated oilseed rape within a site. Patches may be located on the boundaries of fields within a site (e.g. in the UK where each site has many treated fields of oilseed rape) of in a sprayed off area within a large field of oilseed rape.

DBE / BAE: Days before exposure / after exposure.
Separation distance: This refers to the physical separation between sites within blocks and blocks within countries. Within each replicate block sites are separated by at least 3.2 km. Replicate blocks within a country are separated by at least 10 km.

Exposure tent: net tent erected over both the oilseed rape crop and a honeybee hive to force bees to forage only on the crop and so allow a direct assessment of the expression of neonicotinoids in both the pollen and nectar of this crop.

BBCH: Standardised scale for assessing the growth stage of crops, in this case oilseed rape.