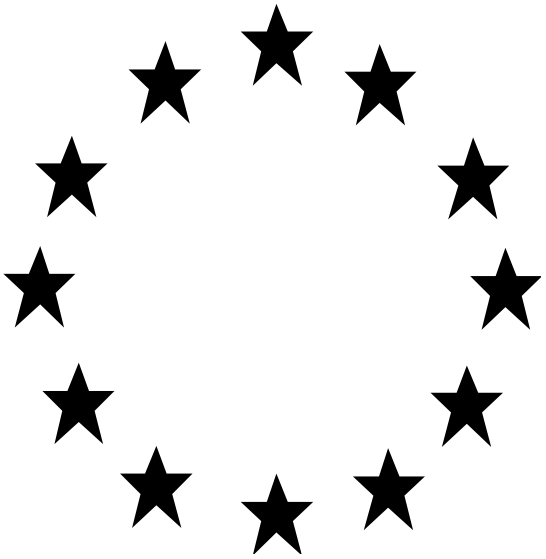

Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State the United Kingdom for the existing active substance

CLOFENTEZINE

of the third stage Part A of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

March 2009

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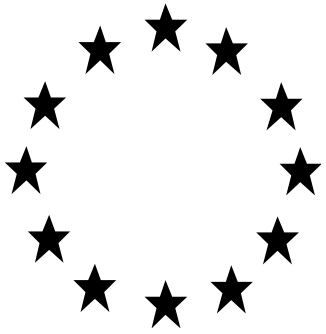
Council Directive 91/414/EEC



Clofentezine

Draft Assessment Report

Addendum 1

Methods of analysis, Toxicology and Ecotoxicology

June 2007

CONTENTS **Page**

This Addendum contains the evaluation of the further data submitted by the Notifier to address the data requirements identified by the RMS (UK) in the Draft Assessment Report for clofentezine dated August 2005.

B.5. METHODS OF ANALYSIS

B.5.1 Analytical methods for technical material (IIA 4.1)

This method was supplied to support the updated technical specification for clofentezine in Volume 4, Annex C (Revised June 2007).

B.5.1.1 Technical active substance (IIA 4.1)

Method A: determination of Clofentezine

Samples were dissolved in acetonitrile and quantified by RP- HPLC-UV against reference standards. Symmetry C18 3.5 µm column, 4.6 x 150mm, λ= 230 nm, isocratic mobile phase 65:35 acetonitrile: 0.2% ortho-phosphoric acid.

Validation data for the analytical method for the active substance are given in Table B.5.1.

B.5.1.2 Impurities (IIA 4.1)

Refer to confidential information – Volume 4, Annex C (Revised June 2007), Section C.1.4.1.

Table B.1 Analytical validation data for determination of clofentezine in technical material.

Matrix	Analyte	LOQ (%w/w))	Recovery fortification level (% w/w)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity	Specificity
Method A	clofentezine	-	-	-	0.9% (n= 5) using standard solution. 0.9% (n=6) for sample.	0.05 – 1.6% w/w. $R^2 \geq 0.99$	No interference shown on chromatograms. Additional PDA analysis confirmed specificity.

(Nudelman, A. 2005a and 2005b)

B.5.4 Analytical methods (residue) in human and animal tissues and fluids (IIA 4.2.5, IIIA 5.2)

B.5.4.1 Residues in animal tissues and products

Enforcement method

f) Residues of clofentezine and metabolites in animal products were analysed using the principal acid hydrolysis, extraction and clean up methods described by Manley, Peatman and Snowden as described in the previous methods. The only differences being the use of n-methyl-n-trimethylsilyltrifluroacetamide (MSTFA) [more acceptable from an occupational health point of view than diazomethane] as the derivatising agent and the resulting extract analysed by GC/MS (not GC/ECD) (monitoring for m/z = 111, 139 and 213), using an Optima-5-MS column. The supporting method validation data are shown in Table B.5.1. Representative chromatograms were submitted.
ILV data were also submitted (see Table B.5.2) for parent clofentezine and its metabolite 4-hydroxyclofentezine (residues definition).
(Witte 2004 and Chambers 2006)

Table B.5.2 Summary of method validation for residues in animal tissues/products

Compound	Substrate	Mean recovery (%)	Number of samples	Precision repeatability (%RSD)	Limit of determination
Method f)					
Clofentezine	Liver (fortification level 0.05 – 0.5 mg/kg)	100 (96-103)	10	2	0.05 mg/kg*
	Muscle (fortification level 0.02 – 0.2 mg/kg)	74 (64-89)	10	12	0.02 mg/kg*
	Fat (fortification level 0.02 – 0.2 mg/kg)	91 (65-101)	10	14	0.02 mg/kg*
	Milk (fortification level 0.01 – 0.1 mg/kg)	105 (95-118)	10	8	0.01 mg/kg*
	Eggs (fortification level 0.01 – 0.1 mg/kg)	96 (90-100)	10	3	0.01 mg/kg*
ILV					
Clofentezine	Liver (fortification level 0.05 – 0.5 mg/kg)	74 (68-82)	7	6	0.05 mg/kg*
	Kidney (fortification level 0.05 – 0.5 mg/kg)	77 (69-85)	6	7	0.05 mg/kg*
	Muscle (fortification level 0.02 – 0.2 mg/kg)	95 (92-96)	6	2	0.02 mg/kg*

Compound	Substrate	Mean recovery (%)	Number of samples	Precision repeatability (%RSD)	Limit of determination
	Fat (fortification level 0.02 – 0.2 mg/kg)	85 (70-98)	7	12	0.02 mg/kg*
	Milk (fortification level 0.01 – 0.1 mg/kg)	91 (71-107)	6	18	0.01 mg/kg*
	Eggs (fortification level 0.01 – 0.1 mg/kg)	93 (90-97)	6	3	0.01 mg/kg*
4-hydroxy-clofentezine	Liver (fortification level 0.05 – 0.5 mg/kg)	75 (66-87)	7	10	0.05 mg/kg*
	Kidney (fortification level 0.05 – 0.5 mg/kg)	84 (78-91)	4	8	0.05 mg/kg*
	Muscle (fortification level 0.02 – 0.2 mg/kg)	98 (91-102)	4	5	0.02 mg/kg*
	Fat (fortification level 0.02 – 0.2 mg/kg)	96 (80-103)	7	10	0.02 mg/kg*
	Milk (fortification level 0.01 – 0.1 mg/kg)	90 (76-111)	4	19	0.01 mg/kg*
	Eggs (fortification level 0.01 – 0.1 mg/kg)	97 (87-111)	4	11	0.01 mg/kg*

*clofentezine equivalents

B.5.5 Evaluation and assessment

Clofentezine was determined in the technical active substance by reverse phase HPLC-UV [230nm; C18 column].

Clofentezine residues in animal products were determined by hydrolysing the samples with hydrobromic acid converting clofentezine and its metabolites containing the 2-chlorophenyl moiety to 2-chlorobenzoic acid. The resulting extracts are extracted with diethyl ether, cleaned up on an anion exchange column and the resulting eluants silylated with MSTFA (addition of $Si(CH_3)_3$ to 2-chlorobenzoic acid) and analysed by GC/MS (monitoring for m/z = 111, 139 and 213), using an Optima-5-MS column. The limits of determinations were milk 0.01 mg/l, eggs 0.01 mg/kg, muscle and fat 0.02 mg/kg and liver and kidney 0.05 mg/kg. Validation and ILV data were submitted.

There are a number of issues with the above method, which question the validity of the method and its acceptance as an enforcement method;

a) The method is a common moiety method, which involves the hydrolysis of clofentezine to 2-chlorobenzoic acid. The issue here is that a number of other pesticides (i.e. clomazone, cumylone, flufenzine) contain this moiety and thus if present in the sample, would give a false positive/inflated result.
b) The use of a derivatising agent in an enforcement method is strongly discouraged. The applicant has tried to address this concern by changing the derivatising reagent from diazomethane to MSTFA, however it is difficult to understand why the HPLC-MS/MS methods use in the environment methods was not modified and employed here (applicant had already shown that HPLC-UV could be used to analyse for 4-hydroxyclofentezine in animal products).
c) The enforcement method was only validated for clofentezine, whereas the residues definition is clofentezine and its metabolite 4-hydroxyclofentezine and no validation data were submitted on kidney. However, the ILV data covered both clofentezine and 4-hydroxclofentezine and kidney, although there is an issue that the ILV data, which did not address the amount of 2-chlorobenzoic acid produce by the two components. In the case of clofentezine, the molecule contains two 2-chlorophenyl groups whereas 4-hydroxyclofentezine contains only one, with the other 2-chlorophenyl ring having an OH group in the 4 position (no indication was give as to whether this would be removed on hydrolysis, which appears unlikely). Therefore, if the OH group is not removed, the retention time may be different and the ions produced during determination by MS may also be different and as SIM is being used, would not be picked up. The result of this would be if 4-hydroxyclofentezine is present in significant amounts and the calibration is based on clofentezine, the residue in the sample would be significantly lower than the true value.

B.5.6 Conclusion

The data submitted by the applicant to addresses the outstanding data required, was correct with regards to the approach taken, however as pointed out above there are a number of major issues associated with the acceptance of the method for the purpose of enforcement and with the associated validation data. Therefore the RMS recommends that a HPLC-MS/MS is developed (along the lines of the environment methods) and validated for clofentezine and its metabolite 4-hydroxyclofentezine (including ILV data) for animal products (milk, eggs, muscle, liver, kidney and fat).

B.5.7 References relied on

Active Substance: Clofentezine

Annex point / Ref. No.	Author	Year	Title Source (where different from company) Company, Report No. GLP status, published or not	Data protection claimed Y/N	Owner*
IIA 1.11/02	Nudelman, A.	2005a	Determination of active ingredient and impurities present at or above 0.1% in five batches of technical Apollo. Analyst Research Laboratories Ltd. Report 2005-005. Irvita Plant Protection NV, Report no. R-18018. GLP. Not published.	Y	Irvita
IIA 4.1.1/07	Nudelman, A.	2005b	Technical clofentezine (Apollo). Determination of active ingredient and impurities present at or above 0.1% in technical Apollo – Method Validation. Analyst Research Laboratories Ltd Report 2004-035. Irvita Plant Protection NV, Report no. R-18017. GLP. Not published.	Y	Irvita
IIA, 4.2.1.2/08	Witte	2004	Validation of an Analytical Method for the Determination of Clofentezine and Metabolites in Animal Tissues GAB Analytik Report no. 20041042/01-RVAT. Irvita Plant Protection NV, Report no. R-17532. GLP. Unpublished.	Y	Irvita
IIA, 4.2.1.2/09	Chambers	2006	An Independent Laboratory Validation of an Analytical Method for the Determination of Clofentezine and its Metabolites in Animal Tissues Synergy Laboratories Ltd. Report no. SYN0801. Irvita Plant Protection NV, Report no. R-20408. GLP. Unpublished.	Y	Irvita

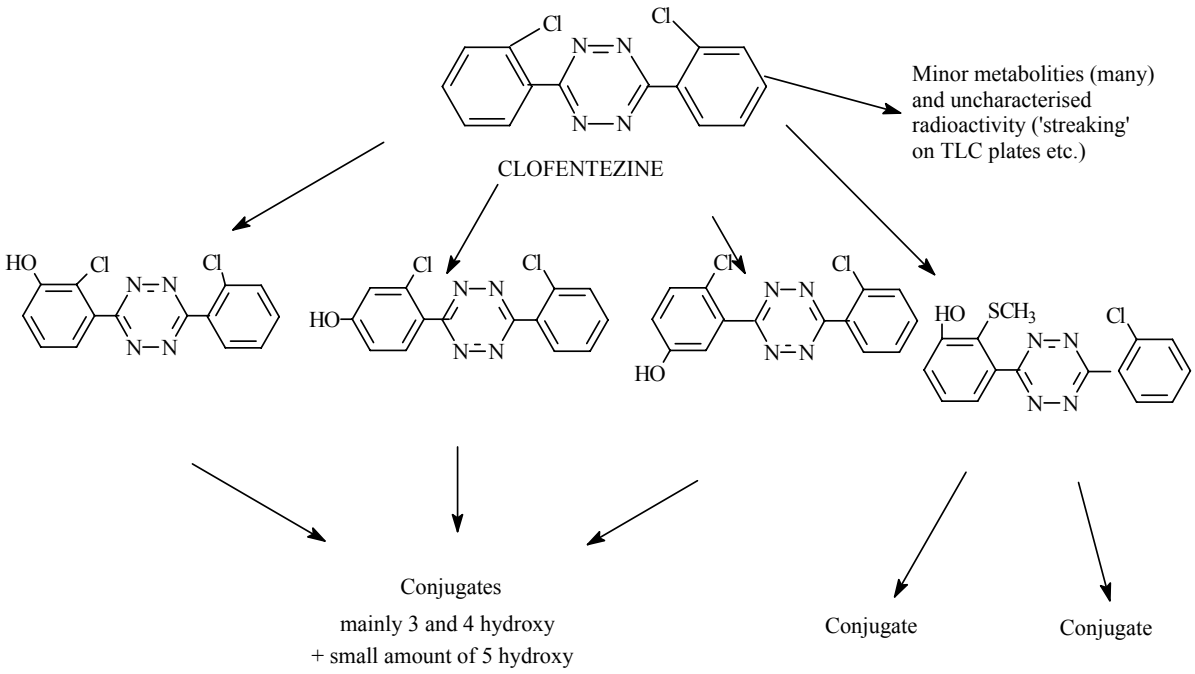
*Irvita Plant Protection, owner of the substance clofentezine, is a Member of Makhteshim-Agan Industries (MAI) group. Referenced studies refer to this ownership by either the abbreviation "MAK" or by "Irvita". As notifier, Irvita is represented in Europe by Makhteshim International Coordination Centre (MAICC), Brussels.

B.6. MAMMALIAN TOXICITY AND METABOLISM

B.6.1 Absorption distribution excretion and metabolism (toxicokinetics) (IIA 5.1)

B.6.1.3 Summary of absorption, distribution, metabolism and excretion

Figure B.6.1. Proposed metabolism of clofentezine in animals



B.6.4 Genotoxicity (IIA 5.4)

B.6.4.1 *In vitro* assays (IIA 5.4.1)

A new bacterial reverse mutation was considered necessary by the RMS due to the inadequacy of the positive controls in the original study submitted.

a. Bacterial reverse mutation

The mutagenic activity of clofentezine (Batch 0418, Purity 98.4%) was investigated in 2005 study using the Ames plate incorporation method with five strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 102, TA 98 and TA 100). The compound was dissolved in dimethyl formamide. The study met the essential requirements of OECD 471.

The dose range was determined by an initial toxicity assay, and in the first experiment was 50 to 5000 µg/plate. The experiment was repeated on a separate day using the same dose range as experiment 1, fresh cultures of the bacterial strains and fresh test materials.

Results

The vehicle control plates gave revertant colonies counts within the normal range. All of the positive controls chemicals gave marked increases in the frequency of revertant colonies, both with and without metabolic activation.

The test material cause no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was therefore tested at a maximum dose level of 5000 µg/plate. A pink patchy precipitate (fibrous in appearance) was observed ≥ 500 µg/plate although it didn't prevent scoring of revertant colonies.

No significant increases in the frequency of revertant colonies were recorded of any bacterial strains, with any test material dose level, both with and without metabolic activation.

Table B.6.1. Average number of revertant colonies per plate with five stains of *Salmonella typhimurium*

Test Sustance	**Amount / plate**	***S. typhimurium* strain (TA..) Average number of revertant colonies/plate**									
		without S-9 mix					**with S-9 mix**				
		100	**1535**	**102**	**98**	**1537**	**100**	**1535**	**102**	**98**	**1537**
positive control*	see notes	ENNG 640	ENNG 353	MNC 1281	4QO 206	9AA 402	2AA 1163	2AA 123	DAN 896	B 182	2AA 236
Clofentezine	0	99	28	239	31	13	98	10	326	26	11
	50	93	33	235	26	9	89	10	308	20	11
	150	126	32	250	21	8	97	12	319	19	8
	500	106 [p]	27 [p]	216 [p]	26 [p]	10 [p]	97	11 [p]	311 [p]	19 [p]	5 [p]
	1500	110 [p]	31 [p]	226 [p]	17 [p]	7 [p]	86	15 [p]	292 [p]	19 [p]	7 [p]
	5000	94 [p]	20 [p]	223 [p]	19 [p]	9 [p]	99	11 [p]	217 [p]	20 [p]	6 [p]

Notes
N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG): 3 µg/plate for TA100 and 5 µg/plate for TA1535
9-Aminoacridine (9AA): 80 µg/plate for TA1537
Mitomycin C (MMC): 0.5 µg/plate for TA102
4-Nitroquinoline-1-oxide (4NQO): 0.2 µg/plate for TA98
2-Aminoanthracene (2AA): 1 µg/plate for TA100, and 2 µg/plate for TA1535 and TA1537
Benzo(a)pyrene (BP): 10 µg/plate for TA102

Conclusion

Clofentezine was not mutagenic at up to 5000 µg/plate.

(Bowles, AJ. (2005))

B.6.15 References relied on

Active Substance: Clofentezine

Annex point / Ref. No.	Author	Year	Title Source (where different from company) Company, Report No. GLP status, published or not	Data protection claimed Y/N	Owner*
IIA 5.4.1/04 Vol. 1 Level 4, point 4.1.	Bowles, AJ	2005	Reverse mutation assay "Ames Test" using Salmonella Typhimurium Safepharm Laboratories Ltd. Report no. 2116/0002. Irvita Plant Protection NV, Report no. R-17812. GLP. Unpublished.	Y	Irvita

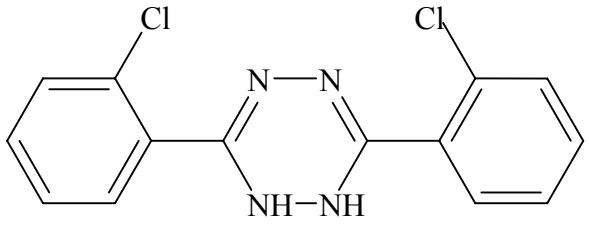
*Irvita Plant Protection, owner of the substance clofentezine, is a Member of Makhteshim-Agan Industries (MAI) group. Referenced studies refer to this ownership by either the abbreviation "MAK" or by "Irvita". As notifier, Irvita is represented in Europe by Makhteshim International Coordination Centre (MAICC), Brussels.

B.7 RESIDUES DATA

B.7.1 Metabolism, distribution and expression of residues in plants (IIA 6.1, IIIA 8.1)

B.7.1.5 Summary/assessment

Fig. B.7.1 Metabolite NC22505



B.9 ECOTOXICOLOGY

Background information

Clofentezine is a selective mite ovicide formulated as Apollo 50 SC, a suspension concentrate containing 500 g/L active substance. The intended uses are pome fruit (apples and pears), stone fruit (plums), strawberries, grapes and roses (ornamentals). Application rates and timings are as outlined in Table B.9.0.1.

Table B.9.0.1 Intended uses of clofentezine

Crop	Max application rate (g a.s./ha)*	Application timings (BBCH growth stages)
Pome fruit (Apples and Pears)	200	Growth stage 08-56
Stone Fruit (Plums)	200	Growth stage 08-75
Strawberries**	200	At occurrence of pest
Grapes	150	Growth stage 11-75
Roses (Ornamentals)**	200	At occurrence of pest

* The maximum number of applications is one.

** Crop and hence use can be either in the field or under glass, however use considered in this assessment is field grown strawberries

B.9.1 Effects on birds

Background

When clofentezine was originally assessed a 'safe use' was identified regarding the acute and short-term risk to birds; however there was still concern regarding the long-term risk. The Notifier had submitted additional data to try and address this concern, however much of the information was qualitative in nature and hence was not sufficiently robust to permit refinements of PT (i.e. proportion of diet obtain from the treated area) or PD (proportion of food types obtained from the treated area). The Notifier has now submitted data to try and address these shortcomings. Presented below are summaries of the studies followed by a detailed risk assessment. It should be noted that the data submitted only cover the long-term risk to birds from the use of clofentezine on **pome and stone fruit and strawberries**. The following also addresses the following points in the reporting table: 5.4, 5.5 and 5.6 (see rev 0 (24 October 2006)).

Data evaluation

Summary of avian feeding ecology study in orchards in southwest France and North Italy.

Report	Schwarz, J. (2006): Generic field monitoring of birds in orchards RIFCon GmbH, unpublished Report No.: RA06016-1 Date: 22 December 2006	
Guideline	Not applicable; the test was especially designed for the purpose of this study.	
GLP	Yes	
Dates of work	Start of experimental phase	21 June 2006
	Completion of experimental phase	08 November 2006

Aim:

The aim of this generic study was to obtain refined dietary estimates for individual wild birds living within or in the close vicinity of pome fruit orchards, for use as revised input data for the exposure and risk assessment.

Objectives:

Habitat choice and determination of the proportion of diet obtained in pome fruit orchards (PT estimate) by radio-tracking individual birds (including visual observations). Food composition and determination of the proportion of different food types in the diet (PD estimates) by analysing content of faeces and/or stomach

Study area:

The study was conducted in the areas of Montauban, France and Zevio (near Verona), Italy. There were three orchards in France and six in Italy and were stated to be representative in terms of size and structure of orchards in that region. The study authors stated that the chosen areas reflect typical pome fruit distribution patterns for Southern Europe.

Methods:

Bird trapping and tagging

The radio-tracking study was carried out during summer 2006 (tracking period 28th June to 13th August 2006). Bird trapping was conducted between 21st June and 6th July in France and 16th July and 12th August in Italy. In order to increase the probability of capturing a bird, trapping was conducted during those times of the day when bird activity was at a peak

(morning or evening hours). If a bird already tagged for this study was recaptured, it was released.

Bird captures were done using mist nets which were set up within the pome fruit orchard. The site selection for setting the nets was based on observation of the presence of birds and the suitability for the net construction.

All captured individuals of the occurring species (France: blackbird, great tit, song thrush, tree sparrow, greenfinch, European robin and melodious warbler; Italy: blackbird, great tit, tree sparrow, greenfinch and blackcap) were tagged with a radio tag and marked with colour rings (on the left and right tarsometatarsus). The unique combination of the colour rings permitted recognition of individuals by visual contact during the tracking sessions of the birds.

Trapped individuals were equipped with a telemetry transmitter which was mounted on their backs. Birds were equipped with tags in such a way that the weight of the tag did not exceed 5% of the bird's body mass. In order to detect any malfunction the transmitters were tested prior to tagging the birds.

Radiotracking

Once the birds had been tagged, they were then 'radio-tracked'. The radio-tracking served two purposes: firstly, to locate the bird in order to observe it ('radio surveillance') and secondly, to continuously follow the bird over a defined period (see below) and to determine every site and behavioural change ('continuous monitoring').

Each tracking session lasted for a whole activity period from dawn to dusk (minimum of 13 hours 5 minutes to maximum 17 hours and 14 minutes, mean 16 hours). During this time the bird was tracked continuously, i.e. the bird was followed non-stop by car or by walking. Every change in behaviour and location (habitat and position) was recorded to the minute.

The use of unidirectional Yagi-antennas made it possible to determine the direction where the tracked individuals were situated. The signal strength permitted an estimation of the distance to the bird. The position was determined by cross bearing and a map-grid-system with a resolution of 50 x 50 m or with a position determination by GPS (Garmin) if a bird left the mapped area. The map grid system was based on aerial photographs of the corresponding region and a grid system generated by using a computer-based geo-information system (GIS) (ArcView 9.1).

In order to describe the behaviour of the tracked bird as accurately as possible and to verify its location, the aim was to keep the bird under observation ('visual contact') with optical devices (scope, binoculars). Recognition of the colour ring combination provided verification that the bird had been accurately identified. However, to ensure that the normal

behaviour of the bird was not affected by the tracker a ‘safety margin’ of 20 m was maintained.

In order to describe the home range used by the birds during each tracking session the ‘minimum convex polygon’ (MCP, Mohr 1947) was applied. This is the area of a *convex* polygon plotted around all locations (in the present case ‘map grids’) the bird used during one tracking session. Every habitat inside the polygon is regarded as being part of the home range. A computer based geo information system (GIS) (ArcView 9.1) was applied for the calculation of the home range sizes.

Every tagged bird was tracked for one tracking session (one daylight period). One blackbird in Italy was tracked twice. The second tracking session was excluded from the analysis. Four tracking sessions (one blackbird, two tree sparrows and one greenfinch) had to be cancelled, because the birds were lost. The data of these incomplete tracking sessions were discarded. One blackcap was not found in commercial pome fruit orchards during interim telemetry checks and during the PT-telemetry. Therefore, the bird was not considered suitable for the PT telemetry study.

Faeces and stomach flushing

A total of 56 faecal and stomach flushing samples were obtained from 53 birds. There were 47 faeces samples and 9 flushing samples resulting sample was then preserved and analysed.

Calculation of PT (proportion of food obtained from the treated area)

For the calculation of PT values it was assumed that a bird takes up the same amount of diet in a pome fruit orchard as it does in the remainder of its home range if the foraging periods are of equal length. Therefore, 'proportion of time potentially foraging' is equal to 'proportion of diet obtained’.

The proportion of diet obtained in pome fruit orchards (PT) by the individual birds was calculated for each of the conducted telemetry sessions as the proportion of time the individual birds spent ‘potentially foraging’ in the pome fruit orchards. The 'time potentially foraging' summarises the time a bird spends for activities classified as 'foraging/feeding', 'feeding young', 'active (details unknown)' and 'behaviour n.s.'. It excludes all instances where the animal was known to be performing any other activity (e.g. reproductive activities like singing, nest building, fighting etc.) or where it was considered to be inactive.

The PT can be calculated based on ‘time potentially foraging‘. The ‘time potentially foraging’ within pome fruit orchards was compared with the total recorded ‘time potentially foraging’ across all habitats for each tracking session (see below).

It should be noted that during some telemetry sessions, the bird's location (i.e. whether the bird was in the pome fruit orchard or not) could not be determined continuously and, therefore, the time period without information on location was recorded as habitat category 'unknown'. Such periods were in most cases rather short (mean 16.6, SD = 16.7 minutes) and were excluded from the data evaluation of the respective tracking session.

It is postulated that the likelihood of determination a bird's position is identical for the different habitat types in an agrarian landscape. Consequently, time periods recorded as habitat 'unknown' are disregarded in the data evaluation since this is not expected to result in data bias.

Definition of behaviour categories (used for calculation of PT)

- foraging/feeding: bird classified as active by radio-tracking signal and visually observed while searching for food
- feeding young: bird classified as active by radio-tracking signal and visually observed while feeding hatchlings
- active (details unknown): bird classified as active by radio-tracking signal but without further details on the purpose of activity
- reproductive behaviour: bird classified as active by radio-tracking signal and visually observed carrying out behavioural elements that are part of reproduction (singing, fighting, breeding …)
- inactive: bird classified as inactive (not moving) by radio-tracking signal and/or by visual contact (thus, foraging can be excluded)
- behaviour not specified: behaviour cannot be specified due to ambiguous signal. This category specifies time periods during which the behaviour of the birds could not be assessed either by interpreting the strength of the radio signals or by visual observations. However it was still clearly possible to determine the location of the bird within the study area.

Scheme for calculating PT

As outlined above, PT can be calculated by comparing the 'time potentially foraging' within pome fruit orchards for each telemetry session with the total recorded 'time potentially foraging' over all habitats for each telemetry session. Since a conservative approach is taken for this study, the 'time potentially foraging' includes the behaviour categories 'foraging/feeding', 'feeding young ', ' active (details unknown)' and 'behaviour n.s.'. In the following, the calculation of PT is shown as an example.

1) Total time a bird is present in all known habitats including pome fruit orchards during an individual tracking session:

Behavioural category	**Duration**	**Sum**
foraging/feeding	1 h	potentially foraging: 9 h
feeding young	1 h	
behaviour n.s.	1.5 h	
active (details unknown)	5.5 h	
reproductive behaviour	2 h	time when foraging behaviour can be excluded: 6 h
inactive	4 h	
total time in all known habitats	15 h	

This results in a 'time potentially foraging' of 9 h for all known habitats.

2) Total time a bird is present in pome fruit orchards during an individual tracking session:

Behavioural category	**Duration**	**Sum**
foraging/feeding	0.5 h	potentially foraging: 4 h
feeding young	0.5 h	
behaviour n.s.	0.5 h	
active (details unknown)	2.5 h	
reproductive behaviour	0 h	time when foraging behaviour can be excluded: 2 h
inactive	2 h	
total time in all known habitats	6 h	

This results in a 'time potentially foraging' for pome fruit orchards of 4 h.

Individual PT calculated as

$$\frac{Potentially\ foraging\ time\ in\ pome\ fruit\ orchards}{Potentially\ foraging\ time\ in\ all\ known\ habitats} = \frac{4\,h}{9\,h} = 0.44$$

Calculation of PD (Proportion of food types in the diet)

Sample analysis

The analytical results obtained for the composition of faeces and stomach content for each individual sample, were pooled to calculate PD values.

Microscopic observations (max. magnification x 400) were used to assign the remains found in the samples to the potential prey or plant ingested by the bird. This allowed the determination of the composition of the diet. In terms of a systematic classification, insect remains could be assigned to the

order. The remains of other invertebrates could mostly be assigned to a class. Seed remains and plant parts were determined as accurately as possible. For the determination of the green plant material, structures of cuticle – particularly stomata – were considered. The determination of seeds was done by analysing husk remains.

The size of characteristic parts of invertebrates and plants (e.g. chitin fragments of arthropods, bristles of earthworms, fragments of seeds, plant material) were measured with a measuring ocular with accuracy of 0.1 mm. The measures were compared to specimens from a reference library and resultant body length was estimated.

Plant material other than seeds could not be quantified in terms of dry or fresh weight proportions, because its more or less amorphous consistency does not allow counting and measuring of food snatches and the calculation of numbers of ingested fruits or leaves. On the other hand the water content of this food type is generally very high, hence, the dry-weight fraction is assumed to be low. Therefore, it was considered, by the study authors, to be negligible and hence excluded from the PD-calculation which uses the dry-weight proportion of the different food types. The number of seeds was obtained by measuring the area of the fragments and dividing this figure by the area of a reference fruit or seed.

In order to quantify the number of invertebrates within the samples the minimum number of individuals required to account for the fragments of each prey type was calculated.

The proportions of different food types ingested by the birds were calculated from their remains found in stomach flushing and faeces samples. For this purpose the following conversions were conducted.

Conversion of the number of individuals in the diet samples to their portion of dry weight of the diet actually ingested (PD)

Different food types are recognised in diet samples in different proportions from those in which they were ingested. Possible reasons are differential passage or digestion times and a differential fragmentation and identification.

In order to address this issue, specific correction factors derived from the open literature were used (see Tables B.9.1.2 and B.9.1.3) were applied to calculate the number of different food types actually ingested by the birds from the number found in the diet samples. From the literature, for example, it can be deduced that the number of Araneida (spiders) ingested is 3.9 times the number subsequently found in the birds' faeces. The number originally eaten by the birds was derived by applying the correction factors to the different invertebrate taxa and plant. Correction factors were applied to the pooled results of the species-specific stomach and faeces samples.

This led to the numerical proportion of different food types in the diet actually ingested by the birds. In order to calculate their mass proportions, the length-weight regressions derived from the literature (Tables B.9.1.4 and B.9.1.5) were used for different invertebrate taxa and plant seeds. Hence, from the length estimations of the food items found in the diet samples their approximate dry weight was calculated.

Table B.9.1.2: Correction factors applied to convert the number of invertebrate items found in the faeces samples to the number of items actually ingested by the bird

Taxonomic group	Factor	Source
Acarina	3.9216	applied after Jenni et al. 1990 as a result of physique analogy
Araneida	3.9216	Jenni et al. 1990
Blattodea	4.2194	applied after Jenni et al. 1990 as a result of physique analogy
Coleoptera	2.445	Jenni et al. 1990
Coleoptera larvae	3.9216	applied after Jenni et al. 1990 according to Green 1984
Dermaptera	2.445	applied after Jenni et al. 1990 as a result of physique analogy
Diptera	3.0581	Jenni et al. 1990
Diptera larvae	3.9216	applied after Jenni et al. 1990 as a result of physique analogy
Gastropoda	2.445	applied after Jenni et al. 1990 as a result of physique analogy
Hemiptera	4.2194	Jenni et al. 1990
Hymenoptera	3.0581	applied after Jenni et al. 1990 according to Green 1984
Lepidoptera	3.9216	applied after Jenni et al. 1990 according to Green 1984
Lepidoptera larvae	3.9216	applied after Jenni et al. 1990 as a result of physique analogy
Lumbricidae	4.4643	Green and Tyler 1989
Myriapoda	4.2194	applied after Jenni et al. 1990 as a result of physique analogy
Neuroptera	3.0581	applied after Jenni et al. 1990 as a result of physique analogy
Opilionida	3.9216	applied after Jenni et al. 1990 as a result of physique analogy
Orthoptera	2.445	applied after Jenni et al. 1990 as a result of physique analogy

Table B.9.1.3: Correction factors applied to convert the number of invertebrate items found in the stomach flushing samples to the number of items actually ingested by the bird

Taxonomic group	Factor	Source
Aphidoidea	38.4615	Jenni et al. 1990
Araneida	5.6818	Jenni et al. 1990
Coleoptera	1.9268	Jenni et al. 1990
Coleoptera larvae	5.6818	applied after Jenni et al. 1990 as a result of physique analogy
Diptera	3.1546	Jenni et al. 1990
Hemiptera	3.9526	Jenni et al. 1990
Hymenoptera	3.1546	applied after Jenni et al. 1990 according to Green 1984
Lepidoptera	5.6818	applied after Jenni et al. 1990 as a result of physique analogy
Lepidoptera larvae	5.6818	applied after Jenni et al. 1990 as a result of physique analogy
Lumbricidae	5.6818	applied after Jenni et al. 1990 as a result of physique analogy
Symphyta larvae	5.6818	applied after Jenni et al. 1990 as a result of physique analogy

Table B.9.1.4: Weight-length regression for estimation of arthropod dry weights (W: weight [mg], L: length [mm]):

Taxonomic group	Regression	Source
Insects adult	$W = 0.0305L^{2.62}$	Rogers et al. 1976
Araneida	$W = 0.076L^{2.245}$	Henschel et al. 1996
Coleoptera larvae	$W = e^{-5.909}L^{3.122}$	Sample et al. 1993
Diptera larvae	$W = e^{-4.486}L^{2.816}$	applied after Sample et al. 1993 as a result of physique analogy
Gastropoda	$\ln(W) = 0.969 + 0.529\ln(L)$	Collins 1992
Lepidoptera larvae	$W = e^{-5.909}L^{3.122}$	Sample et al. 1993
Lumbricidae	$\ln(W) = 2.394 + 0.373\ln(L)$	Collins 1992
Myriapoda	$W = 0.002L^{2.9277}$	applied after Lang et al. 1997 as a result of physique analogy
Opilionida	$W = 0.058L^{2.559}$	Henschel et al. 1996
Symphyta larvae	$W = e^{-4.486}L^{2.816}$	applied after Sample et al. 1993 as a result of physique analogy

Table B.9.1.5: Correction factors to convert the number of seeds found in the diet samples to their dry weight proportion of the actually ingested diet

Group	Factor, regression	Source
Correction factors for the number of digested seeds identified in faeces:		
all seeds	2.004	applied after Jenni et al. 1990 as a result of morphological analogy
Correction factors fort the number of digested seeds identified in stomach flushings:		
all seeds	5.7143	applied after Jenni et al. 1990 as a result of morphological analogy
Weight-length regression for estimation of seed weights (W: weight [mg], L: length [mm]):		
cereal seeds	mean seed weight = 43.75 mg	data for *Triticum aestivum* taken from Klotz et al. 2002
Grossulariaceae	$W = 29.831L - 70.375$	calculated on the base of data taken from Klotz et al. 2002
Poaceae	$W = 0.2673L^{1.8672}$	calculated on the base of data taken from Klotz et al. 2002
Ranunculaceae	W = 0.1766L2.0641	calculated on the base of data taken from Klotz et al. 2002
other seeds	$W = 0.3652L^{1.7646}$	calculated on the base of data taken from Klotz et al. 2002

Preference

An assessment of preference was carried out to try and quantify habitat preferences of the species of concern. The preference indicator relates the PT in pome fruit of each bird to the spatial portion of pome fruit within the home range during the respective tracking session. The analysis of the birds' preference for feeding habitats was done by using the Jacobs' preference index [D] (Jacobs 1974). The Jacobs' index [D] was calculated as:

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

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

The index value for [D] ranges from -1 to 0 for a negative choice and from 0 to +1 for a positive choice ('-1': complete avoidance; '+1': exclusive preference; '0': neutral: no avoidance or preference, i.e. a proportional use of the habitat in relation to its proportion within the home range).

Results

PT – proportion of food obtain in treated area

During the study 36 individuals of eight species were radio-tracked: three blackcaps (*Sylvia atricapilla*), one European robin (*Erithacus rubecula*), seven great tits (*Parus major*), one melodious warbler (*Hippolais polyglotta*), twelve blackbirds (*Turdus merula*), two song thrushes (*Turdus philomelos*), four greenfinches (*Carduelis chloris*) and six tree sparrows (*Passer montanus*).

One blackbird, one blackcap and two tree sparrows left the study area during the tracking session. Thus, the tracking periods for these birds were less than eight hours long and therefore considered by the study authors to be too short to be included in the calculation.

In total, data of 33 tracking sessions were incorporated in the calculation. It should be noted that not all the birds tracked were 'consumers', i.e. some birds did not spend any time in the crop of concern. However, all individuals monitored during this study were trapped in pome fruit orchards, which indicates, that the birds could have used pome fruit orchards as a foraging habitat.

Presented in Table B.9.1.6 are the PT values for all species.

Table B.9.1.6: PT values for birds trapped and subsequently radiotracked in pome fruit orchards in France and Italy.

Potentially foraging							
Individual No.	Behaviour in all known habitats [%]			Behaviour in pome fruit orchards [%]			PT in pome fruit orchards
	All behavioural categories in all known habitats	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	All behavioural categories in orchards	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	
Blackcap							
I14	100.0	11.8	88.2	50.0	0.0	50.0	0.57
I08	100.0	10.6	89.4	25.0	6.7	18.4	0.21
I16	100.0	11.8	88.2	0.0	0.0	0.0	0.00
Great tit							
F09	100.0	14.6	85.4	75.3	11.7	63.7	0.75
F14	100.0	25.2	74.8	69.1	17.5	51.6	0.69
I03	100.0	20.9	79.1	56.9	11.7	45.2	0.57
F13	100.0	1.9	98.1	48.3	0.0	48.3	0.49
F08	100.0	8.1	91.9	18.9	0.2	18.7	0.20
I07	100.0	21.1	78.9	1.7	0.0	1.7	0.02
I05	100.0	21.5	78.5	1.1	0.0	1.1	0.01
Blackbird							
I01	100.0	10.1	89.9	100.0	10.1	89.9	1.00
I02	100.0	30.4	69.6	93.5	26.5	67.0	0.96
I11	100.0	21.4	78.6	90.2	21.4	68.8	0.88
F05	100.0	18.5	81.5	31.2	3.6	27.6	0.34
F07	100.0	5.0	95.0	24.9	0.0	24.9	0.26
F04	100.0	74.3	25.7	4.8	1.1	3.7	0.15
F19	100.0	26.7	73.3	4.2	0.0	4.2	0.06
F01	100.0	7.3	92.7	9.0	4.3	4.7	0.05
F02	100.0	7.0	93.0	1.9	0.0	1.9	0.02
F18	100.0	30.7	69.3	0.0	0.0	0.0	0.00
I12	100.0	7.5	92.5	0.0	0.0	0.0	0.00
Tree sparrow							

Potentially foraging							
Individual No.	Behaviour in all known habitats [%]			Behaviour in pome fruit orchards [%]			PT in pome fruit orchards
	All behavioural categories in all known habitats	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	All behavioural categories in orchards	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	
I18	100.0	10.5	89.5	3.5	2.6	0.9	0.01
I04	100.0	30.4	69.6	27.1	1.8	25.3	0.36
I06	100.0	14.2	85.8	64.1	1.7	62.4	0.73
I09	100.0	11.0	89.0	0.0	0.0	0.0	0.00
Greenfinch							
I10	100.0	11.0	89.0	89.2	11.0	78.2	0.88
I13	100.0	15.0	85.0	93.4	13.8	79.5	0.94
F06	100.0	26.0	74.0	75.8	20.1	55.7	0.75
F15	100.0	32.5	67.5	19.1	6.4	12.7	0.19
Song thrush							
F11	100.0	7.3	92.7	22.0	0.0	22.0	0.24
F12	100.0	16.4	83.6	77.8	13.4	64.4	0.77
Robin							
F17	100.0	2.2	97.8	3.7	0.0	3.7	0.04
Melodious warbler							
F03	100.0	25.2	74.8	5.1	1.7	3.4	0.05

1) All other activities (except those named in foraging, reproductive behaviour and feeding young), 'inactive (resting, preening …)' and 'reproductive behaviour (singing, fighting, breeding, ..)' are combined into 'other behaviour categories (not foraging)'

2) 'foraging/feeding', 'feeding young', 'active (details unknown)' and 'behaviour n.s.' are combined into 'potentially foraging'

From the individual PT values presented in Table B.9.1.6, PT values were calculated for Great tits, Blackbirds, Tree Sparrows and Greenfinch. The 50th percentile and the 90th percentile are presented in Table B.9.1.7.

Table B.9.1.7: 50th and 90th percentile PT values for Great tits, Blackbirds, Tree Sparrows and Greenfinches.

Potentially foraging						
	Behaviour in all known habitats [%]		Behaviour in pome fruit orchards [%]			PT in orchards
	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	All behavioural categories in orchards	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	
Great tit (n = 7)						
50% tile	20.9	79.1	48.3	0.2	45.2	0.49
90% tile	23.0	94.4	71.6	14.0	56.4	0.71
mean	16.2	83.8	38.8	5.9	32.9	0.39
SD	7.8	7.8	28.9	6.9	23.5	0.29
Blackbirds (n = 11)						
50% tile	18.5	81.5	9.0	1.1	4.7	0.15
90% tile	30.7	93.0	93.5	21.4	68.8	0.96
mean	21.7	78.3	32.7	6.1	26.6	0.34
SD	19.1	19.1	39.1	9.0	31.6	0.39
Tree sparrow (n=4)						
50% tile	12.6	87.4	15.3	1.7	13.1	0.19
90% tile	25.5	89.4	53.0	2.4	51.3	0.62
Mean	16.5	83.5	23.7	1.5	22.1	0.28
SD	8.1	8.1	25.6	1.0	25.4	0.30
Greenfinch (n = 4)						
50% tile	20.5	79.5	82.2	12.4	67.0	0.82
90% tile	30.6	87.8	92.1	18.2	79.1	0.92
mean	21.1	78.9	69.4	12.8	56.5	0.69
SD	8.6	8.6	29.7	5.0	27.0	0.30

[1] All other activities (except those named in foraging, reproductive behaviour and feeding young), 'inactive (resting, preening …)' and 'reproductive behaviour (singing, fighting, breeding, ..)' are combined into 'other behaviour categories (not foraging)'

[2] 'foraging/feeding', 'feeding young', 'active (details unknown)' and 'behaviour n.s.' are combined into 'potentially foraging'

It should be noted that in calculating the above PT values both consumers and non-consumers have been considered. Data from two MS have also been combined. It should further be noted that there has been no consideration of the uncertainty surrounding these values and hence the importance of small datasets.

Data were obtained for Song Thrush (*Turdus philomelos*), Robin (*Erithacus rubecula*) and melodius warbler (*Hippolais polyglotta*). The results are presented in Table B.9.1.8.

Table B.9.1.8: PT in pome fruit orchards of two song thrushes, one European robin, and one melodious warbler

Potentially foraging								
Individual No.	Species	Behaviour in all known habitats [%]			Behaviour in pome fruit orchards [%]			PT in pome fruit orchards
		All behavioural categories in all known habitats	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	All behavioural categories in orchards	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	
F11	Song thrush	100.0	7.3	92.7	22.0	0.0	22.0	0.24
F12	Song thrush	100.0	16.4	83.6	77.8	13.4	64.4	0.77
F17	European robin	100.0	2.2	97.8	3.7	0.0	3.7	0.04
F03	Melodious warbler	100.0	25.2	74.8	5.1	1.7	3.4	0.05

1) All other activities (except those named in foraging, reproductive behaviour and feeding young), 'inactive (resting, preening …)' and 'reproductive behaviour (singing, fighting, breeding, ..)' are combined into 'other behaviour categories (not foraging)'

2) 'foraging/feeding', 'feeding young', 'active (details unknown)' and 'behaviour n.s.' are combined into 'potentially foraging'

PD –proportion of different food types obtained from treated area

Results

Great tit

A total of 8 diet samples were obtained, four faeces samples and four stomach flushing. The results were pooled and 57 quantifiable food items

were detected: 49 individuals of twelve different invertebrate taxon groups and eight seeds of at least one plant family were identified. Within the invertebrates, Lepidoptera larvae was the most numerous taxon (21.2% of individual invertebrates by number) followed by Aphidoidea (13.3%), Araneida (10.0%) and Hemiptera (10.0%). Various seeds contributed 15.8% of the total number of items ingested. With regard to the dry weight, Lepidoptera larvae constitute the most important food item (67.8%) followed by Lepidoptera (7.0%) and Hymenoptera (5.5%). All other groups contributed less than 5% of the total dry weight of the diet ingested by great tits. The detailed results are presented in Table B.9.1.9.

Table B.9.1.9: Proportion of different food items contributing to the diet of Great tits (n = 8)

Taxon	Numerical proportion [%]	Dry weight proportion [%] PD
Arthropoda		
Lepidoptera larvae	21.2	67.8
Lepidoptera	5.3	7.0
Hymenoptera	8.5	5.5
Symphyta larvae	5.9	4.5
Araneida	10.0	4.3
Hemiptera	10.0	4.1
Coleoptera	3.2	3.4
Opilionida	1.4	0.5
Coleoptera larvae	2.0	0.4
Aphidoidea	13.3	0.3
Diptera larvae	1.4	0.3
Diptera	2.2	0.1
Subtotal	**84.2**	**98.1**
Plant matter		
other seeds	15.8	1.9
Subtotal	**15.8**	**1.9**
Total	**100.0**	**100.0**

Blackbird

In total, 19 diet samples were obtained consisting of 16 faeces samples and three stomach flushing samples. Within the pooled samples 105 quantifiable food items were detected: 83 items of 14 different invertebrate taxon groups and 22 seeds of at least three different plant families were identified.

Invertebrates contribute 86.6% and plant seeds 13.4% to the number of all diet items ingested by the blackbirds. The total dry weight was composed of 89.9% invertebrates and 10.2% plant seeds. Within the invertebrates earthworms (Lumbricidae) was the most numerous taxon (22.1% of individual invertebrates by number) followed by Hymenoptera (17.8%) and Coleoptera (15.0%). Various seeds contributed to 13.4% of the total number of items ingested. With regard to the dry weight earthworms (Lumbricidae) constituted the most important food item (53.0%) followed by Lepidoptera larvae (15.4%) various seeds (10.2%) and Coleoptera (9.0%). All other groups contribute less than 5% to the total dry weight of the diet ingested by the blackbirds. The results are presented below in Table B. 9.1.10.

Table B.9.1.10: Proportions of different food items contributing to the diet of blackbirds (n = 19, incl. 1 non-GLP)

Taxon	Numerical proportion [%]	Dry weight proportion [%] PD
Arthropoda		
Lepidoptera larvae	7.7	15.4
Coleoptera	15.0	9.0
Hymenoptera	17.8	3.2
Neuroptera	1.9	2.1
Hemiptera	6.3	1.8
Araneida	4.8	1.6
Blattodea	1.3	1.4
Dermaptera	0.7	0.8
Coleoptera larvae	1.2	0.8
Diptera	0.9	0.3
Gastropoda	0.7	0.3
Myriapoda	1.3	0.1
Acarina	4.8	0.0
Subtotal	**64.4**	**36.8**
Lumbricidae	22.1	53.0
Gastropoda	0.7	0.3
Plant matter		
Grossulariaceae seeds	4.9	9.1
other seeds	6.1	0.9

Taxon	Numerical proportion [%]	Dry weight proportion [%] PD
Poaceae seeds	1.8	0.2
Ranunculaceae seeds	0.6	0.0
Subtotal	**13.4**	**10.2**
Total	**100.0**	**100.0**

Tree sparrow

In total, eleven faeces samples were obtained. Within the pooled samples 124 quantifiable food items were detected: 68 individuals of eight different invertebrate taxon groups and 56 seeds of at least eight plant families were identified. Invertebrates contributed 68.1% and plant seeds 31.9% to the number of all diet items ingested by the Tree sparrows. The total dry weight was composed of 55.0% invertebrates and 45.0% plant seeds. Within the invertebrates Hemiptera was the most numerous taxon (31.7% of individual invertebrates by number) followed by Hymenoptera (22.6%) and Coleoptera (4.7%). Various seeds contributed to 26.8% of the total number of items ingested, followed by cereal seeds (3.4%). With regard to the dry weight cereal seeds constituted the most important food item (33.3%) followed by Lepidoptera larvae (23.3%), other seeds (9.9%), Hymenoptera (9.6%) and Orthoptera (9.2%). All other groups contributed less than 8% to the total dry weight of the diet ingested by the tree sparrow. The results are presented in Table B.9.1.11 below

Table B.9.1.11: Proportion of different food items contributing to the diet of Tree sparrows (n = 11)

Taxon	Numerical proportion [%]	Dry weight proportion [%] PD
Arthropoda		
Lepidoptera larvae	3.3	23.2
Hymenoptera	22.3	9.6
Orthoptera	0.7	9.2
Hemiptera	30.8	7.4
Coleoptera	4.1	3.7
Diptera	1.7	1.6
Araneida	1.1	0.2
Acarina	3.3	0.0
Subtotal	**67.4**	**54.9**

Taxon	Numerical proportion [%]	Dry weight proportion [%] PD
Plant matter		
Poaceae seeds (cereal)	3.4	33.2
other seeds	27.6	10.0
Poaceae seeds	1.7	1.9
Subtotal	**32.6**	**45.1**
Total	**100.0**	**100.0**

Due to small sample size no diet data were obtained for the greenfinch or blackcap.

Preference indicators

Blackcap – the home range of the three blackcaps studied ranged from 2.3 to 6.5 ha. Pome fruit was present in all home ranges. The proportion of pome fruit varied from 13.8% to 55.8%. Jacobs index varied from -1.00 to 0.24. (It should be noted that the bird with an index of -1.00 was not observed feeding in pome fruit, i.e. it was not a 'consumer'.)

Great tit – the home range of the seven Great tits ranged from 3.9 to 20.7 ha. Pome fruit orchards were part of all home ranges. The proportion of pome fruit orchards in the home range varied from 12.6% to 76.3%; the mean was 38.0%. The calculated Jacobs' index [D] for pome fruit orchards ranged from -0.95 to 0.81. The mean Jacobs' index was [D] = -0.11, meaning that pome fruit orchards were not positively selected as a feeding habitat by the radio-tracked Great tits in comparison to other available habitats within their home ranges.

Blackbird – the home range of eleven Blackbirds ranged from 1.6 ha to 17.8 ha. The proportion of pome fruit orchards in the home range varied from 0.0% to 85.4%; the mean was 35.9%. The calculated Jacobs' index [D] for pome fruit orchards ranged from -1.00 to 1.00. The mean Jacobs' index was [D] = -0.15. This means that on average pome fruit orchards were not positively selected by Blackbirds during their tracking sessions. However, the blackbirds differed markedly from one session to another (SD = 0.68).

Tree sparrow – The home range of four Tree sparrows ranged from 7.3 ha to 19.6 ha. The mean home range size was 11.5 ha. The proportion of pome fruit orchards in the home range varied from 0.0% to 58.1%; the mean was 30.2%. The calculated Jacobs' index for pome fruit orchards ranged from -0.72 to 0.34. The mean Jacobs' index was = -0.27. This means that pome fruit orchards were on average avoided as a feeding habitat by the radio-tracked Tree sparrows in comparison to other available habitats within their home ranges. One individual totally avoided pome

fruit orchards during its tracking session. However, the tree sparrows differed markedly among the sessions (SD = 0.44).

Greenfinch – The home range of the three greenfinches ranged from 118.0 ha to 8.9 ha. The proportion of pome fruit orchards in the home range varied from 26.3% to 56.2%. The calculated Jacobs' index for pome fruit orchards ranged from -0.69 to 0.92. Two of three Greenfinches preferred pome fruit orchards as a feeding habitat in comparison to other available habitats during their tracking sessions.

Song thrush, Robin and Melodius warbler – the home range of the two song thrushes was 3.6 and 16.9 ha. The proportion of pome fruit orchards in the home range varied from 1.0% to 2.5%. The calculated Jacobs' index for pome fruit orchards was 0.28 and 0.79. This means that pome fruit orchards were preferred as a feeding habitat by the two radio-tracked song thrushes in comparison to other available habitats during their tracking sessions. The home range size of the Robin was 1.6 ha, that of the Melodious warbler was 4.9 ha. The proportion of pome fruit orchards in the home range was 32.5% (Robin) and 59.8% (Melodious warbler). The calculated Jacobs' index [D] for pome fruit orchards was -0.85 for the Robin and -0.94 for the Melodious warbler. This means that pome fruit orchards were avoided as a feeding habitat by both individuals in comparison to other available habitats during their tracking sessions.

(Schwarz (2006))

Summary of a preliminary study to determine potential focal species in strawberry fields in Germany

Report	Riffel, M. & Gießing, B. 2005. Bird species in German strawberry fields – a preliminary survey RIFCON GmbH Hirschberg, unpubl. report no. RC05-002
Guideline	Bibby *et al*. 1992. Bird census techniques. Academic Press
GLP	**No**
Dates of work	Start of experimental work 26.06.2005 Completion of experimental work 05.07.2005

Material and methods:

This generic field study was performed to evaluate which bird species use German strawberry fields between late June and the beginning of July. Twenty strawberry fields in Germany were chosen in three German states, Baden-Wuerttemberg (Rhine valley,10 fields), North-Rhine- Westphalia (Rhineland close to Cologne, 7 fields) and Saxony-Anhalt (close to Madgeburg, 3 fields). To record the bird community, strawberry fields were visited once between the end of June and the first week of July. Contacts of

birds using strawberry fields were assessed using the point count transect method with an observation period of 15 minutes per field. Details of each visit were documented on a form. The data were analysed statistically to derive the endpoints. Species observed outside the field or flying over the field were not included in the analysis.

Endpoints:

- List of bird species observed in strawberry fields
- Dominance of species observed
- Frequency of occurrence of species

Findings:

A total of 20 strawberry fields at late growth stages (harvest, post-harvest) were monitored in 20 point count surveys in 2005 (average duration of observation: 15 minutes). During the course of the survey, a total of 162 individual bird contacts of 17 bird species were recorded which were subjected to further analysis. The species observed in strawberry fields include skylark, yellow wagtail, woodpigeon, carrion crow, starling, house sparrow, yellowhammer, blackbird, linnet, goldfinch, fieldfare, white wagtail, grey partridge, stonechat, black redstart, magpie and pheasant. Combining the data from the three study regions the highest frequency of occurrence figures (defined as the proportion of strawberry fields where the species was observed) in descending order could be calculated for skylark (85%), followed by yellow wagtail (45%). This means that in almost every second field yellow wagtails have been observed. Regarding dominance (defined as the proportion of bird contacts of a species among all bird contacts), the most dominant bird species utilizing strawberry fields at late growth stages across regions was skylark (48.1%) followed by yellow wagtail (14.8%), woodpigeon (14.8%) and carrion crow (6.2%). The results are presented in Table B.9.1.12.

Table B.9.1.12: Frequency of occurrence of bird species in German strawberry fields.

Bird species	Scientific name	Frequency of occurrence [%]	Dominance [%]
skylark	*Alauda arvensis*	85	48.1
yellow wagtail	*Motacilla flava*	45	14.8
woodpigeon	*Columba palumbus*	25	14.8
carrion crow	*Corvus corone*	15	6.2
starling	*Sturnus vulgaris*	15	1.2
blackbird	*Turdus merula*	10	1.9
house sparrow	*Passer domesticus*	10	3.1
white wagtail	*Motacilla alba*	10	1.2
black redstart	*Phoenicurus ochruros*	5	0.6
fieldfare	*Turdus pilaris*	5	1.2
goldfinch	*Carduelis carduelis*	5	1.2
grey partridge	*Perdix perdix*	5	0.6
linnet	*Carduelis cannabina*	5	1.2
magpie	*Pica pica*	5	0.6
pheasant	*Phasianus colchicus*	5	0.6
stonechat	*Saxicola torquata*	5	0.6
yellow hammer	*Emberiza citrinella*	5	1.9

Conclusion

Skylark, yellow wagtail, woodpigeon and carrion crow were those species utilizing German strawberry fields at the end of June and beginning of July. They were characterized by the largest figures for frequency of occurrence and dominance.

(Riffel and Giessing (2005))

Summary of study to determine focal species in German strawberry fields

Report	Dietzen, C. and Scheurig, M. 2006. Bird species in strawberry fields in Germany: Field data for the determination of focal species RIFCon GmbH Hirschberg, unpubl. report no. RC06-036
Guidance	Bibby et al. 1992. Bird census techniques. Academic Press
GLP	**No**
Dates of work	Start of experimental work 28.04.2006 Completion of experimental work 01.06.2006

Aim: The aim of this study was to propose a list of candidate bird species in selected strawberry fields that can be addressed as focal bird species in a refined risk assessment for plant protection products.

Objectives: The objectives of this generic study were to determine the qualitative and quantitative composition of the bird community employing the parameters frequency of occurrence. As part of the study FOfield, FOSurvey and dominance was determined. FOfield denotes the number of fields in which a defined species was recorded as a 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. FOsurvey 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. As regards dominance, this was defined as '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 throughout all species (calculated as arithmetic means over all strawberry fields). Dominance was determined when a respective bird species represented more or equal 5% of the total number of species present.

Study area: The North Rhine-Westphalia and Baden-Wuerttemberg regions of western and southern Germany served as study area and encompassed 20 strawberry fields (average transect length 237 ± 72 m; range 142 – 347 m; median 250 m) selected to represent the average field size and the structure of the landscape. Details of the individual fields were submitted and these indicated that the strawberry fields were situated in open landscape that was dominated by agricultural management and were surrounded by fields of cereals, strawberries, potatoes, onions or other field crops.

Method and parameters: In order to cover different strawberry growth stages, three line transect surveys were conducted in 2006 for each field in late April/early May (inflorescence emergence to early stages of flowering; survey 1), mid May (flowering to development of fruit, survey 2) and late May/early June (flowering to maturity of fruit, survey 3). A standard line transect consisted of an 'in-crop transect band' (a 100 m wide recording band of 50 m to either side of the observer moving along a longitudinal in-crop field transect). For the assessment of the bird community, frequency of occurrence (FOfield and FOsurvey), and dominance were determined.

Strawberry fields were visited between the end of April and early June 2006. Three surveys were conducted in every field within a five weeks period. A standardised strawberry field form and a bird survey form were developed to record a number of parameters during each field survey.

The avifauna of the strawberry fields was surveyed by the line transect method (Bibby *et al*. (1992)). To meet the specific methodological

requirements, the line transect method described by Bibby *et al* (1992) was adapted for this study, as described below.

All bird species were recorded in each strawberry field by walking slowly along a defined longitudinal line transect, allowing for a clear view between the rows of strawberry plants. The length of the line transects was defined by the length of the field. Each of the individual birds visually or acoustically registered was assigned to one of the following areas (see Figure 9.1 for details):

- 'In-crop transect band': birds recorded within a 100 m wide band (50 m to either side of the observer) where the strawberry field was at least 100 m wide. For narrower fields the band considered was narrowed and contained only the in-crop area (i.e. width of the strawberry 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 and off-crop habitat.

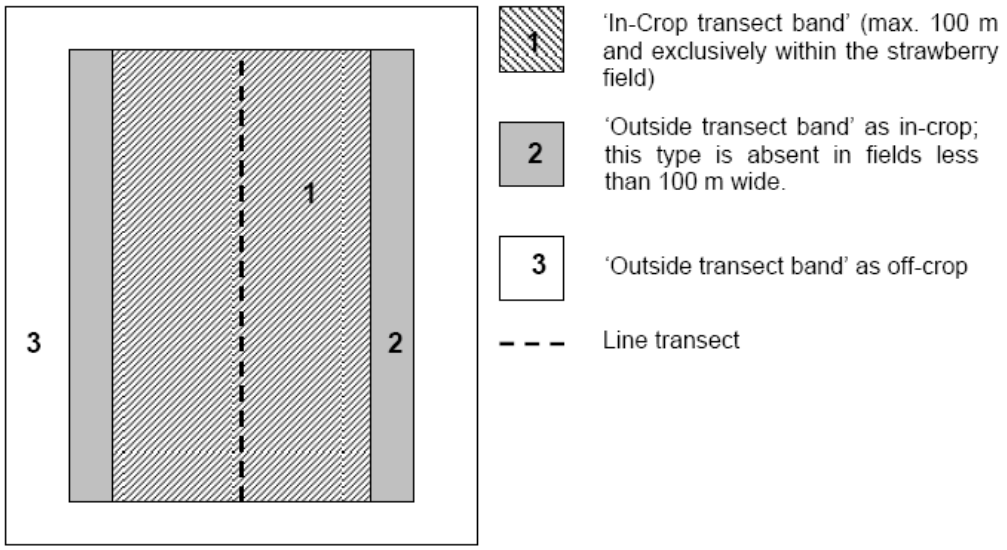


Figure 9.1: Graduation of different areas within defined strawberry fields in Germany as used in this study. The strawberry field consists of the 'in-crop transect band' (1) and – if broader than 100 m – the 'outside transect band in-crop' (2). The 'in-crop band (1) stretches generally 50 m to either side of the transect line. If the field is narrower than 100 m the width of the 'in-crop transect band' is equal to the width of the field. The 'outside transect band' (2/3) includes all off-crop habitats outside the study plot (3) and – in field wider than 100 m – some in-crop habitat.

Only birds present (foraging, roosting, singing) in the 'in-crop transect band' of each strawberry field were included for data analysis. Birds flying up to a height of 5 m above average crop height (e.g. actively hunting swallows, swifts or raptors) were also included in the survey. Birds not

directly associated with the strawberry field, e.g. flying above 5 m over average crop height, were assigned to the 'outside transect band' and ignored for the purposes of this analysis.

Data recording and analysis: Data were entered and analysed using the "Ecology Research Database System" (ERDS). The ranking of species within the list of focal species candidates was carried out in the order of decreasing importance, i.e. FOfield > FOsurvey > dominance. This list of candidates of focal species was then used to affiliate the respective species to defined habitat and foraging guilds in accordance with the SANCO guidance document.

The frequency of occurrence (FO) was determined in two different ways, (1) the total number of fields (field approach; FOfield) a bird species was present on and (2) the total number of surveys (survey approach; FOsurvey) a bird species was present in.

FOfield: denotes the number of fields in which a defined species was recorded 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. A FOfield of 100% for one species indicates that this species was observed in all strawberry fields (n = 20) during at least one survey.

FOsurvey: 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. A FOsurvey of 100% means the species was recorded during each survey (n = 60) in every strawberry field with at least one individual.

The calculation of FOfield and FOsurvey is illustrated with the yellow wagtail in Table B.9.1.13 using data recorded in this study.

Table B.9.1.13: Illustration of the calculation of the frequency of occurrence for the yellow wagtail employing the two different approaches.

Species	Total number of fields	Number of fields with record	FO_{field}	Total number of surveys	Number of surveys with record	FO_{survey}
Yellow wagtail	20	10	**50.0%**	60	18	**30.0%**

Dominance was defined as the relative occurrence of bird species within the bird community. It was reported as the percentage number of individuals of the respective species compared to the total number of individuals throughout all species (calculated as arithmetic means over all strawberry fields):

$$\textbf{Dominance} = \frac{\overline{x}_1}{\sum \overline{x}_i}$$

where xi = x1 → xn and x1 represents the average number of individuals of a given bird species in all 20 strawberry fields analysed.

Dominance was denoted when a respective bird species represented greater or equal to 5% of the total number of species present. For example, a bird species was recorded with 1.4 ± 0.4 individuals (arithmetic mean ± SD) per strawberry field. To calculate dominance this number is divided by the average number of individuals of all bird species per strawberry field (5.0 individuals/strawberry field). Thus, this species shows a dominance value of 100*1.4/5.0 = 28.0%. Dominance was calculated across all survey periods as well as for individual ones.

The dominance parameter is biased by species with a pronounced flocking behaviour which means species that sometimes occur in large numbers but with a rather low overall frequency can nevertheless obtain comparatively high dominance values, e.g. the linnet (*Carduelis cannabina*). To obtain some information on this aspect the dominance during particular survey periods (high values for flocking and uniformly distributed species) was plotted against the frequency of occurrence of the respective period, which resulted in low FO values for flocking species, but high FO values for uniformly distributed species. This is illustrated in Figure 9.2 where dominance and frequency of occurrence are plotted against each other. The dotted lines are arbitrarily chosen, however are based on past ecological work.

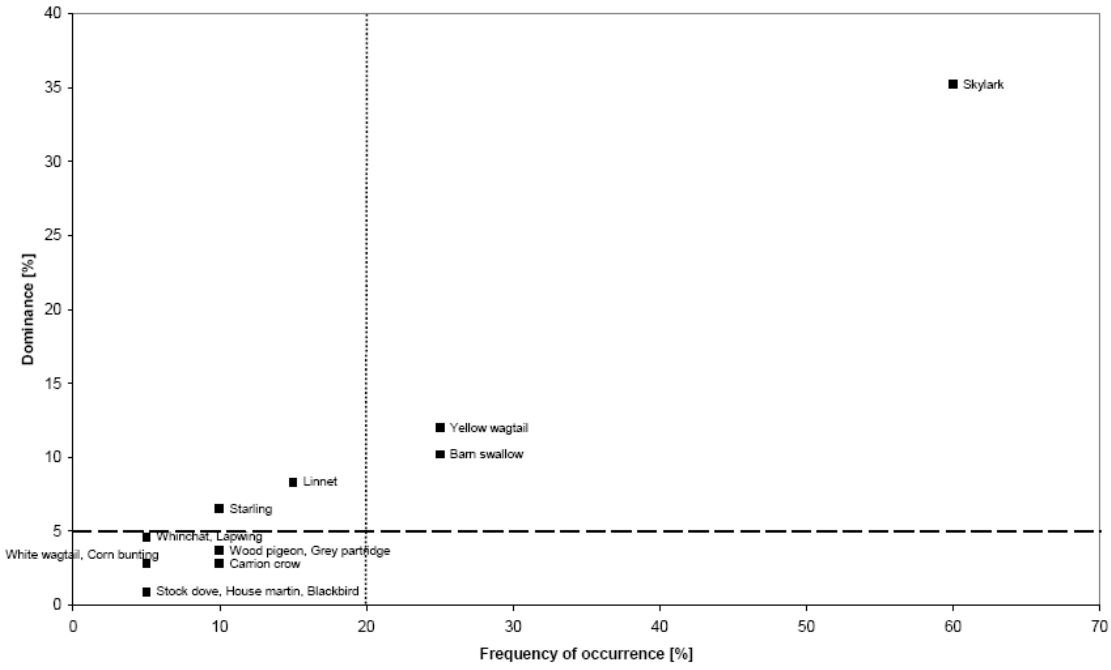


Figure 9.2: Dominance versus frequency of occurrence of bird species recorded during the first period in strawberry fields in Germany. Species being higher ranked according to dominance than to frequency of occurrence (e.g. species in the upper left of the graph) show indication for flocking behaviour. The dashed line indicates the 5% dominance level and the dotted line the 20% frequency of occurrence.

Focal species were determined using information on FO and dominance of birds in the 'in-crop transect band' obtained from the field survey conducted in strawberry fields; and general information on the size and ecology of birds to group the species according to guilds based on size, diet, and foraging stratum

For the ranking of species the authors gave FO and dominance of birds different weight of importance: FOfield > FOsurvey > dominance. The reasons for this weighting scheme are:

The species is characteristic for a given crop at least at a certain period, and there is a high probability that the species occurs on any field of a given crop. This probability is best described by the frequency of occurrence based on fields (FOfield).

The species makes regular use of a given crop, i.e. it is regularly observed over a certain period of time in fields of a given crop. This is best described by the frequency of occurrence based on surveys (FOsurvey). A species characterised by a high FOsurvey value is likely to regularly occur over the complete survey period in a given crop. The FOsurvey approach comprises a long-term perspective, since data from different growth periods are lumped and the overall FOsurvey value denotes the time-weighted occurrence. For the accentuation of seasonal aspects only data from one survey period are considered which quantifies the occurrence during a particular time span or growth stage.

The dominance is considered to distinguish between flocking species that show high dominance but low frequency of occurrence (higher ranked) and species which represent low dominance and low frequency of occurrence (lower ranked).

Consequently, the initial selection of candidates of focal species is primarily based on FOfield values (≥ 20%). A further criterion considered here are FOsurvey values equal to or exceeding 10%. The overall FOsurvey and seasonal aspects are employed for evaluation of growth stage specific occurrence and seasonal changes. FOfield and FOsurvey of one single survey period are identical due to identical sample sizes; consequently the type of FO (field or survey) is not distinguished in this case.

The three general criteria to assign bird species to ecological guilds are the following:

Size: The SANCO guidance document (Anonymous 2002) denotes size classes for birds (small, medium and large species). This has to be considered when deriving focal species for a given crop at a given time. As a general assumption the species characterised by the lowest body weight within its guild group is considered to be at higher risk from the uptake of crop protection products residues since a higher food intake rate per body weight can be assumed compared to species characterised by a higher body weight.

Diet: The SANCO guidance document (Anonymous 2002) categorises birds into three dietary guilds: insectivorous, granivorous and herbivorous. However, the majority of bird species occurring on agricultural land are omnivorous, i.e. they either have a mixed diet at any time or they change their diet seasonally. This circumstance has to be considered within the context of deriving focal species. Four diet guilds are hence proposed: insectivorous, granivorous, herbivorous and omnivorous species.

Foraging stratum: Habitat utilization in terms of foraging strata has to be included when deriving focal species. For instance, a foliage foraging insectivorous bird such as a warbler in a strawberry field might be at high exposure from a spray treatment of the foliage but less exposed from a ground application of a herbicide. The contrary is likely to be true for a ground foraging bird such as e.g. a bunting.

For the assignment of birds to size classes, body weight figures from Dunning (1993) were used.

It should be noted that the allocation of a given species to a particular guild is not exclusive, i.e. some species could be assigned to several guilds depending on season, local circumstances etc. For example, the Blackbird (*Turdus merula*) has a seasonally dependent diet, therefore assignment to insectivore, frugivore or the omnivore guild would be possible. Consequently, the dominant food source during the survey period was the primary determinant for guild allocation of the relevant species.

The first step is the generation of a list of species. The criteria used in this study for inclusion of bird species in the list of candidates of focal species is the FOfield value, basically species with a FOfield of at least 20% were selected.

FOsurvey or dominance values were not considered further as cut-off criteria for inclusion of species in the list but for ranking within the list.

Once the list of candidate species is available, the bird species were grouped as follows:

The selected species were grouped into size classes of small (10 – 50 g), medium (50 – 500 g) and large (> 500 g) birds. Within these size classes, species were grouped according to their predominant diet guild during their

occurrence in strawberry fields (the growing season of the crop). The foraging stratum of each species was considered.

Overall, the candidate species are presented according to size class (body weight), diet and foraging stratum following the categorisation in Table B.9.1.14.

Table B.9.1.14: Listed categories of foraging habitat, diet and size (body weight)

Size	Diet	Stratum
Small (< 50 g)	Insectivorous[1)]	Ground-foraging
Medium (50 – 500 g)	Granivorous	Foliage foraging
Large (> 500 g)	Herbivorous	Combined stratum user
	Omnivorous	

[1)] Includes also species which feed on other invertebrates (e.g. snails, slugs, earth worms, spiders etc.).

Results:

A total of 553 individual bird contacts comprising 23 different species were recorded throughout all surveys within the 'in-crop transect bands'.

FOfield – over the whole study period

The frequency of occurrence (i.e. FOfield) of bird species in strawberry fields in Germany is presented in Table B.9.1.15. The highest frequency of occurrence across study plots was exhibited by the skylark (80.0%), followed by the wood pigeon (55.0%), yellow wagtail (50.0%), barn swallow (50.0%), carrion crow (40.0%), feral pigeon (25.0%), linnet (25.0%), common swift (20.0%) and tree sparrow (20.0%).

Table B.9.1.15: Frequent of occurrence of bird species in relation to the total number of study plots in strawberry fields in Germany. Data collected between 28th April and 1st June 2006.

Species	Number of strawberry fields where the species was observed (n = 20)	FO_{field} [%]
Skylark (*Alauda arvensis*)	16	80.0
Wood pigeon (*Columba palumbus*)	11	55.0
Barn swallow (*Hirundo rustica*)	10	50.0
Yellow wagtail (*Motacilla flava*)	10	50.0
Carrion crow (*Corvus corone*)	8	40.0
Feral pigeon (*Columba livia* f. dom.)	5	25.0
Linnet (*Carduelis cannabina*)	5	25.0
Common swift (*Apus apus*)	4	20.0
Tree sparrow (*Passer montanus*)	4	20.0
Grey partridge (*Perdix perdix*)	3	15.0
Starling (*Sturnus vulgaris*)	3	15.0
Stock dove (*Columba oenas*)	3	15.0
Magpie (*Pica pica*)	2	10.0
Whinchat (*Saxicola rubetra*)	2	10.0
Blackbird (*Turdus merula*)	1	5.0
Corn bunting (*Miliaria calandra*)	1	5.0
Grey heron (*Ardea cinerea*)	1	5.0
House martin (*Delichon urbicum*)	1	5.0
Kestrel (*Falco tinnunculus*)	1	5.0
Lapwing (*Vanellus vanellus*)	1	5.0
Pheasant (*Phasianus colchicus*)	1	5.0
Wheatear (*Oenanthe oenanthe*)	1	5.0
White wagtail (*Motacilla alba*)	1	5.0

FOSurvey over the whole study period

The highest time-weighed occurrence throughout the study period, as indicated by FOsurvey, was recorded for the skylark (68.3%), followed by the yellow wagtail (30.0%), wood pigeon (26.7%), barn swallow (25.0%) and carrion crow (16.7%). Full details are presented in Table B.9.1.16.

Table B.9.1.16: Frequency of occurrence of bird species in relation to the total number of surveys in strawberry field in Germany.

cf. Table 6.

Species	Number of surveys where the species was observed (n = 60)	FO_{survey} [%]
Skylark (*Alauda arvensis*)	41	68.3
Yellow wagtail (*Motacilla flava*)	18	30.0
Wood pigeon (*Columba palumbus*)	16	26.7
Barn swallow (*Hirundo rustica*)	15	25.0
Carrion crow (*Corvus corone*)	10	16.7
Feral pigeon (*Columba livia* f. dom.)	5	8.3
Linnet (*Carduelis cannabina*)	5	8.3
Grey partridge (*Perdix perdix*)	4	6.7
Starling (*Sturnus vulgaris*)	4	6.7
Common swift (*Apus apus*)	4	6.7
Tree sparrow (*Passer montanus*)	4	6.7
Magpie (*Pica pica*)	3	5.0
Stock dove (*Columba oenas*)	3	5.0
Blackbird (*Turdus merula*)	2	3.3
Corn bunting (*Miliaria calandra*)	2	3.3
Grey heron (*Ardea cinerea*)	2	3.3
Whinchat (*Saxicola rubetra*)	2	3.3
White wagtail (*Motacilla alba*)	2	3.3
House martin (*Delichon urbicum*)	1	1.7
Kestrel (*Falco tinnunculus*)	1	1.7
Lapwing (*Vanellus vanellus*)	1	1.7
Pheasant (*Phasianus colchicus*)	1	1.7
Wheatear (*Oenanthe oenanthe*)	1	1.7

Dominance values recorded are shown in Table B.9.1.17. The highest dominance value was recorded for the wood pigeon (31.1%), followed by the skylark (25.5%), yellow wagtail (8.3%), barn swallow (6.1%) and feral pigeon (6.1%). These five species were responsible for 81.1% of all sightings.

Table B.9.1.17: Dominance spectrum of bird species in strawberry field in Germany over the whole study period

Bird species	Total number of individuals	Mean individual number observed per field ± SD	Dominance [%][1)]
Wood pigeon (*Columba palumbus*)	172	2.9 ± 1.1	31.1
Skylark (*Alauda arvensis*)	141	2.4 ± 0.5	25.5
Yellow wagtail (*Motacilla flava*)	46	0.8 ± 0.3	8.3
Barn swallow (*Hirundo rustica*)	34	0.6 ± 0.2	6.1
Feral pigeon (*Columba livia* f. dom.)	34	0.6 ± 0.3	6.1
Tree sparrow (*Passer montanus*)	20	0.3 ± 0.2	3.6
Linnet (*Carduelis cannabina*)	18	0.3 ± 0.1	3.3
Carrion crow (*Corvus corone*)	14	0.2 ± 0.1	2.5
Common swift (*Apus apus*)	13	0.2 ± 0.1	2.4
Starling (*Sturnus vulgaris*)	11	0.2 ± 0.1	2.0
Grey partridge (*Perdix perdix*)	7	0.1 ± 0.1	1.3
Whinchat (*Saxicola rubetra*)	7	0.1 ± 0.1	1.3
White wagtail (*Motacilla alba*)	7	0.1 ± 0.1	1.3
Lapwing (*Vanellus vanellus*)	5	< 0.1	0.9
Corn bunting (*Miliaria calandra*)	5	< 0.1	0.9
Stock dove (*Columba oenas*)	5	< 0.1	0.9
Magpie (*Pica pica*)	4	< 0.1	0.7
Blackbird (*Turdus merula*)	3	< 0.1	0.5
Grey heron (*Ardea cinerea*)	2	< 0.1	0.4
Wheatear (*Oenanthe oenanthe*)	2	< 0.1	0.4
House martin (*Delichon urbicum*)	1	< 0.1	0.2
Kestrel (*Falco tinnunculus*)	1	< 0.1	0.2
Pheasant (*Phasianus colchicus*)	1	< 0.1	0.2
Total	553	9.2	100.0

[1)] Due to rounding errors the sum of dominance values may deviate from 100%.

FO – frequency of occurrence during individual growth stages

Surveys were conducted throughout the growing season and the frequency of occurrence of birds was determined. It should be noted that for these single surveys FOfield and FOsurvey are identical due to identical sample size. Presented in Table B.9.1.18 are the frequency of occurrence of bird species in strawberry field during different growth stages.

Table B.9.1.18: Frequency of occurrence of bird species in strawberry fields during different plant growth stages.

Observation period	28.04. – 12.05.2006	11. – 20.05.2006	24.05. – 01.06.2006
Average growth stage	Inflorescence emergence/ Early stages of flowering	Flowering/ Development of fruit	Flowering/ Development of fruit/ Maturity of fruit
Number of surveys	20	20	20
Bird species	FO [%]	FO [%]	FO [%]
Skylark (*Alauda arvensis*)	60.0	**80.0**	65.0
Yellow wagtail (*Motacilla flava*)	25.0	**35.0**	30.0
Wood pigeon (*Columba palumbus*)	10.0	25.0	**45.0**
Barn swallow (*Hirundo rustica*)	25.0	20.0	**30.0**
Carrion crow (*Corvus corone*)	10.0	**25.0**	15.0
Feral pigeon (*Columba livia* f. dom.)	-	10.0	**15.0**
Linnet (*Carduelis cannabina*)	**15.0**	10.0	-
Grey partridge (*Perdix perdix*)	**10.0**	**10.0**	-
Starling (*Sturnus vulgaris*)	**10.0**	-	**10.0**
Common swift (*Apus apus*)	-	**20.0**	-
Tree sparrow (*Passer montanus*)	-	-	**20.0**
Magpie (*Pica pica*)	-	5.0	**10.0**
Stock dove (*Columba oenas*)	5.0	-	**10.0**
Blackbird (*Turdus merula*)	5.0	-	**5.0**
Corn bunting (*Miliaria calandra*)	**5.0**	**5.0**	-
Grey heron (*Ardea cinerea*)	-	**5.0**	**5.0**
Whinchat (*Saxicola rubetra*)	**5.0**	**5.0**	-
White wagtail (*Motacilla alba*)	**5.0**	**5.0**	-
House martin (*Delichon urbicum*)	**5.0**	-	-
Kestrel (*Falco tinnunculus*)	-	-	**5.0**
Lapwing (*Vanellus vanellus*)	**5.0**	-	-
Pheasant (*Phasianus colchicus*)	-	-	**5.0**
Wheatear (*Oenanthe oenanthe*)	-	**5.0**	-

Dominance of bird species during different plant growth stages in presented in Table B.9.1.19.

Table B.9.1.19: Dominance of bird species in strawberry fields in Germany during different plant growth stages.

Observation period	28.04. – 12.05.2006	11. – 20.05.2006	24.05. – 01.06.2006
Average growth stage	Inflorescence emergence/ Early stages of flowering	Flowering/ Development of fruit	Flowering/ Development of fruit/ Maturity of fruit
Number of study plots	20	20	20
Bird species	Dominance [%]	Dominance [%]	Dominance [%]
Wood pigeon (*Columba palumbus*)	3.7	30.8	**44.2**
Skylark (*Alauda arvensis*)	**35.2**	24.8	21.6
Yellow wagtail (*Motacilla flava*)	**12.0**	10.7	4.3
Barn swallow (*Hirundo rustica*)	**10.2**	3.3	6.9
Feral pigeon (*Columba livia* f. dom.)	-	**9.8**	5.6
Tree sparrow (*Passer montanus*)	-	-	**8.7**
Linnet (*Carduelis cannabina*)	**8.3**	4.2	-
Carrion crow (*Corvus corone*)	2.8	**3.3**	1.7
Common swift (*Apus apus*)	-	**6.1**	-
Starling (*Sturnus vulgaris*)	**6.5**	-	1.7
Grey partridge (*Perdix perdix*)	**3.7**	1.4	-
Whinchat (*Saxicola rubetra*)	**4.6**	0.9	-
White wagtail (*Motacilla alba*)	**2.8**	1.9	-
Lapwing (*Vanellus vanellus*)	**4.6**	-	-
Corn bunting (*Miliaria calandra*)	**2.8**	0.9	-
Stock dove (*Columba oenas*)	0.9	-	**1.7**
Magpie (*Pica pica*)	-	0.5	**1.3**
Blackbird (*Turdus merula*)	**0.9**	-	**0.9**
Grey heron (*Ardea cinerea*)	-	**0.5**	0.4
Wheatear (*Oenanthe oenanthe*)	-	**0.9**	-
House martin (*Delichon urbicum*)	**0.9**	-	-
Kestrel (*Falco tinnunculus*)	-	-	**0.4**
Pheasant (*Phasianus colchicus*)	-	-	**0.4**

On the basis of the above the study authors proposed several potential focal species. These are presented in Table B.9.1.20. Various ecological parameters for the birds presented in Table B.9.1.20 were then considered and as a result the study authors proposed the focal species presented in Table B.9.1.21.

Table B.9.1.20: List of candidates of focal species in strawberry fields, species are ranked according to FOfield>FOsurvey>dominance>size

Species	FO_{field} [1, 2] [%]	FO_{survey} [1, 3] [%]	Dominance [1, 4] [%]	Body weight [5] [g]	Stratum use [6]	Diet guild [7]
Skylark	80.0	68.3	25.5	37.2	ground	omnivorous
Wood pigeon	55.0	26.7	31.1	490.0	ground	herbivorous
Yellow wagtail	50.0	30.0	8.3	17.6	ground	insectivorous
Barn swallow	50.0	25.0	6.1	15.8	aerial	insectivorous
Carrion crow	40.0	16.7	2.5	570.0	ground	omnivorous
Feral pigeon	25.0	8.3	6.1	340.0	ground	herbivorous
Linnet	25.0	8.3	3.3	15.3	ground / foliage	granivorous
Tree sparrow	20.0	6.7	3.6	22.0	ground / foliage	omnivorous
Common swift	20.0	6.7	2.4	37.6	aerial	insectivorous

1) Across the complete study period (3 survey periods)
2) Based on 20 study plots (strawberry fields)
3) Based on 60 surveys
4) Based on the arithmetic mean of the number of individuals of one species per study plot in relation to the mean number of individuals of all species per study plot for a total number of 20 fields
5) According to Dunning (1993). In case sex-specific figures were provided, the lower number was chosen
6) Predominant foraging stratum during growing season according to Perrins (1998)
7) Predominant diet composition during growing season according to Perrins (1998)

Table B.9.1.21: Focal species candidates in strawberry fields in Germany based on FOsurvey data

Guild	Species
Small insectivore (< 50 g)	
Ground dweller	Yellow wagtail
Aerial	Barn swallow
Small omnivore (< 50 g)	
Ground dweller	Skylark
Medium herbivore (50 - 500g)	
Ground dweller	Wood pigeon
Large omnivore (> 500 g)	
Ground dweller	Carrion crow

(Dietzen and Scheurig (2006))

Summary of feeding ecology study of the relevant insectivorous bird species in strawberry field in Germany.

Report	Moosmayer, P. (2006): Feeding ecology of the relevant insectivorous bird species in strawberry fields in Germany RIFCon GmbH, unpublished Report No.: RC06-054, Date: 21. September 2006.
Guidance	Not applicable; the test was especially designed for the purpose of this study.
GLP	Yes
Dates of work	Start of experimental work 27th April 2006 Completion of experimental work 28th July 2006

Aim:

The aim of this generic study was to obtain refined dietary estimates for two focal bird species (yellow wagtail and skylark) in strawberry fields that can be used as revised input data for the recalculation of toxicity-to-exposure ratios (TER) based on the risk of exposure due to foraging preferences.

Objectives:

- to determine the proportion of diet obtained in strawberry fields (PT estimate) by radio tracking individual birds (including visual observations) based on the assumption that the time a bird spends 'active' or, more specifically, foraging in a habitat, is a reliable measure of the proportion of food obtained in this defined area

- to determine the proportion of different food types in the diet (PD estimates) by analysing faeces and/or stomach contents.

Study area:

The study was conducted in two strawberry growing regions in Germany. One was located in North-Rhine Westphalia and one in Baden-Württemberg. The total study area was 512 ha including 66 strawberry fields with a total area of 148 ha. The chosen areas reflect typical strawberry field distribution patterns for Germany.

Methodology:

The two bird species yellow wagtail (*Motacilla flava*) and skylark (*Alauda arvensis*) served as test organisms. All individuals monitored during this study lived in strawberry growing regions and thus had the opportunity to use strawberries as a foraging habitat. The field and statistical methodology was as outlined above in Schwarz (2006). Bird trapping was conducted between 27th April and 31st May and was stopped after 15 individual skylarks and yellow wagtails respectively, had been caught. All skylarks were trapped between 27th April and 16th May and all yellow wagtails between the 3rd and the 31st May. Faeces and stomach flushing as well as determination of PD was carried out as outlined above in Schwarz (2006). In addition, information on habitats, climate and agricultural practice were also presented. Preference indices were also calculated. The methodology used for this was as outlined above in Schwarz (2006).

Results:

PT – proportion of food obtain in treated area

Skylark - The PT for the skylark in strawberry fields was calculated from the proportion of time spent 'potentially foraging' in strawberry fields during 25 tracking sessions of 14 individuals (n = 25). PT ranged from <0.01 to 1 for the individual tracking session. The mean was 0.73 ± 0.33, the median 0.86 and the 90th percentile was 0.99 (see Table B.9.1.22). It should be noted that tracking sessions on the same individual were typically separated by 9 days or more, and for calculations in this study are regarded as statistically independent.

Table B.9.1.22: PT in strawberry fields of radio-tracked skylarks in both study regions (n = 25)

Individual no. /tracking session	PT calculation					PT in strawberries
	Behaviour in all known habitats [%]			Behaviour in strawberries [%]		
	All behavioural categories [%]	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	
1	100	25.7	74.3	0.1	0.3	<0.01
3/a	100	15.2	84.8	0.9	36.4	0.43
3/b	100	28.5	71.5	15.2	56.1	0.79
4/a	100	13.9	86.1	2.4	5.6	0.06
4/b	100	51.1	48.9	32.0	40.2	0.82
5/a	100	18.5	81.5	8.7	67.6	0.83
5/b	100	27.0	73.0	14.9	54.1	0.74
6	100	19.5	80.5	13.9	14.4	0.18
7/a	100	11.4	88.6	5.1	88.6	1.00
7/b	100	29.5	70.5	11.8	12.4	0.18
8/a	100	23.5	76.5	14.6	74.3	0.97
8/b	100	21.3	78.7	16.2	78.4	1.00
9/a	100	25.7	74.3	19.7	71.3	0.96
9/b	100	14.2	85.8	9.6	81.6	0.95
10/a	100	23.7	76.3	14.0	70.4	0.92
10/b	100	17.8	82.2	11.8	77.2	0.94
11/a	100	22.6	77.4	13.9	76.6	0.99
11/b	100	47.6	52.4	34.0	44.8	0.86
12/a	100	25.1	74.9	17.9	54.2	0.72
12/b	100	16.9	83.1	6.5	24.2	0.29
13	100	20.7	79.3	13.5	67.8	0.85
14/a	100	11.6	88.4	5.4	88.4	1.00
14/b	100	36.3	63.7	16.4	56.5	0.89
15/a	100	24.0	76.0	21.3	73.9	0.97
15/b	100	57.2	42.8	56.6	42.0	0.98
50%til		23.5	76.5	13.9	56.5	0.86
90%til		43.1	86.0	27.7	80.4	0.99
mean		25.1	74.9	15.1	54.3	0.73
SD		11.8	11.8	11.9	26.4	0.33

1 active (excluding foraging), inactive and reproduction are combined into 'Other behaviour categories (not foraging)'

2 active (possibly foraging), foraging and behaviour n.s. are combined into 'Potentially foraging'

The PTs of the single tracking sessions were categorised in 10% steps. Eleven out of 25 tracking sessions ranged between 90% - 100% for 'potential foraging time' skylarks spent in strawberry fields. This was

therefore the most frequent class. The following category with regard to frequency was 80% - 90% consisting of 5 tracking session. The median is placed in the same category. Nine tracking sessions were below 80%.

Yellow wagtail

The PT of the yellow wagtail in strawberry fields was calculated from the time spent 'potentially foraging' in strawberry fields during 23 tracking sessions of 13 individuals. The PT value ranged from 0 to 0.96. The mean PT was found to be 0.54 (± 0.34), the median was 0.58 and the 90th percentile was 0.94 (see Table B.9.1.23). Tracking sessions on the same individual were typically separated by 9 days or more, and can be regarded as statistically independent.

Table B.9.1.23: PT in strawberry fields of radio-tracked yellow wagtails in both study regions (n = 23)

Individual no. /tracking session	PT calculation					PT in strawberries
	Behaviour in all known habitats [%]			Behaviour in strawberries [%]		
	All behavioural categories [%]	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	
1/a	100	16.7	83.2	4.3	19.5	0.23
1/b	100	38.2	61.8	0.6	4.5	0.07
2	100	43.1	56.9	34.7	51.5	0.90
3/a	100	29.9	70.1	16.4	66.4	0.95
3/b	100	20.0	80.0	10.0	76.6	0.96
4/a	100	36.9	63.1	19.5	57.6	0.91
4/b	100	43.6	56.4	34.6	35.7	0.63
5	100	17.1	82.9	12.0	78.7	0.95
7/a	100	30.1	69.9	5.3	40.8	0.58
7/b	100	28.0	72.0	15.0	62.4	0.87
8/a	100	11.7	88.3	2.3	70.7	0.80
8/b	100	16.4	83.6	8.3	40.3	0.48
9/a	100	25.9	74.1	4.5	43.1	0.58
9/b	100	21.3	78.7	8.9	19.1	0.24
10	100	17.0	83.0	2.0	10.6	0.13
11/a	100	9.3	90.7	0	0	0
11/b	100	13.3	86.7	0	0	0
13/a	100	16.1	83.9	10.5	64.9	0.77
13/b	100	59.6	40.4	44.6	24.4	0.60
14/a	100	42.8	57.2	23.7	19.3	0.34
14/b	100	9.8	90.2	2.5	25.2	0.28
15/a	100	22.9	77.1	11.0	70.2	0.91
15/b	100	59.8	40.2	58.1	10.1	0.25

Individual no. /tracking session	PT calculation					PT in strawberries
	Behaviour in all known habitats [%]			Behaviour in strawberries [%]		
	All behavioural categories [%]	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	Other behaviour categories (not foraging) [1]	Potentially foraging [2]	
50%til		22.9	77.1	10.0	40.3	0.58
90%til		43.5	88.0	34.7	70.6	0.94
mean		27.4	72.6	14.3	38.8	0.54
SD		14.8	14.8	15.4	26.0	0.34

[1] active (excluding foraging), inactive and reproduction are summarized to other behaviour categories

[2] active (possibly foraging), foraging and behaviour n.s. are summarized to potentially foraging

The PT values of the single tracking sessions were categorised in 10% steps. Six out of 23 tracking sessions ranged between 90% -100% for yellow wagtails and therefore, this was the most frequent class. Regarding frequency, it was followed by the 20% - 30% class (four tracking sessions) which was below the median of 58.4%.

PD – proportion of food types obtained from the treated area

Skylark – In total, 18 diet samples were obtained consisting of 13 faeces samples and 5 samples gained by stomach flushing. The samples were pooled.
Invertebrates were found to contribute 62.53% and plant seeds 37.47% to the number of all diet items ingested by the skylarks. The total dry weight was composed of 56.75% invertebrates and 43.25% plant seeds. The proportion of the different invertebrate and plant seed taxa found in the diet of skylarks are shown below in Table B.9.1.24.

Table B.9.1.24: Proportion different food items contribute to the diet of skylarks

Taxon	Numerical proportion [%]	Dry weight proportion [%] PD
Invertebrates	**62.53**	**56.75**
Lepidoptera larvae	5.30	13.74
Lumbricidae	1.77	12.25
Coleoptera	9.31	8.43
Diptera (adult)	7.71	5.68
Araneida	9.67	4.05
Hemiptera	16.45	3.19
Hymenoptera	5.89	2.51
Diptera larvae	1.27	1.80
Diplopoda	0.97	1.33
Lepidoptera (adult)	0.52	1.13
Dermaptera	0.25	0.89
Isopoda	0.64	0.68
Stylommatophora	0.52	0.55
Formicidae	2.26	0.52
Plant matter	**37.47**	**43.25**
Poaceae seeds (cereal)	4.34	32.54
Poaceae seeds	26.65	9.63
Euphorbiaceae seeds	0.53	0.42
other seeds	4.38	0.34
Brassica seeds	0.26	0.17
Chenopodiaceae seeds	0.79	0.08
Lamiaceae seeds	0.26	0.04
Ranunculaceae capsule	0.26	0.03
TOTAL	**100**	**100**

Within the invertebrates Hemiptera was the most numerous taxon (16.45% of individual arthropods by number) followed by Araneida (9.67%) and Coleoptera (9.31%). Poaceae seeds (excluding cereal seeds) contribute 26.65% of the total number of items ingested. With regard to the dry

weight cereal seeds (Poaceae) represented the most important food item (32.54%,) followed by Lepidoptera larvae (13.74%) and Lumbricidae (12.25%). All other taxa contribute less than 10% to the total dry weight of the diet ingested by the skylarks.

In order to illustrate the food selection of the skylarks with regard to the length of the prey five size classes were defined (see Figure 9.3).

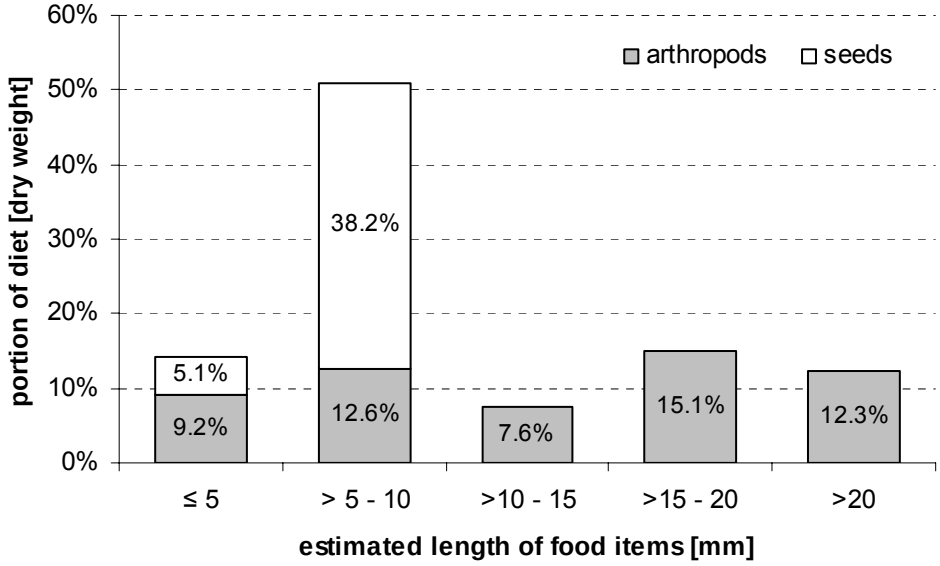


Figure 9.3 Proportion items of different length contribute to the diet of skylarks

Food items between 5 and 10 mm in length compose the most important proportion of dry weight of the diet of skylarks, followed by items between 15 and 20 mm. Only 14.3% of the dry weight was composed of items which were 5 mm in length or smaller. Considering the total part of the skylark diet made up of arthropods, in terms of dry weight 19.73 % were below or equal to 5 mm and 80.27% were greater than 5 m.

Yellow wagtail

A total of 25 faeces samples of yellow wagtails were obtained. The samples were pooled samples and after analysis the proportion of different arthropod taxa contributing to the diet are presented in Table B.9.1.25. Hemiptera (inclusively Aphidae) turned out to be the most numerous taxon (34.15% of individual arthropods by number) followed by Diptera (30.69%), Coleoptera (15.59%) and Araneida (10.46%). The remaining taxa were notably rarer. With regard to the dry weight the Diptera represent the most important taxon (48.65%) followed by Araneida (11.71%) and Hymenoptera larvae (10.98%). Despite their numerous occurrences the Hemiptera contribute only 8.39% to the total dry weight.

Table B.9.1.25: Proportion different invertebrate taxa contribute to the diet of yellow wagtails

Taxon	**Numerical proportion [%]**	**Dry weight proportion [%] PD**
Diptera	30.69	48.65
Araneida	10.46	11.71
Hymenoptera (larvae)	0.83	10.98
Coleoptea (adult)	14.76	8.15
Hemiptera	16.29	7.42
Hymenoptera	3.86	3.73
Neuroptera (adult)	0.64	3.38
Lepidoptera (larvae)	0.55	2.46
Myriapoda	1.48	1.81
Hemiptera (Aphidae)	17.86	0.97
Hymenoptera (Formicidae)	1.20	0.34
Coleoptera (larvae)	0.83	0.25
Neuroptera (larvae)	0.28	0.07
Opilionia	0.28	0.07
TOTAL	**100**	**100**

In order to illustrate the food selection of yellow wagtail regarding the length of the prey four size classes were defined (Figure 9.4).

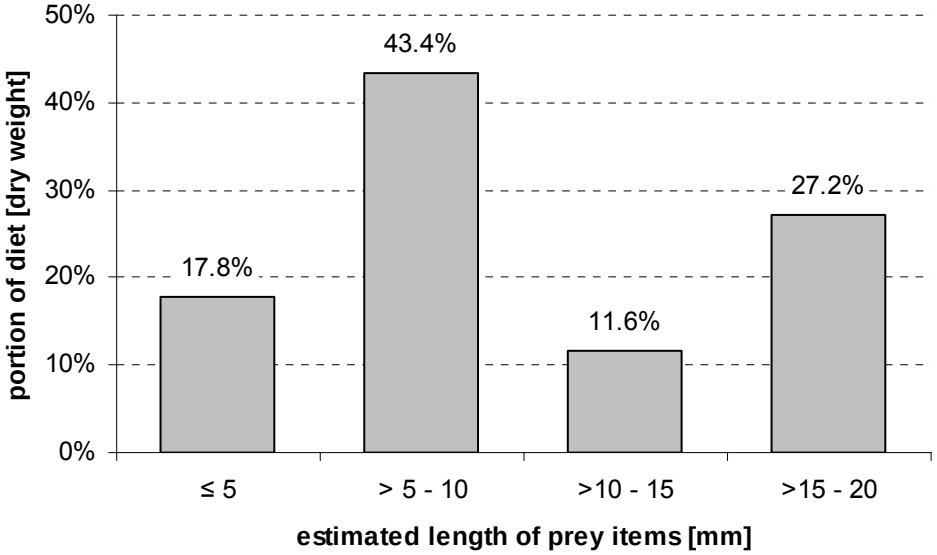


Figure 9.4: Proportion arthropods of different length contribute to the diet of yellow wagtails

No prey items were longer than 20 mm. Arthropods of a length between 5 and 10 mm represented the most important class. Only 17.8% of the dry weight composed of arthropods which are 5 mm in length or smaller.

Preference indicators

Skylark – the home range size of skylarks ranged from 2.1 to 16.5 ha. The mean home range size was 6.3 ha. The proportion of strawberry fields in the home range varied from 13.1% to 93.3%; the mean was 54.4%. The calculated Jacobs' index for strawberry fields ranged from -0.94 to 1 total preference. The mean Jacobs' index was 0.44. This means that strawberry fields were on average preferred as a feeding habitat by the tracked skylarks in comparison to other available habitats within their home ranges. During two tracking sessions (session no. 7a and 14a) skylarks exclusively foraged in strawberry fields. However, the skylarks differed markedly among the sessions (SD = 0.6).

Yellow wagtail – the home range size of yellow wagtails ranged between 1.8 and 124.7 ha. The latter home range was used by a male which obviously was not paired. It flew around and displayed at several different sites to attract a mate. The mean home range size was 22.2 ha. The proportion of strawberry fields in the home range ranged from 0% to 75.9%. The mean proportion of strawberry fields in the home range was 39.3%. The Jacobs' index calculated for strawberry fields ranged between -1 and ≥ 0.9. The mean Jacobs' index was 0.26.

Climate

In the study region in North-Rhine Westphalia, the minimum day temperature during the study was 1.8 °C and the maximum day temperature was 32.1 °C (average temperature 13.8 °C). The maximum precipitation was 17.2 mm/day at the 13th of May. The average precipitation during the study was 1.8 mm/day.

In the study region in Baden-Württemberg, minimum day temperature during the study was 2.0 °C and the maximum day temperature was 31.4 °C (average temperature 15.3 °C). The maximum precipitation was 12.4 mm at the 16th of May. The average precipitation was 1.5 mm.

(Moosmayer P. (2006))

Risk assessment

Background and first tier risk assessment

The following risk assessment is based on the guidance provided in SANCO 4145/2000 (Anon 2002).

Studies were submitted on the long-term/reproductive toxicity of clofentezine to mallard ducks and bobwhite quail and according to the DAR the key endpoint is 7.62 mg a.s./kg bw/day. This endpoint is based on the bobwhite quail. Study summaries and a discussion of the reproductive endpoints is presented in the DAR (See Section B.9.1.4).

It should be noted that in the DAR a potential long-term risk to birds from all proposed uses was highlighted. The Notifier has submitted data to address the long-term risk from the use on strawberries and pome and stone fruit. Due to a lack of further information, the uses on grapes and roses (ornamentals) remain unresolved.

From the introduction it is seen that the proposed uses are pome and stone fruit as well as strawberries. Following the guidance in SANCO 4145/2000, the main route of exposure for birds in orchards and strawberry fields will be via the consumption of treated insects. (It should be noted that strawberry foliage is unpalatable and will not be grazed by birds or mammals.)

Using the assumptions outlined in SANCO 4145/2000, a first tier 'estimated theoretical exposure' (or ETE) of 6.03 mg a.s./kg bw/day is determined. If this is compared to the toxicity endpoint of 7.62 mg a.s./kg bw/day then a long-term TER of 1.26 is produced for both the pome/stone fruit and strawberry use. In the original DAR the Notifier tried to refine the risk, however there were some shortcomings in their approach. They have now submitted ecological data on proportion of food obtain in the treated area (PT) and proportion of different food types obtained from the treated

area (PD) to enable the risk to be refined. Outlined below is an assessment of these data as well as a refined risk assessment.

Use on Strawberries

Determination of a Focal Species (FS)

In trying to refine the risk assessment, the Notifier has chosen to refine the ecological parameters of PD (i.e. proportion of food types in the diet) and PT (the proportion of diet obtain in the treated area). When refining these parameters, it is necessary to determine appropriate focal species. A focal species should be a representative species that occurs in the crop and is both abundant (i.e. the average number of individuals per field) and prevalent (i.e. the average percentage of presence). It should also be noted that when determining a focal species there needs to be a consideration of the weight and feeding guild of the species. It should be further noted that due to a combination of PD and PT it may be necessary to select more than one focal species, to ensure that the risk to birds is fully addressed.

The Notifier has submitted two studies to try and identify appropriate focal species. The first was one by Riffel and Geissing (2005), this was a preliminary study and was not conducted to GLP, due to these reasons, and the fact that a more detailed study has been submitted, this study has not been used for the following risk assessment.

The second study by Dietzen and Scheurig (2006) is considered to a good quality study (although not to GLP) and therefore the findings of this study will be used to determine appropriate focal species. In selecting a study area it is important to ensure that the site is appropriateness of the site in terms of location, time of year and other areas where strawberries are grown. An assessment of these issues is illustrated in Table B.9.1.26.

Table B.9.1.26: Consideration of the relevance of the generic field study on strawberries to determine focal species

Issue	Summary from Dietzen and Scheurig	Conclusion
Appropriateness of site	Study site was in the North Rhine-Westphalia and Baden-Wuerttemberg region of western and southern Germany. Details of the individual fields were submitted and these indicated that the strawberry fields were situated in open landscape that was dominated by agricultural management and were surrounded by fields of cereals, strawberries, potatoes, onions or other field crops.	The sites chosen are considered to be representative of commercial strawberry growing. On the basis of the data submitted it is not possible to extrapolate the findings of this study to other MS.
Appropriateness of the time of the study	The study was conducted between 28th April through to 1st June. Data have been submitted to indicate whether focal species change with respect to time.	The study was carried out over a time that corresponds with the proposed use of clofentezine.

As regards the methodology used, it should be noted that there is currently no agreed methodology for determining focal species. This study used the concept of three line transects in 20 strawberry fields over the course of three months. As a result of this approach outputs are FOfield and FOSurvey as well as an indication of 'dominance'. These key outputs are considered useful, along with other ecological information on weight and feeding guild to determine appropriate focal species. (It should be noted that the term 'dominance' used in the study is, in this instance is interchangeable with the term 'prevalence'.)

On the basis of the above field work the study authors propose several potential focal species. These are detailed in Table B.9.1.21. The RMS is in agreement with these and considers that the refined risk assessment should for this assessment focus on the skylark and yellow wagtail. (It should be noted that these focal species are relevant to strawberry growing in Germany. This information may also be relevant to other MS, however this would need to be justified.)

Determination of PT – proportion of diet obtained in the treated area

On the basis of the above work to determine focal species, the Notifier carried out intensive radiotracking with observation on both the skylark and yellow wagtail. The methodology used involved intensively tracking 14 individual skylarks for 25 tracking sessions and 13 yellow wagtails for 23 tracking sessions. These tracking sessions involved following the same birds more or less from dawn and until dusk. During this time all locations and activities were recorded. Birds that were tracked twice were separated by an interval of at least 9 days. Ideally there should have been no tracking of the same individual twice as this could lead to a bias in the data – for example if a bird was a frequent user of a strawberry field and it was tracked twice then this could give an over estimation of PT in the cropped area. Likewise if an individual was an occasional user of a strawberry field tracking twice would lead to an underestimation. Due to this it is considered that if the variation in two PT values for the same bird is small in comparison with the variation between birds, then the data should not be used. Likewise if the variation within a bird is large in comparison with the variation between birds, then it may be appropriate to use all the data. With this in mind, ideally PT should be recalculated taking account of only one tracking time and then compare this with the above assessment.

With the above in mind and on examining the data for all birds closely that were radio tracked twice (see Table B.9.1.22 and B.9.23) it is considered that tracking the same bird twice is unlikely to have lead to an over or underestimation of the time spent in the treated crop (except for yellow wagtail – see below).

Having obtained data, PT was determined for each bird and then 50^{th} percentile, 90^{th} percentile and a mean value were determined for all radiotracked birds. As regards the skylark data, it is noted that a total of 14 birds were tracked for 25 sessions and that all birds were 'consumers', i.e. they were all users of strawberry fields. When these data are used for risk assessment (see below) the ensemble for the risk assessment will be skylarks that use strawberry fields. This is in contrast to the yellow wagtail where it appears from Table B.9.1.23 that one bird on two separate occasions made no use of strawberry fields, therefore the ensemble will be to different to that for skylarks. (It should be noted that this individual may have had the crop in its home range, however was not recorded visiting it, i.e. it was a 'potential consumer'.) A possible, precautionary, way around this would be to determine the mean, 50^{th} percentile and 90^{th} percentile for yellow wagtails excluding this individual. If this is done, then the 90^{th} percentile increases very slightly from 0.94 to 0.95, whilst the 50^{th} percentile changes from 0.54 to 0.6 and the mean from 0.54 to 0.6. These amended figures will be taken forward for consideration in the risk assessment below.

It should be noted that the PT data have been analysed crudely and various percentiles have been determined. No consideration appears to have been made as to what distribution the data fitted. It should also be noted that

there has been no consideration regarding uncertainty around the about percentiles. This is particularly relevant considering the relative small number of individuals tracked. There is also concern regarding the use of multiple days from the same bird (see above). It is felt that these are *potential* drawbacks to the use of the above data, however they do *not* preclude its' use. These issues will be considered further during the risk assessment.

To conclude, the PT methodology is considered to be appropriate, however the RMS has some reservations regarding the analysis of the data and in particular the lack of the consideration of uncertainty. The RMS also has reservations regarding the inclusion on 'non-consumers' in the determination of PT. Due to this latter point, the RMS proposes the following values for use in the refined risk assessment:

Yellow wagtail – 90^{th} percentile = 0.95 and 50^{th} percentile = 0.6
Skylark – 90^{th} percentile = 0.99 and 50^{th} percentile = 0.86

PD – proportion of food types obtained from the treated area

On the basis of the stomach flushing and faecal analysis the Notifier has proposed diets for both the skylark and the yellow wagtail. The method of collection and the analysis of the data are considered to be acceptable, however the RMS has concerns regarding the pooling of the samples. By pooling the diet information is lost as to what the range of food types are between individual birds. This could underestimate the risk to some individuals and overestimate it to others, for example one individual could consume all one food type whereas another individual consumed another food type – if the data were pooled it could indicate that the birds ate a 50:50 mix.

The faecal and stomach contents obtained in this study are the result of a bird feeding, what is not known is where the particular bird fed to obtain that food. For the yellow wagtail it is noted that all the diet is insectivorous, and it is assumed that this was obtained from within the strawberry field. No assessment has been made to test this assumption, for example by comparing the invertebrate community of a strawberry field with that in yellow wagtail stomachs/faeces. Despite this potential shortcoming, it is assumed that the stomach/faeces contents could have been potentially obtained from a strawberry field.

For the skylark it is noted that the bird is an omnivore and this is reflected in the stomach and faeces analysis. The RMS considers that this needs further consideration. As indicated above, PD is meant to be what food the bird has obtained from the treated area. If it is assumed that the strawberry field contains very few weeds and only strawberries, it is likely that a skylark will mainly obtain insects from the treated field; hence PD should be all insects/invertebrates. On the basis of the PD analysis, several seeds (mainly weed seeds) were found in the stomach/faeces of skylarks. There is no evidence to indicate where the skylark could have obtained this from;

however it is tentatively concluded, by the RMS, that these may have been found in the strawberry field. It is noted from Table B.9.1.27 that cereals seeds formed a large proportion of the diet; again no evidence has been submitted to indicate where these would have been obtained from. However, from examination of photographs submitted with the report it would appear that straw was used around the strawberry plants and hence this *may* account for the presence of these seeds in the stomach of skylarks. Straw tends to be put down approximately one month before harvest and hence it is possible that it may receive a treatment of clofentezine, hence it is appropriate to keep it in the assessment. In conclusion, it is considered that the methodology used to collect data on PD and the data itself are satisfactory and hence can be used for risk assessment purposes.

The Notifier has also analysed the items in the diet according to size, this classification is considered appropriate. This information is then used to ensure that the most 'appropriate' residue data are applied to the invertebrate food items (see below for further details).

So to conclude, the proposed diets of skylark and yellow wagtail feeding in strawberry fields in Germany are presented in Table B.9.1.27.

Table B.9.1.27: Proposed diet for skylark and yellow wagtail

	Proportion of different food types in the diet (PD)		
invertebrate and plant items actually eaten by individuals foraging in and around strawberry fields [proportions of dry weight]	**Food type**	**skylark based on 13 faeces and 5 flushing samples**	**yellow wagtail based on 25 faeces samples**
Invertebrate matter	Insecta* (adult)	0.223	0.728
	Insecta* (larvae)	0.155	0.139
	Araneida	0.041	0.117
	Opiliones	-	0.001
	Isopoda	0.007	-
	Myriapoda	-	0.018
	Diplopoda	0.013	-
	Stylommatophora	0.005	-
	Lumbricidae	0.123	-
	TOTAL	0.567	1
Plant matter	Poaceae seeds (cereal)	0.325	-
	Poaceae seeds	0.096	-
	Euphorbiaceae seeds	0.004	-
	Small seeds (n.s.)+	0.003	-
	Brassica seeds	0.002	-
	Chenopodiaceae seeds	0.001	-
	Lamiaceae seeds	< 0.001	-
	Ranunculaceae seeds	< 0.001	-
	TOTAL	0.433	-

	Proportion of different food types in the diet (PD)		
invertebrate and plant items actually eaten by individuals foraging in and around strawberry fields [proportions of dry weight]	**Food type**	**skylark based on 13 faeces and 5 flushing samples**	**yellow wagtail based on 25 faeces samples**
	Proportion of different item length in the diet (PD)		
Length of food items actually eaten by individuals foraging in and around strawberry fields [proportions of dry weight]	Size class [mm]	skylark based on 13 faeces and 5 flushing samples	yellow wagtail based on 25 faeces samples
Length of food item	≤ 5	0.143	0.178
	> 5 – 10	0.508	0.434
	> 10 – 15	0.076	0.116
	> 15 – 20	0.151	0.272
	> 20	0.123	-

Refined risk assessment

The first tier risk assessment resulted in an ETE of 6.03 mg a.s.kg bw day and a TERlt of 1.26. From the above both the skylark and yellow wagtail are considered appropriate focal species for strawberries.

Yellow wagtail

In the original DAR the Notifier proposed revised food intake rates (FIR) for a 17 g yellow wagtail of **0.88** assuming it was consuming all invertebrates. This figure was considered acceptable and hence will be used in the following assessment.

According to the data on PT, the 90th percentile PT value for the yellow wagtail is **0.95**, whilst the 50th is **0.6**. In selecting an appropriate percentile it is firstly necessary to consider what it actually means – a 90th percentile means that 90% or less of the population of yellow wagtails inhabiting strawberry fields in Germany obtain 95% of their food from strawberry fields. Hence the risk assessment covers 90% of birds that inhabit and feed in strawberry fields, i.e. they are consumers. Likewise if 50th percentile is selected then the resulting risk assessment covers 50% of birds. Strictly speaking the choice of what proportion of the population should be covered by the resulting risk assessment is a risk managers' one. However there is currently no guidance on the desired level of protection. In the absence of such guidance it is proposed to assess the risk using both 50th and 90th percentiles.

The Notifier has proposed that the diet of a yellow wagtail is **17.8%** 'small insects' and **82.2%** 'large insects', this is based on dietary analysis

presented in Table B.9.1.25. It is also based on the assumption that insects less than 5 mm are 'small' and those greater than 5 are 'large'. In the PPR opinion on methamidaphos (see http://www.efsa.europa.eu/en/science/ppr/ppr_opinions/769.html), the Panel proposed that large insects were 3-4 mm, therefore the proposed split by the Notifier is deemed to be acceptable.

Using the above data the ETE can be revised and this is presented in Table B.9.1.28 assuming that the risk manager wishes to assess the risk for 50% of the population inhabiting strawberry fields. The result of assessing the risk for 95% of the population inhabiting strawberry fields is presented in Table B.9.1.29.

Table B.9.1.28: Calculation of long term ETE for 50% of the yellow wagtails potentially foraging in strawberries treated once with clofentezine at a rate of 200 g/ha

Diet proportions	**'Small' arthropods**	**'Large' Arthropods**	
Application rate [kg a.s./ha]	0.20	0.20	
RUD [mg/kg a.s./ha]	29	5.1	
Maximum initial concentration after last application [mg a.s./kg]	5.8	1.02	
Multiple application factor (MAF)	1	1	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.88*	0.88*	
Portion of diet obtained in-crop (PT)	0.6	0.6	
Portion of diet (PD)	0.178**	0.822$	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	0.545	0.442	0.988

* as derived in previously submitted risk assessment (Riffel, 2004), and agreed in the DAR (B.9, p375)
** Proportion of invertebrates considered 'small' i.e. ≤5mm
$ Proportion of invertebrates considered 'large' i.e. >5mm

Table B.9.1.29: Calculation of long term ETE for 90% of the yellow wagtails potentially foraging in strawberries treated once with clofentezine at a rate of 200 g/ha

Diet proportions	**'Small' arthropods**	**'Large' Arthropods**	
Application rate [kg a.s./ha]	0.20	0.20	
RUD [mg/kg a.s./ha]	29	5.1	
Maximum initial concentration after last application [mg a.s./kg]	5.8	1.02	
Multiple application factor (MAF)	1	1	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.88*	0.88*	
Portion of diet obtained in-crop (PT)	0.95	0.95	
Portion of diet (PD)	0.178**	0.822$	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	0.863	0.701	1.563

* as derived in previously submitted risk assessment (Riffel, 2004), and agreed in the DAR (B.9, p375)
** Proportion of invertebrates considered 'small' i.e. ≤5mm
$ Proportion of invertebrates considered 'large' i.e. >5mm

Presented below in Table B.9.1.30 is the revised risk assessment taking on board the above revised ETE.

Table B.9.1.30: Long-term toxicity exposure ratios for focal species foraging in strawberry fields

Species	**Long term NOEC mg/kg b.w./day**	**ETE mg/ kg b.w./day**	**TER**
Yellow wagtail (assuming PT of 0.6)	7.62*	0.988	7.7
Yellow wagtail (assuming PT of 0.95)	7.62*	1.563	**4.9**

*Endpoint agreed by PSD (DAR, B.9, p 361)

From Table B.9.1.30 it can be seen that when the risk assessment covers 50% of the yellow wagtail population inhabiting strawberry fields the TERlt is 7.7 and hence the risk is acceptable. The resulting TERlt when the risk to 90% of the population is assessed is *just* below the Annex VI trigger of 5. It should be noted that there is no consideration of residue decline nor

interception, therefore, this refined ETE is potentially still relatively worst case.

Skylark

The Notifier has proposed a revised food intake rates (FIR) for skylark of 0.7 assuming it was consuming invertebrates and 0.23 if it was consuming seeds. These figures are considered acceptable and hence will be used in the following assessment.

According to the data on PT, the 90th percentile PT value for the skylark is 0.99, whilst the 50th is 0.86. As outlined above, in the absence of such guidance it is proposed to assess the risk using 50th and 90th percentiles.

The Notifier has proposed that the diet of a skylark is **11.2%** 'small insects', **45.5%** 'large insects' and **43.3%** cereal seeds, this is based on dietary analysis presented in Table B.9.1.27. It is also based on the assumption that insects less than 5 mm are 'small' and those greater than 5 are 'large'. This split in to large and small is in line with the PPR opinion on methamidaphos (see http://www.efsa.europa.eu/en/science/ppr/ppr_opinions/769.html) and is therefore considered acceptable. The contribution of cereal seed is considered to originate from the straw placed around the strawberry plants just before fruit is set. It is considered likely that the straw could be present when applications of clofentezine are made and hence the grain could be contaminated with residues. It is assumed that the likely level of contamination is equivalent to that present on large insects (see Appendix II of SANCO 4145).

Using the above data the ETE can be revised and this is presented in B.9.1.31 assuming that the risk manager wishes assess the risk for 50% of the population inhabiting strawberry fields. The result of assessing the risk for 95% of the population inhabiting strawberry fields is presented in Table B.9.1.32.

Table B.9.1.31: Calculation of long term ETE for 50% skylark potentially foraging in strawberry fields treated once with clofentezine at a rate of 200 g/ha

Diet proportions	**'Small' arthropods**	**'Large' invertebrates**	**Seeds (mostly cereals)**	
Application rate [kg a.s./ha]	0.2	0.2	0.2	
RUD [mg/kg a.s./ha]	29	5.1	5.1	
Maximum initial concentration after last application [mg a.s./kg]	5.8	1.02	1.02	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.7*	0.7*	0.23*	
Portion of diet obtained in-crop (PT)	0.86	0.86	0.86	
Portion of diet (PD)	0.112**	0.455$	0.433	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	0.391	0.279	0.087	0.757

* as derived in previously submitted risk assessment (Riffel, 2004)
** Proportion of invertebrates considered 'small' (≤5mm) x PD for invertebrates = 0.1973 x 0.567 = 0.112
$ Proportion of invertebrates considered 'large' (>5mm) x PD for invertebrates = 0.802 x 0.567 = 0.455

Table B.9.1.32: Calculation of long term ETE for 90% skylark potentially foraging in strawberry fields treated once with clofentezine at a rate of 200 g/ha

Diet proportions	**'Small' arthropods**	**'Large' invertebrates**	**Seeds (mostly cereals)**	
Application rate [kg a.s./ha]	0.2	0.2	0.2	
RUD [mg/kg a.s./ha]	29	5.1	5.1	
Maximum initial concentration after last application [mg a.s./kg]	5.8	1.02	1.02	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]	0.7*	0.7*	0.23*	
Portion of diet obtained in-crop (PT)	0.99	0.99	0.99	
Portion of diet (PD)	0.112**	0.455$	0.433	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	0.450	0.322	0.100	0.872

* as derived in previously submitted risk assessment (Riffel, 2004)
** Proportion of invertebrates considered 'small' (≤5mm) x PD for invertebrates = 0.1973 x 0.567 = 0.112
$ Proportion of invertebrates considered 'large' (>5mm) x PD for invertebrates = 0.802 x 0.567 = 0.455

Presented below in Table B.9.1.33 is the revised risk assessment taking on board the above revised ETE.

Table B.9.1.33: Long-term toxicity exposure ratios for focal species foraging in strawberry fields

Species	**Long term NOEC mg/kg b.w./day**	**ETE mg/ kg b.w./day**	**TER**
Skylark (assuming PT of 0.86)	7.62*	0.757	10.1
Skylark (assuming PT of 0.99)	7.62*	0.872	8.74

*Endpoint agreed by PSD (DAR, B.9, p 361)

From Table B.9.1.33 it can be seen that when the risk assessment covers 50% or 90% of the skylark population inhabiting strawberry fields the TERlt is greater than 5 and hence the risk is acceptable.

Use in orchards

When the long-term risk from the use of clofentezine in orchards was originally assessed (see DAR) a potential risk to insectivorous birds was highlighted. The Notifier has refined the risk assessment by using ecological data from orchards in Italy and France (see Schwarz (2006)). An assessment of this study and the resulting information is presented below.

Determination of a Focal Species (FS)

The Notifier has chosen to refine the ecological parameters of PD (i.e. proportion of food types in the diet) and PT (the proportion of diet obtain in the treated area). When refining these parameters, it is necessary to determine appropriate focal species. No study has been carried out to specifically determine the FS for pome fruit in Southern MS. The Notifier has used information in Schwarz (2006) to indicate what species are likely to be FS. This assessment appears to have been based on the number of birds captured and then radiotracked. Whilst not ideal, or in line with the work done for strawberries, it is considered to provide sufficient information to determine suitable FS. Outlined in Table B.9.1.34 is an assessment of the appropriate of the study, the associated sites, location and timings.

Table B.9.1.34: Consideration of the relevance of the generic field study on strawberries to determine focal species

Issue	Summary from Schwarz (2006)	Conclusion
Appropriateness of site	Study site was three commercial pome fruit orchards in Southern France and six commercial pome orchards in Italy. The total study area was 118 ha in France and 2929 ha in Italy. The study sites were located in pome fruit growing areas and they were surrounded by other orchards as well as a mixture of other habitats.	The sites chosen are considered to be representative of commercial pome fruit growing in Southern MS. On the basis of the data submitted it is not possible to extrapolate the findings of this study to other MS (except France and Italy).
Appropriateness of the time of the study	The study was conducted between 21st June and the 12th August.	No information has been presented to indicate whether the time of application corresponds to likely applications of clofentezine to pome fruit. (It should be noted that due to the fact that clofentezine acts against the eggs of target organisms, it tends to be applied earlier in the season that the study was conducted.)

On the basis of Schwarz (2006), the Notifier has proposed that the Great tit is an appropriate FS for insectivorous birds inhabiting SMS pome orchards, the RMS is in agreement with the proposal.

Determination of PT – proportion of diet obtained in the treated area

On the basis of the above study, the Notifier has chosen to focus the risk assessment on the Great tit. A total of 7 Great tits were radiotracked during the course of the study. According to Table B.9.1.7 all of these were 'consumers' and hence the outcome of the risk assessment will be in line with that done for birds using strawberry fields. On the basis of the birds radiotracked the Notifier has indicated that the 50th percentile and 90th percentile are 0.49 and 0.71 respectively. It should however be noted that the Notifer has proposed that these data are *supportive* of the proposal in the initial assessment that PT is 0.61. It should be noted that this latter

figure is in fact based on PT for Bluetits in UK orchards. It should further be noted that when these data were revised the 90th percentile was 0.58 (see http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/ACP/PTFeb06.pdf). Finally, data from two study sites in two separate MS have been collated. Without an in depth consideration of the similarity of the two sites the appropriateness of amalgamating these data is questionable.

To conclude, data have been submitted that indicates that the 90th percentile for Great tits in SMS pome fruit orchards is approximately 0.71, however there is much uncertainty regarding this figure namely – lack of clarity regarding whether the Great tit is an appropriate FS, amalgamation of data from two study sites in different MS and no consideration of the uncertainty surrounding the underlying data. However, it should be noted that the Notifier only wishes the data to be used in a *supportive* manner.

PD – proportion of food types obtained from the treated area

If the data from the Schwarz study is used to determine PD then, according to the analysis presented in Table B.9.1.9 the diet consists of 68% caterpillars, 2.3% small insects/invertebrates, 27.8% large insects/invertebrates and 1.9% seeds. Using this information the revised ETE would be as presented in Table B.9.1.36.

Table B.9.1.35: Proportions of different item lengths in the diet (PD) of the Great tit. Length of food items eaten by individuals foraging in and around pome fruit orchards [proportions of dry weight] (see Table B.9.1.9 for raw data)

Estimated length of food items [mm]	arthropoda	seeds
≤ 5	2.3	1.9
> 5 – 10	26.6	-
>10 – 15	6.5	-
>15 – 20	47.1	-
>20	15.6	-

Table B.9.1.36 Revised ETE for Great tits in SMSs using data on PD from Schwarz

Diet proportions for great tit	**Large arthropods**	**Small arthropods**	**Caterpillars foliage**	**Seeds**	
Application rate [kg a.s./ha]	0.2	0.2	0.2	0.2	
RUD [mg/kg a.s./ha]	5.1	29	5.1	29	
Maximum initial concentration after last application [mg a.s./kg]	1.02	5.8	1.02	5.8	

Diet proportions for great tit	Large arthropods	Small arthropods	Caterpillars foliage	Seeds	
Relative daily food intake (FIR/b.w.) [g fresh weight/g b.w./day]*	0.88	0.88	1.27	0.28	
Portion of diet obtained in-crop (PT)	1	1	1	1	
Portion of diet (PD)	0.278	0.023	0.68	0.019	
Estimated theoretical exposure (ETE) [mg a.s./kg b.w./day]	0.249	0.117	0.881	0.03	
ETE					1.28

* See original DAR for details

Presented in Table B.9.1.37 is the revised risk assessment taking on board the above revised ETE.

Table B.9.1.37: Long-term toxicity exposure ratios for focal species foraging in strawberry fields

Species	Long term NOEC mg/kg b.w./day	ETE mg/ kg b.w./day	TER
Great tit (assuming RMS diet)	7.62*	1.28	6.0

*Endpoint agreed by PSD (DAR, B.9, p 361)

From Table B.9.1.37 it can be seen that the TERlt for Great tits is greater than 5 when using a modified diet . If data on PT were factored in, the resulting risk would obviously be less.

It should be noted that the Notifier has requested use on pome and stone fruit – all the field work and the associated work has been done on pome fruit. No data have been supplied on stone fruit, however it is felt that there is little difference between these two orchards and hence the above assessment for pome fruit can cover stone fruit as well.

Conclusion

Clofentezine is of low acute and dietary toxicity to birds, consequently it also poses a low acute and short term risk to birds.

The first tier risk assessment for the long-term risk assessment for birds indicated a potential concern, i.e. TERlt <5 for all proposed uses. The

Notifier has now submitted data to enable the refinement of the key ecological parameters or PT and PD for strawberries and stone/pome fruit.

On the basis of the additional data revised TERlt for the long-term risk to birds using strawberry fields range from 4.9 to 10.1 depending upon species and the proportion of the population that the risk manager wishes to protect. It should be noted that the TERlt of 4.9 is slightly below the trigger value of 5, however there has been no consideration of residue decline or interception in the calculation of the ETE, therefore this is considered to be still relatively worst case.

As regards the risk to birds in orchards, the underlying data are not as robust as that for strawberries, however it is considered sufficient to enable an adequate risk assessment to be carried out. By using the additional data the revised TERlt is 6.0 for the key focal species.

It should be noted that no additional data have been submitted on the long-term risk to birds in either **grapes or roses (ornamentals),** therefore these areas remain as presented in the original DAR, i.e. further data are required to confirm the assumptions made.

B.9.2 Effects on aquatic organisms

B.9.2.1 Reporting Table, clofentezine (UK) - rev0_24_oct_2006

The aquatic section of this addendum primarily addresses Reporting Table points 5(10) and 5(19) (but see also 5(15).

B.9.2.2 RMS consideration

The Notifier submitted a fish early life stage study to determine an acceptable long term toxicological endpoint for fish. The 'Apollo 50SC' formulation was used in the study to maintain clofentezine at aqueous concentrations above its solubility (see DAR B.2.1.11). The study has been evaluated by the RMS below at 2.3 and refinement of the aquatic risk assessment is discussed at 2.4.

B.9.2.3 Fish early life stage toxicity - 'Apollo 50SC'

Table B.9.2.1 Summary of chronic toxicity of clofentezine to fathead minnow

Test Guideline	GLP	'Apollo 50SC' clofentezine g/L[3]	28d NOEC[2] (ug clofentezine/L)	Reference (report no.)
EPA OPPTS 850.1400, OECD 210	Yes	51.7	1000.0[1]	Cockcroft, 2005 (R-17810) (used in RA)

[1] nominal concentration
[2] based on all parameters
[3] density 1.187 g/mL

An early life-stage toxicity test on the fathead minnow (*Pimephales promelas*) under flow through conditions was undertaken using 'Apollo 50SC' a suspension concentrate formulation (density = 1.187 g/mL) containing clofentezine (517 g/L). The formulation was used to study clofentezine effects at greater than its solubility in water. The study was GLP compliant and conducted in accordance with OECD 210 guidelines. No protocol deviations affecting the study interpretation and integrity were reported by the Notifier and the RMS considered the study to be acceptable for risk assessment.

Nominal aqueous test concentrations of 'Apollo 50SC' of 0, 10, 30, 100, 300 and 1000 ug a.s./L were prepared in duplicate from stock solutions. Groups of 30 (60 per treatment) newly fertilised *Pimephales promelas* eggs were introduced into suspended egg chambers in the pre-hatch period (0-8d). Hatching commenced after 3d, newly hatched larvae were transferred after >90% eggs had hatched (after 8d), egg chambers were removed, and incubation was continued under continuous flow through conditions (nominal 100mL/min) for a further 28d (post hatch). From 0 to 8d larvae were fed twice daily with newly hatched (24h old) brine shrimp (*Artemia* naupli) and from 9d were fed with 48h old shrimp *ad libitum*.

Incubations were maintained under controlled conditions (mean 23^{o}C, 16hL:8hD photoperiod, mean pH 7.3-7.4, mean 7.9-8.1 mg O_2/L) and temperature, dissolved O_2 and pH were recorded daily in vessels. Flow rates from stock solutions and diluent of the aquatic renewal system were measured twice daily.

Analytical verifications were performed from samples of each treatment and control group at the beginning of the test, at weekly intervals until test termination. Samples were extracted by liquid:liquid partition into dichloromethane and residue dissolved in tetrahydrofuran/0.01% aq. orthophosphoric acid (50/50 v/v) prior to analysis by RP HPLC with spectrophotometric detection. The method was validated by analysis of fortified samples.

Effects on hatching success and embryo development, post hatch sublethal effects and mortality of the fry were monitored and, at termination, surviving fry were sacrificed and weighed and measured. Data are summarised in Table B.9.2.2.

Table B.9.2.2 Summary of results of fathead minnow early life stage study with 'Apollo 50SC'

Nominal clofentezine concentration (mean measured) ug/L	% hatch	% post hatch survival at 28d	Mean survivor length at 28d mm (±SD)	Mean survivor dry weight at 28d mg (±SD)
Control	95	84	19.9 (1.5)	21.4 (4.5)
10.0 (11.0)	93	80	19.2 (1.7)	19.7 (5.7)
30.0 (32.0)	93	86	19.9 (1.1)	20.8 (3.7)
100.0 (109.0)	90	93	19.3 (1.4)	19.2 (4.0)
300.0 (323.0)	92	76	20.2 (1.3)	22.9 (5.1)
1000.0 (995.0)	95	82	19.7 (1.6)	21.0 (5.1)
28d NOEC (ug/L)		1000.0	1000.0	1000.0

Mean measured clofentezine concentrations were between 99.5-110% nominal and flow rates were maintained between 90-130% nominal throughout

The validity criteria were met as the control group had >66% hatching success and >70% post hatch survival. There were no statistically significant treatment-related effects on embryo development, egg hatching, post hatch survival and development and surviving fry growth parameters thus the overall NOEC for fathead minnow (*Pimephales promelas*) was 1000.0ug clofentezine/L.

Conclusion:

The long term NOEC for all monitored ELS parameters for *Pimephales promelas* is 1000.0ug clofentezine/L.

B.9.2.4 Aquatic risk assessment

Acute risk assessment

Due to low aqueous solubility of clofentezine, the effects of formulation, 'Apollo 50SC', on aquatic organisms were regarded as more reliable as test concentrations of clofentezine were better maintained. The acute aquatic toxicological endpoints considered appropriate for risk assessment are presented below in Table B.9.2.3 (see DAR B.9.2.3).

Table B.9.2.3 Summary of aquatic formulation ('Apollo 50SC') acute toxicity

Species	Test	mg a.s./L
Rainbow trout	96h LC50[1]	>10
Daphnia magna	48h EC50[1]	>100
Pseudokirchneriella subcapitata	72h EC50[1]	>40

[1] **see DAR B.9.2.3**

The Rainbow trout was the most acutely sensitive to formulation and hence the 96h LC50 (>10 mg a.s./L) was used in the acute risk assessment (see Table B.9.2.4).

Table B.9.2.4 Clofentezine aquatic acute risk assessment

Applic. rate g a.s./ha	Test substance	Crop	*O.mykiss* 96h LC50 mg a.s./L	FOCUSsw Step 1[1] mg a.s./L	TER	Annex VI Trigger
200	'Apollo 50SC'	Pome/stone fruit (early)	>10.0	0.047	>213	100
200	'Apollo 50SC'	Pome/stone fruit (late)	>10.0	0.038	>263	100
150	'Apollo 50SC'	Vine (early)	>10.0	0.022	>455	100
150	'Apollo 50SC'	Vine (late)	>10.0	0.025	>400	100
200	'Apollo 50SC'	Strawberry (<0.5m)	>10.0	0.029	>345	100
200	'Apollo 50SC'	Ornamental (>0.5m)	>10.0	0.033	>303	100

[1] **see DAR B.8.5.2, Table B.8.42**

All TERs in Table B.9.2.4 are >100 at FOCU step1 PECsws and indicate low acute risk to fish and other aquatic organisms from all proposed uses of 'Apollo 50SC'.

Chronic aquatic risk assessment

The chronic aquatic toxicological end points considered acceptable (see DAR B.9.2.3) for use in risk assessment are summarised in Table B.9.2.5. Both the ELS and prolonged fish study were regarded as relevant for the risk assessment. The fathead minnow chronic study is considered to be the more appropriate ELS study for use in the risk assessment since a suitably maintained dose range was employed. This study was preferred to the rainbow trout ELS study which used technical material at one low dose (NOEC = 0.007 mg a.s./L) to overcome solubility problems. Similarly, the

D. magna chronic study using 'Apollo 50SC' in a more natural sediment:water system was selected for risk assessment (see DAR B.9.2.3 for further discussion). Both the ELS and prolonged toxicity fish NOECs were considered appropriate for risk assessment (DAR B.9.2.3).

Table B.9.2.5 Summary of aquatic chronic toxicity

Species	**Test**[1]	**mg a.s./L**
Fathead minnow	ELS 28d NOEC[2]	1.0
Rainbow trout	Prolonged tox 21d NOEC[1]	12.5
Daphnia magna	21d NOEC[1,3]	0.25
Chironomus riparius	28d NOEC[1,4]	0.5

[1] **see DAR B.9.2.3**
[2] **study evaluated above @ 2.3**
[3] **in presence of sediment considered more 'realistic'**
[4] **spiked water study**

The aquatic chronic risk assessment using these data is summarised in Tables B.9.2.6 and 9.2.7.

Table B.9.2.6 Clofentezine chronic aquatic risk assessment - fish

Applic rate g a.s./ha	Crop	Fish NOEC[2] mg a.s./L	FOCUS step 1[1]		Annex VI Trigger
			PECsw mg a.s./L	TER	
200	Pome/ stone fruit (early)	1.0	0.047	21.3	10
		12.5		266	
200	Pome/ stone fruit (late)	1.0	0.038	26.3	10
		12.5		329	
150	Vine (early)	1.0	0.022	45.5	10
		12.5		568	
150	Vine (late)	1.0	0.025	40	10
		12.5		500	
200	Strawberry (<0.5m)	1.0	0.029	34.5	10
		12.5		431	
200	Ornamental (>0.5m)	1.0	0.033	30.3	10
		12.5		379	

[1] **PECsw see DAR B.8.5.2, Table B.8.42**
[2] **see Table B.2.5; Fathead minnow ELS 28d NOEC = 1.0mg a.s./L; Rainbow trout 21d NOEC = 12.5mg a.s./L**

Table B.9.2.7 Clofentezine chronic aquatic risk assessment - aquatic invertebrates

Applic. rate g a.s./ha	Crop	Aq invert NOEC[2] mg a.s./L	FOCUS step 2[1]		FOCUS step 3[3]		Annex VI Trigger
			PECsw mg a.s./L	TER	PECsw mg a.s./L	TER	
200	Pome/ stone fruit (early)	0.25	0.045	***5.6***	0.018	13.9	10
		0.5		11.1		-	
200	Pome/ stone fruit (late)	0.25	0.018	13.9	-	-	10
		0.5		27.8		-	
150	Vine (early)	0.25	0.021	11.9	-	-	10
		0.5		38.1		-	
150	Vine (late)	0.25	0.011	22.7	-	-	10
		0.5		45.5		-	
200	Strawberry (<0.5m)	0.25	0.022	11.3	-	-	10
		0.5		22.7		-	
200	Ornamental (>0.5m)	0.25	0.026	***9.7***	0.001	250	10
		0.5		19.2		-	

[1] total load PECsw see DAR B.8.5.2, Table B.8.45
[2] see Table 2.5, Daphnia 21d NOEC = 0.25 mg a.s./L; *Chironomus* 28d NOEC = 0.5 mg a.s./L
[3] worse case FOCUS Step3 scenarios see DAR B.8.5.2, Tables B.8.48, 8.51a (not total load)

All TERs are >10 for FOCUS step 1 PECsw in Table B.9.2.6 indicating that there is a low chronic risk to fish from all the proposed uses of clofentezine.

Using FOCUS step 2 total load PECsws (see DAR B.8.5.2), TERs >10 were derived for aquatic invertebrates for all crop uses apart from early pome/stone fruit and ornamentals (see Table B.9.2.7). However, with worse case FOCUS Step 3 PECsw scenarios, TERs >10 were obtained indicating low risk to aquatic invertebrates also for these uses.

Together these data indicate that there is a low acute and chronic risk to aquatic organisms from all the proposed uses of clofentezine without requirement for risk mitigation. This is in contrast to the aquatic risk assessment presented in the DAR (B.9.2.3) which was driven by the chronic Rainbow Trout ELS endpoint (NOEC = 0.007 mg a.s./L).

B.9.2.5 Conclusion:

The acute and chronic risk to fish, aquatic invertebrates and algae arising from all proposed uses of clofentezine in 'Apollo 50SC' applications is low and risk mitigation is not required.

B.9.3 Effects on non-target terrestrial vertebrates - mammals

B.9.3.1 Reporting table, clofentezine (UK) - rev0_24_oct_2006

There were no points in the Reporting Table relating to non-target terrestrial vertebrates to be addressed.

B.9.4 Effects on bees

B.9.4.1 Reporting table, clofentezine (UK) - rev0_24_oct_2006

There were no points in the Reporting Table relating to effects on bees to be addressed.

B.9.5 Effects on non-target arthropods

B.9.5.1 Reporting table, clofentezine (UK) - rev0_24_oct_2006

The non-target arthropod (NTA) section of the addendum addresses Reporting Table point 5(22).

B.9.5.2 RMS consideration

On the basis of the NTA toxicity data and risk assessment, the DAR (B.9.5.2a) concludes that there is a low acute risk to adult stages of several non-target arthropod species. However, since clofentezine is a specific contact acaricide primarily with an ovicidal mode of action with some effect on young motile stages, the risk to NTA egg and young motile stages was regarded as in need of further consideration.

Absence of effects on *Folsomia candida* adults and eggs via treated soil provided some indication of low risk to soil invertebrates. However, studies on *Aleochara bilineata* (rove beetle) and *Coccinella septempunctata* (ladybird) where eggs were treated were submitted by the Notifier to further address this requirement and the risk to foliage-dwelling species. These studies are evaluated by the RMS at B.9.5.3 and B.9.5.4 below.

B.9.5.3 *Aleochara bilineata* - extended laboratory study

Table B.9.5.1 Summary effect of clofentezine on reproductive capacity of *Aleochara bilineata* using the formulation 'Apollo 50SC'

Test Guideline	GLP	a.s. content clofentezine g/ha (mg/kg soil)	Mean no. of F1 emergent beetles/female	% deviation from control	repro NOEC g a.s./ha	Reference (report no.) Used in RA
IOBC,BART, EPPO. Candolfi *et al*.,2000	Yes	200 (0.267)	79.9	+1.8	200	Taylor, 2005a (R-17809) Yes

The effects of 'Apollo 50SC', a suspension concentrate formulation (density = 1.187 g/mL) containing clofentezine (517 g/L) on reproductive capacity of adult rove beetles *Aleochara bilineata* was assessed in an extended laboratory study. The a.s. was mixed with soil rather than surface-sprayed to ensure maximum exposure of *Aleochara* eggs which are laid below the surface and potentially more susceptible to the a.s.. No other protocol deviations affecting the integrity and outcome of the study were reported and the RMS considered the study to be acceptable for risk assessment.

Four treatments, water control, 'Apollo 50SC' @ 100 & 200g clofentezine/ha (0.133/0.267 mg a.s./kg soil) and toxic reference 50g dimethoate/ha were prepared. LUFA 2.1 soil (1.23% oc) was treated with 'Apollo 50SC' and water to achieve 35% WHC, the toxic reference was sprayed on the soil surface. Four replicates of 1100g soil (5cm depth)/container were prepared.

Ten pairs of 3d old adult beetles hatched from parasitized *Delia antiqua* pupae were added to each replicate and fed 3x/week with 20 *Musca domestica* pupae/replicate. Approximately 500 *D. antiqua* pupae were added to each replicate and evenly distributed in the soil 7, 14 and 21d after addition of adult beetles. On day 28 F0 adult beetles were removed and, after a further 7d, pupae were removed to suitable collection pots and emergence of F1 adults counted until control treatment emergence fell to <2 beetles/replicate/d. Replicates were incubated and hatched under appropriate controlled temperature, relative humidity and light regimes. Results were expressed as mean F1 adults/female (see Table B.9.5.2).

Table B.9.5.2 Reproductive activity of *Aleochara bilineata* exposed to 'Apollo 50SC' soil treatment

clofentezine g/ha (mg/kg soil)	**Reproduction**	
	Mean no. of F1 emergent beetles/replicate	**Mean no. of F1 emergent beetles/female**
Control (0)	785	78.5
100.0 (0.133)	814	81.4
200.0 (0.267)	799	79.9
Toxic ref. (50g dimethoate/ha)	3	0.3

The significantly reduced F1 beetle emergence in the toxic reference confirmed sensitivity of the test. No statistically significant treatment-related effect on F1 beetle emergence/female compared to control was observed at the highest dose of 200g clofentezine/ha (0.133mg clofentezine/kg soil) indicating absence of effect of clofentezine on *Aleochara bilineata* reproduction.

B.9.5.4 *Coccinella septempunctata* - extended laboratory study

Table B.9.5.3 Summary of clofentezine on reproductive capacity of *Coccinella septempunctata* using the formulation 'Apollo 50SC'

Test Guideline	GLP	a.s. clofentezine g/ha	% Egg hatch (% deviation from control)	% Adult emergence (% deviation from control)	Repro NOEC g a.s./ha	Reference (report no.) Used in RA?
ESCORT 2 Candolfi *et al*.,2001	Yes	200	76 (+14)	98 (-2)	200	Taylor, 2005b (R-17808) Yes

The effects of 'Apollo 50SC', a suspension concentrate formulation (density = 1.187 g/mL) containing clofentezine (517 g/L) on reproductive capacity of the ladybird beetle *Coccinella septempunctata* was assessed in an extended laboratory study by treatment of beetle eggs. No protocol deviations affecting the integrity and outcome of the study were reported and the RMS considered the study to be acceptable for risk assessment.

Six treatments, untreated, water controls 500 and 1000L/ha, 'Apollo 50SC' @ 100 & 200g clofentezine/ha (in 500L/ha and 1000L/ha) and a toxic reference 5g dimethoate/ha (in 200L/ha) were prepared.

Eggs were collected 24h prior to treatment on paper tissue from ladybird cultures held under environmentally controlled conditions. Batches of minimum 20 eggs on paper (representing 1 replicate) were placed on glass plates and sprayed with the treatment solutions and spray deposits were measured. Batches (3 replicates/treatment) were allowed to dry and transferred to Petri dishes lined with filter paper (1 batch/dish). An additional 100g a.s./ha replicate was prepared as a large proportion of eggs in an original replicate were consumed by larvae.

Over a 2d period, immediately after emergence, a minimum of 20 larvae were transferred to small lined Petri dishes (one larva/dish) and monitored to pupation and subsequent adult emergence. Replicates were incubated under appropriate controlled temperature, relative humidity and light regimes. Mortality of both eggs and larvae was assessed at least every 48h through to pupation and subjective assessments of behavioural effects compared to control were recorded. Results were expressed as % egg and larval mortality and % adult emergence after pupation (see Table B.9.5.4).

Table B.9.5.4 Reproductive activity of *Coccinella septempunctata* exposed to 'Apollo 50SC'

Treatment	Reproduction			
	% egg mortality (total treated)	Egg[1] hatch (%)	Larval mortality (% transferred)	Adult emergence (% pupated)
Untreated	0 (96)	58	19	95
Water control (500L/ha)	0 (90)	54	52	95
Water control (1000L/ha)	0 (81)	62	38	100
Apollo 50SC (100 g a.s./ha:500L/ha)	0 (130)[2]	48	42	97
Apollo 50SC (200 g a.s./ha:1000L/ha)	0 (123)	76	25	98
Toxic ref. (5g dimethoate/ha)	100 (75)	0	-	-

[1] reflects eggs consumed by larvae

The sensitivity of the test is confirmed by 100% egg mortality in the toxic reference. Relatively high and variable larval mortality in clofentezine and control treatments may to some extent reflect that younger more fragile larvae had to be transferred to minimise egg cannibalism. Once pupated >95% adult emergence was recorded. Thus no significant treatment-related effects on eggs, larvae, pupation and adult emergence was discernible at 100 and 200g clofentezine/ha indicating absence of effect of clofentezine on *Coccinella septempunctata* reproduction.

B.9.5.5 Conclusions

The data submitted provide further supporting evidence to the conclusion that the general risk to adults, eggs and young motile stages of NTAs from the proposed uses of clofentezine is low.

B.9.6. Effects on earthworms

B.9.6.1 Reporting table, clofentezine (UK) - rev0_24_oct_2006

The earthworm section of the addendum addresses point 5(25) (also relevant to 5(24)) and point 5(26), which also addresses 5(29).

B.9.6.2 RMS consideration - point 5(25) long term NOEC

On the basis of the earthworm toxicity data and risk assessment presented, the DAR (B.9.6.2) concludes that there is a low acute and chronic risk to earthworm from the proposed uses of clofentezine.

However, some concern was expressed regarding the long term risk assessment not being sufficiently addressed, primarily with respect to the selection of the chronic NOEC used in the risk assessment. In the DAR results from two chronic earthworm studies were considered acceptable Staebler, 2002b and Rodgers, 2001. A NOEC of 1.5 kg a.s./ha (based on effects at 3.0 kg a.s./ha) using 'Apollo 50SC' was derived in the former study, whilst a NOEC of 5.5 kg a.s./ha, the only rate tested, using another SC formulation was derived from the latter.

A further case addressing this issue has been provided by the Notifier with refinement of calculation of the soil a.s. concentration from the endpoint (1.5 kg a.s./ha) derived from the Staebler, 2002b study, which has been presented in full below (*in italics*).

Endpoint from earthworm reproduction study: The earthworm reproduction study (Staebler 2002b) has been used as the basis of the long term risk assessment for earthworms. The NOEC from this study is stated in the study report as 2.0 mg a.s./kg soil, which resulted from a spray application to the soil surface in the study at 1.5 kg a.s./ha. The calculation of the concentration in the soil in the study report was based on the generic assumption of a soil depth of 5cm and a soil density of 1.5 g/cm3. However, in accordance with the EU guidance document on terrestrial ecotoxicology (page 30), when the TER is close to the trigger of 5 (as in this case) the actual mass of soil in each test vessel and the surface application rate can be taken into account in the calculation of the NOEC (mg a.s./kg soil). This calculation is as follows:

In this study (Staebler 2002b) the surface dimensions of each vessel was 17 x 12.5 cm, giving a surface area of 212.5 cm^2. The application rate of 1.5 kg a.s./ha equates to a rate of 0.015 mg a.s./cm^2. Hence, the mass of clofentezine applied to each vessel was 212.5 x 0.015 = 3.1875 mg a.s. Each vessel contained a measured dryweight of soil of 0.60 kg. Hence, the concentration in the soil for the purpose of the risk assessment should be 3.1875 mg / 0.6 = 5.3125 mg a.s./kg. This NOEC has to be divided by 2 to allow for the 10% organic matter content of the test soil (which is higher than in the field). Hence, the NOEC for use in the risk assessment should be 5.3125 / 2 = ***2.656 mg a.s./kg****. The maximum PEC used in the risk assessment in the DAR (p427) is* ***0.268 mg a.s./kg****. This gives a* ***TER of 2.656 /0.268 = 9.9****. This is greater than the trigger of 5, indicating a low risk.*

Based on theoretical application rate/soil concentration calculation with a 50% correction for high (10%) organic matter (clofentezine log Kow >2) content in tests, respective NOECs of 1.0 and 3.67 mg a.s./kg soil from the Staebler and Rodgers can be derived. Assuming a maximum PEC of 0.268

mg/ kg soil this derives TERs of 3.7 and 13.7, respectively. Thus, low chronic risk is indicated by the latter TER (>5), but not by the former. It should be noted that the maximum soil PEC is worse case as it assumes highest application rate and no foliar interception, >25% interception would also generate a TER>5 using a NOEC of 1 mg a.s./kg soil. The Notifier has also refined the risk assessment (see above) for the Staebler study endpoint (NOEC = 1 mg a.s./kg soil) by estimating actual a.s. concentration applied to soil which generates a NOEC of 2.7 mg a.s./kg soil and a TER of 10, i.e. also indicative of low risk. Thus RMS considers that overall there is sufficient weight of evidence to indicate a low chronic risk to earthworms.

Conclusion:

The overall conclusion is that the chronic risk to earthworms from proposed uses of clofentezine will be low and the worm field study, not regarded as of sufficient quality for risk assessment, need not be consulted (point 5(24)).

B.9.6.3 RMS consideration - point 5(26) effects of soil metabolite (AE C593600) on earthworm

The DAR does not consider the risk to earthworms from the soil metabolite AE C593600. The Notifier has submitted a case addressing the risk this metabolite presents to soil organisms, which is reproduced in full below *in italics.*

Clofentezine soil metabolite AE C593600: Reasoned case to address risk to non-target soil organisms and processes

The chemical name of AE C593600 is 2-chlorobenzoic (2-chlorobenzylidene) hydrazide. In a soil route of degradation study (Draft Assessment Report (DAR),p291-293, Leake and Arnold, 1983a) the degradate AE C593600 was identified which accounted for a maximum 13% applied radioactivity (%AR) at one time point in only one of the three soils tested. In the other two soils maximum amounts accounted for ca 3% AR. AE C593600 is structurally similar to clofentezine and is formed following cleavage of the tetrazine ring. As it accounted for >10% AR it was concluded by the Rapporteur Member State (RMS) to be the only major metabolite in soil (DAR, p310).

AE C593600 can be formed by both abiotic and biotic degradation and is the first step in the soil degradation pathway. AE C593600 itself is further degraded (DAR, Figure B8.2, p311) by biotic and abiotic processes to the minor metabolites 2-chlorobenzamide and N,N'bis (2-chlorobenzoyl hydrazine). As the metabolite accounted for >10%AR, there is a requirement according the EUguidance document to assess the risk to soil dwelling organisms.

SANCO/10329/2002 rev 2 final accepts that such assessments do not have to be addressed by experimental studies. Studies on the acute toxicity of AE

C593600 to earthworms, soil macro and microorganisms have not been conducted. A rationale using data from the current DAR is presented below.

Worst case predicted environmental concentrations in soil (PECs values) for this metabolite are presented in Table B.8.28 of the DAR (p321). The maximum PECs was 0.027 mg/kg immediately after application. This compares to an initial PECs for clofentezine of 0.213 mg/kg (accumulated PECs used for risk assessment was 0.268mg/kg).

Clofentezine toxicity to earthworms, soil non-target macro organisms and soil microorganisms has been assessed. The corrected 14-day LC50 for earthworms was >215mg/kg and a toxicity exposure ratio (TER) of 800 was calculated (DAR, p427). The corrected NOEC for Apollo 50SC on Folsomia candida was 80 mg/kg giving a TER of 299 (DAR p428-429). It was concluded that even at an exaggerated (10X) rate of 2 kg a.s/ha that clofentezine has no adverse effects on either soil respiration or nitrogen turnover.

Overall it can be concluded that clofentezine is of low toxicity to soil macro and micro fauna. Due to the persistence of clofentezine in some soils a litter bag study was requested in the DAR (level 4) to address the chronic risk to soil fauna. This study (Irvita Report R-17802, Carter, 2006 is submitted to PSD with this statement for evaluation) concluded there was no significant impact on soil organic matter breakdown after application of Apollo 50SC at 200g a.s./ha.

If it is assumed that the degradate AE C593600 is of equal toxicity to clofentezine based on its structural similarity, the TER's can be estimated at >7900 for earthworms and >2900 for Folsomia using the PECs of 0.027 mg/kg. In other words AE C593600 would have to be more than 100 times more toxic than clofentezine to give TER values anywhere near the TER trigger of 5. This can be concluded to be extremely unlikely.

Furthermore the toxicity of the degradate will have been taken into account in the soil microorganism tests and litter bag study, both of which showed no adverse effects. The risk assessments presented in the DAR for soil dwelling organisms do not explicitly consider this metabolite in Annex B9, ecotoxicology. However, the formation of AE C593600 in soil was clearly considered during the evaluation by the RMS given the conclusion on the definition of the residue (DAR, p345, B8.8), which states:
'On the basis of the risk assessments conducted for the metabolite AE C593600 in soil and water the risks to soil dwelling organisms and aquatic indicator species were considered negligible (section B.9). The relevant residue for monitoring in soil, surface water, sediment, groundwater and air would therefore be clofentezine only.' On the basis of the information presented here this conclusion remains valid and should not require any amendment.

References

Draft Assessment Report for clofentezine. Report and proposed decision of the UK made to the European Commission under Article 8(1) of 91/414/EEC. August 2005
Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC. SANCO 10329/2002 rev 2 final

(Dean, 2006)

Conclusion:

The RMS concurs with the Notifier that due to low toxicity of clofentezine (which structurally resembles the soil metabolite AE C593600), approximately 10x less AE C593600 exposure potential in soil than parent and the likely formation of AE C593600 in clofentezine (soil DT50=71.3d) tests on soil microorganisms and soil litter degradation where no adverse effects were recorded, there sufficient evidence to indicate a low risk to soil-dwelling organisms from AE C593600 from its formation in soil following proposed uses of clofentezine. NB this case also addresses Reporting Table points 5(26) and 5(29).

B.9.7 Effects on soil non-target macro-organisms

B.9.7.1 Reporting table, clofentezine (UK) - rev0_24_oct_2006

The earthworm section of the addendum addresses points 5(27) and 5(28).

B.9.7.2 RMS consideration - soil litter bag study

The DAR (B.9.7.2) concluded from the earthworm and Folsomia risk assessment that the risk to soil macro-invertebrates from proposed uses of clofentezine was low. However, since in field studies soil DT90s ranged from 22 to 640.5d (DAR B.8.1.3), according to SANCO/10329/2002 if soil DT90>365d the impact on soil litter (soil litter bag study) should be assessed. The Notifier has since submitted a soil litter bag study which has been evaluated by the RMS at B.9.5.3 below.

B.9.7.3 Effects of clofentezine on soil litter degradation

Table B.9.7.1 Summary of study on effects of clofentezine on soil litter degradation.

Test Guideline	GLP	clofentezine mg a.s./kg DS (nominal)	% straw degradation in treated soil [month post treatment] (% deviation from control)	Reference (report no.)
EPFES Workshop (2002)	Yes	0.08[1] + 0.267[2]	13.47 [1] (-1.5) 37.03 [3] (-3.1) 59.56 [6] (+3.0) 69.05 [12] (+1.2)	Carter *et al*., 2006 (R-17802)

[1] calculated soil plateau accumulation concentration - 10cm soil treated
[2] maximum annual application rate (200g a.s./ha = 0.267mg a.s./kg @5cm)

A study assessing clofentezine effects on soil litter degradation was submitted using the product 'Apollo 50SC', a suspension concentrate (density = 1.187 g/mL) containing clofentezine (517 g/L) as active substance.

The GLP compliant litter bag study was conducted in accordance with EPFES SETAC Workshop (2002)[1] recommendations and validity criteria. No protocol deviations affecting the study integrity and interpretation were reported and the RMS considered the study acceptable for risk assessment.

The field study commenced in late April when six of twelve field plots (5m x 5m) were treated with 'Apollo 50SC' (0.48ml product/L = 0.24g clofentezine/L) to achieve a nominal soil (clay:loam) concentration 0.08mg clofentezine/kg soil (over 10cm depth), the theoretical long term soil plateau a.s. concentration. Plots were then rotavated to a 10cm depth. After 14d, 34 non-degradable 10 x 20cm litter bags (mesh 5 x 5mm) containing 4.0g of dried organic straw (10cm segments) were buried to 5cm depth in the plots followed by a maximum annual spray surface application of 'Apollo 50SC' (200g clofentezine/ha). Control plots were treated with water. Mean earthworm counts at the test site were 61/m^2.

Post spray soil samples (10cm depth) and soil surface filter paper spray intercept samples were taken. Samples were extracted into methanol and partitioned into dicholoromethane prior to analysis by LC-MS/MS and the method was validated by fortified sample analysis. Mean measured clofentezine was 111.3 and 98.6% nominal concentrations after respective first and second soil treatments.

Organic matter breakdown was measured after drying, sieving and combusting ground organic matter from litterbags sampled at 1, 3, 6 and 12 months after treatment (see Table B.9.7.2). Soil moisture (range 15.23-18.05%) was also monitored over the treatment period along with collection of weather data.

Table B.9.7.2 Straw degradation in litter bags after soil treatment with 'Apollo 50SC'

Soil incubation period (month[3])	**Mean straw degraded[1] % total (standard deviation)**		**% difference from control**
	Control soil	**'Apollo 50SC' treated soil[2]**	
1	14.95 (6.52)	13.47 (4.04)	-1.5

[1] EPFES Lisboa 2002, Effects of Plant Protection Products on Functional Endpoints in Soil, (eds. Römbke *et al*.,) SETAC publications 2003

3	40.14 (8.96)	37.03 (8.07)	-3.1
6	56.56 (9.73)	59.56 (8.38)	+3.0
12	67.88 (11.33)	69.05 (10.69)	+1.2

[1] **mean of 6 plots**
[2] **nominal total clofentezine soil concentration (plateau + max. individual dose)**
[3] **after litter bag insertion**

Straw degradation in the control was 60% at 6 months with ≤40% variation, thus fulfilling experimental validity criteria recommended by EPFES (2002). The results of the study show that after 1, 3, 6 and 12 month soil incubation no statistically significant difference could be discerned in straw degradation observed in untreated control soil and in soil treated with 'Apollo 50SC' giving 0.347mg clofentezine/kg soil representing long term exposure from plateau soil clofentezine accumulation with addition of annual maximum application.

B.9.7.5 Conclusion:

The absence of significant effect on soil litter degradation over 12 months in soil treated with predicted maximum soil plateau clofentezine level followed by an annual maximum clofentezine application (worse case as no interception assumed) supports the conclusion that the risk to soil macro-invertebrates and soil degradation processes will be low from all proposed uses of clofentezine.

B.9.11 References relied on

Active substance: Clofentezine

Annex point / Ref. No.	**Author**	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP status, published or not**	**Data protection claimed** **Y/N**	**Owner***
IIA 8.2.2.2/02[c]	Cockcroft, R.	2005	Cofentezine 50SC: Fish early life stage toxicity test for fathead minnow. Huntingdon Life Sciences Report No. IRV099/052509. Irvita Plant Protection NV, Report No. R-17810. GLP. Unpublished.	Y	Irvita

Annex point / Ref. No.	Author	Year	Title Source (where different from company) Company, Report No. GLP status, published or not	Data protection claimed Y/N	Owner*
IIA 8.3.2/26[c]	Taylor, K.	2005	Apollo 50SC: Evaluation of the effect on the eggs of the ladybird beetle *Coccinella*. Huntingdon Life Sciences Report no. IRV094/ 043750. Irvita Plant protection NV Report No. R-17808 GLP. Unpublished	Y	Irvita
IIA 8.3.2/27[c]	Taylor, K.	2005	Apollo 50SC: Evaluation of the effect on the rove beetle, *Aleochara bilineata* in an extended laboratory study. Huntingdon Life Sciences Report no. IRV093/052165. Irvita Plant protection NV Report No. R-17809 GLP. Unpublished	Y	Irvita

*Irvita Plant Protection, owner of the substance clofentezine, is a Member of Makhteshim-Agan Industries (MAI) group. Referenced studies refer to this ownership by either the abbreviation "MAK" or by "Irvita". As notifier, Irvita is represented in Europe by Makhteshim International Coordination Centre (MAICC), Brussels.

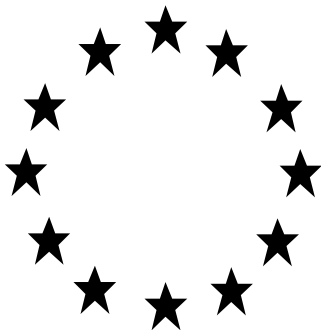
Plant protection product – Apollo 50 SC

Annex point / Ref. No.	Author	Year	Title Source (where different from company) Company, Report No. GLP status, published or not	Data protection claimed Y/N	Owner*
IIIA 10.1.2/01[a]	Riffel, M. & Giessing, B.	2005	Bird species in German strawberry fields – a preliminary survey. Rifcon GmbH Report no. RC05-002. Irvita Plant Protection NV, Report no. R-17819. Non GLP, Unpublished.	Y	Irvita
IIIA 10.1.2/02[a]	Scheurig, M. & Dietzen, C.	2006	Bird species in strawberry fields in Germany: field data for the determination of focal species. Rifcon GmbH Report no. RC06-036. Irvita Plant Protection NV, Report no. R-20182. Non GLP, Unpublished.	Y	Irvita
IIIA 10.1.2/03[a]	Moosmayer, P.	2006	Feeding ecology of relevant insectivorous bird species in strawberry fields in Germany. Rifcon GmbH Report no. RC06-054. Irvita Plant Protection NV, Report no. R-20183. GLP, Unpublished.	Y	Irvita
IIIA 10.1.2/05[c]	Schwarz, A.	2006	Generic field monitoring of birds in orchards. Rifcon GmbH Report no. RA 06016-1. Irvita Plant Protection NV,, Report no. R-21219. GLP, Unpublished.	Y	Irvita

Annex point / Ref. No.	Author	Year	Title Source (where different from company) Company, Report No. GLP status, published or not	Data protection claimed Y/N	Owner*
IIIA 10.6.2/02[a]	Carter, J.	2006	Clofentezine (Apollo 50SC): Breakdown of organic matter in litter bags. Huntingdon Life Sciences Report no. IRV111/063137. Irvita Plant Protection NV, Report no. R-17802. GLP, Unpublished.	Y	Irvita

*Irvita Plant Protection, owner of the substance clofentezine, is a Member of Makhteshim-Agan Industries (MAI) group. Referenced studies refer to this ownership by either the abbreviation "MAK" or by "Irvita". As notifier, Irvita is represented in Europe by Makhteshim International Coordination Centre (MAICC), Brussels.

Council Directive 91/414/EEC



Clofentezine

Volume 4

Annex C

to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8 of Council Directive 91/414/EEC

Confidential Information

Revised June 2007

CONFIDENTIAL BUSINESS INFORMATION:

available at RMS

Clofentezine

Draft Assessment Report

Addendum 2

Methods of Analysis, Mammalian Toxicology, Residues, Environmental Fate and Behaviour, Ecotoxicology

December 2008

CONTENTS **Page**

B.5 Methods of analysis

B.5.2 Analytical methods (residue) for treated plants, plant products, foodstuffs of plant and animal origin and feedingstuffs (IIA 4.2.1, IIIA 5.2)

Plant and plant products

Samples were extracted with acetonitrile and the resulting extracts cleaned up on a solid phase extraction column and analysed by LC/MS/MS (monitoring for the precursor ion m/z 303 and the product ion m/z 138 [and 102 for conformation]), using a C18 column. Limit of determination was 0.01 mg/kg. The supporting method validation data are shown in table B.5.1 . Representative chromatograms were submitted and were acceptable.

(Wiesner and Daneva, 2008)

Table B.5.1 Summary of method description and validation (treated plants samples)

Substrate	Analyte	Dissolution/ extraction	Partition, clean-up	Quantification	Limit of quantif-ication (mg/kg)	Recovery fortifica-tion level (mg/kg)	Recoveries % range (mean)	Repeatabil-ity RSD (%) (n)	Linearity demon-strated (mg/l)	Ref.
Strawberry	Clofentezine	Acetonitrile extraction	Solid phase extraction cartridge	HPLC-MS-MS m/z 138 m/z 102	0.01 0.01	0.01 0.1 0.01 0.1	85-92 (89) 83-101 (89) 91-101 (94) 85-95 (91)	2.9 (5) 7.9 (5) 3.7 (5) 4.9 (5)	0.00025-0.025 0.00025-0.025	Wiesner and Daneva, 2008
Melon peel	Clofentezine	Acetonitrile extraction	Solid phase extraction cartridge	HPLC-MS-MS m/z 138 m/z 102	0.01 0.01	0.01 0.1 0.01 0.1	81-94 (87) 74-87 (81) 89-99 (94) 80-93 (85)	5.6 (5) 6.0 (5) 4.7 (5) 6.4 (5)	0.00025-0.025 0.00025-0.025	Wiesner and Daneva, 2008
Melon pulp	Clofentezine	Acetonitrile extraction	Solid phase extraction cartridge	HPLC-MS-MS m/z 138 m/z 102	0.01 0.01	0.01 0.1 0.01 0.1	86-103 (93) 85-92 (89) 86-103 (94) 83-93 (87)	6.3 (5) 2.6 (5) 6.6 (5) 5.1 (5)	0.00025-0.025 0.00025-0.025	Wiesner and Daneva, 2008
Peach	Clofentezine	Acetonitrile extraction	Solid phase extraction cartridge	HPLC-MS-MS m/z 138 m/z 102	0.01 0.01	0.01 0.1 0.01 0.1	88-115 (101) 84-95 (90) 87-116 (101) 86-93 (90)	11 (5) 4.6 (5) 13 (5) 3.0 (5)	0.00025-0.025 0.00025-0.025	Wiesner and Daneva, 2008
Cucumber	Clofentezine	Acetonitrile extraction	Solid phase extraction cartridge	HPLC-MS-MS m/z 138 m/z 102	0.01 0.01	0.01 0.1 0.01 0.1	84-99 (93) 80-89 (83) 87-103 (95) 83-88 (85)	5.9 (5) 4.1 (5) 7.1 (5) 2.6 (5)	0.00025-0.025 0.00025-0.025	Wiesner and Daneva, 2008
Tomato	Clofentezine	Acetonitrile	Solid phase extraction	HPLC-MS-MS						Wiesner and

		extraction	cartridge	m/z 138 m/z 102	0.01 0.01	0.01 0.1 0.01 0.1	82-92 (86) 81-88 (85) 84-85 (84) 88-99 (89)	6.2 (3) 4.5 (3) 0.7 (3) 3.6 (3)	0.00025- 0.025 0.00025- 0.025	Daneva, 2008
Sweet pepper	Clofentezine	Acetonitrile extraction	Solid phase extraction cartridge	HPLC-MS-MS m/z 138 m/z 102	 0.01 0.01	 0.01 0.1 0.01 0.1	 87-100 (92) 80-85 (82) 92-96 (94) 88-90 (89)	 7.8 (3) 3.2 (3) 2.2 (3) 1.1 (3)	 0.00025- 0.025 0.00025- 0.025	Wiesner and Daneva, 2008
Apricot	Clofentezine	Acetonitrile extraction	Solid phase extraction cartridge	HPLC-MS-MS m/z 138 m/z 102	 0.01 0.01	 0.01 0.1 0.01 0.1	 85-88 (86) 84-89 (86) 87-98 (92) 87-88 (87)	 1.7 (3) 3.0 (3) 6.0 (3) 0.7 (3)	 0.00025- 0.025 0.00025- 0.025	Wiesner and Daneva, 2008

B.5.5 Summary of Methods of Analysis

Clofentezine residues in plant and plant products were determined by extraction with acetonitrile and the resulting extracts analysed by HPLC-MS/MS (monitoring for the precursor ion m/z 303 and the product ion m/z 138 [and 102 for conformation]). The limit of determination was 0.01 mg/kg. Acceptable validation data were submitted.

B.5.7 References relied on

Annex point / Ref. No.	Author	Year	Title Source (where different from company) Company, Report No. GLP status, published or not	Data protection claimed Y/N	Owner*
IIA 4.2.1	Wiesne, F. Daneva, E	2008	Validation of an analytical method for the determination of residues of clofentezine in different plant matrices with high water content; Sudy ref: R-22236	Y	Irvita

B.6 Mammalian toxicology

B.6.1 Absorption distribution excretion and metabolism (toxicokinetics) (IIA 5.1)

B.6.1.3 Summary of absorption, distribution, metabolism and excretion

B.6.1.3.1 Assessment of the Relevance of Plant metabolites

Open point 2.5
Pending on confirmation from the residue experts' meeting, the toxicological relevance of clofentezine metabolites 2-chlorobenzonitrile (and its degradation products 2-chlorobenzoic acid, 2-chlorobenzylalcohol, 2-chlorobenzaldehyde) and (2-chlorobenzoic acid (2-chlorobenzylidene) hydrazide) has to be discussed in a meeting of experts

The main residues in fruit crops are the parent clofentezine, and metabolite 2-chlorobenzonitrile. The levels of 2-chlorobenzonitrile found were <0.05 mg/kg, which was approximately a tenth of those of the parent residue. Based on a residue of 0.05 mg/kg and intakes figures for apples (which are the highest values of the proposed crops), potential consumer intakes of 2-chlorobenzonitrile would be < 0.0007 mg/kg bw/day (>4% of the ADI).

The issue of the degradation products of 2-chlorobenzonitrile appears to have arisen from their mention in a static study on photo degradation in the Physical Properties Section. In the grape metabolism study they were measured as a total 'polar fraction' (i.e. total sum of all degradation products of 2-chlorobenzonitrile). A the field rate application the sum of all all degradation products of 2-chlorobenzonitrile amounted to 0 005 mg/kg or 1.4% of the TRR.

Overall it is considered by the RMS that 2-chlorobenzonitrile or the degradation products of 2-chlorobenzonitrile are of no toxicological significance at these levels, and should not be included in the residue definition.

The Notifier has provided a case to dismiss the relevance of these metabolites which is summarise below:

2-chlorobenzonitrile concentration in plants

The first question to answer is to provide an understanding of the actual concentrations of 2-chlorobenzonitrile reported in the studies of apple, peach and grape metabolism. These have been extensively summarised in the residues section of the DAR[5] (pages 209-216). The concentration presented in the ADME summary (0.05 mg/kg) and used in the consumer risk assessment by the RMS was very much a worst case and conservative in nature.

2-chlorobenzonitrile accounted for in many cases far less than 0.05 mg/kg (and <10% TRR) in plant metabolism studies in apple, peach and grape. Clofentezine

was the only major residue at concentrations 10-20 fold higher than those of the metabolite. There were no other metabolites in any study that accounted for >0.01 mg/kg or >10% TRR. Extensive work was carried out on the fibre bound residue in order to identify metabolites far below 0.01 mg/kg and establish the route of degradation in plants (see further discussion below).

Table 1 summarises the results from 2 apple studies (Kelly, 1985[1] and Edwards, 1987[2], DAR pages 209-219) and provides estimates of the concentration of 2-chlorobenzonitrile based on the fact that, in both studies, it was shown to represent 4% of the extracted radioactivity, correcting the concentration for the difference in molar mass between the parent and metabolite and normalising the concentration to the supported GAP of 0.02% where appropriate. Hence, even in the worst-case example, where the PHI was 25 days (10 days less than the GAP), the highest residue of 2-chlorobenzonitrile is only 0.001 mg/kg. Thus, in reality, residues of 2-chlorobenzonitrile are an order of magnitude below 0.01 mg/kg and nearly 50 times less than the 0.05 mg/kg used to estimate potential human exposure in the DAR example.

Identification of 2-chlorobenzonitrile at these concentrations goes far beyond the requirements of SANCO 7028/VI/95 that requires residues to be characterised and identified that exceed 0.05 mg/kg or 10% total radioactive residue.

In peaches, (Edwards, 1988[3], DAR page 221) 2-chlorobenzonitrile accounted for 0.004 mg/kg (8.4% TRR) after treatment according to the GAP and 0.038 mg/kg (5.4% TRR) at 10 times the GAP. Correcting these values for molar mass and the difference in rate gives actual residues for 2-chlorobenzonitrile of 0.0018 mg/kg at the normal GAP application rate (Table 1). Therefore actual concentrations in peaches are 25 times less than the 0.05 mg/kg used to estimate potential human exposure in the DAR example.

In Grapes, (Campbell, 1989[4], DAR P 225) the 2-chlorobenzonitrile residues accounted for 0.04 mg/kg (9.6 % TRR) at a PHI of 24-25 days and 0.006 mg/kg (5.11% TRR) at a PHI of 45-46 days. Extrapolation to a PHI of 35 days, assuming a linear decline (worst case), would give a residue of 0.02 mg/kg (7.5% TRR). Correcting this value for the difference in molar mass gives a 2-chlorobenzonitrile concentration of 0.009 mg/kg. At 10 times the rate, the normalised and corrected concentrations were 0.008 mg/kg and 0.001 mg/kg at PHIs of 24-25 and 45-46 days respectively (Table.B.6.1). Thus, whilst residues of the metabolite are highest in this crop, they are still at least 6 times lower than the 0.05 mg/kg used to estimate potential human exposure in the DAR example.

Table B.6.1: Actual 2-chlorobenzonitrile concentrations in various plant metabolism studies

Commodity	Treatment rate (kg a.i/ha)(superscript = report reference on p1)	No. of times greater than supported GAP	Total radioactive residue at harvest (mg clofentezine equiv./Kg)	Total radioactive residue normalised to GAP rate (mg clofentezine equiv./Kg)	PHI	Extractable residue (mg clofentezine equiv./Kg)	Concentration of 2-chlorobenzo-nitrile (mg clofentezine equiv./Kg)	Concentration of 2-chlorobenzo-nitrile (mg /kg)[a]	Normalised concentration of 2-chloro-benzonitrile (mg/Kg) to GAP rate
	A	B	C	D	E	F	G	H	I
Apple	0.3[11]	1.5	0.031	0.021	75	0.019	0.0008	0.0004	0.0002
	0.6[2]	3	0.080	0.027	64	0.064	0.0026	0.0011	0.0004
	4.8[2]	24	0.764	0.032	64	0.641	0.0256	0.0118	0.0005
	7.6[1]	38	0.995	0.026	75	0.922	0.0369	0.0170	0.0004
	0.6[2]	3	0.224	0.075	25	0.204	0.0082	0.0038	0.0013
	0.6[2]	3	0.097	0.032	25	0.090	0.0036	0.0017	0.0005
Peach	0.1[3]	1	0.047	0.047	62	0.047	0.0039	0.0018	0.0018
	1.0[3]	10	0.701	0.070	62	0.701	0.0379	0.0174	0.0017
Grape	0.1[4]	1	0.38	0.38	25	0.36	0.04	0.0184	0.0184
					35		0.02[b]	0.0092	0.0092
		1	0.11	0.11	45	0.10	0.006	0.0028	0.0028
	1.0[4]	10	2.52	0.252	25	2.45	0.18	0.0828	0.0083
					35		0.10	0.0490	0.0049
		10	0.45	0.045	45	0.42	0.033	0.0152	0.0015

[a] Corrected by a factor of 0.46 for the difference in molar mass between clofentezine (301.3) and 2-chlorobenzonitrile (137.6)
[b] Calculated by linear regression

Example calculations:
A, B, C, E, F taken from DAR (Study reports)
D = C / B
G = (F x % TRR 2-chlorobenzonitrile) / 100 (for grape value taken from tables in DAR)
H = G X 0.46
I = H / B

Chronic dietary risk assessment for 2-chlorobenzonitrile

In the estimation presented in the DAR (page 82), exposure was estimated at 0.0007 mg/kg bw/day based on the following worst-case figures:

- An estimated 2-chlorobenzonitrile residue of <0.05 mg/kg.
- The 97.5th percentile consumption value for toddlers eating apples (0.2156 kg).

Even using these extremely worst-case figures, it was concluded that 2-chlorobenzonitrile is of no toxicological concern.

This estimation has been refined on the basis of the actual 2-chlorobenzonitrile concentrations shown in Table B.6.1 for the most vulnerable population (toddlers). The results are presented in the table below:

Crop	Maximum residues level (mg/kg)	97.5th percentile consumption (kg/day)	Maximum intake (mg/kg bw/day)	% ADI
Apple	0.0013	0.2156	0.00002	0.1
Peach*	0.0018	0.0312	0.000004	0.02
Grape	0.0090	0.0681	0.00004	0.2

* as representative stone fruit for the intended use "plum"

Thus, examination of the reported data shows that concentrations of 2-chlorobenzonitrile are in reality much less than 0.05 mg/kg and even less than 0.01 mg/kg. Using more realistic residue concentrations, but still worst-case consumption data for the relevant commodity (from the UK chronic dietary exposure model), then the intake by the most vulnerable consumers is by an order of magnitude below the 0.0007 mg/kg bw/day presented in the DAR and concluded by the RMS not of any concern.

Toxicological information on 2-chlorobenzonitrile

From the above, it can be seen that residues of 2-chlorobenzonitrile are <0.01 mg/kg in the representative crops when clofentezine is used according to the supported GAPs.

On this basis alone, it should be concluded that the metabolite is of no toxicological concern and no further consideration of its potential toxicity is required. This is in line with the conclusion made in the DAR.

However, the EFSA, irrespective of the estimated exposure levels, requested information on its potential toxicity.

2-chlorobenzonitrile (EC No.: 212-836-5, CAS No.: 872-32-5) was classified for its hazard by the European Chemical Bureau (19th ATP) as

Xn, R21/22, Xi R36. The chemical is used in various other applications of organic chemistry.

From a commercial MSDS[2], eye irritation in the rabbit at 100 mg for 24 hours is reported as "moderate" and the mouse oral LD50 is >300mg/kg. It is not listed as a carcinogen by ACGIH, IARC, NTP or CA prop 65. No other information was found on its toxicological properties".

Conclusion on toxicological relevance of 2-chlorobenzonitrile

In conclusion, residues of 2-chlorobenzonitrile in crops treated according to the representative GAPs are likely to be much less than 0.01 mg/kg. It should be concluded there is no risk of exposure to consumers from 2-chlorobenzonitrile residues in clofentezine treated fruit, reinforcing the conclusion already made in the DAR by the RMS.

Due to the very low residue concentrations and estimated very low exposure it can be concluded the residue definition for food and feed of plant origin should remain, as concluded in the DAR, to be clofentezine only.

Other metabolites concentrations in plants

As summarised in the DAR (pages 211-212), Kelly[1] reported that for apples treated at 1.5N rate the only other residue with a TRR >0.01 mg/kg (>4% TRR) was the fibre bound residue found in apple peel (0.012 mg/kg). At normal rates this would equate to 0.008 mg/kg. Harsh chemical extraction procedures released *ca* 71% of the fibre radioactivity (0.006 mg/kg), which was found to be predominantly 2-chlorobenzoic acid (2CBA). This compound has probably resulted from the hydrolysis of clofentezine or similar metabolites.

A further study was conducted by Edwards[2] in an attempt to confirm the identity of the extractable residue (clofentezine and 2-chlorobenzonitrile only) and identify / characterise the fibre bound residue more fully. This study is summarised in the DAR (pages 212-216).

The extractable peel residue was shown to be 84% clofentezine and 4.3% 2-chlorobenzonitrile. The remaining 8.5% extractable residue was made up of several minor polar components which did not individually constitute more than 4% TRR (0.001 mg/kg at GAP rate).

The fibre bound residue was investigated by various chemical and enzyme hydrolysis techniques.

After base hydrolysis of the fibre bound residue, it was shown that 20% of the radioactivity present was 2CBA. By comparison to spiking experiments where clofentezine was taken through the extraction procedure, it was concluded that a 20% yield of 2CBA would be obtained if 50% of the fibre

[2] Arcos Organics NV. MSDS for 2-chlorobenzonitrile. https://fscimage.fischersci.com/msds/98636.htm

bound radioactivity were clofentezine. At the 2N rate, fibre bound residue was maximally 0.009 mg/kg of which 0.0045 mg/kg is clofentezine. Therefore at normal rate, the fibre bound residue would account for 0.0045 mg/kg of which 0.00225 mg/kg is clofentezine. It was not possible to identify any of the other polar metabolites extracted in the remainder 0.0045 mg/kg corresponding to 0.00225 mg/kg at the normal rate.

Enzyme hydrolysis released 7.7% of the fibre bound residue (equivalent to 0.0003 mg/kg at normal rates). Of this 43.3% (3.3% TRR or 0.0002 mg/kg fibre bound residue) was identified in a 2:1 ratio as 2CBA (0.0001 mg/kg) and 2-chlorobenzyl alcohol (0.00005 mg/kg). Edwards[2] comments: *"that trace quantities of both the 2-chlorobenzylalcohol and 2-chlorobenzaldehyde were also tentatively identified in other enzyme hydrolysis experiments"*. Thus, it can be assumed that they would each represent <0.0001 mg/kg in apples treated at normal rates. These metabolites, released by chemical and enzyme hydrolysis, were considered to exist in both the free and conjugated forms. Thus, concentrations of these other metabolites are **several** orders of magnitude lower than clofentezine, not one order of magnitude as concluded by the EFSA in their comment 2(14).

Subsequently, it was shown that this fibre bound residue from the Kelly study[1] was not bioavailable when dosed orally to rats. 97% of the administered radioactivity was recovered unabsorbed in the faeces (Needham & Hemmings, 1985[3]. EU data point: Annex II, 5.8.2.5/01, DAR pages 158-160).

On this basis, it can be concluded that the fibre bound residues are of no toxicological concern.

For information purposes, the following toxicological information was found by the notifier for the other very minor plant metabolites:

- 2-chlorobenzaldehyde (EC No.: 201-956-3, CAS No.: 89-98-5) is classified for its hazard by the European Chemical Bureau (19th ATP) as C, R34. The chemical is used in various other applications of organic chemistry. An IUCLID dataset is available for this compound[4]. The rat LD50 is *ca* 2480 mg/kg bw. It is negative in Ames, gene mutation, and micronucleus tests. In rats it is metabolized to 2-chlorobenzoic acid which is conjugated with glycine and excreted as 2-chlorohippuric acid.
- 2-chlorobenzyl alcohol (CAS No.: 17849-38-6) is not classified in Annex I of Directive 67/548/EEC. From a commercial MSDS[5] it is classified *"not hazardous but may cause skin and eye irritation". "It is not listed as a carcinogen by ACGIH, IARC, NTP OSHA. No other information was found on its toxicological properties.*

[3] Needham, D. and Hemmings, P.A., 1985. The bioavailability of clofentezine fibre bound residues in the rat. FBC Ltd., report no. METAB 85/39 62J. Aventis no. A82031 = M42. MAK no. R-12554. GLP, unpublished.

[4] Available at http://ecb.jrc.it/esis-pgm/

[5] Arcos Organics BVBA. MSDS for 2-chlorobenzyl alcohol. http://newsearchch.chemexper.com

- 2-chlorobenzoic acid (CAS No. 118-91-2) is not classified in Annex I of Directive 67/548/EEC. From a commercial MSDS[6] it is given the hazard symbol *"Xi, "moderate eye irritation" in the rabbit at 20 mg for 24 hours and "mild skin irritation" at 500 mg for 24 hours is reported. The rat oral LD50 is 2465 mg/kg bw. It is not listed as a carcinogen by ACGIH, IARC, NTP or CA prop 65. No other information was found on its toxicological properties.*

Conclusion on the toxicological relevance of plant metabolites other than 2-chlorobenzonitrile

In conclusion, using exaggerated application rates, the route of degradation of clofentezine in fruit has been elucidated. However, actual residue levels of
2-chlorobenzaldehyde, 2-chlorobenzyl alcohol and 2-chlorobenzoic acid in fruit after treatment at the representative GAPs would be expected to be many times lower than the LOQ (0.01 mg/kg) for normal analytical methodology. Furthermore, the metabolites were bound to a mixture of fibre components and this fibre residue was shown not to be bioavailable. It can be concluded that exposure to consumers of these residues would be extremely low (several orders of magnitude less than clofentezine) and are of no toxicological concern.

These analyses further support the conclusion in the DAR that the residue definition for food and feed of plant origin should be clofentezine only.

Footnote

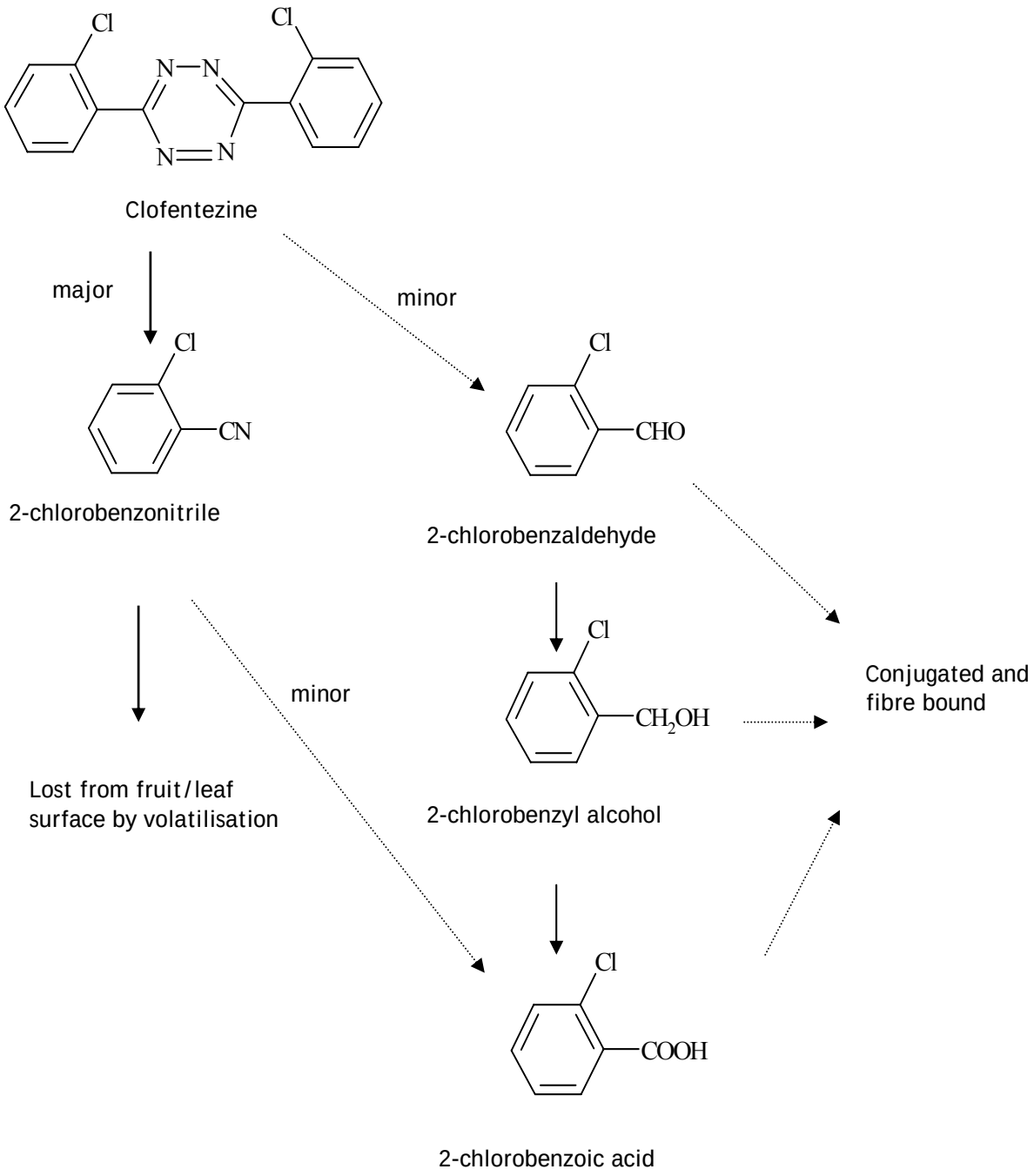
The metabolite (2-chlorobenzoic acid (2-chlorobenzylidene) hydrazide) formed under sterilisation conditions in the standard processing hydrolysis study is discussed in the reporting table under item 3(20) – Please refer to open point 3(8) of the evaluation table rev. 0-0 (03.01.2008).

[6] Arcos Organics BVBA. MSDS for 2-chlorobenzoic acid. http://newsearchch.chemexper.com

B.7 Residues data

B.7.1 Metabolism, distribution and expression of residues in plants (IIA 6.1, IIIA 8.1)

Figure 7.1 – Proposed metabolism of clofentezine in fruit



B.7.2 Metabolism, distribution and expression of the residues in livestock (AII 6.2, IIIA 8.1)

B.7.2.1 Cattle

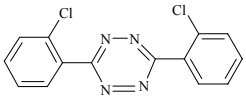
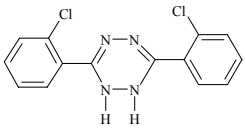
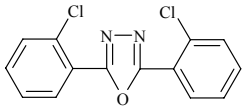
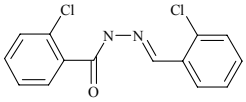
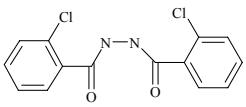
Table B.7.1 Partitioning of extractable radioactivity in milk and tissues (in % of total radioactivity)

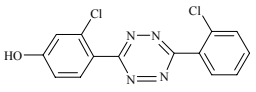
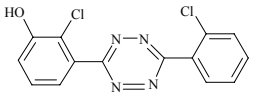
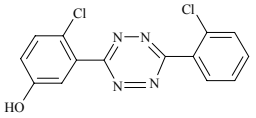
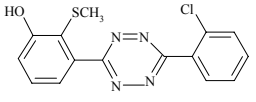
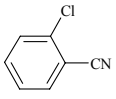
Animal Product and number of days after treatment	Number of doses	Total residue (mg/kg parent equivalent)	Parent residues (mg/kg)	Solvent e xtractable radioactivity (%)	Enzyme extractable radioactivity (%)	Non-extractable radioactivity (%)
Milk						
Day 1 pm	1	0.01	-	-	-	-
Day 2 am		0.11	-	-	-	-
Day 2 pm	2	0.17	-	-	-	-
Day 3 am		0.18	-	-	-	-
Day 3 pm	3	0.16	-	-	-	-
Day 4 am		0.15	-	-	-	-
Extracted sample		0.17	-	93	-	7
Muscle	3	0.02	-	-	-	-
Fat (renal)	3	0.26	-	90	-	10
Fat (subcutaneous)	3	0.02	-	-	-	-
Kidney	3	0.36	-	83	-	17
Liver	3	0.76	-	67	19	14

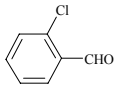
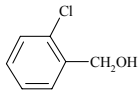
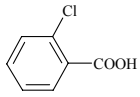
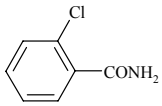
Table B.7.2 Distribution of clofentezine and its metabolites in animal products in % of the total radioactivity (parent equivalent in mg/kg)

	Milk (0.17mg/kg)	Muscle	Fat (renal)	Liver	Kidney
Clofentezine	-	-	-	-	-
4-hydroxy clofentezine	75 (0.13)	-	68 (0.23)	74 (0.57)	83 (0.3)

Summary of degradates and metabolites of clofentezine reported in different matrices

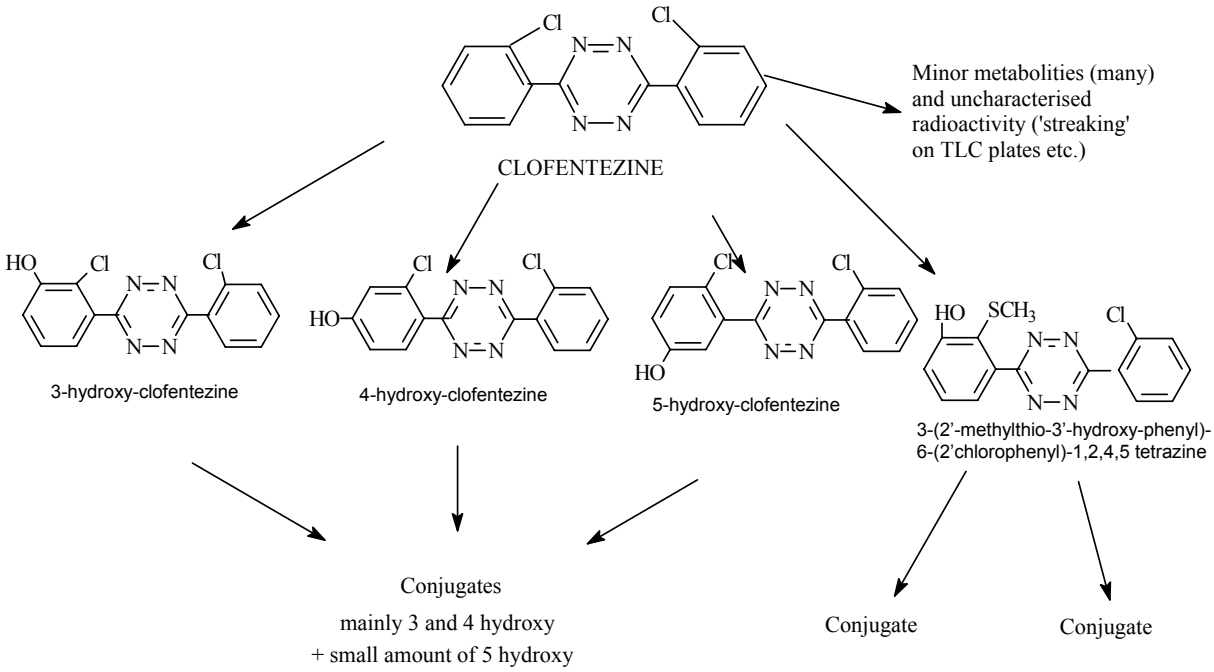
Chemical name/name/code of reference substance*	Chemical structure	Matrix in which detected	Study reference
3,6-bis (2-chlorophenyl)-1,2,4,5-tetrazine Clofentezine Apollo AE B084866, NC21314 SN 84866			
3,6-bis (2-chlorophenyl)-1,2-dihydro-1,2,4,5-tetrazine Tetrazine AE C522505 NC22505		Artifact on plants & in soil, <u>not a genuine metabolite</u>	Warner, 1981 Leake & Arnold, 1983a
2,5-bis (2-chlorophenyl)-1,3,4-oxadiazole Oxadiazole AE C512940 NC12940		Water Sediment	Leake & Arnold, 1983c
2-chlorobenzoic acid (2-chlorobenzylidene) hydrazide Hydrazide-hydrazone AE C593600 FBC 93600		Water Soil Sediment	Van der Gaauw, 2001 Leake & Arnold, 1983b Leake & Arnold, 1983c
N,N'bis (2-chlorobenzoyl)-hydrazine Bis-hydrazide AE C512898		Soil Water Sediment	Leake & Arnold, 1983b Leake & Arnold, 1983c

Chemical name/name/code of reference substance*	Chemical structure	Matrix in which detected	Study reference
4-hydroxy-clofentezine		Rat Baboon Calf Goat Cow Hen	Challis & Needham, 1985 Challis,1983 Needham & Challis, 1985 Campbell, 1987 Phillips & Swalwell, 1989b Phillips & Swalwell, 1988 Phillips & Swalwell, 1989a Creedy & Challis, 1988
3-hydroxy-clofentezine		Rat Baboon Calf Goat Hen	Challis & Needham, 1985 Challis,1983 Needham & Challis, 1985 Creedy & Challis, 1988
5-hydroxy-clofentezine		Rat Calf Goat	Challis & Needham, 1985 Needham & Challis, 1985
3-(2'-methylthio-3'-hydroxy-phenyl)-6-(2'chlorophenyl)-1,2,4,5 tetrazine		Rat Baboon (possible) Calf Goat	Challis & Needham, 1985 Challis,1983 Needham & Challis, 1985
2-chlorobenzonitrile AE F023666		Plants Water Soil (photolysis)	Kelly, 1985c Edwards, 1987 and 1988 Campbell, 1989 Van der Gaauw, 2001c Kelly, 1985b Brice, 2007 Kelly, 1985e

Chemical name/name/code of reference substance*	Chemical structure	Matrix in which detected	Study reference
2-chlorobenzaldehyde AE 0035831		Plants Water	Edwards, 1987 Kelly, 1985b
2-chlorobenzyl alcohol		Plants	Edwards, 1987
2-chlorobenzoic acid AE C500233 NC233		Plants Soil Water Sediment	Edwards, 1987 Leake & Arnold, 1983a & b Leake & Arnold, 1983c Kelly, 1985b Brice, 2007 Leake & Arnold, 1983c
2-chlorobenzamide AE F092117		Water Soil	Van der Gaauw, 2001c Kelly, 1985 Brice, 2007 Leake & Arnold, 1983b

- In individual reports, a Roman numeral is used to refer to some of the reference substances. These are specific to that study and their identity is given within the same report.

Figure 7.2 Metabolic pathway in animals (rat and livestock)



B.8 Environmental Fate and Behaviour

The additional information below has been prepared by the UK RMS to address the open points and points of clarification identified in the clofentezine Evaluation Table (rev. 0-0, 03.01.2008). Where reference is made to the original draft assessment report these references relate to the MS Word version of August 2005.

Point of clarification: 4.1

Applicant to further address the photolysis metabolite 2-chlorobenzonitrile with respect to potential GW contamination.

(EFSA note: According guidance document on assessment of metabolites in GW a metabolite with a max. 5.5 % at the end of a soil degradation study deserves further GW assessment. The photolysis study was performed with natural sunlight in UK (52 °N) between August and September. The study may not be considered to represent worst case EU conditions with respect to photolysis and higher levels could be expected to occur in many EU locations).

See reporting table 4(3)

The Notifier attempted to address this point of clarification in Wiesner and Daneva, 2008 DT_{50} of 1000 d and a K_{oc} of 162 ml/g (estimated value derived from the EPIWIN software). A summary of input values, GAP simulated and first tier results are presented in Tables B.8.1 and 8.2 below.

Table B.8.1 Summary of key substance specific inputs for clofentezine and metabolite 2-chlorobenzonitrile

Parameter	Clofentezine	2-Chlorobenzonitrile
Molecular weight	303.1	137.6
DT_{50} in soil (20°C, pF2)	71.3 days (a)	1000 days (default)
Soil temperature and moisture correction	On (Q_{10}: 2.2)	On (Q_{10}: 2.2)
K_{OC}	1064	162 (EPIWIN)
1/n	0.9	0.9 (default)
Henry constant	0.168 J/mol	Not needed in PELMO
Plant uptake factor	0	0
Metabolite formation fraction	-	5.5%

(a) Calculated using a Q10 of 2.2

Table B.8.2 Summary of first tier FOCUS groundwater simulations for metabolite 2-chlorobenzonitrile based on FOCUS PELMO 3.3.2
(all PECgw values for clofentezine were <0.001µg/l)

	Apples	**Early vines**	**Early strawberries**
Application rate (g a.s./ha) and timing	200 (7 d before emergence)	100 (7 d after emergence)	200 (7 d after emergence)
Crop interception (%)	50	50	30
Maximum a.s./ha reaching soil	100	50	140
Metabolite formation fraction (%)	5.5		
Scenarios	80th percentile annual average concentrations over the 20-year simulation period (µg/l)		
Châteaudun	0.097	0.047	-
Hamburg	0.127	0.062	0.154
Jokioinen	0.077	-	0.037
Kremsmünster	0.143	0.062	0.182
Okehampton	0.115	-	-
Piacenza	0.102	0.046	-
Porto	0.046	0.021	-
Sevilla	0.140	0.099	0.008
Thiva	0.099	0.050	-

Following this initial FOCUSgw assessment, the Notifier carried out a further assessment to establish the highest metabolite DT_{50} that would give PECgw values less than the trigger value of 0.1µg/l. Simulation runs were carried out for all four relevant FOCUS scenarios for the early strawberry application only (the GAP where the worst case PECgw of 0.182µg/l was established at the first tier). For this part of the assessment the Notifier set the formation fraction to 10% and kept the K_{oc} set to 162 ml/g, and manually changed the DT50 to determine what was the longest DT50 that still resulted in an acceptable groundwater exposure assessment via a series of multiple simulation runs. A metabolite formation fraction of 10% was assumed because it was assumed by the Notifier that if it was possible to be kinetically modelled, the formation fraction would likely be greater than the maximum amount formed in the study (i.e. 5.5%). The Notifier also considered that this assumption would address the comment made by EFSA that a greater proportion may be formed under EU conditions other than those prevailing in the soil photolysis study (which was conducted outdoors in the UK in summer). Soil temperature correction was based on a Q_{10} value of either 2.2 (as used by the UK RMS in the original DAR calculations) or 2.58 (in accordance with the latest regulatory guidance from the relevant PPPR Opinion). The RMS considered that this assessment represented a relatively simplistic form of a sensitivity analysis and results of the Notifiers refined assessment are presented in Table B.8.3 below.

Table B.8.3 Summary of refined FOCUS groundwater simulations for metabolite 2-chlorobenzonitrile based on FOCUS PELMO 3.3.2
(all PECgw values for clofentiezine were <0.001µg/l)

	Early strawberries			
Application rate (g ai/ha)	200			
Crop interception (%)	30			
Maximum ai/ha reaching soil	140			
Metabolite formation fraction (%)	10			
Q10	2.2		2.58	
Metabolite DT_{50} (days)	390	400	360	370
Scenario	80th percentile annual average concentrations over the 20-year simulation period (µg/l)			
Hamburg	0.095	0.099	0.095	0.099
Jokioinen	0.017	0.018	0.018	0.019
Kremsmünster	0.097	0.102	0.097	0.102
Sevilla	'0.000'	0.001	'0.000'	'0.000'

Based on a soil temperature correction factor (Q_{10}) of 2.2, the highest DT_{50} of 2-chlorobenzonitrile that would result in acceptable PEC_{gw} values at all four scenarios for early applications to strawberries is approximately 390 days (i.e. when the DT_{50} exceeded 390 d the PECgw would exceed the 0.1µg/l limit as can be seen for the results presented above based on a DT_{50} of 400 d at the Kremsmünster scenario).

Based on a soil temperature correction factor (Q_{10}) of 2.58, the highest DT_{50} of 2-chlorobenzonitrile that would result in acceptable PEC_{gw} at all four scenarios for early applications to strawberries is approximately 360 days (i.e. when the DT_{50} exceeded 360 d the PECgw would exceed the 0.1µg/l limit as can be seen for the results presented above based on a DT_{50} of 370 d at the Kremsmünster scenario).

In the Notifiers submission they also argued that under conditions where 2-chlorobenzonitrile might potentially be formed in amounts >5 %, significant leaching can be excluded as such periods of hot dry sunny weather (especially in Southern Europe) are unlikely to also correspond to periods for any significant groundwater recharge.

For the other supported GAPs and other FOCUS scenarios, including the Southern European scenario, Sevilla, the DT_{50} for 2-chlorobenzonitrile would need to be much greater than one year before groundwater concentrations approached 0.1µg/l in the opinion of the Notifier.

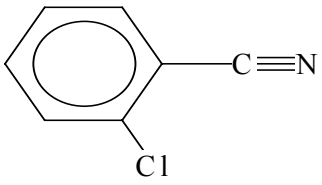
However, in the opinion of the Notifier, it was considered highly unlikely that a small molecule such as 2-chlorobenzonitrile would persist in the environment for any prolonged period of time, and degradation to the amide and subsequently the carboxylic acid was considered likely to occur in much less than one year. On this basis the Notifier proposed that they

had adequately assessed the groundwater leaching potential of the 2-chlorobenzonitrile metabolite.

UK RMS assessment

In the opinion of the UK RMS there are a number of uncertainties in the groundwater exposure assessments provided by the Notifier.

It is noted that the K_{oc} value for the 2-chlorobenzonitrile metabolite is an estimated value only based on the EPIWIN software. In the opinion of the UK RMS, where a FOCUS groundwater assessment is required, it is considered preferable to have experimental sorption data available from a standard batch sorption study (i.e. in compliance with OECD 106) wherever it is technically feasible to conduct such a study. There is no reason to suspect that such a study could not have been performed for the 2-chlorobenzonitrile metabolite. Therefore the reliance on the estimated sorption value is somewhat questionable in this case. However, in the context of Regulation No. 1095/2007 such a study would not be accepted at this stage of the review program. The same restriction prevents the submission of any new data on the potential for soil degradation of the metabolite, which adds further uncertainty. However, although the estimated sorption value based on the EPIWIN software does have a degree of uncertainty associated with it, for such a relatively simple molecular structure as the 2-chlorobenzonitrile metabolite (see Figure B.8.1 below for structure), the degree of uncertainty would be expected to be somewhat less than would be associated with more complex molecules with a higher number of functional groups.



Benzonitrile, 2-chloro-

Figure B.8.1 Structure of 2-chlorobenzonitrile

With respect to the selection of metabolite formation fractions, the Notifier has used a value of 5.5% in their first tier assessment, and a slightly higher value of 10% in their refined assessment. It is clear that the value of 5.5% is too low, since this was simply based on the peak occurrence level of the 2-chlorobenzonitrile metabolite during the soil photolysis study, and by definition the molar formation fraction must exceed the peak occurrence. It should also be noted that the 'peak' occurred at the final sampling point, and therefore it is possible that higher levels could have been determined if the study had been conducted for a longer duration. It is also unclear whether the use of the 10% value in the refined assessment represents an appropriately conservative estimate, and in the absence of a kinetically

derived formation fraction it is considered that it would probably have been more appropriate to assume 100% formation as a worst case. If a formation fraction of 100% had been assumed, the maximum DT_{50} that would have still have resulted in an acceptable PECgw would have been reduced compared with the values currently proposed by the Notifier of 360 to 390 d.

The Notifier has argued that under conditions where the potential photolysis metabolite would form in highest amounts (i.e. hot, dry sunny conditions) these would not also correspond to periods of significant groundwater recharge. Although this argument has some merit, in the absence of reliable information on the persistence of the 2-chlorobenzonitrile metabolite, it cannot be completely excluded that this metabolite could persist in soil into periods of the year where significant groundwater leaching could occur. In addition, some of the proposed crops such as strawberries would be expected to be routinely irrigated as part of normal agricultural practice and this may also enhance the leaching potential irrespective of the prevailing natural climatic conditions at the time of potential 2-chlorobenzonitrile formation.

Overall the UK RMS considered that although the Notifier had made a reasonable attempt to address this point of clarification within the confines of Regulation No. 1095/2007, there was still a large degree of uncertainty in the submitted groundwater exposure assessment. In the original DAR the UK RMS concluded that photolysis was unlikely to be a major route of degradation for clofentezine in the soil environment. Although the metabolite 2-chlorobenzonitrile formed at a level over 5% AR, this only occurred after 31 d of natural exposure outdoors in a thin glass plate exposure system. It would be expected that the experimental system would maximise the potential for formation of photolytic metabolites under the prevailing environmental conditions. Under more natural conditions, shading by the developing crop, leaching out of the upper most soil layers and competing degradation processes may be expected to reduce the overall impact of photolysis. Therefore in the opinion of the UK RMS the point of clarification can be considered sufficiently addressed and no further information is required. However individual MS may still wish to consider the potential for formation of 2-chlorobenzonitrile under very specific National conditions and the conclusion of the EU peer review could include reference to this metabolite for consideration at MS level.

Open point: 4.2

MS experts to discuss the need for further assessment of soil metabolite 2-chlorobenzoic acid.

(Guidance document in the relevance of metabolites in ground water indicates that the % triggers should be considered on a molar basis. Usually this coincides with the % TAR but not in this case. The theoretical maximum transformation of clofentezine in 2-chlorobenzoic acid is 200 %

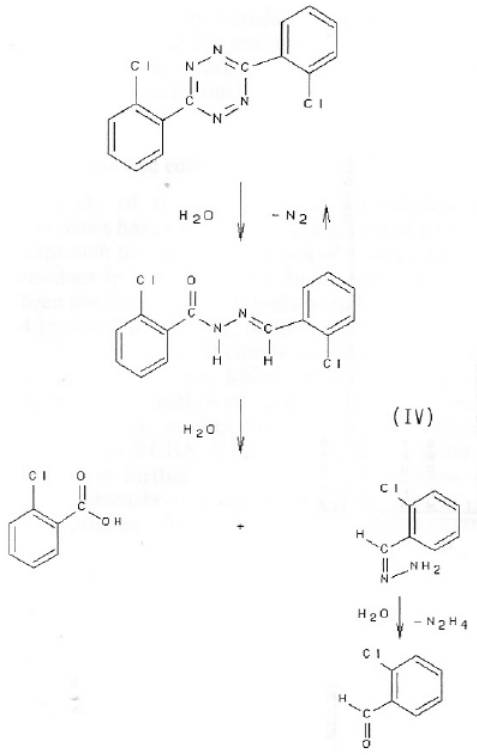
in molar basis but will result only in 100% in TAR. Therefore the observed %TAR values need to be multiplied by 2 in order to obtain the % in molar basis, this will result in exceedance of 10 % in molar basis)

See also 4(2), 4(26), 4(57) and 4(58).

See reporting table 4(11)

The Notifier attempted to address this point of clarification in Attachment IRV1-01. The Notifier provided a brief case based on information generated during the method development of clofentezine. Further supporting information was provided from a published study on clofentezine method development (Snowdon, Whiteoak and Manley (1991) The hydrolysis of clofentezine and related tetrazines as the basis of determination of residues in bovine tissues. *Fresenius J Analyt. Chem*, **339**: 444-447). The possibility that one mole of clofentezine could give rise to two moles of 2-chlorobenzoic acid due to the symmetrical nature of the parent molecule was considered during method development. However this was shown not to be the case due to the fact that molecular symmetry was lost after the initial tetrazine ring was opened via formation of an intermediate hydrazide (see Snowdon, Whiteoak and Manley (1991)). A consistent molar conversion rate for the 2-chlorobenzoic acid of approximately 1:1 was demonstrated (determined via gas chromatography with mass selective detection). A proposed mechanism for hydrolysis of clofentezine is presented in Figure B.8.2 below.

Figure B.8.2 Proposed mechanism for clofentezine hydrolysis (from Snowdon, Whiteoak and Manley (1991))



UK RMS assessment

On the basis of the information provided, the UK RMS accepted that the theoretical maximum transformation of clofentezine into 2-chlorobenzoic acid of 200% on a molar basis outlined in Open point 4.2 would not occur in practice. However it should be noted that the information provided from the method development study was based on an initial step which involved the hydrolysis of clofentezine residues using concentrated hydrobromic acid under laboratory conditions prior to analysis of the liberated metabolites. This step leads to a loss in the symmetry of the molecule and the UK RMS has assumed that a similar loss in symmetry would occur due to the initial hydrolytic reactions in soil.

In the laboratory fate studies this metabolite occurred at a maximum of 6.8% AR and assuming a maximum molar conversion rate of 1:1 no correction for molar formation should be made. This metabolite did not breach any of the triggers in the guidance document on assessing the relevance of metabolites in groundwater and in the opinion of the UK RMS no further information is required.

Open point 4.3

MS to discuss the adequacy of the input parameters used for FOCUS SW calculations that were derived from the water sediment study.

See also 4(36), 4(42), 4(43), 4(48), 4(49), 4(50) and data requirement 4(45).

See reporting table 4(12)

For completeness the UK RMS has repeated their response originally provided in the Reporting Table in response to this comment below.

We agree that the DAR should have included additional statistical data to support the goodness of fit. When evaluating this kinetic fitting, the RMS considered both the statistical data in the original study report, in addition to the graphical outputs of the measured versus observed fits.

For completeness statistical results (in the form of B-values) are presented below:-

		Lode system		*Sadlers Farm*	
		Reac. Rate (d^{-1})	*B-value*[7]	*Reac. Rate (d^{-1})*	*B-value*
A.S. water	*K12*	*0.27*	*0.92*	*0.16*	*0.98*
	K13	*0.096*		*0.14*	
	K15	*0.19*		*0.24*	
A.S. sed.	*K21*	*0.15*	*0.95*	*0.023*	*0.91*
	K24	*<0.001*		*<0.001*	
	K25	*0.013*		*<0.001*	
Met. Water	*K34*	*77.9*	*0.78*	*96.4*	*0.70*
	K35	*<0.001*		*0.11*	
Met. sed	*K43*	*50.2*	*0.97*	*14.9*	*0.90*

[7] For the purposes of the assessment of the acceptability of the kinetic fits the UK RMS assumed that B-values are broadly equivalent to an r^2 value (which would have been used more typically at the time that the DAR was prepared). However the UK RMS accepts that the two values are not strictly interchangeable and neither would be recommended as an appropriate tool according to the latest guidance from the FOCUS kinetics report. The acceptance of the visual fits made in the original DAR was made on the basis of a combination of good visual fits and acceptable statistical measures. This approach is still considered valid in the opinion of the UK RMS for this substance at this stage of the EU review even if not strictly in agreement with the FOCUS kinetics report.

	K45	*0.067*		*0.049*	
Elimination			*0.93*		*0.96*
All data			*0.91*		*0.89*

Since B-values were close to 1, the RMS considered this as evidence of an acceptable fit. In addition visual assessment of the graphical fits were considered acceptable by the RMS.

However we do agree that such complex fitting will be subject to a high degree of uncertainty, particularly due to a high level of correlation between parameters that determine degradation and partitioning between compartments. Such a complex fitting would not now be recommended using the FOCUS degradation kinetics guidance (which was not available to the RMS at the time of DAR preparation). According to FOCUS kinetics it is our understanding that it is not currently possible to calculate individual water and sediment degradation rates for metabolites.

Overall the RMS considered that the values used in the FOCUSsw modelling were acceptable. For the a.s., at Step 2 and 3, the water phase and sediment phase degradation DT50 values were 2.4 and 53.3 d. For the a.s. the hydrolysis DT50 at pH 7 was approximately 1 d. The whole system degradation DT50 values in the water sediment system were between 2 and 7d.

For metabolite AE C593600, a DT50 of 14.1 d was used for all compartments at Step 1 and 2. Although not originally calculated in the DAR, the RMS has estimated the whole system DT50 for this metabolite from the peak of formation onwards (data used from day 7 to day 42 in the clay loam system). This gave a whole system DT50 of 6.4d assuming SFO kinetics ($r^2 = 0.86$). Therefore again we consider the actual values used in the exposure assessments to be appropriate for the purposes of the risk assessment (even if the methods used to derive them may be subject to uncertainty).

Overall the UK RMS concluded that no further information was required to address this point.

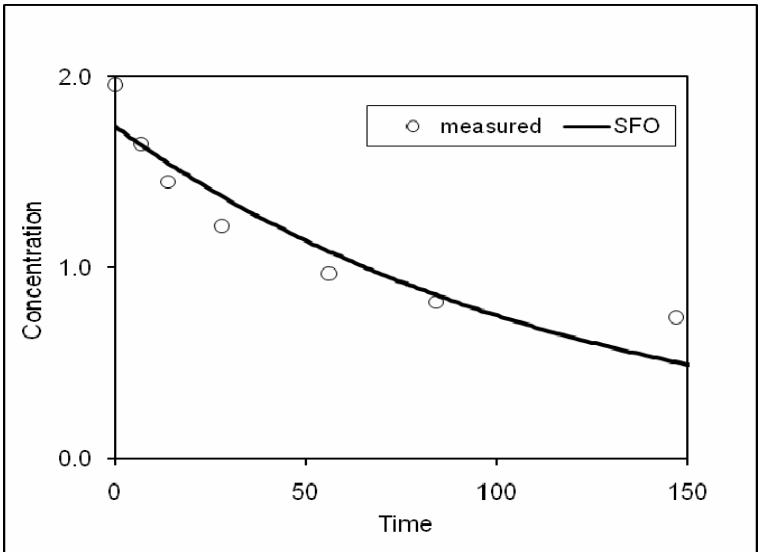
Open point 4.4

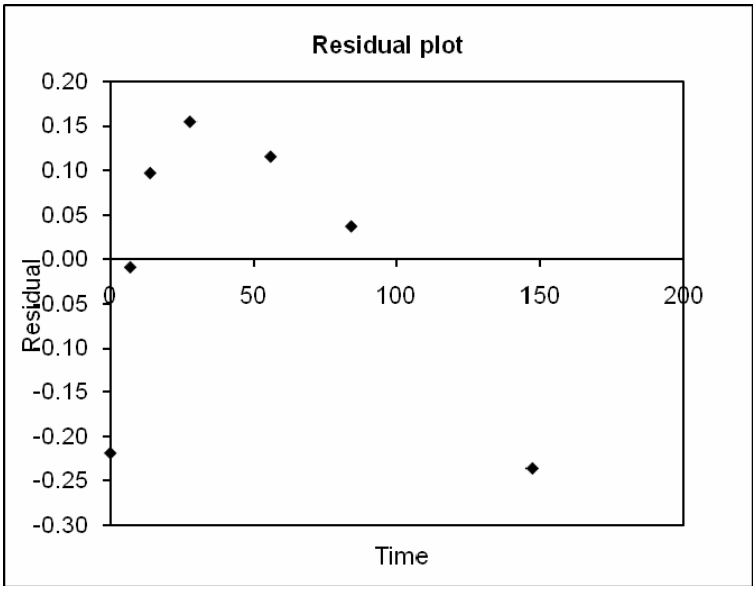
MS to discuss the goodness of fitting of the Speyer 2.2 soil data to first order kinetics. If adequate also discuss the potential effect of the use of this value in the risk assessment and/or the value more appropriate for the list of end points and further assessments.
See also 4(18).
See reporting table 4 (17)

In the original comment in the Reporting Table (see 4(17)) the UK providing details of fitting based on the pre-FOCUS degradation kinetics approach. To aid the discussion the UK RMS has now fitted SFO kinetics to the data for the Speyer 2.2 soil using the simple MS Excel spreadsheet provided by the FOCUS kinetics workgroup in accordance with the latest guidance. Note that the original study evaluation is presented in the August 2005 DAR (see Section B.8.1.2.1 pages 296 and 297; Snowdon, 1982b).

The fitted SFO DT_{50} was 82.1 d (M_0 = 1.74, chi^2 = 9.3). Graphical outputs from the FOCUS kinetics MS Excel spreadsheet are presented in Figure B.8.2 below.

Figure B.8.2 Graphical fit for the Speyer 2.2 soil assuming SFO kinetics (taken from the FOCUS kinetics MS Excel spreadsheet)





In the opinion of the UK RMS, although the chi^2 value from this fit was reasonable, visually the fit was relatively poor with a consistent pattern observed in the residual plot and a poor description of the parent initial concentration.

Even if the UK RMS had ignored the visual assessment and accepted the SFO DT_{50} from this soil on the basis of the chi^2 statistic alone, it is not considered that this value would significantly affect the risk assessment. For example, if the normalised SFO DT_{50} from this soil of 86.5d[8] were included in Table B.8.37 of the original DAR (see page 331 of the August 2005 DAR), the geometric mean would only have increased from 71.3 to 73.6d. This small increase is not expected to significantly alter the exposure assessments based on this value and no further change is proposed by the UK RMS.

Point of clarification 4.2

Applicant to provide scientifically and consistent valid justification for not presenting a soil adsorption desorption study with clofentezine.

See reporting table 4(24)

The Notifier has provided further justification for the non-submission of a soil adsorption desorption study in Attachment IRV4-02 and this information is reproduced below in italics.

The notifier has not attempted to derive the clofentezine adsorption coefficient experimentally for the reason that it is considered practically not possible to design a laboratory study that will meet the current guidelines (SETAC, 1995[9] and OECD 106[10]) given the very low water solubility of clofentezine (ca 0.002 mg/L or 0.000002 mg/mL) and its rapid degradation by hydrolysis.

The following summarises some of the regulatory guideline requirements considered in coming to this conclusion:

- *OECD 106[5] requires the study to be conducted at "below the water solubility" (para. 28).*
- *SETAC[4] requires "concentrations within a range 0.04 - 5 mg/L. However 50% of solubility should not be exceeded" (para. 4.1.4).*

[8] Normalised DT_{50} calculated based on the original study of Snowdon (1982b) being performed at 22°C and 40% MWHC (actual moisture content = 16.52% compared with a default field capacity value of 19% according to the FOCUS groundwater (2002) guidance).

[9] Lynch, M.R., 1995. Procedures for assessing the environmental fate and ecotoxicity of pesticides. Published by: Society of Environmental Toxicology and Chemistry (SETAC-Europe).

[10] Anon., 2000. OECD guideline for the testing of chemicals; adsorption-desorption using a batch equilibrium method. OECD 106.

In addition, consideration to other aspects of the guidelines should be taken into account:

- *OECD 106[5] proposes, "the analytical method LOQ be at least two orders of magnitude below the nominal concentration" (para. 36).*
- *For adsorption isotherms, five test concentrations are used covering preferably two orders of magnitude (para. 72).*
- *OECD 106[5] notes that: "care must be taken to ensure good mixing, and adequate time must be allowed for the system to equilibrate".*
- *OECD 106[5] recommends an alternative approach "to deal with these extreme cases when adequate analytical methodology is missing".*

With respect to solubility of the test substance, the guidance proposes a co-solvent may be used to aid dissolution of poorly soluble substances:

- *SETAC[4] suggests that, "a water miscible organic solvent may be used to add the compound to water" (para. 4.1.4).*
- *OECD 106[5] proposes to "use of a solubilising solvent for poorly soluble substance (<10^{-4} g/L) when it is difficult to dissolve the test substances" (para. 30). The solvent representing <0.1% in the final solutions coming into contact with soil".*

Thus for clofentezine, up to 0.1% organic solvent by volume could be used to prepare solutions, but at no more than 2 µg/L.

The following points are relevant facts to be considered in designing a clofentezine adsorption/desorption study:

- *The very low water solubility of clofentezine is 2.52 µg/L at pH 5 and is < 2 µg/L at pH 7 (DAR[3], page 11).*
- *The rapid hydrolysis of clofentezine is ca 1 day at pH 7 (DAR[3] page 12).*
- *The estimated K_{oc} by calculation is 1064 mL/g (DAR[3], page 313). From this K_{oc}, K_d values of 10 and 42 can be estimated for soils containing 1% and 4% organic carbon. This means that potentially a soil:solution ration of 1:25 will be required to retain sufficient material (>20%) in solution (by reference to Figure 1 in OECD 106[5]).*

A possible experimental design would be as follows:

Starting clofentezine aqueous solution concentrations of 2, 1, 0.2, 0.1 and 0.02 µg/L (covering two orders of magnitude) would be used. This would equate to starting total radioactivity concentrations in the range 779, 389, 79, 39 and 8 dpm/mL since [^{14}C]-Clofentezine is now available at a high specific activity of 6.49 MBq/mg (389400 dpm/µg).
Assuming 5-mL aliquots of aqueous solution are taken for radioassay and assuming the LOQ is taken as twice background (e.g. ca 50 dpm), it would

be possible to accurately quantify total radioactivity in only 4 out of 5 of the starting solutions. After the equilibration period, due to adsorption to the soil (ca 80%) and hydrolytic degradation, the lowest 3 concentrations would be below the LOQ. Furthermore, to monitor clofentezine concentrations, some samples would need to be analysed by radio-HPLC. In a recent photolysis study, clofentezine concentrations in aqueous solutions were accurately determined in the range 2 - 0.2 µg/L (Brice, 2007[11]). That study clearly showed that accurate determination would not be possible below 0.2 µg/L.

The OECD 106[5] guideline says that "care must be taken to ensure good mixing, and adequate time must be allowed for the system to equilibrate". However, for a typical equilibration period of 24 hours, it can be predicted from hydrolysis data that ca 50% of clofentezine will have degraded in soil slurry at ca pH 7.

The OECD 106[5] itself recommends an alternative approach "to deal with these extreme cases when adequate analytical methodology is missing". It suggests the K_{oc} value is predicted by applying estimation techniques, one of which is the Briggs equation[1] as reported by Mackenzie (1999)[2] for clofentezine and evaluated in the DAR[3].

In 2002, the Scientific Committee on Plants suggested: "alternative methods should be triggered if more than 10%/day of the test substance is hydrolysed under the conditions of the batch adsorption test" (SCP opinion: SCP/KOC/002 final).

Whilst a short equilibration time could be used, as suggested by the SCP, to minimise hydrolysis, this would probably lead to incomplete equilibration and an inaccurate determination of $K_{d.}$

UK RMS assessment

In the original DAR the UK RMS accepted the use of the Koc estimation method for clofentezine due to the expected technical difficulties in performing a standard batch sorption study. However for completeness the UK RMS considers that it would have been beneficial if the Notifier had at least provided results of preliminary sorption studies to demonstrate that full experimental studies were not technically feasible.

It should also be noted that in this case the low mobility of clofentezine predicted by the estimated Koc is supported to some extent by the additional column leaching studies submitted (see Section B.8.2.2.1 of the original DAR, page 314 onwards, studies of Snowdon 1982d and Leake and Arnold 1985a and b) and an aged residue column leaching study (see

11 [^{14}C]-Clofentezine photodegradation and quantum yield in water. Covance Laboratories ltd. No. 2614/001. Irvita Plant Protection NV, Report no. R-18905. GLP. Unpublished. The UK RMS notes that although this study is available, it has not been evaluated as part of the review of clofentezine under 91/414

Section B.8.2.2.2 of the original DAR, page 317 onwards, study of Leake 1982). In addition, although it is not a common study type that is routinely seen in modern data packages, a soil TLC study was also available that provided additional supporting information (see Section B.8.2.2.4 of the original DAR, page 319 onwards, study of Leake and Lines 1982). In all of these studies clofentezine was shown to be immobile, with no parent material found in leachates or deeper soil horizons of the column studies, or moved from the origin in the soil TLC study.

Overall the UK RMS accepted that the estimated Koc value for clofentezine was sufficiently validated for use in the exposure assessments, particularly taking into account the low mobility demonstrated in at least 4 other laboratory experimental studies.

Point of clarification 4.4

Applicant to provide further clarification on the low material balance reached in the water sediment studies.

See reporting table 4 (35)

The Notifier has provided further information on the low material balance encountered in the water sediment studies in Attachment IRV4-03 and this information is reproduced below in italics. The original UK RMS evaluation of the water sediment study of Leake and Arnold 1983c was reported in Section B.8.4.4 of the August 2005 DAR, page 324 onwards.

In the sandy clay loam (Lode), recoveries outside the range of 90-110% AR were recorded at 0, 21 and 42 DAT and for the clay loam (Saddlers Farm) at 14 and 21 DAT. The relevant EU guideline (SETAC[12], 1995, 8.2.2) for such experiments was not available at the time this study was conducted. In the study report, the authors wrote "Recoveries were generally better than 85% with an overall mean of 89%. An adequate balance of radioactivity was maintained throughout" and did not further investigate this aspect of the study.

In an attempt answering this question, the raw data has been recalled and examined. Several possibilities exist to explain for the unaccounted radioactivity, both at earlier or later sampling times.

- *One explanation could be that the total radioactivity applied to the test systems may have been overestimated. At zero DAT, recovery from the Lode test system was 86.5% AR. This suggests that an error in the quantification of applied radioactivity may have occurred. However, recovery at zero DAT from the Saddlers Farm test system was 98.5% AR. Given that application to both test systems occurred at the same time and that the total applied radioactivity was calculated from a single set of QC checks of the application solution, it can be concluded that this is unlikely to be the sole*

[12] Lynch, M.R. (1995). Procedures for assessing the environmental fate and ecotoxicity of pesticides. Published by: Society of Environmental Toxicology and Chemistry (SETAC-Europe).

reason for the sporadic low mass balances, especially in the later samples from Sadlers Farm test systems.

- *A second explanation for the low zero DAT recovery in the Lode test system could be that there was an experimental error in the analysis of the water phase, as it contains the largest proportion of applied radioactivity. Whilst movement of clofentezine from the water to sediment was rapid (due to its intrinsic properties), only 2.5% AR was recovered by extraction of the sediment. Therefore, the 17% AR unaccounted for are unlikely to have resulted from inadequate or erroneous analysis of the sediment phase. Indeed, the water phase was not measured by taking aliquots of the water phase directly for radioassay, as is usually the case in this type of study. Here, the water phase was separated from the sediment phase and immediately extracted by liquid/liquid partition in to organic solvent. The increased sample handling and manipulation before measurement of the total radioactivity, compared to simple radioassay of the water phase, provided for more opportunity for radioactivity to be lost. Clofentezine was also shown to stick to the glass vessel walls even after silanisation of the test vessels. Thus a loss from the aqueous phase by binding to other glassware used for extraction of the water phase could have contributed to the zero DAT recovery.*

In conclusion the low zero DAT recovery in one of the test systems, Lode, was probably due to inaccurate quantification of the aqueous phase.

In the later samples, neither underestimation of the total radioactivity in surface water, nor binding of clofentezine to glass surfaces are considered likely to be the reasons for the observed overall low recoveries, due to the relatively low amounts of radioactivity remaining in the aqueous phase at these time points (e.g. 42 DAT recovery in aqueous phase was only 4.3% AR compared to an overall deficit of 21.8% AR).

- *It can be seen from the data in table B8.32 (DAR, p325) that the decline in recovery coincides with the start of mineralisation in the sediment phase. It is possible that there was inadequate trapping of $^{14}CO_2$ or that some of the $^{14}CO_2$ leaked out of the system prior to being trapped. Ethanolamine is known not to mix and uptake well with some liquid scintillants. No water or other adjuvant was added to the aliquots of ethanolamine taken for LSC. It is therefore possible that the $^{14}CO_2$ content could have been systematically underestimated by this assay, especially at latest time points where the concentration was higher.*

An alternative way in which $^{14}CO_2$ may have been accidentally lost is if some had dissolved in the water phase forming a carbonate. This carbonate would generally be more soluble at alkali pH and thus, be accounted for in the aqueous phase. However, during manipulation of the sample e.g. removal of water from the sediment or partition with organic solvent, the equilibrium between CO_2 and its carbonate could be disturbed and $^{14}CO_2$ lost by volatilisation. Adjustment to pH 2 as done in the second partition would almost certainly cause this to happen if any ^{14}C-carbonate was still present.

It can be concluded that underestimation of the amount of mineralisation to $^{14}CO_2$ is a likely reason to account for the low recoveries of total radioactivity observed in later samples.

- *From Table B8.32 (DAR, page 325), it can be seen that at 21 DAT, the recovery of radioactivity as "bound" residue declines relative to the 14 DAT sample before increasing again at 42 DAT. This would not be expected: A trend of increasing bound residue followed by a decrease as the bound residue was mineralised would be more typical. This suggests that there was an experimental error in the determination of the bound residue in, at least, the 21 DAT samples for both sediments. Underestimation of the bound soil residue could arise from the dried sediment not being homogeneous or incomplete oxidation in the sample oxidiser.*

In conclusion, the low recovery of radioactivity at some sampling points in both test systems cannot be explained by a single reason. It is likely that for the Lode zero DAT sample there was an experimental error in the quantification of the water phase. In later samples of both test systems, low quantification of $^{14}CO_2$ and/or the bound residue is considered more probable than an error in the extractable aqueous or sediment radioactivity.

Estimates for clofentezine and degradation products in the water and sediment phases are considered by the notifier to be accurate, apart from the zero DAT Lode sample where it is likely that some clofentezine was unaccounted for. However, this actual starting concentration results in a more conservative estimate of the dissipation rate of clofentezine from the water phase and can be considered as a worst case value.

It can be concluded that the reported low total recoveries do not impact on the validity of key endpoints of the study, e.g. degradation rates and maximum amounts of degradate AE C593600 which have been used in the calculation of surface water PEC's and subsequently the aquatic non target organism risk assessment.
Thus, the notifier supports the view of the RMS that the study endpoints are suitable for exposure and risk assessment.

UK RMS Assessment

The UK RMS considered that the possible reasons for low recovery proposed by the Notifier (i.e. inaccurate quantification of initial water phase concentrations; inadequate recovery of $^{14}CO_2$; underestimation of sediment bound residues) are all plausible explanations which have been encountered in other such studies.

Whilst it would obviously have been preferable to have maintained recoveries within acceptable limits at all sample points, overall the UK RMS considered that in this case the low recoveries did not result in an unacceptable study. Irrespective of the temporal low recoveries, it seems

clear from the available data that clofentezine rapidly dissipates from the water phase via a combination of degradation and partitioning to sediment in both systems tested. The presence of residues absorbed to glassware provides additional evidence of the hydrophobic nature of the active substance. In both sediment systems there was a reasonably clear decline phase by the end of the 42 d study period which cannot be explained by poor recovery alone and must be mainly due to degradation. A clear peak level of metabolite AE C593600 was observed after 7d in the clay loam system when recovery was within acceptable limits (at 91.4% AR). Despite the relatively large number of deficiencies in the water sediment study the UK RMS considered that it was unlikely that significantly different behaviour or results would be obtained from a repeat study.

Overall in the opinion of the UK RMS the endpoints from this study can be relied upon for the purposes of the exposure assessment and no further information is required.

Open point 4.8

MS to discuss the acceptability of the water sediment study for the risk assessment. For the discussion MS also should take into account responses to data requirements in 4(29), 4(35) 4(40) and 4(41).

See also 4(38) and 4(39).

See reporting table 4(37)[13]

The Notifier has provided further information on the acceptability of the water sediment studies in Attachment IRV4-03 and this information is reproduced below in italics. The original UK RMS evaluation of the water sediment study of Leake and Arnold 1983c was reported in Section B.8.4.4 of the August 2005 DAR, page 324 onwards.

The notifier agrees with the statement provided by the RMS (in the Reporting Table). In addition, it is incorrect to draw a conclusion on the sediment:water ratio based on the recorded heights of each phase in the test vessels. Though we agree that the exact sediment:water ratio cannot be established, it can be estimated from the study raw data and shown to be within the guideline requirements. The oven dry weight of the fresh sediment is not reported, nor the total water to sediment on a weight for weight basis. However, the final dry weight of sediment after extraction is recorded and is ca 80g for the sandy clay loam (SCL) and ca 120g for the clay loam (CL). The total volume occupied by water/sediment is 412 cm^3 ($\pi r^2 l$ i.e. π x 2.5^2 x 21). Thus, assuming densities of 1 mL/cm^3 for water and 1.5 g/cm^3 for sediment, this gives sediment volumes of 53 cm^3 (SCL) and 80

[13] the original comment in the Reporting Table 4(37) specifically questioned the high ratio of sediment used during the water sediment study.

cm^3 (CL). Water volumes would be 359 cm^3 (SCL) and 332 cm^3 (CL). Thus, sediment:water ratios were actually approximately 1:7 and 1:4 and therefore in line with guideline requirements.

UK RMS Assessment

The UK RMS was unable to fully validate all the statements provided by the Notifier because information from the raw study data that detailed the dry weights of sediments post extraction were not provided. However the UK RMS accepts that it is not valid to estimate sediment:water ratios simply from recorded heights of each phase only. From the original study report the sediment depths were reported to be 9cm, with an overlying water layer of 12cm. When taking into account the pore space associated with the sediment, it is reasonable to assume that the volumes of water used would have been greater than indicated by the simple 9:12 height ratio alone, and therefore closer to acceptable levels set by the respective SETAC guideline.

Overall the UK RMS considered that the experimental set-up in the water sediment study was likely to be acceptable and considered that no further information was required.

Point of clarification 4.5

Further information on the appropriateness of the formulation used in the water sediment study (WP) to represent the intended SC formulation.

See also open point in 4(37)

See reporting table 4(40)

The Notifier has provided further information on the acceptability of the use of the WP formulation in the water sediment studies in Attachment IRV4-03 and this information is reproduced below in italics. The original UK RMS evaluation of the water sediment study of Leake and Arnold 1983c was reported in Section B.8.4.4 of the August 2005 DAR, page 324 onwards.

The SETAC guidelines propose the application rate "should be high enough to measure the rate of degradation and to identify the products of degradation". In practise, an application rate based on the field rate and assuming 100% overspray was not unusual at that time, for this study type. The water solubility of the compound does not come into the equation as the study is designed to mimic the actual use in the environment and some co-solvent (final maximum concentration 0.1%) is acceptable according to the SETAC guideline.

If the study had been conducted at below the water solubility of clofentezine (e.g. < 2 µg/L), the quality of the study would have been adversely affected. The objectives of the study could not have been met, as the total radioactivity applied would have been far too low. For example, the specific activity of the [^{14}C]clofentezine was 47.7 µCi/mg or 105894 dpm/µg. Thus, the total radioactivity applied to 0.235 L of water would have been only 49770 dpm or 211 dpm/mL in the water phase immediately after application. Assuming an LOQ of 2 x background of 20 dpm, the LOQ would be 40 dpm or nearly 20% AR. Thus, it can be seen that this application rate would have been totally impractical in achieving the objectives of the study. The combination of an exaggerated rate (5X) and formulated test substance with carrier solvent gave the best chance of achieving homogeneous incorporation in the water phase and a measurable concentration of radioactivity in the various compartments that allowed the objectives of the study to be met.

Application of technical clofentezine at the rate used in this study, even with the addition of the permitted volume of co-solvent, would have led to precipitation of the test material and heterogeneous incorporation in the water phase at zero time. This problem was clearly foreseen by the study authors whom used formulation ingredients (of wettable powder formulation type) and a carrier, acetone, to aid the dispersion of the a.i. in the water phase and overcome the problems associated with the low solubility and hydrophobic nature of clofentezine. The use of formulated material was therefore mandatory to properly conduct this study.

By reference to the raw data, the composition of the blank formulation was Reax 45L 10% (a wetter/dispersant, sodium salt of a sulphonated lignin), Neosyl 10% (a precipitated synthetic amorphous silica which helps milling and flow properties) and china clay 80%. This blank formulation was mixed 1:1 w/w with [^{14}C]clofentezine (7.1 mg) and acetone (1 mL) added to dissolve the clofentezine. This was made up with water to a final volume of 10mL (nominal 0.71 mg/mL clofentezine). Aliquots (0.1 mL) were diluted further with water and triplicate aliquots (0.1 mL) taken for LSC. The good replication of these aliquots and closeness to the target concentration demonstrated the homogeneity of the application solution.

The different co-formulants would not be expected to have had any influence either on solubility, rate of dissipation or on degradation of clofentezine. In fact, formulating the a.i. would have enhanced the availability of clofentezine to the water/sediment systems.

The blank formulation was not intended to match a commercial product but to allow the study to be correctly performed as discussed above. Therefore the study has to be considered applicable to the 50 SC or any other formulations.

UK RMS assessment

Overall the UK RMS accepted the Notifiers case that the use of the WP formulation in the water sediment study may have allowed more even mixing in the water phase in the initial period of the study relative to testing

the active substance alone. From the original study it is clear that clofentezine rapidly dissipated from the water phase (less than 5% AR remained as parent clofentezine in the water phase by day 7) even when formulated as a WP. In the opinion of the UK RMS the formulation is unlikely to have had a major adverse impact on the fate and behaviour of the active substance over the duration of the entire water sediment study, and therefore results from this study can be read across to other formulations as appropriate. No further information is considered necessary.

Point of clarification 4.6

Applicant to provide further information on how CO_2 was determined in the water sediment study and separated results for the different volatiles traps if they are available in the raw data of the study.

See also open point in 4(37)

See reporting table 4(41)

The Notifier has provided further information on the results of volatile trapping in the water sediment studies in Attachment IRV4-03 and this information is reproduced below in italics.

The trapping line was made of three traps: successively ethanediol, ethanolamine and sulphuric acid. By reference to the raw data, the separated results for each trap and trapping interval as requested by the EFSA are presented in the table below.

The air stream leaving the test vessels first entered an ethanediol trap. This would have trapped any neutral volatile degradation products including unchanged clofentezine. However based on its physical chemical properties clofentezine would not have been expected to have volatilised from the water under the test conditions. The trap after ethanolamine was sulphuric acid and would have trapped any volatile acidic degradates. Ethanolamine is a standard solvent used to trap CO_2 emissions from gas streams[14] and has been used in the past extensively for environmental fate and animal metabolism studies, sometimes on its own and sometimes in combination with 2-ethoxyethanol. The solvent is rarely assayed further to prove the presence of $^{14}CO_2$. Sodium and potassium hydroxide are alternative CO_2 traps and have the advantage that sodium carbonate can easily be precipitated with barium chloride to form solid barium carbonate. This assay is now used routinely to prove that trapped material is in fact CO_2. Ethanolamine traps CO_2 by forming a carbamate and there is no simple assay for this. In the study, the total radioactivity assayed by LSC in ethanolamine traps was assumed to be $^{14}CO_2$ by the study personnel and no further assay was carried out to prove this conclusion as it is very unlikely that the measured radioactivity was anything else. The conclusion that the

[14] http://pubs.acs.org/cgi-bin/abstract.cgi/ancham/1954/26/i03/f-pdf/f_ac60087a050.pdf?sessid=6006l3

ca 30% AR measured in ethanolamine was CO_2 resulting from the complete mineralisation of clofentezine is a sound scientific one, even if not absolutely proven.

It can be concluded that an adequate and typical trapping line for an experiment of this type was used and would have been expected to capture any radioactive volatile degradation products.

UK RMS Assessment

The UK RMS was unable to fully validate all the statements provided by the Notifier because information from the raw study data that detailed the sampling of the volatile traps were not provided. However the UK RMS accepted the additional information as providing useful supporting information to address this point of clarification and considered that no further information was required.

Evolution of volatile radioactivity from water / sediment test systems treated with [^{14}C]clofentezine

Results are expressed as % total applied radioactivity

Lode, Sandy Clay Loam

Vessel no.	*Day sampled*	*Day 2*			*Day 7*			*Day 14*			*Day 21*			*Day 28*			*Day 35*			*Day 42*			*Total*		
		ED	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*
1009	*2*	*nd*	*0.05*	*nd*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*nd*	*0.05*	*nd*
1008	*7*	*nd*	*0.06*	*nd*	*0.04*	*2.89*	*nd*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*0.04*	*2.95*	*nd*
1007	*14*	*nd*	*0.03*	*nd*	*0.01*	*1.78*	*nd*	*0.01*	*3.86*	*nd*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*0.02*	*5.67*	*nd*
1006	*21*	*nd*	*0.04*	*nd*	*0.01*	*1.38*	*0.01*	*0.02*	*1.62*	*nd*	*0.02*	*1.94*	*nd*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*0.04*	*4.98*	*0.01*
1005	*42*	*nd*	*0.06*	*nd*	*0.02*	*1.67*	*0.01*	*0.02*	*3.98*	*0.01*	*0.02*	*6.04*	*nd*	*0.05*	*5.51*	*0.04*	*0.36*	*7.53*	*0.01*	*0.33*	*4.18*	*0.01*	*0.80*	*28.97*	*0.08*
1004	*(Spare)*	*nd*	*0.06*	*nd*	*0.01*	*1.85*	*nd*	*0.02*	*4.11*	*0.01*	*0.02*	*6.51*	*nd*	*0.03*	*6.04*	*0.01*	*0.03*	*7.93*	*0.01*	*0.02*	*4.28*	*0.01*	*0.13*	*30.78*	*0.04*
1003	*(spare)*	*0.01*	*0.03*	*nd*	*0.01*	*1.41*	*nd*	*0.01*	*1.78*	*0.01*	*0.02*	*5.54*	*nd*	*0.03*	*5.08*	*nd*	*0.31*	*4.92*	*0.01*	*0.32*	*2.87*	*0.01*	*0.40*	*21.95*	*0.03*

ED = ethanediol, neutral volatile trap
EA = ethanolamine, carbon dioxide trap
SA = sulphuric acid, acid volatiles trap
- = No sample
nd = not detected (<2 X background)
In the report the authors assumed all trapped volatiles to be CO_2 i.e total of EA, ED & SA

Source: FBC study 46J raw data

Saddlers Farm clay loam

Vessel no.	*Day sampled*	*Day 2*			*Day 7*			*Day 14*			*Day 21*			*Day 28*			*Day 35*			*Day 42*			*Total*		
		ED	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*	*ED*	*EA*	*SA*
1017	*2*	*nd*	*0.04*	*nd*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*nd*	*0.04*	*nd*
1016	*7*	*nd*	*0.07*	*nd*	*0.01*	*3.38*	*nd*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*0.01*	*3.45*	*nd*
1015	*14*	*nd*	*0.05*	*nd*	*0.01*	*1.40*	*0.01*	*0.01*	*2.11*	*nd*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*0.02*	*3.56*	*0.01*
1014	*21*	*nd*	*0.05*	*nd*	*0.01*	*1.06*	*0.01*	*0.01*	*4.43*	*0.01*	*0.01*	*6.41*	*nd*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*0.03*	*11.95*	*0.01*
1013	*42*	*nd*	*0.05*	*nd*	*0.12*	*1.78*	*0.01*	*0.01*	*4.21*	*nd*	*0.01*	*6.46*	*nd*	*0.01*	*8.15*	*0.01*	*nd*	*8.51*	*nd*	*0.01*	*4.60*	*0.01*	*0.04*	*31.93*[*]	*0.02*
1012	*(Spare)*	*nd*	*0.06*	*nd*	*0.01*	*2.02*	*nd*	*0.01*	*3.65*	*nd*	*nd*	*5.68*	*nd*	*0.02*	*6.28*	*0.01*	*0.02*	*8.25*	*nd*	*0.02*	*6.41*	*0.01*	*0.08*	*32.35*	*0.01*
1011	*(spare)*	*nd*	*0.08*	*nd*	*0.01*	*3.16*	*nd*	*0.01*	*4.88*	*0.01*	*0.02*	*4.70*	*nd*	*0.03*	*8.65*	*0.01*	*0.03*	*7.37*	*nd*	*0.02*	*3.83*	*0.02*	*0.12*	*32.67*	*0.04*

ED = ethanediol, neutral volatile trap
EA = ethanolamine, carbon dioxide trap
SA = sulphuric acid, acid volatiles trap
- = No sample
nd = not detected (<2 X background)
In the report the authors assumed all trapped volatiles to be CO_2 i.e. total of EA, ED & SA
The study director made a calculation error for the total reported volatiles for sample 1013. The totals for Days 2 and 7 were not included in the overall total. This should have been 33.95%AR. This makes no difference to the overall study conclusions or evaluation.

Source: FBC study 46J raw data

Point of clarification 4.7

Applicant to provide further justification of the whole system DT50 calculations including goodness of fitting. (NOTE: difference between PSD and EFSA estimates may come from the consideration or not of the residue attached to the glass)

See also open point in 4(12) and comments 4(43), 4(48), 4(49) and 4(50)

See reporting table 4(45)

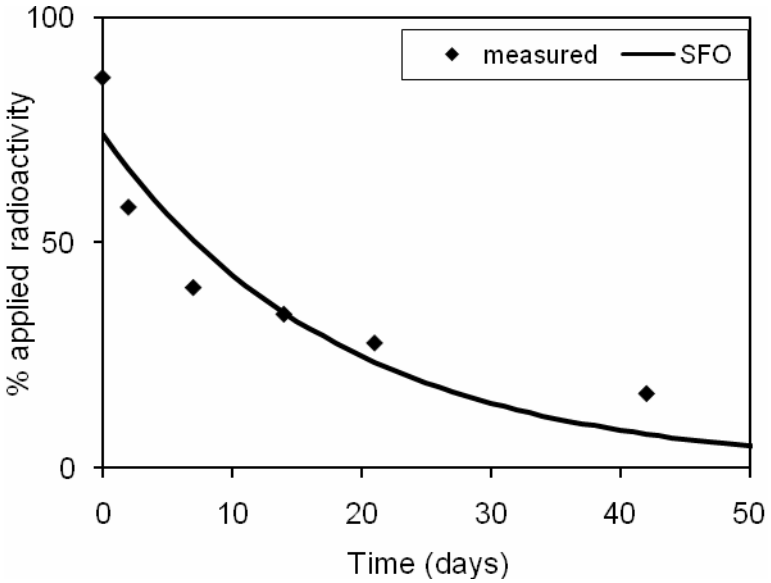
For completeness the UK RMS has re-evaluated the whole system DT_{50} values for clofentezine using the MS Excel worksheet developed by the FOCUS degradation kinetics workgroup. Data were taken from Table B.8.33 on page 326 of the August 2005 DAR and are based on the sum of clofentezine residues detected in the surface water and sediment extracts at each time point. The time zero values were based on the total recoveries presented in Table B.8.32 (page 326 of original DAR). In addition the UK RMS investigated the impact of including results of clofentezine analysis from the glass vessel washing procedure that removed potentially significant amounts of parent material (up to 8.2% AR in the clay loam system). These additional results are included for information purposes only. In the opinion of the UK RMS the inclusion of the glass wash data may not be a valid procedure since this sorbed material may not be fully available for degradation and it could therefore be argued that these data should be excluded from the kinetic calculations. However the UK RMS is not aware of any guidance on how to handle such residues in a consistent manner.

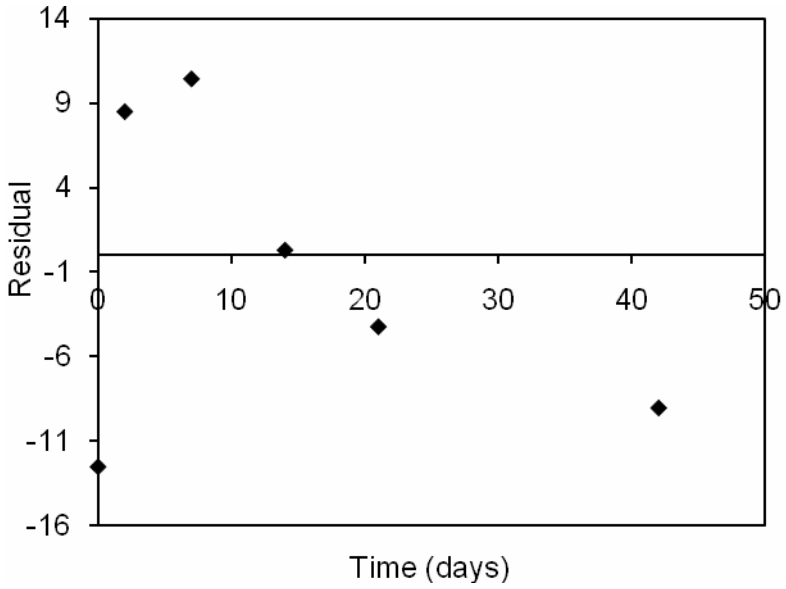
Results are presented in the figures below for each system. In the sandy clay loam system the SFO DT_{50} was 12.7 d (chi^2 = 15.5). In the clay loam system the SFO DT_{50} was 4.2 d (chi^2 = 28.6). If the results of the glass vessel washing procedure are included the DT_{50} values become 13.1 d (chi^2 = 10.1) and 7.1 (chi^2 = 25.4) in the sandy clay loam and clay loam systems respectively. In general in the opinion of the UK RMS the visual fits for the SFO kinetics were relatively poor with or without the glass wash data. This is not unexpected for a substance such as clofentezine that is both rapidly hydrolysed in the water phase and that partitions significantly to sediment, where improved fits with appropriate bi-phasic kinetics may be achieved.

However it should also be noted that the whole system DT_{50} values were only used in the FOCUS surface water Step 1 assessments, and the subsequent risk assessments are based on the maximum initial PECsw values which are unaffected by the actual whole system DT_{50} selected. Therefore although the whole system DT_{50} values do not necessarily represent ideal kinetic fits, a re-working of the assessment to modern guidelines would have no impact on the final assessment as the initial PEC values would be unchanged. On this basis the UK RMS considers that the

original assessment in the DAR is sufficient and proposes that no further work is necessary.

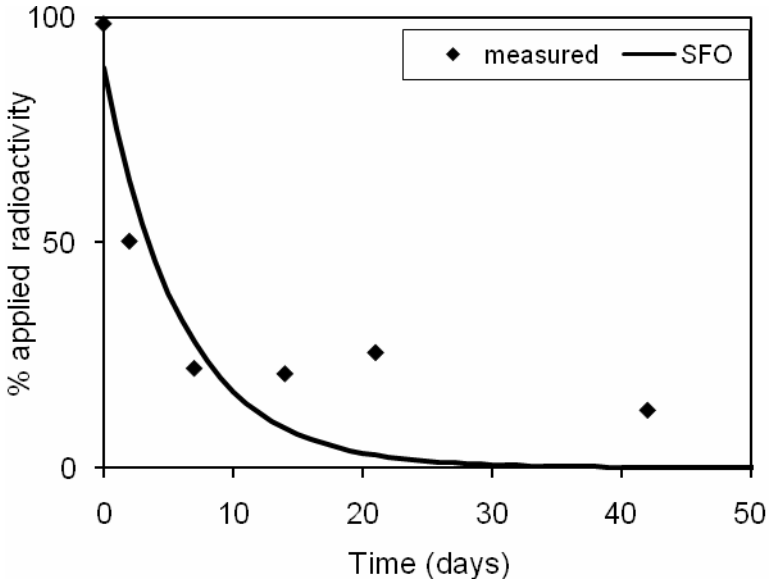
Whole system dissipation behaviour of clofentezine in the sandy clay loam (Lode) system (without inclusion of glass wash data)

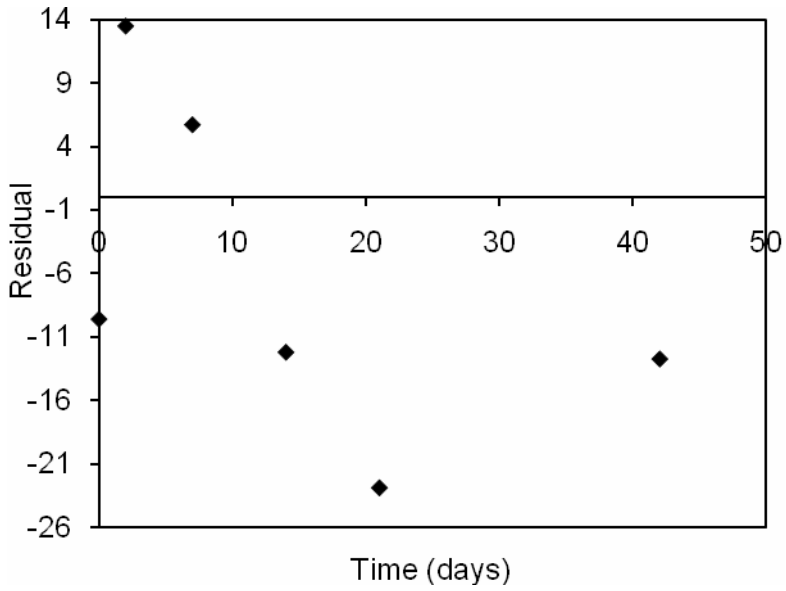




SFO DT_{50} = 12.7 d (chi^2 = 15.5)

Whole system dissipation behaviour of clofentezine in the Clay loam (Sadlers Farm) system (without inclusion of glass wash data)





SFO DT_{50} = 4.2 d (chi^2 = 28.6)

Point of clarification 4.8

Risk assessment based on Step 3 calculations and Step 4 calculations with spray drift mitigation through spray drift buffer zones only should be provided for the EU risk assessment. (Justification: effect of vegetative buffer zones on runoff mitigation is not as straightforward as originally proposed by FOCUS landscape according to the recent EFSA panel opinion).

However, if justified, calculation taking into account run off mitigation may be reported as additional information for MS use.

See reporting table 4(46)

Since this point of clarification was drafted the final report of the FOCUS landscape and mitigation work group has been noted by the Standing Committee.

It should be noted that the original mitigation at Step 4 for the pome/stone fruit (early) applications with implementation of a 35m no spray buffer would seem to result in greater than 95% mitigation of drift which is the upper limit proposed by the FOCUS Landscape report (see Table B.8.49 of the August 2005 DAR, page 339). Spray drift mitigation of late applications to pome/stone fruit, vines and strawberries was within the maximum capped mitigation levels proposed by FOCUS.

For the mitigation of runoff, the Notifier assumed up to 90% reduction of pesticide mass due to the presence of vegetated filter strips up to 35m. It should be noted that these reductions were applied to the pesticide mass only and runoff volumes were considered unaffected by the presence of the vegetated filter strip. The implementation of runoff mitigation would not therefore be consistent with the recommendations of the FOCUS landscape report, where reductions in both pesticide mass and runoff volumes should be applied to all treated fields.

However since the production of the original DAR the effects evaluation has been re-assessed in light of peer review comments and further details are provided in Section B.9 of this Addenda. The UK RMS has proposed that there are two pertinent effects endpoints. A regulatory acceptable concentration of 25µg/l is proposed when exposure is the result of a single spray drift event. A regulatory acceptable concentration of 5µg/l is proposed when exposure is the result of a multiple runoff or drainage events. In the original DAR only the results of the scenarios that gave the maximum Step 3 PECsw values for the pome fruit use were provided (see Table B.8.46 to B.8.48 on pages 337 and 338).

In light of this new assessment it is considered pertinent to re-examine the original exposure assessment.

A revised summary of the maximum initial PECsw values for all Step 3 scenarios and uses are provided below (based on Heimann, 2003b). Details

of the input parameters and model assumptions are provided in Section B.8.5.2 of the original DAR(see page 332 onwards).

Scenario	Apples, pears and plums (early)	
	Water body	Global Maximum (µg/l)
D3	Ditch	15.5
D4	Pond	0.94
	Stream	15.5
D5	Pond	0.94
	Stream	14.8
R1	Pond	0.94
	Stream	12.6
R2	Stream	16.6
R3	Stream	17.7
R4	Stream	12.5

Scenario	Apples, pears and plums (late)	
	Water body	Global Maximum (µg/l)
D3	Ditch	7.3
D4	Pond	0.33
	Stream	7.1
D5	Pond	0.33
	Stream	8.0
R1	Pond	0.33
	Stream	5.6
R2	Stream	7.6
R3	Stream	7.9
R4	Stream	5.8

Scenario	Grapes (early)	
	Water body	Global Maximum (µg/l)
D6	Ditch	0.85
R1	Pond	0.03
	Stream	0.62
R2	Stream	0.82

R3	Stream	0.87
R4	Stream	0.62

Scenario	Grapes (late)	
	Water body	Global Maximum (µg/l)
D6	Ditch	2.57
R1	Pond	0.09
	Stream	1.88
R2	Stream	2.53
R3	Stream	2.65
R4	Stream	1.89

Scenario	Strawberries and ornamentals (early)	
	Water body	Global Maximum (µg/l)
D3	Ditch	1.26
D4	Pond	0.04
	Stream	1.02
D6	Ditch	1.26
R1	Pond	0.13
	Stream	1.39
R2	Stream	1.11
R3	Stream	1.66
R4	Stream	2.55

Scenario	Strawberries and ornamentals (late)	
	Water body	Global Maximum (µg/l)
D3	Ditch	1.26
D4	Pond	0.04
	Stream	0.98
D6	Ditch	1.28
R1	Pond	0.15
	Stream	0.84
R2	Stream	1.12
R3	Stream	1.22
R4	Stream	2.05

From the summary results presented above it is noted that for the grapevine, strawberry and ornamental use the Step 3 PECsw values are lower than both regulatory acceptable concentrations (i.e. 5 and 25µg/l) irrespective of the main route of entry to surface water.

For the apple, pear and plum use, some scenarios exceed the regulatory concentration of 5µg/l, but none exceed the regulatory acceptable concentration of 25µg/l. In the original DAR the RMS investigated the impact of no spray buffer zones on the standard Step 3 PECsw values (i.e. with no mitigation of runoff; see pages 340 and 341 of the original DAR). From these simulations it was clear that for the apple, pear and plum uses, the main route of entry to surface water was via spray drift, since significant mitigation was achieved via no spray buffer zones alone and no significant input via runoff or drainage was noted (see Table B.8.51a, page 341). On this basis the UK RMS concludes that it is appropriate to compare the maximum PECsw values for the apple, pear and plum uses against the higher regulatory acceptable concentration of 25µg/l (appropriate for use against a spray drift driven PECsw values). On the basis that all Step 3 PECsw values are below the appropriate regulatory acceptable concentrations there is now no need to consider the results of the original Step 4 exposure assessments that were presented in the DAR.

The summary Step 3 PECsw values for all uses have been added to the revised list of endpoints.

Open point 4.9

MS experts to discuss the need of further assessment with respect to the air compartment. If considered necessary, the general approach to follow for clofentezine and related substances may need to be discussed as well.

See reporting table 4(56)

In considering this Open point, the UK RMS would like to highlight that the issue of assessments in the air compartment should be seen as a general issue for all substances and not specific to clofentezine. In the comments provided by Sweden in the Reporting table (see 4(56)) there is no additional information provided that is specific to clofentezine.

However the UK RMS accepts that there is a degree of uncertainty in using simple physico-chemical properties to trigger the need for specific assessments of fate in air. This is particularly relevant when this route of exposure can be the result of relatively complex interactions in the environment, including aerosol particle transport (as highlighted in the Reporting table comment). The UK RMS also notes the potential issue regarding the differing physical states of the active substance during the derivation of the Henrys Law constant. However the UK RMS would like to highlight that additional information on the volatilisation of clofentezine from soil and plant surfaces was available in the DAR (see Section B.8.6,

page 344, van der Gaauw, 1990). Over a 24 h period volatile losses were reported to be 1.1-1.8% from plant surfaces and -0.8-1.7% from soil and in the original DAR it was concluded that clofentezine was not volatile from plant or soil surfaces.

It should be noted that the recommendation to use specific vapour pressure triggers in the FOCUS air report was criticised in the PPR Panel Opinion, although it should also be noted that the original DAR was prepared before the finalisation of the FOCUS report.

In the opinion of the UK RMS the existing simplistic assessment of potential for exposure via air for clofentezine is entirely consistent with those assessments that have been performed for many other substances. In the absence of additional substance specific information that indicates that such a simplistic assessment is not sufficient, the UK RMS is of the opinion that no further information can be justified at this stage.

B.9 Ecotoxicology

The additional information below has been prepared by the UK RMS to address the open points and points of clarification identified in the clofentezine Evaluation Table (rev. 0-0, 03.01.2008).

Documents referred to in this assessment are:

The original DAR of 2005 – this will be referred to as **'the DAR'**
The clofentezine Evaluation Table (rev. 0-0, 03.01.2008) – this will be referred to as **'the Evaluation Table'**
The addendum of 2007 – this will be referred to as **'the Addendum'**

In the Evaluation table the following **data gap** was noted:

Applicant to submit:
Information to support the PD values for great tit in pome/stone fruit. justification regarding the focal species in vineyards, PD refinement for cirl bunting and crested lark justification regarding the focal species in strawberries, PD and PT refinement. The risk to insectivorous birds in ornamentals needs to be addressed

See reporting table 5(2)

The revised refined risk assessment is presented in Addendum 1.

This assessment used data on focal species in strawberry fields as well as orchards. This work indicated that for strawberries the focal species were skylark and yellow wagtail. Further data were submitted on the proportion of food obtained from the treated area (PT) as well as the proportion of food types obtained from within the treated area. These data indicate that the 90 percentile of yellow wagtails observed feeding in strawberry fields was 95% (i.e. 90% of the population of consumers obtained 95% of their food from the treated area). The mean was 60%. As regards skylarks the 90th percentile was 99% and the mean was 86%. Data on the proportion of food types obtained from the treated area was obtained and divided in to 'large' and 'small' invertebrates. These refinements were then fed back in to the ETE equation and TERlt of 7.7 were obtained for the yellow wagtail assuming a PT of 0.6 and a TERlt of 4.9 assuming a PT of 0.95. As for skylark the TERlt were 8.74 and 10.1 assuming PT of 0.99 and 0.86 respectively.

Open point 5.1
In the Evaluation Table, the following open point was noted:

RMS to include in an addendum the risk assessment for birds from uptake of contaminated drinking water. See reporting table 5(3).

In order to address this point, the following assessment is proposed:

> Assuming maximum application rate of 200 g/ha, an application volume of 200 L/ha, an acute oral LD50 of >3000 mg a.s./kg and a NOEC of 7.62 mg a.s./kg bw/day, a PECsw of 0.047 mg/l (FOCUS Step 1); the resulting exposure estimates are 53.9 mg a.s./kg bw for a 0.01 kg insectivorous bird. The resulting TERa and TERlt are >55.6 and 601 respectively.

These indicate a low acute and long-term risk to birds

Open point 5.2

In the Evaluation Table, the following open point was noted:

> *RMS to include in an addendum the risk assessment for mammals from uptake of contaminated drinking water. See reporting table 5(8)*

In order to address this point, the following assessment is proposed:

> Assuming maximum application rate of 200 g/ha, an application volume of 200 L/ha, an acute oral LD50 of >5200 mg a.s./kg and a NOEC of 40 mg a.s./kg bw/day, a PECsw of 0.047 mg/l (FOCUS Step 1); the resulting exposure estimates are 53.9 mg a.s./kg bw for a 0.01 kg insectivorous mammal. The resulting TERa and TERlt are >165.7 and 5425 respectively.

These indicate a low acute and long-term risk to mammals.

Open point 5.3

In the Evaluation Table, the following open point was noted:

> *RMS to include the aquatic TERs for all uses in the LoEP. See reporting table 5(10).*

The list of endpoints has been updated.

Open point 5.4

In the Evaluation Table, the following open point was noted:

> *RMS to include in an addendum all details on the studies with aquatic organisms which are required for a transparent and comprehensible evaluation of the endpoints derived from the studies.*
> *If the RMS does not wish to report water parameters, photoperiod, fish size/load it is agreed that it would be enough to state that this was assessed by the RMS as being in accordance with the respective guideline. However key information such as tested concentrations,*

observed mortality/effects at each concentration, observation of sub-lethal effects, statistical methods, confidence intervals, analytical methods, batch no., should always be reported in the study summaries for reasons of transparency and to facilitate the peer-review of the suggested endpoints. See also comment 5(17) See reporting table 5(11)

The RMS acknowledges that further detail in the study summary would have been more useful, however they feel it should be noted that all studies were carried out to standard protocols and hence issues such as temperature, pH, fish loading were all met. It should however further be noted that few of these studies were considered appropriate for risk assessment purposes – see Table B.9.2.16 (a), (b) and (c) in the original DAR for detailed consideration.

Open point 5.5

In the Evaluation Table, the following open point was noted:

RMS to report in an addendum the observations/endpoint from the 21 d chronic daphnia study with the formulation (Barber and Barrett, 1990) and to clarify why the study was considered not acceptable. MSs to discuss in an expert meeting the setting of the NOEC for daphnids. (This may be necessary if the chronic endpoint for fish which is currently triggering the risk assessment is changed to a higher value - see open point 5(19), See reporting table 5(15)

In order to try to address the above open point all the chronic *Daphnia* studies from the DAR are presented in Appendix 1. Presented below is a brief summary of each study as well as a conclusion as to whether they can be used for risk assessment purposes.

Barber and Lattimore (1992) – this was a standard study, however the compound was absorbed on to pumice stone in order to supply dissolved clofentezine to the test chamber. As a result of this study, the NOEC was 0.025 mg a.s./L (This was the only concentration tested). This NOEC is ten times greater than the water solubility[15]. There is concern regarding comparing this endpoint to PECs where both figures are greater than the water solubility, however what this study does indicate is that there are no effects on Daphnia at ten times the water solubility.

Barber and Barrett (1990) – this study was carried out using the formulation, Apollo 50SC. This study was a standard chronic *Daphnia* reproduction study where concentrations were renewed thrice weekly. The NOEC from this study is stated to be 0.1 mg form/L, which is equivalent to 0.05 mg a.s./L or twenty times the water solubility.

[15] The water solubility of clofentezine is 0.00252 mg/L or 2.52 µg/L.

Barber and Barrett (1993) – this was a non-standard study that was aimed at assessing the determining the effects of the formulation. It was concluded that the sub-lethal effect of Apollo 50SC on reproduction is based on a physical effect as the feeding efficiency of the species is inhibited by the particulate nature of the suspended particles.

Mattock (1999) – this study assessed the chronic toxicity of clofentezine to Daphnia in the presence of sediment. Apollo 50SC had no significant effect on survival or growth of Daphnia magna when applied to a water-sediment system at a rate giving initial overlying water concentrations of 0.5 mg form/L, equivalent to 0.25 mg a.s./L.

Clofentezine has a water solubility of 0.00252 mg a.s./L. Due to the low water solubility various methods were used to determine the chronic toxicity of clofentezine. Taking the active substance study first, Barber and Lattimore (1992) used pumice to enable the active substance to remain in water. The maintenance of the active substance in the water phase was also aided by carrying out the study under renewal conditions. As a result, the NOEC, which was also the highest concentration tested, was 0.025 mg a.s./L Studies were also carried out using the formulation. The co-formulants enable the active substance to remain in solution. The study by Barber and Barrett (1990) used the proposed formulation and indicated that the NOEC was at the lowest concentration tested, i.e. 0.1 mg form/L. This concentration is equivalent to 0.05 mg a.s./L. There was one study (Barber and Barrett (1993)) that indicated that the formulation itself played a role in causing a chronic effect. Finally, there is a sediment-water study (Mattock (1999)) that assessed the effects of the formulation in the presence of sediment. This gave a NOEC of 0.5 mg form/L which is equivalent to 0.25 mg a.s./L.

The study by Barber and Barrett (1990) indicates what the chronic toxicity of the active substance is to *Daphnia magna*. The study is complicated, and hence uncertainty introduced, by the fact that pumice was used and hence the effect of this on the overall NOEC is not known. Further uncertainty is introduced by the fact that only one concentration was tested, therefore it is not know whether clofentezine could have caused an effect. These comments do not invalidate the study, they merely highlight the issues that need to be considered. Similarly, the study by Barber and Lattimore (1992) indicates that the NOEC is 0.05 mg a.s./L. The first point to note is that the difference in the NOEC between this study and the one by Barber and Barrett (1990) is a potential artefact as the former study was not carried out at 0.05 mg a.s./L. The study by Barber and Lattimore (1992) is potentially worst case as it assesses the effects of the formulation and there is evidence (see Barber and Barrett (1993)) that the formulation may play a role in the chronic toxicity. Therefore, the endpoint of 0.05 mg a.s./L from this study is considered to be reliable. As regards the Mattock study, this is carried out under potentially more 'realistic' conditions and this gives a NOEC of 0.25 mg a.s./L. Different life stages were assessed and therefore, this study

is considered appropriate for consideration in higher tier assessments, if required.

Before deciding on an appropriate endpoint there needs to be a consideration of what the exposure will actually be; it is clear from the water solubility that clofentezine will not be present in the water phase for any significant time above its water solubility, i.e. 0.00252 mg a.s./L. Clofentezine will exist in the water phase at concentrations above its water solubility when in formulation, however it is considered that formulation will not exist as such for any significant duration. It should also be noted that clofentezine entering via drainflow or runoff will only do so as active substance; entry via spray drift will be via formulation. If it is accepted that clofentezine will not exist above its water solubility then we have a study that indicates that there is no effect at ten times the NOEC (Barber and Lattimore (1992)). Whilst there is some uncertainty regarding the potential influence of pumice in this study, supporting evidence from the study using the formulation indicates that there will not be any effect at 20 times the NOEC. Both of these studies are potentially worst case, in that they are assessing the effect of a compound above its water solubility; which clearly will not happen.

The study by Mattock assessed the potential impact on different life states and indicates that if entry via spray drift is important then the NOEC from this route will be 0.25 mg a.s./L.

From the above, it can be concluded that if the major route of exposure is via drainflow or runoff then the water solubility will be the limiting factor and hence the concentration will be limited to 0.00252 mg a.s./L. On the basis of the Barber and Lattimore (1992) and the Barber and Barrett (1990) study we know that there is unlikely to be any effect at 10-20 times the water solubility, hence it is proposed to use an endpoint of 0.05 mg a.s./L for these routes of exposure. This endpoint has been chosen as it is know that there are no effects at 0.0025 mg a.s./L when the a.s. is absorbed on to pumice and there are also no effects at 0.05 mg a.s./L when the active substances is tested as the formulation.

If the main route of exposure is spray drift, then it is proposed to use the endpoint from the Mattock study of 0.25 mg/L (1999).

In reality, it must be remembered that the concentration of clofentezine in water will not exceed the water solubility and that we have data that indicates that there will be no effects at an artificially maintained concentration of twenty times the water solubility.

Open point 5.7

In the Evaluation Table, the following open point was noted: *RMS to evaluate in an addendum the new fish ELS study with the formulation. See reporting table 5(19).*

This is considered below:

The early-life study was submitted and has been evaluated and is presented in Addendum 1. The study used the formulation and the NOEC was 1000 µg a.s./L or 1.0 mg a.s./L. This endpoint has been used in the risk assessment and the list of endpoints update.

Open point 5.8/Point for clarification 5.1

In the Evaluation Table, the following open point was noted: *It seems that it was not possible for the RMS to assess the field studies with T. pyri since the study reports were either not complete and/or in German language only. Therefore it is suggested to delete the results of the field data from the LoEP. See also data requirement 5(23) and comment 5(29) See reporting table 5(20).* This point is addressed below:

The list of endpoints states that 'field data on *T pyri* indicate that overall effect is less than 50%'. This statement is based on a range of studies. The Notifier has submitted that actual reports and the studies are evaluated below:

Study summaries were presented, these were all relatively brief as it is believed that they followed standardised BBA protocols. No of the studies were to GLP, however all were done before this was a requirement. Details of the study as well as the results are presented below:

Annex Point	Author	Date study conducted	Study Title	Methodology and findings
IIA, 8.3.2/14	Gernoth	1987	*Typhlodromus pyri*. Nebenwirkung auf Raubmilben. Regierungspräsidium Freiburg - Pflanzenschutzdienst No GLP, not published	Mature apple trees were treated with Apollo, following normal agricultural practice. A total of 10 trees were sampled. The study was conducted on two varieties of apples, the application rate for both varieties was 0.61 L/ha. On one variety there was 0% effect on the number of *T pyri* on 50 sampled leaves, this compared to a 79% effect of the toxic standard. On the other variety there was no effect compared to the untreated control. The study summary stated that at the time of application, only adult individuals and eggs were present. It also stated that individuals of the new generation were also noted as the study progressed. The conclusion of the study authors indicated that Apollo did not damage the predator mite population.
IIA, 8.3.2/15	Englert, W.D.	1985	*Typhlodromus pyri*. Untersuchungen zur Auswirkung von Pflanzenschutzmitteln auf die Raubmilbe im Weinbau. BBA Bernkastel-Kues No GLP, not published	The study was conducted on grapevines and used a formulation called 'SCH 11750 A 0.24%' it is assumed that this Apollo 50 SC. It was also unclear what the application rate was. Therefore, the value of this study is limited.
IIA, 8.3.2/16	Kast, W.K.	1989	Versuchsbericht der LVWO Weinsberg für die Prüfung im Zulassungsverfahren: Bekämpfung von Spinnmilben - Nebenwirkung auf Raubmilben. LVWO Weinsberg, Report No. 89-01c-08 No GLP, not published	The study was conducted using 'Apollo (SCH 11750 A) at an application rate of 0.3%. Some limited environmental details were provided, along with an indication of the effects. It appeared from the brief summary that there was no effect on the number *T pyri* present on leaves before and after treatment compared to the control.
IIA,	Schruft, G.	1989	Überprüfung der Auswirkung von	This study appeared to conducted at the same rate and to

Annex Point	Author	Date study conducted	Study Title	Methodology and findings
8.3.2/17			Pflanzenschutzmitteln auf Raubmilben (*Typhlodromus pyri* Scheuten) im Weinbau. Staatliches Weinbauinstitut Freiburg No GLP, not published	the same design as the Kast (1989), and the results indicated the same thing.
IIA, 8.3.2/18	Anonymous	1985b	*Typhlodromus pyri*. Zulassungsprüfung 1985. Landeslehr- und Versuchsanstalt Oppenheim No GLP, not published	Only a summary table was submitted. This indicated that SCH 11750 A was applied at the rate of 0.04% and that there was no adverse effects.
IIA, 8.3.2/19	Anonymous	1985c	*Typhlodromus pyri.* Prüfung 1985 der Auswirkung von Pflanzenbehandlungsmitteln auf Raubmilben im Weinbau. Institut für Phytomedizin und Pflanzenschutz Geisenheim No GLP, not published	The product used was SCH 11750 A (Apollo SC) at the rate of 0.04%. A similar table was submitted for this study and similarly no adverse effects were noted.
IIA, 8.3.2/20	Anonymus	1985d	*Typhlodromus pyri*. Prüfung 1985 der Auswirkung von Pflanzenbehandlungsmitteln auf Raubmilben im Weinbau. Institut für Phytomedizin und Pflanzenschutz Geisenheim No GLP, not published	The product used was SCH 11750 A (Apollo SC) at the rate of 0.04%. A similar table was submitted for this study and similarly no adverse effects were noted.
IIA, 8.3.2/21	Anonymus	1985e	*Typhlodromus pyri*. Ergebnisse der Prüfung von Präparaten gegen Raubmilben 1985. Landes-Lehr- und Forschungsanstalt für	The product used was SCH 11750 A (Apollo SC) at the rate of 0.04%. A similar table was submitted for this study and similarly no adverse effects were noted.

Annex Point	Author	Date study conducted	Study Title	Methodology and findings
			Landwirtschaft, Weinbau und Gartenbau Neustadt No GLP, not published	

From the above table it can be determined that clofentezine as Apollo or SCH 11750 A has little effects on *T pyri*. However, the study summaries are extremely brief and unclear regarding precise application rates (in terms of g/ha) as well as other experimental details. Therefore, it is proposed that these do not add significantly to the available information on clofentezine and that the data and corresponding risk assessment presented in Addendum 1 should be consulted. The list of endpoint has been amended.

Appendix 1

Evaluation of chronic *Daphnia* studies – taken from original DAR

Due to the extremely low solubility of clofentezine in water, the compound was first absorbed to pumice stone that was then used, via a saturation column, to supply dissolved clofentezine to the test chambers. First instar daphnids (less than 24 hours old) were exposed for 21-days to the single concentration of [^{14}C]-clofentezine (purity 99.8%), 0.025 mg a.s./L, which represented the maximum sustainable concentration under the test conditions. The rate of renewal of the test solutions was approximately 25 ml/min, which resulted in greater than ten chamber volume renewals per day. Samples of the test solutions were analysed at intervals throughout the study using appropriate methods to quantify actual [^{14}C]-clofentezine test concentrations. Test organisms were checked daily and the effects on survival and reproduction recorded. At the end of the exposure period, body length of the surviving parental *Daphnia* was measured to assess any effects on growth.

Table B.9.2.9: Results from a *Daphnia magna* 21-day chronic toxicity study

Concentration mg a.s./L	Mortality (%)	Body length (mm)	Number of Live off-spring per adult
0	12.5	3.54 ± 0.04	56.10 ± 11.87
0	10.0	3.91 ± 0.04	99.15 ± 10.57
0.025	12.2	3.80 ± 0.04	82.13 ± 13.91
0.025	19.5	3.85 ± 0.02	92.80 ± 2.44

Concentrations of [^{14}C]-radio-label, in the test solutions indicated that the clofentezine test concentrations were maintained between 0.021 and 0.033 mg a.s./L, with a mean recovery of [^{14}C]-clofentezine of greater than 90%.

Cumulative mortality of the parental daphnids was < 20% in all cases. There was a statistically significant difference in body length. However, analysis by Tukey multiple range tests showed that this was due to slightly smaller body lengths in one of the control groups. The total number of live offspring was not significantly reduced by clofentezine when compared to the control. Results are summarised in Table B.9.2.9.

The results outlined in Table B.9.2.9 indicated that [^{14}C]-clofentezine had limited effects on survival at 0.025 mg a.s./L. There was no effect on growth or reproduction of *Daphnia magna* at the maximum solubility under the conditions of this test. The NOEC from this study was 0.025 mg a.s./L.

The study was conducted according to OECD 202 II (April 1984), EPA 540/9-86-141 (July 1986) – flow-through system methodology and was in compliance with GLP.

(Barber and Lattimore 1992)

b) The chronic toxicity of Apollo 50 SC to *Daphnia magna* was assessed in a 21-day semi-static test. No carriers or vehicles were used. The nominal concentrations of 0, 0.1, 0.35, 1.23, 4.29 and 15.0 mg form/L were tested on 20 animals per concentration, with the solution renewed thrice weekly. Oxygen, pH, temperature and a.s. content were checked on day 0, 7, 14 and 18 on freshly prepared solutions, and on day 2 (48 hour old) and day 21 (72 hour old) on aged solutions. Initial measured concentrations ranged from 95.12% to 100.41%, however mean measured concentrations at renewal ranged from 33.1% to 91.0%. End points are quoted as nominal concentrations. Mortality and number of offspring were checked daily. Statistical analysis was carried out using one-way ANOVA, with $p = 0.05$, and where necessary, Tukey multiple range tests to compare differences between the means for each treatment.

Table B.9.2.10: Results of a sub-lethal toxicity test of Apollo 50 SC to *Daphnia magna*

Concentration (mg form/L)	**% Mortality at day 21[2]**	**Day of first offspring**	**number of live offspring per adult**
0	12.5	9	199.60
0.10	0	9	183.13
0.35	2.5	9	170.79*
1.23	2.5	9	176.41*
4.29	0	9	172.67*
15.00	5	9	164.62*

* Significant difference acc. to ANOVA test. All values based on nominal concentrations
[2] Calculated by reviewer from raw data

The pH, oxygen and temperature were determined to be within the acceptable limits. At 4.29 and 15 mg/L there was some settlement of the test compound over the 48 to 72 hour periods between renewals. It was also noticed at these test concentrations that the digestive tracts of the animals had a distinct pink coloration. At 15 mg/L it was noted that one animal was partially covered in the test compound. This apparently interfered with the animals' ability to swim and feed and the animal remained small throughout the test, only depositing eggs in the brood pouch on day 21. Results are summarised in Table B.9.2.10.

The NOEC of Apollo SC to *Daphnia magna* is 0.1 mg/L, equivalent to 0.05 mg a.s./L; the LOEC is 0.35 mg form/L, equivalent to 0.175 mg a.s./L.

The study was conducted according to OECD 202 II (1984) and was in compliance with GLP.

(Barber and Barrett 1990)

c) Apollo 50 SC is a deep pink colour and contains particles up to 8 µm in diameter. Particles of this size are within the range of particles consumed by

many filter feeding zooplankton. Therefore, a study was conducted to assess the effects of Apollo 50 SC on the reproduction of *Daphnia magna*. The effect of Apollo 50 SC was compared to the effect of comparable concentrations of 'inert' latex beads and a formulation blank.

After 21 days exposure, the number of offspring was determined. In addition, individuals were observed with a light microscope in order to determine whether particles had been ingested.

Latex beads and suspended particles from Apollo 50 SC had similar effects on the number of offspring of *Daphnia magna*. Observations under the light microscope showed that both Latex beads and clofentezine particles had been ingested and were clearly visible in the gut.

It was concluded that the sub-lethal effect of Apollo 50 SC on the reproduction of *Daphnia magna* is based on a physical effect, with the feeding efficiency of the species inhibited by the particulate nature of the suspended clofentezine particles.

The study was conducted to an in-house method, which was based on the OECD 202 II methodology. The study was not conducted to GLP.

(Barber and Barrett 1993)

d) To order to determine the effects of Apollo 50 SC on the reproduction of *Daphnia magna* a modified 21-day chronic toxicity study was used. Ten first instar daphnids were introduced into each of twenty eight test vessels, each containing a 2-cm depth of sediment (loamy sand, 1.1% organic carbon) and approximately 400 ml of overlying water. The test vessels had been aerated and allowed to equilibrate for just over three weeks prior to the start of the study. Four test vessels were used as controls. Of the remaining twenty four vessels, Apollo 50 SC was applied at the equivalent of an overlying concentration of 0.275, 0.5 and 2.5 mg form/L, to four replicate test vessels for each concentration immediately after introduction of the neonates. After six days (once the first brood eggs were visible in the brood pouch), Apollo 50 SC was applied at the same three rates to the remaining vessels. Determination of clofentezine concentrations was performed for a sample of the aqueous stock solutions on each application occasion (day 0 and 6). In addition samples of the overlying water were taken from additional test vessels specifically set up for chemical analysis of the test media. The daphnids in each vessel were observed daily for mortalities, any juveniles present were removed and counted. At the end of the exposure period the body lengths of the surviving parental *Daphnia* were measured to assess any effects on growth.

Mean measured concentrations of clofentezine in the test media on day 0 were 0.109, 0.200 and 1.09 mg a.s./L and were equivalent to 96, 97 and 105% of the nominal concentration, respectively. Initial mean measured concentrations of clofentezine in the test media on day 6 were 0.093, 0.178 and 1.02 mg a.s./L and were equivalent to 82, 86 and 98% of the nominal

concentrations respectively. Therefore, the results indicated that all initial concentrations were within ± 20% nominal at the time of application, and so the effect concentrations were all based on initial nominal Apollo 50 SC test concentrations applied to the test vessels. No data were submitted to indicate whether concentrations were maintained over the duration of the study.

The effect of Apollo 50 SC (NOEC, LOEC and EC/LC50) on survival, growth and reproduction of *Daphnia magna* are summarised in Table B.9.2.11.

Table 9.2.11: Toxicity End points in mg form/L for the effect of Apollo 50 SC on daphnids in a 21-day water/sediment study

	Apollo 50 SC applied on Day 0			**Apollo 50 SC applied on Day 6**		
	NOEC*	**LOEC***	**EC/LC50***	**NOEC***	**LOEC***	**EC/LC50***
Survival	2.5	>2.5	>2.5	2.5	>2.5	>2.5
Growth	2.5	>2.5	>2.5	2.5	>2.5	>2.5
Reproduction	0.5	2.5	>2.5	0.5	2.5	>2.5

*all effect concentrations were based on detection of statistically significant effects (ANOVA, $p < 0.05$)

Statistically significant effects were only detected for reproduction (offspring per female). At the highest rate tested, the reduction in mean offspring production was equivalent to 19% for < 24-hour old adult daphnids, and 21% for 6 day old adult daphnids carrying eggs in the brood sac.

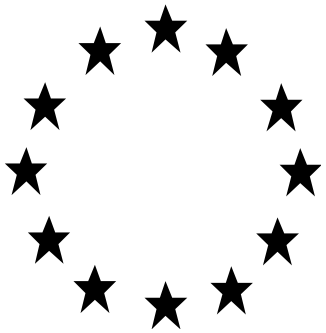
Apollo 50 SC had no significant effect on survival or growth of *Daphnia magna* when applied to a water-sediment test system at rates giving an initial overlying water concentration of 2.5 mg/L. The effects of the product, at the rates tested, was similar irrespective of whether < 24 h old neonates or 6 day old adults carrying the first brood in the brood sac were present at the time the product was applied. However, the product did result in a statistically significant effect on reproduction of *Daphnia magna* at the highest initial concentration tested of 2.5 mg form/L. Therefore, the NOEC is 0.5 mg form/L which is equivalent to 0.25 mg a.s./L.

The study was conducted to OECD 202 II (1984), with the following deviations:
In 2 separate groups the test substance was either added to neonates (OECD 202 II) or when eggs were in the brood sac in order to test the sensitivity of the two most sensitive life stages. In this aspect, the study exceeds the requirements of OECD 202 II. The test was conducted as a water/sediment test, with 2 cm loamy sand as sediment.
The test was conducted with a static test system in order to simulate a single influx of Apollo SC into the aquatic environment. The study was in compliance with GLP.

(Mattock 1999)

B.9.11 References relied on

Annex point / Ref. No.	Author	Year	Title Source (where different from company) Company, Report No. GLP status, published or not	Data protection claimed Y/N	Owner*
IIIA, 10.2.4/02	Barber, I., Barrett, K.L.	1990	. Determination of the effects of Apollo 50 SC on the life-cycle of Daphnia magna. Schering Agrochemicals Ltd., Report No. ENVIR/90/33, Aventis No. W88, MAK No. R-12995 GLP, not published	Y	Irvita
IIA, 8.2.5/01	Barber, I., Lattimore, A.E.	1992	Determination of the effects of [^{14}C]-Clofentezine on the life cycle of Daphnia magna. Schering Agrochemicals Ltd., Report No. ENVIR/92/066, Aventis No. A82572=W93, MAK No. R-12679 GLP, not published	Y	Irvita
IIIA, 10.2.4/03	Barber, I., Barrett, K.L.	1993	The effects of a pesticide suspension concentrate formulation on the reproduction of Daphnia magna Proceedings of the 2nd European Conference on Ecotoxicology - Science of the Total Environment. Supplement 134: 853-858 No GLP, published	N	
IIA, 8.2.5/04 [IIIA, 10.2.4/04]	Mattock	1999	Clofentezine suspension concentrate 50g/L: Reproduction test with Daphnia magna in a water-sediment test system. Covance, Report No. ENVIR/99/009, Aventis No. C003977, MAK No. R-12996 GLP, not published	Y	Irvita



Clofentezine

Draft Assessment Report

Addendum 3

[Residues, Environmental Fate and Behaviour, Ecotoxicology]

February 2009

CONTENTS **Page**

7. Residues data

B.7.2 Metabolism, distribution and expression of the residues in livestock (AII 6.2, IIIA 8.1)

B.7.2.1 Cattle

Following PRAPeR 65 (22 -23 January 2009) there was a request to provide all the available ruminant metabolism data in the residues section.

i) A 1988 study was performed to characterise the [^{14}C] residues in tissues of a fresian cow (bodyweight 480kg) following oral dosing with [^{14}C]-clofentezine for 3 days at a rate of 2.2mg/kg bw/day. Radio-labelled [^{14}C]-clofentezine of radiochemical purity 99% was used in the study, the clofentezine being radio-labelled at one of the two equivalent carbons in the tetrazine ring, as has been the case in the plant metabolism studies. The specific activity of the [^{14}C]-clofentezine used in the study was reduced by addition of unlabelled material; the specific activity of the material was determined to be 2.39 mCi g^{-1}. The cow was dosed orally via capsule to an average dose of 2.21 mg/kg bwday, which is a rate equivalent to approximately 42.5N for beef cattle and 150 N for dairy cattle.

Milk was collected twice daily through the study period until sacrifice. After sacrifice, samples of liver, kidney, renal fat, muscle and sub-cutaneous fat were taken and immediately frozen.

Milk was stored at -20°C for 20 months – on analysis samples were freeze dried then soxhlet extracted with diethyl-ether and methanol. Various clean-up methods, such as hydrolysis and enzyme incubation were used with the extracts then being acidified and extracted with ethyl acetate/hexane.

Levels of radioactivity in milk are shown to plateau at a level of 0.17 µg clofentezine equivalents ml^{-1} at the morning milking of the 3rd day of the test. 93% of the radioactivity was accounted for from the samples. Extracts were chromatographed against potential metabolites found in the rat metabolism study in various TLC systems. It was found that 75% of the radioactivity present in milk could be concluded to be present as 4-OH clofentezine. Small amounts of radioactivity remained unresolved, however there was no evidence of significant quantities of metabolites other than the 4-OH clofentezine.

The majority of radioactive residue found in tissue was found in liver – total detected radioactivity levels for tissue types are shown in Table B.7.1 below:

Table B.7.1 Levels of total radioactivity detected in tissues collected at sacrifice of a lactating cow 16 hours after oral administration of [^{14}C]-Clofentezine at an average rate of 2.2mg/kg/day

Tissue	**TRR (mg clofentezine equivalent/kg)**
Liver	0.760
Kidney	0.357
Muscle	0.016
Renal fat	0.262
Subcutaneous fat	0.020

60% of the total radioactive residue in liver was extracted by soxhlet extraction with diethyl ether and methanol – the methanolic extract containing 53.5% TRR which appeared to correspond to 4-OH clofentezine when subject to TLC using chloroform: methanol: ammonia. The methanolic extract was further extracted with snail juice to liberate 44.1% of the TRR which was further extracted by use of 12.5% ethyl acetate in hexane. This extract was then washed with acetotnitrile, which liberated 14.8% of the residue. This co-chromatographed with 4-OH clofentezine. The ethyl acetate: hexane fraction required further clean up via use of an LC Diol cartridge, acetone precipitation then repeat hydrolysis with snail juice. Of the total residue on a repeated analysis, 68% was found to be solvent extractable. Hydrolysis of this extractable residue with partitioning into ethyl acetate/hexane produced a single component co-chromatographing with 4-OH clofentezine. No evidence was obtained for the presence of metabolites other than 4-OH clofentezine. Note is made that data from previous studies show that the remaining non-solvent extractable residue may be broken down by acid hydrolysis and is composed of ortho chlorobenzoic acid. Further investigation into the 32% unextractable residue was done in study ii below.

(Phillips M.W.A, Swalwell L., 1988)

ii) An additional study was also submitted, determining the level of residues and metabolites of [^{14}C] found in fat, liver, kidney and urine in a lactating dairy cow (as used in the Phillips, Swalwell study above). The investigation is predominantly based on the characterisation of metabolites found in the bound fraction of the liver residue, in addition to characterisation of the residues found in kidney and fat. The samples analysed had been stored at -20°C for approximately 4 months.

Fat:

Renal fat was homogenised in hexane, which was then extracted with acetonitrile, before clean up by LC Diol cartridge. The eluant was precipitated with acetone which was then subject to snail juice hydrolysis with no significant effect. The LC Diol clean up phase was repeated until the majority of activity had been extracted. The extract samples (containing 90.1% of the activity) were subject to TLC with 4-OH Clofentezine in various solvent systems. A minor unidentified peak was noted, which appears to be a

result of hydrolytic breakdown of 4-OH clofentezine during extraction. This peak will account for less than 0.05 mg/kg at 1N rate. The vast majority of residue in fat can therefore be said to be 4-OH clofentezine.

Liver:

60% of the total radioactivity found in liver samples had been extracted by solvent extraction as investigated in the study by Swalwell and Phillips, 1988 shown above. Further extraction of the remaining 40% 'bound' residue was done as follows. Samples of liver were subject to soxhlet extraction, before hydrolysis with collagenase and subtilisin enzymes for periods of between 24 hours and several weeks. The resulting extract was centrifuged and separated into residue and supernatant were treated separately. The residue was soxhlet extracted before being hydrolysed with snail juice and subjected to clean up. The supernatant was also hydrolysed with snail juice before clean up. These extracts were then combined and incubated with subtilisin and pancreatin prior to analysis by TLC. Extracts which had been extracted in the solvent phase accounted for 4.4% of the total residue. Further clean up procedures failed to produce any extracts suitable for TLC determination.

A more gentle extraction method was also carried out in order to improve the efficiency of extraction of bound radioactivity. Samples were homogenised in methanol before being washed with water and incubated with collagenase and subtilisin. The suspension was then hydrolysed with snail juice and extracted with ethyl acetate/ hexane/ acetonitrile. All fractions were then subject to clean-up, via LC Diol cartridge for the acetonitrile fraction or by C18 Mega Bond Elut, but none of the fractions were suitable for analysis by TLC.

The residue was washed with methanol before being combined with the aqueous extracts which were reduced to dryness before being redissolved in phosphate buffer to which was added subtilisin and EDTA before repeated dialysis at 50-60°C for 6 days. Again clean up was via the use of a C18 Bond Elut cleanup column, with eluant solvents of acetone and methanol being used.

This extraction method procured 67.1% of the total radioactivity in the methanol extraction phase which was identified as 4-OH clofentezine. The remaining 32.9% was classed as bound radioactivity which was subjected to enzyme extraction together with snail juice hydrolysis and various solvent extractions. The acetonitrile fraction gave 6% of the total bound radioactivity which was characterised as 4-OH clofentezine by TLC. The aqueous fraction did not produce extracts which could run via TLC. The extracts subjected to digestion by subtilisin in the dialysis sack were characterised as polar and un-polar metabolites which were subject to further analysis, being monitored by TLC in polar and non-polar mediums against potential hydrolysis products of 4-OH clofentezine. Peaks with similar chromatographic properties to 4-OH clofentezine and its hydrolysis products were observed, and accounted for a further 18.3% TRR. The conclusion was made that the main metabolite in liver is 4-OH clofentezine, with the hydrolysis products only resulting due to artificial breakdown as a result of the extraction procedures involved.

Kidney

Kidney was soxhlet extracted with diethyl ether, then methanol liberating a total of 83.2% of the total radioactivity. The ether phase was precipitated in acetone then cleaned up by LC Diol cartridge, before analysis by TLC. The extract co-chromatographed with 4-OH clofentezine. The methanol extract also contained a major peak which co-chromatographed with 4-OH clofentezine, although other components did appear to be present. Further extraction was undertaken as for liver to try and ascertain the identity of these components. The kidney was homogenised in methanol before being washed with hexane and solvent extracted with ethyl acetate:hexane and acetonitrile. The acetonitrile fraction contained 56.5% of the TRR, which was assayed to be similar to 4-OH clofentezine and unidentified components. Similarly the aqueous fraction contained 18.6% TRR which again was found to give similar peaks to 4-OH clofentezine and two unidentified components. Taking into account that a proportion of this radioactivity is 4-OH clofentezine, the unidentified components will account for less than 0.05 mg/kg at 1N rate. Once again, as for liver, the other components appeared to be hydrolysis products of 4-OH clofentezine, resulting from breakdown of 4-OH clofentezine as a result of the extraction procedures used.

The ^{14}C levels found in fractions of milk and tissues are shown in table B.72 and the distribution of clofentezine and metabolites found in milk and tissues are shown in table B.7.3.

Table B.7.2 Partitioning of extractable radioactivity in milk and tissues (in % of total radioactivity)

Animal Product and number of days after treatment	Number of doses	Total residue (mg/kg parent equivalent)	Parent residues (mg/kg)	Solvent e xtractable radioactivity (%)	Enzyme extractable radioactivity (%)	Non-extractable radioactivity (%)
Milk						
Day 1 pm	1	0.01	-	-	-	-
Day 2 am		0.11	-	-	-	-
Day 2 pm	2	0.17	-	-	-	-
Day 3 am		0.18	-	-	-	-
Day 3 pm	3	0.16	-	-	-	-
Day 4 am		0.15	-	-	-	-
Extracted sample		0.17	-	93	-	7
Muscle	3	0.02	-	-	-	-
Fat (renal)	3	0.26	-	90	-	10
Fat (subcutaneous)	3	0.02	-	-	-	-
Kidney	3	0.36	-	83	-	17
Liver	3	0.76	-	67	19	14

Table B.7.3 Distribution of clofentezine and its metabolites in animal products in % of the total radioactivity (parent equivalent in mg/kg)

	Milk (0.17mg/kg)	Muscle	Fat (renal)	Liver	Kidney
Clofentezine	-	-	-	-	-
4-hydroxy clofentezine	75 (0.13)	-	90 (0.23)	74 (0.56)	83 (0.3)

Non-extractable radioactivity	7 (0.01)	-	10 (0.026)	14 (0.11)	17 (0.06)
Non characterised	18 (0.03)	-	-	12 (0.09)	-
TRR – Total radioactive residue	**100** **(0.17)**	**100** **(0.02)**	**100** **(0.26)**	**100** **(0.76)**	**100** **(0.36)**

(Phillips M.W.A., Swalwell L, 1989a)

B.7.2.2 Goat

i) In a 1983 study to determine the presence of clofentezine residues in milk and tissues, a female Saanen lactating goat of between 2 and 3 years old, was dosed with [^{14}C]-Clofentezine as a single oral dose. The [^{14}C]-Clofentezine used had a radiochemical purity of 99.5%, specific activity 47.7 μCi/mg, however no indication was made about the labelling positions of the clofentezine. The dose was administered via a gelatine capsule containing [^{14}C]-Clofentezine at a level equivalent to 22mg/kg in the diet. This level is said in the study to be equivalent to an intake rate of 1N when consideration is made of the quantities of pomace, which may constitute the daily diet of the goat, however this seems somewhat excessive. The N rate is likely to be somewhat higher than that specified. Residues in plasma and milk were monitored for a period of 72 hours after dosing, with tissue samples taken at the end of the study.

Each sample type was prepared and analysed as follows:
Milk and plasma (separated from red cells) were mixed with scintillation cocktail before analysis with LSC. Blood was subjected to combustion analysis, with the $^{14}CO_2$ produced being measured by LSC.
Most tissue types were minced, mixed then samples digested in SHT (clarification is required as to what this refers to) – the samples then neutralised and analysed by LSC. Liver samples were prepared slightly differently, with a whole slice taken across the broadest part of the liver, for its whole length. This slice was then macerated and digested in SHT.

Only limited residues were found in both plasma and milk at all timepoints between 0 and 72 hours after dosing. Highest residues found were a level of 0.040mg/l clofentezine equivalents in goat plasma at a time after dosing of 5.5 hours, and in goat milk a level of 0.049 mg/l clofentezine equivalents at a time after dosing of 24 hours. Residue levels are found to be below 0.001 mg/l 72 days after dosing.

Negligible residues of less than 0.01 mg/kg were found in the majority of tissue samples tested. The exceptions were as follows:

Liver: 0.03 ± 0.005 mg/kg
Kidney: 0.01 ± 0.001 mg/kg
Adrenals: 0.01 ± 0.003 mg/kg
Eyes: 0.03 ± 0.003 mg/kg

The data appears to indicate that excretion of clofentezine is almost complete 72 hours after dosing.

(J.K. Campbell, D. Needham 1983)

ii) A study to investigate the residues of [^{14}C]-Clofentezine in the milk of a lactating goat was carried out in 1987. The goat was dosed with [^{14}C]-Clofentezine at a rate of 2.2 mg/kg bw/day for 7 days, an exaggerated dose rate in order to ensure that quantifiable residues were present in the milk at the time of study. The goat was an Anglo-Nubian breed, weighing approximately 48kg, which was milked twice daily. Radio-labelled clofentezine was used of purity 99% and specific activity 11.82 mCi/g.

Milk samples were freeze dried then extracted with distol diethyl ether and methanol, followed by partition with hexane and analysis by TLC or HPLC. Levels of radioactivity present were quantified by Liquid Scintillation Counting. Further soxhlet extraction of the milk samples was required in order to facilitate analysis.

The TRR in milk are shown in table B.7.4.

Table B.7.4 TRR in milk samples (parent equivalent in mg/kg)

Day number	TRR (mg/kg)	
	Am sampling	PM sampling
1	0	0.044
2	0.177	0.196
3	0.229	0.207
4	0.238	0.219
5	0.163	0.144
6	0.165	0.159
7	0.180	0.118

Overall radioactive residues in milk reached a plateau of 0.2 mg/kg at days 3 and 4 of the test.

Nine of the milk samples above were subjected to the extraction procedure. The Methanol extract accounted for 93 % of the TRR and the ether extract accounted for 0.59 % of the TRR. In the methanol extract the main metabolite identified was 4-hydroxy-clofentezine which accounted for 80 % of the TRR. A further 3.5 % of the TRR was found to be other hydroxyl-clofentezine metabolites (the specific levels and identities are not stated).

No attempt was made to identify the residues in the ether extract or the residual milk solids.

(Campbell J.K, 1987)

The samples which had been used in the Campbell study above were analysed further by Phillips and Swalwell, as conflicting data had been noted between the cow and goat

milk studies. Goat milk samples were extracted using the techniques employed in the Phillips and Swalwell cow study (1988) including the use of snail juice hydrolysis which had not been employed in the original Campbell study. In addition, it was feared that the goat milk samples may have gone off during the storage period, therefore the study was repeated with dosing as previously, except the goat was only fed for 3 days:

The goat was dosed with [^{14}C]-Clofentezine at a rate of 2.2 mg/kg bw/day for 3 days. The goat was a British saanengoat (body weight 58 kg). Radio-labelled clofentezine was used with a specific activity of 10.49 mCi/g. The radio-labelled purity was not stated.

The TRR in milk are shown in table B.7.5.

Table B.7.5 TRR in milk samples (parent equivalent in mg/kg)

Day number	TRR (mg/kg)	
	Am sampling	PM sampling
1	predose	0.059
2	0.144	0.204
3	0.210	0.141
4	0.174	0.092
5	0.018	0.005
6	0.002	0.001
7	0.001	-

The extractable residue accounted for 94.45 % of the TRR. On this occasion, the extractable radioactive residue co-chromatographed with 4-OH clofentezine. No evidence of any other clofentezine metabolites, nor parent clofentezine were noted. Confirmation was carried out by methylation with methyl iodide and silver oxide. Once again the presence of 4-OH clofentezine was confirmed as a single peak was observed which co-chromatographed with 4-methoxyclofentezine in this case.

(Phillips M.W.A, Swalwell L, 1989b)

iii) An additional study was also submitted, determining the nature of residues in the liver of the goat and calf. This study has been evaluated in section B.6 'Toxicology and metabolism'. The evaluation can be seen in section B.6.1.2 page 78 of the DAR.

A single goat (bodyweight 75 kg) and a single calf (bodyweight 85 kg) were administered a single dose of 20 mg/kg radiolabelled clofentezine. Radio-labelled [^{14}C]-clofentezine of radiochemical purity 99 % was used in the study, the clofentezine being radio-labelled at one of the two equivalent carbons in the tetrazine ring. The specific activity of the [^{14}C]-clofentezine used in the study was 47.7 µCi/mg

The livers were extracted with methanol. Unextracted residues following methanol extraction were converted by hydrobromic acid to ortho-chlorobenzoic acid (OCBA) and quantified.

Goat – The TRR was 1.45 mg/kg. Approximately 50 % of the TRR was extracted with methanol from the liver of the goat killed 19 hours after dosing. This extract proved difficult to analyse by TLC due to the large amounts of endogenous material co-extracted. However, it was possible to see that the components present were similar to those in the rat urine (conjugates of hydroxylated clofentezine). The levels of the individual hydoxylated conjugates were not eludicated further.

Following treatment with HBr a further 23.3 % of the TRR was extracted with ether. TLC analysis showed that 35.7 % of the TRR was ortho-benzoic acid.

Calf – The TRR was 1.51 mg/kg. Approximately 80% of the TRR was extracted with methanol from the liver of a calf killed 12 hours after dosing. Clofentezine accounted for 8 % of the TRR, 43 % of the TRR was hydoxylated clofentezine conjugates (The levels of the individual hydoxylated conjugates were not eludicated further).

After refluxing with HBr a further 12 % of the TRR was extracted. 23.3 % of the TRR was found to be ortho-benzoic acid.

The ^{14}C levels found in the liver of the goat and the live of the calf is shown in table B.6.24 (page 78 of the DAR). The distribution of clofentezine and metabolites found in the liver of the goat and calf are shown in table B.7.6.

Table B.7. Distribution of clofentezine and its metabolites in the liver of goat and calf in % of the total radioactivity (parent equivalent in mg/kg)

	Goat liver	Calf liver
Clofentezine	-	8 (0.12)
Conjugates of hydroxylated clofentezine	49.9 (0.72)	43 (0.65)
Ortho-benzoic acid	35.7 (0.52)	23.3 (0.35)
Unidentified	-	28.7 (0.43)
Non-extractable radioactivity	14.4 (0.21)	-
TRR – Total radioactive residue (by combustion)	**100 (1.45)**	**100 (1.51)**
TRR – (by summation)	**100 (1.45)**	**103 (1.55)**

The chromatographic profiles of liver extracts from goats and calves following oral administration of ^{14}C-clofentezine were qualitatively similar to that of rat urine, with conjugates of 3-, 4-, and 5-hydroxylated clofentezine.

The unextracted residues following methanol extraction could be converted almost quantitatively to orthochlorobenzoic acid by hydrobromic acid reflux, suggesting that the overall clofentezine moiety was still present in the residue.

B.7.2.3 Summary/assessment

Studies were provided for the metabolism of [^{14}C]-Clofentezine in cattle, goat and poultry.

The submitted cattle study was undertaken at an exaggerated rate (approximately 165N) in order to produce quantifiable residues. Levels of radioactivity in milk were shown to plateau at a level of 0.17 μg clofentezine equivalents/ml at a time stage of 3 days after treatment. 93% of the radioactivity was accounted for, of which 75% was shown to be present as 4-hydroxy clofentezine. Residues in tissues were most prevalent in the liver (0.76 mg clofentezine equivalents/kg). 68% (0.52 mg clofentezine equivalents/kg) of the TRR was solvent extractable which was found to be 4-hydroxy clofentezine. Of the remaining activity, a further 6% (0.05 mg equiv/kg) was characterised as 4-hydroxy clofentezine, with 18.3% (0.14 mg equiv/kg) having similar chromatographic properties to 4-hydroxy clofentezine and its hydrolysis products. In renal fat, 90.1% of the TRR (0.24 mg equiv/kg) was confirmed to be 4-hydroxy clofentezine.

Residues in kidney were found to be composed predominantly of 4-hydroxy clofentezine (83.2% TRR, 0.30 mg equiv/kg), with the remaining components appearing to be hydrolysis products of 4-hydroxy clofentezine.

The goat study (Campbell, Needham 1983) appears to show residues in all tissues at levels below 0.05mg/kg, with clofentezine being excreted within 72 hours after dosing. Highest residue was in goat milk where a level of 0.049 mg/l clofentezine equivalents at a time of 24 hours after dosing was confirmed. A study at an exaggerated rate was undertaken, which a plateau being reached at days 3 or 4 of the test, with a maximum residue of 0.2 mg/kg being obtained. Further confirmation of the metabolites found in goat milk was undertaken by Phillips and Swalwell in 1989. Over 95% of the TRR was confirmed as 4-hydroxy clofentezine.

A Further study was evaluated in the toxicology and metabolism section of the DAR. The nature of the residue in the liver of a goat and a calf were investigated. The chromatographic profiles of liver extracts from goats and calves following oral administration of ^{14}C-clofentezine were qualitatively similar to that of rat urine, with conjugates of 3-, 4-, and 5-hydroxylated clofentezine.

The unextracted residues following methanol extraction could be converted almost quantitatively to orthochlorobenzoic acid by hydrobromic acid reflux, suggesting that the overall clofentezine moiety was still present in the residue.

Finally, a poultry study was carried out (the proposed uses do not form part of the diets of poultry). By far the greatest residue was found in fat (3.04 mg/kg equivalents). The majority of each daily dose of clofentezine (71-79%) was excreted daily. The majority of residue found in all tissue samples was parent clofentezine, with varying quantities of both 3 and 4-hydroxy clofentezine. Quantities of residue which were accounted for in liver and muscle were lower than what would be expected at 52.4% and 51.5% respectively. The remaining residue was believed to consist of conjugates of the 3 and 4-hydroxy clofentezine metabolites.

B.7.3 Definition of the residue (IIA 6.7, IIIA 8.6)

Plants

At PRAPeR 64 (21 -23 January 2009) it was decided that there was insufficient toxicological information on the plant metabolite 2-chlorobenzonitrile. It was therefore decided at PRAPeR 65 (22 – 23 January) that this metabolite should be provisionally included in the residue definition for risk assessment.

The provisional residue definition for risk assessment is clofentezine and 2-chlorobenzonitrile.

This metabolite was recovered in the surface washings in proportions of 8.9% in the lemon study and 8.4% in the peach study (at 54 and 62 days PHI respectively), and accounted for 11.3% of the TRR in the dichlomethane grape fractions at 24-25 days PHI. This metabolite raises concerns since it is:

- classified,
- more acutely toxic than the parent,
- recovered at non negligible amounts in the processed commodities,
- not recovered in the rat metabolism and therefore not covered by the mammalian toxicology studies.

This metabolite is expected to be present in portions of *c.a.* 10% of parent clofentezine.

Therefore the conversion factor for parent clofentezine to 2-chlorobenzonitrile is 0.1. When the new residue trials are submitted (see B.7.6) in which both parent clofentezine and 2-chlorobenzonitrle have been determined a more accurate conversion factor can be determined.

The residue definition for monitoring is parent clofentezine only.

Animal

The metabolism data submitted for clofentezine in animal products shows the vast majority of the residue in all cattle and goat products as being composed of 4-hydroxyclofentezine. There is indication that other hydroxyl clofentezine isomers are also present in goat and cattle products.

The poultry studies however show more significant quantities of parent clofentezine, with levels of 3 and 4-hydroxyclofentezine. Quantities of 3 and 4-hydroxy clofentezine are not separated in the poultry study, but quantities of 3-hydroxy clofentezine are certain to be outweighed by the combined totals of parent and 4-hydroxy clofentezine. In addition, fruit pomace does not form part of the diets of poultry.

The provisional residue definition should be the sum of all compounds containing the 2-chlorobenzoyl moiety expressed as clofentezine.

However based on the potential non specific (to clofentezine) nature of this common moiety approach it is considered that additional secondary (confirmatory enforcement) methods of analyses specific to clofentezine and the main metabolite 4-hydroxy clofentezine should be required. Furthermore, the common moiety methodological approaches would need to be validated for all the relevant metabolites expected in food of animal origin.

The residue definitions are only provisional as intakes in the diets of livestock of the metabolites formed on processing may have to be considered.

B.7.4 Use pattern

The original spray concentration given in the GAP table did not correspond with the water volumes specified. An updated GAP table is shown in table B.7.5.

Table B.7.5 Summary of intended GAP

Crop and/or Situation (a)	Member State or Country	Product Name	F, G, or I (b)	Pests or Group of Pests Controlled (c)	Formulation		Application				**Application rate per treatment***			PHI (l)	Remarks (m)
					Type (d-f)	Conc. of as (i)	Method Kind (f-h)	Growth Stage (BBCH) (j)	Number min-max (k)	Interval between Applications (min)	kg as/hl min-max	water L/ha min-max	kg as/ha max		
Pome fruit Apples-Pears	B, E, EL, F, I, NL, POR, UK	Apollo 50 SC	F	Tetranychus ssp., Panonychus ssp., P.ulmi	SC	500	Foliar, air assisted & hydrolic	08 – 56	1	NR	0.007 – 0.05	400-1500	0.1-0.2	35	
Stone fruit: Plums	E, F, UK	Apollo 50 SC	F	Tetranychus ssp., Panonychus ssp., P.ulmi	SC	500	Foliar, air assisted & hydrolic	08 - 75	1	NR	0.007 – 0.05	400-1500	0.1-0.2	35	
Grapes	E, F, I	Apollo 50 SC	F	Tetranychus ssp., Panonychus ssp., P.ulmi	SC	500	Foliar, air assisted & hydrolic	11 – 75	1	NR	0.01 – 0.05	300-1000	0.1-0.15	30	
Strawberries	B, E, F, I, NL	Apollo 50 SC	F/G	E. carpini, P.ulmi	SC	500	Foliar, hydrolic	At occurrence - 85	1	NR	0.007 – 0.04	500-1500	0.1-0.2	3	

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restriction

B.7.5 Identification of critical GAPs

Intended GAPs are shown in Table B.7.5.

It was noted during PRAPeR 65 (22 -23 January 2009) that except for strawberries (outdoor) there were insufficient residue trials on each crop. Most of the trials were conducted at a higher application rate than specified, and with two application rates rather than one. The meeting had a general discussion on whether overdose trials were acceptable. It was agreed that such trials could not be used to set MRLs.

On some occasions, the latest growth stage referenced in the intended GAP and the latest PHI do not correlate. For example, the latest growth stage specified for the use of Apollo 50 SC on pome fruit is specified as BBCH56 (green bud stage), with a latest pre-harvest interval of 35 days. In cases such as this, where the interval from the BBCH56 growth stage to harvest would be greater than the minimum pre-harvest interval, then data to support the proposed PHI are used in the risk assessment. Indeed many of the trials evaluated in Table B.7.20 are conducted at a growth stage in excess of that specified in the proposed GAP, hence data to support PHI values are used.

B.7.6 Residues arising from supervised trials (IIA 6.3; IIIA 8.2)

B.7.6.1 Residue trials data

The residue trials submitted to support the GAPs can be seen in the DAR. Table B.7.6 show the trials that support the GAPs.

Table B.7.6 A summary of supervised residue trials data generated according to critical GAP

Crop/variety	Country (region)/year	Application rate (kg as/ha)	Number of applications	Pre-harvest interval (days)	Days after last application (DALA)	Residue (mg/kg)	Comment	Ref.
Apples Idared	Germany (Tonisvorst – vorst) 1992	0.225 (0.03 kg a.s/hl)	1		0 28 35 42	0.36 0.14 0.11 0.10	SE (6%) formulation used for this selection of trials Application at GS 78-80	Peatman M.H, Wright P, 1993
Golden Delicious	Germany (orsingen – Nenzongen) 1992 (all Northern European trials)	0.210 (0.042 kg a.s/hl)	1		0 14 28 35 43	0.29 0.15 0.10 0.06 0.07	Residues are parent clofentezine only	
Apples Jonagold	Belgium (St. Truiden) 1993	0.216 (0.014 kg a.s/hl)	1		7 14 21 30	0.34 0.31 0.24 0.15	Residues are parent clofentezine only SE formulation @ 60g/l Mature apples	Peatman M.H, 1994
	(Northern Europe)	0.225 (0.015 kg a.s/hl)	1		7 14 21 30	0.24 0.19 0.19 0.17	SC formulation @ 500g/l Mature apples Both trials conducted at same location and at the same time. These cannot be considered as independent trials.	

Crop/variety	Country (region)/year	Application rate (kg as/ha)	Number of applications	Pre-harvest interval (days)	Days after last application (DALA)	Residue (mg/kg)	Comment	Ref.
Apples Imperial	Greece (Nissi, Alexandria)1986 (Southern Europe)	0.01 kg as/hl (0.165 kg as/ha) 0.015 kg as/hl (0.25 kg as/ha)	1 1		0 32 42 0 32 42	0.27 0.02 0.03 0.34 0.04 0.04	Applied at the formed fruit stage, i.e. after BBCH65 therefore worse case than GAP Water volume used was 1650 l/ha Both trials conducted at same location and at the same time. These cannot be considered as independent trials.	Manley, Snowdon, 1986
Strawberries Elvira	Entzheim, Alsace, 2001 (Northern Europe)	0.216 (0.025 kg as/hl)	1		0 1 3 5 7	0.29 0.23 0.24 0.18 0.17	Treatment at BBCH86. Application using 50SC formulation.	Pollmann, B. 2002b
Elsanta	Wissem-bourg, Alsace, 2001 (Northern Europe)	0.189 (0.025 kg as/hl)	1		0 1 3 5 7	0.29 0.21 0.19 0.10 0.10	Application at BBCH86	Pollmann, B. 2002b
Darselect	Blaesheim, Alsace, 2001 (Northern Europe)	0.195 (0.025 kg as/hl)	1		3	0.09	Application at BBCH86	Pollmann, B. 2002b

Crop/variety	Country (region)/year	Application rate (kg as/ha)	Number of applications	Pre-harvest interval (days)	Days after last application (DALA)	Residue (mg/kg)	Comment	Ref.
Elsanta	Stutensee-Staffort, Nordbaden, 2001 (Northern Europe)	0.206 (0.025 kg as/hl)	1		0 1 3 5 7	0.25 0.17 <u>0.16</u> 0.08 0.08	Application at BBCH87	Pollmann, B. 2002b
Elsanta	Eberdingen, Baden-Württemberg, 2001 (Northern Europe)	0.190 (0.025 kg as/hl)	1		0 1 3 5 7	0.18 0.11 <u>0.09</u> 0.07 0.07	Application at BBCH87	Pollmann, B. 2002b
Elsanta	Kleinsachsen-heim, Baden-Württemberg, 2001 (Northern Europe)	0.194 (0.025 kg as/hl)	1		3	<u>0.23</u>	Application at BBCH87	Pollmann, B. 2002b
Oso Grande	Moguer-Huelva, Spain, 1992 (32/92) (Southern Europe)	0.2 (0.02 kg as/hl)	1		0 3 7 14	1.20 <u>0.73</u> 0.44 0.22	Application when fruit were ripened, ready for harvest (BBCH87). Check method for validation.	Godfrey, Peatman, 1993a

Crop/variety	Country (region)/year	Application rate (kg as/ha)	Number of applications	Pre-harvest interval (days)	Days after last application (DALA)	Residue (mg/kg)	Comment	Ref.
Chadler	Moguer-Huelva, Spain, 1992 (33/92) (Southern Europe)	0.2 (0.02 kg as/hl)	1		0 3 7 14	1.10 0.60 0.29 0.21		Godfrey, Peatman, 1993a
Chadler	Moguer-Huelva, Spain, 1992 (34/92) (Southern Europe)	0.2 (0.02 kg as/hl)	1		0 3 7 14	0.93 0.81 0.53 0.25		Godfrey, Peatman, 1993a
Muy	Moguer-Huelva, Spain, 1992 (35/92) (Southern Europe)	0.2 (0.02 kg as/hl)	1		0 3 7 14	1.80 1.10 0.60 0.35		Godfrey, Peatman, 1993a
Oso Grande	Moguer-Huelva, Spain, 1992 (36/92) (Southern Europe)	0.2 (0.02 kg as/hl)	1		0 3 7 14	1.10 0.75 0.43 0.26		Godfrey, Peatman, 1993a
Chadler	Moguer-Huelva, Spain, 1992 (37/92) (Southern Europe)	0.2 (0.02 kg as/hl)	1		0 3 7 14	0.66 0.56 0.24 0.12		Godfrey, Peatman, 1993a

Crop/variety	Country (region)/year	Application rate (kg as/ha)	Number of applications	Pre-harvest interval (days)	Days after last application (DALA)	Residue (mg/kg)	Comment	Ref.
Turla	Moguer-Huelva, Spain, 1992 (38/92) (Southern Europe)	0.2 (0.02 kg as/hl)	1		0 3 7 14	0.87 0.70 0.31 0.17		Godfrey, Peatman, 1993a
Chadler	Moguer-Huelva, Spain, 1992 (39/92) (Southern Europe)	0.2 (0.02 kg as/hl)	1		0 3 7 14	0.97 0.50 0.35 0.13		Godfrey, Peatman, 1993a
Chadler	Los Palacios, Spain, 1992 (48/92) (Southern Europe)	0.2 (0.02 kg/as/hl)	1		0 3 7 15	1.20 0.72 0.43 0.25		Godfrey, Peatman, 1993a
Selva	Castillon, France, 1990 (Southern Europe)	0.2 (0.02 kg/as/hl)	1		0 1 3 7 14	0.36 0.29 0.13 0.06 0.04	Treatment at 12 red fruits (BBCH87). Application using SC formulation.	Godfrey, Peatman, 1991

B.7.6.2 Summary of residues resulting from supervised trials

Apples/pears – A total of three residue trials support the GAP in Northern Europe. In Southern Europe there is only one trial that supports the GAP.

Plums – All the residue trials from the DAR represent overdose trials compared to the proposed GAP.

Grapes – All the residue trials from the DAR represent overdose trials compared to the proposed GAP.

Strawberry (outdoor) – In Northern Europe six residue trials support the GAP. In Southern Europe there are ten trials that support the GAP.

Strawberry (indoor) – No trials were submitted to support the indoor use.

Additional residue trials will be required to support the use on apples/pears in Northern and Southern Europe. A complete set of residue trials will be required to support the use on plums and grapes in Northern and Southern Europe and Strawberry (indoor).

Consideration to the metabolite 2-chlorobenzonitrile must be given in the new residue trials.

B.8 Environmental Fate and Behaviour

The additional information below has been prepared by the UK RMS to address the open points and points of clarification identified in the clofentezine Evaluation Table (rev. 1-1, 30.01.2009). Where reference is made to the original draft assessment report these references relate to the MS Word version of August 2005.

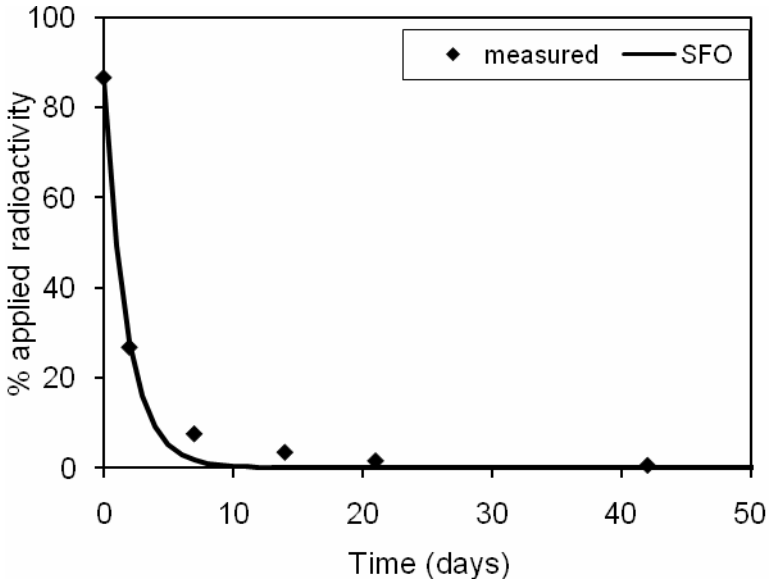
New open point 4.12
RMS to calculate a water dissipation DT50 from the 2 experiments in an addendum (values should not be put in the LoEP).

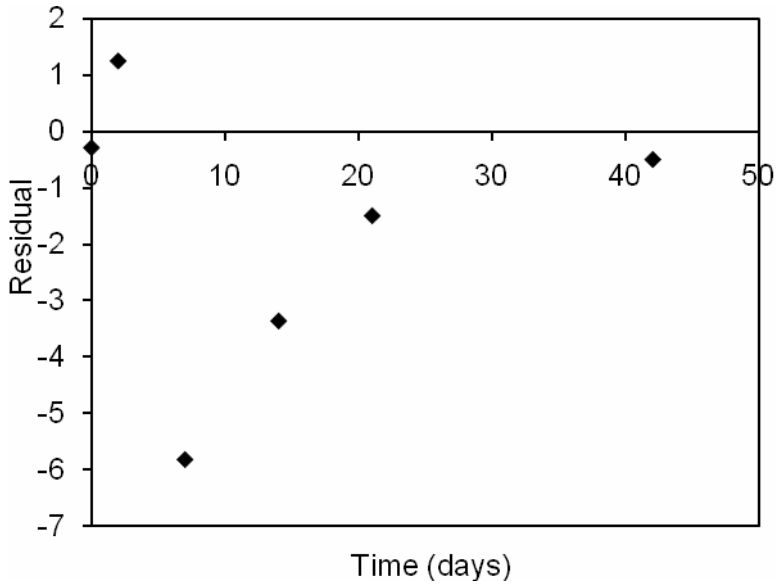
The UK RMS has used the latest FOCUS kinetics guidance to calculate simple water phase dissipation DT_{50} values for the two water-sediment systems reported in the study of Leake and Arnold (1983c) and originally evaluated in the DAR in Section B.8.4.4 (page 324 to 326). Data were taken from Table B.8.33 on page 326 of the August 2005 DAR and are based on the sum of clofentezine residues detected in the surface water and the results of clofentezine analysis from the glass vessel washing procedure that removed potentially significant amounts of parent material (up to 8.2% AR in the clay loam system). The time zero values were based on the total recoveries presented in Table B.8.32 (page 326 of original DAR).

Fitting was performed using the FOCUS_DEGKIN v2.xls spreadsheet available via the FOCUS website. Results are presented in the figures below for each system. In the sandy clay loam system the SFO water phase dissipation DT_{50} was 1.2 d (chi^2 = 10.9). In the clay loam system the SFO water phase dissipation DT_{50} was 1.1 d (chi^2 = 18.6). Although the visual fits were not ideal, in the opinion of the UK RMS the SFO fits adequately described the main period of dissipation from the water phase (i.e. up to the time point where the residues dropped below 10% of initial values at circa 7 d in each system).

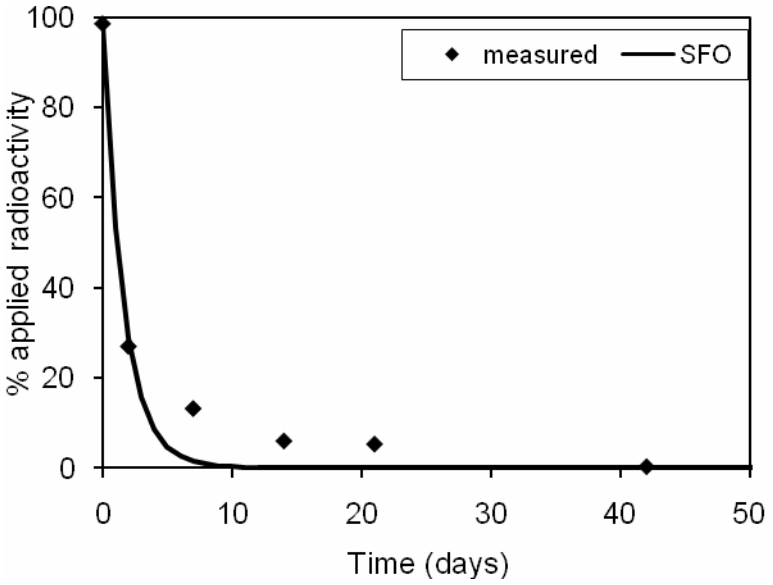
The UK RMS considered that the water phase dissipation values listed above would be acceptable for use at National MS level where individual exposure assessment schemes require such a value. Note that such values are not appropriate for use in the FOCUSsw assessment scheme.

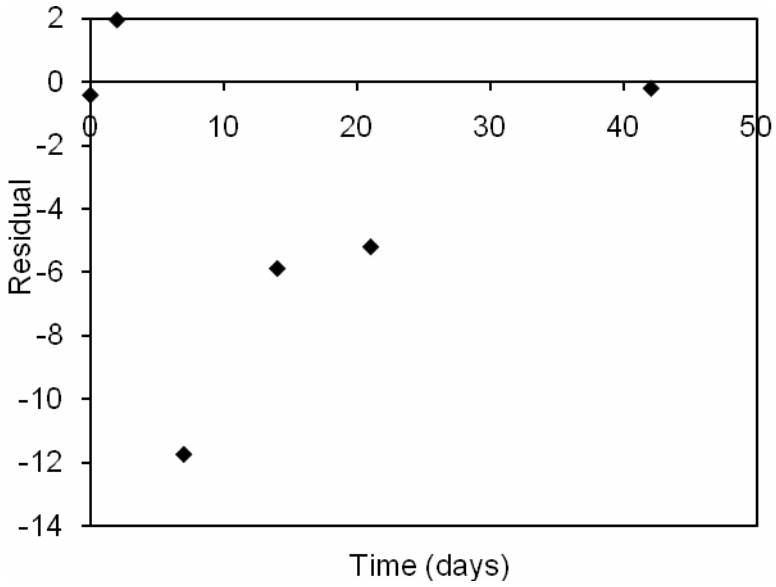
Water phase dissipation behaviour of clofentezine in the sandy clay loam (Lode) system (including glass wash data)





Water phase dissipation dissipation behaviour of clofentezine in the Clay loam (Sadlers Farm) system (inclusing glass wash data)





B.8.9 References relied on

The following studies were relied on in Section B.8 Environmental Fate and Behaviour of the DAR, dated August 2005, but were not included in the list of studies relied Section B.8.9, and have therefore been added to this Addendum for completeness.

Active substance – clofentezine

Annex point / Ref. No.	Author	Year	Title Source (where different from company) Company, Report No. GLP status, published or not	Data protection claimed	Owner
IIA, 2.9.1/01 IIA, 8.4.1	Kelly, I.D.	1985a	The kinetics of the hydrolysis of NC 21314 under acid, neutral and basic conditions. Amended report. FBC Ltd., Report No. METAB/85/11, Aventis No. A82482=W6-2, MAK No. R-12520 GLP, not published	N	Makhteshim Agan
IIA, 2.9.1/02 IIA, 8.4.2	Smith, S., Kelly, I.D.	1985b	Characterisation of the hydrolysis products of Clofentezine in aqueous solution under acid, neutral and basic conditions. FBC Ltd., Report No. METAB/85/11, Aventis No. A82528=W54, MAK No. R-12522 GLP, not published	N	Makhteshim Agan
IIA, 2.9.2/01 IIA, 8.4.2	Kelly, I.D.	1985b	The photodegradation of [14C]-Clofentezine in water under natural sunlight conditions FBC Ltd., Report No. METAB/84/15, Aventis No. A82520=W46, MAK No. R-12521 GLP, not published	N	Makhteshim Agan

9. Ecotoxicology

Following PRAPeR 63 (13-15 January 2009) there was a request for the RMS to provide the key information on the aquatic studies in an addendum.

Presented below are the detailed summaries provided by the Notifier. It is recommended that these are read in conjunction with the assessment in the original DAR and Addendum 1 and 2.

9.1 Acute toxicity of the active substance to fish

9.1.1 Rainbow trout

Report: Hemmings, P.A. (1980) The acute toxicity of technical (unformulated) NC21314 to rainbow trout (*Salmo gairdneri*). (Unformulated NC21314 = CR 20099/5) Fisons, Report No. METAB/80/25, MAK No. R-12643, Aventis No. A82491=W16=T5, MAK No. R-12643

Guidelines: US EPA 660/3-75-009 (1975) – semistatic test

GLP: No

Material and Methods

Test material: NC21314 technical, batch no. CR 20099/5, purity 99.2%

For the test, Clofentezine was mechanically shaken in dilution water to form a fine suspension in excess of the maximum solubility of the compound. This was mixed with dilution water in a glass aquarium to give a suspension of 100 mg Clofentezine/L. Six replicate tanks were prepared each containing 7 litres test solution, together with six replicate control container containing 7 litres dilution water only. Fish, starved 48 hours prior to the start of the bioassay, were randomly transferred to the container to give 5 fish per replicate (30 fish per treatment). Loading of fish to dilution water was 0.9 g/L. The fish were not fed during the bioassay. The water was not aerated during the bioassay. The photoperiod was 16 hours light/ 8 hours dark and the temperature was maintained at 13.5 - 15ºC. The dissolved oxygen range was 59-102% and pH in the Clofentezine treatments varied between 8.2-8.4.

Findings

Table 9.1: Mortality of rainbow trout in an acute toxicity test with Clofentezine

Nominal concentration (ppm)	Mortality (%)			
	24 h	48 h	72 h	96 h
0	0	0	6.7	6.7

Nominal concentration (ppm)	Mortality (%)			
	24 h	48 h	72 h	96 h
100	0	0	3.3	3.3

The mean concentration of dissolved Clofentezine in the water was 5 µg/L at 0 hours and 39 µg/L at 24 hours, although the mean concentration of Clofentezine suspended in the water was 25.92 mg/L at 0 hours and 11.09 mg/L at 24 hours. The lower recoveries of the active ingredient were due to most of the solid either settling on the bottom of the tanks or floating on the water surface.

Mortalities were recorded at 24 hour intervals and indicated a cumulative mortality after 96 hours of 6.7 and 3.3% for the control and treated group, respectively. Surviving fish in both the treatment and control container were healthy at the termination of the bioassay.

Conclusion

The 96 hour LC_{50} of Clofentezine to rainbow trout was therefore > 100 mg/L suspension in water, and greater than the limit of water solubility (which did not exceed 39 µg/L under the test conditions).

9.1.2 Rainbow trout

Report: Barrett, K.L., Arnold, D.J. (1986) Determination of the acute toxicity of [^{14}C]-Clofentezine to rainbow trout (*Salmo gairdneri*) using a dynamic test system. FBC, Report No. METAB/86/2, Aventis No. A82534=W60, MAK No. R-12664

Guidelines: In-house method – continuous flow

GLP: No

Material and Methods

Test material: [^{14}C]-Clofentezine, batch no. CFQ 2874, purity not stated and Clofentezine technical, batch no. CR 200915, purity 98.6%

The 96 hour LC_{50} of [^{14}C]-Clofentezine was assessed under continuous flow conditions. The test was conducted in 25 L volume glass vessels containing 15 L of the test solution with a flow through rate of approximately 4.2 L/hour. Due to the extremely low solubility of Clofentezine in water, the compound was firstly absorbed to pumice which was then used, via a saturation column, to supply a constant level of dissolved [^{14}C]-labelled Clofentezine to the fish. A mean measured concentration of 14.6 µg/L Clofentezine was determined in the test vessels throughout the exposure period.

Three saturation columns were used, two with [^{14}C]-labelled Clofentezine treated pumice and one with untreated pumice as a control. The dilution water passed through each saturation column before entering the test vessels. Fifteen fish were added to each test vessel, i.e. 30 fish exposed to [^{14}C]- Clofentezine, and 15 in the control vessel.

Findings

Table 9.2: Mortality of rainbow trout in an acute toxicity test with Clofentezine

Actual concentration (ppm)	Mortality (%)			
	24 h	48 h	72 h	96 h

Actual concentration (ppm)	Mortality (%)			
	24 h	48 h	72 h	96 h
0	0	0	0	0
0.0146	0	0	0	0

No mortalities were recorded during the exposure period in either treatment or control vessels.

Conclusion

The 96 hour LC_{50} of Clofentezine to rainbow trout is therefore greater than its maximum solubility in water (14.6 µg/L).

9.1.3 Bluegill sunfish

Report: Hill, R.W. (1981) Determination of the acute toxicity of NC21314 to bluegill sunfish (*Lepomis macrochirus*). ICI, Report No. METAB/81/39, Aventis No. A82500=W23=T35, MAK No. R-12649

Guidelines: In-house method – continuous flow

GLP: Yes

Material and Methods

Test material: NC21314 technical, batch no. CR 20099/8, purity 99.8%

The acute toxicity of Clofentezine to bluegill sunfish was determined in freshwater at 22ºC using a continuous flow-through system. The test was conducted with two measured concentrations of Clofentezine suspended in water, 0.25 and 0.12 mg/L. The suspension was aided by first dissolving Clofentezine in acetone/Tween 80.

Findings

Table 9.3: Mortality of bluegill sunfish in an acute toxicity test with Clofentezine

Nominal concentration (mg/L)	Mortality (%)			
	24 h	48 h	72 h	96 h

Nominal concentration (mg/L)	Mortality (%)			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
Solvent control	0	0	5	20
0.15	0	0	0	0
0.30	0	0	5	10

Actual concentrations were within 20% of nominal. Only two mortalities occurred in the twenty fish at 0.30 mg/L and 96 hours, and no deaths occurred at 0.15 mg/L Clofentezine at this time. Four deaths occurred at 96 hours in the solvent control but no deaths occurred in the freshwater control at this time.

Conclusion

The 96 hour LC_{50} for Clofentezine to bluegill sunfish was therefore > 0.25 mg/L (concentration in suspension) and is therefore greater than its maximum solubility in water.

9.1.4 Rainbow trout (metabolite 2-chlorobenzonitrile)

2-chlorobenzonitrile

Report: Wetton, P.M., Mullee, D.M. (2001a) 2-Chlorobenzonitrile: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*). Safepharm Laboratories, SPL No. 1457/005, MAK No. R-12498

Guidelines: 92/69/EEC C.1 = OECD 203 (1992) – semistatic conditions

GLP: Yes

Material and Methods

Test material: 2-chlorobenzonitrile, batch no. R000834, purity 99.9% w/w

The acute toxicity of 2-chlorobenzonitrile to rainbow trout was determined in fresh water using a semi-static system. The test was conducted with 10 / 18 / 32 / 56 / 100 mg/L of 2-chlorobenzonitrile, with concentrations according to a preliminary range-finding test. The number of mortalities and sub-lethal effects were determined 3 and 6 h after exposure, then daily.

Findings

Table 9.4: Mortality of rainbow trout in an acute toxicity test with 2-chlorobenzonitrile

Nominal concentration (mg/L)	Mortality (%)			
	24 h	48 h	72 h	96 h
0	0	0	0	0
10	0	0	0	0
18	14	14	14	14
32	100	100	100	100
56	100	100	100	100
100	100	100	100	100

Actual concentrations were within 97 – 104% (< 20%) of nominal. No sub-lethal effects were observed at 18 mg/L or below.

Table 9.5: Endpoint list for rainbow trout in an acute toxicity test with 2-chlorobenzonitrile

Time	LC_{50} (mg/L)	95% conf. limit (mg/L)
24 h	22	19 – 26
48 h	22	19 – 26
72 h	22	19 – 26
96 h	22	19 – 26

Conclusion

Final mortality rate was reached within 24 hours exposure. The 96 hour LC_{50} for 2-chlorobenzonitrile to rainbow trout was 22 mg/L. The NOEC was 10 mg/L.

9.2 Acute toxicity of the active substance to aquatic invertebrates

9.2.1 Aquatic invertebrates – *Daphnia magna*

Report: Barrett, K.L., Arnold, D.J. (1988a) Determination of the acute toxicity of Clofentezine technical to *Daphnia magna*. Schering Agrochemicals Ltd., Report No. ENVIR/87/47, Aventis No. A82556=W77, MAK No. R-12670

Guidelines: OECD 202 I, US EPA EG1 31: 5007-5009 - static conditions

GLP: Yes

Material and Methods

Test material: Clofentezine technical, batch no. CR 20099/15, purity 99.8%

The toxicity of Clofentezine technical to the freshwater crustacean *Daphnia magna* was assessed over a 48 hour exposure period under static conditions, at a temperature of 20ºC ± 1ºC. Due to the low solubility of the test compound in water, daphnia were exposed to only a single concentration (1.45 µg/L) representing the maximum solubility attainable under the test conditions with the use of 0.5 ml/L acetone/tween 80 (50/50 v/v) solvent concentration. Observations were made over a 48 hour period.

Findings

Table 9.6: Immobilisation of *Daphnia magna* by Clofentezine

Treatment	Immobilised daphnids (%)	
	24 h	48 h
Control	3.3	10.0
Solvent control	6.7	6.7
Clofentezine 1.45 µg/L	16.7	16.7

No toxic effects were observed at the concentration tested and the number of daphnids immobilised in the treatment solution was slightly greater than that recorded in the controls but did not reach an EC_{50} value.

Conclusion

Therefore, as the EC_{50} is greater than the maximum solubility of the compound in water it may be concluded that the compound is of low toxicity to *Daphnia magna*.

9.2.2 Aquatic invertebrates – *Daphnia magna*

Report: Lines, D. (1981) Determination of the acute toxicity of technical NC21314 to the water flea, *Daphnia magna*. FBC, Report No. METAB/81/18, Aventis No. W18=T28, MAK No. R-15417

Guidelines: US EPA 660/3-75-009 (1975), US EPA Guideline draft (1978) – static conditions

GLP: No

Material and Methods

Test material: NC21314 technical, batch no. CR 20099/5, purity 99.0%

The toxicity of Clofentezine to the freshwater crustacean *Daphnia magna* was assessed over a 48 hour exposure period under static conditions, at a temperature of 20ºC ± 1ºC. Nominal concentration was 100 mg/L Clofentezine; however, due to the low solubility of the test compound in water, actual concentration was < 0.1 mg/L. Observations were made over a 48 hour period.

Findings

Table 9.7: Immobilisation of *Daphnia magna* by Clofentezine

Treatment	Immobilised daphnids	
	24 h	48 h
Control	1 / 30	1 / 30
Clofentezine < 0.1 mg/L	2 / 30	2 / 30

The measured concentration of Clofentezine was between 0.01 and 0.14 mg/L. No toxic effects were observed at the concentration tested and the number of daphnids immobilised in the treatment solution was similar to that recorded in the controls but did not reach an EC_{50} value.

Conclusion

Therefore, as the EC_{50} is greater than the maximum solubility of the compound in water it may be concluded that the compound is of low toxicity to *Daphnia magna*.

9.2.3 Aquatic invertebrates – *Daphnia magna* (metabolite 2-Chlorobenzonitrile)

2-Chlorobenzonitrile

Report: Wetton, P.M., Mullee, D.M. (2001b) 2-Chlorobenzonitrile: Acute toxicity to *Daphnia magna*. SafePharm Laboratories, SPL No. 1457/004, MAK No. R-12497

Guidelines: 92/69/EEC C.2 = OECD 202 (1984) – static conditions

GLP: Yes

Material and Methods

Test material: 2-chlorobenzonitrile, batch no. R000834, purity 99.9% w/w

The toxicity of 2-chlorobenzonitrile to the freshwater crustacean *Daphnia magna* was assessed over a 48 hour exposure period under static conditions at concentrations of 1.0 / 1.8 / 3.2 / 5.6 / 10 / 18 / 32 / 56 / 100 mg/L based on a range-finding study. Immobilisation was recorded after 24 and 48 h.

Findings

Table 9.8: Immobilisation of *Daphnia magna* by 2-chlorobenzonitrile

Concentration	Immobilised daphnids (%)	
(mg/L)	24 h	48 h
0	0	0
1.0	0	0
1.8	0	0
3.2	0	0
5.6	0	0
10	10	15
18	75	85
32	100	100
56	100	100
100	100	100

Nominal concentrations are given since actual concentrations were in the range of 103 – 112 % of nominal. The EC_{50} of 2-chlorobenzonitrile to *Daphnia magna* after 48 h was calculated as 13 mg/L (95% conf. interval 12 – 15 mg/L). No aberrant behaviour was recorded.

Conclusion

The EC_{50} of 2-chlorobenzonitrile to *Daphnia magna* after 48 h was 13 mg/L.

9.3 Acute toxicity of the active substance to algae

9.3.1 Toxicity to algae

Report: Oldersma, H, Hanstveit, A.O., Pullens, M.A.H.L. (1983) The effect of the product NC21314 technical on the growth of the green alga *Scenedesmus pannonicus*. TNO, Report No. METAB/83/3, MAK No. R-12642, Aventis No. A82484=W9, MAK No. R-12642

Guidelines: Dutch draft Standard method NEN 6506

GLP: Yes

Material and Methods

Test material: NC21314 technical, batch no. CR 20099/14, purity not stated

Due to the low solubility of the test substance in water, and its slow rate of dissolution, the solvent dimethyl sulphoxide (DMSO) was used at a maximum concentration of 0.1 ml/L, to aid test solution preparation. Actual concentrations of dissolved Clofentezine in the test media were determined, and indicated that most of the nominal concentrations exceeded the limit of water solubility for Clofentezine.

The test concentrations were carried out in duplicate, and compared to a single-background control series of test substance without algae, in square 180-ml culture flasks. A hundred ml of the suspension of algae containing about 10^4 cells/ml was transferred to each flask, and 10 µl of the test substance solution in DMSO added. The concentrations of Clofentezine tested were 0, 0.01, 0.018, 0.032, 0.056, 0.1 and 0.32 mg/L. One sample was taken from each flask once a day on five consecutive days, and the number of algal cells per ml in the samples was determined.

Findings

Table 9.9: Effect of Clofentezine on algal growth. Initial cell count was 1.08 x 10^4 cell/mL.

Concentration (mg/L)	10^4 cells/mL after 94 h
0	37.96
0.01	35.58
0.02	34.43
0.03	34.24
0.06	32.24
0.10	31.39
0.32	30.42

The results showed that Clofentezine in concentrations up to its solubility limit in water did not impair the growth of the alga *Scenedesmus pannonicus* under the conditions of the test. In concentrations exceeding that limit, however, it had a slight effect on growth yield.

Conclusion

The 120-hour EC_{50} was > 0.32 mg/L, indicating that Clofentezine has low toxicity to green algae at its limit of water solubility.

9.3.2 Toxicity to algae (metabolite 2-chlorobenzonitrile)

Report: Mead, C., Mullee, D.M. (2001) 2-Chlorobenzonitrile: Algal inhibition test. SafePharm Laboratories, SPL No. 1457/003, MAK No. R-12496

Guidelines: 92/69/EEC C.3 = OECD 201 (1984)

GLP: Yes

Material and Methods

Test material: 2-chlorobenzonitrile, batch no. R000834, purity 99.9% w/w

Following a preliminary range-finding study, *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) was exposed to an aqueous solution of the test material at concentrations of6.25, 12.5, 25, 50 and 100 mg/L (three replicate flasks per concentration) for 72 hours, under constant illumination and shaking at a temperature of 24 ± 1°C. Samples of the algal populations were removed daily and cell concentrations determined for each control and treatment group, using a Particle Counter.

Findings

Table 9.10: Effect of 2-chlorobenzonitrile

Concentration (mg/L)	Inhibition of growth rate (%)	Inhibition of biomass growth (%)
0	--	--
6.25	1	8
12.5	6	30
25	18	62
50	67	96
100	88	99

Chemical analysis of the test solutions at 0 hours showed measured test concentrations ranging from 93% to 95% of nominal. Analysis of the test solutions at 72 hours showed a marked decline in measured test concentrations ranging from 59% to 62% of nominal. This decline in measured test concentrations was considered to be due to adsorption of the test material to algal cells over the 72 hour study period. Therefore nominal concentrations were chosen.

Conclusion

Exposure of *Pseudokirchneriella subcapitata* to the 2-chlorobenzonitrile gave an E_bC_{50} (72 h) value of 16 mg/L and an E_rC_{50} (0 -72 h) value of 47 mg/L. The No Observed Effect Concentration (NOEC) was 6.25 mg/L.

9.4 Acute toxicity of the preparation to aquatic species

9.4.1 Acute toxicity of the preparation to fish

Report: Arnold, D.J. (1985) The acute toxicity of Apollo 50 SC to rainbow trout (*Salmo gairdneri*). FBC Ltd., Report No. METAB/85/27, Aventis No. W57, MAK No. R-12994

Guidelines: None stated but same method as described in EEC Directive 92/69/EEC Method C.1 except minor deviations

Flow-through system

GLP: No

Material and Methods

Apollo SC (batch no. CL0006 NI 000.6) was tested in a flow-through test on 6 week old rainbow trout. No vehicles were used. The nominal concentrations of 0 / 2.5 / 5.0 / 10.0 mg ai/L were selected on the basis of previous toxicity tests of Clofentezine technical on fish, and tested on 30 animals per concentration. Higher concentrations were also not chosen to maintain minimal visibility of the fish. Oxygen, pH, temperature and a.i. content were checked every 24 h. Mortality was checked after 3, 6, 12, 24, 48, 72 and 96 h.

Findings

The mortality of Apollo SC to rainbow trout is listed in Table 10.2.1-1.

Table 9.12: Acute mortality of Apollo SC to rainbow trout

Nominal concentration (mg ai/L)	Accumulative mortality [%]				
	0 h	24 h	48 h	72 h	96 h
0	0	6.7	6.7	6.7	6.7
2.5	0	3.3	10	10	10
5.0	0	3.3	6.7	6.7	6.7
10	0	0	6.7	6.7	6.7

All data based on nominal concentration.

Observations

Overall recovery rates for the active substances were between 80 and 124%. No adverse sub-lethal effects were observed at any concentration The LC_{50} could not be determined in this test but is > 10 mg a.i./L.

Conclusion

The tested formulation Apollo SC revealed after 96 hours at 20 mg/L (corresponding to 10 mg a.i./L) no discernible toxic effect on the test species (rainbow trout).

Report: Biological part: Hill, R.W., Caunter, J.E. (1988) Apollo 50 SC: Determination of acute toxicity to bluegill sunfish (*Lepomis macrochirus*). ICI, Report No. ENVIR/88/40, 91, Aventis No. A82560=W81, MAK No. R-12674

Analytical part: Arnold, D.J., Barrett. K.L. (1988) Apollo 50 SC: Determination of acute toxicity to bluegill sunfish (*Lepomis macrochirus*) - Analysis of test solutions. Schering Agrochemical Ltd., Report No. ENVIR/88/43, MAK No. R-12675, Aventis No. A82561=W82, MAK No. R-12675

Guidelines: US EPA-540/9-85-006 (1985), US EPA FIFRA subdiv. E Guideline 72-1

Method is equivalent to EEC Directive 92/69/EEC Method C.1

Flow-through system

GLP: Yes

Material and Methods

Apollo SC (batch no. BX CR18298/9) was tested in a flow-through test on 2 – 3.5 cm long bluegill sunfish. No vehicles were used. The nominal concentrations of 0 / 20 / 36 / 64 / 112 / 200 mg/L (formulation) were tested on 20 animals per concentration. The test with 20 mg/L was cancelled after 4.5 h due to a mistake in dosing. Oxygen, pH, temperature and a.i. content were checked every 24 h. Mortality was checked after 24, 48, 72 and 96 h. Behaviour was checked together with mortality as far as low visibility (intense coloration by ai.) allowed.

Findings

The mortality of Apollo SC to bluegill sunfish is listed in Table 10.2.1-2.

Table 9.13: Acute mortality of Apollo SC to bluegill sunfish. Concentrations given are for the ai content.

Nominal concentration (mg/L)	Accumulative mortality [%]				
	0 h	24 h	48 h	72 h	96 h
0	0	0	0	0	0
blank	0	0	0	0	0
20	nd	nd	nd	nd	nd
36	0	0	0	0	0
64	0	0	0	0	5
112	0	0	0	0	5

Nominal concentration (mg/L)	Accumulative mortality [%]				
	0 h	24 h	48 h	72 h	96 h
200	0	0	0	0	0

nd – not determined, test cancelled after 4.5 h. All data based on nominal concentration.

Observations

Overall recovery rates for the active substances was between 80 and 124%. Behavioural changes were not observed on those occasions where fish were visible. The single deaths at 32 and 56 mg ai/L were not attributed to the test substance. The LC_{50} could not be determined in this test but is > 200 mg/L.

Conclusion

The tested formulation Apollo SC revealed after 96 hours at 200 mg/L (corresponding to > 100 mg a.i./L) no discernible toxic effect on the test species (bluegill sunfish).

9.4.2 Acute toxicity of the preparation to aquatic invertebrates

Report: Barrett, K.L., Arnold, D.J. (1988b) Determination of the acute toxicity of Apollo 50 SC to *Daphnia magna*. Schering Agrochemical Ltd.,, Report No. ENVIR/87/41, Aventis No. A82555=W76, MAK No. R-15126

Guideline: OECD Guideline No. 202 (= EEC Directive 92/69/EEC Method C.2) – static conditions

GLP: Yes

Material and Methods

The test substance Apollo SC (Batch No. CR 20663/1) was applied once at test begin (48 hour, static test design). No vehicle was used. Thirty Daphnids per concentration and control were exposed at the following test concentrations: 0 / blank / 3.12 / 6.25 / 12.5 / 25 / 50 / 100 / 200 mg/L (formulation). To avoid that the daphnids were trapped by sedimenting Clofentezine suspension particles, and for easier observation, they were placed in floating plastic "traps" with a porous membrane at the bottom. Three concentrations and the blank formulation were analysed at 0 and 48 h.

Findings

The mortality results are listed in Table 10.2.1-3.

Table 9.14: Results of an acute toxicity test of Apollo SC to *Daphnia magna*

Concentration (mg/L)	Affected daphnids (%) after 24 h	Affected daphnids (%) after 48 h
Control	3.3	10.0
Blank	0	6.6
3.12	0	0
6.25	10.0	10.0
12.50	10.0	10.0
25.00	10.0	10.0
50.00	6.7	6.7
100.00	0	3.3
200.00	3.3	6.7

All values based on nominal concentrations.

Observations

Overall recovery rates for the active substances were 98 to 106% at test start – therefore nominal concentrations are used - and 41 to 56% at test end. The water quality parameters pH and dissolved O_2 measured at 0 and 48 hours were determined to be within the acceptable limits. No further relevant observations were reported. The EC_{50} was not determined but is > 200 mg/L.

Conclusions

The tested formulation Apollo SC revealed after 48 hours at 200 mg/L (corresponding to 100 mg a.i./L) no discernible toxic effect on the test species *Daphnia magna* STRAUS.

9.4.2 Acute toxicity of the preparation to algae

Report: Hanstveit, A.O. (1987) The effects of Apollo 50 SC on the growth of the alga *Selenastrum capricornutum*. TNO, Report No. ENVIR/87/29, Aventis No. A82543=W69, MAK No. R-12665

Guideline: OECD 201 (1984) modified by EG-8 and ES-5[16]

The method is equivalent to EEC Directive 92/69/EEC Method C.3.

Deviation: The centrifugation step before chemical analysis was replaced by filtering followed by sonication of the re-suspended pellet.

GLP: Yes

Materials and Methods

The test substance Apollo SC (batch no. CR 20663/1) was tested in a static test system (duration: 72 hours). No vehicle was used. Three replicates with nominal cell densities of approx. 10^4 cells/ml were tested per concentration (0 / 0.1 / 0.32 / 1.00 / 3.2 / 10 / 32 / 100 mg/L formulation, nominal, or 0 / 0.05 / 0.16 / 0.5 / 1.6 / 5 / 16 / 50 mg ai/L nominal), with 6 replicates for control.

Analytical determination of the ai content: Due to the co-centrifugation of algae and ai particles, the centrifugation step was replaced by filtering followed by sonication of the pellet after suspension in diluted HCl solution (pH 2), followed by ai determination by HPLC-UV.

[16] Algal acute toxicity test, EG-8 and technical support document for algal acute toxicity test ES-5. Chemical Regulation Reporter, 31: 5117-5126 (1983)

Findings

Table 9.15: Results of an acute toxicity test of Apollo SC to *Selenastrum capricornutum*

Effects after 72 h:	Inhibition of biomass growth:	Growth rates related inhibition:
EC_{50}	n.d.*	n.d.*
NOEC	≥ 40 mg ai./L	≥ 40 mg ai./L

* not determined All values based on actual initial concentrations.

Observations

- Overall recovery rates for the active substances were approx. 70 % at test start and approx. 30 - 40% at test end (92 h). However, the determination of the ai content was very difficult since the dispersed particles had similar size as the algae and therefore co-centrifuged. The reliability of the analytical ai determination using the filtering step is accordingly low.
- The pH measured at 0, 24 and 48 hours was determined to be within the acceptable limits.
- Microscopic evaluation of the cells at the start of the incubation revealed no morphological abnormalities.
- At nominal concentrations above 3.2 mg/L formulation, increased cell growth was observed probably due to hydrolytically induced fertilisation with nitrogen-containing compounds, which is the growth-limiting factor in the OECD medium.

Conclusions

The tested formulation Apollo SC revealed no discernible toxic effect on the test alga *Selenastrum capricornutum* at nominal 100 mg/L or actual 80 mg/L (corresponding to 40 mg ai/L).