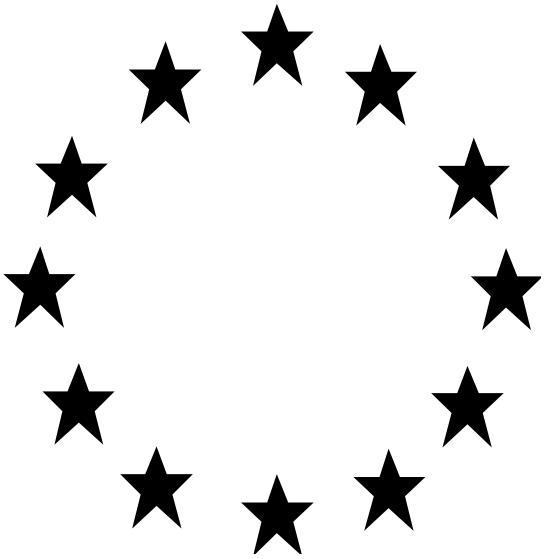


Final addendum to the

Draft Assessment Report (DAR)

- public version -

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

LENACIL

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

July 2009

Table of contents

ANNEX B

Lenacil

B.8 Environmental fate and behaviour

B.8.1 Route and rate of degradation in soil (Annex IIA 7.1.1; Annex IIIA 9.1.1)

B.8.1.1 Route of degradation (Annex IIA 7.1.1.1)

B.8.1.1.1 Aerobic degradation in soil (Annex IIA 7.1.1.1.1)

Lenacil - Fate and Behaviour in Soil (Theis, M., 2003)

Guidelines:
Richtlinien für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren' part IV, 4-1, of the 'Biologische Bundesanstalt für Land- und Forstwirtschaft', Germany and 91/414/EWG Appendix IIA.

GLP:
Yes

Material and Methods:
Test substance:
[4,7a-$^{14}C_2$]-lenacil, Batch: 0183162301, Specific activity: 10.494 MBq/mg, Radiochemical purity: > 97%.

Soils:
Table B.8.1.1.1-1: Characteristics of the test soil

Soil:	**German standard soil 2.2 (lot no.: F 2.23501)**
Sampling site:	Rheinland Pfalz/Hanhofen; Großer Striet, No.585; Soil sampled : 29/8/2001
Soil type:	Loamy sand
Organic carbon:	2.28% ± 0.16%
pH (0.01M $CaCl_2$):	5.8 ± 0.3
Cation exchange capacity [mequivalents/100 g]:	11 ± 2
% sand (0.05 – 2.0 mm):	74.8 ± 1.4
% silt (0.002 – 0.05 mm):	17.0 ± 0.8
% clay (< 0.002 mm):	8.2 ± 1.2
Maximum water holding capacity [g/100 g dry weight]	51 ± 4
Initial microbial biomass (mg BioC/100 g dry weight)	44.2 (mean)

Experimental design:
The fate and behaviour of [4,7a-$^{14}C_2$]-lenacil was investigated in a German standard soil (Speyer 2.2) incubated at 20°C and 40% of maximum water holding capacity. [4,7a-$^{14}C_2$]-lenacil was applied at a concentration of 0.5 mg/kg corresponding to a field rate of 0.375 kg/ha. After addition of [4,7a-$^{14}C_2$]-lenacil to the soil, the systems

were incubated in duplicate for each time point at 20°C in the dark and passively ventilated with CO_2-free air. At intervals of 0, 1, 3, 8, 17, 30, 59, 90, and 120 days of incubation, soils were extracted with methanol, water and hot methanol in a Soxhlet apparatus.

To trap volatile compounds, the incubation flasks were equipped with absorption/ventilation devices. [^{14}C]-carbon dioxide formed during the incubation was absorbed by soda lime. It was liberated by hydrochloric acid and trapped in a stream of nitrogen in gas-washing bottles containing 2M NaOH. Volatile organic compounds were trapped with paraffin oil-coated glass wool which was then extracted with ethyl acetate.

Analytical methods:
Radioactivity in extracts was determined by liquid scintillation counting (LSC). The radioactivity of the Soxhlet extracted soil was measured by incineration of aliquots of the soil and trapping the formed [^{14}C]-CO_2. Analysis of extracts for lenacil and degradation products was performed using reverse phase HPLC with detection by UV at 218 nm and positive ion APCI mass spectrometry.

Findings:

Table B.8.1.1.1-2: Soil parameters and recovery of radioactivity and distribution [%] of metabolites after application of ^{14}C-lenacil to soil and incubation under aerobic conditions

Day	**0**	**3**	**8**	**17**	**30**	**59**	**90**	**120**
Microbial biomass [mg bioC/100 g dry weight]	44.2	n.t	n.t	n.t	n.t	n.t	n.t	31.9
Methanol Water Methanol (Soxhlet)	88.6 3.06 2.10	83.4 3.64 2.84	69.5 5.06 4.07	59.2 5.72 5.12	39.4 5.13 3.32	22.3 4.15 3.21	15.4 3.30 2.85	10.8 2.74 3.05
Total extractable radioactivity	93.8	89.9	78.7	70.1	47.9	29.7	21.6	16.6
Carbon dioxide Volatile compounds	n.t. n.t.	0.50 -	3.93 -	9.35 -	23.15 -	37.9 -	46.6 -	50.8 -
Bound residues	1.05	4.08	12.7	15.3	25.9	27.1	26.9	25.8
Recovery	94.8	94.5	95.3	95.8	97.0	94.7	95.1	93.2
Lenacil (sum) M4.0 M5.0/7.0 M8.5/9.5 M14.0 M15.0 M 20.5 (IN-KF313) M28.0	91.55 - - - - - - -	78.75 - - 0.22 2.01 2.71 2.52 0.90	53.59 0.66 2.17 1.16 4.55 6.11 4.76 1.04	41.91 1.35 4.43 3.89 3.80 5.61 4.72 0.85	22.21 3.05 6.01 4.95 1.79 2.34 4.19 0.62	10.31 3.43 4.71 4.51 0.27 0.63 2.44 0.24	6.44 2.24 4.60 3.04 - 0.24 2.17 -	3.82 2.87 4.35 1.24 - - 1.65 -

n.t.: not tested
-: not detected or not calculated

Conclusions:
Lenacil was degraded under aerobic conditions in a sandy loam soil at 20°C. The mean recovery was 93 to 97% AR. Extractable residues decreased from 94 to 17% AR. Simultaneously, the radioactivity of the non-extractable bound residue increased from 1% AR at the beginning reaching a steady state with 26% AR on Day 30. The amount of CO_2 formed by microbial degradation rose to 51% AR on Day 120. Other volatile compounds were not observed.
In total, 9 metabolites in quantifiable amounts were detected. None reached values higher than 6.1% of applied radioactivity (AR). Using LC-MS analysis of a suitable concentrated methanol extract, the metabolites M14.0, M15.0 (4.55 % AR at day 8) and M20.5 (6.11 % AR at day 8) were characterised as oxo-isomers of lenacil. M20.5 was identified as 5-oxo-lenacil (IN-KF313) (max 4.76% AR at day 8), whereas the metabolites M14.0 and M15.0 were cyclohexanone derivatives (similar to IN-KE121). No structural information was obtained for the metabolites M5.0/7.0 (6.01% AR at day 30) and M8.5/9.5. Besides the quantified metabolites, 2 trace metabolites were detected which were a ketocyclopentapyrimidine and a cyclohexanone derivative of lenacil.
Based on the results from the radio HPLC-analysis and characterisation of metabolites, the major route of metabolism of lenacil is ring-oxidation.

The derivation of the DT50 in this study is discussed below under Point B.8.1.2.1.

Identity of M14/M15 as IN-KE121 in the study by Theis (2003) was indicated by MS analysis but the assignment was not definitive. Conclusion described M14/M15 as oxo-lenacil.
Study by Girkin gives a better understanding of the metabolite profile in soil.
3-cyclohexyl-6,7-dihydro-7-1H-cyclo pentapyrimidine-2,4,5(3H)-trione is the chemical name for IN-KF313.

IN-KF313 (5-oxo-lenacil) results from oxidation of the cyclopentapyrimidine ring moiety. IN-KE121 (7-oxo-lenacil) results from oxidation of the cyclohexyl ring moiety. Both processes can occur simultaneously. Further degradation probably occurs by opening of the pyrimidine ring to produce a number of unidentified polar fragments prior to mineralisation.

B.8.1.1.2 Anaerobic degradation in soil (Annex IIA 7.1.1.1.2)

Considering the supported use as a post-emergence herbicide with spring application on fodder and sugar beet, it is not expected that extended anaerobic conditions will occur and therefore an anaerobic degradation study is not required.

B.8.1.1.3 Soil photolysis (Annex IIA 7.1.1.1.2)

Lenacil - Photodegradation on Soil (Millais, A.J., 2002a)

Guidelines:
SETAC „Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides', March 1995

GLP
Yes

Material and Methods:
Test substance:
[4,7a-$^{14}C_2$]-lenacil, Batch: 0183157901, Specific activity: 10.494 MBq/mg, Radiochemical purity: >97%.

Soils:
Table B.8.1.1.3-1: Characteristics of the test soil

Soil	**Wolston 260**
Particle size distribution (SSEW classification) % sand (63 mm – 2 mm) % silt (2 mm – 63 mm) % clay (<2 mm)	 68.59 19.03 12.38
Classification	Sandy loam
pH (1:5) in water pH (1:5) in 1M KCl pH (1:5) in 0.01M $CaCl_2$ C.E.C. (mEq/100 g) Organic carbon (%) Maximum water holding capacity (%) Water holding capacity at 0.33 bar (%)	6.7 5.8 6.0 15.1 1.8 59.5 17.5
Organism	CFU/g
Bacteria Bacterial spores Actinomycetes Fungi Total aerobic micro-organisms	9.20×10^6 4.20×10^6 2.20×10^5 1.22×10^7

Experimental design:
[4,7a-$^{14}C_2$]-lenacil was applied to layers of sandy loam soil (*ca* 2 mm thick) at a concentration equivalent to an application rate of 500 g/ha. Test soils were irradiated

using a xenon arc simulated sunlight source continuously for periods up to 15 days at 20 ± 3°C (equivalent to a maximum of *ca* 40 days of summer sunlight equivalents at 40°N). Further samples were incubated in the dark to act as controls. Total recoveries of radioactivity including volatile radioactivity were measured for duplicate samples at each analysis time (0, 2, 4, 7, 10 and 15 days of continuous irradiation).

Analytical methods:
Soil samples were extracted sequentially with acetonitrile and acetonitrile:water (3:1 v/v). Radioactivity in extracts was determined by LSC and in the extracted soil by combustion followed by LSC. Soil extracts were analysed by reverse phase HPLC and TLC for unchanged lenacil and photo-degradation products.

Findings:

Table B.8.1.1.3-2: Recovery of radioactivity from control and irradiated soils treated with ^{14}C-lenacil

Sampling interval (days)	Extract	Unextracted	Total volatiles	Total recovery
Irradiated				
0	96.9	7.6	n.s.	104.5
2	85.0	5.2	6.6	96.8
4	83.7	6.0	9.8	99.5
7	87.3	5.6	12.4	105.3
10	75.5	6.0	14.2	95.7
15	74.6	5.7	15.7	96.0
Non-irradiated				
0	96.9	7.6	<0.1	104.5
2	101.1	2.3	<0.1	103.4
4	101.4	1.4	<0.1	102.8
7	101.5	1.3	<0.1	102.7
10	100.2	1.4	<0.1	101.6
15	98.5	1.4	<0.1	99.9

Results expressed as a percent of applied radioactivity and are the mean of duplicate determinations.
n.s. = No sample.

Table B.8.1.1.3-4: Profile of degradation products from irradiated and control soils treated with ^{14}C-lenacil

Sampling interval (days)	Lenacil	H1	H2	H3	Others	Polar
Irradiated						
0	-	-	-	-	-	-
2	72.0	2.9	n.d.	2.4	4.7	3.0
4	64.8	4.4	3.9	1.9	5.2	3.6
7	69.5	5.9	n.d.	1.2	3.1	3.0
10	56.3	7.7	0.4	3.6	4.7	2.9
15	56.8	6.6	0.9	1.7	5.1	3.7
Non-irradiated						
0	91.0	n.d.	n.d.	n.d.	1.8	n.d.
2	94.2	n.d.	n.d.	n.d.	7.0	n.d.
4	97.1	n.d.	n.d.	n.d.	4.3	n.d.
7	97.0	0.4	n.d.	n.d.	4.2	n.d.
10	98.3	0.6	n.d.	n.d.	1.4	n.d.
15	94.6	1.4	n.d.	n.d.	2.5	n.d.

Results expressed as a percent of applied radioactivity and are the mean of duplicate determinations.
n.d. = Not detected.
Others refer to radioactivity not associated with specific components.

The major photolytic degradation product of ^{14}C-Lenacil was $^{14}CO_2$. Only low levels of other degradation products were detected in soil extracts and these were generally more polar than the parent compound. It is therefore not possible to determine a detailed degradation pathway for the photolysis of Lenacil on a soil surface other than the aromatic ring is opened to give smaller molecules, which are then mineralised to carbon dioxide.

The periods of irradiation received by each sample expressed in terms of summer sunlight equivalents (12 hour days) at latitude 40°N were recalculated. DT50 calculation is based on a linear pseudo first order kinetics: k (day^{-1}) = 0.010253, lnC_0 = 80.884, r^2 = 0.712, DT50 = 67.6 days, DT90 = 225 days

Conclusions:
The photodegradation rate of lenacil on soil at 20°C is equivalent to 67.6 days assuming summer sunlight equivalents (12 hour days) at latitude 40°N. For irradiated soil treated with ^{14}C-lenacil, total mean recoveries of radioactivity were in the range of 95.7 to 105.3% AR and for the controls 99.9 to 104.5% AR.

Volatile radioactivity accounted for 15.7% AR at 15 days for the irradiated soil samples of which most (15.6% AR) was carbon dioxide. No significant volatile radioactivity (<0.1% AR) was found in the control samples. No major degradates were detected in soil extracts, although H1 reached a maximum of 7.6%AR. TLC indicated that this radioactivity was associated with more than one component.

B.8.1.2 Rate of degradation (Annex IIA 7.1.1.2.1; Annex IIIA 9.1.1.1.1)

B.8.1.2.1 Aerobic degradation

Degradation Rate of ^{14}C-Lenacil in Soil (Berg, D. S. 1994a)

Guidelines:
Dutch Guidelines G.1 Behaviour in the Soil. Rate of Degradation. Appendix G1.1

GLP:
Yes

Material and Methods:
Test substance:
[2-^{14}C]-lenacil, Specific activity: 17.78 µCi/mg, Radiochemical purity: >98%.

Soils:
Table B.8.1.2.1-1: Characteristics of the test soils

Soil name	Sassafras	Hillsdale	Tama
Origin	Carney`s Point, New Jersey	Quincy, Michigan	Carrolton,Illinois
Soil Type	Sandy Loam	Sandy Loam	Silt Loam
Textural analysis (USDA) [%] 2000 – 50 µm, sand > 50 – 2 µm, silt >2 µm, clay	 64.4 30.4 5.2	 62.4 34.4 3.2	 16.4 70.4 13.2
PH value (KCl)	6.2	6.3	6.6
Organic C [%]	1.3	2.0	2.3
Cation exchange capacity (meq/100g)	5.2	7.7	14.4
OM%	1.3	2.0	2.3
OC% (by calculation)	0.75	1.16	1.33
Maximum Water Capacity (%)	12.1	17.5	28.2

Experimental design:
The aerobic degradation and metabolism of [2-^{14}C]-lenacil was investigated in 3 soils (2 sandy loams, 1 silt loam) incubated at 25°C and pF 2.5 under aerobic conditions. [2-^{14}C]-lenacil was applied at a concentration of 3 mg/kg corresponding to a field rate of 2.3 kg/ha. The test system was connected to a trap with 1M NaOH in order to collect any evolved CO_2.

Analytical methods:
Duplicate samples of each soil type were removed at appropriate intervals and samples were extracted or frozen at –20°C immediately after sampling. Aliquots of the NaOH solutions used to trap CO_2 were combined with scintillation cocktail and

analysed for total radioactivity by LSC. The soil samples were extracted with methanol/water (90:10 v/v) on a shaker for 15 min. After decantation the volume was measured and duplicate aliquots were counted by LSC. Soil extracts were qualitatively and quantitatively analysed by reverse phase HPLC with UV detection for labelled lenacil and metabolites. Extracted soil samples were analysed by combustion/LSC.

Findings:

Table B.8.1.2.1-2: Recovery and distribution of metabolites after application of ^{14}C-lenacil to 3 soils

Soil	Days after appl.	$^{14}CO_2$	Lenacil	IN-KD302	IN-KF313	Met.A *	Met.B **	Other polars ***	Bound Residues	BgK/ others	Total
Sassafras	0	NT	103.12	0	0	0	0	0	0.2	0	103.12
	1	NT	104.7	0	0	0	0	0	0.4	0	105.10
	3	NT	105.57	0	0	0	0	0	0.9	0	106.47
	7	< 0.1	105.26	0	0	0	0	0	1.6	0	106.86
	14	< 0.1	103.33	0	0	0	0	0	2.2	0	105.53
	30	0.2	83.74	0.22	4.64	0	2.14	1.33	9.4	0.72	102.39
	60	1.0	80.50	0.66	5.05	0	2.4	3.63	9.8	0.96	104.00
	100	2.6	67.23	0.24	6.52	0.97	4.19	2.94	14.4	1.3	100.39
Hillsdale	0	NT	106.18	0	0	0	0	0	0.3	0	106.48
	1	NT	100.65	0	1.81	0	0.5	0	0.9	0	103.86
	3	NT	108.04	0	0	0	0	0	2.2	0	110.24
	7	< 0.1	102.37	0	0	0	0	0	3.2	0	105.57
	14	0.2	103.34	0	0	0	0	0	4.8	0	108.34
	30	1.5	92.92	0.25	3.31	0	2.35	5.53	4.8	0.93	111.59
	60	4.6	56.78	0.55	9.96	1.40	3.31	8.21	22.1	0.25	107.16
	100	9.4	50.21	0.43	7.92	1.63	5.54	5.49	18.6	0.37	99.59
Tama	0	NT	100.16	0	0	0	0	0	0.4	0	100.56
	1	NT	93.11	0	0	0	0	0	0.8	0	93.91
	3	NT	102.97	0	0	0	0	0	1.8	0	104.36
	7	< 0.1	101.56	0	0	0	0	0	3.0	0	104.56
	14	0.2	98.39	0	0	0	0	0	3.9	0	102.49
	30	0.7	82.11	0.34	3.45	0	1.87	7.09	8.8	0.55	104.91
	60	2.3	59.78	0.75	6.12	0.85	4.15	11.05	15.6	1.06	101.66
	100	5.2	43.77	0.65	8.89	1.59	5.26	12.78	19.2	0.58	97.92

NT Not tested
* Unknown polar metabolite eluting approx. 1.8 to 2.4 min.
** Unknown polar metabolite eluting approx. 2.7 to 3.6 min.
*** Containing 1 to 3 peaks, with no one peak > 10 % total dpm
BGK/Others are areas of background and/or undesignated activity
Results expressed as a percent of applied radioactivity

Conclusions:

The rate of degradation observed in this study was re-calculated in a modelling study by Shaw, D. (2004) using non-linear first-order regression performed by the

ModelMaker programme. All investigated soils showed an initial lag phase of approximately 14 days.

At a concentration of 3 mg/kg lenacil degraded in 3 different American soils at DT_{50} normalized at 20°C and pF2 of 107 to 185 days. Corresponding DT_{90} are in the range of 355 to 613 days.

IN-KF313 (3-cyclohexyl-6,7-dihydro-7-1*H*-cyclopentapyrimidine-2,4,5(3*H*) -trione) was identified as the principal degradation product. The amount varies from 6.5% AR (Sassafras soil) to 8.9% AR in Tama soil. The presence of IN-KF313 was demonstrated by analysing the samples by HPLC-MS and a peak at m/z 249 (MH^+). The polar metabolite B was found at levels up to 4.19-5.54% AR after 100 days. Up to 2.6-9.4% AR was recovered as CO_2 after 100 days. Up to 14.4-19.2% AR was recovered as bound residue after 100 days.

The notifier has considered that the DT50 derived from the American soils study were not valid due to higher application rate leading to saturation of microbial degradation processes and poor storage of the soils leading to a reduction in microbial biomass. The RMS agreed with that approach and considered that the DT50 derived from the European soils studies are more appropriate to derive the input data for PEC calculations.

Lenacil Aerobic Rate of Degradation in One Soil Type at 10°C and in Four Soils at 20°C
(Girkin, R., 2003)

Guidelines:
SETAC „Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides', March 1995

GLP:
Yes

Material and Methods:
Test substance:
[4,7a-$^{14}C_2$]-lenacil. Batch: 0183157901, Specific activity: 10.494 MBq/mg, Radiochemical purity: > 97%.

Soils:

Table B.8.1.2.1-3: Characteristics of the test soils

Soil name	Wolston	Wolston	Wick	Whimple	Sheringham
Incubation temperature (°C)	10°C	20°C	20°C	20°C	20°C
Particle size distribution:					
% sand (2 mm – 63 μm)	64.54	68.59	82.52	45.37	34.84
% silt (63 μm – 2 μm)	23.20	19.03	8.38	33.33	52.69
% clay (<2 μm)	12.27	12.38	9.10	21.30	12.47
Classification	sandy loam	sandy loam	Loamy sand	clay loam	sandy silt loam
Water content (%)[a]	16.19	17.17	8.92	21.60	14.81
Water capacity (0.33 bar) (%)	16.9	17.5	10.8	30.5	16.5
Maximum water capacity (0bar) (%)[a]	55.01	54.44	37.90	77.46	39.46
PH (1 : 5) in water	6.4	6.7	6.3	7.0	6.4
PH (1 : 5) in 1 M KCl	5.8	5.8	5.4	6.3	5.2
PH (1 : 5) in 0.0 1M $CaCl_2$	6.0	6.0	5.6	6.4	5.4
Cation exchange capacity(mEq/100 g)	16.5	15.1	9.5	20.2	10.6
Organic carbon (%)[b]	1.9	1.8	1.0	3.3	1.2
Organic matter (%)	3.3	3.1	1.7	5.7	2.1
Biomass (Day 0) μgC/g	450	509	476	1122	175
Biomass (Day 0) %	2.36	2.82	4.76	3.4	1.46

Experimental design:
Soil samples were set up in the laboratory and allowed to acclimatise in darkness, at a moisture content of 40% of the maximum water holding capacity and temperatures of 10°C or 20°C, for about one week prior to test substance application. The samples of soil were then treated with ^{14}C-lenacil at a rate of *ca* 2.7 mg/kg dry weight. This was equivalent to an application rate of 2 kg a.s./ha assuming uniform incorporation of the test substance in the top 5 cm of soil and an estimated soil bulk density of 1.5 g/cm^3. The experiments continued for up to 120 days. The test system was connected to a trap containing ethyl digol and 2 traps containing 1M NaOH.

Analytical methods:
At various time intervals, soil samples were extracted twice with acetonitrile and twice with acetonitrile/water (3/1, v/v). If necessary the sample was further extracted by overnight reflux with acetonitrile/water (3/1, v/v) . Radiolabelled volatile metabolites including CO_2 were trapped and quantified using an air flow-through system. Unextractable ^{14}C residues were also quantified and a material balance obtained for each sample.
Radioactivity in extracts was measured by LSC. The proportions of extractable lenacil and its degradation products were determined by reversed phase HPLC.

Findings:

Table B.8.1.2.1-4: Recovery of radioactivity from Wolston soil at 10°C following application of ^{14}C-lenacil at a rate of 2.7 mg/kg

Component	Days after application							
	0	1	7	14	30	60	91 (88)	120
Volatiles	na	0.0	0.1	1.0	3.4	10.5	17.6	24.3
Extractable	96.6	95.2	91.1	91.5	88.1	71.4	63.2	52.8
Polars	0.1	1.9	0.1	0.2	1.3	5.1	4.9	12.5
LN2	nr	Nr	Nr	nr	1.1	2.3	2.0	2.7
LN3	nr	Nr	Nr	0.2	0.4	1.7	1.0	4.0
LN4	nr	Nr	0.5	0.2	0.3	1.6	1.0	3.1
LN5 or IN-KE 121	nr	Nr	3.0	2.4	7.8	7.6	7.0	3.5
IN-KF313	1.0	Nr	3.3	8.3	9.0	9.4	8.1	6.0
Lenacil	94.0	92.3	82.9	77.8	65.4	43.0	37.6	20.1
LN8	nr	Nr	0.2	nr	0.5	nr	nr	nr
LN9	0.1	Nr	0.2	0.1	Nr	nr	nr	nr
LN10	0.1	0.3	0.1	nr	Nr	nr	nr	0.1
Others[a]	1.4	0.7	0.9	2.4	2.3	0.9	1.7	1.0
Bound residues	4.3	4.3	7.6	6.6	7.8	16.7	17.1	20.9
Total recovery	100.9	99.5	98.8	99.1	99.3	98.6	97.9	98.0

na : Not applicable.
Results are expressed as % applied radioactivity

Table B.8.1.2.1-5: Recovery of radioactivity from Wolston soil at 20°C following application of ^{14}C-lenacil at a rate of 0.67 mg/kg

Component	Days after application							
	0	1	7	14	30	60	91 (88)	120
Volatiles	na	0.2	5.8	14.3	33.2	51.1	57.9	61.1
Extractable	96.2	97.5	83.4	69.0	38.4	23.0	16.8	11.8
Polar A	nr	Nr	Nr	nr	Nr	nr	1.5	0.9
Polar B	nr	Nr	1.0	2.7	6.4	9.2	7.0	6.1
LN2	nr	Nr	Nr	2.3	2.0	0.8	nr	Nr
LN6	nr	Nr	Nr	nr	Nr	0.6	0.5	Nr
LN3	nr	Nr	Nr	0.9	1.9	0.7	0.6	0.2
LN4	nr	Nr	Nr	nr	2.0	0.7	0.4	0.1
LN5 or IN-KE 121	nr	Nr	8.9	11.7	4.1	1.1	0.7	0.5
IN-KF313	1.3	6.8	10.7	12.0	8.6	4.9	3.0	1.7
Lenacil	92.9	88.5	61.5	38.2	12.0	4.2	2.6	1.5
LN7	nr	Nr	Nr	nr	Nr	0.2	nr	0.1
LN8	nr	Nr	Nr	nr	0.1	0.1	0.1	Nr
LN9	nr	0.6	Nr	0.1	0.1	0.1	0.1	Nr
LN10	nr	Nr	Nr	nr	0.3	nr	nr	Nr
Others[a]	2.0	1.6	1.3	1.2	0.8	0.4	0.3	0.6
Bound residue	0.9	2.1	5.3	13.9	21.8	22.3	21.7	23.9
Total recovery	97.1	99.8	94.5	97.2	93.4	96.4	96.4	96.8

na : Not applicable.
Results are expressed as % applied radioactivity

Table B.8.1.2.1-6: Recovery of radioactivity from Wick soil at 20°C following application of ^{14}C-lenacil at a nominal rate of 0.67 mg/kg

Component	Days after application							
	0	1	7	14	30	60	91 (88)	120
Volatiles	na	0.2	1.7	6.1	21.8	42.4	50.8	55.5
Extractables	97.5	97.9	92.3	85.1	59.4	37.8	29.6	19.8
Polar A	nr	Nr	Nr	nr	Nr	nr	nr	0.4
Polar B	nr	Nr	0.6	0.7	5.3	14.6	14.5	11.5
LN2	nr	Nr	Nr	0.6	2.3	nr	nr	0.2
LN3	nr	Nr	Nr	0.8	1.6	1.6	1.5	0.3
LN4	nr	Nr	Nr	nr	0.4	0.4	0.4	Nr
LN5 or IN-KE 121	nr	Nr	7.3	13.9	11.3	2.7	1.5	0.9
LN1	nr	Nr	Nr	nr	Nr	1.5	0.8	Nr
IN-KF313	0.8	3.3	11.6	14.7	13.4	8.8	6.0	3.1
LN7	nr	Nr	Nr	nr	Nr	0.3	0.2	Nr
Lenacil	95.1	93.2	70.5	51.6	23.2	6.5	3.8	2.4
LN8	nr	Nr	Nr	nr	Nr	0.3	0.2	0.1
LN9	nr	Nr	0.2	nr	Nr	nr	0.1	0.1
LN10	nr	Nr	0.3	0.4	0.4	nr	nr	Nr
Others[a]	1.7	1.4	1.8	2.5	1.6	1.2	0.7	0.7
Bound residues	0.6	1.7	3.7	6.4	20.4	21.0	20.2	19.4
Total recovery	98.1	99.8	97.7	97.6	101.6	101.2	100.6	94.7

na : Not applicable.
Results are expressed as % applied radioactivity

Table B.8.1.2.1-7: Recovery of radioactivity from Whimple soil at 20°C following application of ^{14}C-lenacil at a nominal rate of 0.67 mg/kg

Component	Days after application							
	0	1	7	14	30	60	91 (88)	120
Volatiles	na	0.2	5.3	13.9	31.7	49.4	56.9	60.7
Extractables	96.6	96.1	79.4	67.8	37.7	25.6	19.0	12.3
Polar A	nr	nr	Nr	nr	nr	nr	nr	1.2
Polar B	nr	nr	0.8	1.5	3.7	4.9	6.8	3.4
LN11	nr	nr	Nr	nr	nr	0.4	nr	0.4
LN2	nr	nr	0.5	1.1	1.3	1.0	0.8	nr
LN3	nr	nr	0.3	0.5	1.2	0.9	0.5	0.4
LN4	nr	nr	Nr	0.2	0.8	0.7	0.3	0.2
LN12	nr	nr	Nr	nr	nr	1.2	nr	nr
LN5	nr	0.7	9.1	9.6	4.3	2.2	1.3	0.7
IN-KF313	0.8	5.3	8.5	8.1	5.3	3.7	2.5	1.8
Lenacil	93.9	88.2	58.6	46.1	20.3	9.8	6.4	3.9
LN8	nr	nr	Nr	nr	0.2	0.1	0.1	0.1
LN9	nr	0.6	0.2	0.1	nr	0.1	0.1	nr
LN10	nr	nr	Nr	nr	0.3	0.2	nr	nr
Others[a]	1.9	1.3	1.4	0.6	0.3	0.4	0.2	0.2
Bound residues	2.4	4.2	11.7	16.2	28.6	28.2	24.4	23.3
Total recovery	99.0	100.5	96.4	97.9	98.0	103.2	100.3	96.3

na : Not applicable. Results are expressed as % applied radioactivity

Table B.8.1.2.1-8: Recovery of radioactivity from Sheringham soil at 20°C following application of ^{14}C-lenacil at a nominal rate of 0.67 mg/kg

Component	Days after application							
	0	1	7	14	30	60	91 (88)	120
Volatiles	na	0.1	0.6	3.5	14.4	31.0	40.2	47.6
Extractables	98.5	98.7	94.9	89.9	66.7	44.2	37.0	28.2
Polar A	nr	nr	Nr	Nr	Nr	Nr	nr	0.8
Polar B	0.1	0.1	0.2	0.6	1.3	8.4	8.2	8.6
LN2	nr	nr	0.3	0.5	1.7	Nr	1.6	1.2
LN3	nr	nr	0.2	0.4	1.3	1.5	1.4	1.1
LN4	nr	nr	Nr	Nr	Nr	0.5	0.7	1.3
LN5 or IN-KE 121	nr	0.4	3.1	8.3	9.2	3.4	2.9	1.5
LN1	nr	nr	Nr	Nr	nr	1.6	nr	nr
IN-KF313	1.1	3.2	12.2	14.0	13.6	10.5	9.4	6.0
LN7	nr	nr	Nr	nr	nr	0.3	nr	nr
Lenacil	95.7	93.5	77.8	64.0	37.7	16.7	12.0	6.9
LN8	nr	nr	Nr	nr	nr	Nr	nr	0.1
LN9	0.3	0.2	0.3	0.3	0.2	0.1	0.1	0.1
LN10	0.1	0.1	Nr	nr	nr	Nr	nr	nr
Others[a]	1.2	1.3	0.8	1.7	1.7	1.1	0.7	0.6
Bound residues	0.7	1.9	2.1	6.6	19.5	27.5	26.5	22.6
Total recovery	99.2	100.7	97.6	100.0	100.6	102.7	103.7	98.4

na : Not applicable.

Results are expressed as % applied radioactivity

Conclusions:

The soil degradation study has investigated the aerobic soil metabolism of [4,7a-$^{14}C_2$]-lenacil in a sandy loam soil conducted at 10°C and at an application rate of 2.7 mg/kg and in 4 soils at 20°C at an application rate of 0.67 mg/kg. These application rates are equivalent to 2 kg/ha and 0.5 kg/ha, respectively.

The main degradation pathways involved oxidation of the cyclopentapyrimidine moiety to IN-KF313 (3-cyclohexyl-6,7-dihydro-7-1H-cyclopentapyrimidine-2,4,5(3H)-trione) and oxidation of the cyclohexane moiety to IN-KE121 followed by oxidation of both degradates to carbon dioxide. The major degradation product Metabolite IN-KF313 reached maximum level of 14.7% AR after 14 days; Metabolite IN-KE121 reached maximum level of 13.9 % AR after 14 days. Polar components were found up to 11.9 % of the applied radioactivity.

The derivation of the DT50 in this study is discussed below under Point B.8.1.2.1.

Degradation Rate of IN-KF313 in Three Soils (Berg, D.S. 1994b)

Guidelines:
Dutch Guidelines G.1 Behaviour in Soil. Rate of Degradation. Appendix G1.1

GLP:
Yes

Material and Methods:
Test substance:
Metabolite IN-KF313, Batch IN-KF313-1, chemical purity not available.

Soils:
Table B.8.1.2.1-9: Characteristics of the test soils

Soil name	**Sassafras**	**Hillsdale**	**Tama**
Origin	Carney`s Point, New Jersey	Quincy, Michigan	Carrolton,Illinois
Soil Type	Sandy Loam	Sandy Loam	Silt Loam
Textural analysis (USDA) [%] 2000 – 50 µm, sand > 50 – 2 µm, silt >2 µm, clay	 73.4 17.1 9.5	 76.5 16.0 7.6	 15.4 62.7 22.0
pH value (KCl)	6.4	6.3	6.8
Organic C [%]	0.9	1.0	2.4
Cation exchange capacity (meq/100g)	4.4	4.7	13.9
OM%	0.9	1.0	2.4
OC% (by calculation)	0.52	0.58	1.39
Maximum Water Capacity (%)	8.5	8.2	23.5

Experimental design:
The aerobic degradation of the metabolite IN-KF313 was investigated in three soils (Sassafras sandy loam, Hilldale sandy loam and Tama silt loam) at the application rate of 6 mg/kg soil.

The test vessels were incubated under aerobic conditions in the dark at approximately 25°C, pF 2.5 (0.33 bar), in duplicate. Samples were taken after 0, 1, 3, 7, 14, 30, 63 and 100 days after treatment. The system was connected to a vacuum manifold capable of fine regulation of the air flow, but no trapping system was present.

Analytical methods:
The samples were extracted with acetonitrile/water (80:20, v/v) and then centrifuged. The supernatant was decanted and the soil re-extracted in the same manner. The extracts were combined and an aliquot was removed for HPLC analysis.

Findings:
Table B.8.1.2.1-10: Recovery of radioactivity from 3 American soils at 25°C following application of ^{14}C metabolite IN-KF313 at a nominal rate of 6 mg/kg

	Sassafras	Hillsdale	Tama
0	100.0	100.0	100.0
1	96.7	103.3	96.7
3	98.3	99.9	95.3
7	95.1	98.3	96.6
14	91.7	91.3	93.3
30	89.1	92.4	95.0
63	83.3	88.6	84.2
100	72.4	82.5	73.7

Conclusions:
At a concentration of 6 mg/kg metabolite IN-KF313 degraded in 3 different American soils at DT_{50} at 25°C and pF2.5 of 237 to 350 days. Corresponding DT_{90} are in the range of 787 to 1162 days.

The notifier has considered that the DT50 derived from the American soils study were not valid due to higher application rate leading to saturation of microbial degradation processes and poor storage of the soils leading to a reduction in microbial biomass. The RMS agreed with that approach and considered that the DT50 derived from the European soils studies are more appropriate to derive the input data for PEC calculations.

Derivation of the DT50 soil used for the PEC calculations

Lenacil

The rates of degradation observed in the aerobic metabolism and aerobic degradations studies were re-calculated in a modelling study by Shaw, D. (2004) using non-linear first-order regression performed by the ModelMaker programme for lenacil. Default optimization and integration settings were used. Model parameters were systematically adjusted to find the best agreement between the model and the experimental data. The standard error enabled the statistical significance of the rate constant to be evaluated by reference to the Student's t distribution, using the residual number of degrees of freedom for the whole model.

Table B.8.1.2.1-11: First order non-linear DT50 a.s. calculations (Berg, D. S. 1994a, Theis, M., 2003, Girkin, R., 2003)

System	Rate constant (day^{-1})	Standard error	Value/error (t)	Degrees of freedom	P	Observed DT_{50} (days)	Observed DT_{90} (days)
Hillsdale	0.00811297	0.00113821	7.1278	6	<0.001	85	284
Sassafras	0.00471988	0.00571027	8.2656	6	<0.001	147	488
Tama	0.00828021	0.000984927	8.4069	6	<0.001	84	278
Speyer 2.2	0.0461931	0.00330780	13.9649	14	<0.001	15	50
Sheringham	0.0282606	0.00159233	17.7480	6	<0.001	25	81
Whimple	0.0501973	0.00490609	10.2316	6	<0.001	14	46
Wick	0.0452566	0.00150159	30.1391	6	<0.001	15	51
Wolston	0.0634932	0.00250699	25.3265	6	<0.001	11	36

The adjustments for water content requires information on the actual water content (on gravimetric basis) of the incubated soil samples.

Table B.8.1.2.1-12: Conversion of the DT50 a.s. to reference conditions (temperature and moisture)

Lenacil Degradation Rates

Soil	**Observed DT_{50}**	**Study conditions**	**Soil Type**	**Moisture content at reference conditions (pF2)**	**Study moisture conditions**	**Correction Factor for moisture**	**Correction Factor for temperature**	**DT_{50} under reference conditions**
Hillsdale	85	25°C 0.33 Bar	sandy loam	19%	15%	0.8475	1.4832	107
Sassafras	147		sandy loam	19%	15%	0.8475	1.4832	185
Tama	84		silt loam	26%	21%	0.8611	1.4832	107
Speyer 2.2	15	20°C 40% MWHC	sandy loam	19%	21.4%	None	None	15
Wolston	11	20°C 40% MWHC	sandy loam	19%	21.8%	None	None	11
Wick	15	20°C 40% MWHC	loamy sand	14%	15.2%	None	None	15

Whimple	14	20°C 40% MWHC	clay loam	28%	31.0%	None	None	14
Sheringham	25	20°C 40% MWHC	sandy silt loam	25%	15.8%	0.7253	None	18

Metabolites IN-KF313 and IN-KE121

The rates of degradation observed in the aerobic metabolism and aerobic degradations studies were re-calculated in a modelling study by Shaw, D. (2004). For the studies performed with the European soils (Theis, M., 2003, Girkin, R., 2003) in which lenacil was the applied substance, degradation times for the metabolites IN-KE121 and IN-KF313 were obtained by simultaneous regression of the parent and two metabolites using the WinNonLin programme. The kinetic fraction of lenacil proceeding to to each metabolite was also calculated.

Table B.8.1.2.1-13: First order non-linear DT50 of lenacil and its metabolites (Theis, M., 2003, Girkin, R., 2003)

	Speyer 2.2.	Sheringham	Whimple	Wick	Wolston
Initial amount of lenacil	88.749424	95.275914	91.477648	96.415177	93.899466
Degradation rate of lenacil	0.046468	0.028329	0.049923	0.045477	0.063771
Kinetic fraction for IN-KF313	0.139793	0.548553	0.735873	0.408532	0.362714
Degradation rate for IN-KF313	0.034481	0.037591	0.226904	0.036534	0.060220
Kinetic fraction for IN-KE121	0.668655	0.348148	0.531210	0.433677	0.401482
Degradation rate for IN-KE121	0.17136	0.056376	0.147514	0.066077	0.111260

Table B.8.1.2.1-14: First order non-linear DT50 of metabolite IN-KF-313 (Berg, D.S. 1994b)

System	Rate constant (day^{-1})	Standard error	Value/error (t)	Degrees of freedom	P	Observed DT_{50} (days)	Observed DT_{90} (days)
Hillsdale	0.00197856	0.000347640	5.69	6	0.001	350	1162
Sassafras	0.00292235	0.000222546	13.1314	6	<0.001	237	787
Tama	0.00263883	0.000306310	8.6149	6	<0.001	263	873

Table B.8.1.2.1-15: Conversion of the DT50 IN-KF313 to reference conditions (temperature and moisture)

IN-KF313 Degradation Rates

Soil	Observed DT_{50}	Study conditions	Soil Type	Moisture content at reference conditions (pF2)	Study moisture conditions	Correction Factor for moisture	Correction Factor for temperature	DT_{50} under reference conditions
Hillsdale	350	25°C 0.33 Bar	sandy loam	19%	15%	0.8475	1.4832	440
Sassafras	237		sandy loam	19%	15%	0.8475	1.4832	298
Tama	263		silt loam	26%	21%	0.8611	1.4832	336
Speyer 2.2	20	20°C 40% MWHC	sandy loam	19%	21.4%	None	None	20
Wolston	12	20°C 40% MWHC	sandy loam	19%	21.8%	None	None	12
Wick	19	20°C 40% MWHC	loamy sand	14%	15.2%	None	None	19
Whimple	3	20°C 40% MWHC	clay loam	28%	31.0%	None	None	3
Sheringham	18	20°C 40% MWHC	sandy silt loam	25%	15.8%	0.7253	None	13

Observed DT_{50} values calculated from the k values reported in Shaw (2004), using Ln2/k.

Table B.8.1.2.1-16: Conversion of the DT50 IN-KE121 to reference conditions (temperature and moisture)

IN-KE121 Degradation Rates

Soil	Observed DT_{50}	Study conditions	Soil Type	Moisture content at reference conditions (pF2)	Study moisture conditions	Correction Factor for moisture	Correction Factor for temperature	DT_{50} under reference conditions
Speyer 2.2	4	20°C 40% MWHC	sandy loam	19%	21.4%	None	None	4
Wolston	6	20°C 40% MWHC	sandy loam	19%	21.8%	None	None	6
Wick	11	20°C 40% MWHC	loamy sand	14%	15.2%	None	None	11
Whimple	5	20°C 40% MWHC	clay loam	28%	31.0%	None	None	5
Sheringham	12	20°C 40% MWHC	sandy silt loam	25%	15.8%	0.7253	None	9

Observed DT_{50} values calculated from the k values reported in Shaw (2004), using Ln2/k.

c) Geometric Mean DT_{50} from EU soils

The geometric mean DT_{50} for lenacil from all EU soils is **14.4 days**.

The geometric mean DT_{50} for IN-KF313 from all EU soils is **11.2 days**.

The geometric mean DT_{50} for IN-KF313 from EU soils, excluding Speyer 2.2 soil, is **9.7 days**.

The geometric mean DT_{50} for IN-KE121 from all EU soils is **6.5 days**.

The geometric mean DT_{50} for IN-KE121 from EU soils, excluding Speyer 2.2 soil, is **7.4 days**.

The DT50 lab with European soils are in line with the DT50 field (18, 25, 28 in Northern European trials, soil temperatures slightly above or slightly below 20°C during the 3 first months of the experiments; 88 days in Spain, experiment characterized by hot soil temperature (26-31°C) and almost no precipation during the 3 first months).
The DT50lab with European soils are also in line with the conclusions of the lysimeter study.

d) Geometric Mean DT_{50} from all soils

The geometric mean DT_{50} for lenacil from all soils tested is **32.7 days**.

The geometric mean DT_{50} for IN-KF313 from all soils tested is **40.9 days**.

e) Average formation fractions

Formation fractions calculated by Shaw (2004) are shown below.

Soil	IN-KE121	IN-KF313
Speyer 2.2	0.6687	0.1398
Wolston	0.4015	0.3627
Wick	0.4337	0.4085
Whimple	0.5312	0.7359
Sheringham	0.3481	0.5486

The mean formation fraction for IN-KF313 from all EU soils is **0.4391**.

The mean formation fraction for IN-KF313 from EU soils, excluding Speyer 2.2 soil, is **0.5139**.

The mean formation fraction for IN-KE121 from all EU soils is **0.4766**.

The mean formation fraction for IN-KE121 from EU soils, excluding Speyer 2.2 soil, is **0.4286**.

For modelling purposes, the lenacil geometric mean DT_{50} of **14.4 days** from all EU soils is appropriate. The soil DT_{50} values obtained from the US soils are considered to be extreme and most likely due to the following factors:

a) High application rate of lenacil (2 kg/ha) leading to microbial saturation.
b) Use of methylene chloride (0.25 mL) as the treatment solvent which may have affected microbial populations.
c) Insufficient aeration of the test soils due to the arrangement of the test vessels (16 flasks linked sequentially).

d) Dry nature of the test soils (MWHC = 12.1, 17.5 and 28.2 g/100g dry weight) likely to result in a low biomass.

Degradation in the US soils was characterised by a lag-phase of 14 days at the start of the incubation period, and this was followed by slow degradation of lenacil for the remainder of the study. A lag-phase was not seen in the study conducted with EU soils and degradation of lenacil was rapid in each soil tested. The factors described above and the observed lag-phase and slow degradation strongly suggest that the US soils had low microbial activity following application of lenacil and that microbial populations were unable to recover sufficiently during the course of the study.

The RMS agreed with that approach and considered that the DT50 derived from the European soils studies are more appropriate to derive the input data for PEC calculations.

For modelling purposes, the IN-KE121 geometric mean DT_{50} of **7.4 days** and formation fraction of **0.43** from the EU soils, excluding the Speyer 2.2 are appropriate. The results from the Speyer 2.2 soil are questionable due to incomplete identification of IN-KE121 in the soil extracts.

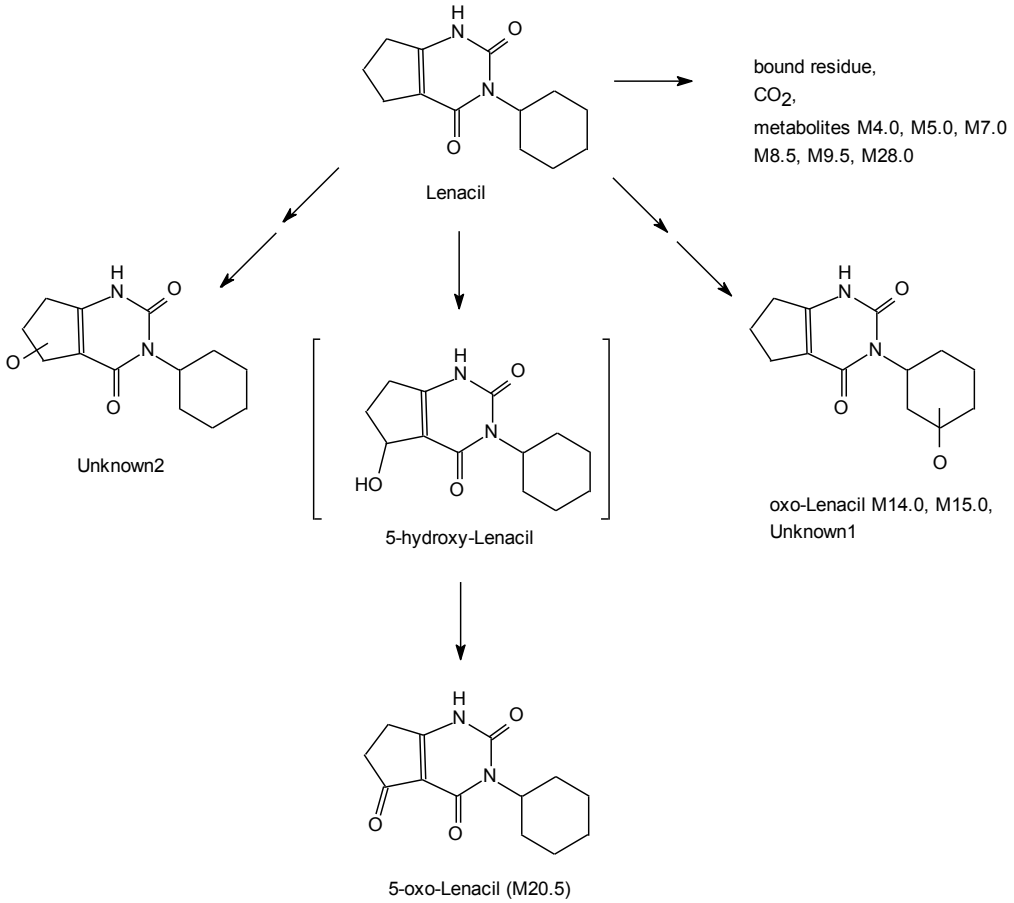
For modelling purposes, the IN-KF313 geometric mean DT_{50} of **11.2 days** from all EU soils is used to give a worst-case assessment for this metabolite. The formation fraction of **0.51** is considered appropriate to be consistent with the approach taken for IN-KE121. The use of IN-KF313 soil degradation data obtained from US soils are not considered appropriate for the reason described above for the study conducted with lenacil.

	DT50 (original dossier)	DT50 (recalculation)
PECgw **lenacil**	9.9	14.4
PECsoil **lenacil**	15	
PECgw **IN-KF313**	11	11.2
PECsoil **IN-KF313**	15	
PECgw **IN-KE121**	4.6	7.4
PECsoil **IN-KE121**	7.7	

B.8.1.2.2 Anaerobic degradation

Considering the supported use as a post-emergence herbicide with spring application on fodder and sugar beet, it is not expected that extended anaerobic conditions will occur and therefore an anaerobic degradation study is not required.

Figure B 8.1-1: Proposed degradation pathway of lenacil in soil



B.8.1.3 Field studies (Annex IIA 7.1.1.2.2; Annex IIIA 9.1.1.2)

B.8.1.3.1 Soil dissipation testing

Venzar 80% WP (containing 80% lenacil) Related Soil Dissipation on Bare Soil, Four Sites in Europe (Pollmann, B., 2003)

Guidelines:
IVA guideline for residue trials (Beutel *et al*., 1992), the BBA guidelines part IV, 4-1 (Schinkel *et al*., 1986), the SETAC guideline (Lynch, 1995).

GLP:
Yes

Material and Methods:
Test substance:
VENZAR 80% WP (batch number NOV00HE037), containing 816 g/kg lenacil.

Soils:
Data from each trial site are contained in the appendix.

Experimental design:
Objective of this study was to determine the residues of lenacil and its main metabolite IN-KF313 in soil at various intervals after application of 625 g/ha (500 g/ha a.s.) VENZAR 80% WP.

The study was carried out at 4 locations in Europe: One trial in Northern Germany, one trial in Southern Germany, one trial in Northern France and one trial in Spain. All trials were located in areas typical for sugar beet cultivation. Before the beginning of the study, samples for soil characterisation (at least 1 kg with spade, 0 - 30 cm), for biomass determination (at least 2 kg with spade, 0-30 cm) and for soil density determination (2 cores for 0-10 cm and 2 cores for 10-30 cm) were taken from each test site. They were shipped at ambient temperatures to IFU Umweltanalytik, Pforzheim (soil characterisation, density) or to GAB Biotechnologie, Niefern (biomass) respectively. The plot size varied from 144 to 288 m². The plots were placed on bare soil.

VENZAR 80% WP was applied once at each trial with a calibrated boom sprayer simulating a commercial application. The actually applied amounts were calculated by measuring the remaining spray solution after each application. Soil samples were taken with a HUMAX soil corer. The inner diameter of the corer was 50 mm. Acetate liners with a length of 30 cm were used. Each core was marked with a black plastic cap at the bottom and a red plastic cap at the top. Per subplot 5 cores were taken. Residue samples were stored deep frozen at the testing facilities and samples for analysis were transported deep-frozen to the analytical laboratory. Treated and non-treated sampes were shipped separately to avoid contamination.

Analytical methods:
The soil processing consisted of two steps: First the field samples of 30 cm length where cut in frozen condition with a circular saw into parts of 0 – 10 cm, 10 – 20 cm

and 20 – 30 cm. Then the parts of 10 cm length were homogenised by milling. Each homogenised sample was divided into two sub-samples of at least 250 g. The samples were analysed for residues of lenacil and the metabolite KF-313 by HPLC-MS/MS. Quantification was performed by means of a calibration curve. The LOQ was 0.02 mg/kg for lenacil and KF-313. The overall mean recovery was 89% for lenacil (7% RSD) and 80% for KF-313 (9% RSD). Mean relative standard deviations ranged from 5 to 6% for lenacil (mean 7%) and 5 to 8% for KF-313 (mean 9%).

Findings:

Table B.8.1.3.1-1: Disappearance of lenacil in the 0-10 cm soil horizon in 4 dissipation trials

	F01N001R		**G01N001R**		**G01N002R**		**S01N002R**	
Interval	**day**	**lenacil mg/kg**	**Day**	**lenacil mg/kg**	**day**	**lenacil mg/kg**	**day**	**lenacil mg/kg**
0	0	0.12	0	0.16	0	0.13	0	0.19
7DALA	7	0.2	7	0.24	8	0.14	6	0.06
14DALA	14	0.18	14	0.13	14	0.07	14	0.1
1MAA	30	0.11	28	0.06	30	0.04	29	0.08
2MAA	62	0.04	59	0.05	64	0.01	61	0.08
3MAA	92	0.02	86	0.03	91	0	92	0.08
6MAA	209	0	176	0.01		-	188	0.03
9MAA	273	0	276	0.01		-	274	0
12MAA		-	363	0		-		-

Table B.8.1.3.1-2: DT_{50} for lenacil in the 0-10 cm soil horizon in 4 dissipation trials

System	Rate constant (day^{-1})	Standard error	Value/error (t)	Degrees of freedom	P	Observed DT_{50} (days)	Observed DT_{90} (days)
F01N001R Alsace, France	0.027291	0.0018078 1	15.0896	4	<0.001	25	84
G01N001R Niedersachsen, Germany	0.0251735	0.0087335 1	2.8824	7	0.024	28	91
G01N002R Baden-Württemberg, Germany	0.0376226	0.0110059	3.4184	4	0.027	18	61
S01N002R Valencia, Spain	0.0079125 9	0.0044761 4	1.7677	6	0.128	88	291

In all 4 trials, lenacil was only found in the 0-10 cm soil layer. The decline kinetics for lenacil was evaluated using the program Model Maker 4. For lenacil a first-order, multi-compartment model was used. If the data input to the model included a time interval when the residue value was below the limit of quantification then a value equal to half the limit of quantification was used (0.01 mg/kg on a dry weight basis). When no peak was present (= n. d.) the concentration was set to zero. The DT50 were not further converted to reference temperature and soil moisture.

The metabolite KF-313 was detected only in 2 trials and only in the 0-10 cm soil layer with residues below the LOQ (0.02 mg/kg). Therefore no decline kinetics were calculated.

No residues of lenacil and KF-313 were detected in the control samples. No residues were detected in the soil layers 10-20 cm and 20-30 cm.

Conclusions:
The dissipation of lenacil and its metabolite IN-KF313 was investigated at 4 different sites in Europe during 2001-2002 (Northern France, Germany and Spain). On bare soil lenacil was found to have a low mobility under field conditions. In all 4 trials lenacil was found only in the 0-10 cm layer. The metabolite IN-KF313 was detected only in 2 trials and only in the 0-10 cm soil layer with residues below the limit of quantification. Under field conditions lenacil has DT_{50} values in the range 18 to 88 days and DT_{90} values in the range 61 to 291 days.
(The DT50 of 88 days observed in the experiment performed in Spain can be considered as an outlier. This experiment is characterized by hot soil temperature (26-31°C) and almost no precipation during the 3 first months).

B.8.1.3.2 Soil residue testing - soil accumulation testing

The $DT_{50\ Lab}$ values (11-18 days) are less than 1/3 of the time between application and harvest (*ca* 160 to 180 days). Therefore no soil residue testing is required.

Under field conditions lenacil has DT_{90} values in the range 61 to 291 days. Therefore no soil accumulation testing is required.

B.8.2 Adsorption, desorption and mobility in soil (Annex IIA 7.1.2 and 7.1.3; Annex IIIA 9.1.2)

B.8.2.1 Adsorption and desorption of the active substance and relevant metabolites (Annex IIA 7.1.2)

Batch Equilibrium (Adsorption/Desorption) Study with [2-^{14}C] Lenacil (Sheftic, G. D. and Priester, T. M., 1992)

Guidelines:
OECD 106.

GLP:
Yes

Material and Methods:

Test substance:

[2-^{14}C]-lenacil with a specific activity of 17.78 µCi/mg and a radiochemical purity of 99%.

Soils:

Table B.8.2.1-1: Characteristics of the test soils

Parameter	Oshtemo	Sassafras	Traver
Textural class	Loamy sand	Sandy loam	Loam
0.050 - 2 mm (sand)	82.4	64.4	50.8
2 µm - 50 µm (silt)	14.4	30.4	40.4
<2 µm (clay)	3.2	5.2	8.8
pH (1:5) in water	5.7	6.2	7.6
Organic matter (%)	0.8	1.3	1.9
CEC (meq/100 g)	2.8	5.2	22

Experimental design:

Adsorption and desorption of ^{14}C-lenacil was studied in three soil types using the batch equilibrium method. Separate test solutions of ^{14}C-lenacil were prepared at four concentration of 0.02; 0.04; 1.0 and 5 mg/L in 0.01M $CaCl_2$. Each dose level was tested in duplicate. 50 mL test solutions were mixed with 10 g of each soil and shaken for 24 hours at 25°C.

The highest concentration soil samples (5 mg/L) were then desorbed 4 times with fresh calcium chloride for 24 hours each time at 25°C.

Analytical methods:

After separation by centrifugation, aliquots of the adsorption and desorption supernatants were analysed by LSC. Recovery of radioactivity in the aqueous solution and soil phase was in the range 94-105% and 94-103% respectively for the adsorption equilibration and for the desorptions sequence.

Findings:

Table B.8.2.1-2: Adsorption/desorption values for lenacil in 3 soils

Soil	Soil type	Organic carbon (%)	Adsorption constant K_f	Adsorption constant K_{foc}	1/n	Desorption constant $K_{foc(des)}$
Oshtemo	Loamy sand	0.46	0.35	75	0.88	136
Sassafras	Sandy loam	0.75	0.61	81	0.86	141
Traver	Loam	1.1	2.8	254	0.91	417

Conclusions:

Freundlich adsorption constants Kfoc for lenacil in the 3 soils were in the range 75 to 254.

Lenacil - Adsorption/Desorption on Soil Girkin, R. (2002a)

Guidelines:
OECD 106.

GLP:
Yes

Material and Methods:
Test substance:
[4,7a-$^{14}C_2$]-lenacil, specific activity 10.494 MBq/mg and purity of >97%.

Soils:
Table B.8.2.1-3: Characteristics of the test soils

Parameter	Wolston	Bottom Watchley	Wick	Elmton
Textural class	Sandy loam	Sandy silt loam	Loamy sand	clay loam
0.063-2 mm(sand)	64.54	34.39	83.13	46.33
2 μm-63 μm (silt)	23.20	46.60	7.83	29.26
<2 μm (clay)	12.27	19.01	9.04	24.41
pH (1:5) in water	6.4	5.9	6.2	8.0
pH (1:5) in 0.01M $CaCl_2$	6.0	5.2	5.4	7.3
Organic carbon (%)	1.9	3.6	0.8	3.2
CEC (mEq/100 g)	16.5	48.2	7.0	28.7

Experimental design:
The adsorption/desorption of ^{14}C-lenacil was studied in four soils using the batch equilibrium method. Initial solution concentrations were 3.0, 1.0, 0.2 and 0.05 mg/L in 0.01 M aqueous calcium chloride. For the adsorption phase 20 mL of the test solutions were mixed with 10 g of each soil and shaken for 24 hours at 20°C in the dark. Soil samples were then desorbed twice with fresh calcium chloride for 24 hours each time.

Analytical methods:
After separation by centrifugation, aliquots of the adsorption and desorption supernatants were analysed by LSC. Aqueous solutions and soil extracts were analysed by HPLC to determine actual concentrations of lenacil present in soil extracts and solutions during the equilibrium phases. Recovery of radioactivity in the aqueous solution, soil extracts and soil residue was in the range 98.7-99.9% and 98.4-103.1% respectively for the adsorption equilibration and after complete adsorption-desorptions sequence.

Findings:

Table B.8.2.1-4: Adsorption/desorption values for lenacil in 4 soils

Soil	Soil type	Organic carbon (%)	Adsorption constant K_f	Adsorption constant K_{foc}	1/n	Desorption constant $K_{foc(des)}$
Wolston	Sandy loam	1.9	1.49	78.4	0.90	104
Bottom Watchley	Sandy silt loam	3.6	7.87	219	0.94	251
Wick	Loamy sand	0.8	0.96	120	0.89	153
Elmton	Clay loam	3.2	2.65	82.8	0.88	158

Conclusions:

Freundlich adsorption constants for lenacil in the four test soils were found to be between 78 and 219.

Mean and median values were calculated for the a.s. considering both available adsorption studies. Where a large number of additional data are available then the median values may be more appropriate than the mean. Both values were calculated.

	Mean	Median
Kfoc	130	83
1/n	0.89	0.89

Batch Equilibrium (Adsorption/Desorption) Study with IN-KF313 (Berg, D. S., 1996c)

Guidelines:

Dutch guideline G1 Behaviour in the soil. Appendix G.1.1

GLP:

Yes

Material and Methods:

Test substance:

Metabolite IN-KF313 (3 cyclohexyl-6,7-dihydro-7-hydroxy-1H-cyclopenta pyrimidine-2,4,5(3H)-trione), purity and batch number not available

Soils:

Table B.8.2.1-5: Characteristics of the test soils

Parameter	Hillsdale	Sassafras	Tama
Textural class	Sandy Loam	Sandy loam	Silt Loam
0.050 - 2 mm (sand)	76.5	73.4	15.4
2 µm - 50 µm (sielt)	16	17.1	62.7
<2 µm (clay)	7.6	9.5	22.0
PH in water	6.3	6.4	6.8
Organic matter (%)	1.0	0.9	2.4
Organic carbon (%)	0.58	0.52	1.39
MWHC	8.2	8.5	23.5
CEC (meq/100 g)	4.7	4.4	13.9

Experimental design:

Adsorption and desorption behaviour of IN-KF313 (3 cyclohexyl-6,7-dihydro-7-hydroxy-1H-cyclopenta pyrimidine-2,4,5(3H)-trione) was studied in three soils using the equilibrium method. Separate test solutions of IN-KF313 were prepared at four concentrations of 3; 4; 5 and 6 mg/L 0.01 M $CaCl_2$. Each dose level was run in duplicate. 50 mL of the test solutions were mixed with 50 g of each soil and shaken for 24 hours at 25°C.

The highest concentration soil samples (6 mg/L) were then desorbed twice with fresh calcium chloride for 24 hours each time at 25°C. The Freundlich desorption isotherm could not be done because IN-KF313 is readily desorbed from the soil.

Analytical methods:

After separation by centrifugation, aliquots of the adsorption and desorption supernatants were analysed by Reversed Phase Liquid Chromatography.

Findings:

Table B.8.2.1-6: Adsorption/desorption values for KF-313 in 3 soils

Soil	Soil type	Organic carbon (%)	Adsorption constant K_f	Adsorption constant K_{foc}	1/n
Sassafras	Sandy Loam	0.52	4.3	823.8	0.69
Hillsdale	Sandy loam	0.58	4.5	769.0	0.99
Tama	Silt Loam	1.39	1.1	79.0	1.00

Conclusions:

Freundlich adsorption constants for IN-KF313 were between 79 and 824 in the three test soils. The mean Kfoc was 557 and the mean value of 1/n was 0.89

IN-KE 121 Adsorption/Desorption on Soil (Kane, T., 2004)

Guidelines:
OECD 106

GLP:
Yes

Material and Methods:
Test substance:
Metabolite IN-KE121, chemical purity: 96.7%, batch no. 7X-0245.

Soils:
Table B.8.2.1-7: Characteristics of the test soils

Parameter	Sheringham	Wick 285	Elmton
Textural class	Loamy sand	Loamy sand	clay loam
0.063-2 mm(sand)	83.24	82.52	46.33
2 μm-63 μm (silt)	7.63	8.38	29.26
<2 μm (clay)	9.13	9.1	24.41
pH in 0.01 M $CaCl_2$	6.4	5.6	7.3
Organic carbon (%)	1.0	1.0	3.2
$CaCO_3$ (g/kg)	0.8	1.1	263.1
CEC (meq/100 g)	12.2	9.5	28.7

Experimental design:
The adsorption properties of IN-KE121 were studied in three soil types using the adsorption/desorption batch equilibrium method. For determination of the Freundlich adsorption and desorption isotherms initial solution concentrations in the range 0.05, 0.1, 0.25, 1.0 to 5.0 mg/l of IN-KE121 in aqueous 0.01 M calcium chloride were studied in soil. 19 mL of the test solutions were mixed with 20 g of each soil and shaken for 8 hours at ca 22°C in the dark . Soils were desorbed once with fresh 0.01M $CaCl_2$ solution for 24 hours.

Analytical methods:
After separation by centrifugation, aqueous solutions were analysed by validated analytical methodology (LC-MS/MS) to determine the actual concentration of IN-KE121 present in the solutions during the equilibration phases. The mean percentage recovery was 102.3%

Findings:

Table B.8.2.1-8: Adsorption/desorption values for IN-KE121 in 3 soils

Soil	Soil type	Organic carbon (%)	Adsorption constant (K_f)	Adsorption constant (K_{foc})	1/n
Wick 285	Loamy sand	1,0	0.435	43.5	0.92
Sheringham	Loamy sand	1,0	0.404	40.4	0.96
Elmton	Clay loam	3,2	0.977	30.5	0.96

Conclusions:

Freundlich adsorption constants for IN-KE121 were in the range 31 to 44 for the 3 test soils. The mean Kfoc was 38 and the mean value of 1/n was 0.94

Revision of Kfoc and 1/n factors used for PECgw calculations

For modelling purposes the lenacil median K_{oc} of **83**, median K_{om} of **48** and the corresponding 1/n value of **0.88** are appropriate based on the results available from seven soil types. The corresponding median study temperature of 20°C was used as a model input. The median K_{oc} and K_{om} values selected represent a conservative assessment as they are at the lower end of the range of the available adsorption data.

For modelling purposes the IN-KE121 mean K_{oc} of **38**, mean K_{om} of **22** and mean 1/n value of **0.95** are appropriate based on the results available from three soil types. The study temperature was 23°C and is used as a model input. The available data show a good correlation to soil organic carbon and the use of mean data are therefore considered to be justified.

For modelling purposes, the IN-KF313 worst-case K_{oc} of **79**, worst-case K_{om} of **46** and the corresponding 1/n value of **1.00** may be considered because the Hillsdale and Sassafras soil are very similar in character (OC, CEC, pH) and as such may not provide true replication. However, it should be noted that adsorption was strong to the Hillsdale and Sassafras soils suggesting that the worst-case values described represents an extreme assessment. Comment from Member States in the reporting table (Point 4(45)) suggested the use of a percentile approach to derive a more realistic adsorption value for the metabolite IN-KF313. P_{10} values of **217** for K_{oc}, of **126** for K_{om} and **0.75** for 1/n were therefore selected for modelling as a realistic assumption. The study temperature of 25°C was used as a model input.

RMS conclusions:

The new Koc proposal for metabolite IN-KF313 takes into account the concern raised by some MS. The evaluation of the Koc of the metabolites must be considered together with the information from the lysimeter study and the toxicological non-relevance assessment.

B.8.2.2 Column leaching studies with the active substance and relevant metabolites (Annex IIA 7.1.3.1; Annex IIIA 9.1.2.1)

Due to the availability of a lysimeter study a column leaching study was not performed.

B.8.2.3 Aged residue column leaching (Annex IIA 7.1.3.2; Annex IIIA 9.1.2.1)

Due to the availability of a lysimeter study an aged column leaching study was not performed.

B.8.2.4 Lysimeter and field leaching studies (Annex IIA 7.1.3.3; Annex IIIA 9.1.2.2)

Lysimeter Study with (^{14}C)-Lenacil (Schnöder, F., 2004)

Guidelines:
BBA-Guideline IV, 4-3 (1990)

GLP:
Yes

Material and Methods:
Test substance:
[2-^{14}C]-lenacil, batch number 2875-016, specific radioactivity 0.6589 MBq/mg, purity >98%.

Soils:
Table B.8.2.4-1: Characteristics of the test soils

horizon	depth	Particle size[1]			pH[2]	organic carbon
		Sand [%]	Silt[%]	Clay[%]	[$CaCL_2$]	[%]
Ap	0-30 cm	76.40	20.25	3.65	5.60	1.32
G_OB_V	30-85 cm	84.65	13.90	0.95	5.85	0.20
G_O	85-130 cm	85.05	13.85	1.10	5.80	0.05

[1] sand = 2 - 0.063 mm; silt = 0.063 - 0.002 mm; clay = < 0.002 mm
[2] ratio of $CaCl_2$ solution to soil is 1:2.5 (v/v)

Experimental design:
Two lysimeters were involved in the study with the test article applied as a formulation similar to Venzar but containing 53% active ingredient [a.s.]. A split application was performed with the first application performed on 5 June 1995 at a rate of 200 g a.s./ha (to sugar beet at growth stage 12-14) and the second application on 19 June 1995 (at growth stage 16 to 18) at a rate of 300 g a.s./ha. The subsequent crop rotation was winter wheat and winter barley. The test article and metabolites were determined over a time period of four years. Undisturbed soil monoliths (Gleysic Cambisol) of 1 m² surface and 110 cm depth were collected on 8 December

1993 from an agricultural field in Münster Handorf and transported to the site of Covance Laboratories GmbH, Kesselfeld 29, D-48163 Münster. Monthly mean temperatures in soil and air were reported. The levels of leachate in the lysimeter outer containers were usually checked in biweekly intervals at least in one lysimeter of this study (in some instances weekly).

Table B.8.2.4-2: Precipitations and amounts leachate

	Annual precipitation + irrigation (mm)	Annual amount leachate in lysimeter ½ (L)	Annual amount leachate in lysimeter 2/2 (L)
Year 1	905	177.0	207.8
Year 2	939	350.9	377.4
Year 3	891	263.7	228.4
Year 4	1085	527.8	526.3

Analytical methods:
Water analysis implies concentration of the leachate by rotary evaporation at <35°C and extraction of the precipitated material by acetonitrile/water. Samples were analysed for lenacil and IN-KF313 by reversed phase HPLC with gradient profile and detection at 266 nm. LOD = 0.05µg /L and LOQ = 0.075µg /L for for lenacil and IN-KF313. Procedural recoveries in the range 90-105%

The soil samples were extracted 3 times with methanol/water (90:10, v/v). Extracts were assayed for radioactivity by LSC. Due to the low amount of extractable radioactivity, no chromatography was performed on the soil extracts.

Findings:
Table B.8.2.4-3: Summary of first monitoring year

Lysimeter 1/1	**Mean conc in µg/L equiv a.s.**	**M1 (RT=3.08)**	**M2 (RT=3.52)**	**M3 (RT=8.16)**	**M4 (RT=9.46)**	**M5 (RT=14.08)**	**M6 (RT=4.28)**	**M7 (RT=11.56)**
Low [µg/L]		0.238	0.489	0.273	0.015	0.000	0.021	-
High [µg/L]	1.19	0.238	0.489	0.273	0.015	0.000	0.021	-
Lysimeter 1/2	**Mean conc in µg/L equiv a.s.**	**M1 (RT=3.08)**	**M2 (RT=3.52)**	**M3 (RT=8.16)**	**M4 (RT=9.46)**	**M5 (RT=14.08)**	**M6 (RT=4.28)**	**M7 (RT=11.56)**
Low [µg/L]		0.256	0.519	0.200	0.023	0.010	0.017	0.000
High [µg/L]	1.03	0.256	0.519	0.213	0.023	0.010	0.017	0.014

The lower value represents the average calculated by assuming all values below the LOQ to be 0.00 µg/L and the higher value is the average calculated assuming all values below the LOQ to be equivalent to the LOQ (0.075 µg/L)

Table B.8.2.4-4: Summary of second monitoring year

Lysimeter 1/1	Mean conc in µg/L equiv a.s.	M1 (RT=3.08)	M2 (RT=3.52)	M3 (RT=8.16)	M4 (RT=9.46)
low [µg/L]		0.160 (0.164) [1]	0.080	0.091	0.032
high [µg/L]	0.46	0.169 (0.173) [1]	0.088	0.104	0.077 (0.080) [1]
Lysimeter 1/2	**Mean conc in µg/L equiv a.s.**	**M1 (RT=3.08)**	**M2 (RT=3.52)**	**M3 (RT=8.16)**	**M4 (RT=9.46)**
low [µg/L]		0.106	0.082	0.033	0.035
high [µg/L]	0.38	0.128 (0.131) [1]	0.086	0.058	0.063

[1] values in parentheses based on a estimate of the maximum concentration for M1/M4

Table B.8.2.4-5: Distribution of radioactivity in the soil monoliths at study termination

Soil depth	Lysimeter 1/1		Lysimeter ½	
	µg/kg	% AR	µg/kg	% AR
0-10 cm	28.93	7.88	19.85	5.29
10-20 cm	16.51	4.55	19.99	4.93
20-30 cm	2.29	0.74	5.27	1.55
30-40 cm	ND	ND	ND	ND
40-50 cm	ND	ND	ND	ND
50-60 cm	ND	ND	ND	ND
60-70 cm	ND	ND	ND	ND
70-80 cm	ND	ND	ND	ND
80-90 cm	ND	ND	ND	ND
90-100 cm	ND	ND	ND	ND
>100 cm	ND	ND	ND	ND
Sum	-	13.17	-	11.76

Values measured at study termination. ND = Not detected (LOD = 1.52 µg/kg).

Neither [2-^{14}C]-lenacil nor IN-KF313 the primary soil metabolite observed in the lenacil soil degradation study (Berg, 1994), were detected in the lysimeter leachate (limit of detection, LOD, about 0.05 µg/L a.s. equivalents) at any time during the four-year study.

Some radioactive components were detected in the leachate (M1, M2, M3) but were of a generally polar nature and could not be conclusively identified despite mass spectrum analysis. MS analysis indicated that M1 would be a ring open structure with the loss of one nitrogen.
Further investigations involving reference material of the metabolite IN-KE121 revealed that this metabolite was also not present in the leachate.

The mean concentrations of total radioactivity in the leachate in a.s. equivalents during the first monitoring year (bi-weekly collection intervals) were 1.19 µg/L for

lysimeter 1/1 and 1.03 µg/L for lysimeter 1/2. The highest concentrations detected were 1.84 µg/L for lysimeter 1/1 and 1.73 µg/L for lysimeter 1/2.

During the second year of monitoring, the mean concentration was significantly lower in both lysimeter 1/1 (0.46 µg/L) and lysimeter 1/2 (0.38 µg/L). In these samples, no lenacil and IN-KF313 was detected (LOD about 0.05 µg/L a.s. equivalents). The mean concentrations were 0.13 and 0.12 µg/L in the third year in lysimeter 1/1 and 1/2, respectively and 0.05 µg/L in both lysimeters in the fourth monitoring year.

The radioactivity present in the leachate represented up to 7 non-identified components. Three of these components exceeded the trigger value of 0.10 µg/L a.s. equivalents in mean of the first year (with maximum 0.21 to 0.52 µg/L a.s. equivalents for individual components). Two peaks (M1 and M2) representing the majority of the radioactivity were characterized as polar material and a third peak (M3) of less polar material was further characterized by LC/MS. However, despite the efforts using LC/MS, it was not possible to identify the structure of the component. In the second year of monitoring, only the most polar component (M1) exceeded a mean value of 0.10 µg/L a.s. equivalents (0.17 µg/L). Selected samples containing the highest equivalent concentrations of the third year were analysed. Because the chromatographic pattern was similar in these samples compared to the samples from the second year and the concentration of radioactivity in the leachate samples became very low, the mean concentration of the polar components was estimated to be below 0.10 µg/L in the third year. No chromatographic analysis was performed on samples from the fourth year, since the mean equivalent concentration was only 0.05 µg/L in this monitoring period in both lysimeters.

The majority of radioactivity in the soil was located in the upper two layers (0-20 cm) with 4.55 to 7.88% of the applied radioactivity. A significantly lower portion was found in the third layer (20-30 cm, 0.74 to 1.55% of the applied). The equivalent concentrations ranged from 16.51 to 28.93 µg/kg in the upper two layers and from 2.29 to 5.27 µg/kg in the third layer. No radioactivity was detectable below a depth of 30 cm.
The total radioactivity recovered in the soil was 13.17% (lysimeter 1/1) to 11.76% (lysimeter 1/2) of the applied radioactivity. Extractable residues in the upper three soil layers represent in total 0.33% AR and 0.31%AR, respectively in lysimeters 1/2 and 2/2. These fractions could not be further investigated. The majority of radioactivity in the soil was found to be non-extractable (bound) residues (12.86%AR and 10.84%AR respectively in lysimeters 1/2 and 2/2.).

Conclusions:
Lenacil was applied as a split application to sugar beets grown on two lysimeters in spring 1995. The first application was performed on 5 June 1995 at a rate of 200 g a.s./ha (at growth stage 12-14) and the second application on 19 June 1995 (at growth stage 16 to 18) at a rate of 300 g a.s./ha.

Neither lenacil nor the main soil metabolite, IN-KF313, were detected in the leachate (LOD 0.050 µg/L as active ingredient equivalent) during four years of monitoring. An unknown component (M3) more polar than lenacil or IN-KF313 was isolated but could not be identified by LC/MS. This unknown component was not the metabolite

IN-KE121. Other polar components were also observed. MS analysis indicated that M1 would be a ring open structure with the loss of one nitrogen.
In the mean of the first year of monitoring, the two polar components (M1 and M2) and the less polar M3 exceeded 0.10 µg/L, while only the mean concentration of M1 (and M3 in one of the lysimeters with 0.104 µg/L) was found to be above 0.10 µg/L in the second year of monitoring. No individual component exceeded 0.10 µg/L in the third and fourth monitoring years.

The total radioactivity recovered in the soil was 13.17% (lysimeter 1/1) to 11.76% (lysimeter 1/2) of the applied radioactivity. Extractable residues in the upper three soil layers represent in total 0.33% AR and 0.31%AR, respectively in lysimeters 1/2 and 2/2. These fractions could not be further investigated. The majority of radioactivity in the soil was found to be non-extractable (bound) residues (12.86%AR and 10.84%AR respectively in lysimeters 1/2 and 2/2).

Position Paper: Unknown Degradation Products of Lenacil in Leachate Water from a Lysimeter Study
(Schnöder, F., 2004)

Position Paper

Title	Unknown Degradation Products of Lenacil in Leachate Water from a Lysimeter Study
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Page Number	1 of 9

AUTHOR'S STATEMENT

I, the undersigned, hereby declare that the this statement represents a faithful review of the literature and information provided on Lenacil and an estimate of the relevance of unknown metabolites in leachate water.

F. Schnöder — 19 Nov 2009

Dr. agr. F. Schnöder
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Date

INTRODUCTION

A lysimeter study was conducted at Covance Laboratories GmbH with ^{14}C-Lenacil (ref. 1) according to the BBA Guideline, Part IV, 4-3, 1990. Two applications were performed to sugar beet with 200 g/ha on 5 June 1995 (2-4 leaves) and with 300 g/ha on 19 June 1995 (6-8 leaves). The soil used in this study was a Gleyic Cambisol meeting the requirements of the BBA Guideline and represented a worst case scenario for the risk of parent or metabolites leaching through the soil cores of a depths of ca. 110 cm. The monitoring period was four years and two monoliths were included in this study.

The study revealed that neither Lenacil nor the two major metabolites IN-KF313 and IN-KE121 were present in the leachate water (LOQ 0.075 µg/L). Two polar components referred to as M1 and M2 as well as a medium polar component (M3) were the most significant compounds in the leachate and the annual average concentrations exceeded 0.1 µg/L in both lysimeters in the first monitoring year and reached maximum annual average concentrations of 0.256 µg/L (M1), 0.519 µg/L (M2) and 0.273 µg/L (M3). None of the two major soil metabolites IN-KF313 and IN-KE121, respectively, co-chromatographed with M1, M2 or M3. Exhaustive efforts to identify M3 by mass spectrometry were not successful and only tentative structures were proposed based on the limited mass spectrometry information obtained from the leachate sample.

The objective of this position paper is to compile the various information on the metabolism and give an estimate of the nature of polar components deriving from Lenacil based on the literature review (see references).

GENERAL METABOLIC PATHWAY OF LENACIL

The metabolic pathway of Lenacil in soil (aerobic condition) (Ref. 2 and 3) and information obtained in the lysimeter study (Ref. 1) were considered for the establishment of a proposed pathway.

The metabolites identified in soil were oxidation products containing an additional keto group in the parent molecule. The metabolite 5-oxo-Lenacil has the keto group in the 5-position of the cyclopentapyrimidine and is referred to as (IN-)KF313. The metabolite LN5 is a cyclohexanone and it is most likely that the keto group is in the *p*-position (this structure corresponds to the reference compound, IN-KE121, Ref. 7). 5-hydroxy-Lenacil was postulated as an intermediate to be further oxidized to 5-oxo-Lenacil. 5- and 7-hydroxy-Lenacil are reported to be formed in sugar beet (Ref. 5). The rat metabolism study (Ref. 4) indicates that mono-hydroxylation in the cyclopentapyrimidine and/or cyclohexanyl moiety occurs and also di-hydroxylation in the cyclopentapyrimidine. The metabolite IN-KD304 was identified in the rat and represents mono-hydroxylated Lenacil in the *p*-position of the cyclohexanyl. This supports the stepwise oxidation of the cyclohexanyl moiety in the *p*-position to form IN-KE121. In general, oxidative processes are the major degradation steps reported for Lenacil in the various matrices.

CONSIDERATIONS ON POTENTIAL NATURE OF POLAR COMPONENTS IN LEACHATE WATER

The applications in the lysimeter study (ref. 1) were performed on 5 and 19 June 1995 and therefore represent a scenario with soil temperatures typically around 20°C in the upper 10 cm of the soil. These conditions are similar to the incubation conditions in the laboratory studies. However, the major soil metabolites IN-KE121 and IN-KF313 were not detected in the leachate water towards the end of the first monitoring year when the leaching of radioactivity started. This is in line with the short half-lives in soil (DT_{50} values reported $\leq$ 16 days for IN-KF313 and $\leq$ 8 days for IN-KE121, respectively, ref. 3) and consequently they were not present in soil anymore four years after application (total extractable $\leq$ 0.62 µg/kg dry mass as a.i. equivalent.

The unknown components found in the leachate of the lysimeter study, M3 and even more the components M1 and M2, were shown to be more polar than IN-KE121 and IN-KF313 on a reversed phase HPLC system. M1 and M2 eluted within the first few minutes of the chromatography. For a conservative assessment, each area was reported as a single peak. However, the visual assessment of the chromatography

demonstrates, that each area is likely to consist of further individual components (compare Appendices 6 and 6 in Ref. 1). This is often observed for such very polar material due to the difficulty to chromatographically separate very polar components in chromatographic systems developed to cover a relatively wide polarity range. Efforts to identify the less polar component M3 by mass spectrometry were not successful and only tentative structures were proposed based on the limited mass spectrometry information obtained from the leachate sample (structures A-D in proposed theoretical degradation pathway). The structures would indicate that ring opening occurs, but the chemical structure of further degradation products is difficult to evaluate, because the only semi-qualitative information available about metabolism of Lenacil following ring opening steps refers to oxidation products with a cyclohexanone moiety (Ref. 2).

However, the formation of a significant proportion of the final mineralization product carbon dioxide was reported (48-61% AR after 120 days at 20°C; ref. 2 and 3) in the laboratory soil degradation studies using [4, 7a-^{14}C]Lenacil. The loss of radioactivity from the total lysimeter test system (only around 16% of the applied radioactivity (AR) recovered from the crops, leachate and soil after four years) also confirms that volatile radioactivity was formed under outdoor conditions. As no organic volatiles were observed in the laboratory soil studies, the vast majority of this volatile radioactivity is considered to be carbon dioxide. Because [2-^{14}C]Lenacil was used in the lysimeter study, it is obvious that the degradation mechanism of Lenacil involves liberation of CO_2 from positions 4, 7a and 2 indicating a complete breakdown of the cyclopentapyrimidine.

The fact that no significant amounts of ring-opening products were detected in the laboratory soil studies supports the argument that the ring opening is the rate-limiting step and further degradation occurs very rapidly. Regardless of the chemical structure, any intermediate would therefore have low concentrations and/or very short half life.

Assuming the formation of IN-KE121 and IN-KF313 as primary metabolites in the lysimeter soil, they would also have the potential to adsorb and/or bind to soil organic matter via their functional groups (e.g. keto-groups). Based on the assessment of the toxicological significance of the ketone metabolites (Ref. 5) it can be concluded that such an association to soil organic matter would not cause any major concerns.

This is supported by a greenhouse herbicide screening test with IN-KF313 (Ref. 6) on nine commercial crops and fourteen economically important weed species (monocots and dicots). The test was performed using pre-emergence and post-emergence application at two rates (1.0 and 0.2 kg ai/ha), while the maximum seasonal recommended use rate for Lenacil is 0.5 kg ai/ha. It is important to note that potential

environmental levels of the IN-KF313 metabolite would be below those evaluated in this test. It was concluded that IN-KF313 is not considered be significantly biologically nor herbicidally active, especially at anticipated environmental concentrations.

The incorporation of low molecular weight degradation products still carrying the radiolabel into soil organic matter is also possible. Particularly the chemical binding of radiolabelled structures containing only C, H, N and O atoms to soil organic matter can be considered as incorporation into natural products. Such components may be mobile in the soil monolith and represent the polar radioactivity found in the leachate water. Such fragments incorporated into natural products would be considered to be of no regulatory concern.

QUANTITATIVE CONSIDERATIONS FOR A REALISTIC FIELD SITUATION

The maximum annual average concentrations of the NIR components M1, M2 and M3 occurred in the first monitoring year with 0.256 µg/L (M1), 0.519 µg/L (M2) and 0.273 µg/L (M3). In the second monitoring year the annual average concentrations were 0.173 µg/L (M1), 0.088 µg/L (M2) and 0.104 µg/L (M3) and all three unknown metabolites were below 0.1 µg/L in the average of the third and fourth year. Due to the chemical nature of very polar components, M1 and M2 may consist of more than one compound. All of the individual NIR components are clearly below an annual average of 0.75 µg/L in any lysimeter and monitoring year.

These results were obtained using a sandy soil meeting the requirements of the BBA Guideline, Part IV, 4-3, 1990; with less than 4% clay in the top soil and around 1% clay in the subsoil (organic carbon 1.3% in topsoil and ≤ 0.2% in subsoil). This approach assumes a worst case scenario and does not reflect representative agricultural practice for sugar beet, because sugar beet are typically grown in Europe on soils with a much higher silt and/or clay content such as e.g. Orthic luvisols. Such soils would typically have a much higher adsorption potential (clay minerals) and, thus, generally a reduced risk for leaching.

CONCLUSION

The components M1, M2 and M3 detected in the leachate water are not identified radioactivity deriving from [2-^{14}C]Lenacil. It is likely that they are formed from Lenacil by oxidative degradation steps via the known environmental metabolites IN-KE121 and/or IN-KF313, but they were shown to be of more polar nature. IN-KE121 and/or IN-KF313 were reported not to have any structural features that would raise concerns due to differences compared to Lenacil (Ref. 5). Further degradation of IN-KE121 and IN-KF313 seems to occur rapidly ($DT_{50} \leq 16$ days) and the final mineralization product CO_2 is reported to be released from all three labelling positions in the parent molecule indicating a complete breakdown. Regardless of the chemical structure of intermediates, any exposure would probably be relatively short. Due to the chemistry of the molecule (C, H, N, and O atoms only), the adsorption, binding and/or incorporation to soil organic matter is likely and the resulting components with similar structures as natural products can be considered to be of no regulatory concern considering the loss of the active groups and/or reduced bioavailability. Under practical agricultural growing conditions for sugar beet, Lenacil would be used on heavier soils than the “worst case” soil used in the lysimeter study which would most likely reduce the leaching to ground water.

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3. Girkin, R; Lenacil aerobic rate of degradation in one soil type at 10°C and in four soil types at 20°C; ACD 042/023664; Huntingdon Life Sciences Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; issued 16 June 2003

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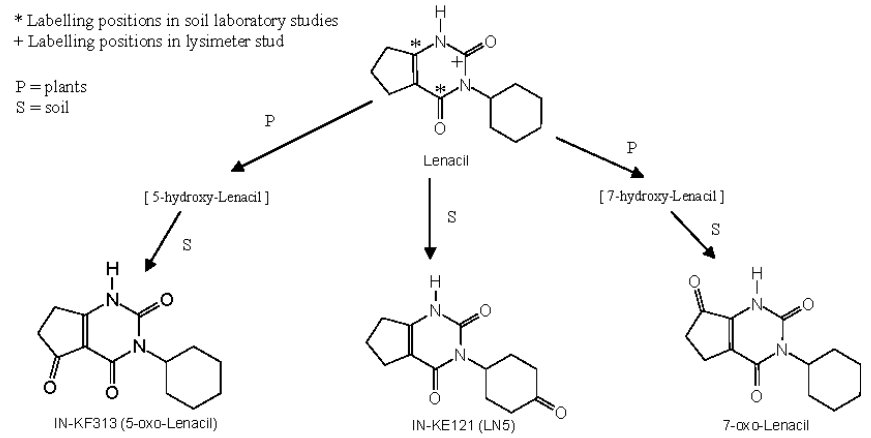
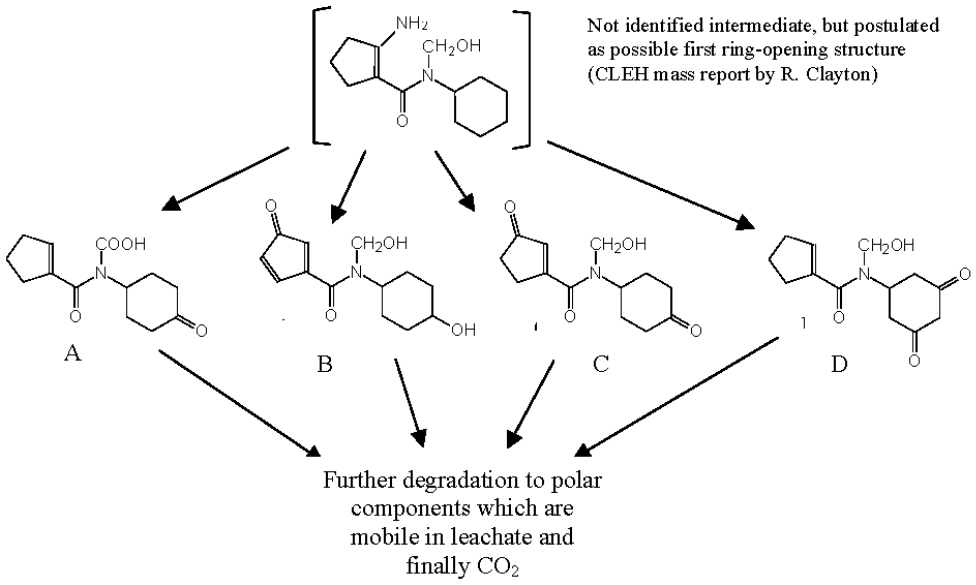
6. Iternal DuPont data base

7. DuPont internal communication, February 2003

Proposed degradation pathway of Lenacil:



Proposed theoretical further degradation pathway:



The possible identity and significance of the polar metabolites M1, M2 and M3 present in lysimeter leachate was discussed in a position paper.

Conclusions:
Although M1, M2 and M3 could not be positively identified, the available information suggests that these polar metabolites are likely to be fragments of the parent molecule resulting from opening of the cyclopentapyrimidine ring and/or low molecular weight fragments incorporated into natural products. Considering the intensive formation of CO2 from carbon in position 4 and 7a, as revealed by the soil metabolism study, it can be expected that these unknown metabolites are further degraded and mineralised.

B.8.3 Predicted environmental concentration in soil (PECs) (Annex IIIA 9.1.3)

Calculation of Predicted Environmental Concentrations of Lenacil and its Metabolites IN-KE121 and IN-KF313 in Soil, Groundwater, Surface Water and Sediment (Shaw, D., 2004)

Scenarios and input data:
Three schemes were considered for lenacil application:

(a) A single 500 g a.s./ha application of lenacil seven days after emergence;
(b) A 300 g a.s./ha application of lenacil seven days after emergence followed by a 200 g a.s./ha application seven days later;
(c) Four 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence.

Predicted environmental concentrations in soil were calculated using the simple model described in „Soil persistence models and EU registration, the final report of the work of the soil modelling work group of FOCUS' (February 1997).

All applications were assumed to have been made to sugar beet with an interception rate of 20% which represents the minimum interception likely under field conditions. This interception rate is recommended in „FOCUS groundwater scenarios in the EU review of active substances,' Report of the FOCUS Groundwater Scenarios Workgroup (Sanco/321/2000). Calculations assumed an even distribution to a depth of 5 cm with a soil bulk density of 1.5 g/cm^3.
Actual and time weighted average (TWA) concentrations were calculated using the worst case DT_{50} (non-linear first order value of **15 days**) from the European laboratory studies at 20°C normalised to pF2.
For the metabolites, IN-KF313 and IN-KE121, worst case normalised DT_{50} values of **15** and **7.7** days respectively were calculated by simultaneous regression of parent and metabolites using computer software WinNonlin 3.3. The application rate for metabolites was corrected for crop interception, kinetic fraction and molecular weight. The kinetic fractions for the conversion of lenacil to IN-KF313 and IN-KE121 were 0.36 and 0.46 respectively.

PEC_{soil} were calculated based on standard equations as follows.

PEC_{soil} (initial, single application) (mg/kg):

$$PEC_{soil,\ initial} = A \times \frac{(1 - f_{int})}{(100 \times depth \times bd)}$$

PEC_{soil} (at time t) (mg/kg):

PEC_{soil} (time-weighted average concentration) (mg/kg):

$$PEC_{soil,\ twa} = PEC_{soil,\ initial} \quad \frac{DT_{50}}{t \times Ln(2)} \left(1 - e^{(-t \times Ln(2)/DT_{50})}\right)$$

where:

A = application rate (g a.s./ha);
f_{int} = fraction intercepted by crop;
depth = mixing depth (5 cm);
bd = bulk density (1.5 g/cm^3);
DT_{50} = time (days) within which the initial concentration is reduced by 50%

Findings:

Table B.8.3-1: Predicted concentrations of lenacil, IN-KE121 and IN-KF313 in soil following a single application of lenacil at 500 g a.s./ha seven days after emergence

Time (days)	Lenacil		IN-KE121		IN-KF313	
	Actual (mg/kg)	TWA (mg/kg)	Actual (mg/kg)	TWA (mg/kg)	Actual (mg/kg)	TWA (mg/kg)
0	0.533	-	0.260	-	0.203	-
1	0.509	0.521	0.238	0.249	0.194	0.198
2	0.486	0.509	0.217	0.238	0.185	0.194
4	0.443	0.487	0.181	0.218	0.169	0.185
7	0.386	0.455	0.138	0.193	0.147	0.173
14	0.279	0.392	0.074	0.148	0.106	0.149
21	0.202	0.341	0.039	0.117	0.077	0.130
28	0.146	0.299	0.021	0.095	0.056	0.114
50	0.053	0.208	0.003	0.057	0.020	0.079
100	0.005	0.114	0.000	0.029	0.002	0.043

Times for the metabolites are from the day of maximum occurrence and not the day of application.

Table B.8.3-2: Predicted concentrations of lenacil, IN-KE121 and IN-KF313 in soil following application of lenacil at 300 g a.s./ha seven days after emergence and 200 g a.s./ha seven days later

Time (days)	Lenacil		IN-KE121		IN-KF313	
	Actual (mg/kg)	TWA (mg/kg)	Actual (mg/kg)	TWA (mg/kg)	Actual (mg/kg)	TWA (mg/kg)
0	0.445	-	0.187	-	0.170	-
1	0.425	0.435	0.171	0.179	0.162	0.166
2	0.406	0.425	0.156	0.171	0.155	0.162
4	0.370	0.406	0.130	0.157	0.141	0.155
7	0.322	0.380	0.100	0.139	0.123	0.145
14	0.233	0.328	0.053	0.106	0.089	0.125
21	0.169	0.285	0.028	0.084	0.064	0.109
28	0.122	0.250	0.015	0.068	0.047	0.095
50	0.044	0.173	0.002	0.041	0.017	0.066
100	0.004	0.095	0.000	0.021	0.002	0.036

Times for the metabolites are from the day of maximum occurrence and not the day of application.

Table B.8.3-3: Predicted concentrations of lenacil, IN-KE121 and IN-KF313 in soil following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence

Time (days)	Lenacil		IN-KE121		IN-KF313	
	Actual (mg/kg)	TWA (mg/kg)	Actual (mg/kg)	TWA (mg/kg)	Actual (mg/kg)	TWA (mg/kg)
0	0.350	-	0.128	-	0.134	-
1	0.334	0.342	0.117	0.122	0.128	0.131
2	0.319	0.334	0.107	0.117	0.122	0.128
4	0.291	0.320	0.089	0.107	0.111	0.122
7	0.253	0.299	0.068	0.095	0.097	0.114
14	0.183	0.258	0.036	0.073	0.070	0.099
21	0.133	0.224	0.019	0.057	0.051	0.086
28	0.096	0.196	0.010	0.047	0.037	0.075
50	0.035	0.136	0.001	0.028	0.013	0.052
100	0.003	0.075	0.000	0.014	0.001	0.029

Times for the metabolites are from the day of maximum occurrence and not the day of application.

Conclusions:
Predicted environmental concentrations in soil were greatest following a single application of lenacil at 500 g a.s./ha. Maximum concentrations were 0.533 mg/kg (lenacil), 0.260 mg/kg (IN-KE121) and 0.203 mg/kg (IN-KF313). Following two applications of lenacil (300 g a.s./ha then 200 g a.s./ha), maximum concentrations were 0.445 mg/kg (lenacil), 0.187 mg/kg (IN-KE121) and 0.170 mg/kg (IN-KF313). Following four 125 g a.s./ha applications of lenacil maximum concentrations were 0.350 mg/kg (lenacil), 0.128 mg/kg (IN-KE121) and 0.134 mg/kg (IN-KF313).

The PECsoil have not been recalculated . The risk assessment for terrestrial organisms exposed to the a.s. and metabolites has been based on the worst case initial PEC. Under those assumptions, an acceptable risk has been identified for all proposed uses.

B.8.4 Fate and behaviour in water (Annex IIA 7.2.1; Annex IIIA 9.2)

B.8.4.1 Hydrolysis rate of relevant metabolites, degradation and reaction products (Annex IIA 7.2.1.1)

^{14}C-Lenacil; Hydrolysis under Laboratory Conditions (Caldwell, E., 2002)

Guidelines:
EEC-Method C7
GLP:
Yes

Material and Methods:
Test substance:
[4,7a-$^{14}C_2$]-lenacil, specific activity: 10.494 Mbq/mg, batch number 0183157901, radiochemical purity: >97%.

Experimental design:
The hydrolysis of ^{14}C-lenacil was determined in buffered aqueous solutions at pH values of 4, 7 and 9 at a nominal concentration of 1 mg/litre. Solutions were incubated in darkness at 50°C under sterile conditions for up to 5 days. At each pH duplicate samples were taken for analysis at zero time and at 2 days and 5 days.

Analytical methods:
Total recoveries of radioactivity were measured and samples analysed by HPLC and TLC.

Findings:
Test conc. 1 mg/L, containing 1% v/v of acetonitrile (cosolvent); Incubation at 50°C for up to 5 days

pH 4 : No significant degradation (<< 10%) after 5 days
pH 7 : No significant degradation(<< 10%) after 5 days
pH 9 : No significant degradation (<< 10%) after 5 days

$\Rightarrow$ Lenacil is hydrolytically stable (DT_{50} at 25°C > 1 year)

Material balance: Total recoveries of applied radioactivity (AR) were in range 95.1 – 103.4%.
Conclusions:
This „preliminary test' at 50°C demonstrates that lenacil is hydrolytically stable within the pH range of 4 to 9. No further tests are required and the hydrolytical DT_{50} at 25°C can be estimated to be greater than 1 year.

B.8.4.2 Direct phototransformation of relevant metabolites, degradation and reactions products in water (Annex IIA 7.2.1.2)

Lenacil quantum yield of direct phototransformation (Millais A., 2002b)

Guidelines:
SETAC 1995.

GLP:
Yes

Material and Methods:
Test substance:
[4,7a-$^{14}C_2$]-lenacil, specific activity: 10.494 Mbq/mg, batch number 0183157901, radiochemical purity: >97%.

Experimental design:
The photolysis of ^{14}C-lenacil was determined in sterile buffered aqueous solutions at pH 5 at a nominal concentration of 1 mg/L. Solutions were maintained at 20°C and irradiated continuously with a xenon arc light for periods up to 7 days. The spectral distribution of the arc light was similar to that of natural sunlight and the intensitiy was such that 7 days irradiation was equivalent to 20.83 days irradiation with natural summer sunlight at a latitude of 40°N. Control samples were maintained in the dark over the same period. Samples were taken for analysis at intervals of 1, 3, 5, 6 and 7 days.

Analytical methods:
Radioactive content was determined by LSC and the amount of lenacil present was measured by HPLC. The quantum yield for lenacil was determined with reference to solutions of a chemical actinometer (p-nitroaceto phenone/pyridine). The lifetime of lenacil in the environment was calculated using the GCSOLAR programme.

Findings:

Table B.8.4.2-1: Recovery and distribution of radioactivity in irradiated and dark control pH5 buffer solutions treated with ^{14}C-lenacil

Incubation time (days)	Total recovery	Lenacil	Met A	Others	Polars
Irradiated					
1	95.8	93.4	n.d.	2.5	n.d.
3	98.5	93.6	2.5	2.2	0.2
5	96.5	83.8	1.5	1.9	9.3
6	101.5	94.1	4.8	2.7	n.d.
7	101.8	95.9	3.6	2.5	n.d.
Dark control					
0	101.5	99.3	n.d.	2.2	n.d.
1	92.5	90.8	n.d.	1.8	n.d.
3	98.8	97.1	n.d.	1.4	n.d.
5	92.2	89.0	n.d.	3.2	n.d.
6	100.3	98.2	n.d.	2.1	n.d.
7	101.1	99.2	n.d.	1.3	n.d.

Results are expressed as % AR and are the mean of duplicate determinations

The mean total recovery of radioactivity from irradiated samples was in the range 95.8 to 101.8% AR. Recoveries from the corresponding dark control samples were 92.2 to 101.5% AR. Degradation of lenacil in the irradiated samples was slow with a mean of 95.93% AR remaining after 7 days. No significant degradation products were formed. Degradation in the dark control samples was negligible. The quantum yield (ϕ) for lenacil in pH 5.0 aqueous buffer was 2.62×10^{-7}, a value which emphasises the stability of this compound to photodegradation in aqueous solution.

Conclusions:

The measured photolytic degradation of lenacil in aqueous buffer at pH5 was negligible. The lifetimes for the photodegradation in the environment (calculated using the GCSOLAR Program) indicate photolysis is unlikely to be a significant route of degradation of lenacil as the values of DT_{50} and DT_{90} are >1 year. The quantum yield (ϕ) for lenacil in pH 5.0 aqueous buffer was 2.62×10^{-7}.

B.8.4.3 Ready biodegradability of the active substance (Annex IIA 7.2.1.3.1)

Lenacil Technical: Assessment of Ready Biodegradability Modified Sturm Test (Barnes, S. P., 2001)

Guidelines:

EC Directive 92/69, C.4-C, „Determination of Ready Biodegradability, CO_2 Evolution Test' (formerly method C5 of EC Directive 84/449). OECD test guideline 301B,

„Ready Biodegradability, CO_2 Evolution Test. OPPTS Method 835.3110 (m), "Carbon Dioxide Evolution Test." (adopted January 1998).

GLP:
Yes

Material and Methods:
Test substance:
Lenacil technical: 98.6%, batch Number: 141712003.

Experimental design:
One day before test substance addition, air-saturated ultrapure water was added to each of six, five-litre amber glass culture bottles followed by the volumes of each of the stock solutions required to prepare three litres of mineral salts medium. Each culture bottle was then inoculated with activated sludge (30 mg Mixed Liquor Suspended Solids/L) obtained from a domestic sewage treatment plant. The bottles were sealed, stirred continuously and aerated overnight.

On Day 0 of the test, ultrasound-treated aqueous mixtures of lenacil technical were prepared in ultrapure water and added to appropriate culture bottles. The reference substance sodium benzoate was added as an aqueous stock solution (1.72 g/l) to one bottle containing the lenacil technical and to one containing inoculated mineral salts medium alone. The vessels contents were continuously flushed, through an air inlet tube reaching approximately 10 cm below the surface of the liquor, for 29 days with treated air at 30 – 100 ml/minute. An air outlet tube passed the air from each vessel to three Dreschel bottles, each containing 0.025N, nominal barium hydroxide (100 ml) connected in series.

Analytical methods:
The residual concentrations of barium hydroxide in the bottles nearest to the test vessels were determined at appropriate intervals by duplicate titration of 20 ml samples with hydrochloric acid (0.05N), using phenolphthalein indicator. On Day 28 of the test, titrations were undertaken and samples (approximately 100 ml) were removed from each test vessel for pH determination. Concentrated hydrochloric acid (1 ml) was then added to each vessel to drive off dissolved inorganic carbon. The contents of the vessels were aerated overnight and the final titrations were carried out on Day 29.

Findings:

Table B.8.4.3-1: Cumulative CO_2 and degradation as a percentage of TCO_2 in reference and test mixtures

Incubation time (days)	Sodium benzoate		Sodium benzoate plus lenacil		Lenacil
	CO_2 (mg)	TCO_2 (%)	CO_2 (mg)	TCO_2 (%)	TCO_2 (%)
1	6.3	6	6.1	5	0
3	35.2	32	33.8	31	0
5	61.1	55	59.1	54	0
7	77.8	71	74.8	68	0
8	85.0	77	-	-	1
11	89.7	81	-	-	1
14	92.1	84	-	-	1
20	97.4	88	-	-	2
28	102.0	93	-	-	2
29	103.1	94	-	-	3

Cumulative CO_2 production in the controls (78.7 and 83.6 mgCO_2) was within the acceptable range for this assay system (recommended maximum for a three litre culture = 120 mgCO_2). The degradation of sodium benzoate was rapid and had achieved 71% of its TCO_2 after 7 days and 94% after 29 days. Sodium benzoate degradation was also rapid in the presence of lenacil technical and had achieved 68% of its TCO_2 after 7 days indicating that lenacil was not inhibitory to biodegradation. Mean cumulative CO_2 production by mixtures containing lenacil technical was negligible and had achieved, at most, 2% of the theoretical value (TCO_2, 110.1 mgCO_2) by the end of the test on Day 29.

Conclusions:

Substances are considered to be readily biodegradable in this test if CO_2 production is equal to or greater than 60% of the theoretical value within ten days of the level achieving 10%. Lenacil technical cannot, therefore, be considered to be readily degradable.

B.8.4.4 Water/sediment study (Annex IIA 7.2.1.3.2)

Lenacil: Fate and behaviour in Water-sediment (Theis, M., 2002a)

Guidelines:

'Richtlinen für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren' part IV, 5-1, of the 'Biologische Bundesanstalt für Land- und Forstwirtschaft', Germany and 91/414/EWG.

GLP:

Yes

Material and Methods:

Test substance:

[4,7a-$^{14}C_2$]- lenacil, specific activity: 10.949 MBq/mg, batch: 0183162301, radiochemical purity: ≥98.5%.

Experimental design:

[4,7a-$^{14}C_2$]-labelled lenacil was used at a concentration of 0.167 mg/l water to investigate the behaviour of lenacil in a water/sediment system the water/sediment systems were reconstituted in 500 mL flasks wityh 1.5-2 cm sediment and about 6 cm heigh of water phase. Two independent water-sediment systems were used. The experiments were performed in duplicate for each time point at 20°C ± 2°C in the dark passively ventilated with CO_2-free air for 120 days.

Sampling of the systems was performed 0.5 - 2 m off-shore. About 10 L of water and 10 L of sediment (max. depth: 20 cm) were taken and stored well ventilated at 4 - 8 °C in the dark for 9 days. The test systems were taken from a pond near 'Schaephysen' (Germany) and from the 'Rückhaltebecken/ Selbeckerbach' at the 'Angertal' (Germany) differing in several properties (e.g. grain size distribution, total organic carbon and microbial biomass).

Table B.8.4.4-1: Water and sediment parameters

Parameters	System „Rückhaltebecken'	System „Schæphysen'
Collection site	Regenrückhaltebecken at Angerbach/Selbeckerbach, Nordhein-Westfalen, Germany	Pond near Schaephysen/hackstein, Nordhein-Westfalen, Germany
Temperature (water)	6.2 °C	5.5°C
pH		
- water (at sampling)	7.2	7.2
- water (at start of the test)	8.3	7.9-8.0
- sediment (at start of the test)	7.5-7.6	7.0-7.1
Oxygen content (water, at sampling)		
- Below the water surface	8.6 mg/L	9.5 mg/L
- Approximately 5 cm above sediment	8.5 mg/L	9.2 mg/L
Oxygen saturation in water at start of the test	93-94%	94-94%
Redox potential [Ag/AgCl]		
- water (at sampling)	260 mV	246 mV
- water (at start of the test)	285 mV	311-308 mV
- sediment (at start of the test)	-227 -97 mV	-193 -145 mV
Total organic carbon		
- sediment (at sampling)	1.2% dry weight	3.6% dry weight
- water (at sampling)	<1%	<1%
- sediment (at start of the test)	98-82 mg/L	110-100 mg/L
- water (at start of the test)	0.9-1.6% dry weight	3.9-3.7% dry weight
Total N		
- sediment	<0.01% dry weight	<0.01% dry weight

- water	6 mg/L	<5 mg/L
Total P - sediment - water	 658 mg/kg dry weight 0.04 mg/L	 321 mg/kg dry weight 0.05 mg/L
Ca	53.9 mg/L	121 mg/L
Mg	9.17 mg/L	11.9 mg/L
Total hardness (water)	1.7 mmol/L 9.7 °dH	3.5 mmol/L 19.7 °dH
Cation Exchange Capacity	220 µmol/g	158 µmol/g
Grain size distribution		
Sand - 2 - >0.63 mm - 0.63 - > 0.2 mm - 0.2 - >0.063 mm	 3.6% 1.7% 3.6%	 15.4% 50.4% 20.9%
Silt - 0.063 - >0.02 mm - 0.02 - >0.006 mm - 0.006 – 0.002 mm	 58.1% 18.8% 3.9%	 4.1% 3.9% 1.0%
Clay - <0.002 mm	 10.4%	 4.3%
Microbial biomass (mg BioC/ 100 g dry weight) - start of the test - end of test (125 days, incubation with a.s.) - end of test (125 days, control)	 11.3-10.4 15.3-17.7 20.2-14.3	 26.5-21.32 9.10-11.3 7.84-9.5

Analytical methods:
After 0, 3, 7/8, 14/15, 30, 58/59, 88/90 and 120/121 days of incubation, the water, methanol extracts of the sediment, $^{14}CO_2$ (NaoH trap) and other volatile [^{14}C]-labeled metabolites (paraffin oil/glass wool)and the bound residue (exhaustive soxhlet extraction with methanol) were analyzed by LSC.
Sediments were extracted twice with methanol. The characterization of the metabolites was performed by LC-MS analysis of methanol extracts. In addition, a UV-detector was used at a wavelength of 218 nm for the analysis of the unlabelled reference item 5-oxo-lenacil.

Findings:

Table B.8.4.4-2: Distribution of radioactivity to lenacil and metabolites [% of applied radioactivity] in the aquatic system 'Rückhaltebecken'

Day	**0**	**3**	**8**	**14**	**30**	**58**	**88**	**120**	**121 Sterile**
Water	94.7	84.5	76.9	65.9	58.2	52.8	48.7	43.3	51.9
Sediment[1]	4.5	15.2	21.2	33.3	40.5	44.9	47.5	50.9	48.1
Bound residue[2]	0.1	0.7	1.6	3.0	6.4	8.4	14.1	16.5	4.3
Carbon dioxide	-	0.0	0.0	0.1	0.3	1.1	1.6	3.8	0.0
Volatile components	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Recovery (mean)	**99.2**	**99.7**	**98.1**	**99.2**	**99.0**	**98.7**	**97.7**	**98.0**	**100.0**
Total recovery (mean)	98.9	98.9	98.9	98.9	98.9	98.9	98.9	98.9	98.9
Distribution in water fraction									
Lenacil	92.8	82.3	74.5	63.3	49.3	38.9	31.8	24.5	49.3
M 5.0	-	-	-	-	-	-	-	0.46	-
M 7.0	-	-	-	-	-	0.49	0.95	0.90	-
M 8.5	-	-	-	-	-	-	-	0.83	-
M 9.5	-	-		-	-	0.79	0.99	1.25	-
M 12.8	-	-	-	-	-	-	-	-	-
M 15.0	-	-	-	-	1.76	2.25	2.95	3.74	-
M 17.3	-	-	-	-	-	-	-	0.42	-
M 19.5					1.38	1.48	1.29	1.57	0.53
M 20.5 (IN-KF313)				0.64	2.86	6.13	7.65	7.84	0.47
Non classified activity	1.9	2.1	2.4	2.3	2.9	2.9	3.1	2.2	1.6
Distribution in methanol extracts of sediment (Extractable)									
Lenacil	3.56	12.99	17.67	27.45	29.98	30.64	26.00	25.23	41.31
M 5.0	-	-	-	-	-	-	-	-	-
M 7.0	-	-	-	-	-	-	-	0.54	-
M 8.5	-	-	-	-	-	-	-	-	-
M 9.5	-	-	-	-	-	-	-	-	-
M 12.8	-	-	-	-	-	-	-	-	-
M 15.0	-	-	-	-	-	0.68	0.89	1.50	-

Day	0	3	8	14	30	58	88	120	121 Sterile
M 17.3	-	-	-	-	-	-	-	-	-
M 19.5	-	-	-	-	-	-	-	-	-
M 20.5 (IN-KF313)	-	-	-	-	0.91	1.55	2.99	2.66	-
Non classified activity	0.8	1.6	1.9	2.8	3.6	3.7	3.5	4.4	2.4
Complete system (water fraction plus methanol extracts)									
Lenacil	96.4	95.3	92.2	90.7	79.3	69.6	57.8	49.8	90.7
M 5.0	-	-	-	-	-	-	-	0.46	-
M 7.0	-	-	-	-	-	0.45	0.95	1.45	-
M 8.5	-	-	-	-	-	-	-	0.83	-
M 9.5	-	-	-	-	-	0.79	0.99	1.25	-
M 12.8	-	-	-	-	-	-	-		-
M 15.0	-	-	-	-	1.76	2.93	3.84	5.24	-
M 17.3	-	-	-	-	-	-	-	0.42	-
M 19.5	-	-	-	-	1.38	1.48	1.29	1.57	0.53
M 20.5 (IN-KF313)	-	-	-	0.64	3.31	7.68	10.64	10.50	0.47
Non classified activity	2.8	3.7	4.2	5.1	6.5	6.6	6.6	6.6	4.0

[1]: calculated as sum from the values of the cold methanol extraction of the sediment and the 'bound residue'
[2]: calculated as difference from the values of the sediment and the methanol soxhlet [%]
- : not detected or not tested

Table B.8.4.4-3: Distribution of radioactivity to lenacil and metabolites [% of applied radioactivity] in the aquatic system 'Schaephysen'

Day	0	3	8	14	30	58	88	120	121 Sterile
Water	92.7	90.7	67.8	47.1	36.7	27.3	22.4	19.3	15.1
Sediment[1]	7.2	8.7	31.4	50.4	60.9	69.3	70.1	70.4	82.6
Bound residue[2]	0.1	0.2	0.8	1.6	3.3	7.4	10.0	10.6	3.8
Carbon dioxide	-	0.0	0.0	0.1	0.5	1.4	4.1	4.8	0.0
Volatile components	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Recovery (mean)	**99.9**	**99.5**	**99.2**	**97.6**	**98.2**	**97.9**	**96.7**	**94.6**	**97.7**
Total recovery (mean)	97.9	97.9	97.9	97.9	97.9	97.9	97.9	97.9	97.9
Distribution in water fraction									
Lenacil	90.61	88.59	65.76	40.93	26.79	13.75	8.05	5.52	13.20
M 5.0	-	-	-	-	-	-	0.45		-
M 7.0	-	-	-	-	0.41	0.77	0.89	0.82	-
M 8.5	-	-	-	-	-	0.43	1.24	0.74	-
M 9.5	-	-	-	-	0.46	-	-	1.30	-
M 12.8	-	-	-	-	-	-	-	-	-
M 15.0	-	-	-	1.45	1.97	2.51	2.30	1.76	-
M 17.3	-	-	-	-	0.50	-	-	-	-
M 19.5	-	-	-	0.68	0.74	1.07	0.82	0.65	-
M 20.5 (IN-KF313)	-		-	2.04	4.36	6.44	7.48	7.10	-
Non classified activity	2.1	2.1	2.0	2.0	1.9	2.5	1.9	2.1	1.9
Distribution in methanol extracts of sediment (Extractable)									
Lenacil	6.02	6.89	28.28	45.55	51.83	50.49	42.00	40.86	75.41
M 5.0	-	-	-	-	-	-	-	-	-
M 7.0	-	-	-	-	-	-	0.82	0.71	-
M 8.5	-	-	-	-	-	-	-	-	-
M 9.5	-	-	-	-	-	-	-	-	-
M 12.8	-	-	-	-	-	-	0.61	0.44	-
M 15.0	-	-	-	-	0.80	1.82	2.20	2.55	-
M 17.3	-	-	-	-	-	-	-	-	-

Day	0	3	8	14	30	58	88	120	121 Sterile
M 19.5	-	-	-	-	-	-	-	-	-
M 20.5 (IN-KF313)	-	-	-	-	1.25	5.04	9.78	10.65	-
Non classified activity	1.1	1.6	2.3	3.3	3.8	4.5	4.8	4.8	3.3
Complete system (water fraction plus methanol extracts)									
Lenacil	96.6	95.5	94.0	86.5	78.6	64.2	50.0	46.4	88.6
M 5.0	-	-	-	-	-	-	0.45	-	-
M 7.0	-	-	-	-	0.41	0.77	1.26	1.52	-
M 8.5	-	-	-	-	-	0.43	1.24	0.74	-
M 9.5	-	-	-	-	0.5	-	-	1.30	-
M 12.8	-	-	-	-	-	-	0.61	0.44	-
M 15.0	-	-	-	1.45	2.8	4.33	4.50	4.31	-
M 17.3	-	-	-	-	0.5	-	-	-	-
M 19.5	-	-	-	0.68	0.7	1.07	0.82	0.65	-
M 20.5 (IN-KF313)	-	-	-	2.04	5.61	11.47	17.26	17.75	-
Non classified activity	3.2	3.8	4.4	5.3	5.7	7.0	6.6	6.9	5.2

[1]: calculated as sum from the values of the cold methanol extraction of the sediment and the 'bound residue'
[2]: calculated as difference from the values of the sediment and the methanol soxhlet [%]
- : not detected or not tested

Table B.8.4.4-4: First order non-linear DT50 of lenacil (whole w/s system)

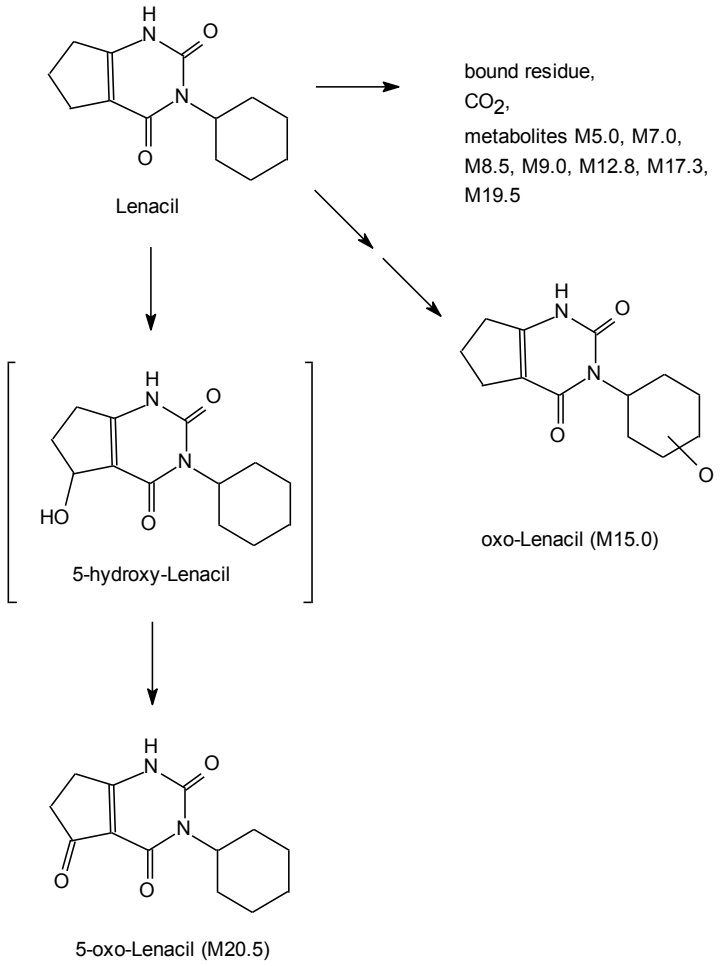
System	Rate constant (day^{-1})	Standard error	Value/error (t)	Degrees of freedom	P	Observed DT_{50} (days)	Observed DT_{90} (days)
Rückhaltebecken	0.00569302	0.000180159	31.6000	14	<0.001	122	405
Schaephysen	0.00674669	0.00357662	18.8633	14	<0.001	103	342

Conclusions:
In both sediment types there was movement of lenacil from the water to the sediment. Evolution of $^{14}CO_2$ was up to 3.8% AR in the Rűckhaltebecken system after 120 days. In the Schaephysen system the $^{14}CO_2$ was slightly greater at 4.8% AR after 120 days. The level of bound residue was 16.5% and 10.6%AR after 120 days, respectively in the Rűckhaltebecken system and the Schaephysen system .

Lenacil accounted for 49.8% AR and 46.4% AR in the whole system after 120 days, respectively in the Rűckhaltebecken system and in the Schaephysen system. In both systems there was only one significant metabolite which accounted for > 10% AR, M20.5 (5-oxo-lenacil, also known as IN-KF313). In the Rűckhaltebecken system IN-KF313 accounted for 10.5% AR after 120 days in the Schaephysen system IN-KF313 reached a maximum of 17.8% AR after 120 days.
The metabolite M15.0 was partially identified as oxo-lenacil.
Lenacil degradation was minimal in the sterile water/sediment systems.

The rate of degradation observed in this study was re-calculated in a modelling study by Shaw, D. (2004) using non-linear first-order regression performed by the ModelMaker programme. The result obtained gave lenacil whole system DT_{50} values of 122 days in the Ruckhaltebecken system and 103 days in the Schaephysen system. Corresponding DT_{90} values were 405 and 342 days. Insufficient data were available to calculate separate degradation rates for the water phase and sediment phase and for the major water sediment metabolite IN-KF313.

Figure B.8.4.4-1: Proposed degradation pathway of lenacil in water/sediment systems



B.8.4.5 Degradation in the saturated zone of active substance, metabolites, degradation and reaction products (Annex IIA 7.2.1.4)

In a lysimeter study, no lenacil and IN-KF313 were not detected in the leachate. Three non-identified components were found above 0.1 µg/L in leachate but consisted mainly of polar material.

A study of the degradation in the saturated zone is therefore not considered necessary.

B.8.5 Impact on water treatment procedures (Annex IIIA 9.2.2)

Assuming that Venzar 80 WP is used in accordance with Good Agricultural Practice and in compliance with label instructions, lenacil is not expected to enter used-water systems and exposure of biological waste-water treatment processes is unlikely to occur.

B.8.6 Predicted Environmental Concentrations in surface water and in groundwater (PECsw, PECgw) (Annex IIIA 9.2.1, 9.2.3)

B.8.6.1 Predicted environmental concentrations in ground water (PECgw) (Annex IIIA 9.21)

New PEC calculations with revised input data have been proposed in May 2009 (See Goodyear A., 2009).

Calculation of Predicted Environmental Concentrations of Lenacil and its Metabolites IN-KE121 and IN-KF313 in Soil, Groundwater, Surface Water and Sediment (Shaw, D., 2004)

Input data:
The main substance parameters used for PEC calculations in groundwater, surface water and sediment are summarised below:

Table B.8.6.1-1: Input data for PEC gw, sw and sediment calculations

Parameter	Lenacil	IN-KE121	IN-KF313
Molecular weight	234.3	248.3	248.3
Vapour pressure	2 x 10^{-7} Pa at 25°C *	1.51 x 10^{-7} Pa at 25°C (EPIWIN estimation)	1.51 x 10^{-7} Pa at 25°C (EPIWIN estimation)
Water solubility	6 mg/litre at 25°C *	1020 mg/litre at 20°C (Kane, 2004)	261.8 mg/litre at 25°C (EPIWIN estimation)
K_{foc} (K_{om})	83 (48)	38 (22)	557 (323)
1/n	0.89	0.94	0.89
DT_{50} (soil) (average)	9.9 days	4.6 days	11 days
DT_{50} (water)	1000 days	1000 days	1000 days
DT_{50} (sediment)	123 days	1000 days	1000 days
Kinetic fraction (molar)	-	0.46	0.36
Crop washoff factor	0.03 cm^{-1} (derived from the water solubility according to FOCUS guidance)	-	-
Plant uptake factor (f)	0.5 (default value of systemic herbicide)	0 (default)	0 (default)

* The difference between Henry's Law constant calculated using Pesticide Manual data (7.8 x 10^{-6}) and Henry's Law constant calculated using Phys Chem data presented in the dossier (1.3 x 10^{-7}) will not significantly affect the PEC values. Both values indicate little or no volatility and losses to air within the models will be negligible.

Models used
PRZM, FOCUS version 2.4.1
PEARL, FOCUS version 2.2.2

Groundwater scenarios
Nine scenarios have been defined for calculation of groundwater PEC values using the FOCUS models. All are defined for sugar beet. The dates of sugar beet growth events, defined within the scenarios, and the resulting actual application dates, are shown below

Table B.8.6.1-2: Dates of sugar beet growth events defined within the FOCUS groundwater scenarios

	Planting	**Emergence**	**Harvest**
Châteaudun	25 March	16 April	15 October
Hamburg	1 April	15 April	8 October
Jokioinen	10 May	25 May	15 October
Kremsmünster	1 April	15 April	10 October
Okehampton	10 April	25 April	25 October
Piacenza	1 March	20 March	15 September
Porto	28 February	15 March	1 August
Sevilla	31 October	10 November	1 July
Thiva	15 April	1 May	30 September

Table B.8.6.1-3: Dates of application in the simulations

	First application	**Second application**	**Third application**	**Fourth application**
Châteaudun	23 April	30 April	7 May	14 May
Hamburg	22 April	29 April	6 May	13 May
Jokioinen	1 June	8 June	15 June	22 June
Kremsmünster	22 April	29 April	6 May	13 May
Okehampton	2 May	9 May	16 May	23 May
Piacenza	27 March	3 April	10 April	17 April
Porto	22 March	29 March	5 April	12 April
Sevilla	17 November	24 November	1 December	8 December
Thiva	8 May	15 May	22 May	29 May

In accordance with FOCUS guidance, a 20% crop interception factor was used throughout and applied to three application schemes.

(a) A single 500 g a.s./ha application of lenacil seven days after emergence;
(b) A 300 g a.s./ha application of lenacil seven days after emergence followed by a 200 g a.s./ha application seven days later;
(c) Four 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence.

The results of the FOCUS groundwater modelling are shown in the following tables.

Table B.8.6.1-4: Predicted concentrations of lenacil, IN-KE121 and IN-KF313 in groundwater following a single application of lenacil at 500 g a.s./ha seven days after emergence (Results are expressed as µg/litre, and each represents the 80th percentile annual average concentration at 1 metre depth over a 20-year simulation period).

Scenario	Lenacil		IN-KE121		IN-KF313	
	PRZM	PEARL	PRZM	PEARL	PRZM	PEARL
Châteaudun	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hamburg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Jokioinen	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Kremsmünster	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Okehampton	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Piacenza	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
Porto	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sevilla	<0.001	<0.001	<0.001	0.005	<0.001	<0.001
Thiva	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table B.8.6.1-5: Predicted concentrations of lenacil, IN-KE121 and IN-KF313 in groundwater following application of lenacil at 300 g a.s./ha seven days after emergence and 200 g a.s./ha seven days later. Results are expressed as µg/litre, and each represents the 80th percentile annual average concentration at 1 metre depth over a 20-year simulation period.

Scenario	Lenacil		IN-KE121		IN-KF313	
	PRZM	PEARL	PRZM	PEARL	PRZM	PEARL
Châteaudun	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hamburg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Jokioinen	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Kremsmünster	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Okehampton	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Piacenza	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
Porto	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sevilla	<0.001	<0.001	<0.001	0.006	<0.001	<0.001
Thiva	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table B.8.6.1-6: Predicted concentrations of lenacil, IN-KE121 and IN-KF313 in groundwater following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence. Results are expressed as µg/litre, and each represents the 80th percentile annual average concentration at 1 metre depth over a 20-year simulation period.

Scenario	Lenacil		IN-KE121		IN-KF313	
	PRZM	PEARL	PRZM	PEARL	PRZM	PEARL
Châteaudun	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hamburg	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Jokioinen	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Kremsmünster	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Okehampton	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Piacenza	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
Porto	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sevilla	<0.001	<0.001	<0.001	0.006	<0.001	<0.001
Thiva	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Conclusions:

Predicted environmental concentrations in groundwater for lenacil, IN-KE121 and IN-KF313 were less than 0.1 µg/litre at all nine FOCUS scenarios defined for sugar beet. Similar concentrations were predicted following each application scheme. Concentrations predicted by PRZM (FOCUS version 2.4.1) for each substance were in all cases <0.001 µg/L. Concentrations predicted by PEARL (FOCUS version 2.2.2) for lenacil and IN-KF313 were in all cases <0.001 µg/L. Concentrations predicted by PEARL for IN-KE121 at the Piacenza and Sevilla scenarios were 0.002 – 0.003 and 0.005 – 0.006 µg/L, respectively (all other scenarios <0.001 µg/L). Overall, the results show that there is very little risk of groundwater contamination by lenacil and its two major soil metabolites IN-KE121 and IN-KF313, following post-emergent application of lenacil to sugar beets at 500 g a.s./ha.

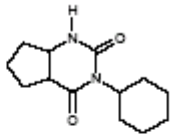
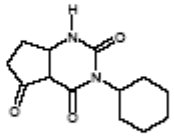
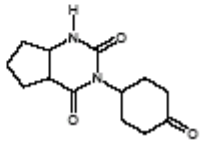
Modelling of the Leaching of Lenacil and its Soil Metabolites IN-KF313 and IN-KE121 to Groundwater Following the use of Lenacil on Sugar Beet (Goodyear A., 2009)

Groundwater modelling was conducted in support of a response to RMS comments on the Annex I dossier submission for lenacil. Specifically, the modelling in this report investigated the effect on predicted groundwater concentrations of lenacil and its metabolites IN-KF313 and IN-KE121, following revision of the soil degradation rate and soil adsorption input parameters for these conpounds.

Modelling information:

Compounds modelled

Table B.8.6.1-7: Structures of lenacil and its soil metabolites IN-KF313 and IN-KE121

Abbreviation	Chemical Name (IUPAC)	Structure
Lenacil	3-cyclohexyl-1,5,6,7-tetrahydrocyclopenta-pyrimidine–2,4(3*H*)-dione	
IN-KF313	3-cyclohexyl-6,7-dihydro-7-hydroxy-1H-cyclopentapyrimidine-2,4,4(3H)-trione	
IN-KE121	3-cyclohexyl-6,7-dihydro-7-1H-cyclopenta-pyrimidine-2,4,5(3H)-trione	

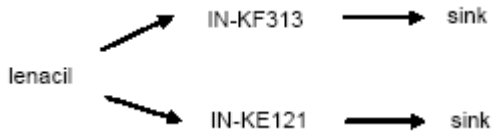
Model background

The groundwater modelling was carried out using PEARL version 3.3.3, which is a model recommended for use with the FOCUS scenarios to simulate leaching to groundwater. Full descriptions of the models are given in the report of the FOCUS Groundwater Scenarios Workgroup (FOCUS, 2000) and so are not repeated here. The models calculate the annual average pore water concentration at 1 m depth over a defined period, and the 80th percentile value is selected for regulatory decision making.

Model assumptions and scenarios

Application of lenacil to sugar beet was modelled using each of the nine FOCUS groundwater scenarios, with treatment made 7 days after emergence, at a rate of 500 g/ha. Application dates were selected automatically within the model according to the 7 days after emergence criteria. The application rate was corrected assuming a FOCUS crop interception value of 20% for early application. The correction was performed as a model input with the net treatment rate (400 g/ha) applied to the soil surface. A cycle of 20 successive seasons of use was modelled including an additional 6 seasons use as a warm-up period for the model.

Route of degradation
In soil, lenacil is degraded by ring oxidation, forming IN-KF313 and IN-KE121 as a major (>10% applied) metabolites. Each metabolite is then degraded to carbon dioxide and non-extractable residues providing a sink for IN-KF313 and IN-KE121. A modelling scheme was constructed according to this degradation profile as shown below.



The formation fractions for IN-KF313 (0.51) and IN-KE121 (0.43) were calculated in a position paper submitted to the RMS for lenacil.

Rate of degradation
The following degradation rates were used as model input values and were derived from an assessment of the available data presented in the position paper shown in Appendix 1.
The lenacil geometric mean DT50 of 14.4 days derived from studies conducted in five EU soil types was considered appropriate.
The IN-KE121 geometric mean DT50 of 7.4 days from a study conducted in four EU soils, was considered appropriate.
The IN-KF313 geometric mean DT50 of 11.2 days from studies conducted in five EU soil types was considered appropriate.

Soil adsorption
The following soil adsorption parameters were used as model input values and were derived from an assessment of the available data presented above.

The lenacil median Kom of 48 and the corresponding 1/n value of 0.88 were used based on the results available from seven soil types. The corresponding median study temperature of 20°C was used as a model input.
The IN-KE121 mean Kom of 22 and mean 1/n value of 0.95 were used based on the results available from three soil types. The study temperature was 23°C and is used as a model input.
As a realistic assumption based on a study in US soils, a 10th percentile value of 126 for Kom and 0.75 for 1/n were selected for IN-Kf313 as model inputs. The study temperature of 25°C was used as a model input. The worst-case Kom value of 46 and the corresponding 1/n value of 1.00 for IN-KF313 were also used for comparative purposes to demonstrate the very low leaching potential for this metabolite.

Chemical Inputs
The physico-chemical properties of lenacil, IN-KF313 and IN-KE121 for input into the model are shown in Table below.

The main changes of the input parameters for this new calculations are the following: DT50 of lenacil: 14.4 days, DT50 of IN-KE121: 7.4 days; K_{foc} of IN-KF313: 217
Very slight changes or the 1/n and kinetic fraction were also taken on board in the new calculations.

Table B.8.6.1-8: Chemical input parameters for FOCUS groundwater modelling

Parameter	**Lenacil**	**IN-KE121**	**IN-KF313**
Molecular weight	234.3	248.3	248.3
Vapour pressure	2 x 10^{-7} Pa at 25°C	1.51 x 10^{-7} Pa at 25°C	1.51 x 10^{-7} Pa at 25°C
Water solubility	6 mg/L at 25°C	1020 mg/L at 20°C	261.8 mg/L at 25°C
K_{foc} (K_{fom})	83 (48)	38 (22)	217 (126)
1/n	0.88	0.95	0.75
DT_{50} (soil)	14.4 days	7.4 days	11.2 days
Kinetic fraction	-	0.43	0.51
Crop was-off factor	0.03 cm^{-1}	-	-
Plant uptake	0.5	0	0

Other model inputs

Other model inputs for PEARL version 3.3.3 are shown in Table below.

Table B.8.6.1-9: Other model inputs for PEARL

Parameter	Value
Molar enthalpy of vaporisation (kJ/mol)	95
Molar enthalpy of dissolution (kJ/mol)	27
Option	Kom
Molar enthalpy of sorption (kJ/mol)	0
Reference conc. in liquid phase (mg/L)	1
Desorption rate coefficient (1/d)	0
Factor relating CofFreNeq and CofFreEql	0
Optimum moisture conditions (pF2/wetter)	Yes
Exponent for the effect of liquid	0.7
Molar activation energy (kJ/mol)	54 (equivalent to Q10 = 2.2)
Reference temperature for diffusion (ºC)	20
Reference diffusion coeff. in water (m2/d)	4.3 x 10-5
Reference diffusion coeff. in air (m2/d)	0.43
Canopy process option	Lumped
Half-life at crop surface (d)	1000000

Results and conclusions:

The predicted environmental concentrations in groundwater of lenacil and IN-KE121 were less than 0.1 µg/L at all nine FOCUS scenarios defined for sugar beet. The concentrations predicted by FOCUSPEARL for lenacil were in all cases <0.01 µg/L and the concentrations for IN-KE121 were no greater than 0.040 µg/L.
The groundwater concentrations predicted by FOCUSPEARL for IN-KF313 were no greater than 0.001 µg/L using a realistic worst-case Kfom value of 126 and a corresponding 1/n value of 0.75. Using the more extreme Kfom value of 46 and the corresponding 1/n value of 1.0, the concentrations predicted for IN-KF313 were in the range <0.001 to 0.036 µg/L for all FOCUS groundwater scenarios except Piacenza. In the Piacenza groundwater scenario, the predicted concentration was 0.105 µg/L.

The results for lenacil, IN-KF313 and IN-KE121 are summarised in Table below.

Table B.8.6.1-10: Predicted groundwater concentrations of lenacil, IN-KF313 and IN-KE121 following use of lenacil on sugar beet

Scenario	80th percentile annual average concentration at 1 m depth (20 year simulation)			
	Lenacil	IN-KE121	IN-KF313	IN-KF313
			Kfom = 126, 1/n = 0.75	Kfom = 46, 1/n = 1.00
Châteaudun	<0.001	0.012	<0.001	0.036
Hamburg	<0.001	0.012	<0.001	0.029
Jokioinen	<0.001	0.003	<0.001	0.008
Kremsmünster	<0.001	0.006	<0.001	0.016
Oakhampton	<0.001	0.010	<0.001	0.028
Piacenza	0.009	0.040	0.001	0.105
Porto	<0.001	<0.001	<0.001	<0.001
Sevilla	<0.001	0.037	<0.001	0.024
Thiva	<0.001	0.004	<0.001	0.014

Using the PEARL groundwater leaching model, normalised geometric mean degradation rates and realistic input values for Kfom, the 80th percentile annual average PECGW values at 1 m depth for lenacil, IN-KE121 and IN-KF313 were less than 0.1 µg/L for all the FOCUS groundwater scenarios relevant to sugar beet.
The modelling results indicate there to be no significant risk to groundwater from lenacil and its soil degradates following use on sugar beet.

B.8.6.2 Predicted environmental concentrations in surface water (PECsw) (Annex IIIA 9.2.3)

Calculation of Predicted Environmental Concentrations of Lenacil and its Metabolites IN-KE121 and IN-KF313 in Soil, Groundwater, Surface Water and Sediment (Shaw, D., 2004)

Input data
See table B.8.6.1-1

Models used
„SWASH' (Surface Water Scenarios Help), version 1.1, incorporating: MACRO, FOCUS version 4.4.2, PRZM, FOCUS surface water version 1.5.6 and TOXSWA, FOCUS surface water version 2.4.2.

Surface water scenarios
Surface water and sediment PECs were calculated at the Step 3 level of the FOCUS scheme. At this level, ten scenarios have been defined for calculation of surface water and sediment PECs. Four scenarios are defined for sugar beet. Two (D3 and D4) are drainage scenarios (inputs from spray drift and drainage) and two (R1 and R3) are runoff scenarios (inputs from spray drift and runoff). All three FOCUS Step 3 water bodies (ditch, pond and stream) are represented.

For the surface water and sediment assessments at Step 3, the actual days of application are calculated by the Pesticide Application Timing (PAT) calculator within the model software. Dates are selected that satisfy pre-set criteria, based on an automatically selected „average' rainfall file, together with the following user-defined information:

1. An application „window', defined by first and last possible days of application;
2. The number of applications;
3. For multiple applications, the minimum interval between applications.

The first possible day of application was set as seven days after emergence. Default dates for emergence for each crop are defined within the models. For the multiple application regimes, the minimum interval between applications was set as seven days. For a single application, the window must be of at least 30 days duration. For two and four applications, with a seven-day interval, the window must be of at least 37 and 51 days duration, respectively. The minimum duration was used in each case. The „windows' differed according to each scenario. The actual dates are shown below.

Table B.8.6.2-1: Actual application dates

Scenario	Actual application dates
D3	4 May, 14 May, 25 May, 21 June
D4	14 May, 21 May, 28 May, 18 June
R1	26 April, 3 May, 15 May, 31 May
R3	28 March, 4 April, 11 April, 22 April

Calculated FOCUS Step 3 PEC_{sw} and PEC_{sed} values are shown in the following tables.

Table B.8.6.2-2: PEC of lenacil in surface water following a single application at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	2.621	0.109	2.172	0.139	2.405	2.551
1	1.298	0.107	0.002	0.137	0.001	0.005
2	0.175	0.106	0.002	0.136	<0.001	0.001
4	0.006	0.104	0.002	0.134	<0.001	<0.001
7	0.001	0.101	0.002	0.131	0.057	<0.001
14	<0.001	0.096	0.002	0.123	0.001	<0.001
21	<0.001	0.090	0.007	0.116	<0.001	<0.001
28	<0.001	0.087	0.013	0.110	<0.001	0.001
42	<0.001	0.079	0.004	0.098	<0.001	<0.001
50	<0.001	0.075	0.004	0.092	<0.001	<0.001
100	<0.001	0.055	0.002	0.062	<0.001	<0.001

Table B.8.6.2-3: PEC of lenacil in surface water following a single application at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	2.065	0.108	0.142	0.138	0.948	2.120
2	1.346	0.107	0.072	0.137	0.474	1.143
4	0.695	0.106	0.037	0.136	0.237	0.573
7	0.399	0.104	0.022	0.134	0.136	0.328
14	0.200	0.101	0.013	0.131	0.109	0.164
21	0.133	0.099	0.011	0.127	0.075	0.109
28	0.100	0.096	0.010	0.124	0.068	0.110
42	0.067	0.092	0.010	0.117	0.046	0.073
50	0.056	0.089	0.009	0.114	0.039	0.062
100	0.028	0.077	0.006	0.102	0.019	0.031

Table B.8.6.2-4: PEC of IN-KE121 in surface water following a single application of lenacil at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	<0.001	0.007	0.024	0.009	0.368	0.607
1	<0.001	0.007	0.020	0.009	<0.001	0.159
2	<0.001	0.007	0.017	0.008	<0.001	0.001
4	<0.001	0.007	0.013	0.008	<0.001	<0.001
7	<0.001	0.007	0.008	0.008	0.009	<0.001
14	<0.001	0.007	0.004	0.008	<0.001	<0.001
21	<0.001	0.006	0.005	0.007	<0.001	<0.001
28	<0.001	0.006	0.004	0.007	<0.001	<0.001
42	<0.001	0.006	0.002	0.006	<0.001	<0.001
50	<0.001	0.006	0.002	0.006	<0.001	<0.001
100	<0.001	0.005	0.001	0.004	<0.001	<0.001

Table B.8.6.2-5: PEC of IN-KE121 in surface water following a single application of lenacil at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.007	0.022	0.009	0.145	0.526
2	<0.001	0.007	0.021	0.009	0.073	0.283
4	<0.001	0.007	0.019	0.008	0.036	0.142
7	<0.001	0.007	0.016	0.008	0.021	0.081
14	<0.001	0.007	0.011	0.008	0.017	0.040
21	<0.001	0.007	0.009	0.008	0.011	0.027
28	<0.001	0.007	0.008	0.008	0.009	0.020
42	<0.001	0.007	0.006	0.007	0.006	0.014
50	<0.001	0.006	0.006	0.007	0.005	0.011
100	<0.001	0.006	0.004	0.006	0.002	0.006

Table B.8.6.2-6: PEC of IN-KF313 in surface water following a single application of lenacil at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	<0.001	0.002	0.002	0.026	0.323	0.402
1	<0.001	0.002	0.002	0.026	<0.001	0.103
2	<0.001	0.002	0.002	0.025	<0.001	0.002
4	<0.001	0.002	0.002	0.025	0.018	0.001
7	<0.001	0.002	0.001	0.024	<0.001	<0.001
14	<0.001	0.002	0.001	0.022	0.009	<0.001
21	<0.001	0.002	0.001	0.021	<0.001	<0.001
28	<0.001	0.002	0.001	0.022	<0.001	<0.001
42	<0.001	0.002	<0.001	0.019	<0.001	<0.001
50	<0.001	0.002	0.001	0.018	<0.001	<0.001
100	Not calculable	0.001	<0.001	0.012	<0.001	<0.001

Table B.8.6.2-7: PEC of IN-KF313 in surface water following a single application of lenacil at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.002	0.002	0.026	0.167	0.348
2	<0.001	0.002	0.002	0.026	0.083	0.187
4	<0.001	0.002	0.002	0.025	0.042	0.094
7	<0.001	0.002	0.002	0.025	0.038	0.054
14	<0.001	0.002	0.001	0.024	0.025	0.027
21	<0.001	0.002	0.001	0.023	0.018	0.018
28	<0.001	0.002	0.001	0.023	0.014	0.015
42	<0.001	0.002	0.001	0.022	0.010	0.011
50	<0.001	0.002	0.001	0.021	0.009	0.009
100	<0.001	0.002	0.001	0.018	0.004	0.005

Table B.8.6.2-8: PEC of lenacil in surface water following two applications (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - Actual concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	1.365	0.086	1.124	0.131	2.643	3.567
1	0.674	0.085	0.002	0.129	0.001	0.941
2	0.091	0.085	0.002	0.128	<0.001	0.008
4	0.003	0.083	0.002	0.126	<0.001	0.002
7	0.001	0.081	0.765	0.124	0.063	0.001
14	0.002	0.076	0.002	0.116	0.001	<0.001
21	<0.001	0.074	0.009	0.110	<0.001	<0.001
28	<0.001	0.072	0.016	0.104	<0.001	<0.001
42	<0.001	0.065	0.005	0.092	<0.001	<0.001
50	<0.001	0.062	0.004	0.086	<0.001	<0.001
100	<0.001	0.045	0.002	0.059	<0.001	<0.001

Table B.8.6.2-9: PEC of lenacil in surface water following two applications (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) – TWA concentrations, µg/L

Time (days)	**Scenario/water body**					
	D3	**D4**	**D4**	**R1**	**R1**	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	1.074	0.086	0.075	0.130	1.042	3.087
2	0.700	0.085	0.038	0.129	0.521	1.664
4	0.362	0.085	0.022	0.128	0.261	0.834
7	0.207	0.084	0.020	0.127	0.162	0.477
14	0.173	0.081	0.016	0.123	0.120	0.239
21	0.115	0.079	0.013	0.120	0.085	0.174
28	0.087	0.078	0.011	0.117	0.070	0.145
42	0.058	0.075	0.011	0.110	0.048	0.097
50	0.049	0.073	0.010	0.107	0.040	0.082
100	0.024	0.064	0.006	0.093	0.020	0.041

Table B.8.6.2-10: PEC of IN-KE121 in surface water following two applications of lenacil (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - Actual concentrations, µg/L)

Time (days)	**Scenario/water body**					
	D3	**D4**	**D4**	**R1**	**R1**	**R3**
	Ditch	**Pond**	**Stream**	**Pond**	**Stream**	**Stream**
Maximum	<0.001	0.007	0.025	0.009	0.303	0.817
1	<0.001	0.007	0.021	0.009	<0.001	0.214
2	<0.001	0.007	0.018	0.009	<0.001	0.001
4	<0.001	0.007	0.014	0.008	<0.001	<0.001
7	<0.001	0.007	0.009	0.008	0.009	<0.001
14	<0.001	0.007	0.004	0.008	<0.001	<0.001
21	<0.001	0.007	0.006	0.007	<0.001	<0.001
28	<0.001	0.007	0.004	0.007	<0.001	<0.001
42	<0.001	0.006	0.002	0.006	<0.001	<0.001
50	<0.001	0.006	0.002	0.006	<0.001	<0.001
100	<0.001	0.005	0.001	0.004	<0.001	<0.001

Table B.8.6.2-11: PEC of IN-KE121 in surface water following two applications of lenacil (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - TWA concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.007	0.023	0.009	0.119	0.707
2	<0.001	0.007	0.022	0.009	0.060	0.380
4	<0.001	0.007	0.020	0.009	0.030	0.190
7	<0.001	0.007	0.017	0.008	0.017	0.109
14	<0.001	0.007	0.012	0.008	0.015	0.054
21	<0.001	0.007	0.009	0.008	0.010	0.036
28	<0.001	0.007	0.008	0.008	0.008	0.027
42	<0.001	0.007	0.007	0.007	0.005	0.018
50	<0.001	0.007	0.006	0.007	0.004	0.015
100	<0.001	0.006	0.004	0.006	0.002	0.008

Table B.8.6.2-12: PEC of IN-KF313 in surface water following two applications of lenacil (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - Actual concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	<0.001	0.002	0.002	0.023	0.278	0.431
1	<0.001	0.002	0.002	0.022	<0.001	0.111
2	<0.001	0.002	0.002	0.022	<0.001	0.003
4	<0.001	0.002	0.002	0.021	0.015	0.001
7	<0.001	0.002	0.001	0.021	<0.001	<0.001
14	<0.001	0.002	0.002	0.019	0.008	<0.001
21	<0.001	0.002	0.001	0.018	<0.001	<0.001
28	<0.001	0.002	0.001	0.019	<0.001	<0.001
42	<0.001	0.002	<0.001	0.016	<0.001	<0.001
50	<0.001	0.002	0.001	0.015	<0.001	<0.001
100	Not calculable	0.001	<0.001	0.010	<0.001	<0.001

Table B.8.6.2-13: PEC of IN-KF313 in surface water following two applications of lenacil (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - TWA concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.002	0.002	0.023	0.144	0.372
2	<0.001	0.002	0.002	0.022	0.072	0.201
4	<0.001	0.002	0.002	0.022	0.036	0.101
7	<0.001	0.002	0.002	0.022	0.033	0.058
14	<0.001	0.002	0.002	0.021	0.022	0.029
21	<0.001	0.002	0.002	0.020	0.015	0.019
28	<0.001	0.002	0.001	0.020	0.012	0.017
42	<0.001	0.002	0.001	0.019	0.008	0.012
50	<0.001	0.002	0.001	0.018	0.007	0.010
100	<0.001	0.002	0.001	0.016	0.004	0.005

Table B.8.6.2-14: PEC of lenacil in surface water following 4 x 125 g a.s./ha applications at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	0.441	0.065	0.375	0.284	4.892	5.217
1	0.255	0.064	0.002	0.282	0.005	1.376
2	0.050	0.064	0.002	0.279	0.001	0.012
4	0.002	0.063	0.002	0.275	0.090	0.003
7	<0.001	0.061	0.352	0.268	0.001	0.001
14	<0.001	0.058	0.006	0.253	0.529	<0.001
21	<0.001	0.055	0.010	0.240	<0.001	<0.001
28	0.198	0.053	0.354	0.227	<0.001	<0.001
42	<0.001	0.049	0.004	0.203	<0.001	<0.001
50	<0.001	0.046	0.003	0.191	<0.001	<0.001
100	<0.001	0.035	0.002	0.128	<0.001	<0.001

Table B.8.6.2-15: PEC of lenacil in surface water following 4 x 125 g a.s./ha applications at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	0.360	0.064	0.032	0.283	2.528	4.515
2	0.250	0.064	0.017	0.282	1.265	2.433
4	0.132	0.064	0.014	0.280	0.633	1.257
7	0.076	0.063	0.013	0.276	0.406	0.719
14	0.071	0.061	0.012	0.269	0.212	0.370
21	0.048	0.060	0.009	0.267	0.203	0.254
28	0.052	0.058	0.008	0.264	0.162	0.195
42	0.035	0.056	0.007	0.264	0.114	0.130
50	0.038	0.055	0.007	0.262	0.101	0.112
100	0.019	0.050	0.005	0.233	0.051	0.056

Table B.8.6.2-16: PEC of IN-KE121 in surface water following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	<0.001	0.006	0.016	0.033	0.465	0.935
1	<0.001	0.006	0.014	0.033	<0.001	0.246
2	<0.001	0.006	0.012	0.033	<0.001	0.001
4	<0.001	0.006	0.009	0.032	0.008	<0.001
7	<0.001	0.006	0.006	0.031	<0.001	<0.001
14	<0.001	0.006	0.003	0.030	0.038	<0.001
21	<0.001	0.006	0.004	0.028	<0.001	<0.001
28	<0.001	0.005	0.003	0.027	<0.001	<0.001
42	<0.001	0.005	0.002	0.024	<0.001	<0.001
50	<0.001	0.005	0.002	0.023	<0.001	<0.001
100	<0.001	0.004	0.001	0.015	<0.001	<0.001

Table B.8.6.2-17: PEC of IN-KE121 in surface water following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.006	0.016	0.033	0.240	0.810
2	<0.001	0.006	0.015	0.033	0.120	0.435
4	<0.001	0.006	0.013	0.033	0.060	0.218
7	<0.001	0.006	0.011	0.032	0.038	0.125
14	<0.001	0.006	0.008	0.031	0.020	0.062
21	<0.001	0.006	0.006	0.031	0.017	0.042
28	<0.001	0.006	0.006	0.030	0.014	0.032
42	<0.001	0.006	0.005	0.028	0.013	0.022
50	<0.001	0.006	0.004	0.028	0.011	0.018
100	<0.001	0.005	0.003	0.024	0.006	0.009

Table B.8.6.2-18: PEC of IN-KF313 in surface water following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	<0.001	0.002	0.002	0.023	0.216	0.350
1	<0.001	0.002	0.002	0.023	<0.001	0.090
2	<0.001	0.002	0.002	0.023	<0.001	0.002
4	<0.001	0.002	0.002	0.022	0.013	0.001
7	<0.001	0.002	0.001	0.021	<0.001	<0.001
14	<0.001	0.002	0.001	0.020	0.011	<0.001
21	<0.001	0.002	0.001	0.019	<0.001	<0.001
28	<0.001	0.002	0.001	0.018	<0.001	<0.001
42	<0.001	0.002	<0.001	0.016	<0.001	<0.001
50	<0.001	0.002	0.001	0.015	<0.001	<0.001
100	Not calculable	0.001	<0.001	0.010	<0.001	<0.001

Table B.8.6.2-19: PEC of IN-KF313 in surface water following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/L

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.002	0.002	0.023	0.112	0.303
2	<0.001	0.002	0.002	0.023	0.056	0.163
4	<0.001	0.002	0.002	0.023	0.028	0.082
7	<0.001	0.002	0.002	0.022	0.027	0.047
14	<0.001	0.002	0.001	0.022	0.018	0.024
21	<0.001	0.002	0.001	0.021	0.013	0.016
28	<0.001	0.002	0.001	0.020	0.010	0.016
42	<0.001	0.002	0.001	0.019	0.009	0.012
50	<0.001	0.002	0.001	0.019	0.008	0.010
100	<0.001	0.002	0.001	0.017	0.004	0.005

Table B.8.6.2-20: PEC of lenacil in sediment following a single application at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	0.575	0.220	0.093	0.292	0.408	0.660
1	0.430	0.220	0.034	0.292	0.171	0.374
2	0.315	0.220	0.027	0.292	0.129	0.282
4	0.225	0.220	0.022	0.292	0.097	0.213
7	0.173	0.219	0.019	0.292	0.086	0.169
14	0.123	0.218	0.016	0.290	0.149	0.125
21	0.100	0.217	0.017	0.287	0.088	0.103
28	0.085	0.217	0.028	0.283	0.070	0.090
42	0.066	0.215	0.024	0.275	0.052	0.072
50	0.059	0.215	0.023	0.272	0.046	0.064
100	0.033	0.210	0.016	0.245	0.026	0.036

Table B.8.6.2-21: PEC of lenacil in sediment following a single application at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	0.548	0.220	0.053	0.292	0.283	0.563
2	0.493	0.220	0.042	0.292	0.221	0.471
4	0.398	0.220	0.033	0.292	0.169	0.369
7	0.318	0.220	0.028	0.292	0.134	0.296
14	0.235	0.220	0.027	0.292	0.114	0.222
21	0.194	0.219	0.026	0.292	0.112	0.187
28	0.169	0.219	0.026	0.291	0.104	0.165
42	0.138	0.219	0.024	0.290	0.090	0.137
50	0.126	0.218	0.024	0.290	0.083	0.126
100	0.086	0.217	0.021	0.282	0.060	0.091

Table B.8.6.2-22: PEC of IN-KE121 in sediment following a single application of lenacil at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	<0.001	0.014	0.012	0.011	0.041	0.103
1	<0.001	0.014	0.012	0.011	0.014	0.052
2	<0.001	0.014	0.011	0.011	0.010	0.038
4	<0.001	0.014	0.011	0.011	0.007	0.028
7	<0.001	0.014	0.011	0.011	0.007	0.021
14	<0.001	0.014	0.009	0.011	0.012	0.015
21	Not calculable	0.014	0.010	0.011	0.007	0.013
28	Not calculable	0.014	0.009	0.011	0.006	0.011
42	Not calculable	0.014	0.008	0.011	0.004	0.009
50	Not calculable	0.014	0.008	0.010	0.004	0.008
100	Not calculable	0.014	0.006	0.009	0.002	0.005

Table B.8.6.2-23: PEC of IN-KE121 in sediment following a single application of lenacil at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/kg

Time (days)	**Scenario/water body**					
	D3	**D4**	**D4**	**R1**	**R1**	**R3**
	Ditch	**Pond**	**Stream**	**Pond**	**Stream**	**Stream**
1	<0.001	0.014	0.012	0.011	0.026	0.085
2	<0.001	0.014	0.012	0.011	0.020	0.069
4	<0.001	0.014	0.012	0.011	0.014	0.053
7	<0.001	0.014	0.011	0.011	0.011	0.041
14	<0.001	0.014	0.011	0.011	0.009	0.030
21	<0.001	0.014	0.010	0.011	0.009	0.025
28	<0.001	0.014	0.010	0.011	0.008	0.021
42	<0.001	0.014	0.010	0.011	0.007	0.018
50	<0.001	0.014	0.010	0.011	0.007	0.016
100	<0.001	0.014	0.008	0.011	0.005	0.011

Table B.8.6.2-24: PEC of IN-KF313 in sediment following a single application of lenacil at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/kg

Time (days)	**Scenario/water body**					
	D3	**D4**	**D4**	**R1**	**R1**	**R3**
	Ditch	**Pond**	**Stream**	**Pond**	**Stream**	**Stream**
Maximum	<0.001	0.023	0.009	0.163	0.131	0.241
1	<0.001	Not calculable	0.009	0.163	0.098	0.187
2	<0.001	Not calculable	0.009	0.163	0.080	0.151
4	<0.001	Not calculable	0.009	0.163	0.077	0.117
7	<0.001	Not calculable	0.009	0.163	0.094	0.094
14	<0.001	Not calculable	0.008	0.163	0.097	0.073
21	<0.001	Not calculable	0.008	0.162	0.068	0.063
28	<0.001	Not calculable	0.008	0.162	0.059	0.073
42	Not calculable	Not calculable	0.008	0.161	0.054	0.062
50	Not calculable	Not calculable	0.007	0.160	0.049	0.055
100	Not calculable	Not calculable	0.006	0.152	0.035	0.040

Table B.8.6.2-25: PEC of IN-KF313 in sediment following a single application of lenacil at 500 g a.s./ha seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.023	0.009	0.163	0.122	0.228
2	<0.001	0.022	0.009	0.163	0.115	0.206
4	<0.001	0.022	0.009	0.163	0.104	0.175
7	<0.001	0.022	0.009	0.163	0.098	0.147
14	<0.001	0.022	0.009	0.163	0.091	0.116
21	<0.001	0.022	0.009	0.163	0.088	0.100
28	<0.001	0.022	0.009	0.163	0.082	0.092
42	<0.001	0.022	0.008	0.163	0.075	0.085
50	<0.001	0.022	0.008	0.163	0.071	0.080
100	<0.001	0.021	0.008	0.161	0.056	0.064

Table B.8.6.2-26: PEC of lenacil in sediment following two applications (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - Actual concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	0.309	0.189	0.054	0.269	0.444	0.934
1	0.232	0.189	0.024	0.269	0.183	0.524
2	0.170	0.189	0.020	0.269	0.138	0.393
4	0.121	0.189	0.017	0.269	0.103	0.294
7	0.093	0.189	0.051	0.269	0.091	0.232
14	0.147	0.188	0.018	0.267	0.159	0.170
21	0.102	0.188	0.018	0.264	0.093	0.139
28	0.083	0.188	0.032	0.261	0.074	0.121
42	0.062	0.187	0.028	0.254	0.055	0.096
50	0.055	0.186	0.027	0.251	0.048	0.085
100	0.030	0.183	0.018	0.227	0.027	0.047

Table B.8.6.2-27: PEC of lenacil in sediment following two applications (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - TWA concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	0.295	0.189	0.035	0.269	0.307	0.795
2	0.265	0.189	0.033	0.269	0.239	0.663
4	0.218	0.189	0.032	0.269	0.181	0.517
7	0.185	0.189	0.032	0.269	0.143	0.413
14	0.168	0.189	0.032	0.269	0.121	0.308
21	0.154	0.189	0.031	0.269	0.119	0.257
28	0.139	0.189	0.030	0.269	0.110	0.226
42	0.117	0.188	0.028	0.268	0.095	0.187
50	0.108	0.188	0.027	0.267	0.088	0.172
100	0.074	0.187	0.024	0.260	0.062	0.118

Table B.8.6.2-28: PEC of IN-KE121 in sediment following two applications of lenacil (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - Actual concentrations, µg/kg

Time (days)	**Scenario/water body**					
	D3	**D4**	**D4**	**R1**	**R1**	**R3**
	Ditch	**Pond**	**Stream**	**Pond**	**Stream**	**Stream**
Maximum	<0.001	0.015	0.012	0.011	0.033	0.137
1	<0.001	0.015	0.012	0.011	0.012	0.069
2	<0.001	0.015	0.012	0.011	0.008	0.051
4	<0.001	0.015	0.012	0.011	0.006	0.037
7	<0.001	0.015	0.011	0.011	0.006	0.029
14	<0.001	0.015	0.010	0.011	0.012	0.021
21	Not calculable	0.015	0.011	0.011	0.006	0.017
28	Not calculable	0.015	0.010	0.011	0.005	0.015
42	Not calculable	0.015	0.009	0.011	0.004	0.012
50	Not calculable	0.015	0.008	0.011	0.003	0.011
100	Not calculable	0.015	0.007	0.009	0.002	0.006

Table B.8.6.2-29: PEC of IN-KE121 in sediment following two applications of lenacil (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - TWA concentrations, µg/kg

Time (days)	**Scenario/water body**					
	D3	**D4**	**D4**	**R1**	**R1**	**R3**
	Ditch	**Pond**	**Stream**	**Pond**	**Stream**	**Stream**
1	<0.001	0.015	0.012	0.011	0.021	0.113
2	<0.001	0.015	0.012	0.011	0.016	0.092
4	<0.001	0.015	0.012	0.011	0.012	0.070
7	<0.001	0.015	0.012	0.011	0.010	0.055
14	<0.001	0.015	0.011	0.011	0.008	0.040
21	<0.001	0.015	0.011	0.011	0.008	0.033
28	<0.001	0.015	0.011	0.011	0.007	0.029
42	<0.001	0.015	0.010	0.011	0.006	0.024
50	<0.001	0.015	0.010	0.011	0.006	0.022
100	<0.001	0.015	0.009	0.011	0.004	0.015

Table B.8.6.2-30: PEC of IN-KF313 in sediment following two applications of lenacil (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - Actual concentrations, µg/kg

Time (days)	**Scenario/water body**					
	D3	**D4**	**D4**	**R1**	**R1**	**R3**
	Ditch	**Pond**	**Stream**	**Pond**	**Stream**	**Stream**
Maximum	<0.001	0.025	0.010	0.142	0.113	0.261
1	<0.001	Not calculable	0.010	0.142	0.084	0.203
2	<0.001	Not calculable	0.010	0.142	0.068	0.164
4	<0.001	Not calculable	0.010	0.142	0.066	0.127
7	<0.001	Not calculable	0.010	0.142	0.081	0.103
14	<0.001	Not calculable	0.009	0.142	0.083	0.080
21	<0.001	Not calculable	0.009	0.141	0.059	0.070
28	<0.001	Not calculable	0.009	0.141	0.050	0.081
42	Not calculable	Not calculable	0.009	0.140	0.047	0.069
50	Not calculable	Not calculable	0.008	0.139	0.042	0.061
100	Not calculable	Not calculable	0.007	0.132	0.030	0.044

Table B.8.6.2-31: PEC of IN-KF313 in sediment following two applications of lenacil (300 g a.s./ha seven days after emergence followed by 200 g a.s./ha seven days later) (FOCUS Step 3) - TWA concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.025	0.010	0.142	0.105	0.246
2	<0.001	0.025	0.010	0.142	0.099	0.223
4	<0.001	0.025	0.010	0.142	0.089	0.189
7	<0.001	0.025	0.010	0.142	0.084	0.159
14	<0.001	0.025	0.010	0.142	0.079	0.126
21	<0.001	0.025	0.010	0.142	0.075	0.109
28	<0.001	0.025	0.010	0.142	0.070	0.101
42	<0.001	0.025	0.010	0.142	0.064	0.093
50	<0.001	0.025	0.009	0.142	0.061	0.088
100	<0.001	0.024	0.009	0.140	0.048	0.070

Table B.8.6.2-32: PEC of lenacil in sediment following four x 125 g a.s./ha applications at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	0.154	0.158	0.033	0.632	0.927	1.353
1	0.128	0.158	0.026	0.632	0.411	0.758
2	0.105	0.158	0.025	0.632	0.310	0.623
4	0.083	0.158	0.023	0.632	0.279	0.442
7	0.069	0.158	0.022	0.631	0.224	0.346
14	0.054	0.158	0.021	0.628	0.637	0.251
21	0.045	0.158	0.019	0.623	0.204	0.204
28	0.110	0.158	0.018	0.619	0.162	0.187
42	0.051	0.157	0.017	0.608	0.151	0.145
50	0.044	0.157	0.016	0.601	0.128	0.128
100	0.023	0.148	0.013	0.554	0.068	0.070

Table B.8.6.2-33: PEC of lenacil in sediment following four x 125 g a.s./ha applications at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	0.150	0.158	0.028	0.632	0.688	1.148
2	0.140	0.158	0.027	0.632	0.542	0.957
4	0.122	0.158	0.025	0.632	0.412	0.761
7	0.104	0.158	0.025	0.632	0.343	0.611
14	0.083	0.158	0.024	0.632	0.269	0.456
21	0.081	0.158	0.023	0.631	0.277	0.381
28	0.075	0.158	0.023	0.631	0.253	0.336
42	0.071	0.158	0.022	0.628	0.229	0.280
50	0.070	0.158	0.021	0.627	0.215	0.257
100	0.056	0.158	0.018	0.614	0.154	0.176

Table B.8.6.2-34: PEC of IN-KE121 in sediment following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	<0.001	0.014	0.010	0.046	0.060	0.158
1	<0.001	0.014	0.009	0.046	0.024	0.080
2	<0.001	0.014	0.009	0.046	0.018	0.059
4	<0.001	0.014	0.009	0.046	0.016	0.044
7	<0.001	0.014	0.009	0.046	0.013	0.034
14	Not calculable	0.014	0.008	0.046	0.033	0.024
21	Not calculable	0.014	0.009	0.045	0.011	0.020
28	Not calculable	0.014	0.008	0.045	0.009	0.018
42	Not calculable	0.014	0.007	0.044	0.012	0.014
50	Not calculable	0.013	0.007	0.043	0.010	0.013
100	Not calculable	Not calculable	0.006	0.037	0.005	0.008

Table B.8.6.2-35: PEC of IN-KE121 in sediment following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.014	0.010	0.046	0.043	0.130
2	<0.001	0.014	0.010	0.046	0.033	0.107
4	<0.001	0.014	0.009	0.046	0.025	0.082
7	<0.001	0.014	0.009	0.046	0.020	0.064
14	<0.001	0.014	0.009	0.046	0.016	0.047
21	<0.001	0.014	0.009	0.046	0.015	0.038
28	<0.001	0.014	0.009	0.046	0.014	0.034
42	<0.001	0.014	0.008	0.046	0.014	0.028
50	<0.001	0.014	0.008	0.046	0.014	0.026
100	<0.001	0.014	0.007	0.045	0.011	0.018

Table B.8.6.2-36: PEC of IN-KF313 in sediment following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - Actual concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
Maximum	<0.001	0.023	0.009	0.155	0.125	0.244
1	<0.001	Not calculable	0.009	0.155	0.107	0.196
2	<0.001	Not calculable	0.009	0.155	0.097	0.164
4	<0.001	Not calculable	0.009	0.155	0.085	0.133
7	<0.001	Not calculable	0.009	0.155	0.077	0.112
14	<0.001	Not calculable	0.008	0.155	0.067	0.092
21	<0.001	Not calculable	0.008	0.155	0.062	0.082
28	<0.001	Not calculable	0.008	0.154	0.061	0.110
42	Not calculable	Not calculable	0.008	0.153	0.056	0.095
50	Not calculable	Not calculable	0.007	0.152	0.052	0.084
100	Not calculable	Not calculable	0.006	0.144	0.040	0.060

Table B.8.6.2-37: PEC of IN-KF313 in sediment following 4 x 125 g a.s./ha applications of lenacil at seven-day intervals, starting seven days after emergence (FOCUS Step 3) - TWA concentrations, µg/kg

Time (days)	Scenario/water body					
	D3	D4	D4	R1	R1	R3
	Ditch	Pond	Stream	Pond	Stream	Stream
1	<0.001	0.023	0.009	0.155	0.119	0.232
2	<0.001	0.023	0.009	0.155	0.113	0.213
4	<0.001	0.023	0.009	0.155	0.103	0.184
7	<0.001	0.023	0.009	0.155	0.094	0.159
14	<0.001	0.023	0.009	0.155	0.083	0.131
21	<0.001	0.023	0.009	0.155	0.077	0.116
28	<0.001	0.023	0.009	0.155	0.075	0.112
42	<0.001	0.023	0.008	0.155	0.074	0.110
50	<0.001	0.023	0.008	0.155	0.073	0.107
100	<0.001	0.022	0.008	0.154	0.064	0.089

Conclusions:
For lenacil, all PEC_{sw} were below 3 µg/L following a single 500 g a.s./ha application (maximum: 2.621 µg/L). Progressing to two to four applications resulted in lower concentrations in the drainage scenarios but generally higher concentrations in the runoff scenarios, especially in streams. Thus, after a 300 / 200 g a.s./ha split application, PEC_{sw} in the R1 and R3 streams were 2.643 and 3.567 µg/L, respectively, and after four 125 g a.s./ha applications, PEC_{sw} in the R1 and R3 streams were 4.892 and 5.217 µg/L, respectively.

Metabolite PEC values were considerably lower than those for lenacil. The application scheme had a less marked effect on predicted concentrations, although differences were still apparent. Highest concentrations of IN-KE121 (in water and sediment) were found in the streams associated with the R1 and R3 scenarios, and these values generally increased from one to two to four applications. Thus, the maximum PEC_{sw} of IN-KE121 was 0.607 µg/L after a single 500 g a.s./ha application of lenacil, 0.817 µg/L after a 300 / 200 g a.s./ha split application and 0.935 µg/l after four 125 g a.s./ha applications. PEC_{sw} in the other water bodies were in all cases below 0.04 µg/L.

Highest concentrations of IN-KF313 in water were also found in the R1 and R3 streams, although the highest PEC_{sw} (0.431 µg/L) occurred following a 300/200 g a.s./ha split application (0.402 µg/L following a single 500 g a.s./ha application and 0.350 µg/L following four 125 g a.s./ha applications). PEC_{sw} in the other water bodies were in all cases below 0.03 µg/L.

PEC_{sed} values were calculated using the computer program „Step 3 in FOCUS', as described for the calculation of concentrations in surface water (PEC_{sed} values were calculated concurrently with PEC_{sw} values by the program.)

For lenacil, all PEC_{sed} values were below 0.7 µg/kg following a single 500 g a.s./ha application of Venzar 80 WP (maximum: 0.660 µg/kg). Multiple applications resulted in lower concentrations in the drainage scenarios but generally higher concentrations in the runoff scenarios, especially in streams. Therefore, after a 300/200 g a.s./ha split application, PEC_{sed} in the R1 and R3 streams were 0.444 and 0.934 µg/kg, respectively, and after 4 x 125 g a.s./ha applications, PEC_{sed} in the R1 and R3 streams were 0.927 and 1.353 µg/kg, respectively. Metabolite PEC_{sed} values were considerably lower than those for lenacil. The maximum PEC_{sed} for IN-KE121 was 0.103 to 0.158 µg/kg and the maximum PEC_{sed} for IN-KF313 was 0.241 to 0.261 µg/kg. These maximum levels were observed in the R3 stream water body in each case.

The PECsw have not been recalculated with new input data.
- the PECsw for the a.s. that have been calculated with slightly too favourable DT50 indicate that several scenarios are acceptable without particular mitigation measures (acceptable TER based on FOCUS step 3 calculations). It is therefore reasonable to consider that the risk evaluation at national level with the new input data will indicate an acceptable risk without mitigations measures or with limited mitigations measures.
- An acceptable risk with very large margins of safety has been identified for metabolites IN-KE121 and IN-KF313

B.8.7 Monitoring data

A selective review of published literature on pesticide monitoring in surface waters was carried out. Martinez, R.C. et al (2000) analysed surface water and groundwater samples in 1998 from the Guarena and Almar river basins in Spain. No lenacil was found (detection limit <0.025 µg/L) in the 18 surface water and 23 groundwater samples analysed.

Beernaerts, S. et al (2003) carried out a 2 year (1998-1999) monitoring study of the Dyle river in Belgium which is representative of a large part of the country. River water samples were taken each month from 8 sites. Peak concentrations of lenacil were less than 2 µg/L immediately after application and declined to undetectable within the next few sampling occasions. The peak values may have been caused by point source contamination associated with the applications.

In summary, a water-monitoring programme in Spain reported that no lenacil was found in agricultural catchment areas while in Belgium transient lenacil residues were found in river water samples only at the time of application indicating point sources of contamination.

Conclusions:
Monitoring results are difficult to interpret because the pesticide use pattern, the pesticide use history, the climatic conditions are not known. These data are given as additional information.

B.8.8 Fate and behaviour in air (Annex IIA, 7.2.2; Annex IIIA 9.3)

Lenacil puregrade: Physico chemical properties (Comb A., 2002)

The low vapour pressure of 1.7×10^{-9} Pascals at 25°C indicates little potential for volatilisation of the active substance and thus it would not be expected to be found in any significant concentration in the air. The Henry's law constant ($H = 1.3 \times 10^{-7}$ $Pa.m^3.mol^{-1}$) calculated from the water solubility value of 3 mg/L and vapour pressure 1.7×10^{-9} Pa at 25 °C indicates that Lenacil is very slightly volatile from water.
The potential persistence of the compound in air has been calculated according to the models developed by Atkinson which estimate the atmospheric oxidative DT_{50} is 2.8 hours.
Therefore lenacil is not expected to be found in the atmosphere.

B.8.9 Summary of behaviour in air and predicted environmental concentrations in air (PECa)
(Annex IIIA 9.3)

Data not submitted. Lenacil is not expected to be found in the atmosphere.

B.8.10 Definition of the residue (Annex IIA 7.3)

Soil

The main degradation pathways in soil involved oxidation of the cyclopentapyrimidine moiety to IN-KF313 (3-cyclohexyl-6,7-dihydro-7-1H-cyclopentapyrimidine-2,4,5(3H)-trione) and oxidation of the cyclohexane moiety to IN-KE121 followed by oxidation of both degradates to carbon dioxide. Both metabolites were formed under aerobic conditions at levels >10%AR.

The soil metabolites IN-KF313 and IN-KE121 are not more toxic to earthworms than the a.s. The ecotoxicological assessment shows that the risk to soils organisms is acceptable. Therefore the metabolites can be excluded from the definition of the residue for monitoring in soil.

Residue definition in soil for risk assessment: lenacil, IN-KF313 and IN-KE121.
Residue definition in soil for monitoring: lenacil

Ground Water

The leaching risk assessment has shown that there is negligible potential for lenacil, IN-KF313 and IN-KE121 to appear in groundwater at level above 0.1 µg/L. Accordingly only lenacil is proposed to be included in the definition of the residue for monitoring in groundwater.

Residue definition in ground water for risk assessment: lenacil, IN-KF313 and IN-KE121.
Residue definition in ground water for monitoring: lenacil

Surface Water and Sediment

No major metabolites have been detected in aqueous photolysis or hydrolysis studies. In a water sediment study, using lenacil, IN-KF313 was the only major metabolite detected reaching a maximum of 17.8% in the total system (water compartment maximum 7.8%). Metabolite IN-KE121 has not been detected at significant level in the aqueous photolysis, hydrolysis or w/s studies.
Metabolites IN-KF313 and IN-KE121 are major metabolites in the soil aerobic studies.
Based upon the above information, lenacil IN-KE121 and IN-KF313 should be defined as the relevant residue in water for risk assessment.

The metabolites IN-KE121 and IN-KF313 are less toxic than the a.s. to a sensitive organism like *Pseudokirchneriella subcapitata* (72-hour E_rC_{50} IN-KE121 : 27800 µg a.s./L; 72-hour E_rC_{50} IN-KF313 : 4270 µg a.s./L; 72-hour E_rC_{50} a.s. : 16 µg a.s./L). The metabolites can be excluded from the definition of the residue for monitoring in water.

Residue definition in surface water for risk assessment: lenacil, metabolite IN-KF313 and IN-KE121
Residue definition in surface water for monitoring: lenacil

Air

Residue definition in air for risk assessment: lenacil
Residue definition in air for monitoring: lenacil

B.8.11 References relied on

Dossier file No.	Author(s)	Year	Title Source Company report no. GLP / GEP status Published	Data Protection	Owner
IIA, 7.1.1.1.1/01	Theis, M.	2003	Lenacil –Fate and behaviour in soil, A&M Labor GmbH, A&M00-077. GLP, Unpublished	Yes	Schirm/ DuPont
IIA, 7.1.1.1.2.2/ 01	Millais, A.J.	2002 a	Lenacil; Photodegradation on Soil, Huntingdon Life Sciences Ltd., ACD 041/023429. GLP, Unpublished	Yes	Schirm/ DuPont
IIA, 7.1.1.2.1.1/ 02 IIA, 7.1.1.2.1.2	Girkin, R.	2003	Lenacil Aerobic Rate of Degradation in one Soil Type at 10°C and in four Soils at 20°C, Huntingdon Life Sciences Ltd, ACD 042/023664. GLP, Unpublished	Yes	Schirm/ DuPont
IIA, 7.1.1.2.2/01 IIA, 7.1.1.2.3/01	Pollmann, B.	2003	Venzar 80 % WP (containing 80% Lenacil) Related Soil Dissipation on Bare Soil, four Sites in Europe, 2001, GAB, 20011048/E1-FSD. GLP, Unpublished	Yes	Schirm/ DuPont
IIA, 7.1.2/01	Sheftic, G. D., Priester, T. M.	1992	Batch equilibrium (adsorption/desorption) study with [2-^{14}C] Lenacil, E.I. DuPont de Nemours, AMR 2332-92. GLP, Unpublished	No	Schirm/ DuPont
IIA, 7.1.2 /02	Girkin, R.	2002 a	Lenacil; Adsorption/Desorption on Soil, Huntingdon Life Sciences Ltd., ACD 044/022152. GLP, Unpublished	Yes	Schirm/ DuPont
IIA, 7.1.2./03	Berg, D. S.	1996 c	Batch equilibrium (adsorption/desorption) study with IN-K 313, E.I. DuPont de Nemours AMR2948-94. GLP, Unpublished	No	Schirm/ DuPont
IIA, 7.1.2/04	Kane, T.	2004	IN-KE 121, Adsorption / Desorption on soil, Huntingdon Life Sciences Ltd, ACD 063/042264. GLP, Unpublished	Yes	Schirm/ DuPont
IIA 7.1.3.3/01	Schnöder, F.	2004	Lysimeter Study with (14C)- Lenacil Revised Final Report, Covance, CLE Study No. 550-022, (AMR3498-95). GLP, Unpublished + Position Paper	Yes	Schirm/ DuPont

Dossier file No.	Author(s)	Year	Title Source Company report no. GLP / GEP status Published	Data Protection	Owner
IIA 7.2.1.1/01	Caldwell, E.	2002	14C-Lenacil; Hydrolysis under Laboratory Conditions, Huntingdon Life Sciences Ltd. ACD046/013764. GLP, Unpublished	Yes	Schirm/ DuPont
IIA 7.2.1.2/01	Millais, A.	2002 b	Lenacil quantum yield of direct phototransformation, Huntingdon Life Sciences Ltd, ACD047/022138. GLP, Unpublished	Yes	Schirm/ DuPont
IIA 7.2.1.3.1/01	Barnes, S.	2001	Lenacil Technical – Assessment of Ready Biodegradability : Modified Sturm Test, Huntingdon Life Sciences, ACD037/013644. GLP, Unpublished	Yes	Schirm/ DuPont
IIA 7.2.1.3.2/01	Theis, M.	2002 a	Lenacil Fate and behaviour in Water-sediment, A&M Labor, A&M00-078. GLP, Unpublished	Yes	Schirm/ DuPont
IIA, 7.2.2/01	Comb, A.L.	2002 a	Lenacil pure grade: Physico-chemical properties, Huntingdon Life Sciences Ltd, ACD025/014039. GLP, Unpublished	Yes	Schirm/ DuPont
IIA, 7.3	Pollard-Langford, A.	2004	Lenacil Definition of the residue in plants and soil, Huntingdon Life Sciences Ltd, Not GLP, Unpublished	Yes	Schirm/ DuPont

Environmental fate and behaviour of the formulations (Annex IIIA 9)

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
IIIA, 9.1, 9.2	Shaw, D	2004	Calculation of predicted environmental concentrations of lenacil and its metabolites IN-KE121 and IN-KF313 in soil, groundwater, surface water and sediment Huntingdon Life Science Ltd., England, report No. ACD043/040080 Non-GLP, unpublished	Yes	Schirm / DuPont

ANNEX B

Lenacil

Appendix 1 – Field studies

Pollmann, B. 2003

Field Trials, Soil Residue (Summary)		Active substance:	Lenacil
Responsible body for reporting (name and address)	GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn Germany	Matrix:	Soil
Country:	France	Submission date:	
Contents of a.s.:	816 g/kg lenacil	Page:	
Formulation:	WP	Indoor/outdoor:	Outdoor
Commercial product (name):	VENZAR 80% WP	Other a.s. in the formulation:	None
Producer of commercial product:	Schirm GmbH and E. I. DuPont de Nemours and Company	Common name and content:	Not applicable.
Residues calculated as:	mg lenacil/kg soil		

1	2	3	4		5	6	7	8	9	10	11
Location, including postal code	Matrix	Soil characteristics (USDA) 1) soil texture 2) pH 3)% org. Carbon 4) CEC meq/100 g	Application rate per treatment		Application date	Days after application	Soil layer (cm)	Residue (mg a.s./kg)	DT_{50} (days)	DT_{90} (days)	Remarks
			kg as/ha	Water (l/ha)							
Trial	Soil	1) Silt	0.514	308	27 June	0	0 – 10	0.12	25	84	-

1	2	3	4		5	6	7	8	9	10	11
Location, including postal code	Matrix	Soil characteristics (USDA) 1) soil texture 2) pH 3)% org. Carbon 4) CEC meq/100 g	Application rate per treatment		Application date	Days after application	Soil layer (cm)	Residue (mg a.s./kg)	DT_{50} (days)	DT_{90} (days)	Remarks
			kg as/ha	Water (l/ha)							
F01N001R Schleithal F-67160 Alsace France		2) 6.1 3) 0.71 4) Not stated			2001	7 14 30 62 92 209 273		0.20 0.18 0.11 0.04 0.02 0.00 0.00			

Pollmann, B. 2003

Field Trials, Soil Residue (Summary)		Active substance:	Lenacil
Responsible body for reporting (name and address)	GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn Germany	Matrix:	Soil
Country:	Germany	Submission date:	
Contents of a.s.:	816 g/kg lenacil	Page:	
Formulation:	WP	Indoor/outdoor:	Outdoor
Commercial product (name):	VENZAR 80% WP	Other a.s. in the formulation:	None
Producer of commercial product:	Schirm GmbH and E. I. DuPont de Nemours and Company	Common name and content:	Not applicable.
Residues calculated as:	mg lenacil/kg soil		

1	2	3	4		5	6	7	8	9	10	11
Location, including postal code	Matrix	Soil characteristics (USDA) 1) soil texture 2) pH 3)% org. Carbon 4) CEC meq/100 g	Application rate per treatment		Application date	Days after application	Soil layer (cm)	Residue (mg a.s./kg)	DT_{50} (days)	DT_{90} (days)	Remarks
			kg as/ha	Water (l/ha)							
Trial	Soil	1) Silty sand	0.487	292	10 July	0	0 – 10	0.16	28	91	-

1	2	3	4		5	6	7	8	9	10	11
Location, including postal code	Matrix	Soil characteristics (USDA) 1) soil texture 2) pH 3)% org. Carbon 4) CEC meq/100 g	Application rate per treatment		Application date	Days after application	Soil layer (cm)	Residue (mg a.s./kg)	DT_{50} (days)	DT_{90} (days)	Remarks
			kg as/ha	Water (l/ha)							
G01N001R Dollern D – 21739 Niedersachsen Germany		2) 5.4 3) 0.56 4) Not stated			2001	7 14 28 59 86 176 276 363		0.24 0.13 0.06 0.05 0.03 0.01 0.01 0.00			

Pollmann, B. 2003

Field Trials, Soil Residue (Summary)		Active substance:	Lenacil
Responsible body for reporting (name and address)	GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn Germany	Matrix:	Soil
Country:	Germany	Submission date:	
Contents of a.s.:	816 g/kg lenacil	Page:	
Formulation:	WP	Indoor/outdoor:	Outdoor
Commercial product (name):	VENZAR 80% WP	Other a.s. in the formulation:	None
Producer of commercial product:	Schirm GmbH and E. I. DuPont de Nemours and Company	Common name and content:	Not applicable.
Residues calculated as:	mg lenacil/kg soil		

1	2	3	4		5	6	7	8	9	10	11
Location, including postal code	Matrix	Soil characteristics (USDA) 1) soil texture 2) pH 3)% org. Carbon 4) CEC meq/100 g	Application rate per treatment		Application date	Days after application	Soil layer (cm)	Residue (mg a.s./kg)	DT_{50} (days)	DT_{90} (days)	Remarks
			kg as/ha	Water (l/ha)							
Trial	Soil	1) Loamy silt	0.544	326	3 July	0	0 – 10	0.13	18	61	-

1	2	3	4		5	6	7	8	9	10	11
Location, including postal code	Matrix	Soil characteristics (USDA) 1) soil texture 2) pH 3)% org. Carbon 4) CEC meq/100 g	Application rate per treatment		Application date	Days after application	Soil layer (cm)	Residue (mg a.s./kg)	DT_{50} (days)	DT_{90} (days)	Remarks
			kg as/ha	Water (l/ha)							
G01N002R Dürrn D – 75248 Baden-Württemberg Germany		2) 6.7 3) 1.23 4) Not stated			2001	8 14 30 64 91		0.14 0.07 0.04 0.01 0.00			

Pollmann, B. 2003

Field Trials, Soil Residue (Summary)		Active substance:	Lenacil
Responsible body for reporting (name and address)	GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn Germany	Matrix:	Soil
Country:	Spain	Submission date:	
Contents of a.s.:	816 g/kg lenacil	Page:	
Formulation:	WP	Indoor/outdoor:	Outdoor
Commercial product (name):	VENZAR 80% WP	Other a.s. in the formulation:	None
Producer of commercial product:	Schirm GmbH and E. I. DuPont de Nemours and Company	Common name and content:	Not applicable.
Residues calculated as:	mg lenacil/kg soil		

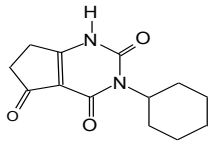
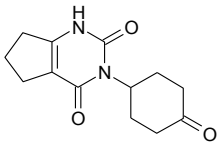
1	2	3	4		5	6	7	8	9	10	11
Location, including postal code	Matrix	Soil characteristics (USDA) 1) soil texture 2) pH 3)% org. Carbon 4) CEC meq/100 g	Application rate per treatment		Application date	Days after application	Soil layer (cm)	Residue (mg a.s./kg)	DT_{50} (days)	DT_{90} (days)	Remarks
			kg as/ha	Water (l/ha)							
Trial	Soil	1) Silty loam	0.493	394	21 June	0	0 – 10	0.19	88	291	-

1	2	3	4		5	6	7	8	9	10	11
Location, including postal code	Matrix	Soil characteristics (USDA) 1) soil texture 2) pH 3)% org. Carbon 4) CEC meq/100 g	Application rate per treatment		Application date	Days after application	Soil layer (cm)	Residue (mg a.s./kg)	DT_{50} (days)	DT_{90} (days)	Remarks
			kg as/ha	Water (l/ha)							
S01N002R Massalaves E – 46292 Valencia Spain		2) 7.5 3) 1.57 4) Not stated			2001	6 14 29 61 92 188 274		0.06 0.10 0.08 0.08 0.08 0.03 0.00			

Lenacil

Appendix 2:

Structure of soil and water metabolites

Common name	a) IUPAC name b) Chemical name	Structure	Occurrence
KF 313-1 5-oxo-lenacil M20.5 IN-KF-313	a) 3-cyclohexyl-6,7-dihydro-7-hydroxy-1H- cyclo pentapyrimidine-2,4,4(3H)-trione b) 3-cyclohexyl-6,7- tributylphenyl)-dihydro-1H-cyclopentapyrimidine-2,4,5(3H)-trione		Soil Water/sediment
LN5 oxo-lenacil M15.0 IN-KE 121	a) 3-cyclohexyl-6,7-dihydro-7-1H- cyclo pentapyrimidine-2,4,5(3H)-trione b) Not available		Soil Water/sediment

ANNEX B

Lenacil

B.6 Toxicology and metabolism
Addendum february 2009
Adapted in April 2009.

Preliminary note: The company provided comments during the period of drafting of the DAR and these comments were included in the DAR. As the RMS disagreed with these comments, the conclusions in the DAR were not modified. The company repeated these comments in the reporting table. Some of them were too large to be included in the reporting table and are therefore reported in this addendum and put in *italic*.

An amendment of this addendum has been made following the late submission of Laboratory Historical Control Data of rat and mouse carcinogenicity studies (rat: mammary tumours, mouse: liver and lung tumours). The changes of April 2009 were highlighted in yellow.

B. 6.3.2.1 Oral 90 day toxicity (rat) (Annex IIA 5.3.2)

Conclusion of the RMS as reported in the DAR:

NOAEL = 500 ppm (40.6 mg/kg bw/d) based on leukopenia, the excretion of proteins in urine of males and increased relative liver weight (21-24%) occurring at 5000 ppm onwards.
From the results reported in this study, at the highest dose of 50000 ppm, target organ in rats seems to be the liver as suggested by the weight increase (however not dose-related) and the centrilobular hepatocyte hypertrophy (reported at top dose). Renal dysfunction seems to occur as suggested by the alteration of electrolytes excretion as well as the increased urinary protein at 5000 ppm onwards. Effects on white blood cells which were not explained were observed at the two high doses. RMS considers that there is no reason to disregard these different effects.

Notifier comment:
Additional histopathological examinations were completed for this study.
Following observation of thyroid changes in the multi-generation reproductive toxicity study additional histopathological examinations of thyroid tissue preserved from a 13 week dietary study in rats were instigated. In the original study (Point 5.3.2.1) thyroids from the control and high dose (50000 ppm) groups were examined. The additional investigation extended the examination to the low and intermediate groups.
The study authors concluded that examination of sections stained with haematoxylin and eosin revealed no changes indicative of any accumulation of pigment in the follicular epithelium or any other change indicative of a response to treatment. Schmorl's staining of the thyroids, however, revealed a background level of Schmorl's positive staining in all groups, particularly in males. Schmorl's positive staining is indicative of lipofuscin in the follicular epithelium. There was a treatment-related increase in the incidence and severity of Schmorl's-positive staining in females given lenacil technical at 50000 ppm, and a slight increase in the severity of this finding in males given 50000 ppm. The slightly increased incidence of Schmorl's-positive staining in females given 5000 ppm was within the background incidence and was, therefore, not attributed to treatment. Following a recovery period of four weeks there were no significant differences in incidence of Schmorl's-positive staining between control and high dose group males or females.
Further thyroid function tests were also completed in female rats dosed for 20 weeks at 250 or 50000 ppm lenacil. Investigations included assessment of T3 and T4 levels, thyroid weights, ^{125}Iodide uptake and displacement. The study concluded that there was no evidence to suggest that lenacil technical at doses of up to 50000 ppm affected the ability of the thyroid to take-up and organify ^{125}Iodide. Measurements of T3 during the study also indicated that lenacil does not act as an inhibitor of the deiodinase which converts T4 to T3. Overall, the results of the study showed that lenacil technical was not directly toxic to the thyroid.
The conclusion to this summary states 500 ppm to be a NOEL. It appears that the RMS has also concluded 500 ppm to be the NOAEL also. From the results presented it is apparent that changes in the two higher dose levels were inconsistent and generally showed no clear dose relationship. While an effect of treatment is clearly apparent at 5000 ppm, this is not the case at the intermediate dose level where reduced monocytes and a slight increase in urinary protein were the only changes of note, both showing recovery after removal of treatment,

indicating no adverse long term effects of lenacil administration. There was no corroborative evidence from macroscopic or microscopic findings to confirm any adverse effects of treatment at 5000 ppm.
The lowest NOEL derived from short-term toxicity studies in rat, mouse and dog was based on the results of the 90-day rat study and set at 40.6 mg/kg/day (500 ppm). The lowest appropriate NOAEL value was derived from the same study as the intermediate dose level of 412 mg/kg/day (5000 ppm). This was based on adaptive liver changes at the highest dose of 50000 ppm, which constituted the LOAEL. The NOAEL was defined by reduced white blood cell numbers at 5000 ppm, considered of uncertain toxicological significance, in that the findings were not consistently seen in the long-term rat study. There were no bodyweight effects at any dosage.
In the opinion of the notifier, the data support the conclusion indicating an NOAEL in the rat 90 day study of 5000 ppm and a NOEL of 500 ppm.

RMS disagrees with the company and maintains its proposal reported in the conclusion of the study.

B6.3.2.2 Oral 90-d toxicity – dog

Conclusion from the RMS as reported in the DAR:

RMS considers that the NOAEL = 1000 ppm (44 mg/kg bw/d) taking into account the increased relative liver weight in female dogs, the increased relative thyroid and parathyroid weight in male and female dogs. Liver centrilobular/midzonal hepatocyte hypertrophy was reported in male dogs at 5000 ppm.

The company considered that: based on the results above the No Effect Level (NOEL) on this study was considered to be 1000 ppm (corresponding to a daily intake of 44 mg/kg in the males and 46 mg/kg/day in the females) based on adaptive histopathological findings in the liver. The highest No Adverse Effect Level (NOAEL) was 25000 ppm (equivalent to 1121 mg/kg/day for males and 1102 mg/kg/day for the females

The notifier proposes including the following additional text in the DAR:
With the exception of increased liver weight, the minor changes noted in various haematological, blood chemistry, urinalysis, organ weight and pathology parameters show no dose relationship, no trends for increasing effect over time or with increasing dose and show no consistency between the sexes. The response in the liver is clearly an adaptive response to increase metabolic workload. The effects on liver weight, alkaline phosphatase and hepatic histopathology are consistent with an adaptive response which does not indicate an adverse effect of treatment.
The findings in the 28 day dog study and 90 day dog study do not show good correlation indicating the minor disturbances are not real toxic changes. The RMS expressed concern about renal dysfunction following the 28 day study but the 90 day study provides no evidence to support the proposition of renal effects. Opposing effects occurred in haematology parameters in the two studies.
Taking the two studies together it is apparent that considerable background variation occurs in a number of parameters following low dose administration of lenacil, without adverse effect on the animals over 4 or 13 weeks. The liver, rather than the kidney, is the target organ and at high doses this organ responds adaptively to the challenge of metabolizing lenacil. The test material is extensively metabolized following oral administration and so the functional liver changes are not unexpected.
Hence the low dose levels can reasonably be assumed to reflect biological variation and the high dose findings indicate an adaptive liver response. Based on these findings, the notifier disagrees with the RMS conclusion and respectfully requests reconsideration of an NOAEL of 25000 ppm.

RMS maintains its conclusions. The effects on hepatic histopathology could indeed be an adaptive response but liver enzyme induction was not measured and therefore not demonstrated.

B.6.3.4-1 Summary of short term toxicity:

According to the RMS, the lowest NOAEL was identified in the 13-week mice study at 100 ppm (15.5mg/kg bw/d) based on blood toxicity at 1000 ppm (157 mg/kg bw/d).

Notifier: A revised table of results is proposed with different endpoints taking into account the adaptive liver response and additional thyroid function tests.

For the 90 day rat study, additional investigations relating to thyroid function demonstrate the non-adverse nature of the findings at the LOAEL defined in table above (5000 ppm).

It is the opinion of the notifier that based on the overall response to 13 weeks administration and evidence of recovery, the appropriate NOAEL derived from short term toxicity studies is 412 mg/kg/day (5000 ppm). This conclusion was based on the occurrence of adaptive liver changes at the highest dose of 50000 ppm, which constituted the LOAEL. The NOAEL was defined by reduced white blood cell numbers at 5000 ppm, considered of uncertain toxicological significance, in that the findings were not consistently seen in the long-term rat study.

Additional histopathological examinations were completed for this study and presented in the revised summary dossier (dated June 2006) at annex point 5.3.2.1.1.

Following observation of thyroid changes in the multi-generation reproductive toxicity study additional histopathological examinations of thyroid tissue preserved from a 13 week dietary study in rats were instigated. In the original study (Point 5.3.2.1) thyroids from the control and high dose (50000 ppm) groups were examined. The additional investigation extended the examination to the low and intermediate groups.

The study authors concluded that examination of sections stained with haematoxylin and eosin revealed no changes indicative of any accumulation of pigment in the follicular epithelium or any other change indicative of a response to treatment. Schmorl's staining of the thyroids, however, revealed a background level of Schmorl's positive staining in all groups, particularly in males. Schmorl's positive staining is indicative of lipofuscin in the follicular epithelium. There was a treatment-related increase in the incidence and severity of Schmorl's-positive staining in females given lenacil technical at 50000 ppm, and a slight increase in the severity of this finding in males given 50000 ppm. The slightly increased incidence of Schmorl's-positive staining in females given 5000 ppm was within the background incidence and was, therefore, not attributed to treatment. Following a recovery period of four weeks there were no significant differences in incidence of Schmorl's-positive staining between control and high dose group males or females.

Further thyroid function tests were also completed in female rats dosed for 20 weeks at 250 or 50000 ppm lenacil. Investigations included assessment of T3 and T4 levels, thyroid weights, ^{125}Iodide uptake and displacement. The study concluded that there was no evidence to suggest that lenacil technical at doses of up to 50000 ppm affected the ability of the thyroid to take-up and organify ^{125}Iodide. Measurements of T3 during the study also indicated that lenacil does not act as an inhibitor of the deiodinase which converts T4 to T3. Overall, the results of the study showed that lenacil technical was not directly toxic to the thyroid.

RMS considers that the liver effects observed in rats at 5000 ppm could not be disregarded as long as enzyme induction was not demonstrated. We agree with the company that some liver parameters suggest an adaptive effect but the investigation was incomplete.
At 5000 ppm, there was an important increase relative thyroid + parathyroid weight.

Thyroid accumulation of lipofuscin: accumulation of lipofuscin in thyroid could suggest that atrophy occurred and that membranes of destroyed organelles were converted within the lysosomes to lipid containing lipofuscin. Lipofuscin is itself not injurious to the cell, but its presence suggests that something adverse has occurred. Moreover, RMS considers that no sufficient information is provided for interpretating changes.

A revised Table B.6.3.4-1 is proposed by the company:

Type of test **Test species**	**Test substance** **purity**	**Results**				**Reference**
		NOEL	**NOAEL (mg/kg bw/day)**	**LOAEL (mg/kg bw/day)**	**Symptoms at LOAEL**	
90 day dietary study , rat+ 4 week recovery Period	Batch n° 141712003 ; purity 98.6%	500ppm (40.6mg/kg bw/d)	5000ppm (412mg/kg bw/d)	50000ppm (5029mg/kg bw/d)	leucopenia, ↑excretion urinary proteins; lipofuscin staining in thyroid follicular epithelium	Thirlwell, 2002b,c
90 day dietary study, mice	Batch n° 9038; purity 98.2%	100 ppm (15.5 mg/kg bw/d)	1000 ppm (157 mg/kg bw/d)	5000 ppm (787 mg/kg bw/d)	leucopenia in male and female mice	Malley,1991
90 day dietary study, dog	Batch n° 141712003 ; purity: 98.6%	1000 ppm (44 mg/kg bw/d)	25000 ppm (1121 mg/kg bw/d)	>25000 ppm (1121 mg/kg bw/d)	Adaptive liver changes: ↑ relative liver weight in female dogs, centrilobular/midzonal hepatocyte hypertrophy	Geary,2002

B. 6.4.1.3 in vitro mammalian cytogenetic test studies (Annex IIA 5.4.1)

As requested by UK, more information is reported on the study of chromosomal aberration test.

- Lenacil technical, in vitro mammalian chromosomal aberration test in human lymphocytes (Allais, 2001)

Metaphase analysis data:

Table 6.4.1.3-1: Summary of results of chromosomal aberrations in human lymphocytes (Test 1)

Exposure period/ S9 mix	**Chromatid type**		**Chromosome type**		**Concentration of Lenacil technical**	**Cells with aberrations Excluding gaps**			**Cells with aberrations Including gaps**			**Relative Mitotic**
-S9 mix	**ctb %**	**cte %**	**csb**	**cse**	**(µg/ml)**	**Individual values (%)**		**Mean (%)**	**Individual values (%)**		**Mean (%)**	**Index (%)**
3 hours	13	1			0 (Culture medium)	1	2	1.5	1	2	1.5	100
	11				625	1	1	1.0	1	1	1.0	82
	11				1250	1	1	1.0	1	1	1.0	82
	26				2500	2	4	3.0	2	4	3.0	68
	1023				5000	7	16	11.5**	7	16	11.5**	54
	1212	42	11		0.2 (Mitomycin C)	17	12	14.5**	17	12	14.5**	-
+ S9 mix												
	1				0 (Culture medium)	1	0	0.5	1	0	0.5	100
					1250	0	0	0.0	1	0	0.5	90
	3				2500	2	0	1.0	2	0	1.0	84

	1	1			5000	1	0	0.5	1	0	0.5	75
	10 11	1 3	2 2		6 (Cyclophosphamide)	12	13	12.5**	12	13	12.5**	-

Statistically significant at **p<0.001; *: p<0.01
Ctb/csb= chromatid /chromosome break
Cte/cse= chromatid/chromosome exchange

Table 6.4.1.3-2: Summary of results of chromosomal aberrations in human lymphocytes (Test 2)

Exposure period/ S9mix	**Chromatid type**		**Chromosome**		**Concentration of Lenacil technical**	**Cells with aberrations Excluding gaps**			**Cells with aberrations Including gaps**			**Relative Mitotic**
- S9mix	**ctb %**	**cte %**	**csb %**	**cse %**	**(µg/ml)**	**Individual values (%)**		**Mean (%)**	**Individual values (%)**		**Mean (%)**	**Index (%)**
3hours	1 1				0 (Culture medium)	1	1	1.0	1	1	1.0	100
	 1				625	0	1	0.5	0	1	0.5	124
	5 6				2500	5	6	5.5*	5	6	5.5*	61
	25 14		1		5000	16	11	13.5**	16	11	13.5**	39
	10 11	4 2			0.1 (Mitomycin C)	13	11	12.0**	13	11	12.0**	-
+ S9mix												
3hours	 1				0 (Culture medium)	0	1	0.5	0	1	0.5	100
			1		1250	1	0	0.5	1	0	0.5	79
	1 2		1 3		2500	2	2	2.0	2	2	2.0	58
	1				5000	1	1	1.0	1	1	1.0	56

	2		1									
	98	1	13	Other11	6 (Cyclophosphamide)	11	11	11.0**	11	11	11.0**	-

Statistically significant at **p<0.001; *: p<0.01
Ctb/csb= chromatid /chromosome break
Cte/cse= chromatid/chromosome exchange

B6.5.2 Carcinogenicity in the rat (Annex IIA5.5)

Conclusion from the RMS as reported in the DAR: from the toxicity study, a NOAEL is proposed at 2500 ppm (139-188 mg/kg bw/d) taking into account the effects reported at 25000 ppm on:
- The thyroid gland (relative weight increase, increased TSH and luminal concretions)
- The liver effects (an increased weight and hepatocellular hypertrophy/vacuolation in both sexes)

At top dose, some effects were reported in the eyes of males (loss of outer nuclear layer bilateral) and females (unilateral lenticular degeneration). Kidney weight and urinary protein excretion were increased and male rats had abnormal blood smears.
The company concluded that the administration of Lenacil technical to Han Wistar rats, via the diet, at concentrations up to 25000 ppm for 104 weeks caused non-specific toxicity in females at 25000ppm and adaptive and toxic change in the liver in males at 25000ppm.

A NOAEL for oncogenicity should be set at 250 ppm (16 mg/kg bw/d) taking into account the increased incidence of for mammary gland malignant adenocarcinoma at 2500 ppm.

In April 2009, the company provided historical histopathological control data for the incidence of selected neoplastic findings in control HAN Wistar rats from recent studies performed at [redacted] These data are reported in the table B.6.5.2-1.

Table B.6.5.2-1: historical histolopathology data in mammary area compared to results reported in the study with lenacil.

Lenacil in diet :	**0 ppm**		**250 ppm**		**2500 ppm**		**25000 ppm**	
Mammary gland		F		F		F		F
Benign adenoma		0		1		0		3**
Fibroadenoma benign		7		12		8		8
Malignant adenocarcinoma		0		2		6** **12**%		5** **10**%
Laboratory background incidence July 96-september 2001:								
Benign mammary adenoma	4 affected /555 females examined = 0.72% range: 0.0-2%							
Benign mammary fibroadenoma	136/555 females affected = 24.5% range : 16.7-33.3%							
Malignant mammary adenocarcinoma	20/555 females affected = 3.6% range : 0.0-**6.7**%							
[redacted] data Wistar Han rats, 2003	Females: 1.82%-13.33%							

Conclusion: based on the reported incidence of historical histopathology data, the results reported in this study suggest that lenacil increases the incidence of malignant

adenocarcinoma in mammary gland at 2500 ppm and 25000 ppm. The results are outside the historical data of the laboratory.
Lenacil should be classified and labelled with Carc. Cat 3, R40.

Notifier: Request for inclusion of additional comment relating to the derivation of the NOEL and NOAEL values in this study.

The notifier agrees with the study author conclusions in relation to endpoints determined for long term studies – based on rat and mouse oncogenicity investigations. The NOEL and NOAEL values proposed were unchanged by the thyroid function assessments. The value proposed for the rat NOEL is 250 ppm (12.0 and 15.9 mg/kg/day in males and females respectively) and for the rat NOAEL is 2500 ppm (118 and 160 mg/kg/day in males and females), based on slightly reduced motor activity in males, and the LOAEL was the highest dose tested, 25000 ppm, where, in our opinion, adaptive liver changes were seen in males and non-specific toxicity in females. There were no neoplastic lesions apparent in the rat and the non-neoplastic liver lesions were indicative of an adaptive response. The neoplastic lesions seen in the mouse were species-specific and not relevant to human risk assessment.

The RMS has concluded from the available data and background information that malignant adenocarcinoma incidence is well within the background incidence for the animal supplier and "in the absence of dosage relationship, the increase in adenocarcinoma is not considered to be associated with the administration of Lenacil" and therefore the responses at 2500ppm and 25000 ppm were deemed equivocal. However, the endpoint subsequently used to set an NOAEL for oncogenicity is below the level of these equivocal findings.

The Notifier suggests that the data support the proposition that the administration of lenacil is not associated with mammary tumour incidence, since the incidence at high dose levels is less than that in background data. The Notifier proposes that the same information is used to set a NOAEL for oncogenicity, where, if lenacil is not associated with induction of any of the tumours observed, as concluded by Notifier and supported by RMS in text above, then 2500 ppm is the appropriate NOAEL.

B.6.5.3 Carcinogenicity study in the mouse (Annex IIA 5.5)

In the DAR, it was reported that:

Microscopy:
Liver: centrilobular hypertrophy was observed in male livers and the incidence was low. This effect was considered by the company to be the result of the induction of smooth endoplasmic reticulum and an increase in SER-associated enzymes but this was not demonstrated, or measured. The centrilobular hypertrophy observed in male mice was not considered as adverse by the company.
Lung: there was no significant statistical increase in the incidence of pulmonary alveolar adenomas or adeno-carcinoma. However, there was a borderline increase in the combined incidence of alveolar adenomas and adeno-carcinoma observed in male mice at top dose. Although this increase was significant by Cochran-Armitage trend test, the increase was not significant by the Fisher exact test. The incidence of various alveolar tumors observed in the concurrent control males was similar to those of historical controls in this laboratory, except at top dose. However, it was not considered compound related based on the following reasons:

1. Incidences of adenoma and adenocarcinoma, taken separately, were not statistically increased.
2. There was no statistical significance with the Fisher exact test at p=0.05 for any dose group.
3. There was no decrease in alveolar tumor latency; most tumors were observed in mice killed at terminal sacrifice.
4. There was no increase in focal hyperplasia of type II alveolar cells.
5. There was no shift in tumor cell anaplasia.

Comment from RMS on the microscopy: the company did not provide the laboratory historical control data for liver tumors and RMS used historical control data published by [redacted] for Crl:CD-1 BR mice, 1995. The incidence of liver cell adenoma multiple reported in males at top dose (16%) is within the maximum range of historical control data at [redacted] (19%).
The incidence of 17/80 (21%) lung alveolar adenomas for males at 7000 ppm is slightly above the maximum range of historical control data at the testing laboratory (16%) and at [redacted] (12%). The incidence of 8/80 (10%) alveolar carcinomas in males at 7000 ppm is above the maximum range of historical control data at the testing facility (0%) but inside [redacted] (21%) and not statistically significant.
The number of any type lung alveolar neoplasms in males receiving 7000 ppm is also slightly increased (26/80, 32%) compared to the concurrent untreated control (18/80, 22.5%), it is statistically significant ($p<0.05$) and is outside the range of the historical controls at the testing facility (18-21%). However, because this increase is small, and did not demonstrate decreased latency compared to controls, it is considered to represent only equivocal toxicologic significance.

In April 2009, the company provided historical control data for male liver tumor as well as for male bronchial alveolar tumors in the mouse. The data are reported in table B.6.5.3-1 and compared with the results reported for lenacil.

Table B.6.5.3.-1: laboratory historical control data for liver tumor and bronchial alveolar tumor data

	N° affected animals:							
Lenacil in diet :	**0**		**100**		**2500**		**7000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Liver: Hepatocellular:								
Centrilobular hypertrophy	-	-	-	-	-	-	**7***	-
Karyomegaly	2	-	2	-	4	-	5	-
Adenoma single	11	2	10	0	10	0	11	1
Adenoma multiple	0	0	5	0	4	0	**13*** 16%**	0
Carcinoma	5	0	3	0	3	0	2	0

	N° affected animals:							
Lenacil in diet :	**0**		**100**		**2500**		**7000 ppm**	
Historical control data from the company on ♂ mice (9 studies, December 1988-February 1995):								
N° mice with adenoma single /total n° mice	59/651 (9.06%) range: 3.75-16.6%							
N° mice with adenoma multiple/total n° mice	9/651 (1.4%) range: 0-**4**%							
Published historical control data for adenoma			Male:0-19%; Female: 0.0-2%					
Lung alveolar:								
Adenoma single	14 **17**%	5	9	5	15	4	**17** **21**%	6
Laboratory historical control* (2 studies, study periods unknown)								
	7-10 male mice/60; range: 11.6-16%							
Laboratory historical control (9 studies, December 1988-February 1995):								
	64/651 (9.8%) range : 5-**17**%							
Adenoma multiple	1 1.3%	1	2	0	0	2	3 3.8%	0
Laboratory historical control* (2 studies, study periods unknown):								
	1-3 male mice/60 (1.6-5%)							
Laboratory historical control (8 studies, December 1988-February 1995):								
	16/571 (2.8%) : range: 0-5%							
Carcinoma single	3 3.8%	3	4	4	4	2	**8** **10%**	2
Laboratory historical control (6 studies, December 1988-February 1995):								
	21/450 (4.6%); range:1.25-**11.25%**							
Carcinoma multiple:	1	1	0	0	2	0	0	0
Laboratory historical control* (2 studies, study periods unknown) 0-0 male mice/60								
Laboratory historical control (6 studies, December 1988-February 1995):								
	3/450 (0.66%); range:0.0-2.5%							
Adenoma + carcinoma	18 22.5%	10	15	8	18	7	**26*** **32.5%**	8
Laboratory historical control (9 studies, December 1988-February 1995):								
	118/651 (18%); range 10-**23%**							

*: data present in the original DAR, superseded by more recently submitted HCD

From the reported table it can be concluded that lenacil at top dose:
- Increases the incidence of multiple liver hepatocellular adenoma outside the laboratory background data.

- Increases the incidence of single adenoma of bronchial alveolar tissue outside the laboratory background data.
- When taken together, the total incidence of bronchial alveolar adenoma + carcinoma reported in males at top dose is higher than the historical control data reported by the company, but this is mainly caused by the slight excess of adenoma incidence (as carcinoma incidence was within HCD range);

RMS considers that the historical control data provided by the company confirm the conclusion of the RMS proposed in the DAR: the increased incidence of tumors at top dose is of equivocal significance but RMS considers that classification / labeling is not required for these effects as the effects are adenoma, and just slightly above the Historical control data and are observed only in males at doses as high as 977 mg/kg bw/d (7000 ppm).
These data do not change the conclusion in the DAR of the RMS concerning the proposed NOAELs

Conclusion proposed in the DAR: a NOAEL for systemic toxicity is proposed at 2500ppm (332 mg/kg bw/d) taking into account the increased liver weight associated with centrilobular hypertrophy.
NOAEL oncogenicity can be set at 2500ppm (332 mg/kg bw/d) taking into account the increased incidence of alveolar tumors in lung, and multiple adenomas in liver.

B.6.5.5 Summary of long-term toxicity and carcinogenicity (Annex IIA 5.5)

Notifier requests that the following information relating to additional thyroid investigations, and additional histopathological examinations, presented in the revised summary dossier (dated June 2006) at annex point 5.3.2.1.1. are considered for inclusion in the DAR.

Following observation of thyroid changes in the multi-generation reproductive toxicity study additional histopathological examinations of thyroid tissue preserved from a 13 week dietary study in rats were instigated. In the original study (Point 5.3.2.1) thyroids from the control and high dose (50000 ppm) groups were examined. The additional investigation extended the examination to the low and intermediate groups.
The study authors concluded that examination of sections stained with haematoxylin and eosin revealed no changes indicative of any accumulation of pigment in the follicular epithelium, or any other change indicative of a response to treatment. Schmorl's staining of the thyroids, however, revealed a background level of Schmorl's positive staining in all groups, particularly in males. Schmorl's positive staining is indicative of lipofuscin in the follicular epithelium. There was a treatment-related increase in the incidence and severity of Schmorl's-positive staining in females given lenacil technical at 50000 ppm, and a slight increase in the severity of this finding in males given 50000 ppm. The slightly increased incidence of Schmorl's-positive staining in females given 5000 ppm was within the background incidence and was, therefore, not attributed to treatment. Following a recovery period of four weeks there were no significant differences in incidence of Schmorl's-positive staining between control and high dose group males or females.
Further thyroid function tests were also completed in female rats dosed for 20 weeks at 250 or 50000 ppm lenacil. Investigations included assessment of T3 and T4 levels, thyroid weights, ^{125}Iodide uptake and displacement. The study concluded that there was no evidence to suggest that lenacil technical at doses of up to 50000 ppm affected the ability of the thyroid to take-up and organify ^{125}Iodide. Measurements of T3 during the study also indicated that lenacil does not act as an inhibitor of the deiodinase which converts T4 to T3. Overall, the results of the study showed that lenacil technical was not directly toxic to the thyroid.

The Notifier disagrees with the conclusion of the RMS to classify the active substance lenacil XnR40 Cat 3 Carcinogen.
The incidence of several lesions has been discussed in the DAR, and with one exception, they are considered by both Notifier and RMS as unrelated to treatment with Lenacil. In the case of malignant mammary adenocarcinoma, the RMS states: 'The incidence of malignant mammary adenocarcinoma in females at top dose (10%) and at intermediate dose (12%) were slightly outside the historical controls of the laboratory (6.7%) and within the data of Charles River laboratories (13.33%), the incidence represents an equivocal finding.'

The relevant legislation is Council Directive 67/548/EEC, as amended by Commission Directive 2001/59/EC, Annex 6 (Annex VI) Section 4.

It is important to note that for other organs and tissues, there are no scientific justifications for classification for carcinogenicity. It is also important to note that in the other rodent species tested, the mouse, and in the short-term studies, all three species, (rat, mouse and dog) there were no indications of adverse effects on female mammary tissue. The RMS notes that the incidence is equivocal.

In considering the two subcategories of category 3 listed under indents a) and b) in Section 4.2.1, category b) does not apply (substances which are insufficiently investigated). The substance Lenacil has been adequately investigated in guideline studies in the required species. The weight of evidence from carcinogenicity and mutagenicity studies is that the substance is not carcinogenic. Further investigations could be anticipated to yield incidences of the finding at or around background. Such findings should not be considered equivocal; this is the purpose of making a comparison with historical control data.

The legislation notes that in distinguishing between category 3 and no classification, one argument in excluding a concern for man is in cases where the only available data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.(Section 4.2.1, last indent).

The finding is present only in one species, and it the neoplasm is one which readily occurs spontaneously. The incidence in the study is actually marginally below the incidence for the finding in the animal supplier's background data. For a finding such as this to be considered eligible for category 3, the incidence would reasonably be expected to be significantly greater than the background incidence in the strain of animals. Strict interpretation of the legislative guidance strongly indicates that the correct conclusion is no classification.

Notifier comment on the results of mice carcinogenicity:
The study authors conclude that effects in the mouse were sex and species specific and, as such, the notifier does not consider the effects valid in the context of the human risk assessment.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

B.6.6.1 Two generation reproductive toxicity in the rat (Annex IIA 5.6.1)

- **Lenacil technical: preliminary study of effects on reproductive performance in Han Wistar rats by dietary administration, [redacted] (Patten, R., 2002)**

RMS reported that the initial birth weight of the F_1 and F_2 offspring was unaffected by maternal treatment but there was a reduction of weight gain at 50000ppm that occurred from day 7 of age for the F_1 offspring and from day 4 of age for the F_2 offspring. This effect occurred before that offspring begin to consume solid food suggesting an effect via lactation. Whether treatment caused a reduction in milk production or quality or whether the offspring were exposed to lenacil via the milk cannot be ascertained in this study.

RMS considers that this effect needs a labelling of lenacil with **R64**. However, this proposal should be discussed.

The Notifier disagrees with the conclusion of the RMS to classify the active substance lenacil with R64.
The relevant legislation is Council Directive 67/548/EEC, as amended by Commission Directive 2001/59/EC, Annex 6 (Annex VI) Section3 2.8 and 4.2.3.3.
- Section 3.2.8 states the criteria for R64 as: For substances and preparations which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child.
In rat metabolism studies, lenacil is primarily excreted via urine as water-soluble hydroxyl metabolites. It is generally considered that the high fat content of milk may lead to fat-soluble substances and metabolites being present in the milk. Residues in the target crop, sugar beet, are also hydroxyls and ketones, and it is predicted that in humans, these will be further hydroxylated and excreted via urine. There is no evidence that lenacil or its metabolites accumulate in the body, such that there is no implication that mobilisation of maternal fat reserves could lead to the presence of lenacil or its metabolites in milk. The ADI for Lenacil is 0,014 mg/kg bw/day. The NOAEL proposed by the RMS is 10,000 ppm or 1,727 mg/kg bw/day. This gives a margin of safety in excess of 120,000. The criterion for R64 includes the words 'in amounts sufficient to cause concern'.
-Furthermore, Section 4.1.3.3 states that 'For the purpose of classification, toxic effects on offspring resulting only from exposure via the breast milk, or toxic effects resulting from direct exposure of children will not be regarded as Toxic to reproduction, unless such effects result in impaired development of the offspring'.
It is accepted that offspring bodyweights were slightly lower than controls in the F0F1 (by 6%) and F1F2 (by 11%) during the lactation period, but offspring survival was not adversely affected, and the bodyweights of the F0F1 pups selected for the F1 generation were not different from controls at the start of the pre-mating maturation period. Also, the behavioural and developmental landmarks assessed prior to and after weaning were not

adversely affected by either maternal treatment or by direct intake of the test material. Any marginal bodyweight effects on offspring prior to weaning are considered transient, and insufficient evidence for adverse effects via maternal milk.

The legislation states: 'This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk'. Where there is an effect on quantity of the milk, there is usually evidence from the immediate post-partum period. The body wall of the newborn rat is translucent, and the technicians can see the presence of milk in the pups' stomach as a whitish crescent in the abdomen. Absence of this crescent is recorded in the data for the study as an indication that the dam is not nursing the pups. It is frequently accompanied by high post natal mortality in pups. Neither finding was made in this study.
The legislation gives further guidance:
R64 would normally be assigned on the basis of:
(a) toxicokinetic studies that would indicate the likelihood that the substance would be present in potentially
toxic levels in breast milk; and/or
(b) on the basis of results of one or two generation studies in animals which indicate the presence of adverse
effects on the offspring due to transfer in the milk; and/or
(c) on the basis of evidence in humans indicating a risk to babies during the lactational period.

The evidence from metabolism studies is that lenacil or its' metabolites would not be preferentially excreted in the milk, and if present at all, would be at a minute fraction of levels considered NOAEL in the rat. The effects on the offspring are minor, transient and there is no indication of impaired development or reduced survival. Finally, there is no evidence in humans.
In conclusion, lenacil should not be classified R64.
In view of the conclusion drawn here in respect of litter data and maturation of the F1 rats, the notifier requests re-evaluation of the proposal for classification with R64. The litter development shows no clear effects leading to impaired growth of maturation in these litters.

B.6.10.1 Acceptable Daily Intake (ADI)

RMS proposes to use the NOAEL from the long-term/carcinogenicity rat study for setting of the ADI, with a NOAEL = 14 (12-16) mg/kg bw/d = 250 ppm. An assessment factor of 100 for inter- and intra-species extrapolation is sufficient.

ADI= 0.14 mg/kg bw/d

Notifier's point of view:
The table of endpoint values for long term studies from which to derive the ADI is set out below. The NOAEL values are those considered appropriate by the Notifier based on an assessment of the occurrence of toxicologically significant adverse effects.

Table 5.10-1: Summary of relevant NOAELs for deriving the ADI

Study	***NOAEL***	
	ppm diet	***mg/kg/day equivalent***
Rat chronic toxicity	*2500*	*Males: 139.1* *Females: 188.5*
Rat oncogenicity	*2500*	***Males: 118*** *Females: 160*
Mouse oncogenicity	*2500 (males)* *7000 (females)*	*Males: 332* *Females: 1358*
Rat multigeneration	*10000 (non-reproductive NOAEL)*	*Dams and progeny 817*

It is the opinion of the notifier that these endpoints adequately take account of minor changes observed in various studies and gives suitable weight to the consideration of adverse and non-adverse toxicological findings. The thyroid effects, adaptive liver changes, tumour incidence below animal supplier's background or sporadic incidence levels and absence of real effects on newborn pups have been discussed in earlier comments.
From this table it is apparent that the lowest NOAEL is 118 mg/kg/day based on the rat oncogenicity study. It is appropriate to apply an uncertainty factor of 100 to the NOAEL of 118 mg/kg/day and the Notifier therefore proposes an ADI of 1.18 mg/kg/day.

RMS does not support the company proposal.

B.6.10.3 Acceptable Operator Exposure Level (AOEL)

Lenacil exhibited low acute toxicity. In a range of tests a mutagenic potential of lenacil was not observable. Regarding the 90-day oral toxicity in rats and mice and the 1-year oral dog study, the mouse is the most sensitive species. The NOAEL of this study was set at 15.5-20 mg/kg bw/d (100 ppm) taking into account the effects on white blood cells observed at 1000 ppm. This value is quite similar to the lowest NOAEL from the long term studies where a NOAEL =12-16 mg/kg bw/d reported in rats. A mean value of 16 mg/kg bw/d is proposed.

Considering the toxicological profile of lenacil, for the determination of the AOELs a safety factor of 100 is considered adequate. As the oral absorption reached 85% of the dose within 48 h, a correction factor for oral absorption is not necessary.

AOEL= 0.16 mg/kg bw/d

Notifier comment:
The most sensitive species, from rat, mouse and dog, tested in short term studies was the rat. It is proposed to set an AOEL based on the No Adverse Effect Level in a 90 day dietary study in the rat of 5000 ppm.
The relevant NOAEL values from short term toxicity and developmental toxicity studies appropriate for derivation of the AOEL are as follows:
Table 5.10-2: Summary of relevant lowest NOELs/NOAELs for derivation of the AOEL

Study type	***NOEL***		***NOAEL***		***References***
	ppm diet	***mg/kg/day***	***ppm diet***	***mg/kg/day***	
13-wk feeding rat	*500*	***40.6***	*5000*	*412*	*5.3.2.1*
13-wk feeding mouse	*1000*	*157*	*10000*	*male 1616 female 2150*	*5.3.2.2*
13-wk feeding dog	*1000*	*44*	*25000*	*male 1121 female 1102*	*5.3.2.3*
Developmental toxicity rat (gavage)	*-*	*1000*	*--*	*1000*	*5.6.2.1*
Developmental toxicity rabbit (gavage)	*-*	*1000*	*--*	*1000*	*5.6.2.2*

The lowest NOEL derived from short-term toxicity studies in rat, mouse and dog was based on the results of the 90-day rat study and set at 40.6 mg/kg/day (500 ppm). The lowest appropriate NOAEL value was derived from the same study as the intermediate dose level of 412 mg/kg/day (5000 ppm). This was based on adaptive liver changes at the highest dose of 50000 ppm, which constituted the LOAEL. The NOAEL was defined by reduced white blood cell numbers at 5000 ppm, considered of uncertain toxicological significance, in that the findings were not consistently seen in the long-term rat study. There were no bodyweight effects at any dosage.
The systemic AOEL ($AOEL_{SYS}$) is derived from the 5000 ppm No Observed Adverse Effect Level (NOAEL) in the rat 90-day repeat dose oral toxicity study which corresponded to an achieved mean daily intake of 412 mg/kg/day. A standard 100-fold safety factor has been used to allow for inter- and intra- species variations without adjustment for either toxicokinetic or toxicodynamic components. This default safety factor provides a high degree of conservatism in the calculation of the AOEL.
Following review of absorption data, no correction for calculation of the systemic (internal) dose was considered appropriate since the estimated oral absorption (circa 74%) did not represent a significant difference between applied and absorbed dose and oral absorption reached 85% of the dose within 48 h. Consequently, the Notifier proposes $AOEL_{SYS}$:

$412/100 \times 1^{a} = 4.12$ *mg/kg/day*

[a]: No correction factor included for oral absorption of at least 74% (estimated by notifier in summary dossier presented in June 2006), estimated from combined urinary and biliary excretion, following single or repeated oral administration to rats or 85% when measured over 48 h (higher mean absorption value of 85% derived by RMS for 48 hour period, the notifier accepts the argument for use of the higher value for absorption). The use of default safety factors for inter and intra species variation (10 fold in each case) provide a highly conservative estimate of the AOEL, not requiring further refinement for systemic availability.

RMS does not support the company proposal.

B.6.12.2 Comparative dermal absorption, in vitro using rat and human skin (Annex IIIA 7.3)

- [^{14}C]-Lenacil –*In vitro* dermal penetration study at two dose levels using human skin (Kane, 2004). HLS, report No.: ACD 073/043372.

Findings**:**

The highest dose was selected as the highest achievable dose concentration which could be accurately applied and had acceptable homogeneity. The low dose was selected as 2.5 g/L corresponding to the in use application rate of the product.
The achieved dose of lenacil was 3131 µg/cell equivalent to 4893 µg/cm^2 (high dose) and 11.13 µg/cell equivalent to 17.39 µg/cm^2.
The company concluded that the total absorbed dose corresponded to 0.018% and 0.400% for the high and low dose levels, respectively. The estimated steady-state absorption rates for radioactivity after application of [^{14}C]-lenacil were very low (between 0.026 and 0.002 µg lenacil equivalents /cm^2/hr at each dose level) showing that lenacil does not rapidly penetrate the skin when applied in a WP formulation.

However, according to the guidance document on Dermal Absorption (SANCO/222/2000 rev. 7), a more acceptable estimation of skin absorption can be obtained by including the amount retained in the different skin levels. So, RMS considers that dermal absorption on human skin represents 2.7% of the concentrate and 34.2% for the diluted formulation.
Log K_{ow} of lenacil = 2.31 and molecular weight =234.3 suggests a 100% value.

Table B.6.12.2-1: distribution of radioactivity in skin.

Endpoints /dose	**3131 µg/cell = 100%**		**11.13 µg/cell**	
Expressed as :	**%**	µg	%	µg
Dose in receptor (0-24 h)	0.013	0.392	0.335	0.036
Skin	0.006	0.178	0.065	0.007
Dose on tape strip 1-2 (surface)	1.82	57.06	18.7	2.080
Dose on tape strip 3-5	0.737	24.99	11.12	1.194
Dose on tape strip 6-8	nd	nd	3.993	0.429
Absorbed dose- dose tape strip1-2:	**0.756**		**15.5**	
Stratum corneum	0.860	23.05	15.11	1.679
Remaining on donor chamber	0.841	26.36	5.637	0.662
Remaining on receptor chamber	Nd	Nd	Nd	Nd
Dose in skin swab (6 hr)	95.20	2981	55.2	6.146
Total recovery	98.62	3088	95.25	10.61
Absorption rate (µg equiv./cm^2/hr)	0.026		0.002	

nd: results within background range.

Conclusion: RMS considers that an acceptable estimation of skin absorption should include the amount retained in the different skin levels giving a dermal absorption rate of 2.7% for the concentrate and of 34.2% for the diluted formulation.
As proposed by different MSs, absorbed dose was calculated not taking into account the dose of tape strip 1-2.
Dermal absorption rate is 0.756 rounded to 1% for concentrate and 15.5% for diluted formulation.

B.6.15 Exposure data (Annex IIIA 7.2)

Venzar 80 WP is a wettable powder formulation containing 80% lenacil as active substance. It is intended for application through hydraulic field crop sprayers to sugar beet. The recommended application rate is a maximum of 500 g a.s./hectare (625 g product/ha), in a minimum spray volume of 200 litres of water/hectare.

B.6.15.1 Estimation of operator exposure (Annex IIIA 7.2.1.1)

New estimations were realized by the RMS using other values of dermal absorption as those proposed in the DAR.
Estimates of operator exposure are based on the UK Predictive Operator Exposure Model (POEM), and the German model.

Data used for the calculation:

Crop type :	Field crops (sugar beet)
Method of application :	Tractor mounted hydraulic boom sprayer (UK POEM) Field crop(German model)
Area treated / day :	50 ha UK POEM ; 20 ha German model
Formulation :	80% WP
Rate of use :	500 g a.s. /ha
Water volume :	200 L/ha
Dermal absorption **- concentrate :**	1 %
- dilution :	15.5 %
AOEL	0.16 mg/kg bw/day
Operator body weight :	60 kg for UK model and 70 kg for German model

The water volume of 200 L/ha represents a minimum recommended volume and therefore provides the worst case scenario for the calculations.

Results of calculations according to the UK POEM or German model are given in Table 6.15.1-1 and 6.15.1-2.

Expected operator exposures:

Table B.6.15.1-1: Estimated operator exposure (mg/person/day) according to the UK POEM

Product/ Application method/ crop	**Dermal absorbed dose (mg/day)**			**Inhalation exposure (mg/ day)**			**Total exposure (mg /day)**
	Mix/load	**Spray**	**Total**	**Mix/load**	**Spray**	**Total**	
Tractor mounted/trailed boom sprayer ; hydraulic nozzles							
Dermal absorption 1% and 15.5%	3.4	16.1	19.5	16.475	0.15	16.62	36.12
Type of protection							
Gloves M/L + A	- 0.034	2.4993	2.53	16.47	0.15	16.62	19.15

Table B.6.15.1-2: Estimated operator exposure (mg/person/day) according to the GERMAN Model

Product/ Application method/ crop	Dermal exposure (mg/day)			Inhalation exposure (mg/ day)			Total exposure (mg /day)
	Mix/load	Spray	Total	Mix/load	Spray	Total	
Tractor field crop							
Dermal absorption of 1 and 15.5%	60	20.4	80.4	0.7	0.01	0.71	81.11
Type of protection							
Gloves M/L + A	0.6	16.63	17.23	0.7	0.01	0.71	17.94

Comparison of estimated and tolerable exposure:

Table B.6.15.1-3: Exposure as a proportion of AOEL- POEM model.

Product/ Application method/ crop	Total systemic exposure – 60 kg person (mg/kg bw/day)		% of AOEL	
	no PPE worn	PPE worn*	no PPE worn	PPE worn
Tractor mounted/trailed boom sprayer ; hydraulic nozzles				
Dermal absorption of 1% and 15.5 %	0.602	0.3193	376	199

* : Gloves M/L + A

Table B.6.15.1-4: Exposure as a proportion of AOEL –German model.

Product/ Application method/ crop	Total absorbed dose – 70 kg person (mg/kg bw/day)		% of AOEL	
	no PPE worn	PPE worn*	no PPE worn	PPE worn
Tractor mounted/trailed boom sprayer ; hydraulic nozzles				
Dermal absorption of 1% and 15.5 %	0.06388	0.0470	39.9	29.4

*: gloves M/L + A

Comments from the company:

The exposure estimated using UK POEM is reduced for operators using RPE during mixing/loading (as permitted in the UK model) in addition to gloves during mixing and loading and application.
The use of gloves during M/L and A and RPE (FFP2, particle filtering mask) during M/L brings a reduction of exposure to below the AOEL.

Conclusions of RMS:

According to the UK POEM model, dermal exposure during application of Venzar 80 WP is important representing 99% of the total exposure. The use of gloves during M/L and A brings a reduction of dermal exposure but still not sufficient to be below the AOEL.

According to the German modeloperator exposure is below the AOEL with or without gloves during M/L and application.

RMS disagrees to use respiratory protection for application of an herbicide considering that this type of additional protection is unrealistic.

B.6.15.2 Measurement of operator exposure (Annex IIIA 7.2.1.2)

Estimates from both the German BBA model indicate exposure to spray operatives to be below the $AOEL_{SYS}$ with and without the need for personal protective equipment. Therefore overall it can be concluded that operator exposure will be at an acceptable level when using Venzar 80 WP on sugar beet as recommended and studies to measure operator exposure are not required.

B.6.15.3 Estimation of bystander exposure (Annex IIIA 7.2.2)

New calculation made by the RMS taking into account a dermal absorption of 15.5%.
Bystanders present at the time of a pesticide application may be subject to dermal and inhalation exposure to the active substance resulting from vapours movement and spray drift. As Venzar 80 WP will only be used in outdoor situations and has a vapor pressure of 2.7×10^{-5} Pa at 25°C, exposure of bystanders is expected to arise primarily as a result of spray drift. The exposure of bystanders would be expected to be of a short/acute duration and unlikely to occur repeatedly to the same individuals.
An estimate of bystander exposure for a downwards spray application to field crops has been calculated based on a study by Lloyd and Bell, 1983. In this study, measurements of simulated bystander exposure were made during field crop spraying operations following a single pass of the sprayer with a bystander located 8 m from the edge of the treatment area.

For risk assessment purposes, the systemic AOEL has been used for comparison to potential exposure since this represents the internal absorbed dose.

Data used for the calculation

PDE = potential dermal exposure = 0.1 ml of spray solution at 8 m (Lloyd and Bell, 1983)
SSC = spray solution concentration (maximum in-use concentration) = 2.5 mg a.s. /ml
DA = dermal absorption (using the value for spray dilution) = 15.5%
AC = concentration of spray in the air = 0.02 ml of spray solution/m^3 (Lloyd and Bell, 1983)
BR = volume of air breathed/min (based on = 3.6 m^3/h) = 0.06 m^3/min
T = Duration of exposure = 5 minutes
BW = body weight = 70 kg

Bystander exposure calculations

$$\text{Systemic exposure} = \frac{(PDE \times SSC \times DA) + (AC \times SSC \times BR \times T)}{BW}$$

$$= \frac{(0.1 \times 2.5 \times 0.155) + (0.02 \times 2.5 \times 0.06 \times 5)}{70}$$

$$= \frac{0.038 + 0.015}{70}$$

= 0.0537 mg/kg bw/day

This exposure is ca 33.5% of the $AOEL_{SYS}$ of 0.16 mg/kg bw/day. Therefore the risk to bystanders from exposure during field spraying with Venzar 80 WP is considered acceptable.

B.6.15.4 Estimation of worker exposure (Annex IIIA 7.2.3.1)

Worker exposure estimation is not required for an herbicide.

Some MSs commented on the need to provide an estimation of worker exposure as well as an estimation of re-entry exposure.

Estimation of re-entry exposure: Estimation is based on the model as developed by the German BBA.

The following parameters were considered:

DFR	3µg/cm2 x kg a.s./ha
Transfer Factor	2500cm^2/h
A (working period)	1 h/day
Penetration Factor clothing	1 (w/o PPE)
Application rate	0.5 kg a.s./ha
Dermal absorption	15.5%
Body weight	60 kg
Potential dermal	3 × 2500 × 1 × 1× 0.5= 3750 µg/worker/1 hour = 62.5 µg/kg

exposure (µg a.s./person/day)	bw/hour = 0.0625 mg/kg bw

Dermal absorbed dose= 0.00968 mg/kg bw/hour =6% of AOEL

For a worker exposed during a 8 hour period, dermal absorbed dose would be = 0.077 mg/kg bw/d= 48% of AOEL.

B.6.15.5 Measurement of worker exposure (Annex IIIA 7.2.3.2)

No data, not necessary.

ANNEX B

Lenacil

Appendix: Estimation of the exposure

UK POEM: tractor mounted trailed boom sprayer: hydraulic nozzles

Product	**VENZAR 80 WP**		
Active substance	lenacil		
Concentration	800 mg/g		
Formulation type	WP		
DERMAL EXPOSURE DURING MIXING AND LOADING			
Hand contamination/kg a.s.	13.6 mg/kg a.s.		
Application dose	0.625 kg product/ha		
Work rate	50 ha/day		
Hand contamination/day	340 mg/day		
Protective clothing	none		
Transmission to skin	100%		
Dermal exposure to a.s.	340 mg/day		
INHALATION EXPOSURE DURING M/L			
Inhalation exposure /kg a.s.	0.659 mg/kg a.s.		
Inhalation exposure/day	16.47 mg/day		
RPE	none		
Transmission through RPE	100%		
Inhalation exposure to a.s.	16.47 mg/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION			
Application technique-tractor-mounted/trailed boom sprayer and nozzles			
Application volume	200 spray/ha		
Volume of surface contamination	10 ml/h		
Distribution	Hands	trunk	Leggs
	65	10	25%
Clothing	none	Permeable	Permeable
	100	5	15%
Dermal exposure	6.5	0.05	0.375 ml/h
Duration of exposure	6 h		
Total dermal exposure to spray	41.55ml/day		
Concentration of a.s.in spray solut	2.5 mg/ml		
Dermal exposure to a.s	103.875 mg/day		
INHALATION EXPOSURE DURING SPRAYING			
Inhalation exposure to spray	0.01 ml/h		
Duration of exposure	6h		
Concentration of as in spray	2.5 mg/ml		
Inhalation exposure to as	0.15 mg/day		
Percent absorbed	100%		
Absorbed dose	0.15 mg/day		

ABSORBED DOSE	**Mix/load**	**Application**
Dermal exposure	340 mg/day	103.87 mg/day
Percent absorbed	-1 %	15.5%
Absorbed dose dermal route	3.4 mg/day	16.1 mg/day
Inhalation exposure to as	16.47 mg/day	0.15 mg/day
Absorbed dose	19.875 mg/day	16.25 mg/day
PREDICTED EXPOSURE		
Total absorbed dose	36.125 mg/day	
Operator body weight	60 kg	
Operator exposure	0.602 mg/kg bw/d	

UK POEM: tractor mounted trailed boom sprayer: hydraulic nozzles- GLOVES

Product	**VENZAR 80 WP**		
Active substance	lenacil		
Concentration	800 mg/g		
Formulation type	WP		
DERMAL EXPOSURE DURING MIXING AND LOADING			
Hand contamination/kg a.s.	13.6 mg/kg a.s.		
Application dose	0.625 kg product/ha		
Work rate	50 ha/day		
Hand contamination	340 mg/day		
Protective clothing	Gloves		
Transmission to skin	1%		
Dermal exposure toa.s.	3.4 mg/day		
INHALATION EXPOSURE DURING M/L			
Inhalation exposure /kg a.s.	0.659 mg/kg a.s.		
Inhalation exposure/day	16.47 mg/day		
RPE	none		
Transmission trhough RPE	100%		
Inhalation exposure to a.s.	16.47 mg/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION			
Application technique-tractor-mounted/trailed boom sprayer and nozzles			
Application volume	200 spray/ha		
Volume of surface contamination	10 ml/h		
Distribution	Hands	trunk	Leggs
	65	10	25%
Clothing	Gloves	Permeable	Permeable
	10	5	15%

Dermal exposure	0.65	0.05	0.375 ml/h
Duration of exposure	6 h		
Total dermal exposure to spray	6.45 ml/day		
Concentration of a.s.in spray solut	2.5 mg/ml		
Dermal exposure to a.s	16.125 mg/day		
INHALATION EXPOSURE DURING SPRAYING			
Inhalation exposure to spray	0.01 ml/h		
Duration of exposure	6h		
Concentration of as in spray	2.5 mg/ml		
Inhalation exposure to as	0.15 mg/day		
Percent absorbed	100%		
Absorbed dose	0.15 mg/day		

ABSORBED DOSE	**Mix/load**	**Application**
Dermal exposure	3.4 mg/day	16.125 mg/day
Percent absorbed	-1%	15.5%
Absorbed dose dermal route	0.034 mg/day	2.499 mg/day
Inhalation exposure to as	16.47 mg/day	0.15 mg/day
Absorbed dose	16.56 mg/day	2.649 mg/day
PREDICTED EXPOSURE		
Total absorbed dose	19.15 mg/day	
Operator body weight	60 kg	
Operator exposure	0.319 mg/kg bw/d	

German model:

Product	VENZAR 80 WP	Active substance	LENACIL
Formulation type	WP	a.s. concentration	800 mg/ml
Method of use	Tractor field crops	Dose(product)	0.625 kg product/ha
Work rate	20 ha/day	Dose (a.s.)	0.5 kg a.s./ha
		Amount handled	10 kg a.s./day

Exposures-mix/loading

	Specific exposures	Estimated exposures	PPE	Estimated exposures
Inhalation	0.07 mg/kg a.s.handled	0.7 mg a.s./day	None	0.7 mg a.s./day
Dermal-hands	6 mg/kg a.s.handled	60 mg a.s./day	gloves	0.6 mg a.s./day

Exposures-application

	Specific exposures	Estimated exposures	PPE	Estimated exposures (PPE)
Inhalation	0.001 mg/kg a.s.handled	0.01 mg a.s./day	None	0.01 mg a.s./day
Dermal-head	0.06 mg/kg a.s.handled	0.6 mg a.s./day	None	0.6 mg a.s./day
Dermal – hands	0.38 mg/kg a.s.handled	3.8 mg a.s./day	Gloves	0.038 mg a.s./day
Dermal-body	1.6 mg/kg a.s.handled	16 mg a.s./day	None	16 mg a.s./day

Total exposures	Estimated exposures	Percent absorbed	Estimated exposures (PPE)
Total potential inhalation	0.71 mg a.s./day	100%	0.71 mg a.s./day
Total dermal-mix	60 mg a.s./day	1%	0.6 mg a.s./day
Total dermal-application	20.4 mg a.s./day	15.5%	16.63 mg a.s./day
Total absorbed dose	4.472 mg a.s./day		3.294 mg a.s./day
Body weight	70 kg		70 kg
Mg/kg bw/d	0.06388 mg/kg bw/d		0.04706 mg/kg bw/d

ANNEX B

Lenacil

B.7 Residue data

(Addendum April 2009)

Open points 3(3) and 3(4) of the reporting tables: Vol.3, B.7.1.1, Metabolism, distribution and expression of residues of Lenacil in Sugar Beets

-Metabolism, distribution and expression of residues of Lenacil in Sugar Beets

-Metabolism of Lenacil in Sugar Beets (Zhang, M. and Glunt, C.D., 1997)
Guidelines:
Commission Directive 96/68/EC amending Council Directive 91/414/EEC.
GLP:
Yes
Material and Methods:
Test substance: ^{14}C-Pyrimidine ring Lenacil (2-^{14}C-DPX-B634).
Specific activity: 8.36 µCi/mg following isotopic dilution with non-radiolabelled Lenacil
Radiochemical purity: > 96 %
Reference standards: Lenacil, IN-G2172(*Z*-isomer), IN-KD304(*E*-isomer), IN-KD305(*E*-isomer), IN-KC939(*Z/E,* 2:1), IN-KQ961, IN-KC943, IN-KE121, IN-KD302 and IN-KF313.
Preparation of the treatment solution:
The ^{14}C-Lenacil treatment solution was prepared by combining ^{14}C-Lenacil with technical Lenacil and other formulation ingredients to simulate 50 % *WP* formulation.
A 50-mL treatment solution for the 2 applications was prepared and the final specific activity of the solution was 8.36 µCi/mg.
The treatment solution was prepared just before the first application. An aliquot of 20 mL of the treatment solution was used for the first application. The remaining 30 mL treatment solution was stored at –20 °C until the second application. Before each application, an aliquot of the treatment solution was analysed by HPLC and by LSC to confirm the purity and the quantity of the test substance.
-Log P*o/w*: 1.70 at pH 4 and 7 at 25 °C.
Experimental design:
The study was performed under greenhouse conditions.
Sugar beet plants (variety: *HM55 Medium*) were grown in containers (*ca* 40 L capacity) filled with a silt loam soil. Two foliar applications were made at post-emergence to the plants, with the first application made at the 4-leaf stage (BBCH 14) and the second application 15 days later at the 6-leaf stage (BBCH 16). The Lenacil test substance was formulated as a 50 % *WP* and applied using a compressed CO_2 sprayer at rates equivalent to 204 and 321 g a.s./ha for the two applications. The total rate applied was 525 g a.s./ha supporting the maximum recommended use rate of 0.5 kg a.s./ha per crop. Untreated plants were grown as controls.
Sugar beet plants (whole) were sampled immediately after the first spray when the treatment solution was dry (0 -day), at 15 -day (immediately after the second treatment solution was dry), and at intervals of 32, 47, 74, 99 and at 130 days

(mature stage) after the first treatment. Plants were separated into foliage and roots prior to analysis.
Extraction procedure:
The total radioactive residues (TRR) in the sugar beet foliage and root samples were quantified by radio combustion analysis and by liquid scintillation counting (LSC) after homogenising in liquid nitrogen.
Samples of foliage and root were extracted using acetonitrile/water (2:1, *v/v*) and analysed by chromatographic comparison in 2 HPLC solvent systems with UV detector against reference standards.
To generate larger amounts of Lenacil metabolites, 7-12 leaf-stage sugar beet leaves were incubated with ^{14}C-Lenacil for 3 to 8 days. LC-MS analyses of the metabolites isolated from excised sugar beet incubation media were confirmed as IN-KC943 and IN-KQ961, respectively.
Metabolite identification was performed mainly on extracts of sugar beet foliage at final harvest (130 days after the first application).
The concentrated 130-day foliage extract was further purified for metabolites isolation using semi-preparative HPLC chromatography.
Identification of the glucose conjugates was performed using HPLC analysis of the aglycons following β-glucosidase hydrolysis of the conjugates. After hydrolysis, the control and enzyme-treated samples were analysed by HPLC.
Structural confirmation of the metabolites IN-KQ961 and IN-KC943 was based on their LC-MS data in comparison with the synthetic standards. These purified radioactive metabolites together with the synthetic standards were used as references for the metabolite identification in this study.
The metabolite profile in the 47-day roots extracts and in the 130-day foliage extracts before and after β-glucosidase hydrolysis was analysed by HPLC.
Elucidation of the structure of the metabolites was achieved using mass spectra analysis.
Findings:
The total radioactive residues and the distribution of radioactivity in sugar beet foliage at each sampling interval are given in Table B 7.1.1-1. The profile of the extractable radioactivity in sugar beet root is summarised in Table B 7.1.1-2.

Table B 7.1.1-1: Extractabilities and investigation of the nature and the amounts of residues of Lenacil in sugar beet foliage following 2 foliar spray applications of the test substance ^{14}C-Pyrimidine ring Lenacil respectively at BBCH growth stage 14 and 16 corresponding to dose rates of application equivalent to 204 and 321 g a.s./ha, respectively – Residues are expressed as % of the total radioactive residues and in mg ^{14}C-Lenacil equiv./kg.

RAC	**Sugar beet foliage**						
Harvest day (day after 1st spray)(1)	**0**	**15**	**32**	**47**	**74**	**99**	**130**
Total radioactive residues expressed as mg ^{14}C-Lenacil equiv./kg							
	7.35	4.71	1.06	1.04	0.69	0.30	0.16

Extractability of the total radioactive residues -% of the TRR and (mg ^{14}C-Lenacil equiv./kg)							
Acetonitrile/water extraction phase	97.4 (7.16)	98.4 (4.63)	99.6 (1.06)	99.5 (1.04)	95.9 (0.66)	95.8 (0.29)	94.4 (0.16)
Elucidation of the radioactive residues -% of the TRR and (mg ^{14}C-Lenacil equiv./kg)							
Parent compound (DPX-B634)	96.0 (7.05)	95.7 (4.51)	88.3 (0.94)	89.9 (0.93)	67.9 (0.47)	52.0 (0.16)	28.4 (0.04)
IN-KC943 (7-hydroxy-Lenacil)	<0.1 (<0.01)	0.3 (0.01)	0.6 (<0.01)	0.3 (<0.01)	<1.0 (<0.01)	1.6 (<0.01)	3.1 (<0.01)
IN-KC943 glucosides(2)	0.5 (0.03)	0.8 (0.04)	4.1 (0.04)	3.6 (0.04)	3.9 (0.03)	5.2 (0.02)	7.7 (0.01)
IN-KC943-glucosyl-conjugate	nd	nd	nd	nd	1.4 (<0.01)	3.6 (0.01)	3.0 (<0.01)
Polar peaks	nd	0.2 (<0.01)	2.1 0.02)	1.5 (0.02)			
Glucose conjugates	nd	nd	nd	nd	<0.1 (<0.01)	2.6 (<0.01)	1.6 (<0.01)
Polar metabolites(3)	Nd	0.2 (<0.01)	2.1 (0.02)	1.5 (0.02)	10.5 (0.07)	18.0 (0.05)	37.9 (0.06)
Unknown metabolites	nd	nd	Nd	nd	6.1 (0.04)	7.3 (<0.03)	12.7(4) (<0.03)
Total identified metabolites	96.6 (7.09)	96.8 (4.56)	93.0 (0.99)	93.8 (0.98)	74.2 (0.52)	62.4 (0.20)	42.2 (0.07)
Unextracted radioactive residues (% of the TRR and mg ^{14}C-Lenacil equiv./kg)							
	2.6 (0.19)	1.6 (0.08)	0.4 (<0.01)	0.4 (<0.01)	4.1 (0.03)	4.2 (0.01)	5.6 (<0.01)
Accountability: extracted phases + residual radioactive residues (% of the TRR)							
	100.0	100.0	100.0	99.9	100.0	100.0	100.0

Remarks:
Nd: Not detectable.
(1): Harvest at 0 day (immediately after the first spray) and at 15 day (immediately after the second treatment) and at 32, 47, 74 and 99 days after the first treatment and at harvest (130 days after the first treatment).
(2): HPLC analyses showed a peak that matched the retention time of IN-KQ961(hydroxylated Lenacil), indicating the presence of this metabolite. Later results indicated that IN-KQ961 showed a similar retention time to that of IN-KC943-glucoside and the peak corresponding to IN-KQ961 could be IN-KC943-glucoside or a mixture of the 2. Therefore, the peak was isolated for further β-glucosidase hydrolysis and this peak matched the retention time of IN-KC943, indicating the existence of IN-KC943 glucose conjugate before hydrolysis with no detectable amount of the metabolite IN-KQ961.
(3): This polar fraction was a mixture of several polar metabolites. These peaks were resolved further in another HPLC system and some of the metabolites in this polar area could be hydrolysed by β -glucosidase suggesting the existence of glucose conjugates among these polar metabolites. It was reported that no single polar metabolite in sugar beet leaves exceeded 10 % of the TRR and therefore no structure confirmations were made on these polar Lenacil metabolites.
(4): This fraction was composed of 3 distinct peaks, with a maximum of 7.5 % of TRR (0.012 mg/kg).

The TRR figures were average results from duplicate solvent extraction analyses.

Table B 7.1.1-2: Extractabilities of the radioactive residues of Lenacil in sugar beet roots following 2 foliar spray applications of the test substance ^{14}C-Pyrimidine ring Lenacil respectively at BBCH growth stages 14 and 16 and at a rate equivalent to 204 and 321 g a.s./ha, respectively – Residues expressed as % of the total radioactive residues and in mg ^{14}C-Lenacil equiv./kg.

RAC	**Sugar beet root**						
Harvest interval (days)	**0**	**15**	**32**	**47**	**74**	**99**	**130**
Total radioactive residues expressed in mg ^{14}C-Lenacil equiv./kg							
	0.02	0.02	0.02	0.03(1)	0.01(1)	0.03(1)	<0.01
Extractability of the total radioactive residues (% of the TRR and mg ^{14}C-Lenacil equiv./kg)							
Acetonitrile/water extraction phase	na	na	na	79.2 (0.02)	66.7 (<0.01)	80.0 (0.01)	na
Unextracted radioactive residues (% of the TRR and mg ^{14}C-Lenacil equiv./kg)							
	na	na	na	20.8 (<0.01)	33.3 (<0.01)	20.0 (<0.01)	na
Accountability: extracted phases + residual radioactive residues (% of the TRR)							
				100.0	100.0	100.0	

Remarks:
Na: not applicable, no solvent extraction analyses were conducted.
(1): TRR values obtained from solvent extraction respectively for the 47-day, 74-day and 99-day root samples. The other TRR figures resulted directly from radio combustion analysis.

The total radioactive residues in the sugar beet foliage declined steadily from 7.35 mg/kg at 0 day to 0.16 mg/kg in the final harvest at 130 days after the first treatment.
In the sugar beet root samples, the level of total residues recovered was low at all sampling intervals, ranging from 0.01 to 0.03 mg/kg from 0 to 99 days after first treatment and <0.01 mg/kg in the mature roots at harvest.

More than 94.4% of the TRR was extractable from the foliage at each sampling interval.
The level of unextractable residues was therefore low at each interval and in the mature foliage this fraction was below 0.01 mg/kg. In the roots, solvent extraction released between 67 % and 80 % of the TRR at the 47, 74 and 99 day intervals and no residue level above 0.01 mg/kg was recovered as the residual radioactive fraction.

-Lenacil metabolites in the early harvested foliage samples (0-day to 47-day harvest), the major part of the extracted radioactivity was recovered as the unchanged parent compound (up to 96 % of TRR). Other peaks did not exceed 10 % of TRR in the foliage extracts and no further analysis was performed on these samples.

-Lenacil metabolites in the later harvest periods (74-day, 99-day and 130-day foliage samples), unchanged parent compound was recovered at a level ranging between 28.4 % of TRR and 67.9 % of TRR along with an increasing polar metabolites fraction. Identified residues in the mature foliage were composed of the 7-hydroxy-Lenacil metabolite - IN-KC943- as an unconjugated metabolite at 3.1% of TRR (<0.01 mg/kg) and as glucoside conjugates at a total level of 10.7 % of TRR (<0.02 mg/kg) in the 130-day foliage sample.
HPLC analysis of the extracts showed a peak matching the retention time to that of metabolite IN-KQ961. However later results indicated that IN-KQ961 showed a similar retention time to that of IN-KC943-glucoside. Therefore the peak corresponding to IN-KQ961 could be IN-KC943-glucoside or a mixture of the two. The peak was isolated for further hydrolysis but no single metabolite exceeded 10%TRR.

-*Polar Lenacil metabolites*: This fraction was a mixture of several polar metabolites, some of which could be hydrolysed by β-glucosidase suggesting the existence of glucose conjugates. No single polar metabolite in sugar beet exceeded 10 % TRR and no further structure elucidation of these polar

metabolites by Mass Spectrometry was attempted. It is not clear whether further tentative characterization/identification of the non identified metabolites in this polar fraction by HPLC was performed (comparison of the retention times to those of the reference standards).

-*Glucose conjugate*: β-glucosidase was used to hydrolyse the 130-day foliage extract for the identification of possible glucose conjugates. This extract contained 3 peaks besides the Lenacil peak.
-*Peak 1* was hydrolysed by B-glucosidase suggesting that this metabolite was a glucose conjugate.
-A peak was formed after the B-glucosidase hydrolysis and this peak matched the retention time of IN-KC943, indicating the existence of IN-KC943 glucose conjugate. This conjugate might have been involved in a further conjugation on the glucose moiety but no further structural elucidation was attempted.
-*Peak 2*: no further effort was made to identify the structure of the metabolite.
-*Peak 3*: matched the retention time of IN-KQ961. Both peaks 1 and 3 were isolated by semi-preparative HPLC and subjected to B-glucosidase hydrolysis. The hydrolysis showed that both peaks could be hydrolysed by B-glucosidase and their degradation compound matched IN-KC943. So, this peak contained mainly IN-KC943 glucoside and no detectable amount of IN-KQ961.

It is not clear whether further tentative characterization/identification of the radioactivity in this polar fraction by HPLC was performed (comparison of the retention times to those of the reference standards).

The total radioactive residues in sugar beet roots were very low (<0.01-0.03 mg/kg). The metabolic profile in root extract was similar to the profile in foliage extract. HPLC analysis of the root showed that no single metabolite in root extracts exceeded 0.01 mg/kg. Therefore, no further tentative metabolites characterization/identification was investigated.

Conclusion:
The parent compound accounted for the majority of the radioactive residues in the early harvested samples (0-day to 47-day foliage samples). Other minor compounds were polar metabolites or conjugates.
In the 130-day foliage samples, the parent accounted for only 28 % TRR and the polar metabolites were recovered at a level of 37.9 % of the TRR. No single metabolite in this polar fraction exceeded 0.01 mg/kg.

The metabolic pathway of Lenacil in sugar beets is depicted in Appendix A to this section.
The following major degradation pathways were observed:
-Hydroxylation of Lenacil on the pentapyrimidine ring to generate the metabolite IN-KC943. Although only tentatively identified by chromatographic comparison, the metabolite IN-KQ961 is also possible as another primary hydroxylated product with other more polar compounds.

-Glucose conjugation of those hydroxylated metabolites. 2 types of IN-KC943 conjugates were observed : the polar IN-KC943 glucose conjugate that might have been a further conjugation on the glucose moiety.
A high ratio of IN-KC943 conjugates/IN-KC943 in the extracts suggested that these hydroxylated products were rapidly transformed to conjugates.

Open points 3(4) and 3(5) in the Evaluation tables: Vol 3, B.7.6 Supervised trials: relevant validation data to be presented fort he following analytical methods:

1) *Study F-95-001-RES* :

Reference: Magnitude of residue of lenacil and triflusulfuron methyl in sugar beet grown in France following application of Venzar® and DPX-MX843-1 – Season 1995 (Tillkes, 1998 – Report No. F-95-001-RES)

GLP:
GLP compliance stated

Principle of the method: DFG Method S19 (with modified extraction)
100 g sample material is extracted with 200 mL acetone. Water is added that takes full account of the natural water content so that the acetone/water ration remains constant at 2:1 (v:v). After addition of ethyl acetate/ cyclohexane (1:1) and repeated mixing excess water is separated. The evaporated residue of an aliquot of the organic phase is cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1:1) as eluant. The residue-containing fraction is concentrated and analysed by GC using a fused silica capillary column (XTI-5, 30m x 0.25mm, 0.25µm) and a mass selective detector (m/z = 153).

Findings :

Specificity interferences :	– No interfering peaks occurred; No lenacil detected (< 30% LOQ) in control (untreated) samples
Linearity :	Calibration range: 0.02 – 2.03 µg/mL (n=8); linear regression line (R^2 = 0.998); approximate corresponding residue concentration range: 0.004 – 0.4 mg/kg
Recovery precision :	– see Table below
Validation by an independent laboratory (ILV) :	Not addressed but not required according to doc. SANCO/3029/99 rev. 4.
Limit of quantification (LOQ) :	0.01 mg/kg

Recovery rates and limits of quantification for lenacil in sugar beet roots (Specht *et al.*, 1998)

Matrix	Analyte	Method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values ± RSD
Sugar beet roots	lenacil	GC-MSD	0.0102	1	86	
			0.102	1	81	

* Limit of quantification

Recovery rates and limits of quantification for lenacil in sugar beet leaves (Specht *et al.*, 1998)

Matrix	Analyte	Method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values ± RSD
Sugar beet leaves	lenacil	GC-MSD	0.0102	1	87	
			0.102	1	79	

Conclusion: Applicability of multi-residue method DFG S19 (GC-MSD) for determination of Lenacil residues in sugar beet was only partly addressed, i.e. 5 determinations should be made at each fortification level according to doc SANCO/3029/99 rev.4.

2) ***Study 20011048/E1-FPSB*** : ***(Trials G 01 N003R, G 01 N004R, G 01 N005R, G 01 N006R)***

Reference**:** Analytical Final Report – Generation of Samples for the determination of Residues of Venzar 80 % WP (containing 80 % Lenacil) in Sugar Beets. Five Sites in Europe, 2001 (Mende, 2002 – Report No. 20011048/E1-FPSB)

GLP:

GLP compliance stated

Principle of the method:

Lenacil was extracted from the sample material with acetone (after adjustment of water content in sample), and the aqueous phase was saturated with sodium chloride. Subsequently, liquid-liquid partitioning into an acetone/ethylacetate/cyclohexane mixture was performed, the extract of which was then concentrated and cleaned-up on a GPC column (Bio-Beads S-X3). Extracts were analysed by HPLC-MS/MS (ThermoHypersil HyPurity C8 column, 150mm x 3mm i.d., 5µm). Quantitation of extracts was performed by monitoring the MS/MS transition of 233 amu to 151 amu and using peak areas of external calibration standards.
ILV has been conducted for sugar beet (roots and leafs), using essentially the same analytical procedure. A second MS/MS transition (233 amu to 107 amu) was also monitored for additional confirmation.

Findings :

Specificity – HPLC-MS/MS is a highly specific technique.

interferences :
- Lenacil was not detectable (< 30 % of LOQ) in all untreated samples of sugar beet leaves and beet; no other interferences observed at retention time of lenacil
- ILV: The specificity of the method was tested using control (untreated) samples of sugar beet root and leaf. The lenacil concentrations in the controls were <30 % of the LOQ.

Linearity : Primary validation: Calibration range: 0.03 – 1 µg/mL (n=8); quadratic regression line (R^2 = 0.9964); approximate corresponding residue concentration range: 0.01 – 0.2 mg/kg (dependent on final extract volume)
ILV: Calibration range: 0.02 – 0.6 µg/mL (n=5); linear regression line (R^2 = 0.9652); approximate corresponding residue concentration range: 0.01 – 0.25 mg/kg

Recovery precision : – see Tables below.
Mende, 2002: only 3 replicates per fortification level. Insufficient information was provided in the report for the fortification level 4.0 mg/kg.

Validation by an independent laboratory (ILV) : First validation by GAB Biotechnology GmbH, Germany (Mende, 2002); ILV by Central Science Laboratory, UK (Turnbull, 2003)
Not required according to doc. SANCO/3029/99 rev. 4.

Limit of quantification (LOQ) : 0.02 mg/kg

Recovery rates and limits of quantification for lenacil in sugar beet samples (Mende, 2002)-Primary validation.

Matrix	Analyte	Method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values ± RSD
Sugar beet leaf	lenacil	HPLC-MS/MS	0.02*	3	80 - 85	83 (3)
			0.20	3	84 - 100	92 (9)
			4.0	*2*	-	*95 (0)*
Sugar beet roots	lenacil	HPLC-MS/MS	0.02*	3	90 - 109	100 (10)
			0.20	3	88 -100	95 (7)

* Limit of quantification

Recovery rates and limits of quantification for lenacil in sugar beet samples (Turnbull, 2003)-ILV validation

Matrix	Analyte	Method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values ± RSD
Sugar beet leaf	lenacil	HPLC-MS/MS	0.02*	5	88 – 97	91 (4)
			0.20	5	73 – 79	75 (3)
Sugar beet roots	lenacil	HPLC-MS/MS	0.02*	5	80 – 111	98 (15)
			0.20	5	82 -93	86 (5)

* Limit of quantification

Conclusion:
The method validation was performed according to SANCO/3029/99.
The HPLC-MS/MS method is suitable for the determination of lenacil in sugar beet (leafs and roots) with a LOQ of 0.02 mg/kg.

3) **Study *20011048/E2-FPSB* : (*Trial P02N001 R*)**
***Reference*:** Analytical Phase Report – Generation of samples for the determination of residues of Venzar 80% *WP* (containing 80 % Lenacil) in Sugar Beets, One site in Europe, 2002 (Hamberger R., 2002 – Report No. 20011048/E2-FPSB)

-Procedure for Lenacil Determination
The extraction and cleanup procedures were performed according to a modified DFG multiresidue method S19 (Specht *et al*., 1995). This method consisted of extraction with acetone/water (2:1, v/v), saturating the aqueous phase with sodium chloride, liquid-liquid

partitioning into cyclohexane/ethyl acetate (1:1) and cleanup by gel-permeation chromatography (GPC). Quantification of Lenacil is performed by HPLC with MS/MS detection.

-*Method validation*:
Recovery samples were prepared by fortification of control samples from the current trial with the reference substance prior to extraction. A full method validation has already been performed in study 20011048/E1-FPSB (Mende, 2002). In the present study, procedural recoveries were analysed to cover the LoQ (0.02 mg/kg).

-Recovery:

Matrix	**Fortification level (mg/kg)**	**Number of tests (n)**	**Recovery (%)**	**Overall mean recovery (%) +/- RSD (%)**
Leaves	0.02	1	84	90 **+/- 7**
Beets	0.02	1	96	

-Blanks:
Lenacil was not detectable (<30 % of LOQ) in all untreated samples of sugar beet and leaves.

-Limit of Quantification: 0.02 mg/kg

-Linearity:
Calibration rate: 0-1000 ng/mL (n=8); R^2=0.9991

-*Specificity*:
Interferences at the retention times were not observed.
HPLC/MS-MS is a highly specific technique.

Conclusion:
The method validation was performed according to SANCO/3029/99.
The HPLC-MS/MS method is suitable for the determination of lenacil in sugar beet (leafs and roots) with a LOQ of 0.02 mg/kg.

4) ***Study 688479*** **: (Tests 1/2)**
Reference: Analytical Phase Report – Decline of lenacil residues in sugar beet (root and tuber vegetables) following a single application of Venzar 80 WP (lenacil) – Southern Europe, season 2005 (Witte, 2006 – Report No. 20051414/01-RSB)

GLP:
GLP compliance stated

Principle of the method:
The samples were analysed for the residues of lenacil in sugar beets. Homogenised sugar beet samples (roots and leaves) were extracted with acetonitrile/water (50:50, v/v). The extract was filtered and analysed by HPLC (Thermo HyPurity Aquastar C18 column, 150mm x 3mm i.d., 5µm) with MS/MS detection (transition of 233 amu to 151 amu).

Findings :

Specificity interferences : – - HPLC-MS/MS is a highly specific technique.
- No interfering peaks occurred; No lenacil detected (< 30% LOQ) in control (untreated) samples

Linearity : Matrix-matched calibration range: 0.5 to 500 ng/mL (n=8); linear regression line (R^2 > 0.999); approximate corresponding residue concentration range: 0.005 – 5 mg/kg

Repeatability The RSD per fortification level ranged from 1 % to 7 %.

Recovery – see Table below

precision :

Validation by an independent laboratory (ILV) : Not provided and not required according to doc. SANCO/3029/99 rev. 4.

Limit of quantification (LOQ) : 0.02 mg/kg was defined as the lowest fortification level with mean recoveries ranging from 70 % and 110 % at a RSD of <20% and blanks not exceeding 30 %.
The Limit of detection for Lenacil was defined as 30 % of the Limit of Quantification (0.006 mg/kg).

Recovery rates and limits of quantification for lenacil in sugar beet samples (Witte, 2006)

Matrix	Analyte	Method	Fortification level [mg/kg]	n	Recovery rates [%]	
					Range	Mean values ± RSD
Sugar beet leaf	lenacil	HPLC-MS/MS	0.02*	5	93 – 110	105 (7)
			0.20	3	101 – 107	105 (3)
			2.0	5	103 – 105	104 (1)
			20.0	5	96 – 107	100 (5)
Sugar beet roots	lenacil	HPLC-MS/MS	0.02*	5	100 – 107	103 (3)
			0.20	5	95 – 108	103 (5)

* Limit of quantification

Conclusion:

The method validation was performed according to SANCO/3029/99.
The HPLC-MS/MS method described above appears to be suitable for the determination of lenacil in sugar beet (leafs and root) with a LOQ of 0.02 mg/kg. It should be noted that this method only differs from the method by Mende, 2002 and Turnbull, 2003 (see further above) in that it uses another HPLC-column (C18 instead of C8).

Open point 3(26) of the Reporting tables: Vol. 1, List of End points (p. 50), Vol. 3, B.7.11, Estimates of the potential and actual exposure through diet and other means

		Lenacil								
		Status of the active substance:		Code no.						
		LOQ (mg/kg bw):	0,02	proposed LOQ:						
		Toxicological end points								
		ADI (mg/kg bw/day):	**0,14**	ARfD (mg/kg bw):						
		Source of ADI:	**DAR**	Source of ARfD:						
		Year of evaluation:	**2009**	Year of evaluation:						
Chronic risk assessment										
		TMDI (range) in % of ADI minimum - maximum								
		No of diets exceeding ADI: ---								
Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)		

	0,3	UK Toddler	0,3	Sugar beet (root)		FRUIT (FRESH OR FROZEN)	FRUIT (FRESH OR FROZEN)	0,3
	0,1	UK Infant	0,1	Sugar beet (root)		FRUIT (FRESH OR FROZEN)	FRUIT (FRESH OR FROZEN)	0,1
	0,1	UK Adult	0,1	Sugar beet (root)		FRUIT (FRESH OR FROZEN)	FRUIT (FRESH OR FROZEN)	0,1
	0,1	UK vegetarian	0,1	Sugar beet (root)		FRUIT (FRESH OR FROZEN)	FRUIT (FRESH OR FROZEN)	0,1

ANNEX B

Addendum April 2009

Lenacil

B.9 Ecotoxicology

B.9.2 Effects on aquatic organisms (fish, aquatic invertebrates, algae) (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.12 Microcosm and mesocosm study (Annex IIIA 10.2.2)

During the Peer Review, the notifier submitted following position paper regarding the outdoor microcosm study.

Position paper in respect of Jenkins (2005): Lenacil (Venzar 80 % WP); Effects on primary productivity and macrophyte biomass in field-based microcosms (HLS report ACD 072/043691). (Don Bealing, 2009).

Introduction :

According to SANCO/3268/2001 rev. 4 (final), p. 31, "the NOEAEC *(from a microcosm or mesocosm study)* may be used for a direct comparison with the relevant PEC if uncertainty has been reduced considerably and the result of the study is relevant for decision making". Jenkins (2005): Lenacil (Venzar 80 % WP); Effects on primary productivity and macrophyte biomass in field-based microcosms (HLS report ACD 072/043691) describes an outdoor ditch mesocosm study whose primary focus was the impact of lenacil on phytoplankton and macrophyte communities. Since lenacil is a herbicide and the mesocosm study addresses the effects of exposure on the groups of organisms that drive the risk assessment, the result of the study is considered to be relevant for decision making.

The Ecotoxicology section of the evaluation table raises an open point relating to uncertainties associated with the mesocosm study. The Notifier's proposal for addressing these and the open point stated in the evaluation table is set out below.

1. Nominal and measured lenacil concentrations :

The application of Venzar 80 % WP to the ditch mesocosms was by spraying, rather than as a direct introduction beneath the water surface. However, the spraying was done at close range, with the nozzle held just above the water surface (Report ACD 072/043691, vol 1, p. 156, Plate 6) and drift losses should therefore have been minimal. Reported exposure concentrations and endpoints are expressed in terms of nominal target concentrations of lenacil in the water column. The first samples analysed for lenacil were taken 3 h post-application, but recoveries at that time amounted to between only < 25 % and 39 % of nominals. Maximum concentrations, corresponding to between 29 % and 58 % of nominals were measured in water samples taken at the next sampling point, on day 3 (Report ACD 072/043691, vol 2, p. 186, Table 2). It is uncertain whether the day-3 concentrations represent peak exposure or whether higher concentrations may have occurred, undetected, on days 1 and 2 when no samples were taken.

The reason(s) for the substantial shortfall between nominal and measured initial concentrations of lenacil in the water column are unknown. Samples of the spray mixes prepared for each treatment were analysed for lenacil and were generally between 86 % and 90 % of nominal. Spray deposits on plastic drift screens and liners temporarily fitted to shield the inner wall surfaces of the microcosms were rinsed down immediately after application. Spray deposits on emergent macrophyte foliage were left *in situ*, but leaf cover at the water surface at the time of application (Report ACD 072/043691, vol 1, p. 154, Plate 4) appears to have been insufficient to cause ≥ 60 % spray interception and is unlikely to account for the low recoveries at 3 h. Whilst it would appear that the mesocosms were correctly dosed and each received its intended Venzar 80 % WP application, the available data do not provide the necessary level of confidence that all the lenacil was dispersed throughout the water column at the same time.

In view of the large discrepancy between nominal and initial or peak measured concentrations of lenacil in the water column, the use of endpoints based on uncorrected nominal exposure appears untenable. At the reported NOEAEC of 22.1 µg a.s./L, the 3-h and day-3 measured concentrations were 7.66 and 10.17 µg a.s./L, respectively. The RMS' safety factor of ×3 is therefore sufficient to cover this uncertainty.

2. Application timing :

The study was performed in Cambridgeshire, UK (N-EU) and Venzar 80 % WP was applied to the ditch mesocosms at the end of July. This is a little later than guidance recommends and is also later than is implied by the GAP for Venzar 80 % WP: weed control by early-season treatment of sugar/fodder beet before the crop foliage has closed between rows.

However, earlier application of lenacil according to GAP may be expected to allow a longer, productive recovery period under field conditions than was available for the experimental mesocosms.

3. Key endpoint :

Charophyta
Superficially, *Charophyta* appears to have been the macrophyte species most sensitive to lenacil. However, *Charophyta* was not one of the species deliberately introduced when the mesocosms were initially stocked with macrophytes and its distribution was not carefully balanced between all replicates mesocosms ahead of the Venzar 80 % WP application. It was accidentally present in some replicates and not others and evidently also grew more vigorously in some replicates than in others, as indicated by the very wide variation between the individual control units (completely absent in one unit). Wherever present, wet and dry biomass weights for this species at study termination were significantly lower than the control in all lenacil-treated mesocosms, but the magnitude of this apparent effect showed no dose-relationship and it is unclear whether lower abundance and vigour of *Charophyta* in Venzar-treated replicates was a real effect of treatment or simply a reflection of its random distribution. In the notifier's opinion, the apparent effect on *Charophyta* is not sufficiently robust to provide the basis of the derivation of the key endpoint from the mesocosm study, though it seems appropriate to recognise that it does warrant accommodation in the overall safety factor applied to the key endpoint.

Elodea
Elodea canadensis was the most sensitive of the macrophyte species deliberately introduced to the ditch mesocosms: it was significantly and immediately affected, relative to the controls, in the 22.1 and 83.7 µg a.s./L (nominal) treatments. However recovery occurred within 8 weeks and this treatment therefore defined the no-observed-ecologically-adverse-effect-concentration (NOEAEC) of 22.1 µg a.s./L (nominal).

In the notifier's opinion, the NOEAEC of 22.1 µg a.s./L nominal serves as the key endpoint for the impact of lenacil on primary producer communities in aquatic systems, allowing for recovery. It should also be borne in mind that Venzar 80 % WP applications are confined to a relatively narrow window early in the growing season, which means that under practical use conditions the opportunity for recovery is longer than that which was available in the mesocosm study.

4. Multiple applications :

Some commentators have noted that the mesocosm study involved only one application, whereas agricultural practice may entail multiple applications, and have therefore questioned whether the outcome of the mesocosm study gives adequate protection for the multiple application use.

The GAP table for the use of Venzar 80 % WP in sugar/fodder beet includes a low-dose programme, where up to four applications (each at 125 g a.s./ha) may be made at minimum intervals of 7 days, timed to coincide with successive flushes of weed seedlings whilst at the most susceptible early pre-emergence growth stage.

The mesocosms were treated with a single application of Venzar 80 % WP according to a dose-response design, and with the selection of nominal dose levels unconnected to field application rates. The possible impact of multiple applications was not examined.

Nevertheless, taking account of the observed dissipation of lenacil from the water column of the microcosms, it is possible to make a rough estimate of the peak lenacil concentration in mesocosms of identical design and under the same conditions if they had been sprayed four times at a rate simulating the off-field (1 m) deposition corresponding to an in-field application of 125 g a.s./ha.

Parameter	Data source	
Ditch mesocosm length and width:	Report ACD 072/043691, vol 1, p. 21	1.8 m (l) and 0.9 m (w).
Final water depth after adjustment:	Report ACD 072/043691, vol 1, p. 21	0.3 m
Exposed water surface area:	Calculation	$(1.8 \times 0.9/10^4) = 1.62 \times 10^{-4}$ ha
Water volume:	Calculation	$(1.8 \times 0.9 \times 0.3 \times 10^3) = 490$ L
Worst-case off-field deposition rate at 1 m (1.85% of the field rate for field crops):	BBA (2000) basic drift values for four applications (74%-ile).	$125 \times 0.0185 = 2.3$ g a.s./ha
Deposition on water surface:	Calculation	$2.3 \times 1.62 \times 10^{-4} \times 10^3 =$ 0.373 mg a.s.
Concentration in water column after single application at 125 g a.s./ha field rate:	Calculation	$0.373 \times 10^3/490 = 0.76$ µg a.s./L
Observed dissipation between peak (day-3) measured concentrations and day-7 residues	Report ACD 072/043691, vol 1, p. 47	15 to 38%

Assuming a 15 % reduction (the lowest, most conservative figure observed in the mesocosm study) occurs during each 7-day interval between applications, the estimated peak lenacil concentrations in the water column immediately after each treatment are as follows:

After treatment #1: 0.76 µg a.s./L;
After treatment #2: $0.76 + (0.76 \times 0.85) = 1.41$ µg a.s./L;
After treatment #3: $0.76 + (1.41 \times 0.85) = 1.96$ µg a.s./L;
After treatment #4: $0.76 + (1.96 \times 0.85) = 2.43$ µg a.s./L.

Since lenacil is a photosynthesis-inhibitor herbicide, the ecotoxicologically relevant concentration is considered more likely to be the maximum peak predicted concentration (2.43 µg a.s./L) that follows the final application than a time-weighted average. Nevertheless, the peak concentration estimated to occur in mesocosms treated to simulate four spray-drift episodes is below the NOEAEC (nominal and peak measured) of the rate-response study. By coincidence, the estimated concentration equals the peak measured lenacil concentration at the reported no-observed-effect treatment (5.81 µg a.s./L, nominal, 2.43 µg a.s./L, day-3 measured).

Based on this comparison, therefore, the low-dose programme with up to four applications at minimum 7-day intervals appears to be accommodated within the rate-response mesocosm study. A large increment in the safety factor to address uncertainty over this aspect appears to be unjustified.

Conclusions of the notifier :

In conclusion, the Notifier proposes adoption of the reported NOEAEC of 22.1 µg a.s./L (nominal) as the key endpoint for the impact of lenacil on primary producer communities, based on the significant, but recoverable effects seen with *E. Canadensis* in the ditch mesocosm study. The Notifier proposes that this endpoint be applied in conjunction with a safety factor of ×5 to accommodate the adjustment required between nominal and measured exposure and, additionally to accommodate some uncertainty regarding inter-species sensitivity as well as exposure following multiple applications.

Conclusions of the RMS :
The RMS agrees with the conclusions of the notifier; the endpoint NOEAEC of 22.1 µg a.s./L is maintained and a safety factor of 5 in stead of 3 can be applied (nominal and measured exposure, inter-species sensitivity, multiple applications).
The RMS is of the opinion that effects were observed for Charophyta at lower doses, but these did not impair the functioning of the mesocosm.

ANNEX B

Lenacil

B.6 Toxicology and metabolism
Addendum Amended in May 2009

Preliminary note: Following the expert meeting round 14 (PRAPeR 69, 04-08.2009), the AOEL was revised upwards. In the proposal of the RMS, the AOEL was based on the 90d mouse NOAEL, which was reconsidered.
As a consequence, the AOEL was based upon the 90d rat NOAEL, which was 40.6 mg/kg b.w./d.
Applying a 100× AF, the AOEL was calculated on this basis: 40.6 ÷ 100 = 0.4 mg/kg b.w./d.

RMS provides an Addendum to the DAR with revised operator, worker and bystander exposure, recalculated taking into account the slightly altered input parameters (UK POEM version 2007) and the agreed AOEL by the meeting.
The altered values are highlighted in green.

B.6.15 Exposure data (Annex IIIA 7.2)

Venzar 80 WP is a wettable powder formulation containing 80% Lenacil as active substance. It is intended for application through hydraulic field crop sprayers to sugar beet. The recommended application rate is a maximum of 500 g a.s./hectare (625 g product/ha), in a minimum spray volume of 200 litres of water/hectare.

B.6.15.1 Estimation of operator exposure (Annex IIIA 7.2.1.1)

New estimations were realised by the RMS using agreed values of dermal absorption. Estimates of operator exposure are based on the UK Predictive Operator Exposure Model (POEM, version 2007), and the German model.

Data used for the calculation:

Crop type :	Field crops (sugar beet)
Method of application :	Tractor mounted hydraulic boom sprayer (UK POEM) Field crop(German model)
Area treated / day :	50 ha UK POEM ; 20 ha German model
Formulation :	80% WP
Rate of use :	500 g a.s. /ha
Water volume :	200 L/ha
Dermal absorption - concentrate :	1 %
- dilution :	15.5 %
AOEL	0.4 mg/kg bw/day
Operator body weight :	60 kg for UK model and 70 kg for German model

The water volume of 200 L/ha represents a minimum recommended volume and therefore provides the worst case scenario for the calculations.

Results of calculations according to the UK POEM or German model are given in Table 6.15.1-1 and 6.15.1-2.

Expected operator exposures:

Table B.6.15.1-1: Estimated operator exposure (mg/person/day) according to the UK POEM

Product/ Application method/ crop	**Dermal absorbed dose (mg/day)**			**Inhalation exposure (mg/ day)**			**Total exposure (mg /day)**
	Mix/load	**Spray**	**Total**	**Mix/load**	**Spray**	**Total**	
Tractor mounted/trailed boom sprayer ; hydraulic nozzles							
Dermal absorption 1% and 15.5%	3.4	16.1	19.5	5.25	0.15	5.4	24.9
Type of protection							
Gloves M/L + A	0.034	2.499	2.53	5.25	0.15	5.4	7.93

Table B.6.15.1-2: Estimated operator exposure (mg/person/day) according to the GERMAN Model

Product/ Application method/ crop	Dermal exposure (mg/day)			Inhalation exposure (mg/ day)			Total exposure (mg /day)
	Mix/load	Spray	Total	Mix/load	Spray	Total	
Tractor field crop							
Dermal absorption of 1 and 15.5%	60	20.4	80.4	0.7	0.01	0.71	81.11
Type of protection							
Gloves M/L + A	0.6	16.63	17.23	0.7	0.01	0.71	17.94

Comparison of estimated and tolerable exposure:

Table B.6.15.1-3: Exposure as a proportion of AOEL- POEM model.

Product/ Application method/ crop	Total systemic exposure – 60 kg person (mg/kg bw/day)		% of AOEL	
	no PPE worn	PPE worn*	no PPE worn	PPE worn
Tractor mounted/trailed boom sprayer ; hydraulic nozzles				
Dermal absorption of 1% and 15.5 %	0.415	0.132	104	33

* : Gloves M/L + A

Table B.6.15.1-4: Exposure as a proportion of AOEL –German model.

Product/ Application method/ crop	Total absorbed dose – 70 kg person (mg/kg bw/day)		% of AOEL	
	no PPE worn	PPE worn*	no PPE worn	PPE worn
Tractor mounted/trailed boom sprayer ; hydraulic nozzles				
Dermal absorption of 1% and 15.5 %	0.06388	0.0470	16	12

*: gloves M/L + A

Conclusions of RMS:

The application of Lenacil, formulated as Venzar 80 WP is safe for operators, even in the absence of PPE in the German model. However, as the a.s. is classified Carc. Cat. 3 (R40), the use of PPE will be recommended anyway.

B.6.15.2 Measurement of operator exposure (Annex IIIA 7.2.1.2)

Estimates from both the German BBA model indicate exposure to spray operatives to be below the $AOEL_{SYS}$ with and without the need for personal protective equipment. Therefore overall it can be concluded that operator exposure will be at an acceptable level when using Venzar 80 WP on sugar beet as recommended and studies to measure operator exposure are not required.

B.6.15.3 Estimation of bystander exposure (Annex IIIA 7.2.2)

New calculation made by the RMS taking into account a dermal absorption of 15.5%.
Bystanders present at the time of a pesticide application may be subject to dermal and inhalation exposure to the active substance resulting from vapours movement and spray drift. As Venzar 80 WP will only be used in outdoor situations and has a vapor pressure of 2.7×10^{-5} Pa at 25°C, exposure of bystanders is expected to arise primarily as a result of spray drift. The exposure of bystanders would be expected to be of a short/acute duration and unlikely to occur repeatedly to the same individuals.
An estimate of bystander exposure for a downwards spray application to field crops has been calculated based on a study by Lloyd and Bell, 1983. In this study, measurements of simulated bystander exposure were made during field crop spraying operations following a single pass of the sprayer with a bystander located 8 m from the edge of the treatment area.

For risk assessment purposes, the systemic AOEL has been used for comparison to potential exposure since this represents the internal absorbed dose.

Data used for the calculation

PDE = potential dermal exposure = 0.1 ml of spray solution at 8 m (Lloyd and Bell, 1983)
SSC = spray solution concentration (maximum in-use concentration) = 2.5 mg a.s. /ml
DA = dermal absorption (using the value for spray dilution) = 15.5%
AC = concentration of spray in the air = 0.02 ml of spray solution/m^3 (Lloyd and Bell, 1983)
BR = volume of air breathed/min (based on = 3.6 m^3/h) = 0.06 m^3/min
T = Duration of exposure = 5 minutes
BW = body weight = 60 kg

Bystander exposure calculations

$$\text{Systemic exposure} = \frac{(PDE \times SSC \times DA) + (AC \times SSC \times BR \times T)}{BW}$$

$$= \frac{(0.1 \times 2.5 \times 0.155) + (0.02 \times 2.5 \times 0.06 \times 5)}{60}$$

$$= \frac{0.039 + 0.015}{60} \text{ mg kg b.w./day}$$

$$= 0.00089 \text{ mg kg b.w./day}$$

This exposure is ca 0.225% of the $AOEL_{SYS}$ of 0.4 mg/kg bw/day. Therefore the risk to bystanders from exposure during field spraying with Venzar 80 WP is considered negligible.

Table B.6.15.1-5: Calculation of the Bystander exposure, according to the model of Lloyd and Bell.

Lloyd and Bell model: BYSTANDER exposure	**FIELD CROP**	
ACTIVE SUBSTANCE	**Lenacil**	
PRODUCT	**Venzar 80 WP**	
PARAMETERS		
	Dermal exposure	***Inhalation exposure***
Volume of spray solution dermally intercepted (mL)	0,1	-
Volume of spray solution intercepted by inhalation (mL/m^3)	-	0,02
Spray volume (L)	200	200
Breathing rate (m^3/hour)	-	3,6
Number of hours worked/day	-	0,08333
Application rate (g/ha)	500	500
Percent absorbed (%)	15,5	100
CALCULATIONS		
	Dermal exposure	***Inhalation exposure***
Dermal intercepted	0,000050%	
Inhalation intercepted		0,000002880%
Amount active intercepted (mg)	0,25000000	0,0149994
Absorbed dose (mg)	0,03875000	0,0149994
Bystander weight (kg)	60	60
Absorbed dose (mg a.s./kg bw/d)	0,0006458333	0,00024999
Total systemic	0,0008858333	
AOEL (mg/kg bw/d)	0,4	
Exposure as % of AOEL:	**0,225%**	

B.6.15.4 Estimation of worker exposure (Annex IIIA 7.2.3.1)

Worker exposure estimation is not required for an herbicide.

Some MSs commented on the need to provide an estimation of worker exposure as well as an estimation of re-entry exposure.
Estimation of re-entry exposure: based on the model as developed by the German BBA.

The following parameters were considered:

DFR	3 µg/cm² × kg a.s./ha
Transfer Factor	2500 cm^2/h
A (working period)	2 h/day
Penetration Factor clothing	1 (w/o PPE)
Application rate	0.5 kg a.s./ha
Dermal absorption	15.5%
Body weight	60 kg
Potential dermal exposure (µg a.s./person/day)	3 × 2500 × 2 × 1× 0.5= 7500 µg/worker/ hour = 125 µg/kg bw/hour = 0.125 mg/kg bw

Dermal absorbed dose (taking into account a dermal absorption of 15.5%)
= 0.019375 mg/kg bw/hour =4.8% of AOEL

For a worker exposed during a 8 hour period, the dermal absorbed dose would be = 0.0775 mg/kg bw/d= 19% of AOEL. However, as the intended use is post-harvest application in sugar beet (not harvested by hand) for now, this figure is of lesser concern.

B.6.15.5 Measurement of worker exposure (Annex IIIA 7.2.3.2)

No data, not necessary.

ANNEX B

Lenacil

Appendix: Estimation of the exposure

THE UK-POEM WITH GERMAN MODEL MIX/LOAD DATA (75th PERCENTILE)

Estimate 1: no gloves during neither Mixing/loading nor application

DERMAL EXPOSURE DURING MIXING AND LOADING				
Hand contamination/kg a.s.	13,6	mg/kg a.s.		
Hand contamination/day	340	mg/day		
Protective clothing	None			
Transmission to skin	100	%		
Dermal exposure to a.s.	340	mg/day		
INHALATION EXPOSURE DURING MIXING AND LOADING				
Inhalation exposure/kg a.s.	0,21	mg/kg a.s.		
Inhalation exposure/day	5,25	mg/day		
RPE	None			
Transmission through RPE	100	%		
Inhalation exposure to a.s.	5,25	mg/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	200	spray/ha		
Volume of surface contamination	10	mL/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	5%	15%	
Dermal exposure	6,5	0,05	0,375	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	41,55	mL/day		
Conc. of a.s. in spray solution	2,5	mg/ml		
Dermal exposure to a.s.	103,875	mg/day		
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure to spray	0,01	mL/h		
Duration of exposure	6	h		
Concentration of a.s. in spray	2,5	mg/ml		
Inhalation exposure to a.s.	0,15	mg/day		
Percent absorbed	100	%		
Absorbed dose	0,15	mg/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure to a.s.	340	mg/day	103,875	mg/day
Percent absorbed	1	%	15,5	%
Absorbed dose (dermal route)	3,4	mg/day	16,100625	mg/day
Inhalation exposure to a.s.	5,25	mg/day	0,15	mg/day
Absorbed dose	8,65	mg/day	16,250625	mg/day
PREDICTED EXPOSURE				
Total absorbed dose	24,900625	mg/day		
Operator body weight	60	kg		
Operator exposure	0,415010417	mg/kg bw/day		
AOEL	0,4			
% of AOEL	103,75%			

THE UK-POEM WITH GERMAN MODEL MIX/LOAD DATA (75th PERCENTILE)

Estimate 2: Gloves during both Mixing/loading and application

DERMAL EXPOSURE DURING MIXING AND LOADING				
Hand contamination/kg a.s.	13,6	mg/kg a.s.		
Hand contamination/day	3,4	mg/day		
Protective clothing	Gloves			
Transmission to skin	1	%		
Dermal exposure to a.s.	3,4	mg/day		
INHALATION EXPOSURE DURING MIXING AND LOADING				
Inhalation exposure/kg a.s.	0,21	mg/kg a.s.		
Inhalation exposure/day	5,25	mg/day		
RPE	None			
Transmission through RPE	100	%		
Inhalation exposure to a.s.	5,25	mg/day		
DERMAL EXPOSURE DURING SPRAY APPLICATION				
Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	200	spray/ha		
Volume of surface contamination	10	mL/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	5%	15%	
Dermal exposure	0,65	0,05	0,375	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	6,45	mL/day		
Conc. of a.s. in spray solution	2,5	mg/ml		
Dermal exposure to a.s.	16,125	mg/day		
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure to spray	0,01	mL/h		
Duration of exposure	6	h		
Concentration of a.s. in spray	2,5	mg/mL		
Inhalation exposure to a.s.	0,15	mg/day		
Percent absorbed	100	%		
Absorbed dose	0,15	mg/day		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure to a.s.	3,4	mg/day	16,125	mg/day
Percent absorbed	1	%	15,5	%
Absorbed dose (dermal route)	0,034	mg/day	2,499375	mg/day
Inhalation exposure to a.s.	5,25	mg/day	0,15	mg/day
Absorbed dose	5,284	mg/day	2,649375	mg/day
PREDICTED EXPOSURE				
Total absorbed dose	7,933375	mg/day		
Operator body weight	60	kg		
Operator exposure	0,132222917	mg/kg bw/day		
AOEL	0,4			
% of AOEL	33,06%			

GERMAN MODEL:
Estimate 3: Gloves during both Mixing/loading and application

Product	VENZAR 80 WP	Active substance	LENACIL		
Formulation type	WP	a.s. concentration	800 mg/mL		
Method of use	Tractor field crops	Dose(product)	0.625 kg product/ha		
Work rate	20 ha/day	Dose (a.s.)	0.5 kg a.s./ha		
		Amount handled	10 kg a.s./day		
Exposures-mix/loading					
	Specific exposures	Estimated exposures	PPE	Estimated exposures	
Inhalation	0.07 mg/kg a.s.handled	0.7 mg a.s./day	None	0.7 mg a.s./day	
Dermal-hands	6 mg/kg a.s.handled	60 mg a.s./day	gloves	0.6 mg a.s./day	
Exposures-application					
	Specific exposures	Estimated exposures	PPE	Estimated exposures	
Inhalation	0.001 mg/kg a.s.handled	0.01 mg a.s./day	None	0.01 mg a.s./day	
Dermal –head	0.06 mg/kg a.s.handled	0.6 mg a.s./day	None	0.6 mg a.s./day	
Dermal –hands	0.38 mg/kg a.s.handled	3.8 mg a.s./day	Gloves	0.038 mg a.s./day	
Dermal –body	1.6 mg/kg a.s.handled	16 mg a.s./day	None	16 mg a.s./day	
Total exposures		Estimated exposures	Percent	Estimated exposures	
Total potential		0.71 mg a.s./day	100%	0.71 mg a.s./day	
Total dermal-		60 mg a.s./day	1%	0.6 mg a.s./day	
Total dermal-		20.4 mg a.s./day	15.5%	16.63 mg a.s./day	
Total absorbed		4.472 mg a.s./day		3.294 mg a.s./day	
Body weight		70 kg		70 kg	
Mg/kg bw/d		0.06388 mg/kg bw/d		0.04706 mg/kg bw/d	
AOEL		0.4		0.4	
% of AOEL		15,87%		11.77%	