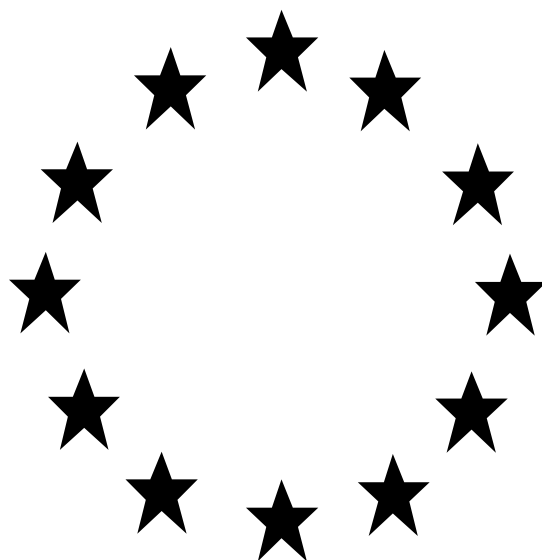




Draft Assessment Report (DAR)

- public version -

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

CYMOXANIL

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

Volume 3, Annex B, part 4, B.8

October 2007

Annex B

Cymoxanil

B.8 Fate and behaviour in the environment

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WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.8 Fate and behaviour in the environment

Background information

The Annex II dossier for **cymoxanil** is prepared collectively (shared) by E.I. du Pont de Nemours and Company (DuPont) and Oxon Italia S.p.A (Oxon). Therefore, available Annex II data of both companies are handled as a collective irrespective of the notifier. Annex III data were prepared and handled separately by both notifiers for the formulations TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon).

Cymoxanil is a cyanoacetamide that is chemically unrelated to any other commercial disease control agent.

Cymoxanil is intended for use as an agricultural and horticultural fungicide for control of pathogens belonging to the order Peronosporales, namely *Phytophthora*, *Plasmopara* and *Peronospora* spp., which cause downy mildew and blight in a wide range of crops such as lettuce, potatoes, tomatoes and grapes.

Representative formulation

Cymoxanil may be formulated as a wettable powder, water-dispersible granule or suspension concentrate alone or in combination with other fungicidal active substances. In the EU it is applied post emergence to target crop via tractor-mounted, ground directed broadcast sprayers or airblast high volume sprayers.

Table B.8.1-1: Intended uses of cymoxanil as TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon).

Crop	Member state or country	Product name	Use	Method	BBCH code	Crop interception [%] ^a	Number of applications	Application rate [g ai ha ⁻¹]	Interval [d]	Notifier
Lettuce	South EU	CYMOXANIL 50 % WP	F	Spray	40 - 49	70	3 – 4	240	7	Oxon
Potato	South EU	CYMOXANIL 50 % WP	F	Spray	21 - 95	50 - 70	5	120	7	Oxon
		TANOS 50 WG ^b	F	Spray	21 - 95	50 - 70	6 – 8	175	7 – 10	DuPont
	North EU	CYMOXANIL 50 % WP	F	Spray	21 - 95	50 - 70	4	120	7 – 10	Oxon
		TANOS 50 WG ^b	F	Spray	21 - 95	50 - 70	6 – 8	175	7 – 10	DuPont

^a Default FOCUS values according to BBCH codes

^b TANOS 50 WG = DPX KP481 50 WG (cymoxanil/famoxadone 250/250 g/kg)

Labelling

Cymoxanil, radio-labelled exclusively in the cyanoacetamide-2 position, was used to evaluate the fate of the active substance in the environment. The ^{14}C radiolabel position was chosen as the most stable position in the cymoxanil molecule as indicated in Figure B.8-1.

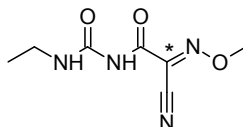


Figure B.8-1: Radio-labelling of cymoxanil used for environmental fate studies.

Labelling in the cyanoacetamide-2 position is not capable to follow the fate of ethyl urea, which is likely to be released together with metabolite IN-W3595 owing to hydrolytic cleavage of the parent molecule. However, ethyl urea and possible degradation products of ethyl urea e.g. urea or amino ethane are considered non-relevant compounds according to SANCO/221/2000, rev. 10 (2003) guidance document on the assessment of the relevance of metabolites in groundwater.

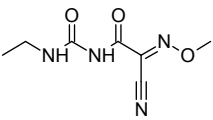
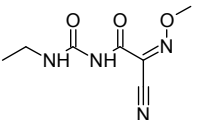
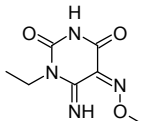
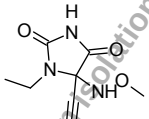
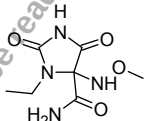
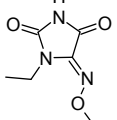
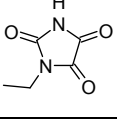
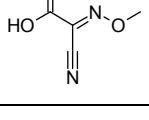
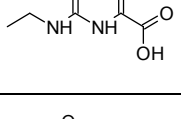
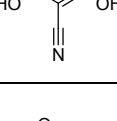
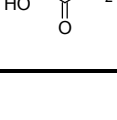
Isomerism

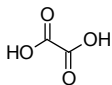
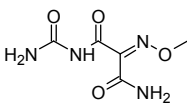
Cymoxanil has an O-alkyloxime group which may be present either in the E or in the Z configuration. O-alkyloxime isomers are known to be relatively stable but may switch to each other e.g. under the influence of light. Cymoxanil exclusively represents the E-configuration which is also considered to be the more favourable structure owing to sterical reasons. The Z-isomer (IN-Q8761) is handled as metabolite. Standards of both isomers were available and used in one water/sediment study (Trabue and Lydick, 2001) and in the soil photolysis study (Berg, 1996). In the water/sediment study, there was no evidence that the E isomer switched to the Z isomer. In the photolysis study, minor formation (< 7 % of AR) of the Z isomer was observed under irradiation but also in dark samples (no significant difference between both treatments). In general, formation of the Z-isomer could not be linked to any environmental impact. Therefore, the Z-isomer (IN-Q8761) of cymoxanil is considered of no concern in the environmental fate assessment.

This E/Z isomerism is also true for all metabolites, which still carry the O-alkyloxime group, these are IN-U3204, IN-R3273, IN-W3595, IN-R3274 and one identified compound of metabolite fraction M5. The isomer stability and the ratio of the E/Z isomers of these metabolites under environmental conditions are unknown. Since no further information on the isomer ratio of these compounds is available, E/Z isomerism was not considered further for environmental risk assessment.

The metabolites IN-JX915 and IN-KQ960 exist as two stereo isomers (R/S configuration). In case of abiotic cyclisation reactions (hydrolysis), both isomers are likely to be formed at almost equal molar rates (1/1) since no steric preference can be deduced from the molecular structure. The ratio of R/S formation is unknown under the assumption of biotic (enzyme driven) reactions (there might be a steric preference for one of the two isomers owing to enzyme-substrate-complexes). However, from soil degradation and water/sediment studies, it has to be assumed that formation of IN-JX915 and IN-KQ960 is predominately driven by abiotic processes (hydrolysis) and both compounds are expected to be present at a molar ratio of approx. 1/1 under environmental conditions. Since no further information on the isomer ratio of these compounds is available, stereo isomerism was not considered further for environmental risk assessment.

Table B.8-2: Overview on parent compound, metabolites and identified degradates mentioned in the section 'fate and behaviour in the environment'.

Code	Chemical name	Chemical structure	Mass [g mol ⁻¹]	Occurrence (maximum amount observed) [% of AR]
Cymoxanil, DPX-T3217	2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide (E-configuration)		198.2	Parent
IN-Q8761	2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide (Z-configuration)		198.2	Lab. soil: - Soil photolysis: 6.2 ^a Hydrolysis: - Photolysis: - Water/sediment: -
IN-U3204	1-ethyl-6-iminodihydropyrimidine-2,4,5(3H)-trione 5-(O-methyloxime) (E-configuration)		198.2	Lab. soil: 24.7 Soil photolysis: 2.2 Hydrolysis (pH 7): 52.7 Photolysis (pH 5): 0.6 Water/sediment: 24.7
IN-JX915	3-ethyl-4-(methoxyamino)-2,5-dioximidazolidine-4-carbonitrile (stereomer racemate)		198.2	Lab. soil: 7.6 Soil photolysis: 10.9 Hydrolysis (pH 7): 7.2 Photolysis (pH 5): 52.6 Water/sediment: 8.5
IN-KQ960	3-ethyl-4-(methoxyamino)-2,5-dioximidazolidine-4-carboxamide (stereomer racemate)		216.2	Lab. soil: 6.3 Soil photolysis: < 1.0 Hydrolysis (pH 7): 9.0 Photolysis (pH 5): - Water/sediment: 14.3
IN-R3273	1-ethylimidazolidine-2,4,5-trione 5-(O-methyloxime) (E-configuration)		171.2	Lab. soil: 2.4 Soil photolysis: < 1.0 Hydrolysis (pH 7): 10.2 Photolysis (pH 5): 35.4 Water/sediment: 5.0
IN-T4226	1-ethylimidazolidine-2,4,5-trione		142.1	Lab. soil: 1.7 Soil photolysis: 1.2 Hydrolysis (pH 7): 5.4 Photolysis (pH 5): 6.7 Water/sediment: 12.0
IN-W3595	Cyano(methoxyimino)acetic acid (E-configuration)		128.1	Lab. soil: 10.1 Soil photolysis: 2.3 Hydrolysis (pH 7): 22.6 Photolysis (pH 5): - Water/sediment: 27.5
IN-KP533	{[(ethylamino)carbonyl]amino} (oxo)acetic acid		160.1	Lab. soil: 2.7 Soil photolysis: < 1.0 Hydrolysis (pH 7): 57.4 Photolysis (pH 5): 7.9 Water/sediment: 26.0 ^b
IN-R3274	Cyano(hydroxyimino)acetic acid		114.1	Lab. soil: < 2.6 Soil photolysis: - Hydrolysis (pH 7): - Photolysis (pH 5): - Water/sediment: ?
IN-18474^c	Amino(oxo)acetic acid, oxamic acid		89.1	Lab. soil: 7.8 Soil photolysis: 4.4 Hydrolysis (pH 7): < 10 Photolysis (pH 5): 2.0 Water/sediment: ?

Code	Chemical name	Chemical structure	Mass [g mol ⁻¹]	Occurrence (maximum amount observed) [% of AR]
Oxalic acid^c	Oxalic acid		90.0	Lab. soil: < 2.6 Soil photolysis: - Hydrolysis (pH 7): < 10 Photolysis (pH 5): - Water/sediment: ?
Metabolite fraction M5^d	N-(aminocarbonyl)-2-(methoxyimino)malonamide (E-configuration)		188.1	Lab. soil: - Soil photolysis: - Hydrolysis (pH 7): - Photolysis (pH 5): - Water/sediment: 22.9

^a Also observed in photolysis dark control samples with 6.9 % of AR, not considered relevant for environmental risk assessment.

^b In the water/sediment study from Slangen and Willems (2000) IN-KP533 is part of the highly polar HPLC fraction M1 which is likely to additionally comprise IN-W3595, IN-R3274, oxamic acid (IN-18474) and oxalic acid. Individual amounts of these compounds are not known, with the exception of IN-W3595. Therefore, the sum of these compounds, with the exception of IN-W3595, is used as worst-case assessment.

^c According to SANCO/221/2000, rev. 10 (2003) a compound of no concern owing to molecular structure

^d Metabolite fraction M5 (observed in the water/sediment study from Slangen and Willems, 2000) comprises at least 2 compounds, one is considered to be represented by the structure shown in the table, the other compound(s) is/are unknown

Table B.8-3: Summary on metabolites of cymoxanil [maximum occurrence in % of AR, mean of replicates] considered relevant for environmental risk assessment (PEC calculations).

Compartment	Soil	Groundwater	Surface water	Sediment
Trigger	> 10 %	> 10 % or 2 consecutive samples > 5 % or steady increase	> 10 % of AR	> 10 % of AR
IN-U3204	24.7	24.7	24.7	<
IN-W3595	10.1	10.1	27.5	<
IN-JX915	10.9 ^a	7.6	52.6 ^b	<
IN-KQ960	<	6.3	14.3	<
IN-T4226	<	<	12.0	<
IN-R3273	<	<	35.4 ^b	<
IN-KP533	<	<	26.0 ^c	<
Metabolite fraction M5	-	-	22.9	<

< denotes 'observed below the trigger'

- denotes 'not observed'

^a Observed > 10 % of AR in soil photolysis study only

^b Observed > 10 % of AR in water photolysis study only

^c Worst-case assessment, individual amounts of co-eluting compounds not known

WARNING: This document forms part of an EC evaluation and should not be used for other purposes.

B.8.1 Route of degradation (Annex IIA, point 7.1.1.1)

B.8.1.1 Aerobic degradation (Annex IIA, point 7.1.1.1.1)

The route of degradation (metabolism) of cymoxanil in soil was established in 4 laboratory studies (which were also used for degradation rate calculations):

- Aerobic degradation of cymoxanil in one soil (20 °C) – Oxon
- Aerobic degradation of cymoxanil in one soil (25 °C) – DuPont
- Aerobic degradation of cymoxanil in one soil under viable and sterile conditions (25 °C) – DuPont
- Aerobic degradation of cymoxanil in four soils (20 °C) – DuPont

Reference: Cymoxanil aerobic soil metabolism (route of degradation)

Author(s), year:	Aikens P. J., 1998
Report/Doc. number:	OXN 224/982398
Guideline(s):	SETAC (1995), EEC Commission Directive 95/36/EC (1995)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, 2.62 MBq mg ⁻¹ , > 99 % radiochemical purity (TLC), batch CFQ9873
Reference substances:	Cymoxanil (non-labelled)
Experimental system:	Moisture adjusted soil (40 % of MWHC), 250 g d.w. equivalent, sieved to 2 mm
Equilibration time:	7 days
Test concentration:	0.19 mg ai kg ⁻¹ (in 5 mL of high purity water), equivalent to 143 g ai ha ⁻¹ assuming a soil depth of 5 cm and a soil density of 1.5 g cm ⁻³
Co-solvent:	None
Incubation conditions:	20 ± 2 °C, dark, 90 days
Volatile traps:	2-(2-ethoxyethoxy)ethanol (1x), 1 M KOH (1x), 2-aminoethanol/2-ethoxyethanol (1/3, v/v) (1x), identity of ¹⁴ CO ₂ confirmed by precipitation
Sample processing:	Soil samples were extracted three times with acetonitrile/water pH 4.4 (4/1, v/v), four times with acetonitrile/water pH 4.4 (1/1, v/v) and finally refluxed one time with 2-aminoethanol. Soil extracts were individually radio-assayed by LSC, combined and analysed by HPLC and by TLC after sample concentration (Rotavapor). Sub-samples of soil debris remaining after extraction were separated into humin, humic acid and fulvic acid fractions. Remaining soil debris was radio-assayed by combustion LSC.
Analytical techniques:	LSC, HPLC-UV/RAD (fraction collector), TLC (including methods <i>TLC1</i> , called 'C' in the study report and <i>TLC2</i> , called 'B') Note: <i>TLC1</i> and <i>TLC2</i> were re-investigated by Brands and Van Noorloos (2006) to determine the R _f /RT values of several cymoxanil metabolites (for details refer to hydrolysis section, B.8.7).
Additional experiments:	A pilot study (identical application rate) was carried out prior to the main study to provide samples for analytical method development and to gain some knowledge of the decay rate of the substance.
Kinetic evaluation:	None

Table B.8.1.1-1: Soil Characteristics.

Soil	'Arrow'
Batch	18/797A
Location	UK
Texture (USDA)	Sandy loam
Sand (USDA) [%]	61.7
Silt (USDA) [%]	25.8
Clay (USDA) [%]	12.6
Organic C [%]	2.1
pH	6.0
CEC [meq kg ⁻¹]	170
WHC (maximum) [%]	46.4
Microbial biomass ^a [µg C _{mic} g ⁻¹] - start / 40 DAT / 86 DAT	197 / 261 / - ^b
Microbial biomass ^a [% C _{org}] - start / 40 DAT / 86 DAT	0.94 / 1.24 / - ^b

^a Fumigation extraction method

^b Measurement of microbial biomass by 86 DAT failed

Findings:

Overall findings from the pilot study (identical application rate) were close to the main study. Therefore, in the following section only data from the main study are presented.

Radiochemical purity of the test substance in the application solution was > 97 %.

Total mean recoveries of radioactivity were in the range of 91.2 – 101.3 % of AR. Total extractable radioactivity declined to a mean of 2.6 % of AR by 90 DAT. NER rapidly increased to a mean of 50.3 % of AR by 2 DAT declining thereafter to 38.7 % of AR by 90 DAT. The humin fraction contained the majority of the radioactive residue in all samples except that measured by 0.5 DAT. Formation of ¹⁴CO₂ accounted for 35.8 % of AR by 1 DAT, further increasing to 56.7 % of AR by study termination (90 DAT). Formation of organic volatiles was negligible.

After application cymoxanil was rapidly degraded with amounts falling from 86.9 % of AR (mean) at application to 9.8 % of AR by 0.5 DAT, degradation of cymoxanil continued to a mean of 2.1 % of AR by 2 DAT. Owing to the rapid decline DT₅₀ of cymoxanil was estimated to be less than 0.5 days. The DT₅₀ could not be calculated. Four minor radioactive degradation products of cymoxanil, each representing less than 6 % of AR, were quantified by TLC¹. All these unidentified metabolite fractions were highly transient, by 2 DAT no unidentified metabolite fractions was observed > 3 % of AR.

Table B.8.1.1-2: Distribution of radioactivity [% of AR] after application of cymoxanil (0.19 mg ai kg⁻¹) to 'Arrow' soil (mean of duplicate samples).

DAT	Total extracted	NER	CO ₂	Other volatiles	Total
0	99.0	2.4	na	na	101.3
0.5	25.9	40.9	28.1	0.07	94.9
1	15.3	40.5	35.8	< 0.05	91.7
2	10.0	50.3	40.5	< 0.05	100.7
5	6.0	40.8	47.0	< 0.06	93.8
10	5.0	40.1	47.9	< 0.07	93.0
20	4.4	36.5	49.9	< 0.07	91.2
40	3.2	37.4	53.3	< 0.07	93.8
61	3.0	37.0	57.2	< 0.07	97.0
90	2.6	38.7	56.7	< 0.07	98.0

na denotes not applicable

Table B.8.1.1-3: Characterization of total extractable radioactivity [% of AR] after application of

cymoxanil (0.19 mg ai kg⁻¹) to 'Arrow' soil (TLC1).

DAT	Replicate	Cymoxanil	Undefined regions				Others
			1	2	3	4	
0	A	88.8	4.4	1.8	4.5	0.0	0.4
	B	85.0	5.1	1.6	6.4	0.0	0.0
0.5	A	11.0	1.1	2.2	6.3	5.3	1.4
	B	8.7	0.7	2.7	5.1	6.5	0.9
1	A	3.4	0.8	2.3	4.7	4.2	0.0
	B	3.5	0.9	1.8	4.4	4.4	0.4
2	A	1.9	0.4	1.6	2.8	2.3	1.1
	B	2.3	0.2	1.7	3.0	2.6	0.0
R _f values	-	0.56	0.66	0.74	0.82	0.89	-

Table B.8.1.1-4: Characterization of NER [% of AR] after application of cymoxanil (0.19 mg ai kg⁻¹) to 'Arrow' soil.

DAT	Humins	Humic acid	Fulvic acid
0.5	13.9	4.3	20.9
1	20.1	4.9	15.0
2	26.9	7.1	19.7
5	19.7	5.9	16.2
10	21.6	6.5	14.0
20	19.0	6.0	11.7
40	17.6	6.2	11.3
61	19.6	5.8	12.1
90	20.9	7.9	12.2

Conclusion:

A rapid degradation of the active substance was observed under aerobic conditions. The major degradation products were CO₂ (56.7 % of AR by 90 DAT) and NER, which reached maximum amounts by 2 DAT (50.3 % of AR), decreasing to 38.7 % of AR by 90 DAT. The humin fraction contained the majority of the NER. Minor polar products identified by TLC did not exceed 6 % of AR each (mean) and dropped below 3 % of AR by 2 DAT.

Comments (RMS):

- DegT₅₀ and DegT₉₀ of the active substance were recalculated by the notifiers and the RMS and are summarized in B.8.2.6 (Malekani, 2003).
- Based on mean values of replicates, the undefined TLC1 metabolite fraction 'region 3' (5.5 % and 5.7 % of AR by 0 and 0.5 DAT) slightly exceeds the trigger for groundwater risk assessment ('2 consecutive sample points > 5 % of AR'). However, it is unlikely that this unknown and short living fraction (DT₅₀ approx. 1.5 days) is not covered by identified metabolites observed in the subsequent soil metabolism studies. Based on Brands and Van Noorloos (2006), the unidentified regions are considered at least partly identical to the following commonly observed soil metabolites of cymoxanil:
 - Region 1 (R_f = 0.66, DT₅₀ approx. 0.3 days): IN-U3204, IN-JX915, IN-R3273
 - Region 2 (R_f = 0.74, DT₅₀ approx. 2.6 days): IN-T4226
 - Region 3 (R_f = 0.82, DT₅₀ approx. 1.5 days): IN-KQ960
 - Region 4 (R_f = 0.89, DT₅₀ approx. 1.2 days): IN-W3595, IN-KP533 and other polars

Reference: Aerobic soil metabolism of ¹⁴C-cymoxanil

Author(s), year: Anderson, J. J., 2001
Report/Doc. Number: AMR 3438-95, Revision No. 1
Guideline(s): US EPA 162-1 (1982)
GLP: Yes
Deviations: None
Validity: Study considered acceptable

Material and methods:

Test substance: [Cyanoacetamide-2-¹⁴C]-cymoxanil, 2.82 MBq mg⁻¹, > 97 % radiochemical purity (HPLC), batch HOTC 425
Reference substances: Cymoxanil (non-labelled), IN-R3274, oxamic acid (IN-18474), oxalic acid
Experimental system: Moisture adjusted soil (75 % of 1/3 bar), 50 g d.w. equivalent, sieved to 2 mm
Equilibration time: Not stated in the report
Test concentration: 1.2 mg ai kg⁻¹ (in 60 µL of acetonitrile), equivalent to 900 g ai ha⁻¹ assuming a soil depth of 5 cm and a soil density of 1.5 g cm⁻³
Incubation conditions: 25 ± 2 °C, dark, 92 days
Volatile traps: 1 M NaOH (5x) and ethane-1,2-diol (1x), identity of ¹⁴CO₂ confirmed by precipitation
Sample processing: Soil samples were extracted twice with 100 mL of methanol/water pH 4.6 (80/20, v/v), followed by multiple extraction using methanol/water pH 4.6 (50/50, v/v). Soil extracts were individually radio-assayed by LSC, afterwards combined, concentrated using cell evaporation (TurboVap, 30 °C), radio-assayed (LSC) and analysed by HPLC. Remaining soil debris were radio-assayed by combustion LSC.
Analytical techniques: LSC, HPLC-UV/RAD (fraction collector)
Additional experiments: None
Kinetic evaluation: Parent: First order multi compartment (FOMC) kinetics (ModelManager 1.1)

Table B.8.1.1-5: Soil Characteristics.

Soil	'Sassafras'
Location	Penns Grove, New Jersey, USA
Texture (USDA)	Sandy loam
Sand (USDA) [%]	73.2
Silt (USDA) [%]	18.0
Clay (USDA) [%]	8.8
Organic C [%]	0.46
pH	6.4
CEC [meq kg ⁻¹]	38
WHC (1/3 bar) [%]	8.1
Bacterial biomass [10 ⁶ CFU g ⁻¹] start / end	5.1 / 11.0
Fungal biomass [10 ⁶ CFU g ⁻¹] start / end	2.0 / 5.4

Findings:

Total mean recovery of radioactivity was in a range of 86.0 – 114.9 % of AR.

Formation of ¹⁴CO₂ accounted for 60.4 % of AR by study termination (92 DAT), NER peaked with a maximum of 36.8 % of AR by 2 DAT, decreasing thereafter to 22.1 % of AR by 92 DAT.

Cymoxanil rapidly declined to less than 27 % of AR by 2 DAT. Degradation of cymoxanil was not following SFO kinetics, according to FOMC kinetics, DT₅₀ of cymoxanil was determined to be 1.2 days with a DT₉₀ of

18.3 days. Amounts of polars increased to about 8.0 % of AR by 0.33 DAT, steadily decreasing thereafter. Using a second HPLC system, this polar fraction was found to be composed of several compounds, all < 2.6 % of AR. RT values were partly consistent with those of reference standards oxalic acid and IN-R3274.

Table B.8.1.1-6: Distribution of radioactivity [% of AR] after application of cymoxanil (1.2 mg ai kg⁻¹) to 'Sassafras' soil (mean of duplicate samples).

DAT	Extracted	NER	CO ₂	Total recovery
0	101.0	2.4	nd	103.3
0.17	92.8	6.0	1.7	100.4
0.33	83.2	12.6	5.4	101.2
1	72.1	13.1	9.7	94.8
2	36.0	36.8	24.0	96.7
3	34.2	33.4	31.9	99.5
6	48.3	25.4	41.3	114.9
9	21.6	33.2	49.3	103.9
13	15.3	33.1	53.5	102.0
21	10.8	31.7	56.4	98.8
30	4.8	28.6	58.7	92.0
59	4.3	28.5	60.0	92.7
92	3.5	22.1	60.4	86.0

nd denotes not analysed

Table B.8.1.1-7: Characterization of extractable radioactivity [% of AR] after application of cymoxanil (1.2 mg ai kg⁻¹) to 'Sassafras' soil (HPLC).

DAT	Replicate	Polars ^a	Cymoxanil	Others
0	A	nd	100.6	nd
	B	nd	101.3	nd
0.17	A	2.7	88.2	nd
	B	nd	94.6	nd
0.33	A	8.9	74.1	0.0
	B	7.1	76.3	nd
1	A	8.6	61.9	0.2
	B	5.6	63.4	4.5
2	A	4.5	24.5	1.7
	B	5.8	29.6	5.8
3	A	3.8	24.4	1.1
	B	6.3	30.9	2.1
6	A	2.3	73.6	1.9
	B	3.7	15.0	0.1
9	A	3.4	5.4	nd
	B	7.4	26.7	0.2
13	A	6.2	2.6	nd
	B	6.9	14.9	nd
21	A	4.1	3.5	0.1
	B	6.4	7.1	0.2
30	A	3.4	3.4	nd
	B	2.2	2.2	0.9

nd denotes not detectable (< 1 % of AR)

^a Polar compounds including oxalic acid and IN-R3274, shown to be < 2.6 % of AR individually using a second HPLC system (at least 6 peaks)

Table B.8.1.1-8: Calculated DT₅₀ and DT₉₀ of cymoxanil in 'Sassafras' soil after application of 1.2 mg ai kg⁻¹ (FOMC kinetics).

Soil	Compound	DT ₅₀ [days]	DT ₉₀ [days]	R ²	Kinetics
'Sassafras'	Cymoxanil	1.2	18.3	0.86	FOMC

Conclusion:

A rapid degradation of the active substance was observed under aerobic conditions. Following FOMC kinetics, DT₅₀ of cymoxanil was estimated to be 1.2 days with a DT₉₀ of 18.3 days. The major degradation products were CO₂ (60.4 % of AR by 92 DAT) and NER, which reached maximum amounts by 2 DAT (36.8 % of AR), declining to 22.1 % of AR by 92 DAT. Minor polar products (total max. 8.0 % of AR by 0.33 DAT) including oxalic acid, IN-R3274 and unidentified substances did not exceed 2.6 % of AR each.

Comments (RMS):

- DegT₅₀ and DegT₉₀ of the active substance were recalculated by the notifiers and the RMS and are summarized in B.8.2.6 (Malekani, 2003).

Reference: Aerobic soil metabolism of [2-¹⁴C]DPX-T3217 (cymoxanil)

Author(s), year: Boucher, C. R., 1993
 Report/Doc. number: AMR 1988-91
 Guideline(s): US-EPA 161-2 (1982), BBA IV 4-1 (1986)
 GLP: Yes
 Deviations: None
 Validity: Study considered acceptable

The material and methods, analytical methods, findings and conclusions are included in the following supplement report:

Reference: Aerobic soil metabolism of [2-¹⁴C]DPX-T3217 (cymoxanil)

Author(s), year: Major, L. J., 1993
 Report/Doc. number: AMR 1988-91, Supplement No. 1
 Guideline(s): US-EPA 161-2 (1982), BBA IV 4-1 (1986)
 GLP: Yes
 Deviations: None
 Validity: Study considered acceptable

Material and methods:

Test substance: [Cyanoacetamide-2-¹⁴C]-cymoxanil, 0.52 MBq mg⁻¹, > 95 % radiochemical purity, batch HOTC 376
 Reference substances: Cymoxanil (non-labelled), IN-U3204, IN-R3273, IN-W3595, IN-KQ960, oxamic acid (IN-18474)
 Experimental system: Moisture adjusted soil (50 % of MWHC), 50 g d.w. equivalent, sieved to 2 mm
 • Viable conditions
 • Sterile conditions (sterilized at 120 °C for 60 min using an autoclave unit)
 Equilibration time: Not mentioned in the report, soil freshly sampled
 Test concentration: 5.0 mg ai kg⁻¹ (in 2.82 mL water/acetone, ~ 9/1, v/v), equivalent to 3750 g ai ha⁻¹ assuming a soil depth of 5 cm and a soil density of 1.5 g cm⁻³
 Incubation conditions: 25 ± 2 °C, dark, 15 days
 Volatile traps: 1 M NaOH (100 mL, 1x)
 Sample processing: Soil samples were extracted twice with 100 mL of methanol/water (95/5, v/v),

followed by multiple extraction using methanol/water (50/50, v/v) until no more radioactivity could be extracted. Soil extracts were individually radio-assayed (LSC), combined and analysed after sample concentration (Rotavapor, 35 °C) by HPLC. Metabolite identification and confirmation was obtained through LC-MS. Remaining soil debris was radio-assayed by combustion LSC.

Analytical techniques: LSC, HPLC-UV/RAD (fraction collector), LC-MS, GC-MS

Additional experiments: Supplemental report: Metabolite fraction B and D were collected from sterile soil samples by multi-injection onto the HPLC system. Isolates were analyzed by HPLC, TLC and LC/MS. Metabolite B required derivatization (silylation with MSTFA) and analysis by GC/MS.

Kinetic evaluation: Parent: Simple first order (SFO) kinetics, data transformation (ln-transformation of amounts observed)

Table B.8.1.1-9: Soil Characteristics.

Soil	'Black Andosol'
Location	Ushiku, Ibaraki, Japan
Texture (USDA)	Sandy clay loam ^a
Sand (USDA) [%]	50.0
Silt (USDA) [%]	20.2
Clay (USDA) [%]	28.4
Organic C [%]	2.01
pH	6.8
CEC [meq kg ⁻¹]	225
WHC (maximum) [%]	83.6
Microbial biomass start / end	nd / nd

nd denotes not determined

^a Designated as 'light clay' according to Japanese soil classification

Findings:

Total mean recoveries of radioactivity were in the range of 71.4 – 100.9 % of AR in the viable and 93.7 – 102.2 % of AR in the sterile samples. Lower recovery in non-sterile samples could be explained by insufficient trapping of ¹⁴CO₂, since only one trap was used per sample.

Viable conditions:

Formation of ¹⁴CO₂ accounted for 28.6 % of AR by study termination (15 DAT), NER peaked by 2 DAT with a maximum occurrence of 50.8 % of AR. Under viable conditions, most of NER was associated with fulvic acids and humins (15 DAT sample).

Cymoxanil declined from 59.8 % of AR (mean) by 0 DAT to a non detectable level (< 1 % of AR) by 5 DAT. Intermediate degradation products identified on the pathway to carbon dioxide were IN-R3273 (maximum 7.6 % of AR by 0 DAT, mean of two replicates), IN-U3204 (24.7 % of AR by 0.33 DAT), oxamic acid (IN-18474, 1.4 % of AR) and two unidentified metabolites: metabolite B (10.1 % of AR by 1 DAT) and metabolite D (9.9 % of AR by 2 DAT). These two metabolites were later identified in the supplemental report as IN-W3595 and IN-KQ960, respectively.

Sterile conditions:

Formation of ¹⁴CO₂ and organic volatiles were not detectable (< 1 % of AR), apart from a measurement of 1 % of AR by 2 DAT. NER gradually increased to 48.7 % of AR by study termination (15 DAT). Under sterile conditions, NER were almost exclusively associated with fulvic acids (15 DAT sample).

Degradation of cymoxanil was slower in the sterilized soil, but overall still rapid. Cymoxanil residues were not detectable (< 1 % of AR) by 10 DAT. The same intermediate metabolites were observed as under viable

conditions: IN-R3273 (maximum 6.5 % of AR, mean of two replicates), IN-U3204 (22.7 % of AR), oxamic acid (IN-18474, 5.8 % of AR), IN-W3595 (28.4 % of AR) and IN-KQ960 (19.0 % of AR).

Table B.8.1.1-10: Distribution of radioactivity [% of AR] after application of cymoxanil (5.0 mg ai kg⁻¹) to 'Black Andosol' soil (mean of replicate samples).

Conditions	DAT	Total extracted	NER	CO ₂ /Volatiles	Total recovery
Viable	0	95.8	5.1	Nd	100.9
	0.17	86.1	14.5	Nd	100.6
	0.25	79.8	18.7	Nd	98.5
	0.33	74.2	24.3	Nd	98.5
	1	45.5	40.9	1.0	87.4
	2	32.0	50.8	1.5	84.3
	3	20.7	50.4	4.5	75.6
	5	12.1	49.2	14.8	76.1
	7	8.2	49.2	17.4	75.1
	10	6.1	46.9	17.0	71.4
	15	3.5	47.0	28.6	80.6
Sterile	0	91.9	2.1	nd	94.0
	0.17	90.5	6.2	0.4	97.1
	0.25	89.8	8.3	0.1	98.2
	0.33	87.2	9.6	0.4	97.2
	1	85.3	16.4	0.5	102.2
	2	74.9	24.0	0.7	99.6
	3	65.8	31.1	0.6	97.5
	5	61.2	31.9	0.6	93.7
	7	56.8	41.3	0.8	98.9
	10	49.0	47.2	na	96.2
	15	47.7	48.7	na	96.3

nd denotes non detectable (< 1 % of AR)

na denotes not analyses

Table B.8.1.1-11: Characterization of extractable radioactivity [% of AR] after application of cymoxanil (5.0 mg ai kg⁻¹) to 'Black Andosol' soil (HPLC).

Conditions	DAT	Repl.	IN-W3595 ^a	IN-KQ960 ^b	IN-U3204	IN-R3273 ^c	Cymoxanil	Others
Viable	0	A	6.9	nd	24.3	8.4	54.7	nd
		B	4.7	nd	17.0	6.8	64.8	nd
	0.17	A	3.6	nd	17.2	3.9	60.4	nd
		B	5.1	nd	18.9	4.9	55.2	nd
	0.25	A	4.8	1.7	15.2	2.8	53.4	nd
		B	10.4	2.1	25.7	8.3	32.1	nd
	0.33	A	8.7	2.1	22.6	5.9	33.3	nd
		B	9.9	2.2	26.8	6.3	28.0	nd
	1	A	9.0	3.7	17.1	3.8	7.5	nd
		B	11.1	3.4	15.3	5.4	5.6	nd
	2	A	3.8	15.0 ^d	5.3	1.2	2.5	nd
		B	6.8	4.7	10.0	3.0	5.6	nd
	3	A	5.4	7.4	3.3	1.5	1.6	nd
		B	5.3	5.1	5.2	2.5	2.0	nd
	5	A	2.1	5.5	1.5	1.7	nd	nd
		B	2.2	5.2	1.1	1.5	nd	nd
	7	A	1.2	4.5	nd	1.7	nd	nd
		B	2.4	4.7	nd	1.9	nd	nd
	10	A	nd	3.8	nd	2.1	nd	1.2
		B	nd	3.9	nd	2.3	nd	1.8

Conditions	DAT	Repl.	IN-W3595 ^a	IN-KQ960 ^b	IN-U3204	IN-R3273 ^c	Cymoxanil	Others
	15	A	nd	nd	nd	1.5	nd	1.1
		B	nd	2.2	nd	3.3	nd	1.8
Sterile	0	A	nd	nd	4.5	1.6	84.9	nd
		B	nd	nd	5.7	1.7	81.4	nd
	0.17	A	3.0	nd	10.5	2.2	71.0	nd
		B	3.4	nd	14.5	3.4	69.6	nd
	0.25	A	6.0	nd	19.8	4.4	56.7	nd
		B	5.0	nd	15.6	3.0	65.0	nd
	0.33	A	5.5	nd	16.8	4.0	57.3	nd
		B	6.4	nd	13.5	2.4	60.7	nd
	1	A	15.9	4.6	24.5	5.5	34.3	nd
		B	14.5	3.5	20.8	4.7	38.6	nd
	2	A	21.8	7.1	19.4	6.5	18.8	nd
		B	20.5	7.6	20.2	6.5	19.2	nd
	3	A	24.8	8.8	14.9	5.6	11.9	nd
		B	23.6	8.9	13.9	5.9	10.5	nd
	5	A	27.7	9.9	11.3	6.6	3.5	nd
		B	28.3	10.6	10.9	6.3	2.2	nd
	7	A	28.2	18.5	nd	5.9	nd	nd
		B	28.5	19.5	nd	5.6	1.1	nd
	10	A	29.5	16.6	nd	4.6	nd	nd
		B	27.2	14.3	nd	5.7	nd	nd
	15	A	29.5	16.6	nd	4.6	nd	nd
		B	26.5	13.5	nd	4.6	nd	nd

nd denotes non detectable (< 1 % of AR)

^a IN-W3595 refers to metabolite B in the original report

^b IN-KQ960 refers to metabolite D in the original report

^c This HPLC fraction is likely to comprise IN-R3273 and IN-JX915, the latter dominating the initial phase (see comments)

^d Sample replicate not considered relevant by RMS for risk assessment (see comments)

Table B.8.1.1-12: Characterization of NER [% of AR] after application of cymoxanil (5.0 mg ai kg⁻¹) to 'Black Andosol' soil (mean of replicate samples).

Conditions	DAT	Humin	Humic acid	Fulvic acid
Viable	15	14.9	4.4	27.8
Sterile	15	0.3	2.3	46.1

Table B.8.1.1-13: Calculated DT₅₀ and DT₉₀ of cymoxanil after application (5.0 mg ai kg⁻¹) to viable and sterile 'Black Andosol' soil (SFO kinetics, including data transformation).

Soil	Compound	Conditions	DT ₅₀ [days]	DT ₉₀ [days]	R ²	Kinetics	Data transformation
'Black Andosol'	Cymoxanil	Viable	0.6	1.9	0.91	SFO	In-transformation of amounts
		Sterile	1.1	3.7	1.00	SFO	

Conclusion:

Under **viable** aerobic conditions a rapid degradation of the active substance was observed (DT₅₀ = 0.6 days). The major degradation products were NER, which reached maximum amounts by 2 DAT (50.8 % of AR), hardly decreasing afterwards. Formation of CO₂ accounted for 28.6 % of AR by 5 DAT. Identified major metabolites were IN-U3204 (max. 24.7 % of AR by 0.33 DAT) and IN-W3595 (max. 10.1 % of AR by 1 DAT). Both metabolites declined to undetectable amounts by 10 DAT. Minor metabolites identified (IN-KQ960 and IN-R3273) reached maximum amounts of 9.9 % of AR (2 DAT) and 7.6 % of AR (0 DAT), respectively.

Under **sterile** aerobic conditions degradation of the active substance was slower (DT₅₀ = 1.1 days), formation of NER accounted for 41.3 % of AR (by 7 DAT). Almost no mineralization to CO₂ occurred,

indicating that mineralization to CO₂ under viable conditions is strongly mediated by soil microbial activity. Major metabolites observed were IN-U3204 (maximum 22.7 % of AR by 1 DAT), IN-W3595 (28.4 % of AR by 7 – 10 DAT) and IN-KQ960 (19.0 % by 7 DAT). The two latter one tended to stay constant towards the end of incubation. Metabolite IN-R3273 occurred at minor rates (< 7 % of AR).

The identities of the metabolites IN-W3595 and IN-KQ960 were confirmed by the supplemental report.

Comments (RMS):

- DegT₅₀ and DegT₉₀ of the active substance and of metabolites were recalculated by the notifiers and the RMS and are summarized in B.8.2.6 (Malekani, 2003).
- Microbial biomass of the soil was not measured, however, increasing amounts of ¹⁴CO₂ compared to sterile samples (¹⁴CO₂ < 1 % of AR) indicate microbial viability throughout the study.
- Total recovery of the viable soil samples was below 90 % of AR by 1 DAT and afterwards. Since both viable and sterile experimental set-ups were identical, it seems reasonable that lower recovery of radioactivity in the non-sterile samples may be attributed to the insufficient trapping of CO₂ extensively formed under viable conditions.
- **Relevant maximum occurrence of IN-KQ960 in the viable soil samples is considered to be 6.3 % of AR** (by 3 DAT, mean of replicates) instead of 9.9 % of AR mentioned in the study. One single replicate of the 2 DAT samples, showing 15 % of AR, is considered as an outlier by the RMS.
- The temporal pattern of the metabolite fraction, designated as 'IN-R3273' in the study report, indicates that this fraction is composed of two compounds, likely to be IN-R3273 and IN-JX915. Metabolite IN-R3273 was never found immediately after onset of a soil degradation experiment with cymoxanil (IN-R3273 is typically slowly increasing during degradation to values ≤ 2 % of AR observed by study termination), whereas the transient metabolite IN-JX915 (one of the precursors of IN-R3273) is commonly formed immediately after application of cymoxanil decreasing rapidly thereafter. Such a metabolite pattern was also observed by Trabue (2003), who investigated the same soil batch in a second degradation study (after a soil storage period of 2 years). Both substances have a similar RT time under conditions of HPLC, in this study IN-JX915 was not used as reference substance (not recognised as a possible metabolite of cymoxanil). In this respect, the RMS proposes to attribute the initial phase of this metabolite fraction to **IN-JX915 (maximum occurrence 7.6 % of AR)** and the subsequent phase to **IN-R3273 (maximum occurrence 2.4 % of AR)** similar to Fig. B.8.1.1-1. For kinetic evaluation of the initial phase (attributed to IN-JX915) data are cut-off by 3 DAT. In accordance with other soil degradation studies, IN-R3273 is considered rather stable in soil.

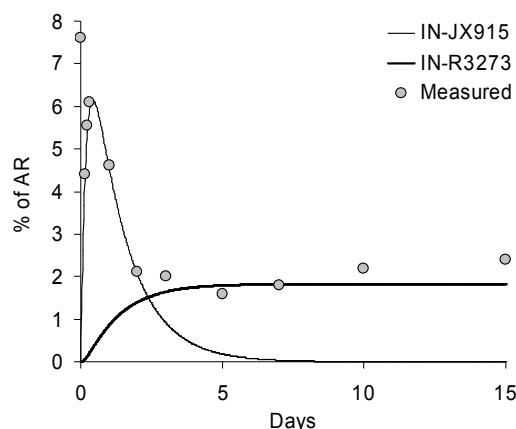


Figure B.8.1.1-1: Proposed partitioning of metabolite fraction 'IN-R3273' into metabolites IN-JX915 (initial phase) and IN-R3273 (final phase).

Reference:	Degradation rate of [¹⁴C]cymoxanil on four soils
Author(s), year:	Boucher, C. R., 1994
Report/Doc. number:	AMR 2869-93
Guideline(s):	BBA IV 4-1 (1986)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

The material and methods, analytical methods, findings and conclusions are included in the following supplement report.

Reference:	Degradation rate of [¹⁴C]cymoxanil on four soils
Author(s), year:	Trabue, S. L., 2003
Report/Doc. number:	AMR 2869-93, Supplement No. 1
Guideline(s):	BBA IV 4-1 (1986)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, 2.82 MBq mg ⁻¹ , > 99 % radiochemical purity (HPLC), batch HOTC 425
Reference substances:	Cymoxanil (non-labelled), IN-U3204, IN-W3595, IN-R3273, IN-KQ960, IN-KP533, IN-JX915, oxamic acid (IN-18474)
Experimental system:	Moisture adjusted soil (50 % of MWHC), 50 g d.w. equivalent, sieved to 2 mm
Equilibration time:	7 days
Test concentration:	1.5 mg ai kg ⁻¹ (in 75 µL of acetonitrile), equivalent to 1125 g ai ha ⁻¹ assuming a soil depth of 5 cm and a soil density of 1.5 g cm ⁻³
Incubation conditions:	20 ± 1 °C, dark, 100 days
Volatile traps:	1 M NaOH (1x), no data on ¹⁴ CO ₂ formation reported
Sample processing:	Soil samples were extracted twice with methanol/water (95/5, v/v), followed by multiple extraction using methanol/water (50/50, v/v) until no more radioactivity could be extracted. Soil extracts were individually radio-assayed by LSC and analysed by HPLC after sample concentration using closed cell evaporation at 30 °C (TurboVap).
Analytical techniques:	LSC, HPLC-UV/RAD
Additional experiments:	None
Kinetic evaluation:	Parent: Simple first order (SFO) and first order multi compartment (FOMC) kinetics

Table B.8.1.1-14: Soil Characteristics.

Soil	'Propstei'	'Sermoise'	'Evensham'	'Black Andosol' ^a
Location	DE	F	UK	J
Texture (USDA)	Sandy loam	Sandy loam	Sandy clay loam	Sandy clay loam ^b
Sand (USDA) [%]	59.4	68.1	64.7	50.0
Silt (USDA) [%]	27.5	15.1	10.3	20.2
Clay (USDA) [%]	13.1	16.7	25.0	28.4
Organic C [%]	0.99	1.69	0.99	2.01

Soil	'Propstei'	'Sermoise'	'Evensham'	'Black Andosol' ^a
pH	6.5	7.8	5.7	6.8
CEC [meq kg ⁻¹]	101	202	137	225
WHC (1/3 bar) [%]	13.5	18.7	18.4	83.6
Bacterial biomass [10 ⁵ CFU g ⁻¹] - start / end	5.5 / 1.3	8.9 / 3.4	4.1 / 11	1.1 / 2.8
Fungal biomass [10 ⁵ CFU g ⁻¹] - start / end	2.7 / 0.23	1.9 / 0.46	6.0 / 0.32	0.023 / 0.52

^a The andosol had been in storage for over 2 years prior to being used in this study (freshly sampled soil was used in Major, 1993)

^b Designated as 'light clay' according to Japanese soil classification

Findings:

Since this study was preliminary designed as parent rate study, no effort was undertaken to trap volatiles and to establish mass balance.

No data on formation of ¹⁴CO₂, organic volatiles and NER are available.

In the original report not all the metabolites detected were reported. In the supplement report the degradation profile of each soil is stated regardless of the level of detection.

In the '**Propstei**' soil the DT₅₀ and DT₉₀ of cymoxanil were 1.5 and 12 days (FOMC kinetics), respectively. No metabolite was detected > 10 % of AR at any time point of the study. Two metabolites, IN-U3204 and oxamic acid (IN-18474) had peak levels between 5 and 10 % of AR. IN-U3204 was detected sporadically with peak level by 5 DAT (7.9 % of AR), oxamic acid (IN-18474) peaked by 31 DAT (7.9 % of AR) before declining to less than 4.5 % of AR at study termination. The metabolites IN-JX915 and IN-T4226 were detected sporadically with no levels of detection occurring above 3.0 % of AR.

The DT₅₀ of cymoxanil in the '**Sermoise**' soil, which was the most alkaline of the soils (pH 7.8), was very short (< 1 day) with a DT₉₀ of approx. 3 days (SFO kinetics). The metabolite IN-U3204 was the only metabolite to be formed over 10 % of AR reaching a peak level of 22.9 % of AR by 0.17 DAT declining to less than 5 % of AR by 5 DAT. Three metabolites, IN-W3595, IN-JX915 and oxamic acid (IN-18474) had peak levels between 5 and 10 % of AR. The metabolites IN-W3595 and IN-JX915 both formed rapidly with peak levels occurring by 2 DAT (8.4 % of AR) and 0.25 DAT (7.9 % of AR), respectively, before each metabolite declined to less than 2 % of AR by 7 DAT and 0.33 DAT, while the oxamic acid (IN-18474) peak level occurred by 9 DAT (5 % of AR), declining thereafter to less than 4 % of AR at study termination. The metabolites IN-R3273, IN-T4226, IN-KP533 and IN-KQ960 formed sporadically during the study with no detection > 4 % of AR.

The respective DT₅₀ and DT₉₀ values of cymoxanil in the more acidic '**Evensham**' soil resulted in 1.6 and 35 days (FOMC kinetics). The DT₉₀ was much longer than that of the other three soils. The metabolites IN-U3204 and oxamic acid (IN-18474) were the two only metabolites detected. IN-U3204 was detected sporadically and in only one of the two replicated soils with a peak level of 13.0 % of AR (0.33 DAT), oxamic acid (IN-18474) peak level occurred by 3 DAT at 9.4 % of AR declining thereafter to less than 3.0 % of AR by 62 DAT.

The DT₅₀ of cymoxanil in the '**Black Andosol**' was about 1 day, the DT₉₀ value averaged almost 7 days (FOMC kinetics). The 'Black andosol' used in this study had been in storage for over 2 years prior to treatment with cymoxanil. Consequently, cymoxanil metabolites that degrade biotically are expected to have longer half-lives due to reduction in the viable soil biomass. The metabolites IN-U3204 and IN-W3595 were the only metabolites to be formed > 10 % of AR reaching peak levels of 18.7 % of AR (by 3 DAT) and 18.1 % of AR (by 7 DAT), respectively, before declining to less than 2 % of AR by 9 and 62 DAT. Three metabolites, IN-JX915, IN-KQ960 and oxamic acid (IN-18474) had peak levels between 4 and 10 % of AR. IN-JX915 formed rapidly with a peak level occurring by 0.33 DAT (8.2 % of AR) before declining to less than 1 % of AR

by 5 DAT, while the metabolites IN-KQ960 and oxamic acid (IN-18474) peak level occurred by 15 DAT (5.5 % of AR) and 62 DAT (5.7 % of AR), respectively, declining thereafter. IN-R3273 was formed less than 2.0 % of AR during the entire study.

Table B.8.1.1-15: Characterization of extractable radioactivity [% of AR] after application of cymoxanil (1.5 mg ai kg⁻¹) to 'Propstei' soil (HPLC).

DAT	Replicate	Oxamic acid (IN-18474)	IN-T4226	IN-U3204	IN-JX915	Cymoxanil
0	A	nd	nd	2.8	nd	89.8
	B	nd	nd	nd	2.5	92.5
0.17	A	nd	nd	nd	nd	92.8
	B	nd	nd	nd	nd	89.5
0.25	A	nd	nd	nd	nd	87.3
	B	2.5	nd	2.1	nd	86.7
0.33	A	nd	nd	nd	nd	89.2
	B	nd	nd	nd	nd	89.0
1	A	2.5	nd	nd	nd	74.4
	B	1.9	nd	nd	nd	72.7
2	A	6.8	nd	nd	nd	54.7
	B	6.4	nd	nd	nd	51.7
3	A	nd	2.8	nd	nd	47.4
	B	nd	nd	6.7	nd	33.6
5	A	nd	nd	7.9	nd	25.1
	B	nd	nd	nd	nd	28.6
7	A	6.1	nd	nd	2.4	14.7
	B	7.3	nd	nd	nd	7.8
9	A	3.3	1.3	3.5	nd	19.1
	B	4.2	nd	1.6	nd	18.0
15	A	3.0	nd	nd	nd	12.4
	B	3.3	nd	nd	nd	14.2
31	A	5.8	nd	nd	nd	1.4
	B	7.9	nd	nd	nd	6.6
62	A	4.2	nd	nd	nd	0.7
	B	6.3	nd	nd	nd	2.7
100	A	1.7	nd	nd	nd	9.3
	B	4.5	nd	nd	nd	1.0

nd denotes < 1 % of AR.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Table B.8.1.1-16: Characterization of extractable radioactivity [% of AR] after application of cymoxanil (1.5 mg ai kg⁻¹) to 'Sermoise' soil (HPLC).

DAT	Repl.	Oxamic acid (IN-18474)	IN-W3595	IN-KP533	IN-T4226	IN-KQ960	IN-U3204	IN-JX915	IN-R3273	Cymoxanil
0	A	nd	nd	nd	nd	nd	12.2	0.0	nd	72.7
	B	nd	nd	nd	nd	nd	5.8	6.7	nd	83.3
0.17	A	nd	nd	nd	nd	nd	22.9	2.8	nd	65.1
	B	nd	nd	nd	nd	nd	22.1	6.0	nd	61.4
0.25	A	nd	nd	nd	nd	nd	nd	7.9	nd	76.6
	B	nd	nd	nd	nd	nd	nd	nd	nd	86.8
0.33	A	nd	nd	nd	nd	nd	12.0	nd	nd	70.8
	B	nd	nd	nd	nd	nd	13.2	nd	nd	68.5
1	A	nd	5.1	nd	nd	nd	7.3	nd	nd	39.3
	B	nd	nd	nd	2.3	nd	4.8	nd	nd	40.0
2	A	4.4	6.4	1.6	nd	nd	10.8	1.1	nd	nd
	B	3.2	8.4	3.7	nd	nd	19.5	nd	nd	nd
3	A	5.9	3.3	nd	nd	1.2	6.3	nd	nd	1.5
	B	3.2	2.9	nd	nd	nd	3.9	0.8	nd	1.3
5	A	7.2	1.5	nd	1.0	nd	nd	0.9	nd	nd
	B	4.6	2.7	nd	nd	nd	4.0	nd	nd	1.1
7	A	7.3	1.1	nd	nd	0.4	1.9	nd	nd	nd
	B	7.0	0.5	nd	nd	0.6	1.5	nd	nd	nd
9	A	7.1	0.5	nd	nd	nd	2.3	nd	nd	nd
	B	8.0	1.3	nd	nd	nd	4.2	nd	nd	nd
15	A	6.5	nd	nd	nd	nd	0.7	nd	nd	nd
	B	5.8	nd	nd	nd	nd	0.8	nd	nd	nd
31	A	4.6	0.2	nd	nd	nd	0.1	0.2	0.1	nd
	B	4.6	1.6	nd	nd	nd	2.9	0.7	0.3	nd
62	A	2.9	0.2	nd	nd	nd	1.3	0.3	0.1	0.1
	B	4.9	nd	nd	nd	nd	0.1	0.2	0.1	nd
100	A	3.0	nd	nd	nd	nd	nd	nd	nd	nd
	B	3.1	nd	nd	nd	nd	nd	nd	nd	nd

nd denotes < 1 % of AR

Table B.8.1.1-17: Characterization of extractable radioactivity [% of AR] after application of cymoxanil (1.5 mg ai kg⁻¹) to 'Evensham' soil (HPLC).

DAT	Replicate	Oxamic acid (IN-18474)	IN-U3204	Cymoxanil
0	A	nd	nd	87.7
	B	nd	nd	94.5
0.17	A	nd	nd	90.7
	B	nd	nd	89.2
0.25	A	nd	nd	89.9
	B	nd	nd	89.1
0.33	A	nd	13.0	72.5
	B	nd	nd	88.5
1	A	nd	nd	73.3
	B	nd	nd	74.7
2	A	2.2	nd	49.5
	B	2.2	nd	49.2
3	A	6.2	nd	46.9
	B	9.4	nd	38.5

DAT	Replicate	Oxamic acid (IN-18474)	IN-U3204	Cymoxanil
5	A	1.9	1.9	38.8
	B	3.5	nd	27.3
7	A	3.4	nd	27.6
	B	7.2	nd	18.7
9	A	3.3	nd	20.0
	B	3.9	nd	20.4
15	A	2.1	nd	15.0
	B	4.1	nd	22.4
31	A	6.1	nd	16.4
	B	4.2	nd	10.5
62	A	2.8	nd	8.0
	B	2.8	nd	5.0
100	A	2.5	nd	11.8
	B	2.4	nd	7.0

nd denotes < 1 % of AR

Table B.8.1.1-18: Characterization of extractable radioactivity [% of AR] after application of cymoxanil (1.5 mg ai kg⁻¹) to 'Black Andosol' soil (HPLC).

DAT	Repl.	Oxamic acid (IN-18474)	IN-W3595	IN-KQ960	IN-U3204	IN-JX915	IN-R3273	Cymoxanil
0	A	nd	nd	nd	nd	5.9	nd	87.9
	B	nd	nd	nd	nd	4.2	nd	89.2
0.17	A	nd	nd	nd	13.0	nd	nd	77.0
	B	nd	nd	nd	12.9	nd	nd	73.7
0.25	A	nd	nd	nd	9.0	6.2	nd	72.8
	B	nd	nd	nd	8.2	nd	nd	73.4
0.33	A	nd	nd	nd	13.3 ^a	8.9	nd	65.1 ^a
	B	nd	5.2	nd	9.6 ^a	7.4	nd	57.7 ^a
1	A	nd	nd	nd	11.9	nd	nd	58.5
	B	nd	5.6	nd	10.2	4.8	nd	60.1
2	A	nd	14.3	nd	14.1	nd	nd	47.1
	B	1.0	12.3	nd	17.2	3.2	nd	44.8
3	A	nd	15.4	nd	23.3	nd	nd	35.7
	B	nd	12.8	nd	14.1	nd	nd	33.1
5	A	1.5	15.7	3.5	10.0	1.0	nd	14.5
	B	1.3	13.8	3.3	8.6	nd	nd	18.1
7	A	2.5	17.8	3.7	6.4	nd	nd	6.9
	B	2.7	18.4	4.9	3.5	nd	nd	5.3
9	A	4.6	19.3	6.0	1.7	nd	nd	4.9
	B	2.5	14.0	4.4	1.8	nd	nd	4.0
15	A	4.6	15.4	5.8	0.8	0.7	1.1	4.6
	B	4.0	16.5	5.2	1.6	nd	1.0	3.2
31	A	4.8	15.8	4.3	0.3	0.3	1.1	2.0
	B	4.3	12.6	1.7	nd	0.3	1.0	0.5
62	A	7.7	1.4	nd	nd	nd	0.6	nd
	B	3.7	0.5	nd	nd	0.4	1.2	nd
100	A	3.4	1.8	nd	nd	0.3	2.0	0.2
	B	1.5	0.3	nd	nd	0.3	0.9	0.8

nd denotes < 1 % of AR.

^a Data from cymoxanil and IN-U3204 obviously interchanged in the study report (corrected by the RMS)

Table B.8.1.1-19: Calculated DT₅₀ and DT₉₀ of cymoxanil in four soils after application of 1.5 mg ai kg⁻¹ using FOMC and SFO kinetics (partly after data transformation).

Compound	Soil	DegT ₅₀ [days]	DegT ₉₀ [days]	R ²	Kinetics	Data transformation
Cymoxanil	'Probstei'	1.5	12.3	0.913	FOMC	None
	'Sermoise'	0.9	3.1	0.711	SFO	In-transformation of amounts
	'Evensham'	1.6	34.5	0.968	FOMC	None
	'Black Andosol'	1.3	6.5	0.948	FOMC	None

Conclusion:

A rapid degradation of the active substance under aerobic conditions was observed in all four soils. According to best fit kinetics (in most cases FOMC), DT₅₀ values were in a range of 0.9 – 1.6 days ($R^2 > 0.7$). Respective DT₉₀ values were in a range of 3.1 – 34.5 days. In consistence to the hydrolysis study, degradation of cymoxanil was depending on the soil pH, showing faster degradation in more alkaline soils. The 'Sermoise' soil and the 'Black Andosol' had the most extensive metabolite degradation profile, while the acidic 'Evensham' soil had the least one. Formation of the major metabolite IN-U3204 was most pronounced in 'Sermoise' soil (maximum 22.5 % of AR) and 'Black Andosol' (maximum 18.7 % of AR) soil. Metabolite IN-W3595 exceeded 10 % of AR in the 'Black Andosol' (18.1 % of AR), no further major metabolite was observed. The minor metabolites IN-JX915, IN-KQ960 and oxamic acid (IN-18474) showed amounts between 5 and 10 % of AR, the metabolites IN-R3273, IN-T4226 and IN-KP533 did not exceed 5 % of AR throughout the study.

Comments (RMS):

- DegT₅₀ and DegT₉₀ of the active substance and of metabolites were recalculated by the notifiers and the RMS and are summarized in B.8.2.6 (Malekani, 2003).
- No material balance is given. However, this study is preliminary considered as degradation kinetic study.
- Since the Japanese 'Black Andosol' had been in storage for over 2 years prior to being used in this study, degradation rate and formation rate of cymoxanil and metabolites are not considered valid by the RMS for the overall risk assessment. The same soil batch, freshly sampled, was used in the soil degradation study from Major (1993). The degradation of cymoxanil and metabolites (at 25 °C but at much higher application rate) was significantly faster than observed in this study. The microbial population was obviously severely compromised by the long storage time (bacterial and fungal biomass were significantly lower in comparison to the other soils tested in this study). In fact, the degradation pattern observed in the 'Black Andosol' in this study is similar to the degradation pattern observed under sterile conditions with freshly sampled soil (Major, 1993). Therefore, results from the 'Black Andosol' in this study were only used to confirm the route of degradation in soil but are **not considered valid for rate calculation (parent and metabolites) and determination of maximum occurrence of metabolites**.

B.8.1.2 Anaerobic degradation in soil (Annex IIA, point 7.1.1.1.2a)

Study not considered essential. Cymoxanil does not have an autumn application and will not be used in crops where anaerobic conditions will persist. In the case of partial/local anaerobic conditions in soil, cymoxanil is likely to extensively degrade during aerobic phases and is not considered to be persistent long enough to move into the saturated zone.

B.8.1.3 Soil photolysis (Annex IIA, point 7.1.1.1.2b)

Soil photolysis of cymoxanil was investigated in two studies:

- **Soil photolysis** in one soil – Oxon
- **Soil photolysis** in one soil – DuPont

Reference:	Photodegradation of cymoxanil on soil surfaces
Author(s), year:	Willems, H., 1998
Report/Doc. number:	NOTOX project 211095
Guideline(s):	US-EPA N 161-3 (1982), SETAC (1995)
GLP:	Yes
Deviations:	None
Validity:	Study not considered valid (see comments)

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, 0.0132 MBq mg ⁻¹ , > 98 % radiochemical purity, batch CFQ9873
Reference substance:	Cymoxanil (non-labelled)
Test system:	A soil/water (3/1, v/v) slurry was evenly spread over glass plates leaving a soil surface of approximately 25 cm ² , dried for 2 hours at ambient temperature and further 3 days at 30 °C
Test concentration:	90 µg ai plate ⁻¹ (in 480 µL of water/methanol, 99.4 /0.6, v/v), equivalent to 360 g ai ha ⁻¹
Test conditions:	20 ± 3 °C, irradiated and dark control, 1 day
Irradiation:	Xenon burner (300 – 800 nm, 700 W m ⁻²), lamp intensity (from 300 – 400 nm) was measured at the beginning and at the end of irradiation with a XenoCal UV sensor
Volatile traps:	2-methoxy-ethanol (1x), 2 M NaOH (2x)
Sample processing:	Soil samples were extracted three times with 10 mL of acidified dichloromethane (pH 5, acetic acid). Soil extracts were combined, concentrated on a rotavapor at 40 °C and radio-assayed by LSC. Following the initial dichloromethane extraction, soil samples were further extracted with 10 mL of hexane and the extracts radio-assayed by LSC. Soil extracts were analysed by TLC and HPLC. Soil residues were analysed by LSC after combustion.
Analytical techniques:	LSC, HPLC-UV/RAD, TLC (method <i>TLC2</i>) Note: <i>TLC2</i> was re-investigated by Brands and Van Noorloos (2006) to determine the R _f /RT values of several cymoxanil metabolites (for details refer to hydrolysis section, B.8.7).
Kinetic evaluation:	Parent: simple first order (SFO), no further details stated

Table B.8.1.3-1: Soil Characteristics.

Soil	'Arrow'
Texture	Sandy loam
Sand [%]	61.7
Silt [%]	25.8
Clay [%]	12.6
C _{org} [%]	2.09
pH	6.0
CEC [meq kg ⁻¹]	170
Microbial biomass (start) [µg C _{mic} g ⁻¹] – start/end	Not determined

Findings:

Radiochemical purity of the stock solution was 99.8 % of AR by TLC.

Light intensity at the soil surface in the wavelength of 300 – 400 nm was equal to 45.6 W m⁻². The solar intensity, measured outside on a sunny day at NOTOX (50 °N) on 13 May 1998 at 13:00 was approx. 42 W m⁻².

Moisture content of the irradiated samples (3.7 %, decreasing to 1.9 % by 1 DAT) was throughout lower than the non-irradiated samples (13.5 %, decreasing to 2.8 % by 1 DAT).

Mass balance was in the range of 88.7 – 109.1 % of AR. Mineralization of cymoxanil to CO₂ was negligible under irradiation and small (< 4 % of AR) in dark control samples. This may be related to the slightly higher moisture content in the dark control samples.

TLC and HPLC analysis indicated that all the activity in the extracts was under the form of cymoxanil. No significant metabolites > 10 % of AR were observed.

Table B.8.1.3-2: Distribution of radioactivity [% of AR] after application of cymoxanil (360 g ai ha⁻¹) to 'Arrow' soil, kept under irradiation or in the dark (no replicates).

Conditions	DAT	Extractable ^a	NER	CO ₂	Organic volatiles	Total
Irradiated	0	108.0	1.1	na	na	109.1
	0.13	73.5	15.2	0.0	nd	88.7
	0.25	66.7	30.3	0.0	nd	96.9
	1	37.0	55.3	0.6	nd	92.5
Dark control	0	93.1	3.7	na	na	96.8
	0.13	65.8	25.2	0.1	nd	91.1
	0.25	49.9	44.9	0.3	nd	95.0
	1	15.8	75.9	3.7	nd	95.2

na denotes not analysed

nd denotes not determined

^a Hexane extractable amounts were < 1.7 % of AR in irradiated samples and < 3.7 % of AR in dark control samples

Table B.8.1.1.2-3: Calculated photodegradation half-life of cymoxanil (expressed as 'extractables') on 'Arrow' soil based on SFO kinetics.

Conditions	DT ₅₀ [days]	R ²	Kinetics
Irradiated	0.71	0.913	SFO
Dark control	0.42	0.979	SFO

Conclusion:

Soil surface irradiation of cymoxanil with artificial sunlight did not alter the pattern of metabolism (mainly bound residues and CO₂) or increase the dissipation rate of cymoxanil in soil compared to non-irradiated samples. Degradation of cymoxanil was rapid in both irradiated and non-irradiated samples with a DT₅₀ of 0.71 and 0.42 days, respectively. Photodegradation is not considered to play a significant role in the overall fate of cymoxanil in soil.

Comments (RMS):

This study is **not considered valid** by the RMS. Based on Brands and Van Noorloos (2006), TLC method TLC2 is not capable to adequately separate several metabolites from the parent. This is in particularly true for the photolysis metabolites IN-JX915 and IN-R3274, which are predominately formed under the influence of irradiation (see valid soil photolysis study from Berg, 1996, and photolysis study in water, Anderson et al., 1993a). Owing to the short RT of cymoxanil (4.6 min)

under the conditions of the HPLC method applied, this alternative analytical approach is also not suitable to adequately separate the large number of metabolites expected. **In this respect, no valid information about the occurrence of metabolites in soil under irradiation can be deduced from this study.**

- In respect to the extensive formation of NER in dark control samples, it seems questionable if the extraction procedure (acidified dichloromethane) is adequate to extract (more polar) metabolites of cymoxanil.

Reference:	Photodegradation of radiolabelled cymoxanil on soil under simulated sunlight
Author(s), year:	Berg, D. S., 1996
Report/Doc. number:	DuPont Report No. AMR 3582-95
Guideline(s):	US-EPA N 161-3 (1982)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, 2.82 MBq mg ⁻¹ , radiochemical purity > 99.5 % (HPLC), batch HOTC 425
Reference substances:	Cymoxanil (non-labelled), IN-Q8761, IN-KP533, IN-W3595, IN-R3274, IN-U3204, IN-R3273, IN-JX915, IN-KQ960, IN-T4226
Test system:	A soil/water slurry (soil 2 mm sieved) was evenly distributed over glass plates leaving a soil surface of approximately 13 cm ² , dried overnight at room temperature
Test concentration:	0.149 mg ai tray ⁻¹ (in 800 µL of methylene chloride), equivalent to 1164 g ai ha ⁻¹
Test conditions:	25 ± 1 °C, irradiated and non-irradiated, 15 days (equivalent to 43.7 days of summer sunlight in Wilmington, Delaware, 39 °N)
Irradiation:	Xenon lamp (>290 nm, 32.8 W m ² hr ⁻¹), approx. 24 cm above samples, light intensity measured with an UV radiometer
Volatile traps:	1 M NaOH (1x), ethane-1,2-diol (1x), identity of ¹⁴ CO ₂ confirmed by precipitation
Sample processing:	Soil samples were extracted three times with 15 – 20 mL of methanol/0.01 M potassium phosphate buffer (pH 4.8 – 5.0) (80/20, v/v). Combined extracts were radio-assayed by LSC, filtered and analysed by HPLC.
Analytical techniques:	LSC, HPLC-UV/RAD
Kinetic evaluation:	Parent: Simple first order (SFO) kinetics (ln transformation of amounts)

Table B.8.1.3-3: Soil Characteristics.

Soil	'Sassafras'
Batch	DuPont ID No. E1602-124
Texture	Sandy loam
Sand [%]	73.2
Silt [%]	18.0
Clay [%]	8.8
C _{org} [%]	0.46
pH	6.4
CEC [meq kg ⁻¹]	38

Findings:

Overall recovery was in a range of 92.3 – 101.4 % of AR. A maximum of 14.0 % of AR was collected as ¹⁴CO₂ in the irradiated samples by 15 DAT. Formation of ¹⁴CO₂ in non-irradiated samples was negligible.

The only degradate exceeding > 10 % of AR was IN-JX915 (10.9 % of AR by 7 DAT).

Table B.8.1.3-4: Distribution of radioactivity [% of AR] after application of cymoxanil (1164 g ai ha⁻¹) to 'Sassafras' soil, kept under irradiation or in the dark (mean of duplicate samples).

Conditions	DAT	¹⁴ CO ₂	Organic volatiles	Extractable	NER	Total recovery
Irradiated	0	0.0	0.0	97.6	2.4	100.0
	1	0.9	< 0.1	94.4	4.4	99.7
	2	2.0	< 0.1	84.4	5.9	92.3
	4	4.1	0.1	86.8	6.0	97.0
	7	5.9	0.1	85.2	5.6	96.7
	11	9.3	0.1	74.5	8.7	92.7
	13	12.8	0.1	69.3	12.7	94.8
	15	14.0	0.2	74.2	6.0	94.5
Non-irradiated	1	0.1	0.0	97.4	3.9	101.4
	2	0.1	< 0.1	92.2	5.2	97.4
	4	0.1	< 0.1	93.9	5.5	99.6
	7	0.1	< 0.1	95.8	4.2	100.1
	11	0.1	< 0.1	86.2	7.2	93.6
	13	0.1	< 0.1	92.4	6.8	99.4
	15	0.1	< 0.1	92.4	4.5	97.1

Table B.8.1.3-5: Characterization of extractable radioactivity [% of AR] after application of cymoxanil (1164 g ai ha⁻¹) to 'Sassafras' soil, kept under irradiation or in the dark (HPLC, mean of duplicate samples).

Conditions	DAT	IN-18474	IN-W3595	IN-T4226	IN-U3204	IN-JX915	Cymox-anil	IN-Q8761	Others ^a
Irradiated	0	0.0	0.0	0.0	0.0	0.6	95.1	0.0	2.5
	1	0.0	0.0	0.0	0.0	8.4	83.0	0.0	3.1
	2	0.0	0.0	0.0	0.0	7.8	69.3	6.2	1.1
	4	0.4	0.0	0.0	1.8	9.2	67.2	5.8	2.4
	7	2.5	0.0	0.0	0.9	10.9	58.3	4.6	7.9
	11	4.4	1.0	0.0	2.2	9.3	47.4	2.9	6.0
	13	2.3	2.2	0.2	1.5	2.0	49.8	3.8	6.5
	15	4.2	2.3	1.2	0.0	5.8	44.6	5.3	8.3
Non-irradiated	1	0.0	0.0	0.0	1.4	0.7	93.6	0.0	1.7
	2	0.0	0.0	0.0	0.9	0.8	87.5	1.5	2.0
	4	0.4	0.0	0.0	3.2	0.0	86.0	2.6	1.8
	7	0.4	1.7	0.0	2.0	0.9	85.2	3.0	2.6
	11	2.2	2.2	0.0	2.0	0.0	70.8	6.7	2.2
	13	1.6	1.7	0.0	3.0	0.0	73.7	6.9	5.6
	15	1.8	2.4	0.0	1.3	1.3	74.0	5.4	6.0

^a Others may include noise, IN-KP533, IN-KQ960 or IN-R3273

Table B.8.1.1.2-3: Calculated photodegradation half-life of cymoxanil on 'Sassafras' soil based on SFO kinetics.

Conditions	k [days ⁻¹]	DT ₅₀ [days]	R ²	Kinetics	Data transformation
Irradiated	0.0460	15.1	0.80	SFO	In-transformation of amounts
Dark control	0.0186	37.3 ^a	0.77	SFO	
Net	0.0274	25.3	-	-	-

^a Interpolated beyond the study termination

Conclusion:

Under irradiation cymoxanil degraded with a DT₅₀ of 15.1 days, while under non-irradiated conditions the DT₅₀ was 37.3 days. After correction for processes occurring in the dark, the half-life owing to irradiation averaged 25.3 days, which is equivalent to 73.9 days of natural sunlight (39 °N). The major degradates under irradiation conditions up to 15 DAT were ¹⁴CO₂ (14.0 % of AR by 15 DAT) and IN-JX915 (10.9 % of AR by 7 DAT). Non-irradiated samples had no individual degradates greater than 10 % of AR.

Comments (RMS):

- Degradation data were re-calculated by the RMS according to current guidance documents (SANCO/10058/2005, ver.1.0, 2005):

Table B.8.1.1.2-3: Re-calculated photodegradation half-life of cymoxanil on 'Sassafras' soil based on SFO kinetics (RMS approach).

Conditions	k [days ⁻¹]	DT ₅₀ [days]	DT ₉₀ [days]	R ²	χ ² error [%]	Kinetics
Irradiated	0.0493	14.1	46.7	0.90	6.4	SFO
Dark control	0.0180	38.5	128	0.87	2.9	SFO
Net	0.0313	22.1	73.6	-	-	-

Re-calculated DT₅₀ under conditions of natural sunlight (39 °N) is 64.5 days.

- The same batch of 'Sassafras' soil was used in the aerobic degradation study from Anderson (2001), both studies were initiated in 1996. As can be clearly seen from the DT₅₀ in the aerobic degradation study (1.2 days), the incubation under conditions of laboratory soil irradiation (air dried soils) severely reduced the degradation of cymoxanil (DT₅₀ in dark control samples 38.5 days). In this respect, it is not expected that IN-JX915 will exceed 10 % of AR in *viable and moist* soils under environmental conditions. For conservative reasons only, **IN-JX915** (which is a common photolysis metabolite of cymoxanil but never exceeded 10 % of AR in any dark degradation study) **is considered a major soil metabolite** by the RMS.

B.8.1.4 Summary on route of degradation in soil

Note: One of the 6 soils investigated to establish the degradation pathway of cymoxanil in soil, is a Black Andosol originating from Japan. This soil, which was investigated in two separate studies (using the same soil batch; Major, 1993, and Trabue, 2003), exhibited the most pronounced metabolite pattern of all soils investigated. This soil type may be considered at least partly relevant for the EU region since andosols are also found in (formally active) volcanic areas in the EU (e.g. central France, central Italy including Sardinia and Sicily). Therefore, results on degradation rates and occurrence of metabolites of the study from Major (1993) were included into the overall environmental risk assessment for conservative reasons. However, before onset of the second study (Trabue, 2003), this soil was stored outdoors over a period of 2 years. In comparison with the first study (Major, 1993), using freshly sampled soil, the microbial activity of the soil was likely to be severely comprised by the long storage time. Therefore, results on degradation rates of cymoxanil and metabolites (including maximum occurrence of metabolites) from the Black Andosol obtained in the second experiment (Trabue, 2003) were omitted from the final risk assessment.

The **aerobic route of soil degradation** of cymoxanil was investigated in total 6 soils (4 studies) with a representative range of properties (pH, organic carbon, texture, origin), at varying temperature (20 and 25 °C) and varying incubation conditions (viable and sterile) using cyanoacetamide-2 labelled cymoxanil.

The degradation of cymoxanil under **aerobic, viable conditions** is characterized by an extensive mineralization to CO₂, which has to be considered the major degradation product of cymoxanil in aerobic, viable soils. After an incubation period of 10 days, 17.0 – 53.0 % of AR (*n* = 4) was found to be released as ¹⁴CO₂. Two of these studies were conducted for a longer time period, levels of released ¹⁴CO₂ steadily increased towards study termination (56.7 and 60.4 % of AR by 90 and 92 DAT, respectively). In two further (degradation rate) experiments, conducted for only 3 days and 1 day, formation of ¹⁴CO₂ was pronounced, too, accounting for 28.7 and 45.7 % of AR (!), respectively.

In soil degradation experiments, which allowed to adequately account for the maximum formation (*n* = 3), peak levels of NER were observed in a range of 36.8 – 50.8 % of AR, occurring by 2 DAT and decreasing thereafter until study termination. In 3 further experiments, maximum formation of NER was observed by study termination (i.e. 10, 3 and 1 DAT) with levels of 35.6, 43.5 and 30.3 % of AR, respectively.

Once in contact with soil water (soil moisture and pore water) at neutral and alkaline conditions, cymoxanil undergoes rapid hydrolysis by (partly reversible) cyclisation processes, leading to the highly transient metabolites IN-U3204 (six-member ring system) and IN-JX915 (five-member ring system), and by cleavage (hydrolysis) of the parent leading to equimolare release of IN-W3595 and ethyl urea (Fig. B.8.1.4-1). Ethyl urea was never quantified in soil degradation studies, since the labelling of the parent (cyanoacetamide-2 position) did not allow to follow the fate of this cleavage product. However, according to SANCO/221/2000, rev. 10 (2003), ethyl urea and further degradation products of ethyl urea are considered compounds of no concern. Metabolite IN-U3204 is highly unstable in soil, rapidly degrading into IN-KP533, IN-T4226 and IN-KQ960. IN-T4226 is a further transient metabolite rapidly degrading into IN-KP533 by ring cleavage. The highly transient metabolite IN-JX915 further degrades into IN-KQ960 and IN-R3273, which in turn degrade to IN-T4226. The hydrolysis end products IN-W3595, IN-KQ960, IN-R3273 and IN-KP533 are considered rather stable under sterile soil conditions (as demonstrated in one study) but are extensively degraded further in viable soils owing to soil microbial activity into oxamic acid (IN-18474), oxalic acid and, finally, CO₂.

Formation of the Z-isomer of cymoxanil (IN-Q8761, observed only in the soil photolysis study, minor amounts in irradiated and dark control samples, Berg, 1996) could not be linked to any environmental impact. Therefore, IN-Q8761 is considered of no concern in the environmental fate assessment.

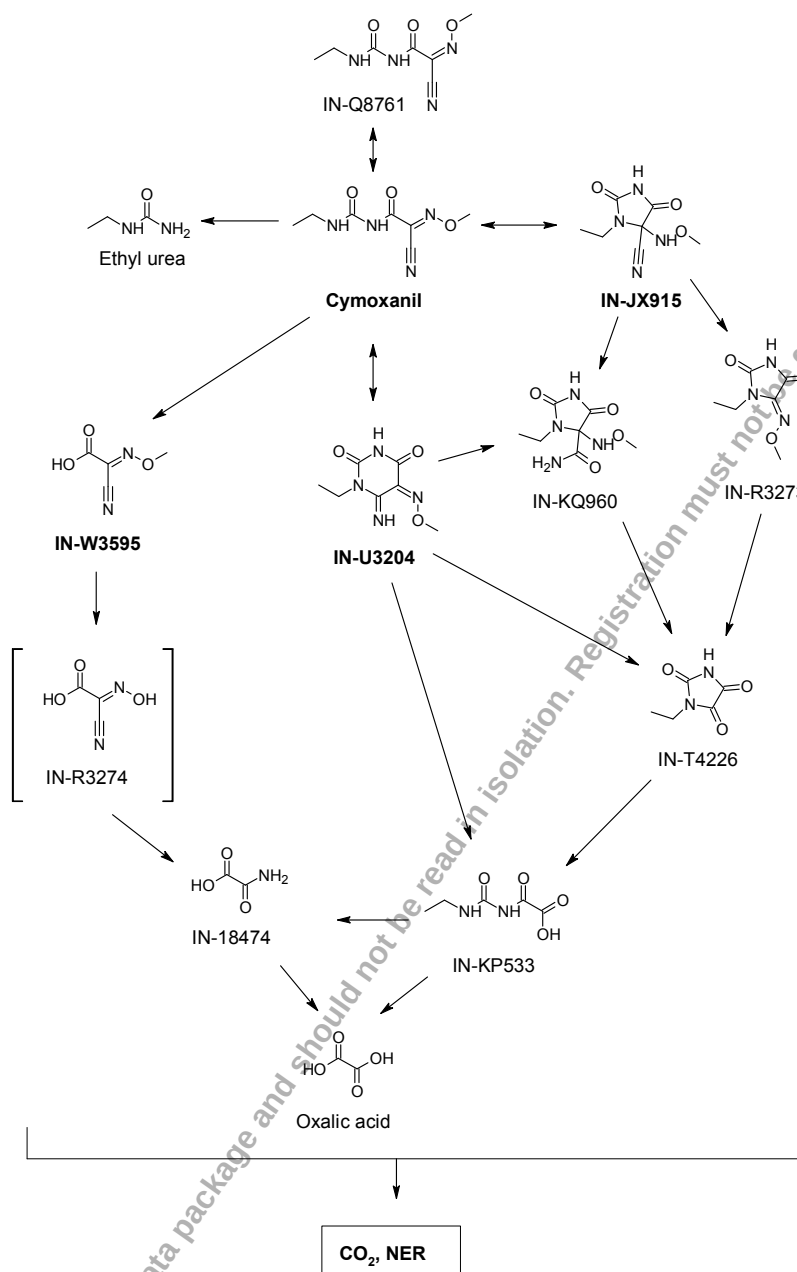


Figure B.8.1.4-1: Proposed aerobic and photolytic degradation pathway of cymoxanil in soil (metabolites in bold exceed 10 % of AR).

Under **dark aerobic viable conditions** two metabolites have to be considered as major metabolites (> 10 % of AR): IN-U3204 (maximum occurrence 24.7 % of AR) and IN-W3595 (maximum 10.1 % of AR). Under the reasonable assumption that the unidentified metabolite fraction Met IV (observed in a degradation rate study, Melkebeke, 1999) is at least partly identical to IN-U3204, this highly transient metabolite was observed > 10 % of AR in 4 of 9 soil degradation studies. Metabolite IN-W3595 was only observed in the Japanese 'Black Andosol' (study with freshly sampled soil) slightly above 10 % of AR and > 5 % of AR in 'Sermoise' soil. On the basis of available data, IN-W3595 is not considered to exceed 5 % of AR in any other soil tested.

The highly transient metabolite IN-JX915 (maximum occurrence 7.6 % of AR in the Japanese 'Black Andosol') exceeded 5 % of AR in 2 of 9 soils degradation studies. Metabolite IN-KQ960 was only found > 5 % of AR in the Japanese 'Black Andosol' (maximum 6.3 % of AR), IN-KQ960 is not considered to exceed 5 % of AR in any other soil tested.

Metabolite IN-18474 (oxamic acid, maximum occurrence of 7.8 % of AR) is a naturally occurring molecule and, based on molecular structure, a degradation product of no concern (SANCO/221/2000, rev. 10, 2003).

No other metabolite or metabolite fraction exceeded 5 % of AR under aerobic, viable conditions.

No data are available on the **route of degradation under anaerobic conditions**. Degradation under anaerobic conditions is not considered relevant for cymoxanil owing to its use pattern in lettuce and potatoes. Cymoxanil degrades so fast in an aerobic environment that it would not persist long enough to be exposed to extensive anaerobic conditions.

One experiment on the **aerobic degradation under sterile conditions** is available, conducted with the Japanese 'Black Andosol'. Under sterile conditions, mineralization of cymoxanil to CO₂ was found to be negligible. Formation of NER was distinct slower than under viable conditions accounting for 48.7 % of AR by 15 DAT (study termination). However, rapid degradation of cymoxanil occurred owing to abiotic hydrolysis processes. In fact, the metabolite pattern formed was almost identical to the metabolite pattern observed in sterile hydrolysis studies. Metabolite IN-U3204 peaked with 22.7 % of AR, decreasing thereafter rapidly. Metabolites IN-W3595, IN-KQ960 and IN-R3273 were observed at maximum levels of 28.4, 19.0 and 6.5 % of AR, respectively, slowly decreasing thereafter in case of IN-R3273 and IN-KQ960.

Under **conditions of soil photolysis**, metabolite pattern formed was similar to dark conditions. However, the degradation pathway is shifted to formation of the common photolysis metabolite IN-JX915 (the degradation pathway via IN-JX915 is also most pronounced during photolysis in sterile water), which has to be considered major in soil photolysis (maximum occurrence 10.9 % of AR). However, IN-JX915, a highly transient metabolite in moist and viable soils, is unlikely to reach this level under real outdoor conditions even under the impact of irradiation. Cymoxanil is applied post emergence (BBCH 40 for lettuce and BBCH 21 for potatoes) and any soil surface photolysis will be significantly reduced due to the presence of crop canopy. In this respect, photolysis may be considered a minor route of degradation for cymoxanil in soil. However, IN-JX915 is included into the list of 'major' soil metabolites for conservative reasons.

Table B.8.1.4-1: Summary on maximum occurrence of soil metabolites of cymoxanil (mean of replicates) occurring in laboratory degradation studies [% of AR] and day of maximum occurrence (numbers in brackets).

Metabolite	Aerobic soil degradation (in viable soils)	Soil photolysis
IN-U3204 ^a	24.7 (0.33)	2.2 (11)
IN-W3595	10.1 (1)	2.3 (15)
Oxamic acid (IN-18474) ^b	7.8 (3)	4.4 (11)
IN-JX915	7.6 (0)	10.9 (7)
IN-KQ960	6.3 (3) ^c	< 1.0
IN-KP533	2.7 (2)	< 1.0
IN-R3274	< 2.6	-
Oxalic acid ^b	< 2.6	-
IN-R3273	2.4 (15) ^d	< 1.0
IN-T4226	1.7 (1)	1.2 (15)
IN-Q8761	-	6.2 (2) ^e

^a Including the unidentified metabolite fraction Met IV (Melkebeke, 1999), occurring at maximum 13.5 % of AR, which is considered to be at least partly identical to IN-U3204

^b Considered a degradation product of no concern (according to SANCO/221/2000, rev. 10, 2003)

^c One sample replicate with 15.0 % of AR not considered relevant, since all other samples were consistently ≤ 7.4 % of AR

^d Value stated according to the final phase of a metabolite fraction which is considered to comprise two compounds. The initial phase is attributed to IN-JX915.

^e IN-Q8761 (Z-isomer of cymoxanil) also observed in dark control samples with 6.9 % of AR (by 13 DAT)

B.8.2 Rate of degradation in soil – laboratory studies (Annex IIA, point 7.1.1.2.1, Annex IIIA, point 9.1.1.1)

B.8.2.1 Aerobic degradation rate of the active substance at 20 °C (Annex IIA, point 7.1.1.2.1a, Annex IIIA, point 9.1.1.1a)

Data on the aerobic degradation rate of cymoxanil are available from degradation route studies (B.8.1.1). One additional study was conducted to investigate the aerobic rate of degradation of cymoxanil at 20 °C in 3 UK soils.

- Rate of aerobic degradation in three soils (20 °C) – Oxon

Reference:	Determination of the degradation rate of cymoxanil in three soils
Author(s), year:	Melkebeke, T., 1999
Report/Doc. number:	NOTOX project 257737
Guideline(s):	CD 95/36/EC (1995), SETAC (1995), OECD (1997)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

amended with supportive study:

Reference:	Determination of TLC R_f values of U3204 and cymoxanil
Author(s), year:	Van Noorloos, B., 2006
Report/Doc. number:	NOTOX project 453443
Guideline(s):	Supportive study - not applicable
GLP:	Yes
Deviations:	Supportive study - not applicable
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, 4.19 MBq mg ⁻¹ , > 99 % radiochemical purity, batch 3304.265
Reference substances:	Cymoxanil (non-labelled), IN-U3204 (supportive study only)
Experimental system:	Moisture adjusted soils (40 % of MWHC), 100 g d.w. equivalent, sieved to 2 mm
Sample storage:	Stored outdoors in well-drained open plastic containers for approx. 18 months before onset of the experiment
Equilibration time:	14 – 16 days
Test concentration:	0.32 mg ai kg ⁻¹ (in 660 µL of acetone), equivalent to 240 g ai ha ⁻¹ assuming a soil depth of 5 cm and a soil density of 1.5 g cm ⁻³
Co-solvent:	0.8 % acetone (m/m)
Incubation conditions:	20 ± 2 °C, dark, 1 - 10 days (as long as to reach DT ₉₀)
Volatile traps:	2 M NaOH (2 x 50 mL) and 2-methoxyethanol (1x)
Sample processing:	Soil samples were extracted three times with 100 mL of acetonitrile/0.01 M acetate buffer pH 4.0 (80/20, v/v). Combined soil extracts were radio-assayed by LCS and analysed by TLC after sample concentration (Rotavapor, 40 °C). Residual activity remaining after extraction was determined by combustion LCS.
Analytical techniques:	LSC, TLC (methods <i>TLC1</i> , 'reversed phase TLC' in the report, and <i>TLC2</i> , 'straight phase TLC') Note: <i>TLC1</i> and <i>TLC2</i> were re-investigated by Brands and Van Noorloos (2006) to determine the R _f /RT values of several cymoxanil metabolites (for details refer to

hydrolysis section, B.8.7).

Additional experiments: In the supportive study (Van Noorloos, 2006), R_f values for IN-U3204 were determined using the same two chromatographic systems (*TLC1* and *TLC2*) as used in the original study and in Brands and Van Noorloos (2006) in order to elucidate the identity of metabolite fraction Met IV, observed in the original study.

Kinetic evaluation: Parent: 1st order, 1.5st order, Timme & Frehse

Table B.8.2.1-1: Soil Characteristics.

Soil	'Cranfield 230'	'Cranfield 164'	'Cranfield 115'
Location	UK	UK	UK
Texture (USDA)	Sandy loam	Silt loam	Clay loam
Sand (USDA) [%]	71.2	13.0	44.9
Silt (USDA) [%]	18.9	73.8	23.1
Clay (USDA) [%]	10.0	13.2	32.2
C _{org} [%]	0.8	2.0	1.6
pH (H ₂ O)	5.1	7.2	8.1
pH (CaCl ₂)	4.3	6.4	7.5
CEC [meq kg ⁻¹]	106	202	259
WHC (maximum) [%]	38.4	55.2	64.7
WHC (1/3 bar) [%]	12.5	40.4	28.6
Microbial biomass ^a [µg C _{mic} g ⁻¹] – start / end	37 / 65	121 / 135	84 / 106
Microbial biomass ^a [% of C _{org}] – start / end	0.46 / 0.80	0.60 / 0.68	0.52 / 0.66

^a Substrate induced respiration

Findings:

Mass balance was in a range of 99.0 – 121.7 % of AR for 'Cranfield 230' soil, 82.6 – 105.9 % of AR for 'Cranfield 164' soil and 87.3 – 105.3 % of AR for 'Cranfield 115 soil'. Mineralization of the test item accounted for 28.7 – 53.0 % of AR by study termination (1, 3 and 10 DAT). No organic volatiles were found. NER increased to 30.3 – 43.5 % of AR by the end of study.

Degradation of cymoxanil was very fast. This decrease was mainly attributed to the extensive formation of CO₂ and NER and to a lesser extent to the formation of more polar degradation products. Apart from cymoxanil, up to five unknown metabolite fractions were quantified in the extracts. The unknown metabolite fraction Met IV was present at concentrations > 10 % of AR in 'Cranfield 115' soil only. All other metabolites never exceeded 5 % of AR.

Table B.8.2.1-2: Distribution of radioactivity [% of AR] after application of cymoxanil (0.32 mg ai kg⁻¹) to three soils (no replicates).

Soil	DAT	Extractable	NER	CO ₂ ^a	Total
'Cranfield 230'	0	104.5	1.4	na	105.9
	0.04	104.1	2.6	0.1	106.7
	0.13	103.7	2.1	0.1	105.9
	0.25	97.9	3.2	0.2	101.3
	0.38	98.1	4.1	0.3	102.5
	0.50	104.3	5.0	0.2	109.5
	1	93.2	8.1	1.5	102.8
	3	55.0	28.5	19.0	102.5
	6	50.0	30.6	18.5	99.0
	10	33.1	35.6	53.0	121.7
'Cranfield 164'	0	100.6	3.8	na	104.3
	0.04	101.2	4.7	0.0	105.9
	0.13	97.1	5.9	0.1	103.0

Soil	DAT	Extractable	NER	CO ₂ ^a	Total
	0.25	91.2	9.6	0.4	101.2
	0.38	85.9	12.8	1.8	100.5
	0.50	79.3	20.2	3.7	103.2
	1	58.7	26.8	8.3	93.7
	3	10.3	43.5	28.7	82.6
'Cranfield 115'	0	101.9	3.4	na	105.3
	0.04	98.1	7.1	0.1	105.3
	0.13	91.0	10.5	0.7	102.3
	0.25	71.7	17.2	2.9	91.8
	0.38	48.4	23.3	15.6	87.3
	0.50	40.5	28.3	22.9	91.6
	1	13.1	30.3	45.7	89.1

na denotes not analysed

^a Organic volatiles = 0.0 % of AR

Table B.8.2.1-3: Characterization of extractable radioactivity [% of AR] after application of cymoxanil (0.32 mg ai kg⁻¹) to three soils (TLC1 and TLC2, no replicates).

Soil	DAT	Cymoxanil			Met I	Met II	Met III	Met IV ^a	Met V
		TLC1	TLC2	Mean	TLC2				
'Cranfield 230'	0	104.5	104.5	104.5	nd	nd	nd	nd	nd
	0.04	104.1	104.1	104.1	nd	nd	nd	nd	nd
	0.13	103.7	103.7	103.7	nd	nd	nd	nd	nd
	0.25	97.9	97.9	97.9	nd	nd	nd	nd	nd
	0.38	98.1	98.1	98.1	nd	nd	nd	nd	nd
	0.50	104.3	104.3	104.3	nd	nd	nd	nd	nd
	1	93.2	93.2	93.2	nd	nd	nd	nd	nd
	3	55.0	55.0	55.0	nd	nd	nd	nd	nd
	6	46.7	45.0	45.8	1.6	0.4	nd	1.4	nd
	10	28.6	28.2	28.4	2.0	0.3	nd	2.3	nd
'Cranfield 164'	0	100.6	99.6	100.1	nd	nd	nd	nd	nd
	0.04	101.2	101.2	101.2	nd	nd	nd	nd	nd
	0.13	97.1	93.6	95.3	nd	nd	nd	nd	nd
	0.25	86.5	91.2	88.9	nd	nd	nd	4.7	nd
	0.38	81.3	76.9	79.1	nd	nd	nd	4.6	nd
	0.50	74.0	72.4	73.2	nd	nd	nd	5.3	nd
	1	50.2	49.2	49.7	2.9	nd	nd	5.5	nd
	3	6.4	7.0	6.7	2.1	nd	nd	1.8	nd
'Cranfield 115'	0	101.9	101.9	101.9	nd	nd	nd	nd	nd
	0.04	93.4	92.7	93.0	nd	nd	nd	3.2	1.5
	0.13	79.2	78.4	78.8	nd	nd	nd	7.5	4.3
	0.25	52.1	52.3	52.2	nd	nd	2.7	13.5	3.4
	0.38	25.3	30.3	27.8	2.1	3.1	3.5	12.4	2.2
	0.50	22.4	25.2	23.8	4.4	nd	4.5	9.2	nd
	1	5.1	6.7	5.9	2.6	nd	2.2	3.3	nd
R _f value (approx.)		0.55	0.65	-	0.03	0.05	0.25	0.45	?

^a Likely to be identical to IN-U3204 (owing to results from supportive study)

Table B.8.2.1-4: Calculated best-fit DT_{50} and DT_{90} of cymoxanil in three soils (mean of both TLC methods) according to Timme & Frehse.

Soil	DegT ₅₀ [days]	DegT ₉₀ [days]	Modified r ²	Kinetics
'Cranfield 230'	4.5	23 ^{ab}	0.97	1.5 st order
'Cranfield 164'	0.7	2.5	0.98	1 st order
'Cranfield 115'	0.2	0.8	0.99	1 st order

^a Extrapolated from DT_{50} ($DT_{90} = 5.2 \times DT_{50}$)

^b Interpolated beyond the study termination

Conclusion:

A rapid degradation of the active substance cymoxanil under aerobic conditions was observed in all three soils. The major degradation products were NER, which reached maximum amounts at the end of incubation (30.3 – 43.5 % of AR). Final formation of CO₂ accounted for 28.7 – 53.0 % of AR. In 'Cranfield 115' soil the unidentified metabolite Met IV exceeded 10 % of AR and reached 13.5 % of AR after 6 hours, declining rapidly thereafter to amounts < 5 % of AR by DAT 1. According to a supplemental study (comparison of TLC-Rf values) Met IV is likely to be identical with IN-U3204. Further unidentified metabolites did not exceed 5 % of AR.

Comments (RMS):

- Microbial biomass of the soils was < 1 % of C_{org} throughout the incubation, likely owing to the long storage time of the soil outdoors in containers. As shown in the 10 °C degradation study from Van Noorloos and Slangen (2001), freshly sampled 'Cranfield 164' soil had a microbial biomass of 1.5 % of C_{org}. In this respect, the study demonstrates that extensive degradation and mineralization of cymoxanil is likely to also occur in soils of low microbial activity (if moist conditions are ensured).
- The low recovery of total RA (< 90 % of AR) by 3 sampling dates is not likely to effect the overall conclusion of the study.
- According to temporal pattern and R_f values obtained in the supplemental study (Noorloos, 2006), metabolite Met IV is likely to mainly comprise IN-U3204.
- Based on Brands and Van Noorloos (2006), TLC method *TLC2* is not capable to adequately separate several metabolites from the parent. However, this is achieved by TLC method *TLC1* which is subsequently used by the RMS to re-calculate degradation data of cymoxanil in line with current guidance documents (SANCO/10058/2005, ver.1.0, 2005).

Table B.8.2.1-5: Re-calculated DT_{50} and DT_{90} values of cymoxanil according to SFO and FOMC kinetics based on *TLC1* data only (RMS approach).

Soil	DT ₅₀ [days]	DT ₉₀ [days]	R ²	χ ² error [%]	Kinetics
'Cranfield 230'	4.7	15.6 ^a	0.97	4.8	SFO
'Cranfield 164'	0.9	3.1	0.99	2.8	
'Cranfield 115'	0.2	0.8	0.98	6.8	
'Cranfield 230'	4.3	25.2 ^a	0.97	4.4	FOMC
'Cranfield 164'	0.9	3.1	0.99	3.0	
'Cranfield 115'	0.2	0.8	0.98	7.3	

^a Interpolated beyond the study termination

B.8.2.2 Aerobic degradation of the active substance at 10 °C (Annex IIA, point 7.1.1.2.1b, Annex IIIA, point 9.1.1.1a)

The rate of aerobic degradation of cymoxanil at 10 °C was investigated in one study:

- Rate of aerobic degradation in one soil at 10 °C – Oxon

Reference:	Determination of the degradation rate of cymoxanil at 10 °C in one soil
Author(s), year:	Van Noorloos, B., Slangen, P. J., 2001
Report/Doc. number:	NOTOX report 308756
Guideline(s):	SETAC (1995), OECD (1997), OECD (2000)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, 4.19 MBq mg ⁻¹ , > 99 % radiochemical purity
Reference substances:	Cymoxanil (non-labelled)
Experimental system:	Moisture adjusted soils (40 % of MWHC), 100 g d.w. equivalent, sieved to 2 mm
Soil storage:	Approx. 6 weeks after sampling at 5 °C
Equilibration time:	15 days
Test concentration:	0.32 mg ai kg ⁻¹ (in 643 µL of acetone), equivalent to 240 g ai ha ⁻¹ assuming a soil depth of 5 cm and a soil density of 1.5 g cm ⁻³
Incubation conditions:	9 ± 2 °C, dark, 7 days
Volatile traps:	2 M NaOH (2 x 50 mL) and 2-methoxyethanol (1x)
Sample processing:	Soil samples were extracted three times with 100 mL of acetonitrile/0.01 M acetate buffer pH 4.0 (80/20, v/v). Combined soil extracts were radio-assayed by LCS and analysed by TLC after sample concentration (Rotavapor, 40 °C). Residual activity remaining after extraction was determined by combustion LCS.
Analytical techniques:	LSC, TLC (methods <i>TLC1</i> , 'reversed phase TLC' in the report, and <i>TLC2</i> , 'straight phase TLC') Note: <i>TLC1</i> and <i>TLC2</i> were re-investigated by Brands and Van Noorloos (2006) to determine the R _f /RT values of several cymoxanil metabolites (for details refer to hydrolysis section, B.8.7).
Additional experiments:	None
Kinetic evaluation:	Parent: Simple first order (SFO) kinetics (ModelManager)

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Table B.8.2.2-1: Soil Characteristics.

Soil	'Cranfield 164'
Location	UK
Texture (USDA)	Silt loam
Sand (USDA) [%]	16.0
Silt (USDA) [%]	72.9
Clay (USDA) [%]	11.1
C _{org} [%]	3.0
pH (H ₂ O)	7.1
pH (CaCl ₂)	6.5
CEC [meq kg ⁻¹]	181
WHC (maximum) [%]	72.8
WHC (1/3 bar) [%]	41.8
Microbial biomass ^a [µg C _{mic} g ⁻¹] – start/end	435 / not determined
Microbial biomass ^a [% of C _{org}] – start/end	1.45 / not determined

^a Substrate induced respiration

Findings:

Mass balance was in a range of 95.4 – 104.7 % of AR. Formation of CO₂ accounted for 38.6 % of AR by study termination (7 DAT), NER peaked with 55.2 % of AR by 5 DAT.

Degradation of cymoxanil at 10 °C was fast. The decrease was mainly attributed to the formation of CO₂ and NER and to a lesser extends to the formation of less polar, unidentified metabolites. None of the unidentified metabolite fractions exceeded 6 % of AR.

Table B.8.2.2-2: Distribution of radioactivity [% of AR] after application of cymoxanil (0.32 mg ai kg⁻¹) to 'Cranfield 164' soil incubated at 10 °C (no replicates).

DAT	Extracted	NER	CO ₂ ^a	Total
0	98.1	3.4	na	101.5
0.13	95.9	8.6	0.1	104.7
0.25	93.4	7.9	0.4	101.6
0.67	76.3	22.9	3.4	102.6
1	67.4	21.6	6.4	95.4
2	41.6	38.5	15.9	96.0
5	12.8	55.2	33.1	101.1
7	9.3	53.0	38.6	100.9

na denotes not analysed.

^a Organic volatiles = 0.0 % of AR

Table B.8.2.2-3: Characterization of extractable radioactivity [% of AR] after application of cymoxanil (0.32 mg ai kg⁻¹) to 'Cranfield 164' soil incubated at 10 °C (TLC2, no replicates).

DAT	Gymoxanil	Met I	Met II	Met III	Met IV	Met V
0	96.5	0.0	0.0	0.0	1.6	0.0
0.13	93.8	0.0	0.0	0.0	2.1	0.0
0.25	91.0	0.0	0.0	0.0	2.3	0.0
0.67	69.7	1.4	1.2	1.1	3.0	0.0
1	59.5	2.4	0.0	2.4	3.1	0.0
2	36.6	0.0	0.0	5.1	0.0	0.0
5	9.2	0.0	0.0	1.6	0.0	2.0
7	6.5	0.0	0.0	0.0	0.0	2.8
R _f value	0.62	0.21	0.26	0.39	0.49	0.00

Table B.8.2.2-4: Calculated DT₅₀ and DT₉₀ of cymoxanil in one soil incubated at 10 °C based on SFO kinetics.

Soil	DegT ₅₀ [days]	DegT ₉₀ [days]	R ²	χ ² error ^a [%]	Kinetics
'Cranfield 164'	1.4	4.7	1.00	2.8	SFO

^a Calculated by the RMS

Conclusion:

A rapid degradation of the active substance was observed under aerobic conditions at 10 °C (DT₅₀ = 1.4 days following SFO kinetics, R² = 1.00). The major degradation products were NER (maximum 55.2 % of AR at day 5) and CO₂ (38.6 % of AR at the end of incubation). Unidentified metabolites did not exceed 6 % of AR.

Comments (RMS):

- Based on Brands and Van Noorloos (2006), TLC method TLC2 is not capable to fully separate several metabolites from the parent. In this respect, the obtained DT₅₀ of cymoxanil is considered a conservative estimate.

B.8.2.3 Aerobic degradation of relevant metabolites at 20 °C (Annex IIA, point 7.1.1.2.1c, Annex IIIA, point 9.1.1.1a)

Data on the aerobic degradation and formation rate of the soil metabolites of cymoxanil were obtained from degradation studies with cymoxanil as parent (B.8.1.1). Details on kinetic evaluation are given in the supplemental report from Malekani (2003), B.8.2.6.

B.8.2.4 Anaerobic degradation rate of the active substance (Annex IIA, point 7.1.1.2.1d, Annex IIIA, point 9.1.1.1b)

This study is not required because cymoxanil will be not used in crops where anaerobic conditions will persist. Such anaerobic conditions would be unacceptable for the culture of lettuce and potatoes. Cymoxanil degrades so rapidly in an aerobic environment that it would not persist long enough to move to the saturated zone even in the presence of local/temporal anaerobic conditions.

B.8.2.5 Anaerobic degradation rate of relevant metabolites (Annex IIA, point 7.1.1.2.1e, Annex IIIA, point 9.1.1.1b)

This study is not required because cymoxanil will be not used in crops where anaerobic conditions will persist. Such anaerobic conditions would be unacceptable for the culture of lettuce and potatoes. Cymoxanil degrades so rapidly in an aerobic environment that it would not persist long enough to move to the saturated zone even in the presence of local/temporal anaerobic conditions.

B.8.2.6 Supplemental studies

Reference:	Calculated half-lives of cymoxanil and its metabolites in environmental fate laboratory studies
Author(s), year:	Malekani, K., 2003
Report/Doc. number:	DuPont-11575, Revision No. 1
Guideline(s):	Calculations - not applicable
GLP:	Calculations - not applicable
Deviations:	Calculations - not applicable
Validity:	Study considered partly acceptable, fully re-calculated by the RMS

Note: For reasons of compactness, the RMS amended this section with information which is stated elsewhere in the dossier, e.g. normalization of soil degradation data.

Material and methods:

The software program ModelManager was used by the notifier to calculate the first order rate constants and DT₅₀ values of cymoxanil and its metabolites in various laboratory environmental fate studies. This section only refers to laboratory soil degradation studies (Aikens, 1998; Anderson, 2001; Major, 1993; Boucher, 1994; Melkebeke, 1999; Van Noorloos and Slangen, 2001).

Data were completely re-processed by the RMS to account for current guidance (SANCO/10058/2005, ver. 1.0, 2005):

- Degradation of the parent was based on **SFO** and **FOMC** kinetics (best fit decision based on χ^2 error, R² and visual assessment)
- 0 DAT amounts of metabolites were added to the parent
- Samples after the last measurement < LOD were cut-off
- In cases where the position within the degradation pathway is clear (e.g. IN-U3204, IN-W3595, IN-JX915), metabolites were directly linked to the parent with all compartments following SFO kinetics and additional pathways to the sink
- In cases where the position within the degradation pathway is not clear or too complex for modelling, metabolites were examined separately following SFO kinetics starting from the maximum occurrence (e.g. IN-18474, IN-R3273)
- Degradation of metabolite IN-KQ960 was fitted to measured data using a multi-compartment model (in line with the soil degradation pathway) with cymoxanil, IN-U3204 and IN-KQ960 linked in series (all compartments following SFO kinetics, additional pathways to sink)
- Maximum formation fraction was set to 1.00 (= 100 %)
- Quality of curve fit was examined by R², χ^2 error and visual assessment, individually for each compartment (parent and metabolites)
- Moisture normalization was based on FOCUS default values for both, study moisture and reference moisture at pF2
- Temperature normalization is based on a Q₁₀ value of 2.2
- Curve fitting was achieved by ModelMaker 4.0

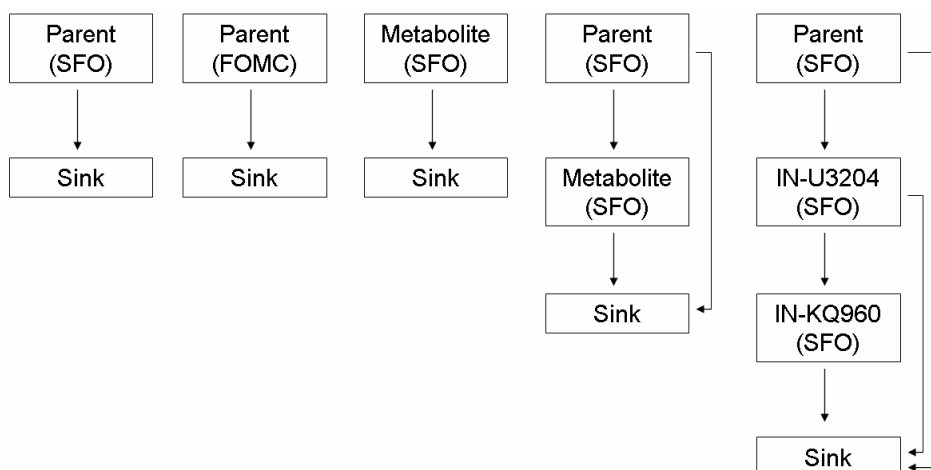


Figure B.8.2.6-1: Multi-compartment models used for curve fitting of cymoxanil and metabolites to measured data (RMS approach).

Findings:

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Table B.8.2.6-1: Summary on basic soil characteristics and incubation conditions of aerobic laboratory soil degradation experiments conducted with cymoxanil as parent.

Soil No.	Soil	Texture	Origin	pH	C _{org} [%]	Clay [%]	Application		T [°C]	Moisture	Source
							[mg kg ⁻¹]	[g ha ⁻¹]			
1	'Arrow'	Sandy loam	UK	6.0	2.1	12.6	0.19	143	20	40 % MWHC	Aikens, 1998
2	'Sassafras'	Sandy loam	USA	6.4	0.5	8.8	1.2	900	25	75 % 1/3 bar	Anderson, 2001
3	'Black Andosol'	Sandy clay loam	J	6.8	2.0	28.4	5.0	3750	25	50 % MWHC	Major, 1993
4	'Probstei'	Sandy loam	DE	6.5	1.0	13.1	1.5	1125	20	50 % MWHC	Trabue, 2003
5	'Sermoise'	Sandy loam	F	7.8	1.7	16.7	1.5	1125	20	50 % MWHC	
6	'Evensham'	Sandy clay loam	UK	5.7	1.0	25.0	1.5	1125	20	50 % MWHC	
7	'Black Andosol' ^a	Sandy clay loam	J	6.8	2.0	28.4	1.5	1125	20	50 % MWHC	Melkebeke, 1999
8	'Cranfield 230'	Sandy loam	UK	4.3	0.8	10.0	0.32	240	20	40 % MWHC	
9	'Cranfield 164'	Silt loam	UK	6.4	2.0	13.2	0.32	240	20	40 % MWHC	
10	'Cranfield 115'	Clay loam	UK	7.5	1.6	32.2	0.32	240	20	40 % MWHC	

^a Soil (batch identical to 'Black Andosol', no. 3) was stored for 2 years prior to testing. Data not considered valid by the RMS.**Table B.8.2.6-2: Summary on normalization factors (moisture and temperature correction) of aerobic laboratory soil degradation experiments conducted with cymoxanil as parent.**

Soil No.	Soil	Texture	Origin	Study moisture	Study MC ^a [%]		MC at 10 kPa (pF2) ^a [%]	Temp. [°C]	f _{Moist}	f _{Temp}	F _{Total}
					Θ	Θ _{ref}					
1	'Arrow'	Sandy loam	UK	40 % MWHC	9.6	19	19	20	0.62	1.00	0.62
2	'Sassafras'	Sandy loam	USA	75 % 1/3 bar	11.3	19	19	25	0.69	1.48	1.02
3	'Black Andosol'	Sandy clay loam	J	50 % MWHC	17.0	22	22	25	0.83	1.48	1.23
4	'Probstei'	Sandy loam	DE	50 % MWHC	13.5	19	19	20	0.79	1.00	0.79
5	'Sermoise'	Sandy loam	F	50 % MWHC	13.5	19	19	20	0.79	1.00	0.79
6	'Evensham'	Sandy clay loam	UK	50 % MWHC	14.0	22	22	20	0.73	1.00	0.73
7	'Black Andosol' ^a	Sandy clay loam	J	50 % MWHC	14.0	22	22	20	0.73	1.00	0.73
8	'Cranfield 230'	Sandy loam	UK	40 % MWHC	10.8	19	19	20	0.67	1.00	0.67
9	'Cranfield 164'	Silt loam	UK	40 % MWHC	12.8	27	27	20	0.59	1.00	0.59
10	'Cranfield 115'	Clay loam	UK	40 % MWHC	12.8	28	28	20	0.58	1.00	0.58

^a FOCUS default values based on soil type (texture)^a Soil (batch identical to 'Black Andosol', no. 3) was stored for 2 years prior to testing. Data not considered valid by the RMS.

Table B.8.2.6-3: Summary on laboratory DegT₅₀, DegT₉₀ and normalized DegT₅₀ values of cymoxanil, re-calculated by the RMS based on simple first order kinetics (SFO kinetics).

No.	Soil	Kinetics	DegT ₅₀ [days]	DegT ₉₀ [days]	R ²	χ ² error [%]	Visual assessment	Norm. DegT ₅₀ [days]	Best fit
1	'Arrow'	SFO	0.2	0.5	1.00	4.6	Good	0.1	No
2	'Sassafras'	SFO	2.3	7.5	0.82	26.0	Medium	2.3	No
3	'Black Andosol'	SFO	0.2	0.7	0.98	5.9	Good	0.2	No
4	'Probstei'	SFO	2.7	9.1	0.98	8.8	Medium	2.1	No
5	'Sermoise'	SFO	0.7	2.3	0.97	20.0	Good	0.6	No
6	'Evensham'	SFO	3.5	11.5	0.96	12.9	Bad	2.6	No
7	'Black Andosol' ^a	SFO	2.1	7.1	0.98	11.8	Good	1.5	No
8	'Cranfield 230'	SFO	4.7	15.6	0.97	4.8	Good	3.1	No
9	'Cranfield 164'	SFO	0.9	3.1	1.00	2.6	Good	0.5	Yes
10	'Cranfield 115'	SFO	0.2	0.8	0.99	5.7	Good	0.1	Yes
Geometric mean			0.9	3.1				0.8	
Worst-case			4.7	15.6				3.1	

^a Soil (batch identical to 'Black Andosol', no. 3) was stored for 2 years prior to testing. Data not considered valid/not included by the RMS.

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Table B.8.2.6-4: Summary on laboratory DegT₅₀, DegT₉₀ and normalized SFO-DegT₅₀ values of cymoxanil, re-calculated by the RMS based on first order multi compartment (FOMC kinetics).

No.	Soil	Kinetics	DegT ₅₀ [days]	DegT ₉₀ [days]	SFO-DT ₅₀ ^a [days]	R ²	χ ² error [%]	Visual assessment	Norm. recalc. SFO-DegT ₅₀ ^a [days]	Best fit
1	'Arrow'	FOMC	0.1	0.5	0.2	1.00	1.4	Good	0.1	Yes
2	'Sassafra'	FOMC	1.2	18.8	5.7	0.86	17.6	Good	5.8	Yes
3	'Black Andosol'	FOMC	0.2	0.8	0.3	0.98	5.9	Good	0.4	Yes
4	'Probstei'	FOMC	2.3	13.1	3.9	0.99	6.9	Good	3.1	Yes
5	'Sermoise'	FOMC	0.7	2.3	0.7	0.95	16.7	Good	0.6	Yes
6	'Evensham'	FOMC	2.5	33.3	10.0	0.98	6.5	Good	7.3	Yes
7	'Black Andosol' ^b	FOMC	2.1	7.5	2.3	0.98	12.1	Good	1.7	Yes
8	'Cranfield 230'	FOMC	4.3	25.2	7.6	0.97	4.4	Good	5.1	Yes
9	'Cranfield 164'	FOMC	0.9	3.1	0.9	0.99	3.0	Good	0.5	No
10	'Cranfield 115'	FOMC	0.2	0.8	0.2	0.98	7.3	Good	0.1	No
Geometric mean			0.8	4.3	1.3				1.0	
Worst-case			4.3	33.3	10.0				7.3	

^a SFO-DegT₅₀ recalculated from FOMC-DegT₅₀ by dividing with 3.32 (i.e. ln(2)/ln(10))

^b Soil (batch identical to 'Black Andosol', no. 3) was stored for 2 years prior to testing. Data not considered valid/not included by the RMS.

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Table B.8.2.6-5: Summary on laboratory DegT₅₀, DegT₉₀, formation fractions and normalized DegT₅₀ values of metabolites of cymoxanil, re-calculated by the RMS based on multi-compartment modelling (all SFO kinetics).

Compound	No.	Soil	Maximum occurrence [% of AR] ^a	Kinetic approach	DegT ₅₀ [days]	DegT ₉₀ [days]	Formation fraction [0 ... 1]	R ²	χ ² error [%]	Visual assessment	Norm. degT ₅₀ [days]
IN-U3204 (Met IV)	3	'Black Andosol' ^c	24.7	P _{SFO} → M _{SFO}	0.6	1.9	0.48	0.93	11.0	Good	0.7
	5	'Sermoise' ^b	22.5	P _{SFO} → M _{SFO}	0.6	2.1	0.44	0.30	91.9	Medium	0.5
	7	'Black Andosol' ^c	18.7	P _{SFO} → M _{SFO}	0.8	2.8	1.00	0.74	37.2	Good	0.6
	9	'Cranfield 164' ^c	5.5	P _{SFO} → M _{SFO}	0.4	1.3	0.24	0.88	26.2	Good	0.2
	10	'Cranfield 115' ^c	13.5	P _{SFO} → M _{SFO}	0.2	0.6	0.36	0.95	12.2	Good	0.1
	Geometric mean Worst-case Arithmetic mean				0.4	1.1	-				0.2
IN-W3595	3	'Black Andosol' ^c	10.1	P _{SFO} → M _{SFO}	1.7	5.5	0.15	0.85	14.5	Good	2.1
	5	'Sermoise' ^d	7.4	P _{SFO} → M _{SFO}	2.8	9.4	0.07	0.60	69.3	Good	2.2
	7	'Black Andosol' ^c	18.1	P _{SFO} → M _{SFO}	24.7	82.0	0.28	0.89	19.9	Good	18.0
	Worst-case Arithmetic mean				2.8	9.4	-				2.2
	3	'Black Andosol' ^c	6.3 ^e	P _{SFO} → M1 _{SFO} → M2 _{SFO}	7.6	25.2	0.16	0.84	19.2	Good	9.3
	7	'Black Andosol' ^c	5.5	P _{SFO} → M1 _{SFO} → M2 _{SFO}	24.8	82.5	0.69	0.81	46.8	Good	18.1
IN-KQ960	Worst-case Arithmetic mean				7.6	25.2	-				9.3
	3	'Black Andosol' ^c	2.4 ^f	M _{SFO}	Stable	Stable	-	-	-	-	Stable
IN-R3273	Worst-case				Stable	Stable					Stable
	3	'Black Andosol' ^c	7.6 ^g	P _{SFO} → M _{SFO}	0.6	1.9	0.10	0.73	27	Good	0.7
	5	'Sermoise' ^b	4.4	P _{SFO} → M _{SFO}	< 0.1	0.1	0.10	0.34	102	Bad	< 0.1
	7	'Black Andosol' ^c	8.2	P _{SFO} → M _{SFO}	0.1	0.4	1.00	0.37	106	Bad	0.1
	Worst-case Arithmetic mean				0.6	1.9	-				0.7
	4	'Probstel' ⁱ	6.7	M _{SFO}	63.8	212	-	0.62	3.6	Good	50.4
Oxamic acid (IN-18474)	5	'Sermoise' ^j	7.6	M _{SFO}	54.5	181	-	0.74	10.2	Good	39.8
	6	'Evensham' ^j	7.8	M _{SFO}	65.4	217	-	0.81	8.5	Good	47.7
	7	'Black Andosol' ^c	4.3	M _{SFO}	80.6	268	-	0.61	2.9	Good	54.0
	Geometric mean Worst-case				61.0	203	-				45.7
	Arithmetic mean				-	-	-				50.4
	Worst-case				-	-	-				-

^a Mean of two replicates

^b Not considered valid by the RMS owing to low R², high χ² error and bad visual fit

^c Soil (batch identical to 'Black Andosol', no. 3) was stored for 2 years prior to testing. Data not considered valid/not included by the RMS.

^d Considered valid by the RMS owing to acceptable visual fit (despite the low R² and high χ² error)

^e In the original report (Major, 1993) a maximum occurrence of 9.9 % of AR is stated. Not considered relevant by the RMS (outlier excluded)

^f Value refers to final phase of the metabolite fraction 'IN-R3273' in the original report (for details see Major, 1993)

^g Value refers to initial phase of the metabolite fraction 'IN-R3273' in the original report (for details see Major, 1993)

Table B.8.2.6-6: Summary on laboratory DegT₅₀ and DegT₉₀ values of cymoxanil and metabolites in 'Black Andosol' under sterile conditions and 25 °C, re-calculated by the RMS based on multi-compartment modelling (all SFO kinetics).

No.	Soil	Metabolite	Maximum occurrence [% of AR] ^a	Kinetics	DegT ₅₀ [days]	DegT ₉₀ [days]	Formation fraction [0 ... 1]	R ²	χ ² error [%]	Visual assessment
3	'Black Andosol', sterile conditions	Cymoxanil	-	P _{SFO}	0.9	2.8	-	0.98	8.6	Good
		IN-U3204	22.7	P _{SFO} → M _{SFO}	0.7	2.5	0.94	0.85	23.0	Good
		IN-W3595	28.4	P _{SFO} → M _{SFO}	Stable	Stable	0.33	0.99	2.9	Good
		IN-KQ960	19.1	P _{SFO} → M1 _{SFO} → M2 _{SFO}	Stable	Stable	0.19	0.84	32.6	Medium
		IN-R3273	6.5	M _{SFO}	20.4	67.6	-	0.81	2.1	Good

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Conclusion:

According to best fit (FOMC in most cases), DegT₅₀ of cymoxanil in aerobic soils was calculated to be in a range of 0.1 – 4.3 days with a geometric mean of 0.8 days (χ^2 error ≤ 17.6). In order to derive adequate modelling endpoints, SFO-DT₅₀ was recalculated from FOMC-DT₉₀ by division with 3.32 following FOCUS recommendations (SANCO/10058/2005, ver. 1.0, 2005). Normalized recalculated SFO-DT₅₀ of cymoxanil was in a range of 0.1 – 7.3 days at 20 °C and pF2 with a geometric mean of 1.0 days.

Degradation half-lives (following SFO kinetics, geometric mean in case of $n \geq 3$, otherwise worst-case value) of metabolites were calculated to be 0.4, 2.8, 7.6, 0.6 and 61.0 days for metabolites IN-U3204, IN-W3595, IN-KQ960, IN-JX915 and oxamic acid (IN-18474). No reliable DT₅₀ could be determined for the metabolite IN-R3273 owing to the low occurrence of this metabolite in aerobic soil degradation studies (maximum 2.4 % of AR). Following normalization to 20 °C and pF2, DT₅₀ of IN-U3204, IN-W3595, IN-KQ960, IN-JX915 and oxamic acid (IN-18474) was calculated to be 0.2, 2.2, 9.3, 0.7 and 45.7 days, respectively.

Comments (RMS):

- Data were fully re-calculated by the RMS to adequately account for current guidance documents (SANCO/10058/2005, ver. 1.0, 2005).

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B.8.2.7 Summary on rate of degradation in laboratory soil studies

Note: One of the 9 soils investigated to establish the rate of degradation of cymoxanil in soil, is a Black Andosol originating from Japan. This soil, which was investigated in two separate studies (using the same soil batch; Major, 1993, and Trabue, 2003), exhibited the most pronounced metabolite pattern of all soils investigated. This soil type may be considered at least partly relevant for the EU region since andosols are also found in (formally active) volcanic areas in the EU (e.g. central France, central Italy including Sardinia and Sicily). Therefore, results on degradation rates of metabolites of the study from Major (1993) were included into the overall environmental risk assessment for conservative reasons only. However, before onset of the second study (Trabue, 2003), this soil was stored outdoors over a period of 2 years. In comparison with the first study (Major, 1993), using freshly sampled soil, the microbial activity of the soil was likely to be severely comprised by the long storage time. Therefore, results on degradation rates of cymoxanil and metabolites from the Black Andosol obtained in the second experiment (Trabue, 2003) were omitted from the final risk assessment.

The **laboratory soil degradation rate** of cymoxanil was investigated in total 9 soils (5 studies) with a representative range of properties (pH, organic carbon, texture, origin), at varying temperature (10, 20 and 25 °C) and varying incubation conditions (viable and sterile) using cyanoacetamide-2 labelled cymoxanil:

- pH: 4.3 – 7.8
- Organic carbon: 0.5 – 2.1 %
- Clay content: 8.8 – 32.3 %
- Origin: EU, USA, Japan
- Temperature: 10, 20 and 25 °C
- Incubation conditions: Aerobic viable and aerobic sterile

Under **aerobic and viable conditions** and temperatures of 20 – 25 °C cymoxanil rapidly degraded with a half-life time in a range of 0.1 – 4.3 days (based on best fit, $n = 9$, $R^2 \geq 0.86$, χ^2 error ≤ 17.6 %) with a geometric mean of 0.8 days. Respective DT_{90} values were in a range of 0.5 – 33.3 days with a geometric mean of 4.3 days. Degradation of cymoxanil mainly followed FOMC kinetics.

No impact of the microbial biomass on the degradation rate could be observed at all. However, since degradation of cymoxanil is mainly driven by pH depending hydrolysis, a significant impact ($p < 0.05$) of the soil pH on the degradation rate (best-fit DT_{50}) could be obtained (at lower pH values degradation of cymoxanil was less rapid, Figure B.8.2.7-1). In addition, degradation of cymoxanil has to be considered to slow down at higher application rates.

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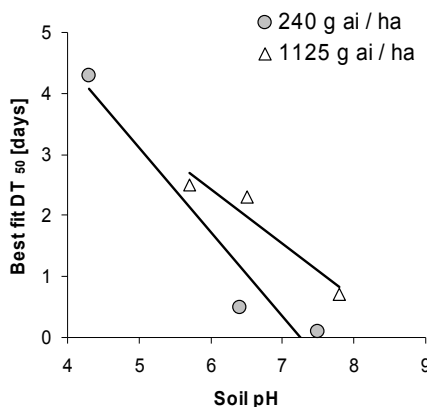


Figure B.8.2.7-1: Impact of soil pH on the DT₅₀ (best fit DT₅₀, not normalized) of cymoxanil at different application rates (two separate studies).

Since degradation of cymoxanil mainly follows FOMC kinetics, normalization of the DT₅₀ was based on the re-calculated SFO-DT₅₀ obtained from the FOMC-DT₉₀ by division with 3.32 in order to derive adequate modelling endpoints (SANCO/10058/2005, ver. 1.0). Normalized re-calc. SFO-DT₅₀ values were in a range of 0.1 – 7.3 days with a geometric mean of 1.0 days. This geometric mean was considered appropriate for groundwater risk assessment. Nevertheless, to account for the impact of the soil pH on the degradation rate of cymoxanil, additional groundwater modellings were performed using the maximum re-calc. SFO-DT₅₀ of cymoxanil observed in acidic soils (7.3 days). Soil risk assessment was based on the worst-case normalized re-calc. SFO-DT₅₀ of 7.3 days.

At 10 °C degradation half life of cymoxanil in one soil was 1.4 days ($n = 1$) following SFO kinetics ($R^2 = 1.00$, χ^2 error = 2.8 %). Respective DT₉₀ was 4.7 days.

Soil degradation studies, which were characterized by significant formation of metabolites (in particularly 'Black Andosol' and 'Sermoise' soil), were subjected to multi-compartment modelling analysis to obtain reliable degradation half-lives for major (> 10 % of AR) metabolites and metabolites considered relevant for groundwater risk assessment (> 10 % of AR, 2 consecutive samples > 5 % of AR or steady increase).

The highly transient major soil metabolite IN-U3204 (which is considered identical to the metabolite fraction Met IV in the study from Melkebeke, 1999), resulting from a (partly reversible) cyclisation of the parent, showed DT₅₀ values in a range of 0.2 – 0.6 days ($n = 3$, all compartments SFO kinetics, $R^2 \geq 0.88$, χ^2 error ≤ 26.2 %) with a formation fraction in a range of 0.24 – 0.48 (arithmetic mean 0.36). Following normalization, DT₅₀ of IN-U3204 was in a range of 0.1 – 0.7 days with a geometric mean of 0.2 days.

The degradation rate of the major metabolite IN-W3595 (observed in only one soil slightly > 10 % of AR) could only be determined in two soils. Directly linked to the parent, the DT₅₀ of IN-W3595 was in a range of 1.7 – 2.8 days ($n = 2$, all compartments SFO kinetics, $R^2 \geq 0.60$, χ^2 error ≤ 69.3 %) with a formation fraction of 0.07 – 0.15. Statistical fit parameters (R^2 and χ^2 error) were rather bad in one of the two soils. However, based on visual assessment the modelling was considered acceptable. Following normalization, DT₅₀ of IN-W3595 was in a narrow range of 2.1 – 2.2 days.

Based on the overall degradation pathway of cymoxanil in soil, degradation of IN-KQ960, a minor metabolite in soil but considered relevant for groundwater risk assessment, was calculated using a multi-compartment model (parent, IN-U3204 and IN-KQ960 in series, all SFO kinetics). Since IN-KQ960 was only observed in the Japanese 'Black Andosol' soil above the trigger (2 consecutive samples > 5 % of AR), only one reliable

DT₅₀ could be obtained, i.e. 7.6 days (n = 1, R² = 0.84, χ^2 error = 19.2 %) with a formation fraction of 0.16 (from IN-U3204), following normalization DT₅₀ was 9.3 days.

The photolysis metabolite IN-JX915, which was shown to exceed 10 % of AR under conditions of soil photolysis but not in dark incubated soils, has to be considered as a highly transient metabolite with a DT₅₀ ≤ 0.6 days (formation fraction from parent 0.10). Following normalization, DT₅₀ of IN-JX915 was determined to be 0.7 days.

Under **aerobic sterile conditions** (study conducted only with the 'Black Andosol' soil, Japan, at 25 °C) cymoxanil rapidly degraded according to abiotic hydrolysis with a half-life of 0.9 days (SFO kinetics, R² = 1.98, χ^2 error = 8.6 %) and a DT₉₀ of 2.8 days. In contrast to viable conditions, formation of metabolites was more pronounced, the transient metabolite IN-U3204 degraded with a DT₅₀ of 0.7 days, IN-R3273 degraded with a DT₅₀ of 20 days, IN-W3595 and IN-KQ960 were almost stable under sterile conditions. In general, under sterile soil conditions degradation of cymoxanil and formation of metabolites closely follows hydrolysis observed in sterile water.

No data are available on the **anaerobic degradation rate** of cymoxanil. However, cymoxanil is not intended to be used in soil with extensive anaerobic conditions. In the eventuality that cymoxanil will be subjected to temporarily or local anaerobic conditions, it is likely that cymoxanil will rapidly degrade owing to hydrolysis (at least under neutral and alkaline conditions) and during aerobic conditions.

Under conditions of **photolysis on the soil surface** (one valid study conducted with air dried soil), cymoxanil degraded with an experimental half-life of 14.1 days (SFO kinetics, R² = 0.90, χ^2 error = 6.4 %) on 'Arrow' soil of pH 6.4. Based on the degradation rate of cymoxanil in the dark control samples of 38.5 days, a net soil photolysis of 22.1 days could be obtained. Taking into account that cymoxanil will degrade rapidly in moist and viable soils, photolysis on the soil surface is considered to be of minor impact onto the overall dissipation of cymoxanil under environmental conditions.

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Table B.8.2.7-1: Summary on laboratory DT₅₀ and DT₉₀ and normalized DT₅₀ of cymoxanil in soil based on SFO and FOMC kinetics.

Soil	Texture	Origin	pH	C _{org} [%]	Application rate [g ai ha ⁻¹]	SFO			FOMC			Best fit kinetics ^b
						DT ₅₀ [days]	DT ₉₀ [days]	Norm. DT ₅₀ (pF2, 20 °C) [days]	DT ₅₀ [days]	DT ₉₀ [days]	Norm., re-calc. SFO-DT ₅₀ ^a (pF2, 20 °C) [days]	
'Arrow'	Sandy loam	UK	6.0	2.1	143	0.2	0.5	0.1	0.1	0.5	0.1	FOMC
'Sassafras'	Sandy loam	USA	6.4	0.5	900	2.3	7.5	2.3	1.2	18.8	5.8	FOMC
'Black Andosol'	Sandy clay loam	J	6.8	2.0	3750	0.2	0.7	0.2	0.2	0.8	0.4	FOMC
'Probstei'	Sandy loam	DE	6.5	1.0	1125	2.7	9.1	2.1	2.3	13.1	3.1	FOMC
'Sermoise'	Sandy loam	F	7.8	1.7	1125	0.7	2.3	0.6	0.7	2.3	0.6	FOMC
'Evensham'	Sandy clay loam	UK	5.7	1.0	1125	3.5	11.5	2.6	2.5	33.3	7.3	FOMC
'Cranfield 230'	Sandy loam	UK	4.3	0.8	240	4.7	15.6	3.1	4.3	25.2	5.1	FOMC
'Cranfield 164'	Silt loam	UK	6.4	2.0	240	0.9	3.1	0.5	0.9	3.1	0.5	SFO
'Cranfield 115'	Clay loam	UK	7.5	1.6	240	0.2	0.8	0.1	0.2	0.8	0.1	SFO
Geometric mean						0.9	3.1	0.7	0.8	4.3	1.0	

^a SFO-DT₅₀ re-calculated from FOMC-DT₉₀ by division with 3.32

^b Based on R², χ^2 error and visual assessment

Table B.8.2.7-2: Summary on laboratory DT₅₀ and DT₉₀ and normalized DT₅₀ of metabolites of cymoxanil in soil (all SFO kinetics).

Metabolite	Soil	Texture	Origin	pH	C _{org} [%]	Clay [%]	DT ₅₀ [days]	DT ₉₀ [days]	Formation fraction [0 ... 1]	Norm. DT ₅₀ (pF2, 20 °C) [days]
IN-U3204	'Black Andosol'	Sandy clay loam	J	6.8	2.0	28.4	0.6	1.9	0.48	0.7
	'Cranfield 164'	Silt loam	UK	6.4	2.0	13.2	0.4	1.3	0.24	0.2
	'Cranfield 115'	Clay loam	UK	7.5	1.6	32.2	0.2	0.6	0.36	0.1
	Geometric mean						0.4	1.1	0.36^a	0.2
IN-W3595	'Black Andosol'	Sandy clay loam	J	6.8	2.0	28.4	1.7	5.5	0.15	2.1
	'Sermoise'	Sandy loam	F	7.8	1.7	16.7	2.8	9.4	0.07	2.2
	Worst-case						2.8	9.4	0.11^a	2.2
IN-KQ960	'Black Andosol'	Sandy clay loam	J	6.8	2.0	28.4	7.6	25.2	0.16	9.3
IN-JX915	'Black Andosol'	Sandy clay loam	J	6.8	2.0	28.4	0.6	1.9	0.10	0.7
Oxamic acid (IN-18474)	'Probstei'	Sandy loam	DE	6.5	1.0	13.1	63.8	212	-	50.4
	'Sermoise'	Sandy loam	F	7.8	1.7	16.7	54.5	181	-	39.8
	'Evensham'	Sandy clay loam	UK	5.7	1.0	25.0	65.4	217	-	47.7
	Geometric mean						61.0	203	-	45.7

^a Arithmetic mean

B.8.3 Field studies (Annex IIA, point 7.1.1.2.2, Annex IIIA, point 9.1.1.2)

B.8.3.1 Soil dissipation testing (Annex IIA, point 7.1.1.2.2a, Annex IIIA, point 9.1.1.2a)

Field soil dissipation studies are not required because cymoxanil consistently showed DT_{50} values at both 10 and 20 °C very much shorter than 60 days under laboratory conditions ($DT_{50} \leq 4.3$ days according to best fit).

B.8.3.2 Soil residues testing (Annex IIA, point 7.1.1.2.2b, Annex IIIA, point 9.1.1.2b)

Soil residues studies on cymoxanil are not required since the DT_{50} under laboratory conditions (geometric mean $DT_{50} = 0.8$ days according to best fit) is typically less than one third of the proposed interval between application and harvest of lettuce (PHI = 7 days) and potatoes (PHI = 7 – 10 days).

B.8.3.3 Soil accumulation testing (Annex IIA, point 7.1.1.2.2c, Annex IIIA, point 9.1.1.2c)

Owing to the fast degradation of cymoxanil observed in laboratory degradation studies ($DT_{50} \leq 4.3$ days and $DT_{90} \leq 33.3$ days according to best fit), the potential of accumulation is considered to be negligible even after multiple application.

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B.8.4 Mobility studies

B.8.4.1 Adsorption and desorption of the active substance (Annex IIA, point 7.1.2a, Annex IIIA, point 9.1.2)

The adsorption/desorption of cymoxanil was investigated in one batch equilibrium study (four soils).

Reference:	Adsorption/desorption of cymoxanil on soil
Author(s), year:	Slangen, P. J., 1999
Report/Doc. number:	NOTOX project 257748
Guideline(s):	ECCD 95/36/EC (1995), OECD 106 (1997), US-EPA N 163-1 (1982), US-EPA, OPPTS 835:1220 (1996), SETAC (1995)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, 4.19 MBq mg ⁻¹ , radiochemical purity ≥ 99 % (TLC, HPLC), batch 3304.265
Reference substances:	Cymoxanil (non-labelled)
Pre-equilibration time:	12 hrs in 0.01 M CaCl ₂
Test temperature:	20 ± 2 °C
Pre-tests:	- Determination of adsorption/desorption equilibrium: - Test concentration: 1 mg L⁻¹ in 0.01 M CaCl₂ - Sampling times: up to 48 hrs - Soil/solution ratio: 1/2 (m/v) - Blank control (stability of test substance, wall adsorption)
Main-test:	Test concentration: 0.04, 0.4, 1.0, 2.0 and 5.0 mg L ⁻¹ Equilibration time: 3 hrs adsorption / 3 hrs desorption Soil/solution ratio: 1/2 (m/v)
Sample processing:	Supernatants were radio-assayed by LSC. Soil slurries were extracted with 15 mL of acetonitrile/0.01 M buffer sodium acetate/acetic acid, pH 4 (4/1, v/v), radio-assayed by LSC and TLC. Remaining soil debris is radio-assayed by combustion-LSC.
Analytical techniques:	LSC, TLC (method TLC2) Note: TLC2 was re-investigated by Brands and Van Noorloos (2006) to determine the R _f /RT values of several cymoxanil metabolites (for details refer to hydrolysis section, B.8.7).
Calculations:	Amounts of test item adsorbed to the soil were determined by calculating the difference between initial and equilibrium concentrations of the aqueous solution. Adsorption coefficients were calculated according to the Freundlich equation.

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Table B.8.4.1-1: Soil Characteristics.

Soil	'Speyer 2.1'	'Midwest 1'	'Cranfield 115'	'Cranfield 164'
Location	DE	Nebraska, USA	UK	UK
Horizon [cm]	0 - 20	Topsoil	0 - 20	15 - 22
Texture (USDA)	Sand	Sandy loam	Clay loam	Silty loam
Sand (USDA) [%]	89.5	62	44.9	13.0
Silt (USDA) [%]	8.0	22	23.1	73.8
Clay (USDA) [%]	2.5	16	32.2	13.2
C _{org} [%]	0.59	1.0	1.6	2.0
pH (H ₂ O)	6.9	5.7	8.1	7.2
pH (CaCl ₂)	6.0	na	7.5	6.4
CEC [meq kg ⁻¹]	40	107	259	202
WHC (maximum) [%]	29	na	64.7	55.2
WHC (at 1/3 bar) [%]	na	13.1	28.6	40.4

Findings:

Cymoxanil was stable (102.0 – 106.1 % of AR) in the test solution in the absence of soil (up to 48 hrs) and no adsorption of cymoxanil onto the container wall was observed during the experiments.

After an experimental time of 48 hrs adsorption and 48 hrs desorption, only 66.6 – 75.5 % of AR was recovered in the total system (only conducted on 'Cranfield 164' and 'Midwest 1' soil). No indication for any metabolite was found by TLC analysis. Therefore, equilibration time tests were repeated with a maximum of 6 hrs for adsorption and desorption. Subsequent, total recovery was in a range of 91.7 – 107.6 % of AR (for all soils). For three samples of the 6 hrs experiment, a metabolite was found by TLC with a R_f value of 0.50 (R_f of cymoxanil 0.56). Amounts of this unknown metabolite ranged from 13 – 19 % of AR. Finally, adsorption and desorption time for the definitive test (main test) was restricted to 3 hrs to minimize biodegradation.

Table B.8.4.1-2: Adsorption/desorption parameter for cymoxanil (mean of two replicates).

Soil	Adsorption				Desorption			
	K _F [L kg ⁻¹]	K _{FOC} [L kg ⁻¹]	1/n	R ²	K _F [L kg ⁻¹]	K _{FOC} [L kg ⁻¹]	1/n	R ²
Speyer 2.1	0.090	15.1	0.882	0.996	nd	nd	nd	nd
Midwest 1	0.910	87.1	0.871	0.999	0.317	30.3	0.572	0.972
Cranfield 115	0.462	28.9	0.806	0.999	nd	nd	nd	nd
Cranfield 164	0.856	43.4	0.867	0.996	0.486	24.6	0.515	0.938
Arithmetic mean	-	43.6	0.857	-	-	27.5	0.544	-

nd denotes not determinable

Conclusion:

Cymoxanil can be considered slightly to moderately mobile in soils with a mean K_{OC} of 43.6 L kg⁻¹. The mean 1/n value is 0.857.

Comments (RMS):

- Based on Brands and Van Noorloos (2006), the metabolite observed (R_f value 0.50) is likely to be IN-U3204, a common and transient metabolite of cymoxanil in soil.

B.8.4.2 Adsorption and desorption of relevant metabolites (Annex IIA, point 7.1.2b, Annex IIIA, point 9.1.2)

The adsorption/desorption of metabolites of cymoxanil was investigated in one batch equilibrium study and, owing to partly instability of several metabolites, by HPLC.

- Adsorption/desorption of IN-W3595, IN-R3273, IN-KQ960, IN-JX915 and IN-U3204 to soil (batch study, four soils) – DuPont
- K_{OC} value of IN-U3204, IN-KQ960, IN-T4226 and IN-KP533 (HPLC method) – DuPont

Reference:	Soil batch equilibrium study of cymoxanil degradates
Author(s), year:	Hausmann, S. M., Adams, G. M., 1996
Report/Doc. number:	DuPont Report No. AMR 3722-95
Guideline(s):	US-EPA 163-1 (1982)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substances:	• IN-W3595, purity 95.8 % • IN-R3273, purity 92.1 % • IN-KQ960, purity 99.8 % • IN-JX915, purity 86 % • IN-U3204, purity 97.6 %
	All non-labelled
Test concentration:	• IN-W3595, IN-R3273: 1 mg L⁻¹ individually in 0.01 M CaCl₂ solution • IN-KQ960, IN-JX915: 5 mg L⁻¹ individually in 0.01 M CaCl₂ solution • IN-U3204: 5 mg L⁻¹ individually in 0.01 M CaCl₂ solution containing 0.075 % of methanol (v/v)
Test systems:	10 g soil (oven dry weight equivalent) + 10 mL of test solution or blank 0.01 M CaCl ₂ solution; soil/solution ration 1/1 (m/v)
Test temperature:	25 ± 2 °C
Equilibration time:	24 hrs of adsorption / 24 hrs of desorption (2 desorption cycles)
Sample processing:	Aliquots of decanted supernatants and control samples were stored at -10 to -31 °C. For analysis samples were filtered (0.2 to 0.45 µm) and analysed by capillary electrophoresis
Analytical techniques:	Capillary electrophoresis (CE) with UV detection
	LOQ: 2 % of total applied (IN-JX915, IN-U3204)
	5 % of total analyte applied (IN-W3595, IN-R3273)
	10 % of total analyte applied (IN-KQ960)
Stability monitoring:	To obtain <u>control water samples</u> , each soil was mixed with pure 0.01 M CaCl ₂ in a ratio of 1/1 (m/v), shaken at 25 °C for 24 hrs and centrifuged. Duplicate supernatant samples were fortified with IN-W3595 and IN-R3273 (final concentration 2.0 mg L ⁻¹) and IN-KQ960, IN-JX915 and IN-U3204 (5.0 mg L ⁻¹). Fortified samples were equilibrated along with the treated soils samples for 24 hrs.
Calculation:	Amounts of analyte adsorbed to the soil matrix were calculated by subtracting the concentration found in the solution (corrected for degradation based on fortified control water samples in case of IN-JX915) from the initial concentration applied.

Table B.8.4.2-1: Soil Characteristics.

Soil	'Bayboro'	'Chino'	'Fargo'	'Sassafras'
Location	USA	USA	North Dakota, USA	New Jersey, USA
Texture (USDA)	Silty loam	Loam	Silty clay loam	Sandy loam
Sand (USDA) [%]	13.2	40.8	15	73.2
Silt (USDA) [%]	74.0	40.4	56	18.0
Clay (USDA) [%]	12.8	18.8	29	8.8
C _{org} [%]	2.3	0.99	3.2	0.46
pH	4.6	7.6	7.8	6.4
CEC [meq kg ⁻¹]	112	173	443	38
WHC (maximum) [%]	na	na	na	na
WHC (at 1/3 bar) [%]	24.3	23.5	41	8.1
WHC (at 15 bar) [%]	8.0	10.3	22	3.3

Findings:

Mean recovery for all analytes (except IN-U3204) from freshly fortified control water samples (0.1 and 2.0 mg L⁻¹) was 91 – 109 % of applied (analysed for 'Bayboro' and 'Fargo' soil only) with a relative standard deviation of 8 – 23 %. Recover of IN-U3204 was very low, averaging 50 % of applied. This was due to the high instability of IN-U3204 in aqueous solutions. Recovery of IN-U3204 immediately measured after fortification (additional experiment) was 94 ± 5 % of applied (mean of all soils).

IN-W3595 and IN-R3273 did not show significant degradation in fortified control water samples equilibrated for 24 hrs, IN-JX915 degraded tentatively to IN-R3273 (except 'Bayboro' soil) and was corrected for degradation (57 to 68 % recovery). IN-KQ960 was also partly unstable degrading to an unknown metabolite, which could not be fully separated from IN-KQ960 by CE. IN-U3204 was extremely unstable in fortified samples and tentatively degraded to IN-R3273 in 'Bayboro' soil. However, in remaining soils no IN-R3273 was detected. No degradation correction could be performed for IN-U3204. All test substances were stable under freezing storage conditions.

Table B.8.4.2-2: Adsorption [% of applied] of investigated metabolites to the soil after equilibrium time of 24 hrs (test concentration 1 mg L⁻¹, mean of two replicates).

Soil	C _{org} [%]	IN-U3204	IN-W3595	IN-R3273	IN-KQ960	IN-JX915
'Bayboro'	2.3	Unstable	39	37	0 (0)	8
'Chino'	0.99	Unstable	3	33	0 (-15)	25
'Fargo'	3.2	Unstable	7	60	0 (-5)	40
'Sassafras'	0.46	Unstable	2	17	0 (-16)	2

Table B.8.4.2-3: Adsorption K_d values [L kg⁻¹] of investigated metabolites at 1 mg L⁻¹ (mean of two replicates).

Soil	C _{org} [%]	IN-U3204	IN-W3595	IN-R3273	IN-KQ960	IN-JX915
'Bayboro'	2.3	Unstable	0.63	0.59	0.0 (0.0)	0.13
'Chino'	0.99	Unstable	0.026	0.49	0.0 (-0.13)	0.34
'Fargo'	3.2	Unstable	0.074	1.5	0.0 (-0.05)	0.66
'Sassafras'	0.46	Unstable	0.020	0.21	0.0 (-0.14)	0.021

Table B.8.4.2-4: Adsorption K_{OC} values [$L\ kg^{-1}$] of investigated metabolites (mean of two replicates).

Soil	pH	IN-U3204	IN-W3595	IN-R3273	IN-KQ960	IN-JX915
'Bayboro'	4.6	Unstable	27.4	25.7	0.0 (-0.1)	5.4
'Chino'	7.6	Unstable	2.6	49.5	0.0 (-14)	34.3
'Fargo'	7.8	Unstable	2.3	46.9	0.0 (-1.5)	20.6
'Sassafras'	6.4	Unstable	4.3	45.7	0.0 (-30)	4.4
Arithmetic mean		Unstable	9.2	41.9	0.0	16.3

Conclusion:

IN-U3204 was unstable in the test system and consequently no sorption data were reported. The K_{OC} values for IN-W3595 were in a range of 2.3 – 27.4 $L\ kg^{-1}$, strongly depending on soil pH. Since IN-W3595 is an acidic compound this behaviour is likely to result from dissociation under more alkaline conditions. The K_{OC} values for IN-R3283 and IN-JX915 ranged from 25.7 – 49.5 $L\ kg^{-1}$ (arithmetic mean 41.9 $L\ kg^{-1}$) and 4.4 – 34.3 $L\ kg^{-1}$ (arithmetic mean 16.3 $L\ kg^{-1}$), respectively. Owing to partly instability of IN-KQ960 in contact with soil, resulting in a degradation product, which could not be fully separated from IN-KQ960 by CE, no reliable K_{OC} value could be determined (virtually no adsorption measured).

Comments (RMS):

- Data presented on metabolite IN-KQ960 in treated soil samples, include IN-KQ960 and an unknown degradation product, which could not be fully separated from IN-KQ960 by CE. Since quantification was based on UV-detection, it can not be concluded that this degradate disproportionately contributes to the CE peak area used for quantification. This might have resulted in a higher 'amount' of IN-KQ960 found in the solution and might therefore result in virtually no adsorption or even negative values (as reported). Based on typical HPLC-retention times of IN-KQ960 and on the subsequent study (Mattson, 2003), in which sorption data for several metabolites were generated using the HPLC method, it is highly unlikely that IN-KQ960 will not significantly adsorb to the soil matrix. Further evidence for significant adsorption of IN-KQ960 to soil and sediment can be obtained from the water/sediment study (Trabue and Lydick, 2001). Based on 8 DAT, 21 DAT and 28 DAT samples from the 'Brandywine Creek' system a mean K_{OC} of 22.0 $L\ kg^{-1}$ could be estimated by the notifier.
- Adsorption of the acidic metabolite IN-W3595 was shown to depend on soil pH with higher K_{OC} values found in acidic soils. Such a pH-dependent adsorption behaviour is frequently observed for acidic compounds and mainly results from a change of the polarity of the molecule owing to dissociation processes. The soil pH depending adsorption behaviour is based on the following context:

$$K_{FOC}(pH) = \frac{K_{FOC-acid} + K_{FOC-base} \left(\frac{M_{base}}{M_{acid}} \right) 10^{pH - pK_a - \Delta pH}}{1 + \left(\frac{M_{base}}{M_{acid}} \right) 10^{pH - pK_a - \Delta pH}},$$

whereby $K_{OC-acid}$ is the K_{OC} value under acidic conditions, $K_{OC-base}$ under alkaline conditions, M_{base} is the mol mass of the non-dissociated, M_{acid} the mol mass of the dissociated molecule. The pK_a of the substance represents the inflection point and ΔpH is a constant, which shifts the S-shaped curve into more alkaline or acidic conditions (ΔpH is set to zero in this case). Using the EXCEL solver tool, this formula (with the three unknowns $K_{OC-acid}$, $K_{OC-base}$ and pK_a) was fitted to the measured K_{OC} values of IN-W3595 from soil equilibrium batch experiments. The obtained values for $K_{OC-acid}$, $K_{OC-base}$ and pK_a values were 33.3 $L\ kg^{-1}$, 2.3 $L\ kg^{-1}$, and 5.2, respectively.

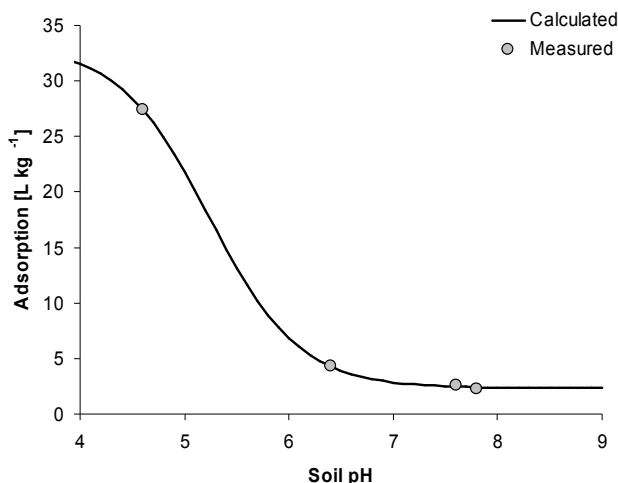


Figure B.8.4-1: Modelled vs. measured K_{FOC} values of IN-W3595 depending on soil pH.

Reference: Estimation of adsorption coefficients (K_{oc}) of cymoxanil metabolites, IN-U3204, IN-KQ960, IN-T4226 and IN-KP533 using HPLC

Author(s), year: Mattson, S. L., 2003

Report/Doc. number: DuPont-11758

Guideline(s): OECD 121 (2001)

GLP: Yes

Deviations: None

Validity: Study considered acceptable, data recalculated by the notifier

Material and methods:

Test substances:

- IN-U3204, purity 94.4 %
- IN-KQ960, purity 98.4 %
- IN-T4226, purity 99.1 %
- IN-KP533, purity 99.7 %
- IN-W3595, purity 93.7 % (in the original study, IN-W395 was considered as reference substance, after re-vision by the notifier, IN-W3595 was included into the list of test items)

All non-labelled

Reference substances: Saccharin, atrazin, IN-R3273, IN-JX915 and cymoxanil (all non-labelled)

Test system:

Reference standards and test substance were each dissolved in an appropriate amount of methanol for a concentration of approximately 1 g L^{-1} . The standards and test substance were run on an HPLC equipped with a Zorbax CN (cyanopropyl) column. The HPLC method employed an isocratic elution consisting of a mobile phase of methanol/0.01 M citrate-buffer pH 5.9 (30/70, v/v). The K_{oc} is deduced from the k' value using a calibration plot of $\log K_{oc}$ versus $\log k'$ of the reference substances:

$$k' = \frac{t_R - t_0}{t_0}$$

where:

t_R : HPLC retention time of test and reference substance (minutes)

t_0 : HPLC dead time (acetonitrile, 2.3 minutes)

The $\log k'$ data of the reference substances are plotted against their $\log K_{oc}$ values

obtained in batch equilibrium experiments and from literature. Using this plot, the $\log k'$ value of a test substance is then used to determine its $\log K_{oc}$ value.

Findings:

Linear regression of reported $\log K_{oc}$ values and measured retention times of the reference standards gave an R^2 of 0.85, and an equation describing the line of $\log(K_{oc}) = 0.534 \times \log(k') + 1.45$.

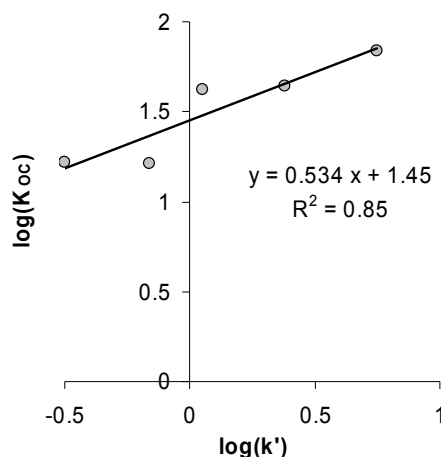


Figure B.8.4.2-2: Linear regression between $\log(k')$ and $\log(K_{oc})$ for reference substances used.

Table B.8.4.2-5: K_{oc} values, $\log(K_{oc})$, measured retention times (t_R) and calculated $\log(k')$ for reference substances used.

Compound	K_{oc} [L kg ⁻¹]	Log(K_{oc})	t_R [min]	Log(k')	Source
Cymoxanil	43.6	1.64	7.84	0.38	Slangen (1999)
Atrazine	69.2	1.84	15.2	0.75	Literature [1]
Saccharin	16.5	1.22	3.02	-0.50	Yeomans (1999) [2]
IN-JX915	16.3	1.21	3.89	-0.16	Hausmann and Adams, 1996
IN-R3273	41.9	1.62	4.86	0.05	Hausmann and Adams, 1996

[1] Kordel, W., Kotthoff, G., Miller, J. (1995) HPLC-screening method for the determination of the adsorption-coefficient on soil – result of a ring test. Chemosphere **30**, 1373-1384.

[2] Yeomans, P (1999) [¹⁴C]IN-00581: adsorption/desorption in soil. DuPont-1806, E. I. du Pont de Nemours and Company, Wilmington, DE, USA

Table B.8.4.2-6: K_{oc} values, $\log(K_{oc})$, measured retention times (t_R) and calculated $\log(k')$ for test substances.

Compound	t_R [min]	Log(k')	Log(K_{oc})	K_{oc} [L kg ⁻¹]
IN-U3204	4.55	-0.010	1.44	27.9
IN-KQ960	3.70	-0.216	1.33	21.6
IN-W3595	2.90	-0.584	1.14	13.8
IN-T4226	3.26	-0.379	1.25	17.7
IN-KP533	2.83	-0.637	1.11	12.9

Conclusion:

According to HPLC method, K_{oc} values of cymoxanil degradates IN-U3204, IN-KQ960, IN-W3595, IN-T4226 and IN-KP533 were estimated to be 27.9, 21.6, 13.8, 17.7 and 12.9 L kg⁻¹, respectively.

Comments (RMS):

- K_{OC} values for all test compounds were re-calculated by the notifier owing to the following reasons:
 - The K_{OC} value of cymoxanil used in the original study (120 L kg⁻¹) is based on the DuPont study Horne, P.: Batch equilibrium (adsorption/desorption) studies of [¹⁴C]DPX-T3217 (1992), which is not included in the submitted dossier. This study is superseded by the Oxon study Slangen (1999) which determined a K_{OC} value of 46.3 L kg⁻¹ for cymoxanil.
 - IN-W3595, formally considered as reference substance, is now included into the list of test items

B.8.4.3 Column leaching with the active substance (Annex IIA, point 7.1.3.1a, Annex IIIA, point 9.1.2.1)

Reliable sorption data have been generated for the active substance and metabolites. Therefore column leaching studies on cymoxanil and any degradation product are not required.

B.8.4.4 Column leaching with relevant degradation metabolites, degradation and reaction products (Annex IIA, point 7.1.3.1b, Annex IIIA, point 9.1.2.1)

Reliable sorption data have been generated for the active substance and metabolites. Therefore column leaching studies on cymoxanil and any degradation product are not required.

B.8.4.5 Aged residue column leaching (Annex IIA, point 7.1.3.2, Annex IIIA, point 9.1.2.1)

Reliable sorption data have been generated for the active substance and metabolites. Therefore aged residue column leaching studies on cymoxanil and any degradation product are not required.

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B.8.4.6 Lysimeter and field leaching studies (Annex IIA, point 7.1.3.3, Annex IIIA, point 9.1.2.2)

Reference:	Outdoor lysimeter study on cymoxanil
Author(s), year:	Hassink, J., 1998
Report/Doc. number:	DuPont Report No. AMR 3897-96
Guideline(s):	BBA IV, 4-3, 1990 (including the modification dated June 1991), Lysimeteruntersuchungen zur Verlagerung von Pflanzenschutzmittel in den Untergrund, German Federal Biological Research Centre for Agriculture and Forestry
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, 2.82 MBq mg ⁻¹ , > 96 % radiochemical purity (TLC), batch 2891-226
Reference substances:	Cymoxanil (non-labelled), IN-R3273, IN-U3204, IN-T4226, oxamic acid (IN-18474), IN-R3274, oxalic acid
Soil:	Two undisturbed soil monoliths were sampled in autumn 1992 from a field near Borstel, Neustadt am Rübenberge (Lower Saxony, Germany), installed into the outdoor-facilities and kept fallow before planting in spring 1993.
Lysimeter design:	The surface of the two lysimeter was approximately 1 m ² each, the test substance was applied on a central area of approx. 0.9 m ² to avoid edge effects. The soil surrounding the lysimeters (approx. 10.5 m ²) was maintained and cultivated the same as the lysimeters in order to achieve sufficient microclimate like on the field. The depth of the lysimeters was 1.2 m.
Application:	Lysimeter 1: 1993: 3 × 320 g ai ha⁻¹ (30 June, 6 July, 14 July), total 960 g ai ha⁻¹ 1994: 3 × 320 g ai ha⁻¹ (30 June, 7 July, 14 July), total 960 g ai ha⁻¹ Lysimeter 2: 1993: 3 × 320 g ai ha⁻¹ (30 June, 6 July, 14 July), total 960 g ai ha⁻¹ 90 mL of application solution (35.6 mg ¹⁴C-cymoxanil and 35.6 mg blank formulation Curzate 50 WP in 100 mL of water) were sprayed onto the plants and the surface of the lysimeter using an automatic jet system. Application was performed under a plastic tent to prevent contamination of surrounding area.
Crop cover:	1993: Potatoes (growing stage at application: beginning till ending of flowering), winter rye 1994: Potatoes (growing stage at application: beginning till ending of flowering), winter barley
Irrigation:	1996: April: 80 L m ⁻² , May: 110 L m ⁻² , June: 80 L m ⁻²
Leachate handling:	Radioactive content of the leachate (approx. 20 – 50 L each) was quantified by LSC. Samples containing > 50 Bq L ⁻¹ (corresponding to 0.02 µg ai equiv. L ⁻¹) were subjected to further purification. Samples were stored at 4 °C for usually no longer than 4 weeks prior to purification (see sample processing).
Lysimeter sampling:	Soil samples were taken after the end of each vegetation period, on 22 September 1993, 7 September 1994 and 13 September 1995. Additionally, samples from the topsoil were taken on 3 May 1994, before the second treatment of lysimeter 1. At each sampling date five samples were taken from each lysimeter and divided into 0 – 10 and 10 – 20 cm layers (diameter 2 cm each). For further

analysis the five samples of each layer were combined. Afterwards the lysimeter soil was either mixed to a depth of 15 – 20 cm before seeding, or the soil cores were filled with untreated Borstel topsoil (in case of May 1994).

The lysimeter were removed from the test site at the end of study (18 July 1995, lysimeter 2; 16 July 1996, lysimeter 1) and divided into 10-cm-layers starting from the 110 – 120 cm layer.

Sample processing:

One litre of leachate samples, collected over each month, was assayed for $^{14}\text{CO}_2$, concentrated by freeze drying and resolved in methanol and after method modification (from January 1994 onwards) in acetonitrile/0.2 % formic acid in water (40/60, v/v). Recovery of radioactivity after resolving in methanol was only 30 to 40 %, therefore resolving procedure was changed (recovery > 90 % for cymoxanil and 70 – 90 % in general). Analysis of resolved leachate samples was done by reversed phase C8 HPLC including fraction collection and LSC. Polar fractions were combined to form representative yearly combined samples and were analysed by an additional HPLC method (Supelcogel C610H). Individual peaks were identified by spiking with non-labelled reference substances under UV-detection.

Soil analysis was conducted on 50 g damp sample, which were extracted twice with 100 mL of methanol/water (95/5, v/v) and three times with 100 mL of methanol/water (50/50, v/v) for 30 min. Supernatants were radio-assayed by LSC, combined, concentrated by slight vacuum (35 °), once again radio-assayed by LCS and analysed by HPLC. Remaining soil samples were extracted twice with 100 ml 0.5 M NaOH to quantify the amount of radioactive material bound to humic and fulvic acids. The remaining soil debris was radio-assayed by combustion-LSC.

The uptake of radioactive material by plants was quantified by combustion of plant material.

Analytical techniques:

- LSC, HPLC (RP-C8, Supelcogel C-610H) coupled with a fraction collector (LSC)
- Selected leachate samples were analyzed for cymoxanil, IN-T4226, IN-U3204 und IN-R3273 using GC/MS after silylation with MSTBFA
- LOQ (cymoxanil and metabolites) = $0.01 \mu\text{g L}^{-1}$

Note: HPLC methods used in this study are comparable to HPLC methods used in the soil degradation study from Boucher (1996)

Table B.8.4.6-1: Properties of the Borstel field (source of lysimeter soil) in September 1994.

Depth [cm]	Sand [%]	Silt [%]	Clay [%]	Texture	C _{org} [%]	pH [CaCl ₂]	CEC [meq kg ⁻¹]	MC at field capacity [%]
0 – 30	75.2	21.2	3.6	Loamy sand	1.29	5.4	80	15.7
30 – 50	75.9	21.1	3.0	Loamy sand	0.89	5.5	50	13.4
50 – 70	77.6	19.4	3.0	Loamy sand	0.27	5.6	30	11.8
70 – 90	89.7	10.2	0.1	Sand	0.08	5.6	30	6.0
90 – 110	97.9	2.0	0.1	Sand	0.03	5.8	< 10	3.4
110 – 130	98.8	1.1	0.1	Sand	0.03	5.6	<10	2.5

Table B.8.4.6-2: Actual properties of the topsoil of the lysimeter at the beginning of the study (May 1993) and microbial biomass of the topsoil in May 1993 and May 1994.

Depth [cm]	C _{org} [%]	pH [CaCl ₂]	CEC [meq kg ⁻¹]	Microbial biomass [µg C _{mic} g ⁻¹]	
				May 1993	May 1994
0 – 30	1.4	5.4	50	270	250

Table B.8.4.6-3: Timetable of application, soil sampling, lysimeter maintenance and crop cultivation.

Year	Date	Seeding	Harvest	Fertilizer ^a [kg ha ⁻¹]	PPP	Application [g ai ha ⁻¹]		Soil sampling
						Lys. 1	Lys. 2	
1993	13 May	Potatoes ^b						
	26 May			800				
	30 June					320	320	
	6 July					320	320	
	14 July					320	320	
	1 September		Potatoes					
	22 September							Soil sampling
1994	4 October	Winter rye		400				
	3 May	Potatoes ^b	Winter rye	800				Soil sampling
	30 June					320		
	7 July					320		
	14 July					320		
	5 September		Potatoes					
	7 September							Soil sampling
1995	12 September	Winter barley		300				
	13 April				Folicur ^c			
	8 May				Folicur ^c			
	31/12 ^d July		Winter barley					
	19 July							Disassembling of lysimeter 2
	13 September							Soil sampling (lysimeter 1)
1996	14 September	Winter rye		400				
	15 July		Winter rye					
	17 July							Disassembling of lysimeter 1

^a N/P/K/Mg (12/12/17/2) fertilizer

^b 8 plants/lysimeter

^c Folicur: 250 g tebuconazole L⁻¹, 1.25 L Folicur in 300 L water ha⁻¹

^d Lysimeter 1: July 31, lysimeter 2: July 12

Table B.8.4.6-4: Climatic conditions during test period.

Test period	Annual air mean temp. [°C]	Annual soil (10 cm) mean temp. [°C]	Annual precipitation [mm]
1 st year	11.9	8.9	1171
2 nd year	9.9	8.9	1176
3 rd year	8.5	9.3	850 ^a
Mean 1990 - 1996	9.0	8.2	936

^a Including artificial irrigation

Findings:**Table B.8.4.6-5: Total amount of precipitation (including irrigation, [mm]) and of leachate [mm]**

Parameter	1 st year		2 nd year		3 rd year
	Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1
Precipitation + irrigation [mm]	1171		1176		850 ^a
Leachate [mm]	903	885	758	718	497

^a including artificial irrigation*Leachates:*

The highest absolute concentrations of radioactive material in individual leachate samples of lysimeter 1 were detected in the first winter months of the treatment year: 0.68 µg ai equiv. L⁻¹ on 18 November 1993 after the first and 1.22 µg ai equiv. L⁻¹ on 23 December 1994 after the second year of treatment. The highest concentration of RA in the second lysimeter accounted for 0.63 µg ai equiv. L⁻¹ (13 December 1993), decreasing thereafter. Based on monthly leachate samples (after work-up) maximum concentration of total RA in lysimeter 1 was found to be 0.68 and 0.96 µg ai equiv. L⁻¹ (appearing close to November/December 1993 and December 1994), in lysimeter 2 maximum monthly mean concentration of AR accounted for 0.52 µg equiv. L⁻¹ (close to November/December 1993). Based on annual mean concentrations, amounts of total RA found in the leachates were in a range of 0.19 – 0.56 µg ai equiv. L⁻¹ (lysimeter 1) and 0.11 – 0.26 µg ai equiv. L⁻¹ (lysimeter 2).

Cymoxanil did not occur > LOQ in the leachate samples at any time (LOQ = 0.01 µg L⁻¹).

Leachates of both lysimeters were characterized by a large polar fraction (RP-C8 HPLC, RT about 2 – 4 min), which comprises the majority of radioactive compounds throughout the study period. In lysimeter 1 maximum monthly mean amounts of polars were found in December 1993 (0.11 µg ai equiv. L⁻¹) and in December 1994 (0.28 µg ai equivalent L⁻¹). In lysimeter 2, maximum monthly mean amounts were found in December 1993 (0.18 µg ai equiv. L⁻¹). Amounts of polars present in the leachates significantly decreased after maximum occurrence. Annual mean concentration of polars were in a range of 0.07 – 0.15 µg ai equiv. L⁻¹ (lysimeter 1) and 0.03 – 0.10 µg ai equiv. L⁻¹ (lysimeter 2).

The polar fraction (monthly aliquots combined to annual samples) obtained by the RP-C8 HPLC analysis was further characterized by a second HPLC method (Supelcogel C610H, including co-chromatography of authentic reference standards). The metabolites IN-R3274 and oxalic acid could be found at a maximum annual mean concentration of 0.02 and 0.03 µg L⁻¹. No oxamic acid (IN-18474) was found in the leachate samples (< LOQ). The great majority of polar compounds remained unidentified with annual mean concentrations of 0.02 – 0.11 µg ai equiv. L⁻¹.

Beside the large polar fraction detected by RP-C8 HPLC analysis, only minor amounts of less polar metabolites were observed in the leachates. In both lysimeter maximum monthly mean amounts of IN-T4226, IN-R3273 and IN-U3204 accounted for 0.12, 0.02 and 0.01 µg L⁻¹ (only sporadic findings > LOQ for each metabolite). In the great majority findings of IN-T4226, IN-R3273 and IN-U3204 were < LOQ resulting in annual mean concentrations ≤ 0.02 µg L⁻¹, ≤ 0.01 µg L⁻¹ and < 0.01 µg L⁻¹, respectively.

In the leachate samples, which were analysed for cymoxanil, IN-T4226, IN-U3204 and IN-R3273 using GC/MS (after silylation) only IN-R3273 could be detected once in a sample. Therefore, metabolite identification was solely based on co-chromatography with reference substances.

Maximum monthly mean amounts of ¹⁴CO₂ present in the leachates did never exceed 0.06 µg L⁻¹ (lysimeter 1 and lysimeter 2).

The great majority of radioactivity (approx. 80 – 90 %) present in the leachates could not be identified. Approx. 28 % of radioactivity was lost during sample work-up, when lyophilized leachate aliquots (1 L) were re-constituted in organic solvents for HPLC analysis, the remaining 50 – 60 % comprise unidentified radioactivity obtained by RP-C8 HPLC and unidentified polars obtained by Supelco C610H HPLC. Annual mean concentrations of total unidentified RA was in a range of 0.16 – 0.46 $\mu\text{g ai equiv. L}^{-1}$ (lysimeter 1) and 0.10 – 0.10 $\mu\text{g ai equiv. L}^{-1}$ (lysimeter 2). Based on monthly mean samples, maximum amounts of RA lost during sample work-up were 0.38 $\mu\text{g ai equiv. L}^{-1}$ (lysimeter 1) and 0.38 $\mu\text{g ai equiv. L}^{-1}$ (lysimeter 2), when re-constituting lyophilized samples in methanol. After switching to acetonitrile/0.2 % formic acid in water (40/60, v/v) as re-constituting solvent from January 1994 onwards, maximum monthly mean losses were 0.25 $\mu\text{g ai equiv. L}^{-1}$ (lysimeter 1) and 0.13 $\mu\text{g ai equiv. L}^{-1}$ (lysimeter 2).

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Table B.8.4.6-6: Annual mean concentrations (based on 1-year, 2-year and 3-year periods) of total radioactivity, ^{14}C , cymoxanil and metabolites in leachate samples (expressed as $[\mu\text{g ai equivalent L}^{-1}]$ and $[\% \text{ of AR}]$).

		1 st year		2 nd year		3 rd year		2-year period		3-year period	
		Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2
Total radioactivity	$[\mu\text{g ai equiv. L}^{-1}]$	0.27	0.26	0.56	0.11	0.19	0.19	0.40	0.19	0.35	0.35
	$[\% \text{ of AR}]$	0.26	0.25	0.23	0.09	0.05	0.05	0.49	0.33	0.54	0.54
^{14}C	$[\mu\text{g ai equiv. L}^{-1}]$	0.03	0.04	0.04	0.01	0.01	0.01	0.03	0.03	0.03	0.03
	$[\% \text{ of AR}]$	0.03	0.04	0.02	0.01	< 0.01	< 0.01	0.05	0.04	< 0.06	< 0.06
Loss before RP-C8 HPLC owing to work-up procedure	$[\mu\text{g ai equiv. L}^{-1}]$	0.10	0.07	0.13	0.03	0.05	0.05	0.11	0.05	0.10	0.10
	$[\% \text{ of AR}]$	0.10	0.07	0.05	0.02	0.02	0.02	0.15	0.09	0.17	0.17
	$[\% \text{ of total leachate RA}]$	37.0	26.9	23.2	27.3	26.3	26.3	27.5	26.3	28.6	28.6
All (RP-C8 HPLC)	Cymoxanil	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	$[\mu\text{g ai equiv. L}^{-1}]$	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	$[\% \text{ of AR}]$	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	IN-U3204	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	$[\mu\text{g ai equiv. L}^{-1}]$	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	$[\% \text{ of AR}]$	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	IN-T4226	< LOQ	< LOQ	0.02	0.01	< LOQ	< LOQ	0.01	0.01	0.01	0.01
	$[\mu\text{g ai equiv. L}^{-1}]$	< LOQ	< LOQ	0.02	0.01	< LOQ	< LOQ	0.01	0.01	0.01	0.01
	$[\% \text{ of AR}]$	< LOQ	< LOQ	0.01	0.01	< LOQ	< LOQ	0.01	0.01	0.01	0.01
	IN-R3273	< LOQ	< LOQ	0.01	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Polars ^a (Supelcogel C610H)	$[\mu\text{g ai equiv. L}^{-1}]$	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	$[\% \text{ of AR}]$	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Polars (unidentified) ^a	0.08	0.10	0.15	0.03	0.07	0.07	0.11	0.06	0.10	0.10
	$[\mu\text{g ai equiv. L}^{-1}]$	0.07	0.09	0.06	0.02	0.02	0.02	0.13	0.11	0.15	0.15
	$[\% \text{ of AR}]$	0.07	0.07	0.22	0.05	0.06	0.06	0.14	0.06	0.12	0.12
	Unidentified RA	0.07	0.06	0.09	0.04	0.02	0.02	0.16	0.10	0.18	0.18
	$[\mu\text{g ai equiv. L}^{-1}]$	< LOQ	< LOQ	0.02	< LOQ	< LOQ	< LOQ	0.01	< LOQ	0.01	0.01
	$[\% \text{ of AR}]$	< LOQ	< LOQ	0.01	< LOQ	< LOQ	< LOQ	0.01	< LOQ	0.01	0.01
	IN-18474	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	$[\mu\text{g ai equiv. L}^{-1}]$	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Polars ^a (Supelcogel C610H)	$[\mu\text{g ai equiv. L}^{-1}]$	0.02	0.02	0.03	< LOQ	0.02	0.02	0.02	0.01	0.02	0.02
	$[\% \text{ of AR}]$	0.02	0.02	0.01	< LOQ	< 0.01	< 0.01	0.03	0.02	< 0.03	< 0.03
	Oxalic acid	0.06	0.07	0.11	0.02	0.05	0.05	0.08	0.05	0.07	0.07
	$[\mu\text{g ai equiv. L}^{-1}]$	0.05	0.07	0.04	0.02	0.01	0.01	0.09	0.09	0.10	0.10
	$[\% \text{ of AR}]$	0.23	0.21	0.46	0.10	0.16	0.16	0.33	0.16	0.29	0.29
	Sum of unidentified radioactivity ^b	0.22	0.20	0.18	0.08	0.05	0.05	0.40	0.28	0.45	0.45
	$[\mu\text{g ai equiv. L}^{-1}]$	85.2	80.8	82.1	90.9	84.2	84.2	82.5	84.2	82.9	82.9
	$[\% \text{ of total leachate RA}]$										

LOQ = 0.01 $\mu\text{g L}^{-1}$

^a Detailed characterization of unidentified polars (RP-C8 HPLC) with 2nd HPLC method (Supelcogel C610H)

^b Sum of loss during work-up, non-identified RA of RP-C8 HPLC and unidentified polars (Supelcogel C610H)

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Soil samples:

Total radioactivity present in the top soil (0 – 30 cm) after the study period (3 years in case of lysimeter 1, 2 years in case of lysimeter 2) was 20.8 % of AR (lysimeter 1) and 15.9 % of AR (lysimeter 2). Amounts of RA in soil layers below 30 cm were < 0.1 of AR. The great majority of total RA present in soil after the study period comprises NER (associated with fulvic/humic acids and humins), only 1.1 – 1.3 % of AR could be extracted. Cymoxanil accounted for < 0.1 % of AR in lysimeter 1 (0 – 5 cm), no cymoxanil could be found in other soil samples after disassembling of the lysimeters. The great majority of extractable RA comprises unidentified RA (0.7 – 0.9 % of AR), no metabolite was found > 0.1 % of AR.

During the study period, total RA present in the top soil (0 – 20 cm) of lysimeter 1 decreased from 59.4 % of AR (22 September, 1993) to 42.1 % of AR (3 May, 1993). After the second application period, amounts of total RA were found to be 65.5 % of AR (7 September, 1994), decreasing thereafter to 33.9 % of AR (13 September, 1995) and 19.7 % of AR (17 July, 1996, disassembling of lysimeter). In the lysimeter 2, treated only for one period, total RA present in the top soil decreased from 73.1 % of AR (22 September, 1993) to 44.6 % of AR (3 May, 1994), hardly changing thereafter until 7 September 1994 and, finally, decreasing to 14.9 % of AR by study termination (19 July, 1995).

Maximum extractable RA in the top soil (0 – 20 cm) was found to be 5.9 % of AR in lysimeter 1 and 5.4 % in lysimeter 2 in the first year of application (22 September, 1993). In lysimeter 2 a second maximum of 5.7 % of AR was found to be extractable in 1994 (7 September) owing to the application in the second year.

Maximum amounts of cymoxanil present in the first soil sampling after application (84 days after the first application) were 0.3 % of AR, further decreasing thereafter. No metabolites could be found < LOQ at all. The great majority of extractable RA from soil samples comprises unidentified polars (maximum 2.3 % of AR) and unidentified RA maximum (3.5 % of AR).

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Table B.8.4.6-7: Distribution of radioactivity [% of AR] in lysimeter soil samples (0 – 20 cm, during study) and after disassembling of lysimeters (0 – 120 cm, end of study).

Sampling date	DAFT ^a	Depth [cm]	Total		Extractable		NER					
							Fulvic/humic acids		Humins		Total	
			Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2
22 September, 1993	84	0 - 10	57.3	71.9	5.6	5.1	41.8	40.6	20.7	21.1	62.5	61.7
		10 - 20	2.1	1.2	0.3	0.3	1.2	0.7	0.4	0.2	1.6	0.9
3 May, 1994	307	0 - 10	24.0	22.9	1.5	1.9	7.7	10.4	8.6	11.3	16.3	21.7
		10 - 20	18.4	21.7	1.8	1.8	8.8	10.3	8.9	11.7	17.7	22.0
7 September, 1994	434	0 - 10	59.9	29.3	5.2	1.7	35.7	14.0	14.7	11.3	50.4	25.3
		10 - 20	5.6	15.3	0.5	1.1	3.0	8.5	2.2	6.8	5.2	15.3
13 September, 1995	805	0 - 10	17.4		1.1	-	7.4	-	9.7	-	17.1	-
		10 - 20	16.5	-	1.1	-	7.5	-	9.2	-	16.7	-
17 July, 1996 (Lys. 1) 19 July, 1995 (Lys. 2) (after lysimeter disassembling)	1113/ 749	0 - 10	11.7	7.2	0.6	0.5	4.4	3.0	5.5	4.0	9.9	7.0
		10 - 20	8.0	7.7	0.6	0.5	3.7	3.4	4.2	4.7	7.9	8.1
		20 - 30	1.1	1.0	<0.1	0.1	0.5	0.6	0.5	0.7	1	1.3
		30 - 40	0.2	0.1	<0.1	<0.1	0.1	0.1	0.1	0.1	0.2	0.2
		40 -	≤0.1	≤0.1	na	na	na	na	na	na	na	na
Total		21.8	16.8	na	na	na	na	na	na	na	na	

^a DAFT denotes day after first treatment

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Table B.8.4.6-8: Characterization of extractable radioactivity [% of AR] in lysimeter soil samples (0 – 20 cm, during study) and after disassembling of lysimeters (0 – 120 cm, end of study).

Sampling date	DAFT ^a	Depth [cm]	¹⁴ C-Cymoxanil		IN-T4226		IN-U3204		IN-R3273		Non-identified polars		Unidentified RA	
			Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2	Lys. 1	Lys. 2
September 22, 1993	84	0 - 10	0.2	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	2.3	2.1	3.2	2.7
		10 - 20	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.1	0.2	0.5	0.2
May 3, 1994	307	0 - 10	0.1	< 0.1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.4	0.5	1.0	1.4
		10 - 20	< LOQ	0.1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.7	0.5	1.2	1.3
September 7, 1994	434	0 - 10	0.1	< 0.1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.5	0.5	3.5	1.2
		10 - 20	< LOQ	< 0.1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.2	0.3	0.3	0.7
September 13, 1995	805	0 - 10	< 0.1	-	< LOQ	-	< LOQ	-	< LOQ	-	0.4	-	0.7	-
		10 - 20	< 0.1	-	< LOQ	-	< LOQ	-	< LOQ	-	0.3	-	0.8	-
July 17, 1996 (Lys. 1) July 19, 1995 (Lys. 2) (after lysimeter disassembling)	1113/ 749	0 - 10	< 0.1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.1	0.1	0.4	0.3
		10 - 20	< LOQ	< LOQ	< LOQ	0.1	< LOQ	< LOQ	< LOQ	< LOQ	0.1	0.1	0.4	0.3
		20 - 30	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< 0.1	< 0.1	0.1	0.1
		30 - 40	< LOQ	na	< LOQ	na	< LOQ	na	< LOQ	na	< 0.1	na	< 0.1	na
		40 -	na	na	na	na	na	na	na	na	na	na	na	na

na denotes not analysed

^a DAFT denotes day after first treatment

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Plants:

Because the application of the test substance was performed to the potatoes in the growth stage of beginning till end of flowering, 5.9 % (lysimeter 1) and 6.7 % (lysimeter 2) of AR were detected in the potato tubers, 19.5 % of AR (lysimeter 1) and 24.0 % (lysimeter 2) occurred in the leaves. After the second application in 1994 (harvest date 5 September), 1.9 % of AR were found in tubers and 7.4 % of AR in leaves potatoes of lysimeter 1. In lysimeter 2 (no treatment in the second year) less than 0.1 % of AR was detected in potatoes tubers and leaves.

Harvest of cereals did not yield any radioactivity > 0.01 % of AR in the plant material.

Table B.8.4.6-9: Total radioactivity [% of AR] found in potatoes and cereals.

Harvest date	Crop	Tissue	Lysimeter 1	Lysimeter 2
1 September 1993	Potatoes	Tubers	5.9	6.7
		Leaves	19.5	24.0
3 May 1994	Winter rye	Total	< 0.01	< 0.01
5 September 1994	Potatoes	Tubers	1.9	< 0.1
		Leaves	7.4	< 0.1
12/31 July 1995 ^a	Winter barley	Total	< 0.01	< 0.01
15 July 1996	Winter rye	Total	< 0.01	-

^a 12 July: lysimeter 2, 31 July: lysimeter 1

Mass balance:

From the balance of the radioactivity applied and the total radioactivity recovered at the end of the study a loss of 43.0 % of AR (lysimeter 1) and 52.1 % (lysimeter 2) owing to volatilization and mineralization processes can be established.

Table B.8.4.6-10: Total material balance [% of AR] at the end of experiment.

Compartment	Total radioactivity		Cymoxanil	
	Lysimeter 1 (3-year period)	Lysimeter 2 (2-year period)	Lysimeter 1 (3-year period)	Lysimeter 2 (2-year period)
Soil	21.8	16.8	< 0.2	< 0.1
Leachate	0.54	0.33	< LOQ	< LOQ
Plants	34.7	30.8	No quantification performed	
Loss ^a	43.0	52.1	No quantification performed	

LOQ = 0.01 µg L⁻¹

^a Likely owing to volatilisation and mineralization.

Conclusion:

Leachates:

Annual mean amounts of total radioactivity present in leachates of both lysimeters were in a range of 0.11 – 0.56 µg ai equiv. L⁻¹, 0.01 – 0.04 µg ai equiv. L⁻¹ of total radioactivity could be attributed to dissolved ¹⁴CO₂. During further work-up of leachates a loss of radioactivity of approx. 30 % occurred (accounting for 0.02 – 0.10 µg ai equiv. L⁻¹). Cymoxanil did not occur > LOQ in the concentrated leachate samples at any time (LOQ = 0.01 µg L⁻¹). The investigated metabolites IN-U3204, IN-R3273, IN-T4226, IN-R3274, oxamic acid (IN-18474) and oxalic acid did not exceed annual mean amounts of 0.05 µg L⁻¹ (values not corrected for loss during work-up). Unidentified radioactivity, which was diffusely distributed over the chromatogram, was in a range of 0.05 – 0.22 µg ai equiv. L⁻¹, annual mean concentrations of unidentified polar substances were in a range of 0.02 – 0.11 µg ai equiv. L⁻¹ (both not corrected for loss). It can be assumed that all unknown fractions consist of several different compounds with a concentration below 0.1 µg L⁻¹ each.

Soil:

At the end of the experimental period the soil contained about 21.8 % of AR (lysimeter 1 after three years) and 16.8 % of AR (lysimeter 2 after two years), concentrated within the 0 – 20 cm of soil depth. Below a depth of 60 cm only 0.6 % of AR were present in the soil of both lysimeter. About 1.1 % of AR was extractable from the 0 – 20 cm top soil. Cymoxanil was only detectable in the uppermost soil layer (0 – 10 cm) showing amounts ≤ 0.1 % of AR. Investigated metabolites in soil samples (IN-U3204, IN-R3273 and IN-T4226) were not detectable throughout the profile. Non-identified polar substances and radioactivity, diffusely distributed over the chromatogram, did not exceed 0.8 % of AR from 0 - 20 cm of soil depth. Total radioactivity found in the soil was almost exclusively attributed to non-extractable residues (NER), including fulvic and humic acids (approx. 8 % of AR) and humins (approx. 10 % of AR), concentrated within the topsoil (0 – 20 cm).

Mass balance:

From the mass balance of the radioactivity applied and the total radioactivity recovered at the end of the study a loss of 43.0 % of AR (lysimeter 1) and 52.1 % of AR (lysimeter 2) owing to volatilization and mineralization processes can be established.

Comments (RMS):

- Owing to the soil pH of 5.4, the lysimeter soil is considered a worst-case situation in respect to the degradation half-life of cymoxanil (significant slower degradation in more acidic soil).
- The application rate of $3 \times 320 \text{ g ai ha}^{-1}$ (single application equivalent to $0.28 \text{ g ai kg}^{-1}$ assuming a soil depth of 5 cm, a soil density of 1.5 g cm^{-3} and a crop interception of 33 %) onto each lysimeter is considered a worst-case situation in respect to the intended use of cymoxanil. The worst-case PEC_s was calculated to be 0.24 mg kg^{-1} (compare to B.8.5).
- Annual mean amounts of unidentified polars on the Supelcogel C610H HPLC system slightly exceeded $0.1 \text{ } \mu\text{g ai equiv. L}^{-1}$ in one annual sample ($0.11 \text{ } \mu\text{g ai equiv. L}^{-1}$). However, even under the consideration of the loss during work-up (approx. 28 %), it is highly unlikely that one single polar compound will exceed $0.1 \text{ } \mu\text{g L}^{-1}$ individually considering the low molecular weight of the polar metabolites of cymoxanil.
- Metabolite identification was mainly based on co-chromatography with reference substances. Some commonly observed and mobile soil metabolites, e.g. IN-W3595 or IN-KQ960, were not investigated. Owing to the high number of soil degradates and the rather poor separation under conditions of HPLC, the final metabolite identification stated in the study has to be considered somewhat indicative. However, this has no impact on the overall outcome of the study (i.e. no individual metabolite $> 0.1 \text{ } \mu\text{g L}^{-1}$).

B.8.4.7 Summary on mobility in soil

Reasonable adsorption/desorption coefficients according to Freundlich isotherms (K_{FOC} , $1/n$ values) were determined for cymoxanil in soil batch experiments using 4 soils (3 EU, 1 USA) with a representative spectrum of soil properties. Owing to the high instability of cymoxanil in soil studies, time for adsorption had to be restricted to 3 hrs. Resulting K_{FOC} values of cymoxanil were in a range of $15.1 - 87.1 \text{ L kg}^{-1}$ with an arithmetic mean of 43.6 L kg^{-1} . Arithmetic mean $1/n$ value was 0.86. No impact of soil pH on the adsorption could be found.

Metabolites IN-U3204, IN-W3595, IN-R3273, IN-JX915 and IN-KQ960 were investigated in batch equilibrium experiments (only one concentration tested) using 4 soil (all US) with a representative spectrum of soil properties. Owing to the high instability of IN-U3204 in aqueous solutions, no reliable K_{OC} value could be stated for this metabolite. K_{OC} values for IN-JX915, also instable in aqueous solutions (data corrected for degradation), were in a range of $5.4 - 34.4 \text{ L kg}^{-1}$, with an arithmetic mean of 16.3 L kg^{-1} . Metabolite IN-R3273 was stable during equilibration time, K_{OC} values were calculated to be in a range of $25.7 - 49.5 \text{ L kg}^{-1}$, arithmetic mean 41.9 L kg^{-1} . Adsorption of IN-R3273 is considered to be lower in more acidic soils.

Adsorption of IN-W3595, an acidic compound which was stable during the test, was strongly depending on soil pH likely owing to dissociation processes under more alkaline conditions. K_{OC} values were in a range of $2.3 - 27.4 \text{ L kg}^{-1}$ (arithmetic mean 9.2 L kg^{-1}) with lowest adsorption found in the most alkaline soil (pH 7.8). This behaviour of the major soil metabolite IN-W3595 was adequately taken into account for groundwater risk assessment. In case of IN-KQ960 (virtually no adsorption observed, compound partly instable during the test) no reliable K_{OC} value could be stated owing to analytical reasons (degradation product of IN-KQ960 during the test could not be fully separated from IN-KQ960). Since no Freundlich isotherms could be calculated owing to the test design, $1/n$ values were set to the FOCUS default value of 0.9 for all metabolites.

In an additional study, metabolites IN-U3204 (unstable under conditions of soil batch experiments), IN-KQ960 (no reliable K_{OC} could be calculated in the batch experiment), IN-T4226, IN-W3595 and IN-KP533 were investigated in a HPLC method according to OECD guideline 121. On the basis of their retention times in comparison to the reference substances cymoxanil, atrazine, saccharin, IN-R3273 and IN-JX915, the K_{OC} values of IN-U3204, IN-KQ960, IN-T4226, IN-W3595 and IN-KP533 were estimated to be 27.9, 21.6, 17.7, 13.8 and 12.9 L kg^{-1} , respectively. $1/n$ values were set to the FOCUS default value of 0.9.

Table B.8.4.7-1: Summary on adsorption coefficients of cymoxanil and metabolites.

Compound	$K_{OC}/K_{FOC}^a [\text{L kg}^{-1}]$		$1/n^b$	n	Method	pH dependency
	Range	Arithmetic mean				
Cymoxanil	15.1 – 87.1	43.6	0.86	4	Batch	No
IN-U3204	-	27.9	0.9	1	HPLC	-
IN-W3595	2.3 – 27.4	9.2	0.9	4	Batch	Yes
	-	13.8		1	HPLC	-
IN-JX915	5.4 – 34.3	16.3	0.9	4	Batch	No
IN-R3273	25.7 – 49.5	41.9	0.9	4	Batch	Yes
IN-KQ960	0.0 ^c	0.0 ^c	0.9	4	Batch	-
	-	21.6		1	HPLC	-
IN-T4226	-	17.7	0.9	1	HPLC	-
IN-KP533	-	12.9	0.9	1	HPLC	-

^a Only in case of cymoxanil K_{FOC} (according to Freundlich isotherm), all metabolites K_{OC}

^b In case of metabolites FOCUS default value used

^c Batch experiment not considered relevant

In a **field lysimeter study**, conducted in Lower Saxony, Germany, [cyanoacetamide-2-¹⁴C] labelled cymoxanil, formulated as Curzate 50 WP, was applied three times at 320 g ai ha⁻¹ (7 day interval) onto a loamy sand soil with a pH of 5.4 in the top soil layer. At application, lysimeter were planted with potatoes (flowering stage), application was repeated in one of the two lysimeter in the second year.

In respect to the lysimeter soil pH and the application rate, the lysimeter study is considered a worst-case situation.

Cymoxanil was never found in leachate samples > LOQ (i.e. 0.01 µg L⁻¹).

A significant fraction of radioactivity found in the leachates could be attributed to polar compounds, which were detected with a maximum annual mean concentration of 0.15 µg ai equiv. L⁻¹. This polar fraction was shown to comprise oxalic acid at maximum annual mean amounts of 0.03 µg L⁻¹ and metabolite IN-R3274 at maximum annual mean amounts of 0.02 µg L⁻¹. However, the majority of polars remained unidentified with a maximum annual mean concentration of 0.11 µg ai equiv. L⁻¹. From the chromatographic behaviour and from the low molecular weight of polar metabolites, it is unlikely that unidentified polar compounds exceeded 0.1 µg L⁻¹ individually. None of the other investigated, less polar metabolites (IN-U3204, IN-T4226 and IN-R3273) exceeded 0.05 µg L⁻¹ individually.

The great majority (approx. 85 %) of radioactivity found in the leachates, accounting for a maximum annual mean concentration of 0.46 µg ai equiv. L⁻¹, could not be identified. This amount results from loss of radioactivity during work-up before HPLC (maximum 0.13 µg ai equiv. L⁻¹), unidentified radioactivity distributed over the HPLC chromatogram (maximum 0.22 µg ai equiv. L⁻¹) and unidentified polars (maximum 0.11 µg L⁻¹).

B.8.5 Predicted environmental concentrations in soil for the active substance and relevant metabolites (PEC_s) (Annex IIIA, point 9.1.3)

Reference:	Predicted environmental concentrations of cymoxanil and major metabolites in surface water, sediment, soil, and air after application of Tanos 50 WG to potatoes for the European Union
Author(s), year:	Ball, M.A., 2004
Report/Doc. number:	DuPont-11941, Revision No. 1
Guideline(s):	Calculation - not applicable
GLP:	Calculation - not applicable
Deviations:	Calculation - not applicable
Validity:	Study considered partly acceptable, fully re-calculated by the RMS (see comments)

and

Reference:	Cymoxanil 50 % WP, Plant protection product, Annex IIIA: Section 5: Fate in the environment, Summaries and assessment (Tier II – Document M-III)
Author(s), year:	Cicotelli, M., 2004
Report/Doc. number:	OXON CYMOA3S5T2 2004
Guideline(s):	Calculation - not applicable
GLP:	Calculation - not applicable
Deviations:	Calculation - not applicable
Validity:	Calculations considered partly acceptable, fully re-calculated by the RMS (see comments)

Material and methods:

Predicted environmental concentrations in soils (PEC_s) of cymoxanil and major metabolites in soil (> 10 % of AR) were calculated for the intended use of TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon) in potatoes and lettuce. Calculations were carried out using a simple Excel spreadsheet approach.

Degradation of each compound in soil was described using worst-case degradation DT₅₀ values from laboratory degradation studies. The bi-phasic (FOMC) degradation of the parent substance was adequately addressed using the worst-case re-calc. SFO-DT₅₀ (SFO-DT₅₀ re-calculated from FOMC-DT₉₀ by division with 3.32). All metabolites were assumed to follow SFO kinetics. In order to adequately account for different incubation temperatures (20 and 25 °C), worst-case degradation is based on normalized degradation data.

Table B.8.4.7-1: Application data of cymoxanil applied as TANOS 50 WG (DuPont) or CYMOXANIL 50 % WP (Oxon) formulation.

Product	TANOS 50 WG (DuPont)	CYMOXANIL 50 % WP (Oxon)	
Crop	Potatoes	Potatoes	Lettuce
Application rate [g ha ⁻¹]	175	120	240
Number of application	8	4 (North EU) 5 (South EU) ^b	4
Interval [days]	7	7	7
Crop interception [%]	50 ^a	50 ^a	70 ^a
BBCH stage	21 - 95	21 - 95	40 - 49
Soil depth [cm]	5	5	5
Soil density [kg L ⁻¹]	1.5	1.5	1.5

^a FOCUS default value according to BBCH stages

^b Application number of 5 used for calculations (worst-case assessment)

Table B.8.5-2: Input parameters for cymoxanil and major (> 10 % of AR) soil metabolites.

Parameter	Cymoxanil	IN-U3204	IN-W3595	IN-JX915 ^a
DegT ₅₀ [d] – laboratory worst-case, normalized	7.3 ^b	0.7	2.2	0.7
Maximum occurrence [% of AR]	-	24.7	10.1	10.9
Molecular weight [g mol ⁻¹]	198.2	198.2	128.1	198.2

^a Major only in soil photolysis, conservative risk assessment

^b Worst-case SFO-DT₅₀ recalculated from worst-case FOMC-DT₉₀ by division with 3.32

Findings:

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Table B.8.5-3: Actual PEC_s and time-weighted average concentrations (TWAs) in soil for cymoxanil after single and multiple application of TANOS 50 WG (DuPont) or CYMOXANIL 50 % WP (Oxon) to potatoes or lettuce.

Application	Single application						Multiple application					
	TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)			TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)		
Crop	Potatoes			Lettuce			Potatoes			Lettuce		
Days after last application	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]
0	117	-	80	-	96	-	239	-	159	-	184	-
1	106	111	73	76	87	92	217	228	144	152	167	176
2	96	106	66	73	79	88	198	218	131	145	152	168
4	80	97	55	67	66	80	164	200	109	133	126	153
7	60	86	41	59	49	70	123	176	82	117	95	135
14	31	65	21	45	25	54	63	134	42	89	49	103
21	16	51	11	35	13	42	33	105	22	70	25	81
28	8	42	6	29	7	34	17	85	11	57	13	66
50	1	25	1	17	1	21	2	51	1	34	2	39
100	0	13	0	9	0	10	0	26	0	17	0	20

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Table B.8.5-4: Actual PEC_s and time-weighted average concentrations (TWAs) in soil for IN-U3204 after single and multiple application of TANOS 50 WG (DuPont) or CYMOXANIL 50 % WP (Oxon) to potatoes or lettuce.

Application	Single application						Multiple application					
	TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)			TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)		
Crop	Potatoes			Lettuce			Potatoes			Lettuce		
Days after last application	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]
0	29	-	20	24	-	-	29	-	20	24	-	-
1	11	20	7	9	16	16	11	20	7	9	14	16
2	4	14	3	3	12	12	4	15	3	3	10	12
4	1	9	0	0	7	7	1	9	0	0	6	8
7	0	6	0	0	5	5	0	6	0	0	4	5
14	0	3	0	0	3	3	0	3	0	0	2	3
21	0	2	0	0	2	2	0	2	0	0	1	2
28	0	2	0	0	1	1	0	2	0	0	1	1
50	0	1	0	0	1	1	0	1	0	0	1	1
100	0	0	0	0	0	0	0	0	0	0	0	0

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Table B.8.5-5: Actual PEC_s and time-weighted average concentrations (TWAs) in soil for IN-W3595 after single and multiple application of TANOS 50 WG (DuPont) or CYMOXANIL 50 % WP (Oxon) to potatoes or lettuce.

Application	Single application						Multiple application					
	TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)			TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)		
Crop	Potatoes		Lettuce		Potatoes		Potatoes		Potatoes		Lettuce	
Days after last application	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]
0	8	-	5	-	6	-	9	-	6	-	7	-
1	6	7	4	4	5	5	6	7	4	5	5	6
2	4	6	3	4	3	5	5	6	3	4	4	5
4	2	4	1	3	2	4	2	5	2	3	2	4
7	1	3	1	2	1	3	1	4	1	2	1	3
14	0	2	0	1	0	2	0	2	0	1	0	2
21	0	1	0	1	0	1	0	1	0	1	0	1
28	0	1	0	1	0	1	0	1	0	1	0	1
50	0	1	0	0	0	0	0	1	0	0	0	1
100	0	0	0	0	0	0	0	0	0	0	0	0

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Table B.8.5-6: Actual PEC_s and time-weighted average concentrations (TWAs) in soil for IN-JX915 after single and multiple application of TANOS 50 WG (DuPont) or CYMOXANIL 50 % WP (Oxon) to potatoes or lettuce.

Application	Single application						Multiple application					
	TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)			TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)		
Crop	Potatoes		Lettuce		Potatoes		Potatoes		Potatoes		Lettuce	
Days after last application	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]	Actual PEC _s [µg kg ⁻¹]	TWA PEC _s [µg kg ⁻¹]
0	13	-	9	-	10	-	13	-	9	-	10	-
1	5	9	3	6	4	7	5	9	3	6	4	7
2	2	6	1	4	1	5	2	6	1	4	1	5
4	0	4	0	3	0	3	0	4	0	3	0	3
7	0	3	0	2	0	2	0	3	0	2	0	2
14	0	1	0	1	0	1	0	1	0	1	0	1
21	0	1	0	1	0	1	0	1	0	1	0	1
28	0	1	0	0	0	1	0	1	0	0	0	1
50	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0

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Conclusion:

Table B.8.5-7: Summary on maximum initial PEC_s [µg kg⁻¹] after multiple application of cymoxanil according to the intended uses.

Product	Crop	Cymoxanil	IN-U3204	IN-W3595	IN-JX915
TANOS 50 WG (DuPont)	Potatoes	239	29	9	13
CYMOXANIL 50 % WP (Oxon)	Potatoes	159	20	6	9
	Lettuce	184	24	7	10

Comments (RMS):

- After complete evaluation of the dossier, some changes regarding the input parameter of cymoxanil and metabolites for PEC_s calculation occurred. To minimize data confusion and to keep relevant endpoints as concise as possible, the RMS re-calculated PEC_s on the basis of validated endpoints.

However, in some cases, input parameter suggested by the notifier based on non-validated endpoints are more conservative in comparison to the RMS risk assessment (e.g. degradation rates and maximum occurrence of metabolites in the 'Black Andosol', study from Trabue, 2003, were included). For comparison, the input parameters suggested by the notifier are given in the following table.

Table B.8.5-8: PEC_s input parameters for cymoxanil and soil metabolites, notifier approach.

Compound	Parameter	Value	Remarks (RMS)
Cymoxanil	DegT ₅₀ [d] – laboratory worst-case	4.6	Value according to SFO kinetics, bi-phasic degradation of cymoxanil (FOMC kinetics) not considered by notifier
IN-U3204	DegT ₅₀ [d] – laboratory worst-case	0.8	Data from 'Black Andosol' (Trabue 2003) included by notifier, notifier assessment more conservative
IN-W3595	DegT ₅₀ [d] – laboratory worst-case	23.8	Data from 'Black Andosol' (Trabue 2003) included by notifier, notifier assessment more conservative
	Maximum occurrence [% of AR]	18.1	Data from 'Black Andosol' (Trabue 2003) included by notifier, notifier assessment more conservative
IN-KQ960	DegT ₅₀ [d] – laboratory worst-case	14.5	Data from 'Black Andosol' (Trabue 2003) included by notifier, notifier assessment more conservative
	Maximum occurrence [% of AR]	9.9	Outlier (value of 15 % of AR) included by notifier, not considered relevant by RMS, not considered as major metabolite in soil, notifier assessment more conservative
IN-JX915	Not considered relevant by notifier	-	Major in soil photolysis (10.9 % of AR)

B.8.6 Predicted environmental concentrations in groundwater (PEC_{GW}) (Annex IIIA, point 9.2.1)

Reference:	Predicted environmental concentrations of cymoxanil and major metabolite, IN-U3204, in groundwater after application of Tanos 50WG to potatoes in the European Union
Author(s), year:	Ball, M. A., 2004
Report/Doc. number:	DuPont-11942, Revision No. 1
Guideline(s):	Calculation, not applicable
GLP:	Calculation, not applicable
Deviations:	Calculation, not applicable
Validity:	Study considered partly acceptable, fully re-calculated by the RMS (see comments)

and

Reference:	Cymoxanil 50 % WP, Plant protection product, Annex IIIA: Section 5: Fate in the environment, Summaries and assessment (Tier II – Document M-III)
Author(s), year:	Ciccotelli, M., 2004
Report/Doc. number:	OXON CYMOA3S5T2 2004
Guideline(s):	Calculation, not applicable
GLP:	Calculation, not applicable
Deviations:	Calculation, not applicable
Validity:	Calculations considered partly acceptable, fully re-calculated by the RMS (see comments)

Material and methods:

The leaching behaviour of cymoxanil and soil metabolites, relevant for groundwater risk assessment (i.e. IN-U3204, IN-W3595, IN-KQ960 and the soil photolysis metabolite IN-JX915) was investigated for the use of TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon) in potatoes and lettuce according to the European GAP. Model calculations were carried out with FOCUS PELMO 3.3.2 and FOCUS PEARL 3.3.3 following FOCUS guidance.

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Table B.8.6-1: Application parameters for TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon).

Product	TANOS 50 WG (DuPont)	CYMOXANIL 50 % WP (Oxon)		
Crop	Potatoes	Potatoes		Lettuce ^a
Region	North/South EU	North EU	South EU	South EU
Application rate [g ai ha ⁻¹]	175	120	120	240
Number of applications	8	4	5	4
Interval [d]	7	7	7	7
First application	7 days after leaf emergence	28 days pre harvest	35 days pre harvest	31 days pre harvest
BBCH stage	21 - 95	21 - 95	21 - 95	40 - 49
Crop interception ^b [%]				
1 st Application	50	50	50	70
2 nd Application	50	50	50	70
3 rd Application	50	50	50	70
4 th Application	50	80	80	70
5 th Application	80	-	80	-
6 th Application	80	-	-	-
7 th Application	80	-	-	-
8 th Application	80	-	-	-

^a For modelling purposes, lettuce is substituted by 'cabbage'

The groundwater risk assessment for cymoxanil and relevant metabolites is exclusively based on laboratory soil degradation data (normalized to 20 °C and pF2). Modelling of cymoxanil and metabolites was conducted individually, the application rate (application onto the soil surface) was corrected for crop interception, maximum occurrence in soil and molecular weight.

Since degradation of cymoxanil is considered to significantly depend on soil pH (slower degradation observed in acidic soils), additional modellings were conducted for cymoxanil only, using the worst-case normalized DT₅₀ of cymoxanil observed in acidic soils (i.e. 7.3 days). For simplification, this (extreme worst-case) modelling was conducted for all groundwater scenarios irrespective of the actual soil pH of the individual scenario.

Adsorption of metabolite IN-W3595 is depending on the soil pH following a sigmoid curve characterized by a maximum K_{OC} at low pH (33.3 L kg⁻¹) and a minimum K_{OC} at higher pH (2.3 L kg⁻¹). The inflection point of the curve is determined by the pK_a value of IN-W3595, which was estimated to be 5.2 (for details refer to Hausmann and Adams, 1996, B.8.4.2). These parameters were directly implemented into FOCUS PEARL 3.3.3 modelling ('Option: Kom, pH dependent'), for FOCUS PELMO 3.3.2 modelling, individual K_{OC} values for each FOCUS groundwater scenario were calculated on the basis of the sigmoid curve according to the top soil pH (in KCl) (Table B.8.6-2).

Table B.8.6-2: Obtained K_{OC} values for IN-W3595 depending on the pH of the topsoil of each FOCUS groundwater scenario (used as input variable in FOCUS PELMO 3.3.2).

FOCUS scenario	Topsoil pH (KCl)	IN-W3595 - K _{OC} [L kg ⁻¹]
Châteaudun	7.3	2.6
Hamburg	5.7	10.2
Jokioinen	5.5	13.2
Kremsmünster	7.0	2.9
Okehampton	5.1	20.1
Piacenza	6.3	4.8
Porto	4.2	30.6
Sevilla	6.6	3.6
Thiva	7.0	2.9
Arithmetic mean K _{OC} from batch experiments (n = 4)	-	9.2

Table B.8.3-1: Modelling input parameters for cymoxanil and metabolites.

Compound	Parameter	Value	Remarks
Cymoxanil	DegT ₅₀ [d]	1.0	Standard risk assessment, geometric mean of normalized laboratory SFO-DT ₅₀ (<i>n</i> = 9), SFO-DT ₅₀ recalculated from FOMC-DT ₉₀ by division with 3.32
		7.3	Additional risk assessment accounting for slower degradation in acidic soils, worst-case of normalized laboratory SFO-DT ₅₀ observed in acidic soils, extreme worst-case assessment
	Molecular weight [g mol ⁻¹]	198.2	
	K _{FOC} [L kg ⁻¹]	43.6	Arithmetic mean (batch experiment, <i>n</i> = 4)
	1/ <i>n</i>	0.86	Arithmetic mean (batch experiment, <i>n</i> = 4)
	Water solubility (pH 7, 20 °C) [mg L ⁻¹]	783	
	Vapour pressure (20 °C) [Pa]	1.5 x 10 ⁻⁴	
	Plant uptake	0.5	FOCUS default value for systemic compound
IN-U3204	DegT ₅₀ [d]	0.2	Geometric mean of normalized laboratory DT ₅₀ (<i>n</i> = 3)
	Maximum occurrence in soil [%]	24.7	
	Molecular weight [g mol ⁻¹]	198.2	
	K _{OC} [L kg ⁻¹]	27.9	HPLC method, Single value
	1/ <i>n</i>	0.9	FOCUS default value
	Water solubility (pH 7, 25 °C) [mg L ⁻¹]	783	Unknown, value from parent used
	Vapour pressure (20 °C) [Pa]	0.0	Unknown, worst-case assessment
	Plant uptake	0.0	No uptake, worst-case assessment
IN-W3595	DegT ₅₀ [d]	2.2	Worst-case of normalized laboratory DT ₅₀ (<i>n</i> = 2)
	Maximum occurrence in soil [%]	10.1	
	Molecular weight [g mol ⁻¹]	128.1	
	K _{OC} [L kg ⁻¹]	pH depending: K _{OCacid} = 33.3, K _{OCbase} = 2.3, pK _a = 5.2	Scenario specific K _{OC} value
	1/ <i>n</i>	0.9	FOCUS default value
	Water solubility (pH 7, 20 °C) [mg L ⁻¹]	783	Unknown, value from parent used
	Vapour pressure (20 °C) [Pa]	0.0	Unknown, worst-case assessment
	Plant uptake	0.0	No uptake, worst-case assessment
IN-JX915	DegT ₅₀ [d]	0.7	Single value, normalized laboratory DT ₅₀
	Maximum occurrence in soil [%]	10.9	Value from soil photolysis (conservative assessment)
	Molecular weight [g mol ⁻¹]	198.2	
	K _{OC} [L kg ⁻¹]	16.1	Arithmetic mean (batch experiment, <i>n</i> = 4)
	1/ <i>n</i>	0.9	FOCUS default value
	Water solubility (pH 7, 20 °C) [mg L ⁻¹]	783	Unknown, value from parent used
	Vapour pressure (20 °C) [Pa]	0.0	Unknown, worst-case assessment
	Plant uptake	0.0	No uptake, worst-case assessment
IN-KQ960	DegT ₅₀ [d]	9.3	Single value, normalized laboratory DT ₅₀
	Maximum occurrence in soil [%]	6.3	
	Molecular weight [g mol ⁻¹]	216.2	
	K _{OC} [L kg ⁻¹]	21.6	HPLC method, Single value
	1/ <i>n</i>	0.9	FOCUS default value
	Water solubility (pH 7, 20 °C) [mg L ⁻¹]	783	Unknown, value from parent used
	Vapour pressure (20 °C) [Pa]	0.0	Unknown, worst-case assessment
	Plant uptake	0.0	No uptake, worst-case assessment

Findings:

Table B.8.6-3: Predicted 80th percentile annual average groundwater concentration [$\mu\text{g L}^{-1}$] of cymoxanil (input $\text{DT}_{50} = 1.0$ days) and metabolites after application of TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon) to potatoes and lettuce according to GAP at 1 m depth using FOCUS PELMO 3.3.2.

Crop / product / region	FOCUS scenario	Cymoxanil [$\mu\text{g L}^{-1}$]	IN-U3204 [$\mu\text{g L}^{-1}$]	IN-W3595 [$\mu\text{g L}^{-1}$]	IN-JX915 [$\mu\text{g L}^{-1}$]	IN-KQ960 [$\mu\text{g L}^{-1}$]
Potatoes / TANOS 50 WG (DuPont)	Châteaudun	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Hamburg	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Jokioinen	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Kremsmünster	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Okehampton	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Piacenza	< 0.001	< 0.001	< 0.001	< 0.001	0.001
	Porto	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Sevilla	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Thiva	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Potatoes / CYMOXANIL 50 % WP (Oxon) / North EU	Châteaudun	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Hamburg	< 0.001	< 0.001	< 0.001	< 0.001	0.001
	Jokioinen	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Kremsmünster	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Okehampton	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Potatoes / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	< 0.001	< 0.001	< 0.001	< 0.001	0.006
	Porto	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Sevilla	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Thiva	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Lettuce / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	No cabbage (used as surrogate for lettuce)				
	Porto	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Sevilla	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Thiva	< 0.001	< 0.001	0.001	< 0.001	< 0.001

Table B.8.6-4: Predicted 80th percentile annual average groundwater concentration [$\mu\text{g L}^{-1}$] of cymoxanil (input $\text{DT}_{50} = 1.0$ days) and metabolites after application of TANOS 50 WG and CYMOXANIL 50 % WP to potatoes and lettuce according to GAP at 1 m depth using FOCUS PEARL 3.3.3.

Crop / product / region	FOCUS scenario	Cymoxanil [$\mu\text{g L}^{-1}$]	IN-U3204 [$\mu\text{g L}^{-1}$]	IN-W3595 [$\mu\text{g L}^{-1}$]	IN-JX915 [$\mu\text{g L}^{-1}$]	IN-KQ960 [$\mu\text{g L}^{-1}$]
Potatoes / TANOS 50 WG (DuPont)	Châteaudun	< 0.001	< 0.001	< 0.001	< 0.001	0.004
	Hamburg	< 0.001	< 0.001	< 0.001	< 0.001	0.009
	Jokioinen	< 0.001	< 0.001	< 0.001	< 0.001	0.001
	Kremsmünster	< 0.001	< 0.001	< 0.001	< 0.001	0.004
	Okehampton	< 0.001	< 0.001	< 0.001	< 0.001	0.007
	Piacenza	< 0.001	< 0.001	< 0.001	< 0.001	0.014
	Porto	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Sevilla	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Thiva	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Potatoes / CYMOXANIL 50 % WP (Oxon) / North EU	Châteaudun	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Hamburg	< 0.001	< 0.001	< 0.001	< 0.001	0.006
	Jokioinen	< 0.001	< 0.001	< 0.001	< 0.001	0.002
	Kremsmünster	< 0.001	< 0.001	< 0.001	< 0.001	0.001
	Okehampton	< 0.001	< 0.001	< 0.001	< 0.001	0.003
Potatoes / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	< 0.001	< 0.001	< 0.001	< 0.001	0.012
	Porto	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Sevilla	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Thiva	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Lettuce / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	No cabbage (used as surrogate for lettuce)				
	Porto	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Sevilla	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Thiva	< 0.001	< 0.001	0.002	< 0.001	0.011

Table B.8.6-5: Predicted 80th percentile annual average groundwater concentration [$\mu\text{g L}^{-1}$] of cymoxanil (input $\text{DT}_{50} = 7.3$ days) after application of TANOS 50 WG and CYMOXANIL 50 % WP to potatoes and lettuce according to GAP at 1 m depth using FOCUS PEARL 3.3.3 – additional worst-case risk assessment accounting for maximum degradation half-life of cymoxanil observed under acidic conditions.

Crop / product / region	FOCUS scenario	Cymoxanil [$\mu\text{g L}^{-1}$]
Potatoes / TANOS 50 WG (DuPont)	Châteaudun	< 0.001
	Hamburg	< 0.001
	Jokioinen	< 0.001
	Kremsmünster	< 0.001
	Okehampton	< 0.001
	Piacenza	0.001
	Porto	< 0.001
	Sevilla	< 0.001
	Thiva	< 0.001
Potatoes / CYMOXANIL 50 % WP (Oxon) / North EU	Châteaudun	< 0.001
	Hamburg	< 0.001
	Jokioinen	< 0.001
	Kremsmünster	< 0.001
	Okehampton	< 0.001
Potatoes / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	0.003
	Porto	< 0.001
	Sevilla	< 0.001
	Thiva	< 0.001
Lettuce / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	No cabbage (used as surrogate for lettuce)
	Porto	< 0.001
	Sevilla	< 0.001
	Thiva	0.001

Conclusion:

The modelled PEC_{GW} (annual average groundwater concentrations at 1 m soil depth) for cymoxanil and the soil metabolites IN-U3204, IN-W3595, IN-KQ960 and IN-JX915 were predicted to be clearly below the limit of $0.1 \mu\text{g L}^{-1}$ in all European standard scenarios after application of TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon) to potatoes and lettuce according to GAP. Even under consideration of extreme worst-case degradation rates observed in acidic soils (cymoxanil is considered to degrade significantly slower in acidic soils), cymoxanil is not expected to leach to shallow groundwater.

Comments (RMS):

- After complete evaluation of the dossier by the RMS, some changes regarding the input parameter of cymoxanil and metabolites for groundwater modelling occurred. To minimize data confusion and to keep relevant endpoints as concise as possible, the RMS re-calculated PEC_{GW} on the basis of validated endpoints.

However, in some cases input parameter suggested by the notifier based on non-validated endpoints are more conservative in comparison to the RMS risk assessment (e.g. degradation rates and maximum occurrence of metabolites in the 'Black Andosol', study from Trabue, 2003, were included). For comparison, the input parameter suggested by the notifier and the PEC_{GW} results obtained by the notifier approach are given in the following tables. Even under consideration of these more conservative (non-validated) input data, cymoxanil and metabolites were not predicted to occur $> 0.1 \mu\text{g L}^{-1}$ in shallow groundwater (1 m).

Table B.8.3-1: Modelling input parameters for cymoxanil and metabolites proposed by the notifier (only values different from the RMS approach are shown).

Compound	Parameter	Value (suggested by notifier)	Remarks (RMS)
Cymoxanil	DegT ₅₀ [d]	1.0	Geometric mean of normalized laboratory SFO data (including 'Black Andosol' from Trabue, 2003), value identical to geometric mean of normalized re-calc. SFO-DT ₅₀ (excluding 'Black Andosol'), no change
IN-U3204	DegT ₅₀ [d]	0.5	Value revised by RMS, notifier assessment more conservative
	K _{OC} [L kg ⁻¹]	27.5	Revised by RMS, minor change
IN-W3595	DegT ₅₀ [d]	3.4	Data from 'Black Andosol' (Trabue, 2003) included, geometric mean (<i>n</i> = 3), notifier assessment more conservative
	Maximum occurrence [% of AR]	18.1	Data from 'Black Andosol' (Trabue, 2003) included, notifier assessment more conservative
	K _{OC} [L kg ⁻¹]	pH depending (linear regression)	Scenario specific K _{OC} value (K _{OC} = -0.331 × pH(H ₂ O) + 2.89), calculation based on pH measured in water, notifier assessment more conservative
IN-JX915	Not considered relevant for groundwater risk assessment by notifier	-	> 5 % at two consecutive sample points, major in soil photolysis
IN-KQ960	DegT ₅₀ [d]	14.5	Data from 'Black Andosol' (Trabue, 2003) included, worst-case used (<i>n</i> = 2), notifier assessment more conservative
	Maximum occurrence [% of AR]	9.9	Outlier (one replicate with 15 % of AR) included, notifier assessment more conservative
	K _{OC} [L kg ⁻¹]	21.4	Revised by RMS, minor change

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. It must be read in conjunction with the basis of this document.

Table B.8.6-6: Predicted 80th percentile annual average groundwater concentration [$\mu\text{g L}^{-1}$] of cymoxanil and metabolites after application of TANOS 50 WG and CYMOXANIL 50 % WP to potatoes and lettuce according to GAP at 1 m depth using FOCUS PELMO 3.3.2 – notifier approach.

Crop / product / region	FOCUS scenario	Cymoxanil [$\mu\text{g L}^{-1}$]	IN-U3204 [$\mu\text{g L}^{-1}$]	IN-W3595 [$\mu\text{g L}^{-1}$]	IN-KQ960 [$\mu\text{g L}^{-1}$]
Potatoes / TANOS 50 WG (DuPont)	Châteaudun	nc	nc	nc	nc
	Hamburg	nc	nc	nc	nc
	Jokioinen	nc	nc	nc	nc
	Kremsmünster	nc	nc	nc	nc
	Okehampton	nc	nc	nc	nc
	Piacenza	nc	nc	nc	nc
	Porto	nc	nc	nc	nc
	Sevilla	nc	nc	nc	nc
	Thiva	nc	nc	nc	nc
Potatoes / CYMOXANIL 50 % WP (Oxon) / North EU	Châteaudun	< 0.001	< 0.001	< 0.001	< 0.001
	Hamburg	< 0.001	< 0.001	< 0.001	0.007
	Jokioinen	< 0.001	< 0.001	0.002	0.001
	Kremsmünster	< 0.001	< 0.001	< 0.001	0.001
	Okehampton	< 0.001	< 0.001	< 0.001	0.003
Potatoes / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	< 0.001	< 0.001	0.002	0.040
	Porto	< 0.001	< 0.001	< 0.001	< 0.001
	Sevilla	< 0.001	< 0.001	< 0.001	< 0.001
	Thiva	< 0.001	< 0.001	< 0.001	< 0.001
Lettuce / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	No cabbage (used as surrogate for lettuce)			
	Porto	< 0.001	< 0.001	< 0.001	0.001
	Sevilla	< 0.001	< 0.001	< 0.001	< 0.001
	Thiva	< 0.001	< 0.001	0.011	0.003

nc denotes not calculated using FOCUS PELMO 3.3.2

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Table B.8.6-7: Predicted 80th percentile annual average groundwater concentration [$\mu\text{g L}^{-1}$] of cymoxanil and metabolites after application of TANOS 50 WG and CYMOXANIL 50 % WP to potatoes and lettuce according to GAP at 1 m depth using FOCUS PEARL 2.2.2 – notifier approach.

Crop / product / region	FOCUS scenario	Cymoxanil [$\mu\text{g L}^{-1}$]	IN-U3204 [$\mu\text{g L}^{-1}$]	IN-W3595 [$\mu\text{g L}^{-1}$]	IN-KQ960 [$\mu\text{g L}^{-1}$]
Potatoes / TANOS 50 WG (DuPont)	Châteaudun	< 0.001	< 0.001	< 0.001	0.050
	Hamburg	< 0.001	< 0.001	< 0.001	0.066
	Jokioinen	< 0.001	< 0.001	< 0.001	0.015
	Kremsmünster	< 0.001	< 0.001	< 0.001	0.037
	Okehampton	< 0.001	< 0.001	< 0.001	0.066
	Piacenza	< 0.001	< 0.001	< 0.001	0.088
	Porto	< 0.001	< 0.001	< 0.001	< 0.001
	Sevilla	< 0.001	< 0.001	< 0.001	< 0.001
	Thiva	< 0.001	< 0.001	< 0.001	0.014
Potatoes / CYMOXANIL 50 % WP (Oxon) / North EU	Châteaudun	nc	nc	nc	0.007
	Hamburg	nc	nc	nc	0.068
	Jokioinen	nc	nc	nc	0.034
	Kremsmünster	nc	nc	nc	0.026
	Okehampton	nc	nc	nc	0.050
Potatoes / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	nc	nc	nc	0.094
	Porto	nc	nc	nc	< 0.001
	Sevilla	nc	nc	nc	< 0.001
	Thiva	nc	nc	nc	0.003
Lettuce / CYMOXANIL 50 % WP (Oxon) / South EU	Piacenza	No cabbage (used as surrogate for lettuce)			
	Porto	nc	nc	nc	nc
	Sevilla	nc	nc	nc	nc
	Thiva	nc	nc	nc	nc

nc denotes not calculated using FOCUS PEARL 2.2.2

B.8.6.1 Impact on water treatment procedures (Annex IIIA, point 9.2.2)

In the readily biodegradability study the test showed that cymoxanil had no inhibitory effect on microbial activity in an activated sludge media. Furthermore, the activated sludge respiration test gave a cymoxanil EC_{50} of 19.4 mg L^{-1} . Results of modelling indicate that maximum predicted concentrations of cymoxanil in surface water will not exceed $2.2 \mu\text{g L}^{-1}$, indicating a large margin of safety. The concentration in groundwater does not exceed the maximum acceptable concentration of $0.1 \mu\text{g L}^{-1}$. It can therefore be concluded that cymoxanil is unlikely to impact adversely onto water treatment procedures.

B.8.7 Hydrolysis (Annex IIA, point 7.2.1.1)

The hydrolysis of cymoxanil in sterile buffer solutions was investigated in two studies:

- Hydrolysis in sterile buffer solutions at pH 5, 7 and 9 at 25 °C (DuPont)
- Hydrolysis in sterile buffer solution at pH 4, 7 and 9 at 20 °C (Oxon)

Reference:	Hydrolysis of cymoxanil (DPX-T3217) in buffer solutions of pH 5, 7 and 9
Author(s), year:	Lawler, S. M., 1996
Report/Doc. number:	DuPont Report No. AMR 3677-95
Guideline(s):	US-EPA 161-1 (1982)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

including relevant kinetic re-calculations from:

Reference:	Calculated half-lives of cymoxanil and its metabolites in environmental fate laboratory studies
Author(s), year:	Malekani, K., 2003
Report/Doc. number:	DuPont-11575, Revision No. 1
Guideline(s):	Calculations - not applicable
GLP:	Calculations - not applicable
Deviations:	Calculations - not applicable
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, 0.52 MBq mg ⁻¹ , radiochemical purity > 95 % (HPLC), batch HOTC 376 [Cyanoacetamide-2- ¹³ C]-cymoxanil (purity > 97 %) was used to assist in characterization of hydrolysis products (¹³ C/ ¹⁴ C-cymoxanil approx. 1/1; only for pH 7 and pH 9 samples)
Reference substances:	Cymoxanil (unlabelled), oxamic acid (IN-18474), oxalic acid, IN-W3595, IN-KP533, IN-T4226, IN-KQ960, IN-U3204, IN-JX915, IN-R3273
Test medium:	• pH 5: 0.01 M sodium acetate buffer • pH 7: 0.01 M phosphate buffer • pH 9: 0.01 M borate buffer All buffer solutions filter sterilized (0.22 µm), sterility checked by 0, 15 and 30 DAT (pH 7 and pH 9) and by 10 and 30 DAT (pH 5)
Test concentration:	• pH 5: 25 mg L⁻¹ • pH 7 and 9: 50 mg L⁻¹
Co-solvent:	0.5 - 1 % acetonitrile
Test system:	Static system
Temperature:	25 ± 1 °C
Volatile traps:	No volatile traps
Sample processing:	Samples from pH 7 and pH 9 treatment were adjusted to a pH between 4 and 5 with 1 M formic acid immediately after sampling
Analytical techniques:	LSC, HPLC-UV/RAD (two independent methods), metabolite identity was confirmed by LC-MS and GC-MS (in case of IN-W3595, after derivatization with MSTFA)
Kinetic analysis:	• Parent (re-calculated, Malekani, 2003): Simple first order (SFO) kinetics • Metabolites (Malekani, 2003):

- IN-U3204 and IN-R3273: Two-compartment model linking each metabolite separately to the parent in series (all compartments SFO kinetics with additional pathway to sink)

All calculations using ModelManager 1.1

Findings:

Overall mass balance was in a range of 95.7 – 105.0 % of AR at pH 5, 94.3 – 105.0 % of AR at pH 7 and 86.9 – 135.8 % of AR at pH 9 (single replicates). Owing to the complete mass balance, formation of CO₂ is considered to be negligible. No significant change in the pH of test solutions occurred. Samples were shown to be sterile throughout the incubation.

Hydrolysis of cymoxanil was strongly depending on the pH. Cymoxanil degraded very rapidly at pH 9 and 7 and relatively slowly at pH 5 with calculated half-lives of 0.02 days (31 minutes), 1.1 days and 144 days, respectively, all following SFO kinetics ($R^2 \geq 0.67$, χ^2 error ≤ 2.4 %).

At **pH 5**, no hydrolytic degradate exceeded 10 % of AR throughout the 30 day incubation. IN-U3204 reached 9.1 % of AR by 7 DAT, the DT₅₀ of this metabolite at pH 5 was calculated to be 25.8 days (with a poor statistic significance).

At **pH 7**, metabolite IN-U3204 accounted for max. 52.7 % of AR by 2 DAT declining thereafter to < 9 % of AR by 15 DAT. IN-KP533, IN-W3595 and IN-R3273 steadily increased during hydrolysis and accounted for max. 57.0 %, 16.2 % and 10.2 % of AR close to study termination. No other degradate was observed > 10 % of AR. The DT₅₀ of IN-U3204, IN-JX915 (both linked to the parent via multi-compartment modelling) and IN-T4226 (calculated from maximum occurrence) were calculated to be 2.6, 1.1 and 7.2 days, respectively. IN-KP533, IN-W3595, IN-KQ960 and IN-R3273 are considered stable under conditions of sterile hydrolysis at pH 7.

At **pH 9**, metabolite pattern was similar to pH 7 conditions. Metabolite IN-U3204 was observed at maximum amounts of 60.8 % of AR by 0.17 DAT, rapidly declining to < 10 % of AR by 1 DAT. Amounts of metabolites IN-KP533, IN-W3595 and IN-R3273 did hardly change after maximum formation and accounted for max. 31.6 %, 39.0 % and 13.5 % of AR. No other degradate was observed > 10 % of AR. The DT₅₀ of IN-U3204, IN-JX915 and IN-T4226 were calculated to be 0.4, 1.7 and 2.0 days, respectively. IN-KP533, IN-W3595, IN-KQ960 and IN-R3273 are considered stable under conditions of sterile hydrolysis at pH 9.

Table B.8.7-1: Composition of radioactivity [% of AR] in sterile buffer solution at pH 5 (HPLC, mean of duplicate samples).

DAT	IN-18474	IN-W3595	IN-KP533	IN-U3204	IN-JX915	IN-R3273	Cymoxanil	Others ^a
0	nd	nd	nd	nd	nd	nd	96.8	3.3
0.17	0.9	nd	nd	3.1	1.1	0.4	93.0	1.6
1	nd	nd	nd	5.1	1.6	nd	90.3	3.0
2	0.5	0.5	nd	3.6	1.1	nd	92.7	1.7
3	nd	1.3	nd	4.8	1.3	nd	89.8	2.8
7	nd	1.2	nd	9.1	1.8	0.4	84.2	3.4
10	0.1	1.0	0.8	4.4	0.5	0.6	91.1	1.6
15	nd	2.0	nd	7.5	1.5	0.6	84.8	3.6
30	nd	2.3	nd	7.3	1.0	0.9	81.3	7.4

nd denotes not detected (< 0.1 % of AR)

^a Other minor radioactive components

Table B.8.7-2: Composition of radioactivity [% of AR] in sterile buffer solution at pH 7 (HPLC, mean of duplicate samples).

DAT	Pol. ^a	IN-W3595	IN-KP533	IN-T4226	Met ^b	IN-KQ960	IN-U3204 ^c	IN-JX915	IN-R3273	Cym-oxanil	Oth. ^d
0	nd	nd	nd	nd	nd	nd	nd	nd	nd	100.0	nd
0.042	0.8	nd	nd	nd	nd	nd	1.2	0.8	nd	97.2	nd
0.083	1.6	nd	nd	nd	nd	nd	4.7	1.5	nd	91.7	0.4
0.17	1.3	0.7	nd	nd	nd	nd	8.0	2.0	nd	88.0	nd
0.25	1.8	2.0	nd	nd	nd	nd	11.7	2.1	nd	82.5	nd
0.33	nd	3.1	nd	nd	nd	nd	19.5	2.5	nd	75.0	nd
1	0.6	5.4	nd	0.2	nd	nd	40.8	4.2	nd	48.6	0.3
1.08	1.4	6.4	nd	0.2	nd	nd	39.3	5.0	0.7	47.1	nd
2	1.9	7.2	3.2	3.4	nd	0.6	52.7	1.8	2.4	26.3	0.5
3	0.7	10.8	4.2	3.5	1.2	0.3	49.8	3.8	5.1	18.7	1.7
7	3.2	14.6	24.3	5.2	5.9	4.9	24.5	2.0	9.0	3.3	3.2
10	3.1	16.0	38.9	5.4	6.1	7.8	9.5	nd	9.6	nd	3.5
15	4.7	16.2	50.7	4.2	2.2	7.7	nd	1.6	10.2	nd	2.6
30	6.4	14.4	57.0	nd	nd	9.0	nd	3.1	10.1	nd	nd

nd denotes not detected (< 0.1 % of AR)

^a Polar degradates including oxamic acid (IN-18474), oxalic acid and 3 unknowns

^b Unknown metabolite (RT ~ 21 min)

^c IN-U3204 is composed of geometric isomers

^d Other minor radioactive components

Table B.8.7-3: Composition of radioactivity [% of AR] in sterile buffer solution at pH 9 (HPLC, mean of duplicate samples).

DAT	Polars ^a	IN-W3595	IN-KP533	IN-T4226	IN-KQ960	IN-U3204 ^b	IN-JX915	IN-R3273	Cym-oxanil	Others ^c
0	nd	nd	nd	nd	nd	nd	0.5	nd	99.5	nd
0.004	1.4	3.1	nd	nd	nd	8.4	2.7	nd	84.5	nd
0.007	0.6	5.8	nd	nd	nd	15.7	2.6	nd	75.2	nd
0.010	1.4	7.1	nd	nd	nd	20.0	3.1	nd	68.4	nd
0.014	1.6	9.2	nd	nd	nd	23.6	3.8	nd	61.8	nd
0.017	1.8	10.7	nd	nd	0.5	30.8	3.8	nd	52.4	nd
0.021	1.5	11.1	nd	nd	nd	32.8	4.5	nd	50.3	nd
0.028	1.7	13.5	nd	nd	nd	39.0	4.9	nd	40.0	0.9
0.042	1.3	17.2	nd	nd	nd	51.0	5.7	nd	24.9	nd
0.083	1.8	22.5	1.5	0.5	nd	59.5	6.9	nd	7.2	nd
0.13	2.0	25.4	3.3	nd	nd	59.2	8.1	nd	1.8	0.2
0.17	1.9	24.2	2.4	0.5	0.2	60.8	7.4	nd	2.1	0.5
0.25	3.8	28.5	4.8	1.4	1.7	50.0	8.2	0.4	0.5	0.8
0.33	2.7	28.7	4.9	1.4	2.3	49.0	7.3	1.0	nd	2.9
1	5.1	36.9	15.2	9.8	11.0	9.9	6.5	3.4	nd	2.2
1.17	7.2	38.7	18.4	5.5	12.9	5.7	6.0	4.8	nd	1.1
2	8.2	38.9	21.0	7.2	13.3	0.5	3.5	5.6	nd	1.9
3	7.6	39.0	25.4	4.4	13.4	nd	2.0	6.2	nd	2.1
7	11.0	36.8	29.3	nd	13.5	nd	nd	7.2	nd	2.2
10	10.8	36.8	31.6	nd	12.1	nd	nd	6.8	nd	2.0
15	11.8	35.9	30.5	nd	12.9	nd	nd	6.4	nd	2.7
30	16.6	34.9	27.6	nd	12.2	nd	nd	7.0	nd	3.2

nd denotes not detected (< 0.1 % of AR)

^a Polar degradates including oxamic acid (IN-18474), oxalic acid and 3 unknowns (each < 10 % of AR)

^b IN-U3204 is composed of geometric isomers

^c Other minor radioactive components

Table B.8.7-4: Hydrolysis half-life of cymoxanil, IN-U3204 and IN-JX915 in sterile buffer solution at pH 4, 7 and 9 based on two-compartment modelling (all compartments SFO, re-calculated by the notifier).

Test conditions	Substance	DT ₅₀ [days]	DT ₉₀ [days]	Formation fraction [0 ... 1]	R ^{2a}	χ ² error ^a [%]	Kinetics
pH 5	Cymoxanil	144	479	-	0.67	2.4	P _{SFO}
	IN-U3204	25.8	85.6	1.00	0.42	52.7	P _{SFO} → M _{SFO}
pH 7	Cymoxanil	1.1	3.5	-	1.00	3.2	P _{SFO}
	IN-U3204	2.6	8.5	0.99	1.00	6.8	P _{SFO} → M _{SFO}
	IN-JX915	1.1	3.7	0.12	0.52	48.4	P _{SFO} → M _{SFO}
	IN-T4226 ^b	7.2	23.8	-	0.94	16.4	M _{SFO}
pH 9	Cymoxanil	0.02	0.07	-	1.00	3.0	P _{SFO}
	IN-U3204	0.4	1.3	0.77	0.99	6.7	P _{SFO} → M _{SFO}
	IN-JX915	1.7	5.7	0.09	0.94	11.3	P _{SFO} → M _{SFO}
	IN-T4226 ^b	2.0	6.6	-	0.81	21.3	M _{SFO}

^a Re-calculated by the RMS for each compartment separately

^b Calculated by the RMS

Conclusion:

Hydrolysis of cymoxanil in sterile buffer solutions was strongly depending on the pH. Cymoxanil degraded very rapidly at pH 9 and 7 and relatively slowly at pH 5 with calculated half-lives of 0.02 days (31 minutes), 1.1 days and 144 days, respectively. All degradates occurring > 10 % of AR were identified: IN-W3595, IN-KP533, IN-KQ960, IN-U3204 and IN-R3273. The metabolites IN-W3595, IN-KP533, IN-R3273 and IN-KQ960 are considered stable after maximum formation at each pH, whereas all other metabolites rapidly degraded further. Quantitative formation pattern of metabolites was strongly depending on the pH. Based on multi-compartment modelling (all compartments following SFO kinetics), hydrolysis DT₅₀ of IN-U3204 was calculated to be 25.8, 2.6 and 0.4 days at pH 5, 7 and 9, respectively. Hydrolysis DT₅₀ of IN-JX915 and IN-T4226, both minor metabolites in hydrolysis, was calculated to be 1.1 and 1.7 days (IN-JX915) and 7.2 and 2.0 days (IN-T4226) at pH 7 and 9, respectively.

Comments (RMS):

None

Reference:

Aqueous hydrolysis of cymoxanil

Author(s), year: Willems, H., Slangen, P. J., Hoitink, M., 2003

Report/Doc. number: NOTOX project 308734

Guideline(s): SETAC (1995), OECD 111 (1981)

GLP: Yes

Deviations: None

Validity: Study considered acceptable, metabolite identification revised by the RMS

Material and methods:

Test substance: [Cyanoacetamide-2-¹⁴C]-cymoxanil, radiochemical purity 99.0 % (TLC) and 99.8 % (HPLC), 4.19 MBq mg⁻¹, batch 3304.265

Reference substances: Cymoxanil (unlabelled), IN-W3595 (AS 329)

Test medium:

- pH 4: 0.05 M acetate buffer
- pH 7: 0.05 M phosphate buffer
- pH 9: 0.05 M borate buffer

All buffer solutions sterilized by autoclaving and filtration (0.2 µm), sterility checked by 0, 13 and 30 DAT

Test concentration: 5.97 mg L⁻¹
 Co-solvent: 0.1 % acetonitrile
 Test system: Flow through system with humified N₂ gas (0.2 µm filtered)
 Temperature: 20 ± 1 °C
 Volatile traps: 2 x 50 mL of 2 M NaOH (¹⁴CO₂), 1 x 50 mL of 2-methoxyethanol (organic volatiles)

Analytical techniques:

- Three TLC methods (*TLC1*, *TLC2*, *TLC3*)
- Two HPLC methods (*HPLC1*, *HPLC2*), *HPLC2* was only used for additional metabolite identification work
- LSC

Note: These TLC and HPLC methods were re-investigated by Brands and Van Noorloos (2006) to determine the R_f/RT values of several cymoxanil metabolites (for details refer to hydrolysis section, B.8.7).

Additional experiments: Additional metabolite identification work was performed on samples obtained after incubation of unlabelled and radio-labelled cymoxanil (500 mg L⁻¹) at pH 9 (0.05 M borate buffer) for approx. 2 days. After sampling, pH was adjusted to pH 4 and samples were measured by *TLC3* and *HPLC2*. Afterwards, samples were partitioned with 100 mL of dichloromethane (3 times), finally the total dichloromethane and remaining water phase were concentrated. Aliquots of concentrated dichloromethane phase and the freeze dried water phase were separated using *TCL3*, spots were scraped of, extracted with acetonitrile and subjected to additional *TLC3* and *HPLC2* analysis. LC-MS/MS analysis was performed on the whole acidified sample prior to extraction and on the remaining water phase (additional HPLC method similar to *HPLC2*).

Kinetic evaluation: Parent: simple first order (SFO) kinetics, ModelManager

Findings:

Radiochemical purity of the test substance was confirmed to be > 97.7 % (*TLC1*, *TLC2*, *TLC3*, *HPLC1*). The pH did not significantly change during the experiment. Samples taken from pH 4 and pH 7 solutions were sterile throughout the test period, samples taken from pH 9 solutions were not sterile by 13 DAT and 30 DAT. Based on the fact that the test was conducted under anaerobic conditions and the fact that the metabolite profile of the test at pH 9 was similar to the metabolite profile of the test at pH 7, it is believed that microbial degradation did not play a significant role in the test at pH 9.

Mass balance was in a range of 97.3 – 101.8 % of AR for all experiments, formation of any volatiles was negligible (< 0.2 % of AR) at all pH values.

Cymoxanil was stable at a pH of 4, but underwent rapid hydrolysis at pH 7 and 9 with hydrolytic DT₅₀ values of 2.2 and 0.05 days (1.2 hrs) following SFO kinetics (R² ≥ 0.99).

At **pH 4**, degradation of cymoxanil was negligible, no degradates were observed > 3 % of AR.

At **pH 7**, three metabolite fractions (based on *HPLC1*) exceeded 10 % of AR: H-4, H-6 and H-3. H-3 is identical to the transient metabolite IN-U3204 (maximum 28.0 % of AR), the highly polar fraction H-4 (maximum 81.7 % of AR) comprises IN-W3595, IN-KP533, IN-R3274, oxamic acid (IN-18474) and oxalic acid. IN-W3595 and IN-KP533 could be adequately separated by *TLC3*, maximum occurrence of these metabolites were 22.6 % and 57.4 % of AR, respectively. Remaining unidentified polars did not exceed 10 % of AR in sum. Metabolite fraction H-6 comprises IN-KQ960 (maximum 6.4 % of AR, adequately separated by *TLC2*) and 2 other unknown metabolite fractions, individually not exceeding 10 % of AR. Metabolites IN-R3273 (identical to H-9) and IN-JX915 (identical to H-2) were observed at minor amounts. Several metabolite fractions < 10 % of AR remained unidentified.

At **pH 9**, four metabolite fractions (based on *HPLC1*) exceeded 10 % of AR: H-4, H-6, H-3 and H-2. IN-U3204 (H-3) reached maximum levels of 35.4 % of AR, IN-W3595 and IN-KP533 (both parts of H 4) peaked with 41.5 % and 57.4 % of AR, respectively. Remaining unidentified polars of H-4 are not expected to exceed 10 % of AR individually. IN-KQ960 (part of H-6) was observed with maximum amounts of 14.1 % of AR, remaining unknown compounds of H-6 is not expected to exceed 10 % of AR individually. IN-JX915 (H-2) reached a maximum occurrence of 11.0 % of AR. Metabolite IN-R3273 (H-9) was observed at a maximum occurrence of 5.4 % of AR. Several metabolite fractions, each individually < 10 % of AR, remained unidentified.

Table B.8.7-5: Characterization of radioactivity [% of AR] in sterile buffer solutions at pH 4 (based on HPLC1).

DAT	H-4	H-6	H-5	H-3	H-7	H-8	H-10	H-9	H-2	Cymoxanil	H-1
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	97.1	2.9
0.125	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	95.5	2.8
0.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	96.2	2.1
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	96.1	2.3
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	94.8	2.9
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	94.8	2.7
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	96.1	1.3
13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	97.5	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	95.6	0.0
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	98.1	0.0
RT _{UV} ^a [min]	1.9	2.7	3.0	3.6	3.9	4.2	5.9	6.8	7.9	13.5	14.0
Metabolite identification ^b	IN-W3595, IN-KP533, other polars	IN-KQ960, unknowns	Unknown	IN-U3204	Unknown	Unknown	Unknown	IN-R3273	IN-JX915	Cymoxanil	Unknown

^a RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^b Metabolite identification proposed by the RMS (see comments)

Table B.8.7-6: Characterization of radioactivity [% of AR] in sterile buffer solutions at pH 7 (based on HPLC1).

DAT	H-4	H-6	H-5	H-3	H-7	H-8	H-10	H-9	H-2	Cymoxanil	H-1
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	96.7	0.0
0.125	2.7	0.0	0.0	3.4	0.0	0.0	0.0	0.0	3.8	91.2	0.0
0.25	2.6	0.0	0.0	6.7	0.0	0.0	0.0	0.0	4.6	87.5	0.0
1	5.7	0.0	0.0	18.5	0.0	0.0	0.0	0.0	6.9	70.3	0.0
2	11.5	4.1	2.0	25.9	3.0	0.0	0.0	1.8	4.6	49.0	0.0
3	15.6	3.9	4.5	28.0	2.5	0.0	1.2	2.8	7.2	35.3	0.0
4	20.8	5.2	8.3	25.6	4.1	1.4	1.8	3.8	6.1	24.3	0.0
13	58.3	14.3	3.9	7.2	0.0	2.6	2.4	8.1	1.3	2.6	0.0
21	79.0	11.2	0.0	0.0	0.0	0.0	0.0	9.8	0.0	0.0	0.0
30	81.7	9.3	0.0	0.0	0.0	0.0	2.8	6.4	0.0	0.0	0.0
RT _{UV} ^a [min]	1.9	2.7	3.0	3.6	3.9	4.2	5.9	6.8	7.9	13.5	14.0
Metabolite identification ^b	IN-W3595, IN-KP533, other polars	IN-KQ960, unknowns	Unknown	IN-U3204	Unknown	Unknown	Unknown	IN-R3273	IN-JX915	Cymoxanil	Unknown

^a RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^b Metabolite identification proposed by the RMS (see comments)

Table B.8.7-7: Characterization of radioactivity [% of AR] in sterile buffer solutions at pH 9 (based on HPLC1).

DAT	H-4	H-6	H-5	H-3	H-7	H-8	H-10	H-9	H-2	Cymoxanil	H-1
0	17.9	0.0	0.0	23.5	0.0	0.0	0.0	0.0	6.7	51.9	0.0
0.125	37.8	6.3	2.3	35.4	0.0	0.0	0.0	0.0	11.0	5.6	0.0
0.25	39.1	11.7	5.0	31.3	0.0	0.0	0.0	0.0	10.1	1.3	0.0
1	45.4	29.1	5.2	9.8	0.0	0.0	0.0	1.7	7.2	0.0	0.0
2	52.1	29.9	5.9	3.0	0.0	0.0	0.0	2.7	5.3	0.0	0.0
3	58.4	22.5	7.6	1.8	0.0	0.0	0.0	4.6	2.9	0.0	0.0
4	62.4	22.0	4.6	0.0	0.0	0.0	1.6	5.0	3.1	0.0	0.0
13	79.3	13.0	0.0	0.0	0.0	0.0	1.7	4.6	0.0	0.0	0.0
21	78.3	13.9	0.0	0.0	0.0	0.0	1.3	5.4	0.0	0.0	0.0
30	82.3	14.1	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0
RT _{UV} ^a [min]	1.9	2.7	3.0	3.6	3.9	4.2	5.9	6.8	7.9	13.5	14.0
Metabolite identification ^b	IN-W3595, IN-KP533, other polars	IN-KQ960, unknowns	Unknown	IN-U3204	Unknown	Unknown	Unknown	IN-R3273	IN-JX915	Cymoxanil	Unknown

^a RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^b Metabolite identification proposed by the RMS (see comments)

WARNING: This document contains data that is not to be read in isolation. Registration must not be granted on the basis of this document.

Table B.8.7-8: Composition of *HPLC1* metabolite fraction H-4 [% of AR] in sterile buffer solution at pH 7 and pH 9 (based on *TLC3*).

pH	DAT	H-4 (<i>HPLC1</i>)	T3-7 (<i>TLC3</i>)	T3-4 (<i>TLC3</i>)	Unidentified fraction of H-4 (<i>HPLC1</i>)
pH 7	0	0.0	0.0	0.0	0.0
	0.125	2.7	0.0	1.3	1.4
	0.25	2.6	0.0	2.2	0.4
	1	5.7	0.0	5.9	0.0 (-0.2)
	2	11.5	0.0	9.4	2.1
	3	15.6	1.3	11.7	2.6
	4	20.8	3.2	13.9	3.7
	13	58.3	34.3	22.6	1.4
	21	79.0	54.6	18.9	5.5
	30	81.7	57.4	18.5	5.8
pH 9	0	17.9	0.0	4.4	13.5
	0.125	37.8	0.0	33.6	4.2
	0.25	39.1	0.0	40.6	(0.0) -1.5
	1	45.4	2.3	41.4	1.7
	2	52.1	8.9	41.5	1.7
	3	58.4	14.6	39.8	4.0
	4	62.4	20.7	40.9	0.8
	13	79.3	34.3	39.9	5.1
	21	78.3	31.8	40.2	6.3
	30	82.3	28.8	41.5	12.0
RT _{UV} [min] ^a / R _f		1.9	0.15	0.35	1.9
Metabolite identification ^b		IN-W3595, IN-KP533, other polars	IN-KP533	IN-W3595	Other polars ^c

^a RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^b Metabolite identification proposed by the RMS (see comments)

^c Several polar compounds (likely to comprise IN-R3274, oxamic acid (IN-18474) and oxalic acid), not expected to exceed 10 % of AR individually (see comments)

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Table B.8.7-9: Composition of *HPLC1* metabolite fractions H-6 [% of AR] in sterile buffer solution at pH 7 and pH 9 (based on *TLC2*).

pH	DAT	H-6 (<i>HPLC1</i>)	T2-3 (<i>TLC2</i>)	Unidentified fraction of H-6 (<i>HPLC1</i>)
pH 7	0	0.0	0.0	0.0
	0.125	0.0	0.0	0.0
	0.25	0.0	0.0	0.0
	1	0.0	0.9	0.0 (-0.9)
	2	4.1	1.2	2.9
	3	3.9	2.3	1.6
	4	5.2	6.4	0.0 (-1.2)
	13	14.3	5.9	8.4
	21	11.2	6.4	4.8
	30	9.3	6.1	3.2
pH 9	0	0.0	0.0	0.0
	0.125	6.3	3.5	2.8
	0.25	11.7	3.8	7.9
	1	29.1	11.5	17.6
	2	29.9	13.1	16.8
	3	22.5	12.4	10.1
	4	22.0	11.6	10.4
	13	13.0	11.8	1.2
	21	13.9	14.1	0.0 (-0.2)
	30	14.1	- ^a	-
RT _{UV} [min] ^b / R _f		2.7	0.40	2.7
Metabolite identification ^c		IN-KQ960, unknowns	IN-KQ960	Unknowns ^d

^a Original value of 1.3 % of AR likely to be an error, therefore excluded

^b RT denotes retention time. For higher comparability with supplemental study from Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^c Metabolite identification proposed by the RMS (see comments)

^d At least two unknown compounds, individually < 10 % of AR, co-eluting with IN-KQ960 under conditions of *HPLC1* (see comments)

Table B.8.7-10: Hydrolysis DT₅₀ and DT₉₀ of cymoxanil in sterile buffer solution at pH 4, 7 and 9 based on SFO kinetics.

Conditions	DT ₅₀ [days]			DT ₉₀ [days]			R ²		
	<i>HPLC1</i>	<i>TLC1</i>	<i>TLC3</i>	<i>HPLC1</i>	<i>TLC1</i>	<i>TLC3</i>	<i>HPLC1</i>	<i>TLC1</i>	<i>TLC3</i>
pH 4	Stable	362	714	Stable	1202	2371	-	0.77	0.33
pH 7	2.1	2.1	2.4	7.0	7.0	8.0	1.00	1.00	1.00
pH 9	0.04	0.05	0.05	0.13	0.17	0.17	1.00	1.00	0.99

Conclusion:

Cymoxanil was stable at a pH of 4, but underwent rapid hydrolysis at pH 7 and 9 with hydrolytic DT₅₀ values of 2.2 and 0.05 days (1.2 hrs) following SFO kinetics (R² ≥ 0.99). A large number of hydrolysis products was observed in the test solutions at pH 7 and pH 9. A steady state situation occurred by 21 DAT (pH 7) and 13 DAT (pH 9). At steady state, IN-KP533, IN-W3595 and IN-KQ960 exceeded 10 % of AR and can be considered as stable. Metabolites IN-U3204, IN-JX915 and a unknown metabolite fraction (co-eluting with IN-KQ960 under conditions of *HPLC1*) were transient metabolites exceeding 10 % of AR, rapidly decreasing thereafter to non-detectable amounts. Based on TLC-HPLC comparison, the unknown metabolite fraction (co-eluting with IN-KQ960 under conditions of *HPLC1*) is likely to consist of at least 2 compounds non of them exceeding 10 % of AR individually.

Comments (RMS):

- After complete revision, observed pattern, occurrence and time progress of observed metabolites are consistent with data observed in the respective DuPont study (Lawler, 1996).
- Owing to the high number of metabolites occurring during the hydrolysis of cymoxanil, none of the analytical methods applied in the study (*HPLC1*, *HPLC2*, *TLC1*, *TLC2*, *TLC3*) was capable to fully separate all metabolites. Since no authentic reference material was used, identification of metabolites in the original report was exclusively based on MS data. Owing to miss-interpretations of MS data and inconsistencies in the original report, the RMS requested further information and complete revision of the report. The notifiers submitted additional data on the RT/R_f values of metabolites under the conditions of TLC and HPLC methods used in the original studies (Brands and Van Noorloos, 2006) and subjected all Oxon studies (hydrolysis, photolysis, water/sediment) to thorough revision (results summarized in the position paper from Goodyear, 2006).

After complete evaluation of the dossier, the RMS proposes the following chromatographic systems to be most appropriate for individual (major) metabolites in Oxon aquatic studies (NOTOX projects):

Table B.8.6.1-11: Most appropriate chromatographic analysis systems for metabolite separation in Oxon aquatic studies (NOTOX projects) proposed by the RMS.

Compound	Analytical system	Alternative peak naming in respective studies	Approx. RT _{UV} [min]	Approx. R _f value	Reliability ^a
IN-U3204	<i>HPLC1</i>	H-3 (hydrolysis), M3 (water/sediment)	3.6	-	High
IN-KQ960	<i>TLC2</i>	T2-3 (hydrolysis), MVII (water/sediment)	-	0.40	High
IN-W3595	<i>TLC3</i>	T3-4 (hydrolysis)	-	0.35	High
	<i>TLC2</i>	MVI (water/sediment)	-	0.06	High
IN-KP533	<i>TLC3</i>	T3-7 (hydrolysis)	-	0.15	High
IN-JX915	<i>HPLC1</i>	H-2 (hydrolysis), M9 (water/sediment)	8.2	-	High
IN-R3273	<i>HPLC1</i>	H-9 (hydrolysis), M8 (water/sediment)	6.9	-	High

^a Reliability based on metabolite identification work presented in the hydrolysis study (MS data), temporal pattern of metabolite fractions under conditions of individual HPLC and TLC methods, RT/R_f values according to Brands and Van Noorloos (2006) and comparison to data from respective DuPont studies

- ***HPLC1* fraction H-6** consists of at least 3 compounds (including IN-KQ960), which are separated as metabolite fractions T3-1 and T3-6 under conditions of *TLC3* (data not shown). T3-1 comprises IN-KQ960 (confirmed by R_f values of IN-KQ960, Brands and Van Noorloos, 2006) and a second unknown fraction (never exceeding 10 % of AR). The composition of T3-6 (never exceeding 10 % of AR, too) is unknown. The notifier proposed that this fraction may be at least partly identical to IN-T4226 (see position paper Goodyear, 2007). However, this assumption is considered wrong by the RMS. Concluding, none of the unknown compounds of *HPLC1* fraction H-6 exceeds 10 % of AR individually under hydrolysis at pH 7 and pH 9.
- On the basis of RT values (Brands and Van Noorloos, 2006), the highly polar ***HPLC1* fraction H4** comprises IN-W3595, IN-KP533, IN-R3274 and several other polar metabolites, likely to be oxamic acid (IN-18474) and oxalic acid. IN-W3595 and IN-KP533 could be adequately separated by *TLC3* (metabolite fractions T3-4 and T3-7, respectively). The remaining other polar compounds (slightly exceeding 10 % of AR by 0 DAT and 30 DAT) are not considered to exceed 10 % of AR individually based on comparison to available data from the respective DuPont study (Lawler, 1996).
- The RMS conducted additional kinetic calculations (following current guidance, SANCO/10058/2005, ver. 1.0, 2005) using a two-compartment model for IN-U3204 and IN-JX915 (both separately linked to the parent with additional pathways to the sink) based on SFO kinetics for all compartments (ModelMaker 4.0):

Table B.8.7-12: Calculated hydrolysis DT_{50} of IN-U3204 and IN-JX915 in sterile buffer solution at pH 4, 7 and 9 based on two compartment modelling (all compartments SFO) – RMS assessment.

Test conditions	Substance	DT_{50} [days]	DT_{90} [days]	Formation fraction [0 ... 1]	R^2	χ^2 error [%]	Model
pH 7	IN-U3204	2.3	7.7	0.75	0.99	6.9	$P_{SFO} \rightarrow M_{SFO}$
	IN-JX915	0.5	1.6	0.55	0.70	37	$P_{SFO} \rightarrow M_{SFO}$
	IN-KP533	Considered stable		-	-	-	-
	IN-W3595	Considered stable		-	-	-	-
	IN-KQ960	Considered stable		-	-	-	-
	IN-R3293	Considered stable		-	-	-	-
pH 9	IN-U3204	0.5	1.5	0.57	1.00	5.1	$P_{SFO} \rightarrow M_{SFO}$
	IN-JX915	1.7	5.6	0.15	0.99	9.1	$P_{SFO} \rightarrow M_{SFO}$
	IN-KP533	Considered stable		-	-	-	-
	IN-W3595	Considered stable		-	-	-	-
	IN-KQ960	Considered stable		-	-	-	-
	IN-R3293	Considered stable		-	-	-	-

Reference:	Determination of TLC RF values and HPLC retention times of cymoxanil and eight reference substances
Author(s), year:	Brands, C., Van Noorloos, B., 2006
Report/Doc. number:	NOTOX project 457818
Guideline(s):	Supplemental study - not applicable
GLP:	Yes
Deviations:	Supplemental study - not applicable
Validity:	Study considered acceptable

Material and methods:

Test substances:	Cymoxanil, IN-U3204, IN-KQ960, IN-KP533, IN-W3595, IN-R3274, IN-JX915, IN-T4226, IN-R3273, all non-labelled, purity > 93 %
Test concentration:	All reference substances were individually dissolved in acetonitrile and diluted by a factor of at least 100 in 0.05 M phosphate buffer (pH 7) and 0.05 M borate buffer (pH 9). A mixture of all substances were also prepared in the same buffer solutions. Test solution were subjected to TLC/HPLC without further extraction/concentration.
Analytical techniques:	• HPLC1: Mobile phase milli-Q water/acetonitrile (75/25, v/v) adjusted to pH 3 with formic acid, Supersphere 60 RP-select B (250 x 4 mm) HPLC column, room temperature, 1 ml min⁻¹, 227 nm detection wave-length • HPLC2: Mobile phase A: 0.1 % TFA in milli-Q water, mobile phase B: 0.1 % TFA in acetonitrile, linear gradient, Lichrospher 60 RP select B (250 x 4 mm), 30 °C, 0.75 ml min⁻¹, 227 nm detection wave-length • TLC1: Mobile phase methanol/formic acid 0.2 % (70/30, v/v), RP-18 F₂₅₂ S 20 x 20 cm TLC plate • TLC2: Mobile phase methanol/ethylenacetate/n-hexane 95 % (15/60/30, v/v/v), Si-60 F₂₅₄ 20 x 20 cm TLC plate • TLC3: Mobile phase n-butanol/formic acid/milli-Q water (70/7/7, v/v/v), Si-60 F₂₅₄ 20 x 20 cm TLC plate

Findings:

The retention times (HPLC) and R_f values (TLC) of cymoxanil and the metabolites IN-U3204, IN-KQ960, IN-KP533, IN-W3595, IN-R3274, IN-JX915, IN-T4226 and IN-R3273 were re-analysed on three TLC systems

and 2 HPLC systems which were used in the hydrolysis, photolysis and water/sediment studies of the same laboratory (Willems et al., 2003, Willems, 2000, Slangen and Willems, 2000).

Table B.8.6.1-13: Retention times (HPLC) and R_f values (TLC) of cymoxanil and reference substances in buffer solution pH 7.

Compound	Retention times [min]		R_f value		
	HPLC1	HPLC2	TLC1	TLC2	TLC3
IN-KP533	1.7	8.7	0.88	0.01	0.12
IN-R3274	1.7	4.2/4.5 ^d	0.94	0.03	0.55
IN-W3595	1.8	8.2	0.93	0.06	0.50
IN-KQ960	2.7 ^c	10.4	0.84	0.48 ^a	0.66 ^{ab}
IN-U3204	3.6	10.0	0.65	0.56	0.49
IN-T4226	4.2	10.3	0.75	0.68	0.82 ^a
IN-JX915	6.7/7.9 ^{de}	15.5 ^e	0.63	0.70	0.83
IN-R3273	6.7 ^e	15.5 ^e	0.62	0.68	0.83
Cymoxanil	13.8	18.4	0.54	0.66	0.76

^a Additional spot at origin

^b Additional spot with R_f 0.81

^c Additional peak (peak area 15 %) at 4.2 min

^d 2 major peaks

^e Additional peaks (peak area ≤ 5 %) at 4.2 and 5.8 min (HPLC1) or 10.3 and 14.9 min (HPLC2)

Table B.8.6.1-14: Retention times (HPLC) and R_f values (TLC) of cymoxanil and reference substances in buffer solution pH 9.

Compound	Retention times [min]		R_f value		
	HPLC1	HPLC2	TLC1	TLC2	TLC3
IN-KP533	1.8	8.7	0.86	0.00	0.14
IN-R3274	1.6	4.5	0.90	0.02	0.57
IN-W3595	1.6	8.3	0.92	0.06	0.53
IN-KQ960	2.7 ^c	10.4	0.82	0.48 ^a	0.62 ^a
IN-U3204	3.6	10.0	0.62	0.55	0.48
IN-T4226	4.2 ^f	10.3 ^f	0.90 ^f	0.17 ^f	0.82 ^f
IN-JX915	6.8/8.1 ^{de}	15.5 ^e	0.64	0.71 ^a	0.83 ^b
IN-R3273	6.8 ^e	15.5 ^e	0.65	0.67	0.82
Cymoxanil	13.7 ^f	18.4 ^f	0.52 ^f	0.67 ^f	0.73 ^f

^a Additional spot at origin

^b Additional spot with R_f 0.89

^c Additional peak (peak area 7 %) at 4.2 min

^d 2 major peaks

^e Additional peaks (peak area ≤ 6 %) at 4.2 and 5.8 min (HPLC1) or 14.9 min (HPLC2)

^f Additional spots/peaks observed on TLC/HPLC (see text)

In HPLC chromatograms of cymoxanil in buffer solution pH 9, additional peaks (peak area > 5 %) were observed at the position of IN-U3204 (RT 3.6 and 10.0 min, HPLC1 and HPLC2) and IN-W3595 (RT 1.6 and 8.3 min). Also on TLC plates additional spots were observed. These results are attributed to the distinct instability of cymoxanil under alkaline conditions.

In HPLC chromatograms of reference substance IN-T4226 in buffer solution pH 9, two peaks were observed (RT 1.5 and 4.2 min for HPLC1 and 8.7 and 10.3 min for HPLC2), whereas only one peak was observed at pH 7, suggesting that IN-T4226 is unstable at higher pH. On TLC additional spots were observed with a R_f value of 0.78 (TLC1), 0.00 (TLC2) and 0.08 and 0.30 (TLC3).

Table B.8.6.1-15: Possible matches between major metabolites determined in the hydrolysis study of cymoxanil (Willems *et al.*, 2003) and reference substances.

Metabolite	HPLC1	HPLC2 ^a	TLC1	TLC2	TLC3
IN-U3204	H-3	T3-3, T3-5	T1-2, T1-1	T2-1	T3-3
IN-KQ960	H-6	T3-3, T5-5	T1-6, T1-4	T2-3	T3-1
IN-KP533	H-4	T3-7, T3-4	T1-6, T1-4	T2-6, T2-5	T3-7
IN-W3595	H-4	T3-7, T3-4	T1-6, T1-4	T2-6, T2-5, T2-4	T3-3
IN-R3274	H-4	?	T1-6, T1-4	T2-6, T2-5	T3-3
IN-JX915	H-2	T3-2	T1-2, T1-1	T2-1	T3-5, T3-2
IN-T4226	H-7 ^b , H-8 ^b	T3-3, T3-5	T1-1, T1-6, T1-4	T2-4, T2-1	T3-5, T3-2
IN-R3273	H-9 ^b	T3-2	T1-2, T1-1	T2-1	T3-5, T3-2

^a Based on RT values obtained from isolated TLC3 metabolites (metabolite identification work in NOTOX project 308734)

^b Information added by the RMS based on RT values

Conclusion:

The retention times and R_f values of cymoxanil and eight reference substances were successfully determined using two HPLC and three TLC methods that were used in NOTOX project 308734 (hydrolysis of cymoxanil, Willems *et al.*, 2003).

Comments (RMS):

- The RMS requested this additional information, owing to the high uncertainties and misleading interpretations according to metabolite identification in the hydrolysis study (Willems *et al.*, 2003). Study results were used by the RMS to support revision of metabolite identification in relevant Oxon studies (NOTOX projects).

Reference:

Identification of cymoxanil aquatic degradation products

Author(s), year:	Goodyear, A., 2006
Report/Doc. number:	TSGE 4-3-4.PP1
Guideline(s):	Position paper - not applicable
GLP:	Position paper - not applicable
Deviations:	Position paper - not applicable
Validity:	Study considered partly acceptable (see comments)

Material and methods:

The notifiers made an effort to give a fully revised synopsis on metabolite occurrence and identification in the following Oxon studies (NOTOX projects):

- 308734 (hydrolysis of cymoxanil, Willems *et al.*, 2003)
- 257759 (photolysis of cymoxanil in water, Willems, 2000)
- 257761 (water/sediment study, Slangen and Willems, 2000)
- 366784 (additional water/sediment study to regenerate M-5, Willems and Hoitink, 2003)
- 310859 (comparison of test media, Willems and Slangen, 2003)

Findings:

Table B.8.6.1-16: Suggested linkage of unidentified metabolite fractions in Oxon studies to identified metabolites in DuPont studies proposed by the notifiers (only metabolite fractions > 10 % of AR considered).

Type of study	Method used	Unidentified metabolite fraction in original study	Maximum occurrence [% of AR]	Suggested linkage to DuPont metabolite	Remarks by the RMS
Hydrolysis (Willems et al., 2003)	TLC3	T3-1	19.5	IN-KQ960	Not considered correct, metabolite fraction T3-1 comprises at least 2 compounds, one of them is IN-KQ960, the other one is unknown (not exceeding 10 % of AR individually), IN-KQ960 is adequately separated by TLC2 (Brands and Van Noorloos, 2006)
		T3-2	10.1	IN-JX915	-
		T3-3	38.0	IN-U3204	-
		T3-4	41.5	IN-W3595	-
		T3-5	14.9	IN-R3273	Not considered correct, T3-5 comprises at least 3 compounds (none of them exceeding 10 % of AR individually), one of them is IN-R3273, the other ones are unknown, IN-R3273 is adequately separated by HPLC1
		T3-7	57.4	IN-KP533	-
Photolysis (Willems, 2000)	TLC1	MI	18.5	IN-R3273	Under conditions of TLC1, IN-U3204 co-elutes with IN-JX915 (Brands and Van Noorloos, 2006). However, since amounts of IN-U3204 under conditions of photolysis are considered to be minimal (DuPont study, Anderson et al., 1993), peak identification proposed by the notifiers is considered valid in this case only
		MII	52.6	IN-JX915	
Water/ sediment study (Slangen and Willems, 2000)	HPLC1	M-1	35.0	IN-W3595	Not considered correct, metabolite fraction M-1 comprises several polar metabolites, i.e. IN-W3595, IN-KP533, IN-R3274, oxamic acid (IN-18474) and oxalic acid, IN-W3595 is adequately separated by TLC3, no final conclusion can be drawn on the (maximum) occurrence of the other polar compounds
		M-2	14.3	IN-T4226	Not considered correct, M-2 is likely to mainly comprise IN-KQ960 (based on RT values, Brands and Van Noorloos, 2006)
		M-3	24.7	IN-U3204	-
		M-5	22.9	Not seen	M5 is a metabolite fraction likely to comprise at least 2 or 3 compounds

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Conclusion:

Overall, it can be concluded that the route of hydrolysis degradation of cymoxanil observed in NOTOX studies is essential the same as that observed in the DuPont studies where identification of products was achieved using authentic reference standards. None of the hydrolysis metabolites exceed 10 % of AR in NOTOX or DuPont studies remain unidentified.

Comments (RMS):

- The conclusions of the position paper made on metabolite identification in the respective Oxon studies (all NOTOX reports) are only considered partly valid by the RMS, see statements in the table.

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B.8.8 Photolysis (Annex IIA, point 7.2.1.2)

The photolysis of cymoxanil was investigated in two studies:

- Photolysis in sterile buffer solution at pH 5 at 25 °C (DuPont)
- Photolysis in sterile buffer solution at pH 5 at 25 °C (Oxon)

Additional studies were conducted on the

- Quantum yield of cymoxanil (DuPont)
- Calculated theoretical lifetime of cymoxanil (DuPont)
- Calculated theoretical lifetime of cymoxanil (Oxon)
- UV/VIS absorption of IN-R3273 and IN-JX915 (major photolysis metabolites) (DuPont)
- Quantum efficiency and calculated theoretical lifetime for IN-R3273 and IN-JX915 (DuPont)

Reference:	Photodegradation of [2-¹⁴C]-DPX-T3217 (cymoxanil) in pond water and sterile buffer pH 5
Author(s), year:	Anderson, J. J., Horne, P., Lawler, S. M., Swain, R. S., 1993a
Report/Doc. number:	DuPont Report No. AMR 1990-91
Guideline(s):	US-EPA 161-2 (1982)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

including supplement:

Reference:	Quantum yield of DPX-T3217 (cymoxanil) and LS/MS confirmation of unknown degradates in sterile buffer at pH 5
Author(s), year:	Anderson, J. J., Lawler, S. M., Swain, R. S., 1993b
Report/Doc. number:	DuPont Report No. AMR 1990-91, supplement report No. 1
Guideline(s):	US-EPA 161-2 (1982)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

including relevant kinetic re-calculations from:

Reference:	Calculated half-lives of cymoxanil and its metabolites in environmental fate laboratory studies
Author(s), year:	Malekani, K., 2003
Report/Doc. number:	DuPont-11575, Revision No. 1
Guideline(s):	Calculations - not applicable
GLP:	Calculations - not applicable
Deviations:	Calculations - not applicable
Validity:	Study considered acceptable

Material and methods:

Test substance:	• [Cyanoacetamide-2-¹⁴C]-cymoxanil, 0.52 MBq mg⁻¹, > 97 % radiochemical purity (TLC), batch HOTC 376 • Cymoxanil technical (supplemental study only)
Reference substances:	Cymoxanil (unlabelled), IN-R3273, IN-U3204, IN-T4226, IN-KP533, oxamic acid (IN-18474), IN-JX915 (the latter supplemental study only)
Test systems:	• Sterile buffer solution (0.01 M acetate buffer, pH 5), filter sterilized (2 µm) • Pond water (natural water, pH 8.7, adjusted to pH 7.0, non-sterile)

Test concentration:	25 mg L ⁻¹
Co-solvent:	0.5 % acetonitrile (v/v)
Test temperature:	25 ± 1 °C
Test duration:	15 days (irradiated), 30 days (dark controls)
Irradiation system:	Xenon arc lamp (Suntest, > 290 nm), approx. 370 W m ⁻² (corresponding to approx. 138 % of natural sunlight energy, 270 W m ⁻² , measured at noon on a clear midsummer day in Wilmington, Delaware, USA, 39 °N), irradiation measured by a spectroradiometer, 1 days of experimental irradiation equivalent to approx. 2.5 and 2.7 Wilmington summer days for sterile pH 5 and non-sterile pH 7 pond samples, respectively
Sample processing:	None, samples directly injected into HPLC
Volatile traps:	None
Analytical techniques:	LSC, HPLC-UV/RAD (two independent methods), TLC (three methods), metabolites confirmed by LC/MS
Additional experiments:	- In the supplemental report (Anderson et al, 1993b) the quantum yield for cymoxanil (unlabelled) was determined using p-nitroacetophenone (PNAP) as actinometer (investigated in a range of 300 – 335 nm). Solutions of cymoxanil (25 mg L⁻¹ = 1.3 x 10⁻⁴ M) and PNAP (2.1 x 10⁻⁵ M) were prepared in sterile 0.01 M acetate buffer (pH 5.0), experiments were conducted at the same conditions as stated above for 2 days. The actinometer quantum yield (Φ) was calculated to be 0.000992. - In the supplemental report (Anderson et al, 1993b) samples from the original study were re-investigated by HPLC-UV/RAD, TLC and LC/MS for metabolite identification
Kinetic evaluation:	- Parent (re-calculated, Malekani, 2003): Simple first order (SFO) kinetics - Metabolites (Malekani, 2003): - IN-JX915 and IN-R3273: Multi-compartment model linking parent, IN-JX915 and IN-R3273 in series (all compartments SFO kinetics with additional pathways to sink)

ModelManager 1.1

Findings:

All pH 5 samples were shown to be sterile. Overall mass balance (including non-sterile pH 7 pond water samples) was in a range of 91.8 – 106.0 % of AR, indicating that no significant amounts of volatiles were formed (pH 5 and pH 7 samples).

Under irradiation in sterile solutions at pH 5 (where hydrolysis is slowest), cymoxanil rapidly degraded with a half-life of 1.7 days (following SFO kinetics, R² = 0.94, χ² error = 13.9). Respective degradation under dark conditions was 110 days. The net photolysis rate in the lab was calculated to be 1.7 days which corresponds to 4.3 days under environmental conditions (mid summer day, 39 °N).

At pH 7, in non-sterile pond water, cymoxanil degraded with a half-life of 0.23 days owing to photolysis and hydrolysis. Accounting for the fast hydrolysis at neutral conditions (hydrolysis DT₅₀ = 0.5 days, dark control samples), net photolysis rate was calculated to be 0.42 days, which is equivalent to 1.1 days under environmental conditions.

Degradation of cymoxanil at pH 5 under dark conditions was slow (no metabolite observed > 10 % of AR, metabolite pattern almost identical to pattern observed in hydrolysis study). Under irradiation, metabolite IN-JX915 was rapidly formed, representing 51.7 % of AR by 4 DAT declining thereafter to 25.4 % of AR by 15 DAT. Metabolite IN-R3273 continuously increased to 35.4 % of AR by study termination (15 DAT). No other metabolite was observed > 10 % in the irradiated test solution. Minor metabolites identified were oxamic acid

(IN-18474, maximum 2.0 % of AR), IN-KP533 (7.9 % of AR), IN-T4226 (6.7 % of AR) and IN-U3204 (0.6 % of AR). Metabolites were not identified in the pH 7 pond water. In the supplemental report degradates E and D were shown to be consistent with IN-JX915 and IN-T4226.

Based on multi-compartment modelling (all compartments following SFO kinetics), DT₅₀ of IN-JX915 was calculated to be 6.7 days in sterile buffer solution at pH 5, calculated DT₅₀ of IN-R3273 was 32.7 days (including photolysis and hydrolysis processes for both compounds).

Cymoxanil has an absorption maximum of 238 nm and the absorption extends to 330 nm. The quantum yield Φ^c of cymoxanil photolysis is given by

$$\Phi^c = \frac{k_p^c \cdot \sum \epsilon_\lambda^a \cdot L_\lambda}{k_p^a \cdot \sum \epsilon_\lambda^c \cdot L_\lambda} \cdot \Phi^a$$

where Φ^a is the quantum yield of the actinometer solution and k_p^a and k_p^c are the photolysis rate constants for the photo-degradation of the actinometer and cymoxanil solutions, respectively, corrected for dark controls, determined by first-order kinetics. ε_λ^a and ε_λ^c are the molar absorptivities of the actinometer and cymoxanil solution, respectively, L_λ is the irradiance of the light source in units of Einsteins cm⁻² of the actinometer and of cymoxanil, respectively (the same for both systems). The quantum yield (Φ) of cymoxanil photolysis was determined to be 0.0052.

Table B.8.8-1: Composition of radioactivity [% of AR] in sterile buffer at pH 5 under irradiated and dark conditions (HPLC, mean of duplicate samples).

Conditions	DAT	IN-18474	IN-KP533	IN-T4226 ^a	IN-U3204	IN-JX915 ^a	IN-R3273	Cymoxanil	Others ^b
Irradiated	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	100.0	< 0.1
	0.08	< 0.1	< 0.1	< 0.1	< 0.1	3.1	< 0.1	96.7	0.3
	1.04	< 0.1	< 0.1	< 0.1	< 0.1	6.9	< 0.1	93.2	< 0.1
	1	0.2	1.9	2.6	0.2	28.4	1.6	63.8	1.2
	1.17	0.5	2.8	2.3	0.3	30.9	2.0	59.2	2.0
	1.42	0.9	2.6	2.6	0.3	35.4	2.8	52.9	2.5
	2	0.9	2.8	2.7	0.5	41.5	4.3	43.2	4.2
	4	1.1	4.7	4.4	0.3	51.7	10.6	20.7	6.6
	7	2.0	5.3	4.6	0.6	46.7	18.9	9.9	12.1
	10	2.0	7.1	5.8	0.3	36.6	28.9	6.6	12.8
	15	1.5	7.9	6.7	< 0.1	25.4	35.4	3.5	19.8
Dark	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	96.8	3.3
	0.17	0.9	< 0.1	< 0.1	3.1	1.1	< 0.1	93.0	1.6
	1	< 0.1	< 0.1	< 0.1	5.1	1.6	< 0.1	90.3	3.0
	2	0.5	< 0.1	0.5	3.6	1.1	< 0.1	92.7	1.7
	3	< 0.1	< 0.1	1.3	4.8	1.3	< 0.1	89.8	2.8
	7	< 0.1	< 0.1	1.2	9.1	1.8	0.4	84.2	3.4
	10	0.1	0.8	1.0	4.4	0.5	0.6	91.1	1.6
	15	< 0.1	< 0.1	2.0	7.5	1.5	0.6	84.8	3.6
	30	< 0.1	< 0.1	2.3	7.3	1.0	0.9	81.3	7.4

^a Identity of degradates was confirmed in the supplemental report (degradate E = IN-JX915, degradate D = IN-T4226).

^b Minor radioactive components, none which is > 10 % of AR

Table B.8.8-2: Amounts of cymoxanil [% of AR] in non-sterile pond water at pH 7 under irradiated and dark conditions (HPLC, mean of duplicate samples).

Irradiated		Dark	
DAT	Cymoxanil	DAT	Cymoxanil
0	95.1	0	75.9
0.01	86.1	0.01	88.6
0.03	88.2	0.03	86.6
0.04	81.3	0.04	86.7
0.06	79.1	0.06	79.4
0.07	77.0	0.07	81.6
0.08	74.5	0.08	82.8
0.17	57.4	0.17	58.4
1	1.1	1	23.3
2	0.4	2	5.9
4	< 0.1	3	2.0
7	< 0.1	4	0.8
10	< 0.1	7	0.1
15	< 0.1	10	< 0.1
		15	< 0.1
		30	< 0.1

Table B.8.8-3: Amounts of cymoxanil [% of applied] in sterile buffer at pH 5 under irradiated and dark conditions (HPLC, mean of duplicate samples) – supplemental study.

Irradiated		Dark	
DAT	Cymoxanil	DAT	Cymoxanil
0	100.0	0	100.0
0.08	91.5	0.08	98.8
0.21	80.6	0.21	99.5
1	57.2	1	99.0
1.17	54.0	1.17	98.9
1.7	41.4	1.4	98.8
2	37.4	2	98.0

Table B.8.8-4: Net photolysis half-life of cymoxanil in sterile buffer solution at pH 5 and in non-sterile pH 7 pond water (re-calculated by the notifier).

Compound	Test medium	Conditions	k [day ⁻¹]	DT ₅₀ [days]	DT ₉₀ [days]	R ²	χ^2 error ^a [%]
Cymoxanil	Sterile buffer, pH 5	Irradiated	0.416	1.7	5.6	0.94	13.9
		Dark	0.0063	110	365	0.68	2.6
		Net	0.410	1.7	5.6	-	-
		38 °N, midsummer	-	4.3	14.2	-	-
	Non-sterile pond water, pH 7	Irradiated	3.03	0.23	0.78	0.99	2.8
		Dark	1.38	0.50	1.7	0.99	7.9
		Net	1.65	0.42	1.4	-	-
		38 °N, midsummer	-	1.1	3.5	-	-

^a Calculated by the RMS

Table B.8.8-5: Photolysis/hydrolysis half-life of metabolites IN-JX915 and IN-R3273 in sterile buffer solution at pH 5 based on two-compartment modelling (all compartments SFO, re-calculated by the notifier).

Compound	Test medium	Conditions	DT ₅₀ [days]	DT ₉₀ [days]	Formation fraction [0 ... 1]	R ^{2a}	χ ² error ^a [%]	Model
IN-JX915	Sterile buffer, pH 5	Irradiated	6.7	22.2	0.77	0.87	17.8	P _{SFO} → M _{SFO}
IN-R3273			32.7	109	0.73	1.00	6.3	P _{SFO} → M1 _{SFO} → M2 _{SFO} ^b

^a Re-calculated by the RMS for each compartment individually^b M1 = IN-JX915, M2 = IN-R3273**Conclusion:**

Under irradiation, cymoxanil rapidly degraded at pH 5 in sterile buffer solution (conditions under which chemical hydrolysis is slowest). Laboratory net degradation half life of cymoxanil under sterile conditions at pH 5 was calculated to be 1.7 days (following SFO kinetics), which is equivalent to 4.3 days under environmental conditions (midsummer day, 38 °N). However, experiments on pond water solutions indicated that at neutral pH, under non-sterile conditions, hydrolysis and microbial processes were much more important (net photolysis half-life of 0.42 days, equivalent to 1.1 days under environmental conditions). Metabolites occurring > 10 % of AR in sterile buffer solution at pH 5 were IN-JX915 (maximum 51.7 % of AR) and IN-R3273 (maximum 35.4 % of AR). Based on multi-compartment modelling (all compartments following SFO kinetics), DT₅₀ of IN-JX915 owing to photolysis and hydrolysis was calculated to be 6.7 days in sterile buffer solution at pH 5, calculated DT₅₀ of IN-R3273 was 32.7 days.

The quantum yield (Φ) of cymoxanil photolysis was determined to be 0.0052.

Comments (RMS):

None

Reference:**Photodegradation of cymoxanil in water**

(including Report Amendment number 1)

Author(s), year: Willems, H., 2000

Report/Doc. number: NOTOX project 257759

Guideline(s): US-EPA 161-2 (1982), SETAC (1995)

GLP: Yes

Deviations: None

Validity: Study considered acceptable, metabolite identification revised by the notifiers

Material and methods:

Test substance: [Cyanoacetamide-2-¹⁴C]-cymoxanil, radiochemical purity 99.0 % (TLC), 99.8 % (HPLC), 4.19 MBq mg⁻¹, batch 3304.265

Reference substances: Cymoxanil (unlabelled), IN-W3595 (AS 329), IN-R3274 (AS 330), IN-T4226 (AS 331), CAU (AS 336), Oxime (AS 335), hippuric acid (B076)

Test system: Sterile 0.05 M phosphate buffer at pH 5.0, sterility of the test system was checked throughout the study

Test concentration: 0.62 mg L⁻¹

Co-solvent: 0.01 % acetone

Test temperature: 25 ± 1 °C

Irradiation system: Suntest CPS+ system, xenon burner, > 290 nm, 700 W m²

Irradiation time: 1 day of laboratory exposure equivalent to 4 days of natural summer sunlight at 40 °N (study was determined after 6 days because DT₅₀ was reached)

Volatile traps: Humified, CO₂ free air passing the test system was passed through 2 x 2 M NaOH

- (¹⁴CO₂) and 1 x 2-methoxyethanol (organic volatiles)
- Actinometry: Intensity of xenon burner was determined using a ferri-oxalate actinometer (absorbs light in the range of 290 – 510 nm with a quantum yield of 1.1)
- Analytical techniques:
- Two TLC methods (*TLC1*, called '*TLC2*' in the report, *TLC2*, called '*TLC1*' in the report)
 - One HPLC methods (*HPLC1*)
 - LSC
- Note:** These TLC and HPLC methods were re-investigated by Brands and Van Noorloos (2006) to determine the *R_f*/RT values of several cymoxanil metabolites (for details refer to hydrolysis section, B.8.7).
- Additional experiments: Irradiated samples were partitioned three times with dichloromethane. Concentrated dichloromethane phase (evaporated) and remaining water phase were subjected to MS/MS.
- Kinetic evaluation: Parent: Simple first order (SFO) kinetics

Findings:

Radiochemical purity of the cymoxanil stock solution was > 98.0 % of AR (*TLC1*, *TLC2*, *HPLC1*). Sterility of the test system was confirmed, no changes in pH occurred either in the irradiated or in the dark control samples. Cymoxanil does not absorb any light > 350 nm.

The number of photons absorbed by the actinometer solution was $2.34 \times 10^{18} \text{ sec}^{-1}$ at the beginning and $2.56 \times 10^{18} \text{ sec}^{-1}$ at the end of the test. The average was used for xenon lamp intensity calculation.

Mass balance was in a range of 96.6 – 101.1 % of AR in both test systems, only negligible amounts of ¹⁴CO₂ (maximum 0.5 % of AR) were formed. *TLC2* (in the study text called '*TLC1*') was not able to separate metabolites, which co-elute with cymoxanil. Peak performance under *HPLC1* conditions was rather bad, however from RT values for peak 1 (7.0 min) and peak 2 (8.3 min) it can be concluded that these two *HPLC1* peaks refer to IN-R3273 (maximum of 18.5 % of AR by study termination) and IN-JX915 (52.6 % of AR). These two substances were adequately separated using *TLC1* (called '*TLC2*' in the study report) method.

Quantum yield of cymoxanil was calculated to be 0.00058.

Table B.8.8-6: Characterization of radioactivity [% of AR] of irradiated and dark control samples after application of cymoxanil to sterile buffer solution at pH 5.0 based on HPLC1 and TLC1.

Conditions	DAT	<i>TLC1</i> ^a				
		Cymoxanil	M I	M II	M III	M IV
Irradiated	0	100.0	0.0	0.0	0.0	0.0
	0.17	95.9	0.0	4.9	0.0	0.0
	0.83	83.3	0.0	17.3	0.0	0.0
	1.13	76.5	0.0	24.0	0.0	0.0
	2.13	63.9	0.0	35.5	0.0	0.0
	3	43.5	7.5	45.0	4.2	0.0
	6	25.1	18.5	52.6	0.0	0.0
Dark	0	100.0	0.0	0.0	0.0	0.0
	2.13	100.0	0.0	0.0	0.0	0.0
	6	91.6	0.0	6.9	0.0	2.3
RT _{UV} [min] ^c / R _f		0.48	0.56	0.65	0.76	?
Metabolite identification ^c		0.54	IN-R3273	IN-JX915	Unknown	Unknown

^a In the report *TLC1* is called '*TLC2*', *TLC2* is called '*TLC1*'

RT denotes retention time. For higher comparability with supplemental study from Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^c Metabolite identification proposed by the notifiers (position paper from Goodyear, 2006)

Table B.8.8-7: Net photolysis half-life of cymoxanil in sterile buffer solution at pH 5 based on SFO kinetics.

Substance	Conditions	k [day ⁻¹]	DT ₅₀ [days]	DT ₉₀ [days]	R ²
Cymoxanil	Irradiated	0.236	2.9	9.8	0.990
	Dark	0.00679	102	339	0.877
	Net	0.229	3.0	10.0	-
	40 °N, midsummer	-	12.1	40.2	-

Conclusion:

A photolytic DT₅₀ of cymoxanil was obtained by subtracting the dark rate constant from the irradiated rate constant. The experimental net photolytic DT₅₀ of cymoxanil was 3.0 days, which is equivalent to 12.1 days under natural conditions (40 °N, midsummer day). The quantum yield was 0.00058. The cyclisation products IN-JX915 and IN-R3273 were observed > 10 % of AR, both accumulating towards study end.

Comments (RMS):

- After revision by the notifiers, observed pattern, occurrence and time progress of observed metabolites are consistent with data observed in the respective DuPont study.
- The RMS conducted additional kinetic calculations according to current guidelines (SANCO/10058/2005, ver. 1, 2005) for the photolysis metabolites IN-JX915 and IN-R3273 using a three-compartment model linking the metabolites to the parent in series (all compartments following SFO kinetics, with additional pathways from the parent to the sink, ModelMaker 4.0).

Table B.8.8-8: Photolysis/hydrolysis DT₅₀ of IN-JX915 and IN-R3273 in sterile buffer solution at pH 5 based on multi-compartment modelling (all compartments SFO kinetics).

Substance	Conditions	DT ₅₀ [days]	DT ₉₀ [days]	Formation fraction [0 ... 1]	R ²	χ ² error [%]	Kinetics
IN-JX915	Irradiated	6.5	21.5	1.00	1.00	2.2	P _{SFO} → M _{SFO}
IN-R3273		Considered stable		0.74	0.96	31.6	P _{SFO} → M1 _{SFO} → M2 _{SFO} ^a

^a M1 = IN-JX915, M2 = IN-R3273

Reference:	Calculated theoretical lifetime for cymoxanil in the top layer of aqueous systems
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Author(s), year:	Hatzenbeler, C. J., Moore, L. A., 2004
Report/Doc. number:	DuPont-12330
Guideline(s):	Calculations - not applicable
GLP:	Calculations - not applicable
Deviations:	Calculations - not applicable
Validity:	Study considered acceptable

Material and methods:

The US-EPA photolysis model GC SOLAR was used to calculate quantum efficiencies for cymoxanil in water (pH 5). The estimated rate of photolysis, light intensities and molar absorptivities were used to obtain quantum efficiency. Input parameters were: a photolysis half-life of 1.7 days (recalculated by Malekani, 2003, based on Anderson *et al.*, 1993) and a molar absorption of 2127 M⁻¹ cm⁻¹ at 290 nm, decreasing to 8 M⁻¹ cm⁻¹ at 400 nm.

Findings:

The theoretical half-life of cymoxanil in the top layer on an aqueous system integrated over a full day ranged from 5.2 days in the summer at N 40° latitude to 132 days at N 60° latitude in winter.

Conclusion:

The theoretical half-life of cymoxanil in the top layer on an aqueous system (at pH 5) integrated over a full day in the summer at N 40° latitude was 5.2 days.

Comments (RMS):

None

Reference: **Aquatic photolysis of cymoxanil. Estimation of lifetime in the top layers of aqueous system (GC SOLAR calculations)**

Author(s), year: Willems, H., 2003
 Report/Doc. number: NOTOX project 397439
 Guideline(s): Calculations - not applicable
 GLP: Calculations - not applicable
 Deviations: Calculations - not applicable
 Validity: Study considered acceptable

Material and methods:

The US-EPA photolysis model GC SOLAR 1.20 was used to calculate quantum efficiencies for cymoxanil in water (pH 5). The estimated rate of photolysis, light intensities and molar absorptivities were used to obtain quantum efficiency.

Findings:

The theoretical half-life of cymoxanil in an aqueous system integrated over a full day in the summer at N 40° latitude ranged from 17.3 days in the top layer to 18.4 days in a depth of 1.0 m.

Conclusion:

The theoretical half-life of cymoxanil in the top layer on an aqueous system (at pH 5) integrated over a full day in the summer at N 40° latitude was 17.3 days.

Comments (RMS):

None

Reference: **UV/Visible absorption and molar absorptivity of IN-R3273**

Author(s), year: Moore, L. A., 2002a
 Report/Doc. number: DuPont-5778
 Guideline(s): OECD 101 (1981), EPA 830.7050
 GLP: Yes
 Deviations: None
 Validity: Study considered acceptable

Material and methods:

Photolysis metabolite IN-R3273 was solved (1.47×10^{-4} M) in 0.01 M acetate buffer pH 5.1 and photometrically scanned from 200 to 400 nm at 25 ± 1 °C.

Findings:

Absorbance peak maxima were seen at 227 and 290 nm. No absorbance maxima were detected above 290 nm. Absorptivity (molar extinction coefficient, ϵ) was $7.86 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ ($\log(\epsilon) = 3.90$) at 227 nm and $9.57 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ ($\log(\epsilon) = 3.98$) at 290 nm.

Table B.8.4.1.1-1: Absorptivity (molar extinction coefficient, ϵ) and $\log(\epsilon)$ of IN-R3273 in aqueous solution, pH 5.1.

Wave length	Concentration [M]	$\epsilon [\text{L mol}^{-1}\text{cm}^{-1}]$	$\log(\epsilon)$
224 nm	1.47×10^{-4}	7.86×10^3	3.90
290 nm	1.47×10^{-4}	9.57×10^3	3.98

Conclusion:

Absorptivity (molar extinction coefficient, ϵ) of IN-R3273 was $7.86 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ ($\log(\epsilon) = 3.90$) at 227 nm and $9.57 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ ($\log(\epsilon) = 3.98$) at 290 nm.

Comments (RMS):

None

Reference: UV/Visible absorption and molar absorptivity of IN-JX915

Author(s), year: Moore, L. A., 2003b
 Report/Doc. number: DuPont-5777
 Guideline(s): OECD 101 (1981), EPA 830.7050
 GLP: Yes
 Deviations: None
 Validity: Study considered acceptable

Material and methods:

Photolysis metabolite IN-JX915 was solved ($1.55 \times 10^{-4} \text{ M}$ and $3.87 \times 10^{-4} \text{ M}$) in 0.01 M acetate buffer pH 5.1/methanol (70/30, v/v) and photometrically scanned from 200 to 400 nm at $25 \pm 1^\circ\text{C}$.

Findings:

Absorbance peak maxima were seen at 224 and 290 nm. No absorbance maxima were detected above 290 nm.

Table B.8.4.1.1-1: Absorptivity (molar extinction coefficient, ϵ) and $\log(\epsilon)$ of IN-JX915 in aqueous solution, pH 5.1.

Wave length	Concentration [M]	$\epsilon [\text{L mol}^{-1}\text{cm}^{-1}]$	$\log(\epsilon)$
224 nm	1.55×10^{-4}	3.00×10^3	3.48
	3.87×10^{-4}	3.01×10^3	3.21
290 nm	1.55×10^{-4}	1.61×10^3	3.48
	3.87×10^{-4}	1.62×10^3	3.21

Conclusion:

Absorptivity (molar extinction coefficient, ϵ) of IN-JX915 was $3.00 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ (mean $\log(\epsilon) = 3.35$) at 227 nm and $1.62 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ (mean $\log(\epsilon) = 3.35$) at 290 nm.

Comments (RMS):

None

Reference:	Quantum efficiency for IN-R3273 and IN-JX915 in water
Author(s), year:	Moore, L. A., 2003c
Report/Doc. number:	DuPont-9318, Revision No. 1
Guideline(s):	US-EPA 161-2 (1982), SETAC (1995)
GLP:	Not applicable (calculation)
Deviations:	Not applicable (calculation)
Validity:	Study considered acceptable

Material and methods:

The US-EPA photolysis model GC SOLAR was used to calculate quantum efficiencies for cymoxanil photolysis metabolites IN-JX915 and IN-R3273 in water (pH 5). For each metabolite the estimated rate of photolysis, light intensities and molar absorptivities were used to obtain quantum efficiency.

Modelling of the degradation rates for IN-R3273 and IN-JX915 used the parent compound to the first two metabolites in series kinetic model in ModelManager. The photolytic half-life time for cymoxanil at pH 5 obtained using the modelling procedure was 1.7 days, the half life time estimated for IN-R3273 and IN-JX915 were 33 and 6.7 days, respectively (Malekani, 2003).

Molar absorptivities are based on results from Moore (2002a) and Moore (2003b).

Findings:

The quantum yield for IN-R3273 was 2.41×10^{-5} . The quantum yield for IN-JX915 was 5.04×10^{-4} . The theoretical half-life of IN-R3273 in the top layer on an aqueous system integrated over a full day in the summer ranged from 4.3 days at N 30° latitude to 5.4 days at N 50° latitude. The theoretical half-life of IN-JX915 in the top layer on an aqueous system integrated over a full day in the summer ranged from 20.0 days at N 30° latitude to 23.3 days at N 50° latitude.

Conclusion:

The theoretical half-lives of IN-R3273 and IN-JX915 in the top layer on an aqueous system integrated over a full day in the summer at N 40° latitude were 4.7 and 21.2 days, respectively.

Comments (RMS):

- Half life time used for calculation of environmental half-life includes photolysis and hydrolysis processes for both, IN-JX915 and IN-R3273. IN-R3273 is considered to be stable under conditions of hydrolysis. Therefore, calculation is considered valid for IN-R3273. In case of IN-JX915, which is unstable under conditions of hydrolysis at neutral and alkaline conditions, no information about the hydrolysis rate at pH 5 is available. In this respect, environmental half-life of IN-JX915 is considered to include both processes.

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B.8.9 Ready biodegradability of the active substance (Annex IIA, point 7.2.1.3.1)

Reference:	Determination of 'ready' biodegradability: Carbon dioxide (CO₂) evolution test (modified Sturm test) with cymoxanil technical
Author(s), year:	Luit, R. J., 2001
Report/Doc. number:	NOTOX project 308778
Guideline(s):	OECD 301B (1992)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	Cymoxanil technical, purity 98.9 %, batch 8980028
Reference substance:	Sodium acetate
Inoculum:	Activated sludge micro-organisms obtained from a municipal sewage treatment plant at 'Waterschap de Maaskant', 's-Hertogenbosch', the Netherlands
Test systems:	• Test substance (approx. 48 mg L⁻¹, equivalent to 10 mg TOC L⁻¹) and inoculum • Inoculum only (inoculum blank) • Reference substance (approx. 40 g L⁻¹ sodium acetate, equivalent to 12 mg TOC L⁻¹) and inoculum (positive control) • Test substance, reference substance and inoculum (toxicity control)
Test procedure:	The test solutions (pH approx. 7.6, Temp. 21.0 – 23.5 °C) were continuously stirred during the test duration of 10 days. Carbon dioxide produced in each test bottle was reacted with barium hydroxide contained in a gas scrubbing bottle and was precipitated as barium carbonate. The amount of carbon dioxide produced was determined by titrating the remaining barium hydroxide with 0.05 M standardized HCl.

Findings:

The relative degradation values calculated from the measurements performed during the test revealed no significant degradation (< 10 %) of cymoxanil technical. In the toxicity control, more than 25 % degradation occurred within 14 days (based on theoretical CO₂). Therefore, the test substance was found to have no inhibiting effect on microbial activity at a concentration of ca 48 mg L⁻¹.

Conclusion:

Under the conditions of the modified Sturm test, cymoxanil is not considered to be readily biodegradable.

Comments (RMS):

None

B.8.10 Degradation in aquatic systems**B.8.10.1 Water/sediment studies (Annex IIA, point 7.2.1.3.2)**

The fate of cymoxanil in water/sediment systems was investigated in 2 studies (in total 4 systems):

- Aerobic degradation of cymoxanil in two water/sediment systems - DuPont
- Aerobic degradation of cymoxanil in two water/sediment systems - Oxon

Reference:	Degradation of cymoxanil in two water/sediment systems
Author(s), year:	Trabue, S. L., Lydick, T. M., 2001
Report/Doc. number:	DuPont-2695
Guideline(s):	SETAC (1995), OECD guideline proposal (1999), US-EPA 162-4 (1982)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

including relevant kinetic re-calculations from:

Reference:	Calculated half-lives of cymoxanil and its metabolites in environmental fate laboratory studies
Author(s), year:	Malekani, K., 2003
Report/Doc. number:	DuPont-11575, Revision No. 1
Guideline(s):	Calculations - not applicable
GLP:	Calculations - not applicable
Deviations:	Calculations - not applicable
Validity:	Study considered acceptable

Material and methods:

Test substance:	[Cyanoacetamide-2- ¹⁴ C]-cymoxanil, batches HOTC No. 425, 376 and 505, radiochemical purity > 95 % (all batches), 2.82 MBq mg ⁻¹ (batches HOTC No. 425, 505) and 0.52 MBq mg ⁻¹ (batch HOTC No. 376)
Reference substances:	Cymoxanil (unlabelled), IN-Q8761, IN-U3204, IN-W3595, IN-R3274, IN-R3273, IN-KP533, IN-JX915, IN-KQ960, IN-T4226, oxamic acid (IN-18474), oxalic acid
Test concentration:	250 µg L ⁻¹ (equivalent to overspraying with 750 g ai ha ⁻¹ and a water depth of 30 cm), additional application at 10fold rate for metabolite identification
Co-solvent:	< 0.025 % of methanol (v/v)
Test system:	Approx. 60 g wet weight sediment (2 mm sieved), approx. 140 mL water layer, in 250 mL centrifuge bottles, sediment/water ratio approx. 1/3.5 (m/m) based on sediment dry weight
Test temperature:	20 ± 2 °C
Test duration:	127 days in the dark
Equilibration time:	Not stated in the report
Monitoring data:	Redox potential, dissolved O ₂ content, pH (at each sampling)
Volatile traps:	Humified air passing the test system was passed through 2 x 100 mL of 1 M KOH (¹⁴ CO ₂), no traps used for organic volatiles, ¹⁴ CO ₂ identity was proven by precipitation
Sample processing:	Decanted water layer was brought to pH 5 (20 mM sodium acetate buffer) and radio-assayed by LSC. The water phase was combined with an equal volume of methanol, concentrated to approx. 1 mL (TurboVap, 40 °C and finally under a gentle stream of N ₂) and reconstituted with approx. 4 mL of 20 mM sodium acetate buffer (pH 5).

The remaining **sediment layer** was extracted once with 100 mL of methanol/20 mM sodium acetate solution (95/5, v/v) and several times (typically 2 times) with 100 mL of methanol/20 mM sodium acetate solution (50/50, v/v) unless extracted RA was less than 5 % of AR. Extracts were individually radio-assayed by LSC, pooled extracts were concentrated to approx. 1 mL (TurboVap, 40 °C and finally under a gentle stream of N₂) and reconstituted with approx. 4 mL of 20 mM sodium acetate buffer (pH 5).

Unextractable residues were radio-assayed by combustion-LSC.

Analytical techniques: LSC, HPLC-UV/RAD (two methods), TLC (two methods)

LOD < 0.2 % of AR

- Additional experiments:
- Metabolite were isolated, analysed on different HPLC and TLC systems and compared with non-labelled reference substances
 - A separate test was conducted to demonstrate methane production: After passing the test system, the humidified air was passed through 1 x 100 mL of ethane-1,2-diol (organic volatiles), 1 x 100 mL of 1 M KOH (¹⁴CO₂), a combustion tube (set to 600 °C) containing cupric oxide to combust methane and other volatile organics (not trapped in the ethane-1,2-diol) to ¹⁴CO₂ and, finally, 1 x 100 mL of 1 M KOH (to trap ¹⁴CO₂ formed in the combustion unit)
- Kinetic evaluation:
- Parent (re-calculated, Malekani, 2003):
 - Simple first order (SFO) kinetics in the water layer (dissipation) and in the entire system (degradation)
 - Metabolites (re-calculated by Malekani, 2003):
 - Two-compartment modelling in the entire system (degradation), linking the metabolite to the parent in series (both compartments SFO kinetics, additional pathway to sink)
 - SFO kinetics in the entire system (degradation) starting from the time of maximum occurrence

All calculations performed with ModelManager 1.1

Table B.8.10.1-1: Physical and chemical properties of the two test systems.

Compartment	Parameter	Location	
		'Brandywine Creek'	'Lums Pond'
Water	pH at sampling	7.4	7.6
	Redox at sampling [mV]	156.8	103.4
	O ₂ -concentration at sampling [mg L ⁻¹]	5.7	6.1
	pH	7.9	6.7
	Total hardness [mg equivalent CaCO ₃ L ⁻¹]	107	54
	Total organic carbon [mg L ⁻¹]	3.3	6.8
	Total nitrogen [mg L ⁻¹]	3.5	4.2
	Phosphate phosphorus [mg L ⁻¹]	0.1	0.0
	Sodium [mg L ⁻¹]	21	17
	Calcium [mg L ⁻¹]	26	10
	Magnesium [mg L ⁻¹]	10	7
	Total dissolved solids [mg L ⁻¹]	232	162
Sediment	pH at sampling	7.0	6.7
	Redox at sampling [mV]	-321.6	-247.6
	pH	6.6	6.0
	Organic carbon [%]	0.8	0.1
	Total nitrogen [%]	0.063	0.013
	Olsen phosphorus [mg kg ⁻¹]	19	9
	Cation exchange capacity [cmol ⁺ kg ⁻¹]	4.7	3.6

Compartment	Parameter	Location	
		'Brandywine Creek'	'Lums Pond'
	Moisture (1/3 bar) [%]	14.4	5.8
	Initial microbial biomass ^a [$\mu\text{g C}_{\text{mic}} \text{kg}^{-1}$]	85.4	31.9
	Final microbial biomass ^a [$\mu\text{g C}_{\text{mic}} \text{kg}^{-1}$]	36.3	17.4
	Texture (USDA) / particle size distribution:	Sand	Sand
	Sand [%]:	90	94
	Silt [%]:	6	4
	Clay [%]:	4	2

^a Fumigation extraction method

Findings:

During incubation, dissolved oxygen in the water layer of the 'Brandywine Creek' test system was in a range of 4.4 – 6.4 mg L⁻¹ (mean 5.8 mg L⁻¹) indicating aerobic conditions in the water layer throughout the incubation. Respective redoxpotential was rather low with values in a range of +17 – +165 mV (mean +104 mV). The pH in the water layer was fairly constant in a range of 6.7 – 8.3 with a mean of 7.4. In the sediment layer pH values in the range of 6.5 – 7.9 with a mean of 7.0 were measured, redox potential was low with a range of -202 – +56 mV (mean -83 mV), indicating anaerobic conditions in the sediment.

In the water layer of the 'Lums Pond' test system dissolved oxygen was in a range of 5.0 – 6.5 mg L⁻¹ (mean 6.0 mg L⁻¹), the water layer can be considered as aerobic. Redox potential was in a range of +122 – +302 mV with a mean of +211 mV. The pH in the water layer was in a range of 4.7 – 6.2 with a mean of 5.3, indicating more acidic conditions than in the 'Brandywine Creek' system. In the sediment layer the pH value scattered in a range of 4.9 – 5.4 (mean 5.1). The redox potential was highly variable with a range of -182 – 281 mV (mean +33 mV).

Total recovery of AR was in a range of 66.3 – 101.8 % of AR (average 79.1 % of AR) for the 'Brandywine Creek' system and 65.3 – 107.7 % of AR (average 80.2 % of AR) for the 'Lums Pond' system. The low RA recovered after 3 DAT and onwards was attributed to the formation of ¹⁴C-methane which was not trapped in the KOH trapping solution. Evidence for methane production via methanogenesis was based on low redox potentials recorded in both systems. Additional evidence for methane production was substantiated in a separate experiment with the 'Brandywine Creek' system, in which volatiles passing the KOH trapping solution were combusted to ¹⁴CO₂ and trapped in an additional KOH solution. Using this experimental set-up total radioactivity was adequately recovered over a period of 28 days. By 28 DAT 12.8 % of AR were trapped in the KOH solution after combustion, likely owing to the formation of methane.

¹⁴CO₂ released during the study steadily increased to 45.6 % of AR by 127 DAT ('Brandywine Creek') and 39.6 % of AR by 70 DAT ('Lums Pond').

The total radioactivity in the water layer of both systems decreased from > 100 % of AR at the start of incubation to 10.1 % of AR ('Brandywine Creek') and < 2 % of AR ('Lums Pond') by 70 DAT. Maximum amounts of total radioactivity found in the sediment layer were 31.0 % of AR by 28 DAT ('Brandywine Creek') and 38.5 % of AR by 15 DAT ('Lums Pond'), significantly declining thereafter. Extractable radioactivity in the sediment never exceeded 10 % of AR and peaked by 3 DAT declining thereafter.

Peak formation of NER was detected by 28 DAT in both systems with 27.4 % of AR ('Brandywine Creek') and 35.2 % of AR ('Lums Pond') and decreased thereafter.

Cymoxanil rapidly dissipated in the water phase of both systems, by 8 DAT amounts found in the water layer has dropped below 1 % of AR. Cymoxanil did not significantly dissipate into the sediment layer (< 5 % of AR). Dissipation half life of cymoxanil in the water layer was 0.5 and 1.5 days for 'Brandywine Creek' and

'Lums Pond' system, respectively. Owing to the negligible dissipation into the sediment, degradation half-life of cymoxanil in the entire system was in the same range (0.5 and 1.6 days, respectively).

Formation of major metabolites (> 10 % of AR) was only observed in the water layer, IN-U3204 (maximum of 15.7 % of AR in the entire system), IN-W3595 (12.7 % of AR) and IN-T4226 (12.0 % of AR) exceeded 10 % of AR, rapidly decreasing thereafter. Minor metabolites observed were IN-KP533 (maximum 8.0 % of AR), IN-KQ960 (9.6 % of AR), IN-JX915 (8.5 % of AR) and IN-R3273 (3.9 % of AR). No metabolite was observed in the sediment > 3 % of AR. All metabolites are rapidly degrading in the entire system (DT₅₀ in a range of 0.5 – 6.7 days) with the exception of IN-KQ960 showing a DT₅₀ in a range of 45.5 – 154 days.

Table B.8.10.1-2: Distribution of radioactivity [% of AR] in the water/sediment system of 'Brandywine Creek' and 'Lums Pond' system.

System	DAT	¹⁴ CO ₂	Water phase	Sediment phase			Total recovery
				Extractable	NER	Total	
'Brandywine creek'	0	na ^a	101.8	na	na	na	101.8
	1	0.2	76.3	6.4	8.5	14.9	91.4
	3	2.9	59.2	7.2	18.2	25.4	87.6
	8	7.1	40.4	6.0	23.9	29.9	77.4
	15	17.8	29.0	5.8	24.4	30.2	76.9
	21 ^a	20.6	22.8	4.2	18.8	23.0	66.3
	28	24.6	20.7	3.6	27.4	31.0	76.2
	50	30.1	14.7	2.5	25.2	27.7	72.4
	70	30.8	10.1	1.8	25.4	27.2	68.3
	99	40.5	11.2	1.4	22.3	23.7	74.5
	127	45.6	6.9	1.2	23.1	24.3	77.1
'Lums Pond'	0	na	107.7	na	na	na	107.7
	1	1.2	76.9	8.1	6.9	15.0	93.2
	3	4.9	57.3	8.3	18.3	26.6	88.8
	8	13.4	20.3	5.7	32.2	37.9	71.6
	15	30.9	7.7	3.8	34.7	38.5	77.0
	21 ^a	33.7	6.5	2.2	28.3	30.5	70.7
	28	35.5	4.9	1.9	35.2	37.1	77.5
	50	38.4	1.5	1.7	28.1	29.8	69.8
	70	39.6	1.7	1.1	22.8	23.9	65.3
	99	nd	nd	nd	nd	nd	nd
	127	nd	nd	nd	nd	nd	nd

na denotes not applicable

nd denotes not determined

^a Invalid data

Table B.8.10.1-3: Distribution of radioactivity [% of AR] in the water/sediment system 'Brandywine Creek' (additional experiment demonstrating significant formation of organic volatiles not trapped in the main study).

System	DAT	¹⁴ CO ₂	Water phase	Sediment extract	NER	Ethane-1,2-diol	Combustion tube	Total
'Brandywine creek'	11	23.9	32.9	3.6	30.4	0.6	12.2	103.7
	28	37.6	17.1	2.2	23.5	0.7	12.8	93.6

Table B.8.10.1-4: Characterization of radioactivity [% of AR] in the water phase of the 'Brandy Creek' and 'Lums Pond' system after application of Cymoxanil (equivalent to 750 g ai ha⁻¹).

System	DAT	M1 ^a	IN-W3595/ IN-KP533 ^{bc}	IN-W3595 ^d	IN-KP533 ^d	IN-T4226	IN-KQ960	IN-U3204	IN-JX915	IN-R3273	Cymoxanil
'Brandywine Creek'	0	< 0.1	0.6	0.6	< 0.1	0.4	< 0.1	1.6	3.9	0.7	94.2
	1	3.8	11.5	8.4	1.5	7.4	< 0.1	15.6	7.2	2.0	23.7
	3	6.9	23.0	11.0	7.9	5.2	3.7	3.8	4.5	3.5	1.1
	8	12.3	13.2	3.2	1.4	2.0	8.2	< 0.1	1.6	1.7	< 0.1
	15	10.4	6.3	0.4	0.9	< 0.1	8.9	< 0.1	1.7	0.6	< 0.1
	21	8.2	5.6	0.2	1.2	< 0.1	6.7	< 0.1	0.5	0.6	< 0.1
	28	5.6	4.2	< 0.1	1.2	< 0.1	7.6	< 0.1	0.3	0.5	< 0.1
	50	3.5	2.7	na	na	< 0.1	7.4	< 0.1	0.2	0.5	< 0.1
	70	3.0	0.9	na	na	< 0.1	5.2	< 0.1	0.2	0.5	< 0.1
	99	1.6	1.8	na	na	< 0.1	6.2	< 0.1	0.2	0.5	< 0.1
	127	0.1	0.8	na	na	< 0.1	4.8	< 0.1	0.1	0.2	< 0.1
	0	< 0.1	0.8	na	na	0.2	< 0.1	1.7	3.8	0.9	100.2
'Lums Pond'	1	2.3	2.2	1.2	1.0	6.8	< 0.1	1.6	2.9	0.8	58.5
	3	5.6	3.7	na	na	11.1	0.6	0.5	2.5	0.8	28.0
	8	2.8	3.5	0.2	2.6	8.0	1.0	< 0.1	0.8	0.5	0.7
	15	1.4	2.3	0.3	1.1	0.9	1.2	< 0.1	0.3	0.2	< 0.1
	21	1.0	2.1	0.1	0.3	0.6	1.3	< 0.1	0.1	0.2	< 0.1
	28	0.5	1.6	0.2	0.4	0.3	1.3	< 0.1	0.1	0.1	< 0.1
	50	0.3	0.2	na	na	0.1	0.9	< 0.1	< 0.1	0.1	< 0.1
	70	0.2	0.3	na	na	< 0.1	0.6	< 0.1	< 0.1	0.1	< 0.1

na denotes not analysed

^a Mixture of several polar metabolites, none of which > 10 % of AR (likely to include oxamic acid, oxalic acid and IN-R3274)

^b HPLC

^c Mixture of more than two metabolites that was composed almost exclusively of IN-W3595 and IN-KP533

^d TLC

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Table B.8.10.1-5: Characterization of radioactivity [% of AR] in the sediment phase of the 'Brandy Creek' and 'Lums Pond' system after application of Cymoxanil (equivalent to 750 g ai ha⁻¹).

System	DAT	M1 ^a	IN-W3595/ IN-KP533 ^{bc}	IN-W3595 ^d	IN-KP533 ^d	IN-T4226	IN-KQ960	IN-U3204	IN-JX915	IN-R3273	Cymoxanil
'Brandywine Creek'	0	na	na	na	na	na	na	na	na	na	na
	1	0.9	1.0	1.0	< 0.1	0.8	0.1	0.1	1.2	0.3	1.3
	3	1.4	2.3	1.7	0.1	0.6	0.7	0.2	0.9	0.5	0.1
	8	1.9	1.5	0.2	0.2	0.5	1.0	< 0.1	0.5	0.2	0.1
	15	1.9	1.4	< 0.1	0.2	0.4	0.7	< 0.1	0.5	0.2	< 0.1
	21	1.3	0.8	na	na	< 0.1	1.3	< 0.1	0.2	< 0.1	< 0.1
	28	1.1	0.7	na	na	< 0.1	1.3	< 0.1	0.3	0.1	< 0.1
	50	0.6	0.4	na	na	< 0.1	0.8	< 0.1	0.2	0.1	< 0.1
	70	0.5	0.2	na	na	< 0.1	0.6	< 0.1	0.2	0.1	< 0.1
	99	0.2	0.3	na	na	< 0.1	0.8	< 0.1	0.1	< 0.1	< 0.1
	127	0.2	0.2	na	na	< 0.1	0.5	< 0.1	0.1	0.1	< 0.1
	0	na	na	na	na	na	na	na	na	na	na
'Lums Pond'	1	1.7	0.4	0.1	0.1	0.9	< 0.1	0.2	0.4	0.1	3.9
	3	2.2	1.8	0.4	1.0	0.9	0.1	0.5	0.6	0.4	1.6
	8	2.2	1.1	0.1	0.4	1.0	0.3	< 0.1	0.3	0.1	0.2
	15	1.6	0.8	nd	nd	0.2	0.5	< 0.1	0.2	0.2	< 0.1
	21	na	na	na	na	na	na	na	na	na	na
	28	na	na	na	na	na	na	na	na	na	na
	50	na	na	na	na	na	na	na	na	na	na
	70	na	na	na	na	na	na	na	na	na	na

na denotes not analysed

^a Mixture of several polar metabolites, none of which > 10 % of AR (likely to include oxamic acid, oxalic acid and IN-R3274)

^b HPLC

^c Mixture of more than two metabolites that was composed almost exclusively of IN-W3595 and IN-KP533

^d TLC

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Table B.8.10.1-6: Characterization of radioactivity [% of AR] in the entire system of the 'Brandy Creek' and 'Lums Pond' system after application of Cymoxanil (equivalent to 750 g ai ha⁻¹).

System	DAT	M1 ^a	IN-W3595/ IN-KP533 ^{bc}	IN-W3595 ^d	IN-KP533 ^d	IN-T4226	IN-KQ960	IN-U3204	IN-JX915	IN-R3273	Cymoxanil
'Brandywine Creek'	0	< 0.1	0.6	0.6	< 0.1	0.4	< 0.1	1.6	3.9	0.7	94.2
	1	4.7	12.6	9.5	1.5	8.2	0.1	15.7	8.5	2.2	25.0
	3	8.4	25.4	12.7	8.0	5.8	4.4	4.0	5.3	3.9	1.2
	8	14.2	14.7	3.4	1.5	2.5	9.2	< 0.1	2.1	1.9	0.1
	15	12.3	7.7	0.4	1.1	0.4	9.6	< 0.1	2.2	0.8	< 0.1
	21	9.4	6.5	0.2	1.2	< 0.1	8.0	< 0.1	0.8	0.7	< 0.1
	28	6.7	4.9	< 0.1	1.2	< 0.1	8.8	< 0.1	0.6	0.5	< 0.1
	50	4.0	3.1	na	na	< 0.1	8.2	< 0.1	0.5	0.6	< 0.1
	70	3.5	1.1	na	na	< 0.1	5.9	< 0.1	0.4	0.6	< 0.1
	99	1.8	2.1	na	na	< 0.1	7.0	< 0.1	0.2	0.5	< 0.1
	127	0.2	0.9	na	na	< 0.1	5.3	< 0.1	0.1	0.3	< 0.1
	0	< 0.1	0.8	na	na	0.2	< 0.1	1.7	3.8	0.9	100.2
'Lums Pond'	1	4.0	2.6	1.3	1.1	7.6	< 0.1	1.8	3.3	0.9	62.4
	3	7.7	5.4	0.4	1.0	12.0	0.8	0.9	3.1	1.2	29.5
	8	5.0	4.6	0.3	3.0	8.9	1.4	< 0.1	1.0	0.6	0.9
	15	3.0	3.1	0.3	1.1	1.2	1.7	< 0.1	0.5	0.4	< 0.1
	21	1.0	2.1	0.1	0.3	0.6	1.3	< 0.1	0.1	0.2	< 0.1
	28	0.5	1.6	0.2	0.4	0.3	1.3	< 0.1	0.1	0.1	< 0.1
	50	0.3	0.2	na	na	0.1	0.9	< 0.1	< 0.1	0.1	< 0.1
	70	0.2	0.3	na	na	< 0.1	0.6	< 0.1	< 0.1	0.1	< 0.1

na denotes not analysed

^a Mixture of several polar metabolites, none of which > 10 % of AR (likely to include oxamic acid, oxalic acid and IN-R3274)

^b HPLC

^c Mixture of more than two metabolites that was composed almost exclusively of IN-W3595 and IN-KP533

^d TLC

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Table B.8.10.1-7: Degradation (entire system) and dissipation (water layer) half-lives of cymoxanil and metabolites in the water/sediment systems 'Brandywine Creek' and 'Lums Pond'.

System	Compound	Compartment	DT ₅₀ [days]	DT ₉₀ [days]	Formation fraction [0 ... 1]	R ^{2a}	χ ² error ^a [%]	Kinetics
'Brandywine creek'	Cymoxanil	Entire system	0.5	1.7	-	1.00	3.2	P _{SFO}
		Water layer	0.5	1.7	-	1.00	0.5	P _{SFO}
	IN-U3204	Entire system	0.6	2.0	0.43	0.92	11.9	P _{SFO} → M _{SFO}
	IN-W3595	Entire system	3.6	12.1	0.18	0.95	22.0	P _{SFO} → M _{SFO}
	IN-KQ960	Entire system	154	512	-	0.76	7.9	M _{SFO}
	IN-T4226	Entire system	3.9	12.9	-	1.00	4.1	M _{SFO}
	IN-JX915	Entire system ^a	2.5	8.3	0.13	0.79	51.4	P _{SFO} → M _{SFO}
	IN-R3273	Entire system ^a	6.0	19.9	-	0.93	27.5	M _{SFO}
'Lums Pond'	Cymoxanil	Entire system	1.6	5.3	-	1.00	3.6	P _{SFO}
		Water layer	1.5	5.0	-	1.00	5.2	P _{SFO}
	IN-KQ960	Entire system ^a	45.4	151	0.15	0.97	9.2	M _{SFO}
	IN-T4226	Entire system	5.4	17.9	-	0.95	25.8	M _{SFO}
	IN-JX915	Entire system ^a	1.1	3.7	0.12	0.96	19.4	P _{SFO} → M _{SFO}
	IN-R3273	Entire system ^a	6.7	22.2	-	0.98	8.7	M _{SFO}
	IN-KP533	Entire system ^a	3.0	10.0	-	0.97	23.8	M _{SFO}

^a Calculated by the RMS.

Conclusion:

Cymoxanil rapidly degraded in 'Brandywine Creek' and 'Lums Pond' water/sediment environments, with dissipation half-lives in the water layer of 0.5 and 1.5 days, respectively, and DT₉₀ values of 1.7 and 5.0 days. Dissipation in the water layer was almost consistent with degradation in the entire system. Degradation half-lives of cymoxanil in the entire system were 0.5 and 1.6 days. Cymoxanil was not found in the sediment layer > 5 % of AR.

Major metabolites (> 10 % of AR) of cymoxanil to form in the 'Brandywine Creek' system were IN-W3595 (maximum 12.7 % of AR), and IN-U3204 (15.7 % of AR) with DT₅₀ values in the entire system of 3.6 and 0.6 days, respectively. In the 'Lums Pond' system the only major metabolite observed was IN-T4226 (maximum 12.0 % of AR) with a DT₅₀ of 5.4 days in the entire system. No metabolites were observed in the sediments > 5 % of AR.

Mineralization and sediment NER represented the major part of metabolism in each system, reaching up to 39.6 and 35.2 % of AR, respectively, after 70 days. Owing to anaerobic conditions in the sediment layer, significant amounts of volatiles are considered to be released as methane.

Comments (RMS):

None

Reference:	The fate of cymoxanil in two water/sediment systems
Author(s), year:	Slangen, P. J., Willems, H., 2000
Report/Doc. number:	NOTOX report 257761
Guideline(s):	SETAC (1995), BBA IV 5-1 (1995), US-EPA 162-4 (1982), OECD guideline proposal (1997)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable, metabolite identification revised by the RMS

Material and methods:

Test substance:	Cyanoacetatamide-2- ¹⁴ C]-cymoxanil, radiochemical purity 99.0 % (TLC), 99.8 % (HPLC), 4.19 MBq mg ⁻¹ , batch 3304.265
Reference substances:	Cymoxanil (unlabelled), IN-W3595 (AS 329), IN-R3274 (AS 330), IN-T4226 (AS 331), CAU (AS 336), Oxime (AS 335), hippuric acid (B076)
Test concentration:	Approx. 80 µg L ⁻¹ (equivalent to overspraying with 240 g ai ha ⁻¹ and a water depth of 30 cm)
Co-solvent:	< 0.1 % of acetone
Experimental set-up:	2.5 cm sediment layer (approx. 200 g, 2 mm sieved), 6 cm water (approx. 470 mL, 150 µm filtered), in 1 L brown glass bottles
Test temperature:	20 ± 2 °C
Test duration:	100 days in the dark
Equilibration time:	6 weeks
Monitoring data:	Redox potential, O ₂ content, pH (weekly)
Volatile traps:	Humified, CO ₂ free air passing the test system (through the upper 1 cm of the water layer without disturbing the sediment) was passed through 2 x 50 mL of 2M NaOH (¹⁴ CO ₂) and 1 x 50 mL of 2-methoxyethanol (organic volatiles), by 22 DAT and 64 DAT all traps were refreshed, ¹⁴ CO ₂ identity was proven by precipitation
Sample processing:	Decanted water layer was radio-assayed by LSC, then 25 mL of acetate buffer pH 4 was added. The water layer was extracted three times with 100 mL of dichloromethane, the combined fractions were radio-assayed by LSC and after addition of 2 mL 1 % (v/v) glycerol in acetone, combined extractes were concentrated (rotavap, 30 °C) and redissolved in 5 mL of acetonitrile (radio-assayed by LSC). Remaining water layer radio-assayed by LSC, freeze-dried and reconstituted in 7.5 – 10 mL of methanol (radio-assayed by LSC). The remaining sediment layer was extracted three times with 20 mL of acetate buffer (pH 4) and 80 mL of acetonitrile. Supernatants were combined and radio-assayed by LSC. Combined supernatants were concentrated (rotavap, 45 °C), radioassayed by LSC, further concentrate by freeze-drying and reconstituted in methanol. Remaining solids were extracted with 100 mL of n-hexane. The n-hexane phase was separated and radio-assayed by LSC. Unextractable residues were radio-assayed by combustion-LSC. All samples were stored at -20 °C.
Analytical techniques:	• Two TLC methods (<i>TLC1</i>, <i>TLC2</i>) • One HPLC methods (<i>HPLC1</i>) • LSC Note: These TLC and HPLC methods were re-investigated by Brands and Van Noorloos (2006) to determine the R_f/RT values of several cymoxanil metabolites (for details refer to hydrolysis section, B.8.7).
Additional experiments:	The aqueous and organic phase of the water layer of the 'OVP' system obtained by 0.25 DAT and of the 'SW' system obtained by 0.25, 1 and 3 DAT were

investigated by continuous flow injection MS/MS.

Kinetic evaluation: Parent: Simple first order (SFO) kinetics

Table B.8.10.1-8: Physical and chemical properties of the two test systems.

Compartment	Parameter	Location			
		'Oostvaardersplassen' ('OVP')		'Schoonrewoerdsewiel' ('SW')	
		Start of study	End of study	Start of study	End of study
Water	Temperature at sampling [°C]	21		21	
	pH at sampling	8.3		8.9	
	Redox at sampling [mV]	+318		+271	
	O ₂ -concentration at sampling [mg L ⁻¹]	8.5		9.3	
	Total hardness [mg equivalent CaCO ₃ L ⁻¹]	219	297	164	1082 ^a
	Dissolved organic carbon [mg L ⁻¹]	11.4	18.7	18.2	11.7
	Total nitrogen [mg L ⁻¹]	< 0.05	9.8	2.8	19.6
	Total phosphorus [mg L ⁻¹]	0.6	0.6	0.2	0.3
	Suspended solids [mg L ⁻¹]	88	216	52	44
Sediment	pH (H ₂ O)	7.6	8.0	7.6	7.6
	pH (CaCl ₂)	7.3	7.6	7.4	7.5
	Redox at sampling [mV]	-100		-14	
	Organic carbon [%]	4.7	4.0	4.9	6.0
	Total nitrogen [g kg ⁻¹]	3.2	2.5	2.8	4.4
	Total phosphorus [g kg ⁻¹]	0.86	0.89	1.14	1.19
	Cation exchange capacity [meq kg ⁻¹]	238	119	177	152
	ATP [µg kg ⁻¹]	146	214	59.7	113
	Texture	Silty clay loam		Silt loam	
	Sand (63 – 2000 µm) [%]	7.3	5.4	41.5	37.9
	Silt (2 – 63 µm) [%]	63.7	63.0	50.0	51.7
	Clay (< 2 µm) [%]	29.0	31.6	8.5	10.4

^a Maybe a typing error

Findings:

The radiochemical purity of the spike solution was found to be > 99 % of AR (TLC).

Measured redox potentials (+300 – +500 mV) indicate aerobic conditions in the water layer during incubation for both the 'OVP' and the 'SW' system. Anaerobic (reducing) conditions were found in the sediment layer of both systems (-100 – 0 mV). Dissolved oxygen contents were between 6.4 and 8.5 mg L⁻¹ during incubation indicating a high degree of oxygen saturation in the water layer. The pH of the water layers of both systems remained stable during equilibration and incubation period and fluctuated between 7.7 and 8.9.

Mass balance for the 'OVP' system was in a range of 81.5 – 105.1 % of AR and 86.8 – 104.8 % of AR for the 'SW' system. Mass balances by study termination were > 97 % of AR for both systems indicating that recovery rates < 90 % of AR (in total 4 samplings) may be attributed to leakage losses during the incubation.

Formation of CO₂ accounted for 75.5 % of AR ('OVP') and 68.5 % of AR ('SW') by 100 DAT (study termination). Formation of NER accounted for maximum of 22.5 % and 29.7 % of AR in the 'OVP' and 'SW' test system, respectively, decreasing thereafter towards study end.

Cymoxanil rapidly degraded in the entire system with a DT₅₀ of 0.06 and 0.16 days in the 'OVP' and 'SW' test system, respectively. Since no transfer of cymoxanil into the sediment layer was observed, degradation in the entire system is considered identical with dissipation in the water layer, likely almost exclusively owing to hydrolysis.

Formation of major metabolites was restricted to the water layer. The following *HPLC1* fractions were observed to exceed 10 % of AR in the entire system of both test systems: **M1, M2, M3 and M5**.

The highly polar ***HPLC1* fraction M1** comprises several polar compounds, including IN-W3595 (identical to MVI under conditions of *TLC2*) with a maximum occurrence of 27.5 % and 12.4 % of AR in the entire system of 'OVP' and 'SW' test system, respectively, and other polar compounds, likely to be IN-KP533, IN-R3274, oxamic acid (IN-18474) and oxalic acid. The unidentified fraction of M1 (all above mentioned polar compounds except IN-W3595) reached maximum amounts of 26.0 % and 22.9 % of AR in the entire system of 'OVP' and 'SW' system, respectively. On the basis of available data no final conclusion on the individual amounts of these compounds can be drawn.

***HPLC1* fraction M2** is considered almost identical to IN-KQ960 (also mirrored by *TLC2* fraction MVII), reaching maximum amounts of 14.3 % and 14.1 % of AR in the entire system of the 'OVP' and 'SW' system, respectively.

***HPLC1* fraction M3** is identical to IN-U3204 with a maximum occurrence of 24.7 % and 19.8 % of AR in the entire system of the 'OVP' and 'SW' system, respectively.

***HPLC1* fraction M5** peaked with 22.9 % and 15.4 % of AR in the entire system of the 'OVP' and 'SW' system, respectively. The identity of this fraction, which was *per se* not observed in hydrolysis or photolysis studies, is unknown.

Minor metabolites (< 10 % of AR) observed in the entire system of 'OVP' and 'SW' were IN-R3273 (*HPLC1* fraction M8) and IN-JX915 (M9), both individually not exceeding 5 % of AR. Other minor fractions (M4, M6, M7) remained unidentified.

All metabolites (and metabolite fractions) rapidly degraded in both test system, DT_{50} of metabolites in the entire system varied in a range of 0.2 – 15.2 days, based on RMS calculations.

Table B.8.10.1-9: Distribution of radioactivity [%of AR] after application of cymoxanil to two water/sediment systems.

System	DAT	Water phase			Sediment phase			CO ₂	Volatiles	Total recovery
		Organic phase	Aqueous phase	Total	Extractable ^a	NER	Total			
'OVP'	0	98.5	6.6	105.1	0.0	0.0	0.0	0.0	0.0	105.1
	0.125	48.2	46.1	94.3	5.1	3.6	8.7	0.0	0.0	103.0
	0.25	29.8	63.5	93.3	4.5	4.3	8.8	0.0	0.0	102.0
	1.04	11.1	68.3	79.5	8.8	7.9	16.7	0.6	0.0	96.8
	3	4.3	51.4	55.8	8.6	12.3	20.9	4.9	0.0	81.5
	10	1.4	31.6	32.9	10.8	21.5	32.3	23.5	0.0	88.7
	30	0.2	15.4	15.6	6.7	22.5	29.2	53.4	0.0	98.1
	59	0.1	8.3	8.4	4.4	19.2	23.6	64.2	0.0	96.3
	100	0.0	4.3	4.4	1.9	17.9	19.8	75.5	0.0	99.7
'SW'	0	96.2	3.8	100.0	0.0	0.0	0.0	0.0	0.0	100.0
	0.125	75.4	23.7	99.1	2.1	3.6	5.7	0.0	0.0	104.8
	0.25	51.2	43.7	94.8	3.3	5.8	9.1	0.1	0.0	104.0
	1.04	16.5	56.3	72.8	7.4	12.3	19.7	1.3	0.0	93.7
	3	7.6	48.6	56.2	8.5	17.9	26.4	5.9	0.0	88.5
	10	1.8	23.8	25.5	10.1	24.3	34.4	26.8	0.0	86.8
	30	0.3	9.5	9.8	5.2	29.7	34.9	45.4	0.0	90.1
	59	0.3	4.3	4.6	3.0	26.2	29.2	59.1	0.0	93.0
	100	0.1	1.9	2.0	1.4	25.6	27.0	68.5	0.0	97.5

^a Radio activity measured in the n-hexane phase < 0.1 % of AR

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Table B.8.10.1-10: Characterization of extractable radioactivity [%of AR] in the water layer after application of cymoxanil to two water/sediment systems (HPLC¹).

System	DAI	M1	M2	M3	M4	M5	M6	M7	M8	M9	Cymoxanil	Others
'OVP'	0	6.6	0.0	3.4	0.0	0.0	0.0	0.0	0.0	1.0	94.1	0.0
	0.125	26.5	1.8	24.7	1.6	5.0	5.2	2.7	0.0	3.7	23.2	0.0
	0.25	34.0	5.0	18.9	6.4	13.1	4.4	2.8	1.9	3.2	3.6	0.0
	1.04	35.0	13.0	3.2	0.0	22.9	0.0	0.8	1.1	3.5	0.0	0.0
	3	33.1	10.6	0.0	0.0	7.4	0.0	0.0	2.8	1.6	0.0	0.0
	10	22.1	9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
	30	15.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
	59	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	100	4.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0	3.8	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	94.9	0.0
'SW'	0.125	23.7	0.0	9.8	0.0	2.4	0.0	0.0	0.0	1.9	61.3	0.0
	0.25	23.9	3.0	19.3	6.3	4.5	6.6	2.0	0.0	2.3	27.0	0.0
	1.04	25.2	1.3	19.8	1.7	15.4	5.5	0.0	0.8	3.1	0.0	0.0
	3	24.3	10.2	1.6	0.0	6.5	5.3	1.0	5.0	2.2	0.0	0.0
	10	19.7	4.1	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0
	30	4.6	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	59	na	na	na	na	na	na	na	na	na	na	4.6 ^a
RT _{UV} [min] ^b	100	na	na	na	na	na	na	na	na	na	na	2.0 ^a
	1.9	IN-W3295, IN-KP533, other polars	3.1	3.5	3.9	4.4	5.3 - 5.7	5.8 - 6.3	6.9	8.2	13.5	-
Metabolite identification ^c			IN-KQ960	IN-U3204	Unknown	Unknown	Unknown	Unknown	IN-R3273	IN-JX915	Cymoxanil	Unknown

^a In case of samples not further analysed (na), remaining radioactivity is equal to the total extractable radioactivity

^b RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^c Metabolite identification proposed by the RMS (see comments)

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Table B.8.10.1-11: Characterization of extractable radioactivity [%of AR] in the sediment layer after application of cymoxanil to two water/sediment systems.

System	DAT	M1	M2	M3	M4	M5	M6	M7	M8	M9	Cymoxanil	Others
'OVP'	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.125	na	na	na	na	na	na	na	na	na	na	5.1 ^a
	0.25	na	na	na	na	na	na	na	na	na	na	4.5 ^a
	1.04	na	na	na	na	na	na	na	na	na	na	8.8 ^a
	3	na	na	na	na	na	na	na	na	na	na	8.6 ^a
	10	6.1	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	30	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	59	na	na	na	na	na	na	na	na	na	na	4.4 ^a
	100	na	na	na	na	na	na	na	na	na	na	1.9 ^a
	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
'SW'	0.125	na	na	na	na	na	na	na	na	na	na	2.1 ^a
	0.25	na	na	na	na	na	na	na	na	na	na	3.3 ^a
	1.04	na	na	na	na	na	na	na	na	na	na	0.0
	3	7.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	4.6	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	30	4.6	5.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.2 ^a
	59	na	na	na	na	na	na	na	na	na	na	3.1 ^a
	100	na	na	na	na	na	na	na	na	na	na	1.4 ^a
	RT _{UV} [min] ^b	1.9	3.1	3.5	3.9	4.4	5.3 – 5.7	5.8 – 6.3	6.9	8.2	13.5	-
	Metabolite identification ^c	IN-W3295, IN-KP533, other polars	IN-KQ960	IN-U3204	Unknown	Unknown	Unknown	Unknown	IN-R3273	IN-JX915	Cymoxanil	Unknown

^a In case of samples not further analysed (na), remaining radioactivity is equal to the total extractable radioactivity.

^b RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^c Metabolite identification proposed by the RMS (see comments)

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Table B.8.10.1-12: Characterization of extractable radioactivity [%of AR] in the entire system after application of cymoxanil to two water/sediment systems.

System	DAT	M1	M2	M3	M4	M5	M6	M7	M8	M9	Cymoxanil	Others
'OVP'	0	6.6	0.0	3.4	0.0	0.0	0.0	0.0	0.0	1.0	94.1	0.0
	0.125	26.5	1.8	24.7	1.6	5.0	5.2	2.7	0.0	3.7	23.2	5.1
	0.25	34.0	5.0	18.9	6.4	13.1	4.4	2.8	1.8	3.2	3.6	4.5
	1.04	35.0	13.0	3.2	0.0	22.9	0.0	0.8	1.1	3.5	0.0	8.8
	3	33.1	10.6	0.0	0.0	7.4	0.0	0.0	2.8	1.6	0.0	8.6
	10	28.1	14.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
	30	22.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
	59	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5
	100	4.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9
	0	3.8	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	94.9	0.0
'SW'	0.125	23.7	0.0	9.8	0.0	2.4	0.0	0.0	0.0	1.9	61.3	2.1
	0.25	23.9	3.0	19.3	6.3	4.5	6.6	2.0	0.0	2.3	27.0	3.3
	1.04	32.5	1.3	19.8	1.7	15.4	5.5	0.0	0.8	3.1	0.0	0.0
	3	28.9	14.1	1.6	0.0	6.5	5.3	1.0	5.0	2.2	0.0	0.0
	10	24.3	9.6	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0
	30	4.6	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5
	59	na	na	na	na	na	na	na	na	na	na	7.7 ^a
RT _{UV} [min] ^b	100	na	na	na	na	na	na	na	na	na	na	3.4 ^a
	Metabolite identification ^c	IN-W3295, IN-KP533, other polars	3.1	3.5	3.9	4.4	5.3 - 5.7	5.8 - 6.3	6.9	8.2	13.5	-
										IN-JX915	Cymoxanil	Unknown

^a In case of samples not further analysed (na), remaining radioactivity is equal to the total extractable radioactivity.^b RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)^c Metabolite identification proposed by the RMS (see comments)

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Table B.8.10.1-13: Composition of HPLC1 metabolite fraction M1 in the water and sediment layer and in the entire system after application of cymoxanil to two water/sediment systems (based on TLC2).

Compartment		Water layer			Sediment layer			Entire system		
System	DAT	M1 (HPLC1)	MVI (TLC2)	Unidentified fraction of M1 (HPLC1)	M1 (HPLC1)	MVI (TLC2)	Unidentified fraction of M1 (HPLC1)	M1 (HPLC1)	MVI (TLC2)	Unidentified fraction of M1 (HPLC1)
'OVP'	0	6.6	4.0	2.6	0.0	0.0	0.0	6.6	4.0	2.6
	0.125	26.5	21.2	5.3	na	0.9	na	26.5	22.1	4.4
	0.25	34.0	26.1	7.9	na	1.4	na	34.0	27.5	6.5
	1.04	35.0	19.7	15.3	na	2.3	na	35.0	22.0	13.0
	3	33.1	13.7	19.4	na	1.0	na	33.1	14.7	18.4
	10	22.1	1.6	20.5	6.1	0.5	5.6	28.1	2.1	26.0
	30	15.4	1.5	13.9	6.7	0.5	6.2	22.1	2.0	20.1
	59	8.3	0.4	7.9	na	0.0	na	8.3	0.4	7.9
	100	4.3	0.0	4.3	na	na	na	4.3	0.0	4.3
	0	3.8	1.1	2.7	0.0	0.0	0.0	3.8	1.1	2.7
'SW'	0.125	23.7	8.3	15.4	na	0.0	na	23.7	8.3	15.4
	0.25	23.9	9.5	14.4	na	0.3	na	23.9	9.9	14.0
	1.04	25.2	11.5	13.7	7.4	0.9	6.5	32.5	12.4	20.1
	3	24.3	7.3	17.0	4.6	0.7	3.9	28.9	8.1	20.8
	10	19.7	1.4	18.3	4.6	0.0	4.6	24.3	1.4	22.9
	30	4.6	0.6	4.0	na	0.3	na	4.6	0.9	3.7
	59	na	0.3	0.0 (-0.3)	na	0.0	na	na	0.3	0.0 (-0.3)
	100	na	na	na	na	0.0	na	na	na	0.0
	RT _{uv} ^a [min] / R _f value	1.9	0.03 – 0.08	1.9	1.9	0.03 – 0.08	1.9	1.9	0.03 – 0.08	1.9
	Metabolite identification ^b	IN-W3295, IN-KP533, other polars	IN-W3595	IN-KP533, other polars	IN-W3295, IN-KP533, other polars	IN-W3595	IN-KP533, other polars	IN-W3295, IN-KP533, other polars	IN-W3595	IN-KP533, other polars

na denotes not analysed

^a RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006), RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^b Metabolite identification proposed by the RMS (see comments)

^c None of these polar compounds is expected to exceed 10 % of AR individually based on comparison to respective DuPont study (see comments)

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Table B.8.10-14: Calculated degradation half-life of cymoxanil in the entire system of two water/sediment systems based on SFO kinetics.

Substance	System	Compartment	DT ₅₀ [days]	DT ₉₀ [days]	R ²
Cymoxanil	'OVP'	Entire system	0.06	0.20	1.00
	'SW'		0.16	0.52	1.00

Conclusion:

In two water/sediment systems cymoxanil degraded instantaneously with a degradation half-life of 0.06 – 0.16 days for the entire system, respective DT₉₀ values were in a range of 0.16 – 0.52 days (SFO kinetics, R² = 1.00). Since no cymoxanil was observed in the sediment phase dissipation in the water phase is equal to the overall degradation. Cymoxanil did not move into the sediment (no cymoxanil found).

Major metabolites (> 10 % of AR) of cymoxanil to form in the entire system of 'OVP' system were IN-W3595 (maximum 27.5 % of AR), IN-KQ960 (14.3 % of AR), IN-U3204 (24.7 % of AR) and metabolite fraction M5 (22.9 % of AR). In the 'SW' system the same major metabolites were observed: IN-W3595 (maximum 12.4 % of AR), IN-KQ960 (14.1 % of AR), IN-U3204 (19.8 % of AR) and metabolite fraction M5 (15.4 % of AR). The unidentified portion of the polar *HPLC1* fraction M1 (likely to include IN-KP533, IN-R3274, oxamic acid (IN-18474) and oxalic acid) accounts for maximum 26.0 % and 22.9 % of AR in the entire 'OVP' and 'SW' system, respectively. No final conclusion on the individual amounts of these polar metabolites can be drawn from the study results. No metabolite was found in the sediment > 10 % of AR.

Mineralization and sediment NER represented the major products of metabolism in each system, reaching up to 75.5 and 29.7 % of AR, respectively.

Comments (RMS):

- Additional information according to the *HPLC1* peak assignment (RMS assessment):
 - *HPLC1* fraction M1:
The highly polar *HPLC1* fraction M1 comprises IN-W3595 (major compound, maximum of 27.5 % of AR by 0.25 DAT) and several other polar degradates. On the basis of available RT values (Brands and Van Noorloos, 2006), these are IN-KP533, IN-R3274, oxamic acid (IN-18474) and oxalic acid (all not separated under the conditions of *HPLC1*). IN-W3595 was adequately separated by *TLC2* (i.e. metabolite fraction MVI with a R_f value 0.03 – 0.08, this fraction is also considered identical to *TLC3* fraction T3-4, observed in the hydrolysis study with a R_f value of 0.35). Most of the remaining polar substances are likely to be covered by *TLC2* fraction MV with a R_f value < 0.02. However, on the basis of available data from this study it cannot be excluded that one of these compounds (IN-KP533, IN-R3274, oxamic acid (IN-18474) or oxalic acid) exceeds the trigger of 10 % of AR individually. According to SANCO/221/2000, rev. 10, oxamic acid (IN-18474) and oxalic acid are considered of no concern. IN-R3274 was hardly observed in environmental fate studies. From results of Willems and Slangen (2003), who re-analysed 0.25 and 1.04 DAT samples from 'OVP' and 'SW' system using *TLC* method *TLC3*, it can be concluded that IN-KP533 (in the hydrolysis study covered as *TLC3* metabolite fraction T3-7) does not significantly contribute to this polar metabolites at this sampling dates. However, it can not be concluded that IN-KP533, a major hydrolysis metabolite in sterile buffer solutions and also observed in the respective water/sediment study from DuPont (Trabue and Lydick, 2001) with 8.0 % of AR, does not exceed the trigger at later time points. **Therefore, the RMS suggests to consider IN-KP533 a major metabolite in water/sediment studies for conservative reasons**, with a maximum occurrence based on the sum of all polar metabolites (*HPLC1* fraction M1) except IN-W3595 (*TLC2* fraction MVI). Adequate degradation data of IN-KP533 are available from the DuPont water/sediment study (Trabue and Lydick, 2001).

- *HPLC1* fraction M2:
Temporal pattern of *HPLC1* peak fraction M2 closely follows temporal pattern of *TLC2* fraction MVII with a R_f of 0.37 – 0.47. Based on RT/R_f values (Brands and Van Noorloos, 2006), metabolite fractions M2 (*HPLC1*) and MVII (*TLC2*) are likely to mainly comprise IN-KQ960.
- The RMS conducted additional calculations according to current guidance (SANCO/10058/2005, ver. 1.0, 2005) applying a multi-compartment system in case where the position of the metabolite within the degradation pathway is known (linking metabolite to parent in series, all compartments following SFO kinetics, additional pathways to sink). In case where the position within the degradation pathway is too complex or unknown, metabolite data were fitted to measured data starting from the maximum occurrence (following SFO kinetics).

Table B.8.10-15: Degradation half-life of metabolites of cymoxanil in the entire system of two water/sediment systems based on multi-compartment modelling (all SFO kinetics, RMS assessment).

System	Substance	DT ₅₀ [days]	DT ₉₀ [days]	Formation fraction [0 ... 1]	R ²	χ ² error [%]	Kinetics
'OVP'	IN-U3204	0.2	0.5	0.46	0.98	15.3	P _{SFO} → M _{SFO}
	IN-JX915	2.1	7.1	0.05	0.88	20.1	P _{SFO} → M _{SFO}
	IN-W3595	2.7	9.0	0.31	0.99	12.0	P _{SFO} → M _{SFO}
	IN-KQ960	nc	nc	nc	nc	nc	nc
	Metabolite fraction M5	1.2	4.0	-	1.00	0.7	M _{SFO}
'SW'	IN-U3204	0.5	1.7	0.33	0.96	16.2	P _{SFO} → M _{SFO}
	IN-JX915	1.5	5.1	0.046	0.97	11.3	P _{SFO} → M _{SFO}
	IN-W3595	2.7	8.9	0.26	0.98	12.7	P _{SFO} → M _{SFO}
	IN-KQ960	15.2	50.5	-	0.98	8.7	M _{SFO}
	Metabolite fraction M5	1.6	5.2	-	1.00	2.0	M _{SFO}

nc denotes not calculable

DT₅₀ values of IN-W3595 were 2.7 days for the entire system in both test systems, for IN-KQ960 only one valid DT₅₀ (15.2 days) could be established. The highly transient metabolite IN-U3204 showed a DT₅₀ in the range of 0.2 – 0.5 days. Metabolite fraction M5 was shown to rapidly degrade with a DT₅₀ of 1.2 – 1.6 days in the entire system. IN-JX915, a minor metabolite in the test systems, rapidly degraded with a DT₅₀ of 2.1 and 1.5 days in the 'OVP' and 'SW' system, respectively.

B.8.10.2 Supplemental studies

Reference:	Incubation of cymoxanil in one water/sediment system in order to regenerate metabolite M-5 observed during NOTOX project 257761
Author(s), year:	Willems, H., Hoitink, M., 2003
Report/Doc. number:	NOTOX project 366784
Guideline(s):	CD 95/36/EC, SETAC (1995), BBA IV, 5-1 (1995), US-EPA, N, 162-4 (1982), OECD guideline proposal (1997)
GLP:	Yes
Deviations:	None
Validity:	Study considered partly acceptable

Material and methods:

Test substance:	[Cyanoacetamid-2- ¹⁴ C]-cymoxanil, batch 3362-236, radiochemical purity 97.4 % (HPLC), 4.20 MBq mg ⁻¹
Reference substances:	Cymoxanil (unlabelled)
Test concentration:	Approx. 49.7 µg L ⁻¹
Co-solvent:	< 0.1 % of acetone
Experimental set-up:	2.5 – 2.7 cm sediment layer (approx. 200 g fresh weight, 2 mm sieved), 5.4 - 6 cm water (approx. 470 mL, 150 µm filtered), in 1 L brown glass bottles
Test temperature:	20 ± 2 °C
Test duration:	10 days in the dark
Equilibration time:	4.5 weeks
Monitoring data:	Redox potential, O ₂ content, pH
Volatile traps:	Humified, CO ₂ free air passing the test system (through the upper 1 cm of the water layer without disturbing the sediment) was passed through 2 x 50 mL of 2M NaOH (¹⁴ CO ₂) and 1 x 50 mL of 2-methoxyethanol (organic volatiles)
Sample processing:	The decanted water layer (0 and 0.25 DAT) or 20 mL of the water layer (2 and 10 DAT) were adjusted to a pH of 3.5 with 37% HCl, radio-assayed by LSC and analysed using <i>TLC3</i> (on day of sampling), <i>HPLC1</i> , <i>HPLC2</i> methods and LC-MS/MS without further sample concentration. All samples were stored at -18 °C for a maximum time of 20 days. Note: Owing to the study purpose (generation of metabolite fraction M-5) no analyses were performed on the volatile traps or on the sediment layer.
Analytical techniques:	• TLC (<i>TLC3</i>) • HPLC (<i>HPLC1</i> and <i>HPLC2</i>) • LSC • LC-MS/MS Note: These TLC and HPLC methods were re-investigated by Brands and Van Noorloos (2006) in order to determine the R _f /RT values of several cymoxanil metabolites (for details refer to hydrolysis section B.8.7.)
Kinetic evaluation:	Not a study purpose

Findings:

The radiochemical purity of the spike solution was found to be > 97.8 % of AR (TLC).

Measured redox potentials (approx. +200 mV) indicate aerobic conditions in the water layer during incubation. Anaerobic (reducing) conditions were found in the sediment layer (approx. -200 mV). Dissolved oxygen contents were between 7.5 and 8.6 mg L⁻¹ during incubation indicating a high degree of oxygen

saturation in the water layer. The pH of the water layer remained stable during equilibration and incubation period and fluctuated between 7.6 and 8.2.

The amount of radioactivity present in the water layer decreased with time to 49 % of AR by 10 DAT.

Decay of cymoxanil and formation of metabolites in this supplemental study was quite similar to the original water/sediment study (Slangen and Willems, 2000) which was conducted with the same water/sediment system ('SW'). The single peak on the radio-detector with the RT of 4.6 min (considered to be identical with metabolite fraction M5 in the original water/sediment study) was clearly demonstrated to consist of two individual peaks with the RTs of 4.1 and 4.3 min (using UV detector). The second and larger one ('Peak 7') exceeded 10 % of AR by 10 DAT decreasing afterwards to non-detectable amounts (by 10 DAT). The first and smaller peak ('Peak 6') was < 10 % of AR throughout the study.

Based on LC-MS/MS analysis, the major compound of metabolite fraction M5 (i.e. 'Peak 7' in this study) was tentatively identified as 2-(amino)carbonyl-N-[(amino)carbonyl]-2-(methoxyimino)-acetamide (Fig. B.8.10.2-1).

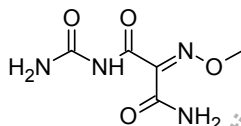


Figure B.8.10.2-1: Proposed structure of the major compound ('Peak 7') of metabolite fraction M5.

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Table B.8.10.2-1: Characterization of total radioactivity [% of AR] in the water layer of 'SW' water/sediment system (HPLC1).

DAT	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6 + 7 ^a	Peak 6	Peak 7	Peak 8	Peak 9	Cymoxanil	Total RA
0.25	15.9	0.0	0.0	1.9	23.4	3.6	1.8	1.8	0.0	7.6	41.7	94.1
1	27.5	4.4	2.9	7.1	23.0	10.0	2.5	7.5	0.0	8.9	1.5	85.2
2	26.0	0.0	4.4	7.0	5.1	20.5	8.2	12.3	3.2	5.5	0.0	71.7
10	33.0	0.0	11.6	0.0	0.0	0.0	0.0	0.0	4.5	0.0	0.0	49.1
RT _{UV} [min] ^b	1.9	2.6	2.9	3.1	3.5	4.1 + 4.3	4.1	4.3	6.9	8.2	14.0	-
Metabolite identification ^c	IN-W3595, IN-KP533 other polars	Unknown	IN-KQ960	Unknown	IN-U3204	Metabolite fraction M5	Minor part of metabolite fraction M5	Major part of metabolite fraction M5	IN-R3273	IN-JX915	Cymoxanil	-

^a Two compounds, not baseline separated by RAD, separated peak areas ('Peak 6' and 'Peak 7') re-estimated by RMS based on RAD signals^b RT denotes retention time. For higher comparability with results from Brands and Van Noorloos (2006) the RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)^c Metabolite identification proposed by the RMS (see comments)

Conclusion:

Metabolite M5, present in the water phase of water/sediment systems, consists of two different components, the largest component with a maximum occurrence of 12.3 % of AR by 2 DAT was tentatively identified as 2-(amino)carbonyl-N-[(amino)carbonyl]-2-(methoxyimino)-acetamide. The smaller part, not exceeding 10 % of AR, could not be identified.

Comments (RMS):

- Estimation of amounts [% of AR] of 'Peak 6' and 'Peak 7', representing the smaller and the larger fraction of metabolite fraction M5, are based on UV-signals in the original report. Since no reference material was available and the UV absorption of these fractions is unknown, this approach is not considered valid. The RMS re-estimated adequate peak areas on the basis of RAD signals (considering peak shoulders, etc.).
- Individual amounts of *HPLC1* peak areas (except metabolite fraction M5) were not converted into [% of AR] in the report. For reasons of better comparison with the original water/sediment study (Slangen and Willems, 2000), these data were added by the RMS based on total RA observed in the water layer. Based on *HPLC1* data, metabolite pattern of this study and the original water/sediment study are consistent.
- It has to be noted, that the identification of M5 is solely based on one LC-MS/MS result (with a highly complex mass spectrum) without co-chromatography of any reference substance. In addition, there are some inconsistencies regarding peak retention times, peak heights and peak positions between the additional LC-MS/MS report and the core study report (e.g. in the LC-MS/MS report the identified peak is the *smaller* one, the distinct *larger* peak clearly consists of two unidentified compounds, HPLC chromatograms of additional LC-MS/MS report and core report are not consistent, etc.), leading to the conclusion, that the final identification of metabolite fraction M5 remains highly indicative. **Therefore, the RMS proposes to handle M5 as 'unidentified' metabolite fraction for further risk assessment.**

Reference:	Comparison of degradation products of cymoxanil formed in ecotox media, buffered aqueous solutions (pH 4, 7, 9), irradiated aqueous solutions (pH 5) and water/sediment systems
Author(s), year:	Willems, H., Slangen, P. J., 2003
Report/Doc. number:	NOTOX 310859
Guideline(s):	Supplemental study - not applicable
GLP:	Yes
Deviations:	Supplemental study - not applicable
Validity:	Study considered acceptable

The main objective of this study was to demonstrate that ecotox tests performed under static and semistatic conditions give simultaneously information on the toxicity of the active ingredient and its relevant aquatic metabolites, in agreement with the 'Guidance Document on Aquatic Ecotoxicology in the frame of Directive 91/414/EEC' (Sanco/3268/'001 rev. 4, October 2002).

Material and methods:

- Test substance: [Cyanoacetamide-2-¹⁴C]-cymoxanil, batch 3304.265, 4.19 MBq mg⁻¹, radiochemical purity ≥ 99.0 % (TLC, HPLC)
- Reference substance: Cymoxanil (unlabelled)
- Test systems:
- ISO-medium (used for fish and daphnia acute toxicity testing, pH 7.9):

Ca ²⁺	80 mg L ⁻¹
Mg ²⁺	12 mg L ⁻¹

	Na^+ 15 mg L ⁻¹ K^{2+} 3 mg L ⁻¹ Cl^- 145 mg L ⁻¹ SO_4^{2-} 49 mg L ⁻¹ HCO_3^- 47 mg L ⁻¹ in Milli-Ro water
	• M2-medium (used for algae acute toxicity, pH 8.1) according to ISO/IS 8692 • Irradiated sterilized buffer solution (0.05 M phosphate buffer, pH 5)
Test concentration:	5.97 mg L ⁻¹
Co-solvent:	< 0.2 % acetonitrile (v/v)
Test temperatures:	• 20 ± 2 °C (ISO- and M2-medium) • 25 ± 1 °C (irradiated sterile buffer solution)
Irradiation:	Only performed on sterilized buffer (ph 5) solution: Xenon burner (Suntest CPS+, > 290 nm), 700 W m ²) over a period of 7 days
Volatile traps:	Only used for M2- and ISO-medium: stream of humified, CO ₂ free air passed through the system, outgoing flow passed through 2 x 50 mL of 2 M NaOH (¹⁴ CO ₂) and 1 x 50 mL of 2-methoxyethanol (organic volatiles)
Analytical techniques:	• TLC method (<i>TLC1</i>, <i>TLC2</i>, <i>TLC3</i>) • One HPLC methods (<i>HPLC1</i>) • LSC Note: These TLC and HPLC methods were re-investigated by Brands and Van Noorloos (2006) to determine the R_f/RT values of several cymoxanil metabolites (for details refer to hydrolysis section, B.8.7).
Additional experiments:	For comparison purposes, the following samples from the water/sediment study (Slangen and Willems, 2000), stored at -15 °C over a period of 19 months, were re-analysed using the above mentioned chromatographic TLC methods (including <i>TLC3</i> , which was not used in the original water/sediment study): • 'OVP' system, 0.25 DAT, organic extract of the water layer • 'OVP' system, 0.25 DAT, remaining aqueous phase of the water layer • 'SW' system, 1.04 DAT, organic extract of the water layer • 'SW' system, 1.04 DAT, remaining aqueous phase of the water layer
Kinetic evaluation:	Not a study purpose

Findings:

Radiochemical purity of the stock solution was > 97.7 % of AR (*HPLC1*, *TLC1*, *TLC2* and *TLC3*) just prior to the start of the experiment.

Measured pH values of the ISO- and M2-medium were 7.9 and 8.3 at the beginning and 8.1 (for both) at the end of incubation (4 and 3 DAT). By study termination the mass balance was 96.8 % of AR (ISO) and 100.7 % of AR (M2). All activity was present in the aqueous phase, no volatiles were formed.

The pH of irradiated sterile solution was 5.1 at the beginning and 5.0 at the end of experiment (by 7 DAT). The test system remained sterile throughout the incubation. No volatile degradation products are expected from photolysis study (Willems, 2000).

Based on *HPLC1*, *TLC1*, *TLC2* and *TLC3*, metabolite patterns observed in the tested media (ISO, M2 and irradiated solution) were highly consistent to metabolite pattern observed in the original hydrolysis study (Willems et al., 2003), water/sediment study (Slangen and Willems, 2000) and in the photolysis study (Willems, 2000).

Table B.8.10.2-2: Characterization of total radioactivity [% of AR] in the test media (HPLC1).

Test medium	DAT	H-4	H-6	H-5	H-3	H-8	H-10	H-9	H-2	Cymoxanil
ISO-medium, pH 7.9	0	3.4	0.0	0.0	7.9	0.0	0.0	0.0	4.2	83.0
	0.125	5.9	0.0	0.0	16.4	0.0	0.0	0.0	6.6	68.8
	0.25	10.6	1.7	1.9	25.4	0.0	0.0	0.0	8.0	49.9
	1	19.2	9.4	4.4	42.2	0.0	0.0	0.0	9.7	12.8
	2	22.8	20.2	10.0	31.1	0.0	0.0	2.0	11.5	0.0
	4	32.2	28.8	14.7	10.6	0.0	1.9	5.0	3.5	0.0
M2-medium, pH 8.1	0	8.0	0.0	0.0	15.7	0.0	0.0	0.0	6.0	76.9
	0.125	12.1	0.0	0.0	27.6	0.0	0.0	0.0	8.3	55.7
	0.25	14.0	2.8	1.8	31.1	0.0	0.0	0.0	7.3	46.8
	1	22.4	8.7	7.3	44.0	0.0	0.0	0.0	11.0	10.2
	2	24.8	20.0	13.9	32.2	0.0	0.0	3.1	9.7	0.0
	3	26.7	30.0	11.1	19.0	0.0	2.2	4.9	6.8	0.0
Irradiated solution, pH 5.0	2 ^a	0.0	0.0	0.0	0.0	5.5	0.0	0.0	45.6	36.7
	7	9.0	5.0	0.0	0.9	7.1	3.2	15.8	54.6	4.6
RT _{UV} [min] ^b		1.8	2.7	3.1	3.5	4.5	5.8	6.8	8.1	13.5
Metabolite identification ^c		IN-W3595, IN-KP533, other polars	IN-KQ960, unknowns	Unknown	IN-U3204	Unknown	Unknown	IN-R3273	IN-JX915	Cymoxanil

^a One additional compound was found at RT_{UV} = 14.7 min (10.2 % of AR), maybe identical to the Z-isomer of cymoxanil (IN-Q8761) owing to irradiation

^b RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006) the RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)

^c Metabolite identification proposed by the RMS (see comments)

Table B.8.10.2-3: Composition of *HPLC1* peak H-4 [% of AR] in the test media and in re-analysed water/sediment samples based on *TLC3*.

Test medium	DAT	H-4 (<i>HPLC1</i>)	T3-7 (<i>TLC3</i>)	T3-4 (<i>TLC3</i>)	Unidentified fraction of H-4 (<i>HPLC1</i>)
ISO-medium, pH 7.9	0	3.4	0.0	0.5	2.9
	0.125	5.9	0.0	4.4	1.5
	0.25	10.6	0.0	7.7	2.9
	1	19.2	0.0	18.0	1.2
	2	22.8	1.8	20.7	0.3
	4	32.2	8.6	20.6	3.0
M2-medium, pH 8.1	0	8.0	0.0	0.8	7.2
	0.125	12.1	0.0	7.9	4.2
	0.25	14.0	0.0	11.8	2.2
	1	22.4	0.0	19.8	2.6
	2	24.8	1.8	22.2	0.8
	3	26.7	4.0	21.4	1.3
Irradiated solution, pH 5.0	2 ^a	0.0	1.0	0.7	0.0 (-1.7)
	7	9.0	1.5	0.8	6.7
'OVP', water phase, OE ^a	0.25	0.0 ^c	0.0	0.0	0.0
'OVP', water phase, AP ^b	0.25	35.0 ^c	0.0	30.7	4.3
'SW', water phase, OE ^a	1.04	0.0 ^c	0.0	0.0	0.0
'SW', water phase, AP ^b	1.04	25.2 ^c	0.0	16.3	8.9
RT _{UV} [min] ^d		1.9	0.15	0.37	1.9
Metabolite identification ^e		IN-W3595, IN-KP533, other polars	IN-KP533	IN-W3595	Other polars ^f

^a OE denotes organic extract of water phase^b AP denotes remaining aquatic phase after organic extraction^c Values from original water/sediment study (Slangen and Willems, 2003)^d RT denotes retention time. For higher comparability with Brands and Van Noorloos (2006) the RT values are based on the UV-signal (approx. 0.3 min before the RAD signal)^e Metabolite identification proposed by the RMS (see comments)^f Likely to include IN-R3274, oxamic acid (IN-18474) and oxalic acid

Conclusion:

Adequate exposure of all relevant aquatic metabolites (hydrolysis, photolysis, water/sediment study), except metabolite fraction M-5, can be assumed in ecotox tests conducted under static conditions. Exposure (although lower) under semistatic conditions (24 hr renewal rate) can also be assumed as all metabolites are formed within 24 hrs.

Re-analysis of 0.25 and 1.04 DAT samples from the water/sediment study (Slangen and Willems, 2000) using *TLC3* method revealed that IN-KP533 does not significantly contribute to *HPLC1* fraction M1 (H-4 in this study) observed in the water/sediment study at these time points.

Comments (RMS):

- Since only 0.25 and 1.04 DAT samples were re-analysed, it can not be excluded that IN-KP533 is more present within metabolite fraction M1 at later time samples. For details see water/sediment study (Slangen and Willems, 2000).

B.8.10.3 Summary on aquatic systems

Hydrolysis

Hydrolysis of cymoxanil was investigated in sterile buffer solutions at pH 4, 5, 7 and 9 in two independent studies (DuPont and Oxon), which gave consistent results.

Once in contact with (sterile) buffer solutions, cymoxanil undergoes extensive hydrolysis strongly depending on the pH of the solution, leading to the formation of numerous metabolites. Cymoxanil is considered stable at a pH of 4 (and below); half-life times at pH 5, 7 and 9 were 144, 1.1 and 0.02 days at 25 °C. At 20 °C half-life times at pH 7 and 9 were determined to be 2.1 and 0.04 days.

Hydrolysis of cymoxanil is driven by three main processes (Figure B.8.10.3-1):

- partly reversible cyclisation into IN-U3204 (six-member ring system) – major process
- partly reversible cyclisation into IN-JX915 (five-member ring system) – minor process
- cleavage of the parent to release IN-W3595 and ethyl urea – major process

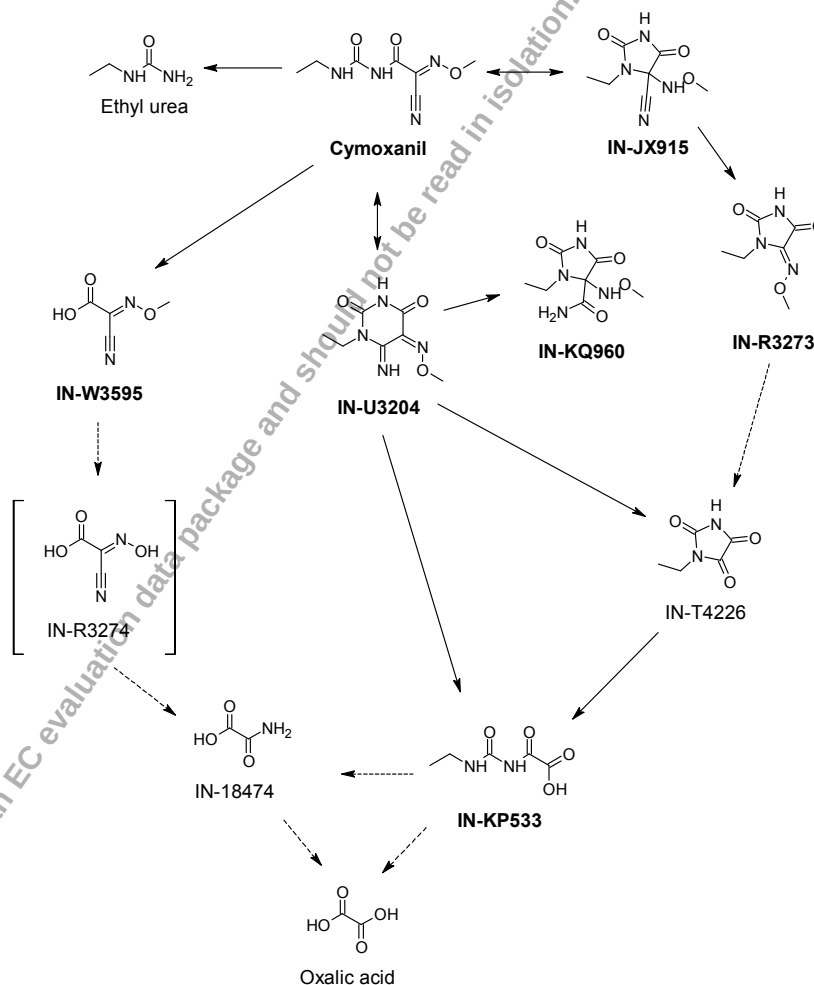


Figure B.8.10.3-1: Proposed hydrolytic degradation pathway of cymoxanil in sterile water (metabolites in bold exceed 10 % of AR at a pH of 7 or 9).

Metabolite IN-U3204 is highly unstable in aqueous solutions, rapidly degrading into IN-KP533, IN-T4226 and IN-KQ960. IN-T4226 is a further transient hydrolysis metabolite rapidly degrading into IN-KP533 by ring cleavage; IN-KQ960 and IN-KP533 have to be considered stable under the conditions of sterile hydrolysis.

Metabolite IN-JX915 rapidly further degrades into IN-R3273, which in turn slowly degrades into IN-T4226.

The parent cleavage product IN-W3595 is considered rather stable under the conditions of hydrolysis in sterile buffer solutions. Ethyl urea, which is likely to be formed together with IN-W3595, was never quantified in environmental fate studies, since the labelling of the parent (cyanoacetamide position) does not allow to follow the fate of this cleavage product. Nevertheless, ethyl urea has to be considered a major degradation product of the hydrolysis of cymoxanil in sterile buffer solutions at neutral and alkaline pH, too. According to SANCO/221/2000, rev. 10, (2003) guidance document on the relevance of metabolites in groundwater, ethyl urea and its degradation products are considered compounds of no concern and therefore not further considered in the environmental risk assessment.

Hydrolysis half-life of the transient metabolites IN-U3204, IN-JX915 and IN-T4226 at pH 7 and pH 9 were estimated to be 2.5 and 0.5 days, 0.7 and 1.7 days, and 7.2 and 2.0 days, respectively. The metabolites IN-W3595, IN-KQ960, IN-R3273 and IN-KP533 have to be considered rather stable under the conditions of sterile hydrolysis at each pH, their amounts remained almost stable once the hydrolysis process has finished (which occurred by approx. 15 DAT at pH 7 and by 7 DAT at pH 9).

Under conditions of sterile hydrolysis, the following metabolites were observed > 10 % of AR (at pH 7 or pH 9): IN-U3204 (maximum of 60.8 % of AR), IN-JX915 (11.0 % of AR), IN-W3595 (41.5 % of AR), IN-KP533 (57.4 % of AR), IN-R3273 (10.2 % of AR) and IN-KQ916 (14.1 % of AR). In the Oxon study (Slangen and Willams, 2003) several degradation products, not exceeding 10 % of AR individually, remained unidentified.

Table B.8.10.3-1: Summary on maximum occurrence of metabolites during hydrolysis of cymoxanil in sterile buffer solutions (number in brackets indicate day of maximum occurrence).

Compound	pH 4	pH 5	pH 7	pH 9	Notifier
IN-U3204	-	9.1 (7)	52.7 (2)	60.8 (0.2)	DuPont
	0.0	-	28.0 (3)	35.4 (0.13)	Oxon
IN-JX915	-	1.8 (7)	5.0 (1.1)	8.2 (0.3)	DuPont
	0.0	-	7.2 (3)	11.0 (0.13)	Oxon
IN-T4226	-	0.0	5.4 (10)	9.8 (1)	DuPont
	0.0	-	nd/ni	nd/ni	Oxon
IN-W3595	-	2.3 (30)	16.2 (15)	39.0 (3)	DuPont
	0.0	-	22.6 (13)	41.5 (2/30)	Oxon
IN-KP533	-	0.8 (10)	57.0 (30)	31.6 (10)	DuPont
	0.0	-	57.4 (30)	34.4 (13)	Oxon
IN-R3273	-	0.9 (30)	10.2 (15)	7.2 (7)	DuPont
	0.0	-	9.8 (21)	5.4 (21)	Oxon
IN-KQ960	-	nd	9.0 (30)	13.5 (7)	DuPont
	0.0	-	6.4 (4/21)	14.1 (21)	Oxon

nd/ni denoted not determined, not identified

Table B.8.10.3-2: Summary on hydrolysis half-life [days] of cymoxanil and metabolites in sterile buffer solutions.

Compound	Temperature [°C]	pH 4	pH 5	pH 7	pH 9	Notifier
Cymoxanil	25	-	144	1.1	0.02	DuPont
	20	Stable	-	2.1	0.04	Oxon
IN-U3204	25	-	25.8	2.6	0.4	DuPont
	20	-	-	2.3	0.5	Oxon
IN-JX915	25	-	-	1.1	1.7	DuPont
	20	-	-	0.5	1.7	Oxon
IN-T4226	25	-	-	7.2	2.0	DuPont
	20	-	-	nd/ni	nd/ni	Oxon
IN-W3595	25	-	-	Stable	Stable	DuPont
	20	-	-	Stable	Stable	Oxon
IN-KP533	25	-	-	Stable	Stable	DuPont
	20	-	-	Stable	Stable	Oxon
IN-R3273	25	-	-	Stable	Stable	DuPont
	20	-	-	Stable	Stable	Oxon
IN-KQ960	25	-	-	Stable	Stable	DuPont
	20	-	-	Stable	Stable	Oxon

nd/ni denoted not determined, not identified

Photolysis

Photolysis of cymoxanil in sterile buffer solution was investigated at pH 5 (where cymoxanil is considered to be almost stable) in two independent studies (DuPont and Oxon), which gave consistent results.

Under the impact of irradiation, degradation of cymoxanil owing to photolysis is strongly driven by formation of the cyclisation metabolite IN-JX915 (five-member ring system, maximum occurrence 52.6 % of AR), which rapidly further degrades to IN-R3273 (maximum occurrence 35.4 % of AR). No other major metabolites were observed. This pathway is clearly the major degradation route of cymoxanil in acidic solutions exposed to irradiation. The alternative hydrolysis processes (cyclisation to IN-U3204 and cleavage of the parent to form IN-W3595) were almost negligible at the investigated pH value. In the dark control samples almost no degradation of cymoxanil was observed.

Net photolysis half-life time of cymoxanil in sterile buffer solution at pH 5 was calculated to be 1.7 and 3.0 days (DuPont and Oxon study, respectively). The experimental net photolysis of cymoxanil corresponds to 4.3 and 12.1 days (DuPont and Oxon study, respectively) under environmental conditions (midsummer day, approx. 40 °N). Additional calculations with GC-SOLAR yielded a theoretical half-life of cymoxanil of 5.2 and 17.3 days (DuPont and OXON study, respectively) in the top layer of an aqueous system integrated over a full day in the summer at 40 °N (at pH 5.0).

As demonstrated in one additional experiment, conducted in non-sterile pond water at pH 7.0, the impact of irradiation on the overall dissipation of cymoxanil in aquatic ecosystems loses its significance at neutral and alkaline conditions owing to the extensive abiotic hydrolysis of cymoxanil at higher pH values.

Quantum yield (Φ) of cymoxanil was calculated to be 0.0052 (DuPont) and 0.00058 (Oxon).

The DT_{50} of IN-JX915, owing to the influence of photolysis and hydrolysis, was calculated to be approx. 6.6 days at the investigated pH of 5.0 (DuPont and Oxon study). However, owing to the highly transient character of IN-JX915 during hydrolysis under neutral and alkaline conditions (hydrolysis $DT_{50} < 2$ days) it is

expected that levels of photolytically formed IN-JX915 will be significantly lower in aquatic systems under environmental conditions (without considering biotic degradation). Based on GC-SOLAR modelling, DT_{50} of IN-JX915 under environmental conditions (pH 5) was estimated to be 21.2 days.

Degradation half-life of IN-R3273 at pH 5.0, owing to the influence of photolysis and hydrolysis, was calculated to be 32.7 days in the DuPont study, in the Oxon study, no reliable half-life time could be calculated for IN-R3273. Based on GC-SOLAR modelling, DT_{50} of IN-R3273 under environmental conditions (pH 5) was estimated to be 4.7 days.

Further minor photolysis products (< 10 % of AR) were IN-T4226 and IN-KP533 which derive from the degradation of IN-JX915 and IN-R3273.

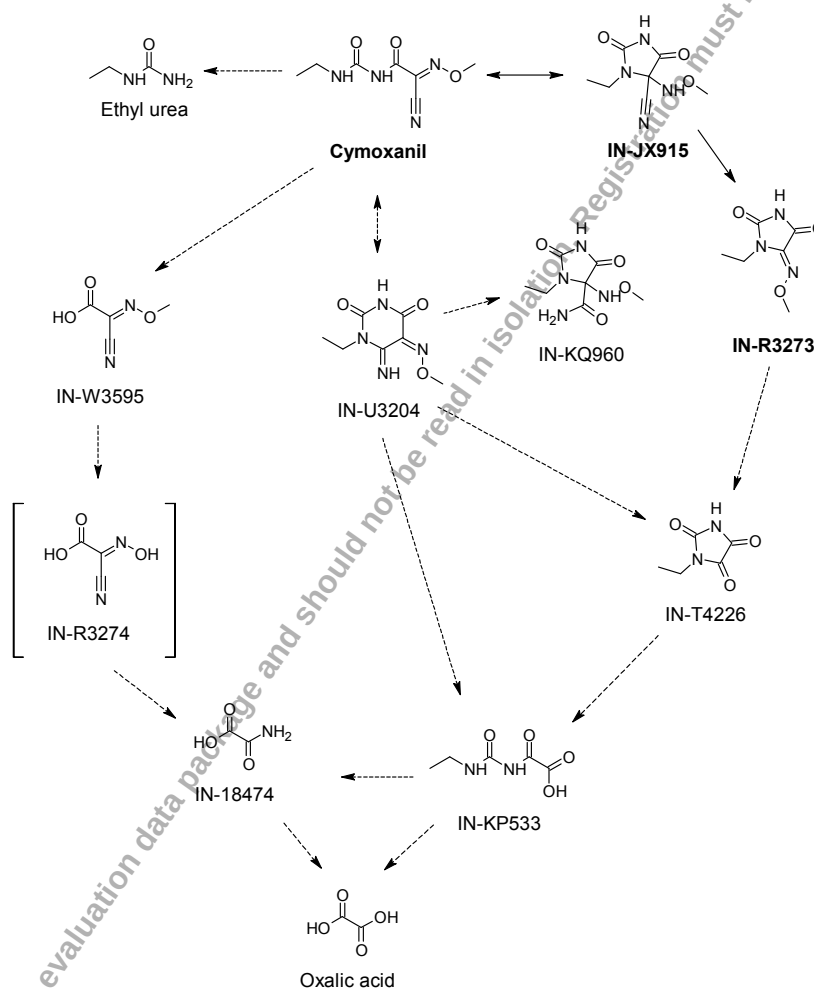


Figure B.8.10.3-2: Proposed photolytic degradation pathway of cymoxanil in sterile water, pH 5 (metabolites in bold exceed 10 % of AR).

Table B.8.10.3-3: Summary on maximum occurrence of metabolites during photolysis of cymoxanil in sterile buffer solutions (pH 5).

Compound	pH 5	Notifier
IN-U3204	0.6 (7)	DuPont
	nd/ni	OXON
IN-JX915	51.7 (4)	DuPont
	52.6 (6)	OXON
IN-T4226	6.7 (15)	DuPont
	nd/ni	OXON
IN-KP533	7.9 (15)	DuPont
	nd/ni	OXON
IN-R3273	35.4 (15)	DuPont
	18.5 (6)	OXON

nd/ni denoted not determined, not identified

Biological degradation

Results of a **readily biodegradability study** indicate that cymoxanil can not be considered readily biodegradable.

The fate and behaviour of cymoxanil in **water/sediment systems** was investigated in four contrasting test systems ('Brandywine Creek', 'Lums Pond', 'OVP' and 'SW') with a representative range of properties in the water and sediment layer.

- 'Brandywine Creek': Sand, 0.8 % organic C, pH_{water} 7.4, pH_{sed} 7.0
- 'Lums Pond': Sand, 0.1 % organic C, pH_{water} 5.3, pH_{sed} 5.1
- 'OVP': Silty clay loam, 4.7 % organic C, pH_{water} 8.3, pH_{sed} 7.5
- 'SW': Silty loam, 4.9 % organic C, pH_{water} 8.3, pH_{sed} 7.5

Dissolved oxygen contents and redox potential values in the water layer indicated aerobic conditions the water layer of all test systems. Anaerobic (reducing) conditions were found in the sediment layers of all systems.

Formation of CO₂ accounted for approx. 41 % of AR by 99 DAT in test systems 'Brandywine creek' and 'Lums Pond', both poor in organic C and microbial activity. Owing to the reductive conditions in these test systems, a significant formation of organic volatiles, likely to be methane, could be demonstrated. In the test systems 'OVP' and 'SW', both with a high amount of organic C in the sediment, formation of CO₂ accounted for 75.5 and 68.5 % of AR by 100 DAT, respectively.

Maximum formation of NER accounted for 22.5 – 35.2 % of AR, with the maximum amount occurring around 28/30 DAT. By 100 DAT, NER have decreased to amounts in a range of 17.9 – 25.6 % of AR.

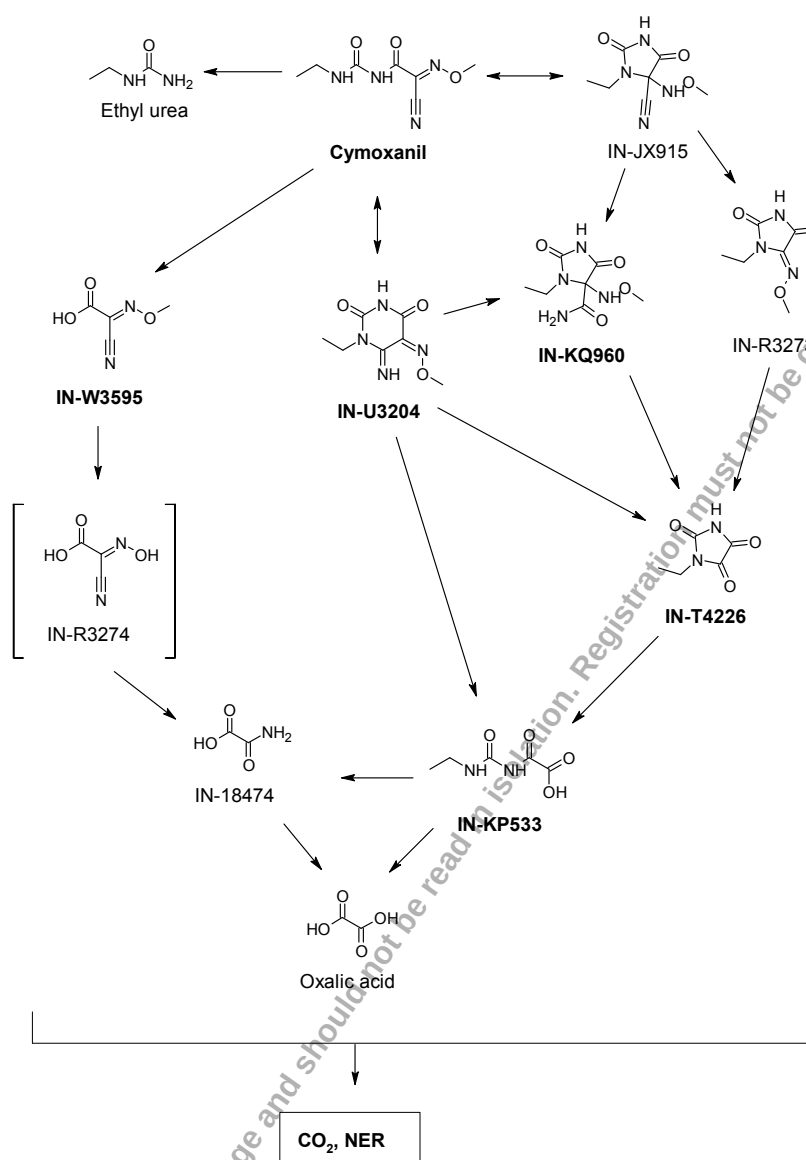


Figure B.8.10.3-3: Proposed degradation pathway of cymoxanil in water/sediment systems (metabolites in bold exceed 10 % of AR).

Degradation of cymoxanil in the entire system was fast with a DegT₅₀ values in a range of 0.1 – 1.5 days following SFO kinetics ($R^2 \geq 0.99$) with a geometric mean of 0.3 days. Respective DT₉₀ values were in a range of 0.2 – 5.0 days, geometric mean 1.0 days. Since transfer of cymoxanil into the sediment layer was negligible, dissipation in the water layer is almost consistent to degradation in the entire system. In accordance to hydrolysis, degradation of cymoxanil in the water/sediment system strongly depends on the water pH with slower degradation observed under more acidic conditions. However, likely owing to non-sterile conditions, degradation of cymoxanil in the rather acidic 'Lums Pond' system (pH in water 5.3) was much faster than expected from hydrolysis alone (conducted under sterile conditions).

Degradation of cymoxanil in the water/sediment system is driven by hydrolysis and microbial turnover. In this respect, formation and occurrence of metabolites is similar to the pattern observed in the hydrolysis study. However, owing to the microbial activity, all metabolites observed to be rather stable under conditions of sterile hydrolysis were rapidly degraded further.

Based on the entire system, the following metabolites are considered major (> 10 % of AR): IN-U3204 (maximum occurrence 24.7 % of AR by 0.1 DAT), IN-W3595 (27.5 % of AR by 0.3 DAT), IN-KQ960 (14.3 %

of AR by 3 DAT), IN-T4226 (12.0 % of AR by 3 DAT), metabolite fraction M5 (22.9 % of AR by 1 DAT) and IN-KP533 (26.0 % of AR by 10 DAT). IN-KP533 is included by the RMS into the list of major water/sediment metabolites owing to conservative reasons. IN-KP533 is part of the polar HPLC fraction M1, observed in one water/sediment study (Slangen and Willems, 2000) with a maximum occurrence of 35.0 % of AR. Metabolite fraction M1 additionally comprises IN-W3595, IN-R3274, oxamic acid (IN-18474) and oxalic acid. IN-W3595 could be adequately separated by an additional TLC system, the individual amounts of the remaining polars contributing to the remaining fraction of M1 (maximum 26.0 % of AR) are not known. In the water/sediment study from Trabue and Lydick, 2001, IN-KP533 was observed with maximum amounts of 8.0 % of AR in the entire system. However, formation of polars was generally smaller in this study in comparison to the water/sediment study from Slangen and Willems (2000). For conservative reasons, the sum of the remaining polars (including IN-KP533) is attributed to IN-KP533. Under conditions of sterile hydrolysis, IN-KP533 was observed up to 57.4 % of AR (at pH 7.0).

No metabolite or metabolite fraction was observed > 10 % of AR in the sediment phases of all test systems investigated.

None of the observed metabolites in the water/sediment studies was persistent. Based on the entire system, geometric mean DegT₅₀ values for the metabolites IN-U3204, IN-W3595, IN-T4226, IN-JX915, IN-KP533 and the metabolite fraction M5 were calculated to be 0.4, 3.0, 4.6, 1.7, 2.6 and 1.4 days, respectively. IN-R3273 and IN-KQ960 degraded slower with geometric mean DegT₅₀ values of 6.3 and 47.4 days, respectively.

Table B.8.10.3-4: Summary on maximum occurrence [% of AR] of cymoxanil and metabolites in the water and sediment layer and in the entire system of four water/sediment systems.

Compound		'Brandywine Creek'	'Lums Pond'	'OVP'	'SW'	Total maximum
Cymoxanil	Sediment	1.3 (1)	3.9 (1)	0.0	0.0	3.9 (1)
IN-U3204	Water	15.6 (1)	1.7 (0)	24.7 (0.13)	19.8 (1)	24.7 (0.13)
	Sediment	0.2 (3)	0.5 (3)	0.0	0.0	0.5 (3)
	Total	15.7 (1)	1.8 (1)	24.7 (0.13)	19.8 (1)	24.7 (0.13)
IN-W3595	Water	11.0 (3)	1.2 (1)	26.1 (0.25)	11.5 (1)	26.1 (0.25)
	Sediment	1.7 (3)	0.4 (3)	2.3 (1)	0.9 (1)	2.3 (1)
	Total	12.7 (3)	1.3 (1)	27.5 (0.25)	12.4 (1)	27.5 (0.25)
IN-KQ960	Water	8.9 (15)	1.3 (21/28)	13.0 (1)	10.2 (3)	13.0 (1)
	Sediment	1.3 (21/28)	0.5 (15)	4.8 (10)	5.5 (30)	5.5 (30)
	Total	9.6 (15)	1.7 (15)	14.3 (10)	14.1 (3)	14.3 (10)
IN-T4226	Water	7.4 (1)	11.1 (3)	nd/ni	nd/ni	11.1 (3)
	Sediment	0.8 (1)	1.0 (8)	nd/ni	nd/ni	1.0 (8)
	Total	8.2 (1)	12.0 (3)	nd/ni	nd/ni	12.0 (3)
IN-KP533^a	Water	7.9 (3)	2.6 (8)	20.5 (10)	18.3 (10)	20.5 (10)
	Sediment	0.2 (8/15)	1.0 (3)	6.2 (30)	6.5 (1)	6.5 (1)
	Total	8.0 (3)	3.0 (8)	26.0 (10)	22.9 (10)	26.0 (10)
Metabolite fraction M5	Water	nd/ni	nd/ni	22.9 (1)	15.4 (1)	22.9 (1)
	Sediment	nd/ni	nd/ni	0.0	0.0	0.0
	Total	nd/ni	nd/ni	22.9 (1)	15.4 (1)	22.9 (1)

^a In case of 'OVP' and 'SW' system, amounts stated for IN-KP533 includes IN-R3274, oxamic acid (IN-18474) and oxalic acid (individual amounts of these polar compounds, including IN-KP533, are not known) – conservative risk assessment

Table B.8.10.3-5: Summary on degradation half-lives of cymoxanil and metabolites in the entire system of four water/sediment systems.

Compound	System	Entire system (degradation)		
		DegT ₅₀ [days]	DegT ₉₀ [days]	R ²
Cymoxanil	'Brandywine Creek'	0.5	1.7	1.00
	'Lums Pond'	1.6	5.3	0.99
	'OVP'	0.1	0.2	1.00
	'SW'	0.2	0.5	1.00
	Geometric mean	0.3	1.0	-
IN-U3204	'Brandywine Creek'	0.6	2.0	0.92
	'Lums Pond'	-	-	-
	'OVP'	0.2	0.5	0.98
	'SW'	0.5	1.7	0.96
	Geometric mean	0.4	1.2	-
IN-W3959	'Brandywine Creek'	3.6	12.1	0.95
	'Lums Pond'	-	-	-
	'OVP'	2.7	9.0	0.99
	'SW'	2.7	8.9	0.98
	Geometric mean	3.0	9.9	-
IN-KQ960	'Brandywine Creek'	154	512	0.76
	'Lums Pond'	45.4	151	0.97
	'OVP'	nc	nc	-
	'SW'	15.2	50.5	0.98
	Geometric mean	47.4	158	-
IN-T4226	'Brandywine Creek'	3.9	12.9	0.99
	'Lums Pond'	5.4	17.9	0.91
	'OVP'	nd/ni	nd/ni	-
	'SW'	nd/ni	nd/ni	-
	Geometric mean	4.6	15.2	-
IN-JX915	'Brandywine Creek'	2.5	8.3	0.79
	'Lums Pond'	1.1	3.7	0.96
	'OVP'	2.1	7.1	0.88
	'SW'	1.5	5.1	0.97
	Geometric mean	1.7	5.8	-
IN-R3273	'Brandywine Creek'	6.0	19.9	0.93
	'Lums Pond'	6.7	22.2	0.98
	'OVP'	nc	nc	nc
	'SW'	nc	nc	nc
	Geometric mean	6.3	21.0	-
IN-KP533	'Brandywine Creek'	2.3	7.5	0.96
	'Lums Pond'	3.0	10.0	0.97
	'OVP'	nc	nc	nc
	'SW'	nc	nc	nc
	Geometric mean	2.6	8.7	-
Metabolite fraction M5	'Brandywine Creek'	nd/ni	nd/ni	-
	'Lums Pond'	nd/ni	nd/ni	-
	'OVP'	1.2	4.0	1.00
	'SW'	1.6	5.2	1.00
	Geometric mean	1.4	4.6	-

B.8.11 Predicted environmental concentrations in surface water (PEC_{SW}) and sediment (PEC_{SED}) for the active substance and relevant metabolites (Annex IIIA, point 9.2.3)

Reference:	Predicted environmental concentration of cymoxanil and major metabolites in surface water, sediment, soil, and air after application of Tanos 50 WG to potatoes for the European Union
Author(s), year:	Ball M., A., 2004
Report/Doc. number:	DuPont-11941, Revision No. 1
Guideline(s):	Calculation, not applicable
GLP:	Calculation, not applicable
Deviations:	Calculation, not applicable
Validity:	Study considered partly acceptable, re-calculated by the RMS (see comments)

and

Reference:	Cymoxanil 50% WP, plant protection product, Annex IIIA: Section 5: Fate in the Environment, Summaries and Assessment (Tier II – Document M-III)
Author(s), year:	Ciccotelli, M., 2004
Report/Doc. number:	OXON CYMOA3S5T2 2004
Guideline(s):	Calculation, not applicable
GLP:	Calculation, not applicable
Deviations:	Calculation, not applicable
Validity:	Calculations considered partly acceptable, re-calculated by the RMS (see comments)

Material and methods:

Predicted environmental concentrations of cymoxanil and relevant metabolites in surface water (PEC_{SW}) and sediment (PEC_{SED}) after application of TANOS 50 WG (DuPont) to potatoes and of CYMOXANIL 50 % WP (Oxon) to potatoes and lettuce were calculated according to FOCUS surface water STEPS 1 & 2 (FOCUS Step 1-2 calculator, version 1.1).

Table B.8.10.3-1: Application data of cymoxanil applied as TANOS 50 WG (DuPont) or CYMOXANIL 50 % WP (Oxon).

Product	TANOS 50 WG (DuPont)	CYMOXANIL 50 % WP (Oxon)	
Crop	Potatoes	Potatoes	Lettuce (Leafy vegetable)
Application rate [g ha ⁻¹]	175	120	240
Number of application	8	4 (North EU) 5 (South EU)	4
Interval [days]	7	7	7
Crop interception [%]	50 (average) ^a	50 (average) ^a	70 (full canopy) ^a
Region	North EU/South EU	North EU/South EU	South EU
Season	March - May	June - September	June - September
BBCH stage	21 - 95	21 - 95	40 - 49

^a FOCUS default value according to BBCH codes

Table B.8.10.3-2: Physiochemical properties and degradation data of cymoxanil and metabolites.

Compound	Property	Units	Value	Remarks
Cymoxanil	Molecular weight	[g mol ⁻¹]	198.2	
	Water solubility	[mg L ⁻¹]	783	
	K _{OC} /K _{FOC}	[L kg ⁻¹]	43.6	Arithmetic mean (n = 4), batch experiment
	Soil DegT ₅₀	[days]	1.0	Geometric mean of normalized recalculated SFO-DT ₅₀ (n = 9)
	Entire system DegT ₅₀	[days]	0.3	Geometric mean (n = 4)
IN-U3204	Molecular weight	[g mol ⁻¹]	198.2	
	Water solubility	[mg L ⁻¹]	783	Unknown, parent value used
	K _{OC} /K _{FOC}	[L kg ⁻¹]	27.9	HPLC, n = 1
	Soil DegT ₅₀	[days]	0.2	Geometric mean of normalized DT ₅₀ (n = 3)
	Entire system DegT ₅₀	[days]	0.4	Geometric mean (n = 3)
	Maximum occurrence (soil)	[% of AR]	24.7	
	Maximum occurrence (water/sediment)	[% of AR]	24.7	
IN-W3595	Molecular weight	[g mol ⁻¹]	128.1	
	Water solubility	[mg L ⁻¹]	783	Unknown, parent value assumed
	K _{OC} /K _{FOC}	[L kg ⁻¹]	9.2	Arithmetic mean (n = 4), batch experiment
	Soil DegT ₅₀	[days]	2.2	Worst-case of normalized DT ₅₀ (n = 2)
	Entire system DegT ₅₀	[days]	3.0	Geometric mean (n = 3)
	Maximum occurrence (soil)	[% of AR]	10.1	
	Maximum occurrence (water/sediment)	[% of AR]	27.5	
IN-KQ960	Molecular weight	[g mol ⁻¹]	216.2	
	Water solubility	[mg L ⁻¹]	783	Unknown, parent value used
	K _{OC} /K _{FOC}	[L kg ⁻¹]	21.6	HPLC, n = 1
	Soil DegT ₅₀	[days]	9.3	Worst-case of normalized DT ₅₀ (n = 2)
	Entire system DegT ₅₀	[days]	47.4	Geometric mean (n = 3)
	Maximum occurrence (soil)	[% of AR]	6.3	
	Maximum occurrence (water/sediment)	[% of AR]	14.3	
IN-T4226	Molecular weight	[g mol ⁻¹]	142.1	
	Water solubility	[mg L ⁻¹]	783	Unknown, parent value used
	K _{OC} /K _{FOC}	[L kg ⁻¹]	17.7	HPLC, n = 1
	Soil DegT ₅₀	[days]	1000	Unknown, worst-case assessment
	Entire system DegT ₅₀	[days]	4.6	Geometric mean (n = 2)
	Maximum occurrence (soil)	[% of AR]	1.7	
	Maximum occurrence (water/sediment)	[% of AR]	12.0	
IN-JX915	Molecular weight	[g mol ⁻¹]	198.2	
	Water solubility	[mg L ⁻¹]	783	Unknown, parent value used
	K _{OC} /K _{FOC}	[L kg ⁻¹]	16.3	Arithmetic mean (n = 4), batch experiment
	Soil DegT ₅₀	[days]	0.7	Normalized, one value (n = 1)
	Entire system DegT ₅₀	[days]	1.7	Geometric mean (n = 4)
	Maximum occurrence (soil)	[% of AR]	10.9	Soil photolysis, worst-case assessment
	Maximum occurrence (water/sediment)	[% of AR]	52.6	Water photolysis, worst-case assessment
IN-R3273	Molecular weight	[g mol ⁻¹]	171.2	
	Water solubility	[mg L ⁻¹]	783	Unknown, parent value used
	K _{OC} /K _{FOC}	[L kg ⁻¹]	41.9	Arithmetic mean (n = 4), batch experiment

Compound	Property	Units	Value	Remarks
	Soil DegT ₅₀	[days]	1000	Unknown, worst-case assessment
	Entire system DegT ₅₀	[days]	6.3	Geometric mean (<i>n</i> = 2)
	Maximum occurrence (soil)	[% of AR]	2.4	
	Maximum occurrence (water/sediment)	[% of AR]	35.4	Water photolysis, worst-case assessment
IN-KP533	Molecular weight	[g mol ⁻¹]	160.1	
	Water solubility	[mg L ⁻¹]	783	Unknown, parent value used
	K _{OC} /K _{FOC}	[L kg ⁻¹]	12.9	HPLC, <i>n</i> = 1
	Soil DegT ₅₀	[days]	1000	Unknown, worst-case assessment
	Entire system DegT ₅₀	[days]	2.6	Geometric mean (<i>n</i> = 2)
	Maximum occurrence (soil)	[% of AR]	2.7	
	Maximum occurrence (water/sediment)	[% of AR]	26.0	Individual maximum occurrence unknown, sum of polar metabolites with the exception of IN-W3595 used, worst-case assessment
Metabolite fraction M5	Molecular weight	[g mol ⁻¹]	198.2	Mole mass of one compound known (i.e. 188 g mol ⁻¹), mole mass of other compounds unknown, worst-case assessment
	Water solubility	[mg L ⁻¹]	783	Unknown, parent value used
	K _{OC} /K _{FOC}	[L kg ⁻¹]	9.2	Unknown, value of IN-W3595 used as worst-case assessment (arithmetic mean, <i>n</i> = 4, batch experiment)
	Soil DegT ₅₀	[days]	1000	Unknown, worst-case assessment
	Entire system DegT ₅₀	[days]	1.4	Geometric mean (<i>n</i> = 2)
	Maximum occurrence (soil)	[% of AR]	0.0	Not observed in soil studies
	Maximum occurrence (water/sediment)	[% of AR]	22.9	

Findings:

Table B.8.10.3-3: Maximum predicted environmental concentrations of cymoxanil and metabolites in surface water (PEC_{SW}) and sediment (PEC_{SED}) after application of TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon) to potatoes and lettuce at FOCUS surface water STEP 1.

Scenario	Application	Single application							
	Formulation	TANOS 50 WG (DuPont)		CYMOXANIL 50 % WP (Oxon)					
	Crop	Potatoes		Potatoes (N-EU)		Potatoes (S-EU)		Lettuce	
	PEC _{SW/SED}	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]
FOCUS STEP 1	Cymoxanil	56.7	24.0	38.9	16.5	38.9	16.5	77.8	33.0
	IN-U3204	14.3	3.88	9.80	2.66	9.80	2.66	19.6	5.32
	IN-W3595	32.4	2.77	11.1	0.95	13.9	1.19	22.2	1.90
	IN-KQ960	33.2	7.05	11.4	2.42	14.2	3.02	22.7	4.83
	IN-T4226	6.66	1.01	2.28	0.35	2.86	0.43	4.57	0.69
	IN-JX915	7.07	1.01	4.85	0.70	4.85	0.70	9.70	1.39
	IN-R3273	13.1	4.84	4.49	1.66	5.61	2.07	8.98	3.32
	IN-KP533	12.7	1.29	4.36	0.44	5.45	0.55	8.72	0.89
	Metabolite fraction M5	0.37	0.02	0.25	0.01	0.25	0.01	0.51	0.03

Table B.8.10.3-4: Maximum predicted environmental concentrations of cymoxanil and metabolites in surface water (PEC_{SW}) and sediment (PEC_{SED}) after application of TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon) to potatoes and lettuce at FOCUS surface water STEP 2.

Scenario	Application Formulation	Single application						Multiple application						
		TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)			TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)			
		Potatoes		Lettuce	Potatoes		Lettuce	Potatoes		Lettuce	Potatoes		Lettuce	
	PEC _{SW/SED}	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]	PEC _{SW} [µg L ⁻¹]	PEC _{SED} [µg kg ⁻¹]	
FOCUS STEP 2 North EU	Cymoxanil		1.61	0.15	1.10	0.10	-	-	0.88	0.15	0.74	0.10	-	-
	IN-U3204		0.40	0.01	0.27	0.01	-	-	0.22	0.01	0.18	0.01	-	-
	IN-W3595		0.29	0.02	0.20	0.01	-	-	0.20	0.02	0.16	0.01	-	-
	IN-KQ960		0.52	0.11	0.36	0.08	-	-	1.42	0.30	0.79	0.17	-	-
	IN-T4226		0.14	0.02	0.10	0.01	-	-	0.61	0.10	0.24	0.04	-	-
	IN-JX915		0.85	0.06	0.58	0.04	-	-	0.49	0.03	0.42	0.03	-	-
	IN-R3273		0.49	0.16	0.34	0.11	-	-	1.21	0.46	0.56	0.21	-	-
	IN-KP533		0.34	0.03	0.23	0.02	-	-	1.06	0.13	0.40	0.05	-	-
	Metabolite fraction M5		0.37	0.01	0.25	0.01	-	-	0.21	0.01	0.18	0.01	-	-
FOCUS STEP 2 South EU	Cymoxanil		1.61	0.30	1.10	0.15	2.21	0.19	0.88	0.30	0.72	0.16	1.49	0.19
	IN-U3204		0.40	0.01	0.27	0.01	0.55	0.02	0.22	0.01	0.18	0.01	0.37	0.01
	IN-W3595		0.33	0.03	0.20	0.01	0.39	0.02	0.32	0.03	0.19	0.02	0.33	0.02
	IN-KQ960		0.81	0.17	0.45	0.10	0.67	0.14	2.12	0.45	1.10	0.23	1.50	0.32
	IN-T4226		0.21	0.03	0.12	0.02	0.19	0.03	1.15	0.20	0.40	0.07	0.44	0.07
	IN-JX915		0.85	0.06	0.58	0.04	1.16	0.08	0.49	0.03	0.40	0.03	0.83	0.06
	IN-R3273		0.53	0.20	0.34	0.12	0.67	0.21	2.11	0.84	0.83	0.31	1.06	0.40
	IN-KP533		0.36	0.04	0.23	0.02	0.46	0.03	2.04	0.26	0.70	0.09	0.74	0.09
	Metabolite fraction M5		0.37	0.01	0.25	0.01	0.51	0.02	0.21	0.01	0.17	0.01	0.35	0.01

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Conclusion:

Table B.8.10.3-5: Summary on global maximum PEC_{SW} [$\mu g L^{-1}$] of cymoxanil and metabolites after application of TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon) to potatoes or lettuce.

Formulation		TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)			
FOCUS STEP	Compound	PEC_{SW} [$\mu g L^{-1}$]	Type of application ^a	Scenario ^b	PEC_{SW} [$\mu g L^{-1}$]	Type of application	Scenario	Crop ^c
STEP 1	Cymoxanil	56.7	-	-	77.8	-	-	Lettuce
	IN-U3204	14.3	-	-	19.6	-	-	Lettuce
	IN-W3595	32.4	-	-	22.2	-	-	Lettuce
	IN-KQ960	33.2	-	-	22.7	-	-	Lettuce
	IN-T4226	6.66	-	-	4.57	-	-	Lettuce
	IN-JX915	7.07	-	-	9.70	-	-	Lettuce
	IN-R3273	13.1	-	-	8.98	-	-	Lettuce
	IN-KP533	12.7	-	-	8.72	-	-	Lettuce
	Metabolite fraction M5	0.37	-	-	0.51	-	-	Lettuce
STEP 2	Cymoxanil	1.61	Single	South EU	2.21	Single	South EU	Lettuce
	IN-U3204	0.40	Single	South EU	0.55	Single	South EU	Lettuce
	IN-W3595	0.33	Single	South EU	0.39	Single	South EU	Lettuce
	IN-KQ960	2.12	Multiple	South EU	1.50	Multiple	South EU	Lettuce
	IN-T4226	1.15	Multiple	South EU	0.44	Multiple	South EU	Lettuce
	IN-JX915	0.85	Single	South EU	1.16	Single	South EU	Lettuce
	IN-R3273	2.11	Multiple	South EU	1.06	Multiple	South EU	Lettuce
	IN-KP533	2.04	Multiple	South EU	0.74	Multiple	South EU	Lettuce
	Metabolite fraction M5	0.37	Single	South EU	0.51	Single	South EU	Lettuce

^a Type of application at which maximum PEC_{SW} was observed

^b Scenario at which maximum PEC_{SW} was observed

^c Crop in which maximum PEC_{SW} was observed

Table B.8.10.3-6: Summary on global maximum PEC_{SED} [$\mu g kg^{-1}$] of cymoxanil and metabolites after application of TANOS 50 WG (DuPont) and CYMOXANIL 50 % WP (Oxon) to potatoes or lettuce.

Formulation		TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)			
FOCUS STEP	Compound	PEC_{SW} [$\mu g kg^{-1}$]	Type of application ^a	Scenario ^b	PEC_{SW} [$\mu g kg^{-1}$]	Type of application	Scenario	Crop ^b
STEP 1	Cymoxanil	24.0	-	-	33.0	-	-	Lettuce
	IN-U3204	3.88	-	-	5.32	-	-	Lettuce
	IN-W3595	2.77	-	-	1.90	-	-	Lettuce
	IN-KQ960	7.05	-	-	4.83	-	-	Lettuce
	IN-T4226	1.01	-	-	0.69	-	-	Lettuce
	IN-JX915	1.01	-	-	1.39	-	-	Lettuce
	IN-R3273	4.84	-	-	3.32	-	-	Lettuce
	IN-KP533	1.29	-	-	0.89	-	-	Lettuce
	Metabolite fraction M5	0.02	-	-	0.03	-	-	Lettuce
STEP 2	Cymoxanil	0.30	Single	South EU	0.19	Single	South EU	Lettuce
	IN-U3204	0.01	Single	South EU	0.02	Single	South EU	Lettuce
	IN-W3595	0.03	Single	South EU	0.02	Single	South EU	Lettuce
	IN-KQ960	0.45	Multiple	South EU	0.32	Multiple	South EU	Lettuce
	IN-T4226	0.20	Multiple	South EU	0.07	Multiple	South EU	Lettuce
	IN-JX915	0.06	Single	South EU	0.08	Single	South EU	Lettuce
	IN-R3273	0.84	Multiple	South EU	0.40	Multiple	South EU	Lettuce
	IN-KP533	0.26	Multiple	South EU	0.09	Multiple	South EU	Lettuce

Formulation		TANOS 50 WG (DuPont)			CYMOXANIL 50 % WP (Oxon)			
FOCUS STEP	Compound	PEC _{SW} [µg kg ⁻¹]	Type of application ^a	Scenario ^b	PEC _{SW} [µg kg ⁻¹]	Type of application	Scenario	Crop ^c
	Metabolite fraction M5	0.01	Single	South EU	0.02	Single	South EU	Lettuce

^a Type of application at which maximum PEC_{SW} was observed

^b Scenario at which maximum PEC_{SW} was observed

^c Crop in which maximum PEC_{SW} was observed

Comments (RMS):

- After complete evaluation of the dossier by the RMS, some changes regarding the input parameter of cymoxanil and metabolites for PEC_{SW}/PEC_{SED} occurred. To minimize data confusion and to keep relevant endpoints as concise as possible, the RMS re-calculated PEC_{SW/SED} on the basis of validated endpoints.
- For aquatic risk assessment, (geometric) mean DT₅₀ values of cymoxanil in the soil and in the water were used. On the basis of FOCUS surface water STEP 2, safe use of cymoxanil applied as TANOS 50 WG (DuPont) or CYMOXANIL 50 % WP(Oxon) could be demonstrated (refer to Vol. 3, B.9, Ecotoxicology). Nevertheless, degradation of cymoxanil in soil and water is considered to significantly depend on the environmental pH. Therefore, PEC_{SW} might be distinct higher if the product is applied onto more acidic soils and/or close to acidic surface water. **In this respect, MS should pay particular attention to the aquatic risk assessment when assessing application for national authorisations in acidic soils and surface water.**

B.8.12 Degradation in the saturated zone (Annex IIA, point 7.2.1.4)

There is no scientific rationale for conducting degradation studies in the saturated zone with cymoxanil since it degrades so rapidly in an aerobic environment that it would not persist long enough to move to the saturated zone.

B.8.13 Rate and route of degradation in air (Annex IIA, point 7.2.2, Annex IIIA, point 9.3)

Neither cymoxanil nor any of its environmental relevant metabolites have significant volatility. The vapour pressure of cymoxanil was 1.5×10^{-4} Pa at 20 °C. There is no guidance available for conducting meaningful studies regarding the potential breakdown of cymoxanil or its relevant metabolites in air. Furthermore, the Henry's law constant of cymoxanil is less than 3.8×10^{-5} Pa m³ mol⁻¹, suggesting little potential for volatilisation in the environment. Based on Atkinson calculations (assuming a 12-hrs day and 1.5×10^6 OH⁻ cm⁻³), half-life of cymoxanil in the air owing to atmospheric oxidation was estimated to be 21.3 hrs (RMS calculation, AopWin v.1.91, EpiSuite v.3.11, US-EPA 2003).

B.8.14 Predicted environmental concentration in the air (PEC_A) (Annex IIA, point 9.3.1)

Reference:	Predicted environmental concentration of cymoxanil and major metabolites in surface water, sediment, soil, and air after application of Tanos 50WG to potatoes for the European Union
Author(s), year:	Ball, M. A. (2004)
Report/Doc. number:	DuPont-11941, Revision No. 1
Guideline(s):	Calculations - not applicable
GLP:	Calculations - not applicable
Deviations:	Calculations - not applicable
Validity:	Study considered partly acceptable

Material and methods:

Volatilization estimation method developed by Dow Chemicals (Thomas, 1982 [1]):

$$k_v = 4.4 \cdot 10^{-7} \cdot \left(\frac{vp}{K_{oc} \cdot S} \right)$$

where

k_v = rate constant for volatilisation [day⁻¹]

vp = vapour pressure [mm Hg]

K_{oc} = organic carbon partition coefficient [L kg⁻¹]

S = solubility in water [mg L⁻¹]

Estimated initial daily loss from a treated field:

$$loss = (1 - e^{-k_v}) \cdot 100$$

where

loss = percent of applied compound volatilized from the soil surface within 1 day after application

k_v = rate constant for volatilisation [day⁻¹]

For volatilization losses less than 20 % of applied within 1 day, the PEC_A is assumed to be negligible.

[1] Thomas, R. G. (1982) Volatilization from soil. Chapter 16. In: Handbook of Chemical property estimation methods. Lyman, W. J., Reehl, W. F., Rosenblatt, D. H. (eds.), American Chemical Society, Washington DC

Findings:

Based on water solubility (780 mg L⁻¹), K_{oc} (43.6 L kg⁻¹) and the vapour pressure (1.5×10^{-4} Pa at 20 °C, equivalent to 1.14×10^{-6} mm Hg) it can be predicted that no more than 0.1 % of applied cymoxanil would be lost from a treated field within 1 day.

Conclusion:

Based on calculation (formula developed Dow Chemicals) volatilization of cymoxanil from a treated field is considered negligible.

Comments (RMS):

None

B.8.15 Definition of residues (Annex IIA, point 7.3)

Environmental occurring metabolites **requiring further assessment by other disciplines** (toxicology and ecotoxicology):

Soil: Cymoxanil, IN-U3204, IN-W3595, IN-JX915 (the latter major in soil photolysis)
Surface water: Cymoxanil, IN-U3204, IN-W3595, IN-KQ960, IN-T4226, IN-KP533, metabolite fraction M5, IN-JX915, IN-R3273 (the latter two major in water photolysis)
Sediment: Cymoxanil
Groundwater: Cymoxanil
Air: Cymoxanil

B.8.16 Monitoring data (Annex IIA, point 7.4)

No monitoring data are available.

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B.8.17 References relied on

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
Annex II Data and Information					
IIA, 7.0/01	Malekani, K.	2003	Position paper for cymoxanil: Calculated half-lives of cymoxanil and its metabolites in environmental fate laboratory studies. DuPont Stine-Haskell Research Center DuPont-11575, Revision No.1 GLP: No Published: No	Y	DuPont
IIA, 7.1.1.1.1/01	Aikens, P.J.	1998	Cymoxanil Aerobic Soil Metabolism (Route of Degradation) Huntingdon Life Sciences Ltd OXN 224/982398 GLP: Yes Published: No	Y	Oxon
IIA, 7.1.1.1.1/02	Anderson, J.J.	2001	Aerobic soil metabolism of ¹⁴ C-cymoxanil DuPont Experimental Station AMR 3438-95, Revision No.1 GLP: Yes Published: No	Y	DuPont
IIA, 7.1.1.1.1/03	Boucher, C.R.	1993	Aerobic soil metabolism of [2- ¹⁴ C]DPX-T3217 (cymoxanil) DuPont Experimental Station AMR 1988-91 GLP: Yes Published: No	N	DuPont
IIA, 7.1.1.1.1/04	Major, L.J.	1993	Aerobic soil metabolism of [2- ¹⁴ C]DPX-T3217 (cymoxanil) DuPont Experimental Station AMR 1988-91 Supplement No.1 GLP: Yes Published: No	N	DuPont
IIA, 7.1.1.1.1/05	Boucher, C.R.	1994	Degradation rate of [¹⁴ C]-cymoxanil on four soils DuPont Experimental Station AMR 2869-93 GLP: Yes Published: No	N	DuPont
IIA, 7.1.1.1.1/06	Trabue, S.L.	2003	Degradation rate of [¹⁴ C]-cymoxanil on four soils DuPont Stine-Haskell Research Center AMR 2869-93, Supplement No. 1 GLP: Yes Published: No	Y	DuPont
IIA, 7.1.1.1.2/01	Willems, H.	1998	Photodegradation of Cymoxanil on Soil Surfaces NOTOX B.V., 's-Hertogenbosch, The Netherlands 211095 GLP: Yes Published: No	Y	Oxon

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
IIA, 7.1.1.1.2/02	Berg, D.S.	1996	Photodegradation of radiolabelled cymoxanil on soil under simulated sunlight DuPont Experimental Station, Wilmington, Delaware, USA AMR 3582-95 GLP: Yes Published: No	Y	DuPont
IIA, 7.1.1.2.1.1/01	Melkebeke, T.	1999	Determination of the Degradation Rate of Cymoxanil in Three Soils NOTOX B.V., 's-Hertogenbosch, The Netherlands 257737 GLP: Yes Published: No	Y	Oxon
IIA, 7.1.1.2.1.1/02	Aitkens, P.J.	1998	Cymoxanil Aerobic Soil Metabolism (Route of Degradation) Huntingdon Life Sciences Ltd OXN 224/982398 GLP: Yes Published: No	Y	Oxon
IIA, 7.1.1.2.1.1/03	Anderson, J.J.	2001	Aerobic soil metabolism of ¹⁴ C-cymoxanil DuPont Experimental Station AMR 3438-95, Revision No. 1 GLP: Yes Published: No	Y	DuPont
IIA, 7.1.1.2.1.1/04	Major, L.J.	1993	Aerobic soil metabolism of [2- ¹⁴ C]DPX- T3217 (cymoxanil) DuPont Experimental Station AMR 1988-91 Supplement No.1 GLP: Yes Published: No	N	DuPont
IIA, 7.1.1.2.1.1/05	Boucher, C.R.	1994	Degradation rate of [¹⁴ C]-cymoxanil on four soils DuPont Experimental Station AMR 2869-93 GLP: Yes Published: No	N	DuPont
IIA, 7.1.1.2.1.1/06	Trabue, S.L.	2003	Degradation rate of [¹⁴ C]-cymoxanil on four soils DuPont Stine-Haskell Research Center AMR 2869-93, Supplement No. 1 GLP: Yes Published: No	Y	DuPont
IIA, 7.1.1.2.1.1/07	Van Noorloos, B., Slangen, J.	2001	Degradation of the Degradation Rate of Cymoxanil at 10°C in One Soil NOTOX B.V., 's-Hertogenbosch, The Netherlands 308756 GLP: Yes Published: No	Y	Oxon
IIA, 7.1.1.2.1.1/08	Van Noorloos, B.	2006	Determination of TLC Rf values of U3204 and cymoxanil NOTOX B.V., 's-Hertogenbosch, The Netherlands 453443 GLP: Yes Published: No	Y	DuPont/Oxon

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
IIA, 7.1.2/01	Slangen, P.J.	1999	Adsorption/Desorption of Cymoxanil on Soil NOTOX B.V., 's-Hertogenbosch, The Netherlands 257748 GLP: Yes Published: No	Y	Oxon
IIA, 7.1.2/02	Hausmann, S.M., Adams, G.M.	1996	Soil Batch Equilibrium Study of Cymoxanil Degradates DuPont Experimental Station AMR 3722-95 GLP: Yes Published: No	N	DuPont
IIA, 7.1.2/03	Mattson, S.L.	2003	Estimation of adsorption coefficients (K _{oc}) of cymoxanil metabolites, IN-U3204, IN-KQ960, IN-T4226, and IN- KP533, using HPLC DuPont Stine-Haskell Research Center DuPont-11758 GLP: Yes Published: No	Y	DuPont
IIA, 7.1.3.3/01	Hassink, J.	1998	Outdoor lysimeter study on cymoxanil Institut Fresenius, Chemische und Biologische/GmbH AMR 3897-96 GLP: Yes Published: No	Y	DuPont
IIA, 7.2.1.1/01	Lawler, S.M.	1996	Hydrolysis of cymoxanil (DPX-T3217) in buffer solutions of pH 5, 7, and 9 DuPont Experimental Station AMR 3677-95 GLP: Yes Published: No	N	DuPont
IIA, 7.2.1.1/02	Willems, H., Slangen, P.J., Hoitink, M.	2003	Aqueous Hydrolysis of Cymoxanil NOTOX B.V., 's-Hertogenbosch, The Netherlands 308734 GLP: Yes Published: No	Y	Oxon
IIA, 7.2.1.1/03	Brands, C., Van Noorloos, B.	2006	Determination of TLC R _f values and HPLC retention times of cymoxanil and eight reference substances. NOTOX B.V., 's-Hertogenbosch, The Netherlands 457818 GLP: Yes Published: No	Y	DuPont/Oxon
IIA, 7.2.1.1/04 IIA, 7.2.1.2/03 IIA, 7.2.1.3.2/05	Goodyear, A	2006	Cymoxanil Position Paper: Identification of Cymoxanil Aquatic Degradation Products. TSGE, Knaresborough, U.K. 4-3-4.PP1 GLP: No Published: No	Y	DuPont/Oxon

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IIA, 7.2.1.2/01	Anderson, J.J., Horne, P., Lawler, S.M., Swain, R.S.	1993a	Photodegradation of [2- ¹⁴ C]DPX-T3217 (cymoxanil) in pond water and sterile buffer pH 5 DuPont Experimental Station AMR 1990-91 GLP: Yes Published: No	N	DuPont
IIA, 7.2.1.2/02	Willems, H.	2000	Photodegradation of Cymoxanil in Water NOTOX B.V., 's-Hertogenbosch, The Netherlands 257759 GLP: Yes Published: No	Y	Oxon
IIA, 7.2.1.2/04	Anderson, J.J., Lawler, S.M., Swain, R.S.	1993b	Quantum yield determination of DPX-T3217 (cymoxanil) and LC/MS confirmation of unknown degradates in sterile buffer pH 5 DuPont Experimental Station AMR 1990-91, Supplement No. 1 GLP: Yes Published: No	N	DuPont
IIA, 7.2.1.2/05	Hatzenbeler, C.J., Moore, L.A.	2004	Calculated Theoretical Lifetime for Cymoxanil in the Top Layer of Aqueous Systems DuPont Stine-Haskell Research Center DuPont-12330 GLP: Not applicable Published: No	Y	DuPont
IIA, 7.2.1.2/06	Willems, H.	2003	Aquatic Photolysis of Cymoxanil Estimation of Lifetime in the Top Layer of Aqueous Systems (GC Solar Calculations) NOTOX B.V., 's-Hertogenbosch, The Netherlands 397439 GLP: Not applicable Published: No	Y	Oxon
IIA, 7.2.1.2/07	Moore, L.A.	2002	UV/visible absorption and molar absorptivity of IN-R3273 DuPont Stine-Haskell Research Center DuPont-5778 GLP: Yes Published: No	Y	DuPont
IIA, 7.2.1.2/08	Moore, L.A.	2003a	UV/visible absorption and molar absorptivity of IN-JX915 DuPont Stine-Haskell Research Center DuPont-5777 GLP: Yes Published: No	Y	DuPont
IIA, 7.2.1.2/09	Moore, L.A.	2003b	Quantum efficiency for IN-R3273 and IN- JX915 DuPont Stine-Haskell Research Center DuPont-9318, Revision No. 1 GLP: Not applicable Published: No	Y	DuPont

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IIA, 7.2.1.3.1/01	Luit, R.J.	2001	Determination of Ready Biodegradability: Carbon Dioxide (CO ₂) Evolution Test (Modified Sturm Test) with Cymoxanil Technical NOTOX B.V., 's-Hertogenbosch, The Netherlands 308778 GLP: Yes Published: No	Y	Oxon
IIA, 7.2.1.3.2/01	Trabue, S.L., Lydick, T.M.	2001	Degradation of cymoxanil in two water/sediment systems DuPont Experimental Station DuPont-2695 GLP: Yes Published: No	Y	DuPont
IIA, 7.2.1.3.2/02	Slangen, P.J., Willems, H.	2000	The Fate of Cymoxanil in Two Water/Sediment Systems NOTOX B.V., 's-Hertogenbosch, The Netherlands 257761 GLP: Yes Published: No	Y	Oxon
IIA, 7.2.1.3.2/03	Willems, H., Hoitink, M.	2003	Incubation of Cymoxanil in One Water/Sediment System in Order to Regenerate Metabolite M-5 Observed during NOTOX Project 257761 NOTOX B.V., 's-Hertogenbosch, The Netherlands 366784 GLP: Yes Published: No	Y	Oxon
IIA, 7.2.1.3.2/04	Willems, H., Slangen, P.J.	2003	Comparison of Degradation Products of Cymoxanil formed in Ecotox Media, Buffered Aqueous Solutions (pH 4, 7, 9), Irradiated Aqueous solutions (pH 5) and Water/Sediment Systems. NOTOX B.V., 's-Hertogenbosch, The Netherlands 310859 GLP: Yes Published: No	Y	Oxon
Annex III Data and Information					
IIIA, 9.1.3./01 IIIA, 9.2.3/01 IIIA, 9.3/01	Ball, M.A.	2004	Predicted environmental concentrations of cymoxanil and major metabolites in surface water, sediment, soil, and air after application of Tanos 50WG to potatoes for the European Union DuPont Stine-Haskell Research Center DuPont-11941, Revision No. 1 GLP: No Published: No	Y	DuPont
IIIA, 9.2.1/01	Ball, M.A.	2004	Predicted environmental concentrations of cymoxanil and major soil metabolite, IN-U3204, in groundwater after application of Tanos 50WG to potatoes in the European Union DuPont Stine-Haskell Research Center DuPont-11942, Revision No. 1 GLP: No Published: No	Y	DuPont

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