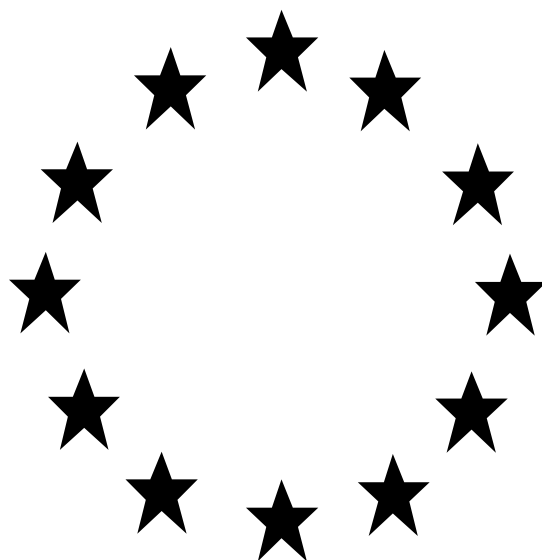




Draft Assessment Report (DAR)

- 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 3, B.7

October 2007

Annex B

Cymoxanil

B.7 Residue data

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.

Contents

	Page
B.7 Residue data	1
B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1; Annex IIIA, 8.1)	4
B.7.1.1 Metabolism in lettuce	4
B.7.1.1.1 Fox G.C., 1999, Metabolism of [2-14C]cymoxanil in lettuce - (DuPont)	4
B.7.1.1.2 Melkebeke T., van Noorloos B., 2003b, Metabolism, distribution, and expression of cymoxanil residues in lettuce - (Oxon)	10
B.7.1.2 Metabolism in potato	14
B.7.1.2.1 Li Y. and Hausmann S.M., 1996, Plant metabolism of [2-14C]Cymoxanil in potatoes - (DuPont)	15
B.7.1.2.2 Melkebeke T., van Noorloos B., 2003a, Metabolism, distribution, and expression of cymoxanil residues in potatoes - (Oxon)	19
B.7.1.3 Overall conclusion on the metabolic fate on crops (DuPont + Oxon)	23
B.7.2 Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2; Annex IIIA, 8.1)	27
B.7.2.1 Anderson J. J., Gaddamidi V., Moghaddam M. F., Swain R. S., Hashinger B.M., Lilly D.E., 1996, The distribution of [2-14C]-DPX-T3217 (cymoxanil) in the lactating goat (nature of residue study to EPA guideline)	27
B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)	41
B.7.4 Use pattern	42
B.7.5 Identification of critical GAPs	46
B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2)	46
B.7.6.1 Cymoxanil residues in lettuce - southern Europe, Oxon	46
B.7.6.2 Cymoxanil residues in potato - northern and southern Europe, DuPont	49
B.7.6.3 Cymoxanil residues in potato - northern and southern Europe, Oxon	53
B.7.6.4 Overall conclusion on supervised residue trials, lettuce, potato - DuPont + Oxon	58
B.7.6.5 Stability of residues prior to analysis (DuPont + Oxon)	59
B.7.6.5.1 Freschi G., 2004, Freezer storage stability of cymoxanil residue in lettuce plants - (Oxon)	60
B.7.6.5.2 Nathan E.C., 1996, Magnitude of residues of cymoxanil in potatoes following application of Curzate M-8 fungicide at maximum label rates and at five times maximum use rates to investigate the need for magnitude of residue data in processed fractions - (DuPont)	62
B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4)	65
B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3)	65
B.7.9 Residues in succeeding crops or rotational crops (Annex IIA 6.6; Annex IIIA 8.5)	65
B.7.9.1 Singles S.K., Strek H.J., Sheftic G.D., 1996, Accumulation of residues in confined rotational crops: Lettuce, wheat, and beets after treatment with [¹⁴ C]cymoxanil - DuPont	65
B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post harvest uses (Annex IIA 6.8; Annex IIIA 8.7)	71
B.7.10.1 Proposed pre-harvest intervals for each relevant crop	71
B.7.10.2 Re-entry period for livestock to areas to be grazed	71
B.7.10.3 Re-entry period for man to crops, buildings or spaces treated	71
B.7.10.4 Withholding period for animal feeding stuffs	71
B.7.10.5 Waiting period between application and handling treated product	72
B.7.11 Community MRL and MRLs in EU Member States (Annex IIIA 12.2)	72
B.7.12 Proposed EU MRLs and justification for the acceptability of those MRLs (Annex IIA 6.7; Annex IIIA 8.6)	72
B.7.12.1 Food of plant origin	72
B.7.12.1.1 Lettuce	72
B.7.12.1.2 Potatoes	72
B.7.12.2 Food of animal origin	72
B.7.13 Proposed EU import tolerances and justification for the acceptability of those residues	72
B.7.14 Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs	72
B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)	73
B.7.15.1 Estimation of the potential and actual exposure through diet	73
B.7.15.1.1 Estimation of chronic exposure through diet	73
B.7.15.1.2 Estimation of acute exposure through diet	74
B.7.15.2 Estimation of the potential and actual exposure through other means	76

B.7.16	Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9)	76
B.7.17	References relied on	80
Annex Ia:	Summary of supervised residue trials of lettuce from Southern-Europe - Oxon	84
Annex Ib:	Summary of supervised residue trials of potato from Northern Europe - DuPont	86
Annex Ic:	Summary of supervised residue trials of potato from Southern Europe - DuPont	88
Annex Id:	Summary of supervised residue trials of potato from northern Europe - Oxon	90
Annex Ie:	Summary of supervised residue trials of potato from southern Europe - Oxon	91
Annex If:	Summary of supervised residue trials of potato from southern Europe - Oxon	93
Annex Ig:	Summary of supervised residue trials of potato from southern Europe - Oxon	94
Annex Ih:	Summary of supervised residue trials of potato from southern Europe - Oxon	95
Appendix 2:	Specific terms and abbreviations	96

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.7 Residue data

Concerning the result of completeness check of submitted dossiers and studies on cymoxanil only the respective studies of DuPont and Oxon will be evaluated in this chapter. DuPont and Oxon formed a data sharing regarding Annex II data exclusively.

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

With respect to intended uses two metabolism studies each were submitted from Oxon and DuPont regarding DG Sanco doc. 7028/VI/95 rev. 3 (072297) for the crop groups leaf vegetables (lettuce, lactuca sativa) and tuber vegetables (potatoes, solanum spp).

B.7.1.1 Metabolism in lettuce

DuPont (B.7.1.1.1) and Oxon (B.7.1.1.2) have submitted one metabolism study each concerning cymoxanil on/in lettuce grown outdoors.

B.7.1.1.1 Fox G.C., 1999, Metabolism of [2-¹⁴C]cymoxanil in lettuce - (DuPont)

Report No.: AMR 4375-97

Guideline: US EPA OPPTS 860.1300, nature of the residue, plant, livestock

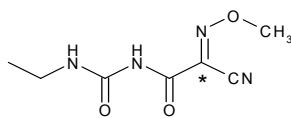
GLP: Yes

Study is considered acceptable for evaluation.

Study characteristics

reference	Fox G.C., 1999 (IIA 6.1/01)	application rate per treatment	equivalent to 0.84 kg as/ha (0.75 lb as/acre)
study title	Metabolism of [2- ¹⁴ C]cymoxanil in lettuce	concentration	equivalent to 1.287 g as/L
type of study	distribution and metabolism	vehicle	water (approx. equivalent to 650 L/ha)
year of execution	1997	number of treatments; dates of foliar applications ^{c)}	4; 7 May 1997 (BBCH about 13-14), 22 May 1997 (about 15-16), 17 June 1997 (about 35), 15 July 1997 (BBCH about 43)
test substance	[cyanoacetamide-2- ¹⁴ C]-cymoxanil ^{a)} , NEN lot 2732-155, radiochem. purity 98.2 % (HPLC), specific activity 14.09 mCi/g	harvest time ^{c)}	18 July 1997 (BBCH about 49)
plant	lettuce (lactuca sativa), variety Prizehead	Intervals between applications	15-28 days
testing facility; test site	DuPont Agricultural Products; Newark (field phase) plus Wilmington (analytical phase), Delaware, USA	total rate	equivalent to 3.36 kg as/ha (3.00 lb as/acre) ^{b)}
soil type	sandy loam	PHI	3 days
area	USA, pots in the field		

- a) Position of radiolabel in ^{14}C -cymoxanil: * Denotes position of ^{14}C radiolabel



CAS: 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide, E isomer
IUPAC: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea
CAS reg. no.: 57966-95-7

Notes: Z-isomer was co-chromatographed by HPLC and separated from E-isomer.

Material and Methods

Study design: Six test pots (surface area approx. 0.061 m² per pot, planted at 22 April 1997) of lettuce plants (variety Prizehead) were used in this outdoor study (Delaware, USA), whereof in 4 pots the lettuce plants have been treated and 2 pots were used as controls in the field. All pots were covered with individual plastic tents (bags) to prevent any contamination of the control pots during application. Lettuce plants were sprayed (using a hand-held atomizer powered by carbon dioxide) four times at 15-28 day intervals with ^{14}C -cymoxanil, formulated with inert ingredients to simulate a wettable powder formulation, at a single rate equivalent to 0.84 kg as/ha.

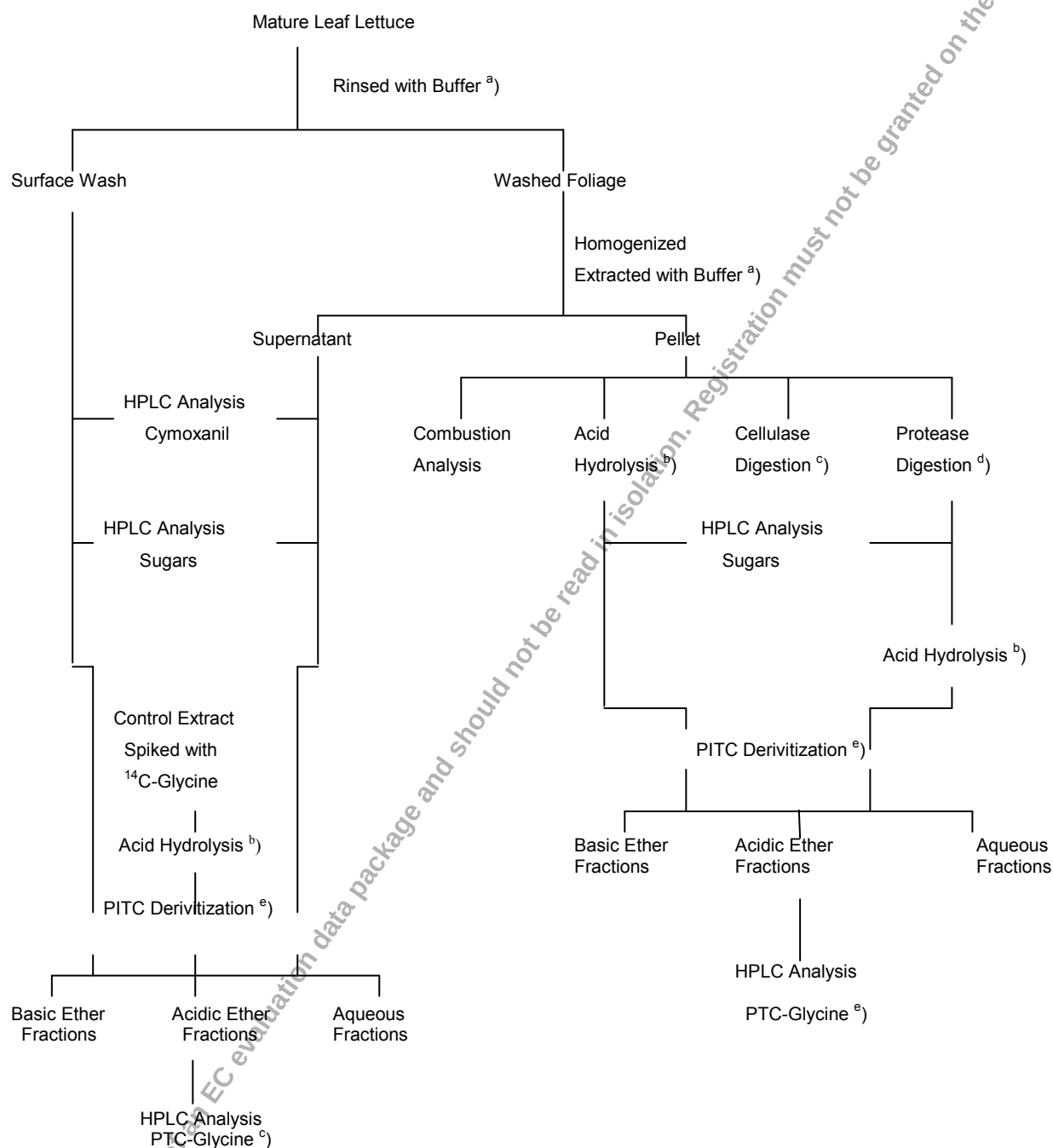
Sample collection: Immature plant samples were collected immediately (after the spray solution was dried) following each application and additionally 7 days after the second application. Mature lettuce plants were harvested 3 days after the final (fourth) application. The lettuce leaves were cut at their base just above the soil surface. The total treated mature lettuce sample weight amounted to 0.386 kg.

Sample preparation: Both control and treated lettuce foliage samples collected at final harvest and at other sampling dates were separately homogenized using liquid nitrogen in the commercial blender. A portion of the final harvest (about 300 g treated lettuce leaves) was surface washed with sodium phosphate buffer (50 mM, pH 5.0) before being homogenized.

Determination of TRR: The total radioactive residue (TRR) of homogenized foliage samples and the surface wash each with triplicate aliquots was determined by combustion/liquid scintillation counting (LSC) and calculated as mg/kg equivalents of ^{14}C -cymoxanil on a fresh weight basis. The limit of detection (LOD) was ≤ 0.01 mg/kg ^{14}C -cymoxanil equivalents. Radioactivity above two times the average background was considered detectable.

Extraction of lettuce leaves homogenate and identification of metabolites: For extraction procedures see Figure B.7.1.1.1-1.

Figure B.7.1.1.1-1 Extraction and characterisation of cymoxanil and metabolites from lettuce (DuPont)



^a) sodium phosphate buffer (50 mM, pH 5.0)

^b) acid hydrolysis (6 M HCl, 100 °C, overnight)

^c) cellulose digestion (37 °C, overnight)

^d) protease digestion (37 °C, overnight)

^e) derivitization with phenylisothiocyanate (PITC) to produce phenylthiocarbamyl-Glycine (PTC-Glycine)

Notes: Cymoxanil, glycine, sugars and other metabolites (closely related to cymoxanil) were examined by several HPLC methods. The extraction procedures and analyses (i.e. LSC, HPLC) of all study samples were done within 4 months after harvest of mature lettuce. The efficiency of the extraction procedure was positive verified with the enforcement extraction method AMR 3705-95.

Findings

Distribution of total radioactive residues (TRR): The TRR in immature lettuce samples at several time intervals were high immediately after application of ^{14}C -cymoxanil (88.03 mg eq/kg) and declined rapidly with time (e.g. 7 days after second application: 10.75 mg eq/kg). The TRR in immature plant material depending on time of application and sampling (see table B.7.1.1.1-1). The immature lettuce samples were not further analysed.

Table B.7.1.1.1-1 Distribution of ^{14}C -residues (TRR) in immature and mature lettuce foliage after application of ^{14}C -cymoxanil at several time intervals

Days after initial treatment	Days after last treatment	Application equivalent total: 4x 0.84 kg as/ha	Growth stage (BBCH)	Radioactive concentration TRR [mg eq/kg] *)
0		1	about 13-14	88.03
15		2	n.a. ^{a)}	60.45
22			n.a. ^{a)}	10.75
41		3	n.a. ^{a)}	50.46
69	0	4	about 43	33.85
72	3	(harvest of mature lettuce)	about 49	10.78 ^{b)}

*) mg eq/kg ... mg equivalents of ^{14}C -cymoxanil based on kg fresh weight of lettuce foliage

^{a)} n.a. ... not available

^{b)} based on wash and extraction of 50.59 gram fresh weight lettuce foliage plus analysis of pellet

The TRR in mature lettuce harvested three days after the final (fourth) application was 10.78 mg/kg ^{14}C -cymoxanil equivalents, which represents the sum of surface wash, extractable and unextractable radioactivity. About 7.5 % of TRR (0.81 mg eq/kg) from mature lettuce was rinsed off with aqueous buffer and additional 64.6 % of TRR (6.96 mg eq/kg) was extractable with aqueous buffer. The extractable radioactivity (calculated from surface wash and aqueous extract) accounted for 72.1 % of TRR (7.77 mg eq/kg). In mature lettuce foliage 27.9 % of the TRR (3.01 mg eq/kg) was unextractable. See table B.7.1.1.1-2.

Table B.7.1.1.1-2 Characterisation of ^{14}C -residues in mature lettuce foliage treated with ^{14}C -cymoxanil (equivalent to 4x 0.84 kg as/ha), PHI: 3 days

Sample	Radioactive concentration	
	[% of TRR]	[mg eq/kg] *)
Extractable residues	72.1	7.77
Surface wash	7.5	0.81
Aqueous extract	64.6	6.96
Unextractable residues (pellet)	27.9	3.01
Total	100.0	10.78

*) mg eq/kg ... mg equivalents of ^{14}C -cymoxanil based on kg fresh weight

Further treatment of separate portions from unextractable residue using 6 M HCl, cellulase and protease enzyme released 15.4 % (1.66 mg eq/kg), 3.4 % (0.36 mg eq/kg) and 12.7 % (1.37 mg eq/kg) of the TRR, respectively. An overview is given in table B.7.1.1.1-3.

Table B.7.1.1.1-3 Characterisation of ^{14}C -residues from pellet (i.e. unextractable residues) of mature lettuce foliage treated with ^{14}C -cymoxanil (equivalent to 4x 0.84 kg as/ha; PHI: 3 days) after separately acid hydrolysis, cellulase and protease digestion

	Released from Pellet		Remained in Pellet (finally unextractable residues)		Total (sum of released + remained residues from pellet)	
	Radioactive concentration					
Treatment	[% of TRR]	mg eq/kg *)	[% of TRR]	mg eq/kg *)	[% of TRR]	mg eq/kg *)
6 M HCl acid hydrolysis	15.4	1.66	12.5	1.35	27.9	3.01
Cellulase digestion	3.4	0.36	24.5	2.65	27.9	3.01
Protease digestion	12.7	1.37	15.3	1.64	28.0	3.01

*) mg eq/kg ... mg equivalents of ^{14}C -cymoxanil based on kg fresh weight

Identification of metabolites: Radioactive HPLC-analysis of surface wash and foliage extracts of mature lettuce (PHI 3 days) indicated rapid degradation of ^{14}C -cymoxanil in lettuce. ^{14}C -cymoxanil was detected only in surface wash (2.1 % of TRR, 0.23 mg eq/kg). In the foliage extract of mature lettuce no ^{14}C -cymoxanil was identified. The major extractable radioactive components from the mature washed lettuce were ^{14}C -glycine (30.6 % of TRR, 3.30 mg eq/kg) after acid hydrolysis and ^{14}C -glucose (19.6 % of TRR, 2.11 mg eq/kg). Further in minor concentration range peaks were identified as IN-KP533 (1.8 % of TRR, 0.20 mg eq/kg) and IN-KQ960 (5.8 % of TRR, 0.62 mg eq/kg) in mature washed lettuce extract, whereas unidentified radioactivity was summarised under "natural products" (6.8 % of TRR, 0.73 mg eq/kg). In surface wash also ^{14}C -glucose (1.6 % of TRR, 0.17 mg eq/kg), IN-KP533 (1.0 % of TRR, 0.11 mg eq/kg), IN-KQ960 (1.6 % of TRR, 0.18 mg eq/kg) and radioactivity incorporated as so called "natural products" (1.2 % of TRR, 0.12 mg eq/kg) were determined. In table B.7.1.1.1-4 the identification of extractable radioactive residues in aqueous fraction of mature lettuce foliage (surface wash and extract) is presented.

Table B.7.1.1.1-4 Identification of extractable ^{14}C -residues from mature lettuce foliage treated with ^{14}C -cymoxanil (equivalent to 4x 0.84 kg as/ha), PHI: 3 days

	Extractable ¹⁴ C-Residues: 72.1 % of TRR, 7.77 mg eq/kg					
Identity of metabolites	Surface wash		Foliage aqueous extract		Total (sum of surface wash + foliage aqueous extract)	
	Radioactive concentration					
	% of TRR	[mg eq/kg] *)	% of TRR	[mg eq/kg] *)	% of TRR	[mg eq/kg] *)
¹⁴ C-Cymoxanil	2.1	0.23	---	---	2.1	0.23

	Extractable ¹⁴ C-Residues: 72.1 % of TRR, 7.77 mg eq/kg					
Identity of metabolites	Surface wash		Foliage aqueous extract		Total (sum of surface wash + foliage aqueous extract)	
	Radioactive concentration					
	% of TRR	[mg eq/kg] *)	% of TRR	[mg eq/kg] *)	% of TRR	[mg eq/kg] *)
¹⁴ C-Glycine ^{a)}	---	---	30.6	3.30	30.6	3.30
¹⁴ C-Glucose	1.6	0.17	19.6	2.11	21.2	2.28
IN-KP533 ^{b), d)}	1.0	0.11	1.8	0.20	2.8	0.31
IN-KQ960 ^{c), d)}	1.6	0.18	5.8	0.62	7.4	0.80
Natural products ^{e)}	1.2	0.12	6.8	0.73	8.0	0.85
Total extractable residues ^{f)}	7.5	0.81	64.6	6.96	72.1	7.77

*) mg eq/kg ... mg equivalents of ¹⁴C-cymoxanil based on kg fresh weight

a) after acid hydrolysis and derivatization to PTC-¹⁴C-glycine

b) IN-KP533 ... [(Ethylamino)carbonyl]amino]oxoacetic acid, CAS reg. no. 105919-00-4

c) IN-KQ960 ... 3-Ethyl-4-(methoxyamino)-2,5-dioxo-4-imidazolidinecarboxamide

d) The metabolite was identified by HPLC retention time with reference substance.

e) Incorporation into multiple minor natural products. No further characterisation or identification was conducted.

f) Values were calculated per each column.

Further characterisation/identification of the unextractable residues after treatment with 6 M HCl, cellulase and protease digestion of mature lettuce pellets was reported. Remarkable radioactivity was released after acid hydrolysis (15.4 % of TRR, 1.66 mg eq/kg) or protease digestion (12.7 %, 1.37 mg eq/kg) and lower radioactivity after cellulase digestion (3.4 % of TRR, 0.36 mg eq/kg). A lot amount of radioactivity released of the lettuce pellets was identified (but not quantified) as ¹⁴C-sugars (glucose, fructose), so called "natural products" and a small amount was identified after acid hydrolysis or protease digestion (plus acid hydrolysis) as ¹⁴C-glycine (≤ 0.9 % of TRR, ≤ 0.10 mg eq/kg). See table B.7.1.1.1-5. Broad areas of several minor radioactive peaks appeared in all HPLC-analysis indicating excessive metabolism and incorporation into natural products.

Table B.7.1.1.1-5 Identification of unextracted ¹⁴C-residues from mature lettuce foliage treated with ¹⁴C-cymoxanil (equivalent to 4x 0.84 kg/ha), PHI: 3 days

Unextractable ¹⁴ C-Residues: 27.9 % of TRR, 3.01 mg eq/kg *)						
	Acid Hydrolysis		Cellulase Digestion		Protease Digestion	
	Radioactive concentration					
	[% of TRR]	[mg eq/kg]	[% of TRR]	[mg eq/kg]	[% of TRR]	[mg eq/kg]
Released from Pellet	15.4	1.66	3.4	0.36	12.7	1.37
¹⁴ C-Glycine ^{a)}	0.8	0.09	n.d.	n.d.	0.9	0.10
¹⁴ C-Sugars (glucose + fructose) ^{b)}	identified, but not quantified	identified, but not quantified	identified, but not quantified	identified, but not quantified	identified, but not quantified	identified, but not quantified
Natural products ^{c)}	identified, but not quantified	identified, but not quantified	identified, but not quantified	identified, but not quantified	identified, but not quantified	identified, but not quantified

*) mg eq/kg ... mg equivalents of ¹⁴C-cymoxanil based on kg fresh weight

- The acid hydrolysis and protease enzyme digestion extracts (plus acid hydrolysis) were derivatized with PITC to form PTC-¹⁴C-glycine. n.d. ... not detected
- The retention times of large peaks corresponded to ¹⁴C-glucose and ¹⁴C-fructose.
- Not further characterised or identified in this study.

Conclusion

In foliage extract of lettuce (*Lactuca sativa*, variety Prizehead) no parent compound was detected, but in foliage surface rinse solution (sodium phosphate buffer, pH 5.0) cymoxanil was identified at a level of 2.1% TRR (0.23 mg eq/kg). Cymoxanil was rapidly and extensively metabolised in lettuce primarily to glycine (surface wash: absent; aqueous foliage extract: 30.6 % of TRR or 3.30 mg eq/kg; pellet: ≤ 0.9 % TRR or ≤ 0.10 mg eq/kg). Minor metabolites were identified in surface wash and aqueous foliage extract, i.e. IN-KQ960 (total: 7.4 % of TRR or 0.80 mg eq/kg) and IN-KP533 (total: 2.8 % of TRR or 0.31 mg eq/kg). A further metabolism of cymoxanil results to incorporation of radioactivity in naturally occurring compounds such as carbohydrates like glucose. 21.2 % of TRR or 2.28 mg eq/kg was detected as glucose in surface wash and aqueous foliage extract. A substantial (but not quantified) amount of radioactivity was released as sugars (glucose and fructose) from the pellet.

B.7.1.1.2 Melkebeke T., van Noorloos B., 2003b, Metabolism, distribution, and expression of cymoxanil residues in lettuce - (Oxon)

Report No.: 257794

Guideline: DG Sanco doc. 7028/Vi/95 rev. 3 (22 July 1997), metabolism and distribution in plants

GLP: Yes

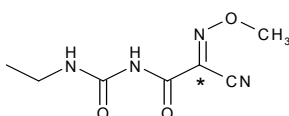
Study is considered acceptable for evaluation.

Study characteristics

reference	Melkebeke T., van Noorloos B., 2003b (IIA 6.1/02)	area	outdoor, container, NL
study title	Metabolism, distribution, and expression of cymoxanil residues in lettuce	application rate per treatment	equivalent to 0.240 kg as/ha (0.204 kg unlabelled + 0.036 kg labelled as/ha)
type of study	distribution and metabolism	concentration	equivalent to 0.160 g as [0.136 g cymoxanil + 0.024 g ¹⁴ C-cymoxanil ^b]/L
report number published	257794 no	vehicle	water (approx. equivalent to 1500 L/ha) ^c
year of execution	1999 (field phase) 1999-2002 (analytical phase)	number of treatments; dates of foliar applications	3; 31 May 1999 (BBCH 12-14), 22 June 1999 (BBCH about 42), 1 July 1999 (BBCH about 47)
test substance	Cymoxanil, batch 89800028 chem. purity 98.8 % (HPLC)+ [cyanoacetamide-2- ¹⁴ C]-cymoxanil ^a , batch 3304.265, radiochem. purity 99.8 % (HPLC), specific activity 22.49 mCi/mmol	harvest time	12 July 1999 (BBCH 49)
plant	Lettuce (<i>Lactuca sativa</i>), variety Patty	intervals between applications	9-22 days
testing facility; test site	NOTOX B.V., 's-Hertogenbosch, NL; Wageningen, NL (field phase),	total rate	equivalent to 0.720 kg as/ha

	's-Hertogenbosch, NL (analytical phase)		
soil type	peat mould	PHI	11 days

- a) Position of radiolabel in ^{14}C -cymoxanil: * Denotes position of ^{14}C radiolabel



CAS: 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide, E-isomer
IUPAC: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea
CAS reg. no.: 57966-95-7

- b) Corresponding 3x 10 MBq total nominally applied radioactivity.
c) Calculated by RMS. 0.240 kg as/ha over 0.00016 kg as/L results to 1500 L/ha.

Materials and methods

Study design: Eight lettuce plants (variety Patty, seedlings planted on 27 May 1997) were grown outdoors in peat mould in a polyethylene test container (small-scale test, surface area approx. 0.54 m², on average 0.068 m²/plant) at the test site. The lettuce plants were sprayed (using a hand sprayer with air pump) three times with an aqueous mixture of blank CYM 50% WP-formulation, cymoxanil and ^{14}C -cymoxanil at 9-22 days spraying intervals and at a rate equivalent to 0.240 kg as/ha per treatment (foliar application).

Sample collection: The harvest of the complete vegetative developed (mature) lettuce plants (BBCH 49) took place 11 days after the last (third) application. All plants were cut from the roots just above the soil surface and transferred to plastic bags. Soil were removed from the roots and also transferred to plastic bags. The total weight of sampled lettuce (without roots) was 5.26 kg (on average 0.658 kg/lettuce).

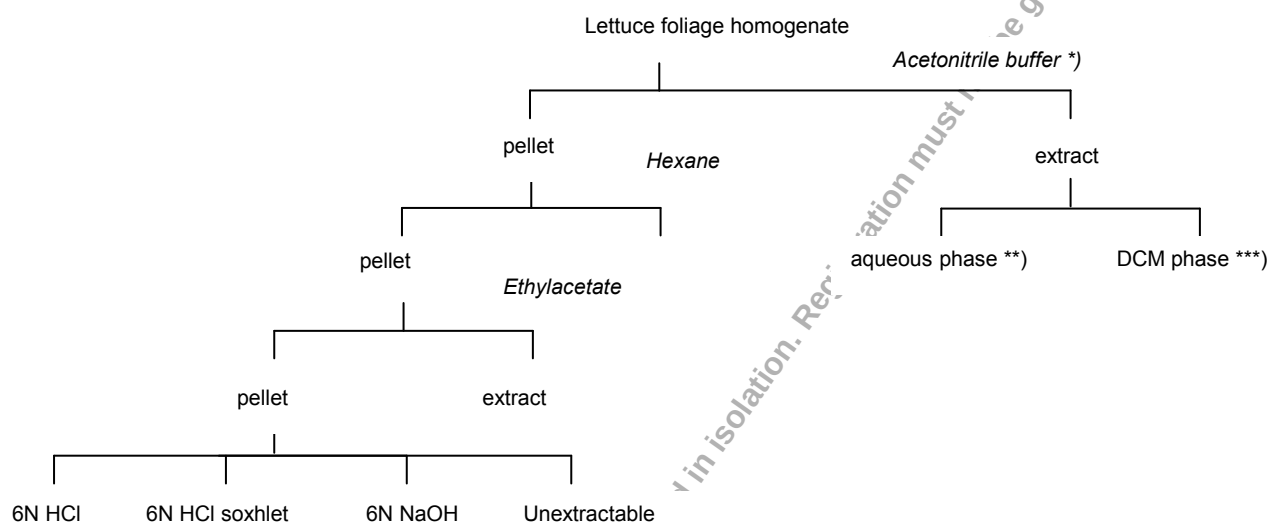
Sample preparation: Four lettuce plants (foliage under cold condition with dry ice) were cut, mixed and homogenized. A subsample of 500 g was macerated completely with dry ice in a food processor. After sublimation of dry ice the homogenized matrix was divided in five approx. 100 g analytical samples and stored in polyethylene boxes in the freezer before analysis.

Determination of TRR: The total radioactive residue (TRR) of homogenized foliage samples was determined by combustion/liquid scintillation counting (LSC) and calculated as mg/kg equivalents of ^{14}C -cymoxanil on a fresh weight basis. The limit of detection (LOD) was not reported in this study. A LOD of approx. 0.001 mg eq/kg was stated by the notifier. No surface wash sample of lettuce was taken for analysis.

Extraction of lettuce leaves homogenate and identification of metabolites: A first extraction on lettuce homogenate (started August 1999) showed a too low radioactivity concentration in the aqueous fraction for the chromatographic evaluation. A modified extraction process (fewer steps and larger - 150 g instead of 100 g - amount of matrix) of lettuce homogenate (performed in November 2000) allowed a chromatographic identification of metabolites without freeze-drying steps. The fractionation pattern during the extraction steps and the TLC chromatograms of the

aqueous fraction from August 1999 and November 2000 matched with each other. All quantitative results were based on the radioactivity in the original (first, August 1999) aqueous extract. An extraction scheme is given in figure B.7.1.1.2-1.

Figure B.7.1.1.2-1 Extraction scheme of (^{14}C)cymoxanil and its metabolites from lettuce matrix (Oxon)



- *) acetonitrile/sodium acetate buffer (1.0 M, pH 4.0)
 **) the aqueous fraction was incubated with β -glucosidase
 ***) DCM ... dichloromethane

Note: The storage stability of cymoxanil in frozen aqueous lettuce extract was shown stable for 20 months with representative TLC radio-chromatograms. The extraction procedure was considered suitable by the notifier, because 94.7 % of TRR was achieved in the mass balance.

The aqueous fraction was analysed on several TLC- and HPLC-systems and co-chromatographed with reference standards. The aqueous extract was further analysed on TLC after incubation with β -glucosidase (January 2001). Analysis for ^{14}C -glycine in aqueous plant extracts was performed applying derivatisation method to glycine (ortho-phthaldialdehyde and N-acetylcysteine under alkaline conditions formed a fluorescent isoindole derivate determined by UV- or radiodetection on HPLC). Additional indicative information on possible identity of metabolites was obtained by LC-MS/MS.

Findings

Distribution/fractionation of total radioactive residues (TRR): The TRR (extractable and unextractable radioactive residues) in mature lettuce (foliage) harvested 11 days after the final application accounted for 1.071 mg/kg ^{14}C -cymoxanil equivalents (12.3 % of total applied radioactivity on lettuce plants). Extraction of mature lettuce (foliage) with acetonitrile/sodium acetate buffer (1.0 M, pH 4.0) removed 67.9% of TRR (0.727 mg eq/kg). After partition of the acetonitrile/buffer-extract with dichloromethane (DCM) 2.5% of the TRR (0.027 mg eq/kg) was obtained in the DCM phase and 64.2% of the TRR (0.688 mg eq/kg) was detected in the aqueous fraction as polar compounds, respectively. The total organosoluble fractions (DCM, hexane and ethyl acetate) accounted for 3.0 % of the TRR (0.032 mg eq/kg). Using strong acid (6 M HCl) and

base (6 M NaOH) hydrolysable conjugates were released 14.1% (0.151 mg eq/kg) and 8.8% (0.094 mg eq/kg) of the TRR, respectively. Unextracted radioactivity after strong acid and base treatment represented 4.6% of the TRR (0.049 mg eq/kg). The distribution of fractionated TRR in mature lettuce leaves homogenate is shown in table 7.1.1.2-1.

Table B.7.1.1.2-1 Distribution/fractionation of ¹⁴C-residues in mature lettuce foliage treated with ¹⁴C-cymoxanil (equivalent to 3x 0.240 kg labelled + unlabelled as/ha), PHI: 11 days

Fractionation	Radioactive concentration	
	[% of TRR]	[mg eq/kg] *)
Extractable residues		
Acetonitrile/buffer	67.9	0.727
Aqueous phase	64.2	0.688
Dichloromethane(DCM) phase ^{a)}	2.5	0.027
Hexane ^{a)}	0.3	0.003
Ethylacetate ^{a)}	0.2	0.002
Unextractable residues		
6 M hydrochloric acid	14.1	0.151
6 M sodium hydroxide	8.8	0.094
Final solid residues ^{b)}	4.6	0.049
Sum of extractable + unextractable residues	94.7	1.014
Lost of residues	5.3	0.057
Total	100.0	1.071

*) mg eq/kg ... mg equivalents of ¹⁴C-cymoxanil based on kg fresh weight

^{a)} Organosoluble fraction (total: 3.0 % of TRR, 0.032 mg eq/kg)

^{b)} After extraction with acetonitrile/buffer, hexane, ethyl acetate plus strong acid and base hydrolysis

Characterisation of residues and identification of metabolites:

In the dichloromethane fraction 2.5 % of TRR or 0.027 mg eq/kg was determined using TLC. The radioactivity in the dichloromethane phase was separated into three zones, whereof only the largest one was identified as ¹⁴C-cymoxanil (1.4 % of TRR, 0.015 mg eq/kg). See table B.7.1.1.2-2.

Table B.7.1.1.2-2 Identification of ¹⁴C-residues (2.5 % of TRR, 0.027 mg eq/kg) in mature lettuce dichloromethane extract *)

Analysis method (best example)	Compounds	Radioactive concentration	
		[% of TRR]	[mg eq/kg] *)
TLC **)	unidentified	0.5	0.006
	¹⁴ C-cymoxanil	1.4	0.015
	unidentified	0.5	0.005

*) mg eq/kg ... mg equivalents of ¹⁴C-cymoxanil based on kg fresh weight

**) Plate: RP-18, 20 x 20 cm; mobile phase: methanol/0.2% formic acid, 7/3 (v/v)

The radioactivity of the aqueous fraction was separated by HPLC into six regions, whereof one peak representing 18.1 % of TRR or 0.193 mg eq/kg ^{14}C -cymoxanil equivalents, was identified as IN-W3595 (2-cyano-2-methoxyiminoacetic acid). No Cymoxanil was identified in the aqueous lettuce fraction. The other HPLC-peaks remained unidentified (≤ 7.9 % TRR, ≤ 0.084 mg eq/kg), except a part of one peak which was identified as ^{14}C -glycine after acid hydrolysis (approx. 13 % of the TRR or 0.139 mg eq/kg). No free ^{14}C -glycine was detected in the aqueous fraction prior acid hydrolysis. Further analysis (HPLC + TLC) on the aqueous fraction after incubation with β -glucosidase released no conjugated ^{14}C -cymoxanil or related metabolites. The characterisation (partial identification by HPLC) of ^{14}C -cymoxanil radioactivity in the mature lettuce aqueous fraction is shown in table 7.1.1.2-3.

Table B.7.1.1.2-3 Characterisation/partial identification of ^{14}C -residues (64.2 % of TRR, 0.688 mg eq/kg) in mature lettuce aqueous extract *)

Analysis method (best example)	Compounds	Radioactive concentration	
		[% of TRR]	[mg eq/kg] *)
HPLC **)	conjugated ^{14}C -glycine ^{a)}	13.0	0.139
	unidentified	6.0	0.064
	unidentified	7.2	0.077
	unidentified	5.6	0.060
	IN-W3595 ^{b)}	18.1	0.193
	unidentified	6.4	0.069

*) mg eq/kg ... mg equivalents of ^{14}C -cymoxanil based on kg fresh weight

**) Column: Lichrospher 60 RP-select B, 250 x 4 mm, dp 5 μm ; mobile phase: methanol/0.01 M TBABr in water, 37.5/62.5 (v/v)

^{a)} After acid hydrolysis (6 M HCl) released. The values of conjugated glycine are approximately. The whole region (peak) contains total 20.9 % of TRR (0.223 mg eq/kg).

^{b)} 2-cyano-2-methoxyiminoacetic acid, CAS reg. no. 78325-17-4

Note: A further identification of ^{14}C -residues was not reported.

Conclusion

Metabolism of ^{14}C -cymoxanil on/in mature lettuce (*lactuca sativa*, variety Patty) grown in field pots resulted mainly to IN-W3595 (2-cyano-2-methoxyiminoacetic acid, up to 18.1 % of TRR or 0.193 mg eq/kg) and ^{14}C -glycine (after acid hydrolysis, approx. 13 % of TRR or 0.139 mg eq/kg), both metabolites obtained in the aqueous fraction. A small amount of parent was identified in the dichloromethane fraction (1.4 % of TRR or 0.015 mg eq/kg). A major amount of radioactivity (range: 5.6-7.9 % of TRR, 0.060 - 0.084 mg eq/kg) remained unidentified, predominately characterised as part of the aqueous fraction or as strong acid or base hydrolysable residues.

B.7.1.2 Metabolism in potato

One metabolism study each concerning cymoxanil in potato tubers grown outdoors were submitted by DuPont (B.7.1.2.1) and Oxon (B.7.1.2.2).

B.7.1.2.1 Li Y. and Hausmann S.M., 1996, Plant metabolism of [2-¹⁴C]Cymoxanil in potatoes - (DuPont)

Report No.: AMR 3408-95

Guideline: US EPA pesticide assessment guidelines, subdivision O, series 171-4(a); others

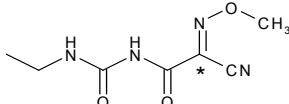
GLP: Yes

Study is considered acceptable for evaluation.

Study characteristics

reference	Li Y. and Hausmann S.M., 1996 (IIA 6.1/03)	area	outdoor, pots, USA
study title	Plant metabolism of [2- ¹⁴ C]Cymoxanil in potatoes	application rate per treatment	equivalent to 0.404 kg as/ha ^{b)}
type of study	distribution and metabolism	concentration	equivalent to 0.432 g as/L ^{c)}
report number, published	AMR 3408-95, no	vehicle	water (approx. equivalent to 935 L/ha)
year of execution	1995 (field phase) 1995 - 1996 (analytical phase)	number of treatments; dates of foliar applications	3; 8 Sept. 1995 (BBCH about 122), 28 Sept. 1995 (BBCH about 401-403), 6 Oct. 1995 (BBCH not reported)
test substance	[2- ¹⁴ C]Cymoxanil ^{a)} , NEN lot no 2732-155, radiochemical purity 95.6 % (HPLC), specific activity 14.09 mCi/g	harvest time	9 Oct. 1995 (BBCH about 409)
plant	potato, variety Red Pontiac	intervals	8-20 days
testing facility, test site	DuPont Agricultural Products, Newark (field phase) plus Wilmington (analytical phase), Delaware, USA	total rate	equivalent to 1.212 kg as/ha
soil type	Sassafras sandy loam soil	PHI	3 days

^{a)} Position of radiolabel in ¹⁴C-cymoxanil: * Denotes position of ¹⁴C radiolabel



CAS: 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide, E isomer

IUPAC: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea

CAS reg. no: 57966-95-7

Notes: Z-isomer was co-chromatographed by HPLC and separated from E-isomer.

^{b)} lb/acre were converted to kg as/ha by RMS.

^{c)} Calculated by RMS: (404 g as/ha) : (935 L/ha) = 0.432 g as/L

Material and Methods

Study design: After uniform germination of potatoes (variety Red Pontiac) in 10 pots (diameter of 36.8 cm and 0.1064 m² surface area per each pot) filled with Sassafras sandy loam soil in a greenhouse, the pots were moved outdoor at the test site. 6 of 10 pots with potato plants were treated (foliar sprayed using a hand-held spray equipment) 3 times with ¹⁴C-cymoxanil, formulated with inert ingredients to simulate a wettable powder formulation, at a rate equivalent to 0.404 kg as/ha per treatment. 4 field pots of potato plants remained untreated as controls.

Sample collection: Three days after the last application mature potato tubers were harvested and composite foliage samples were collected at crop maturity. Control and treated potato tubers were also sampled before the second treatment and 3 days after the last treatment (harvest time). The

adhering soil from the hand-harvested potato tubers was removed by gently hand washing. The dried potato tubers were frozen until analysis. Only the mature potato tubers (without a separate taken washing sample) were analysed. The total amount of sampled treated mature potato tubers add up to 2.808 kg.

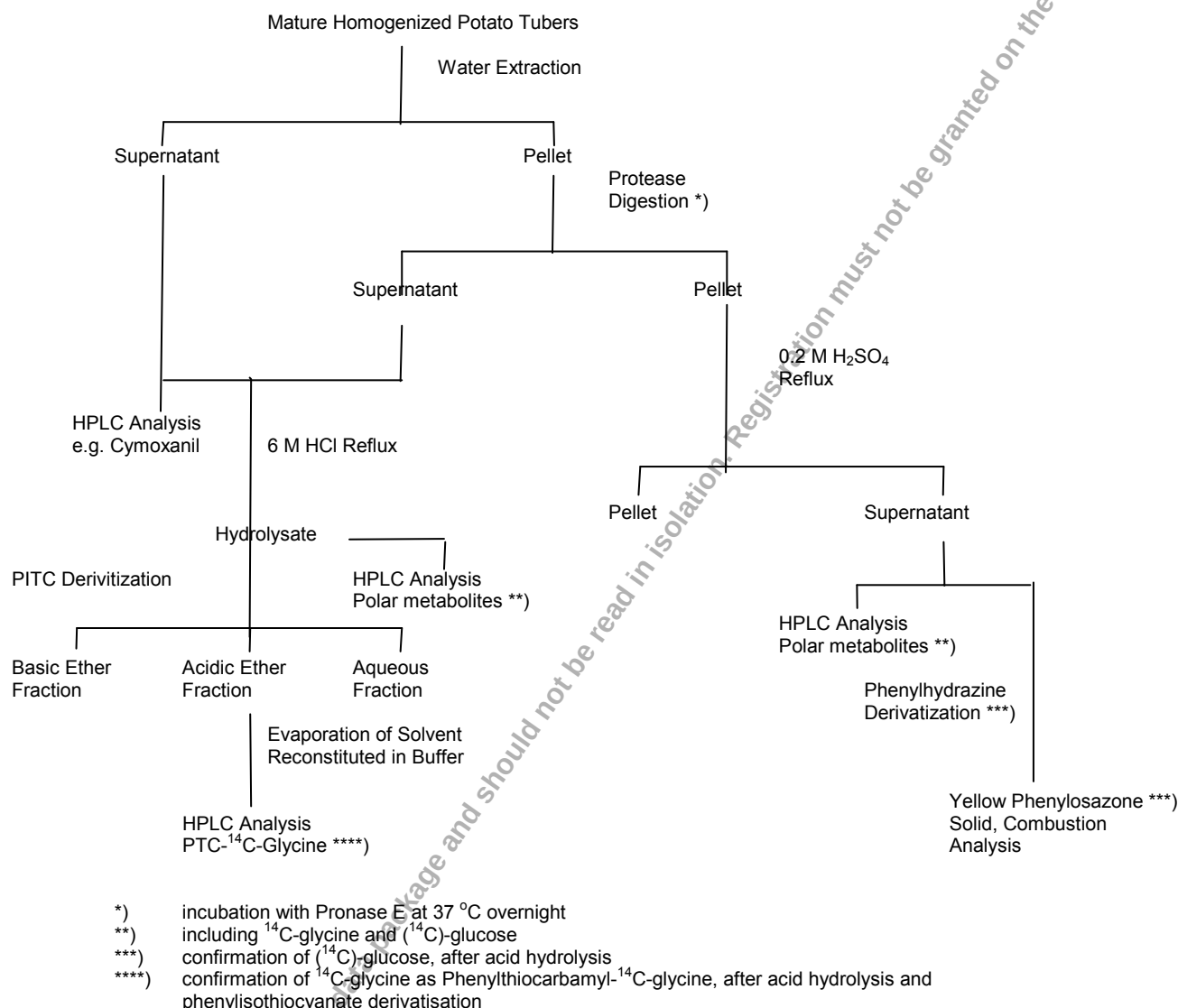
Sample preparation: Homogenization of representative potato tuber samples was performed using an ultra homogenizer. Dry ice was not used in the homogenization process.

Determination of TRR: The total radioactive residue (TRR) of treated or untreated samples were analysed by combustion/liquid scintillation counting (LSC) and calculated as mg/kg equivalents of ^{14}C -cymoxanil on a fresh weight basis. The LOD (limit of detection) for combustion/LSC (or HPLC-radiodetection) was about 0.01 mg eq/kg (radioactivity detected above two times of the average background).

Extraction of potato tuber homogenate and identification of metabolites: The homogenised mature potato tuber containing more than 0.01 mg eq/kg radioactive residue were initially extracted with sodium acetate buffer (20 mM, pH 5.0), substituted later by an extraction step with water (since no ^{14}C -cymoxanil was detected in the buffer extract). In figure B.7.1.2.1-1 an overview of the extraction procedure is given.

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

Figure B.7.1.2.1-1 Extraction scheme of (¹⁴C)cymoxanil and its metabolites for potato matrix (DuPont)



Note: The storage stability of frozen potato tuber extracts was shown stable for at least 6.5 months with representative radio-HPLC data. The extraction procedure was verified by enforced extraction methods (i.e. report no. AMR 3333-95 and AMR 3705-95 with cymoxanil recoveries from 90 to 110 % in freshly fortified control extracts).

The metabolites were characterised and identified by several HPLC methods (UV-, radiochemical detection), whereby ¹⁴C-glycine was confirmed as PTC(phenylthiocarbamyl)-¹⁴C-glycine (after derivatisation procedure with phenylisothiocyanate - PITC). The pellet released after water extraction and protease digestion (combusted to determine the unextractable TRR with LSC) were hydrolysed with diluted mineral acid [0.2 M sulphuric acid under reflux to hydrolyse (¹⁴C)-starch to (¹⁴C)-glucose] for further characterisation of polar metabolites by HPLC, e.g. (¹⁴C)-glucose was confirmed after derivatisation with phenylhydrazine as corresponding phenyllosazone. Untreated mature tubers (control samples) were extracted and analysed in the same manner as treated mature tubers.

Findings

Distribution/Fractionation of total radioactive residues (TRR): 90.0 % of the detected TRR (0.62 mg eq /kg) was obtained in the extractable fraction (aqueous phase), 10 % of the TRR remained in the unextractable residues (pellets). Table B.7.1.2.1.-1 presents the distribution/fractionation of radioactive residues derived from ^{14}C -cymoxanil in mature potato tuber.

Table B.7.1.2.1-1 Distribution/fractionation of ^{14}C -residues in mature potato tubers treated with 3x 0.404 kg ^{14}C -cymoxanil per hectare, PHI: 3 days

Sample fractions	Radioactive concentration	
	[% of TRR]	[mg eq/kg] *)
Extractable residues ^{a)}	90.0	0.62
Unextractable residues	10.0	0.07
Total residues (sum of extractable + unextractable residues)	100.0	0.69

*) mg eq/kg ... mg equivalents of ^{14}C -cymoxanil based on kg fresh weight

^{a)} TRR of water extract and released from the water extracted pellet after protease digestion were combined and calculated together as extractable residues.

Notes: No further refinement of fractionation (e.g. use of an organic solvent system) was reported.

Characterisation/Identification of residues: A very small amount of free ^{14}C -glycine (without acid hydrolysis) was detectable (HPLC radiochromatogram), but not separately quantified. The major compound found in the water extractable radioactivity (plus protease digestion supernatant of pellet) of mature potato tubers after 6 M HCl acid hydrolysis was ^{14}C -glycine (78.5 % of TRR, 0.54 mg eq/kg). No cymoxanil or structurally related metabolites were found in mature potato tuber samples. The unextractable radioactivity was mainly identified as ^{14}C -starch, which was hydrolysed with mineral acid to (^{14}C -)glucose. Non radioactive residue data were reported from potato foliage or surface wash (foliage or tubers) or from the test soil. An extraction procedure using an organic solvent system was not performed in this study. The characterisation and partly identification of radioactivity in mature potato tubers are shown in table 7.1.2.1.-2.

Table B.7.1.2.1-2 Characterisation/partly identification of ^{14}C -residues in mature potato tubers treated with 3x 0.404 kg ^{14}C -cymoxanil per hectare, PHI: 3 days

Sample fraction		Radioactive concentration	
		[% of TRR]	[mg eq/kg] *)
Extractable residues ^{a)}		90.0	0.62
	^{14}C -glycine ^{b)}	78.5	0.54
	unidentified	11.5	0.08
Unextractable residues		10.0	0.07
	^{14}C -starch ^{c)}	8.1	0.06
	unidentified	1.9	0.01
Total residues (sum of extractable + unextractable radioactive residues)		100.0	0.69

- *) mg eq/kg ... mg equivalents of ^{14}C -cymoxanil based on kg fresh weight
a) TRR of water extract and released from the water extracted pellet after protease digestion were combined and calculated as extractable residue.
b) After 6 M HCl hydrolysis a fractionation in basic ether (3 % of TRR), acidic ether (68 % of TRR) and aqueous fraction (29 % of TRR) resulted. The percentage on PTC- ^{14}C -glycine of each fraction was normalised to 100 %.
c) Detected as PTC- ^{14}C -glucose (HPLC) after acid hydrolysis (0.2 M sulphuric acid) of the unextractable potato tuber pellet.

Notes: No further characterisation or identification (e.g. 11.5 % of TRR on unidentified extractable fraction; extraction with organic solvent systems; incubation with β -glucosidase) is reported in this study.

Conclusion

The parent compound was not detected in mature potato tuber (variety Red Pontiac). As main metabolite in the mature potato tuber homogenate glycine was identified (after acid hydrolysis and protease digestion of aqueous fraction: 78.5 % of TRR or 0.54 mg eq/kg). A significant amount of glucose (8.1 % of TRR or 0.06 mg eq/kg) was released from the starch in the pellet of mature potato tuber homogenate after acid hydrolysis. No other structurally related metabolites of ^{14}C -cymoxanil have been detected in mature potato tuber. This study indicates an extensively metabolism of ^{14}C -cymoxanil resulting to glycine and incorporation of radioactivity in naturally compounds (like carbohydrates, peptides or proteins).

B.7.1.2.2 Melkebeke T., van Noorloos B., 2003a, Metabolism, distribution, and expression of cymoxanil residues in potatoes - (Oxon)

Report No.: 257772

Guideline: DG Sanco doc. 7028/Vi/95 rev. 3 (22 July 1997), metabolism and distribution in plants

GLP: Yes

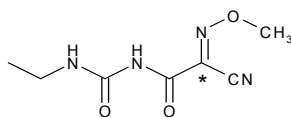
Study is considered acceptable for evaluation.

Study characteristics

reference	Melkebeke T., van Noorloos B., 2003a (IIA 6.1/04)	area	outdoor, container, NL
study title	Metabolism, distribution, and expression of cymoxanil residues in potatoes	application rate per treatment	equivalent to 0.240 kg as/ha (0.186 kg labelled + 0.054 kg unlabelled as/ha)
type of study	distribution and metabolism	concentration	equivalent to e.g. 0.160 g as [0.124 g cymoxanil + 0.036 g ^{14}C -cymoxanil ^{b)}]/L
report number published	257772 no	vehicle	water (approx. equivalent to e.g. 1500 L/ha ^{c)})
year of execution	1999 (field phase) 1999 - 2002 (analytical phase)	number of treatments; dates of foliar applications	8; 22 June 1999 (BBCH 45-50), 1 July 1999 (BBCH about 51-59), 9 July 1999 (BBCH about 60-65), 19 July 1999 (BBCH about 66-69), 4 Aug. 1999 (BBCH about 70-79), 17 Aug. 1999 (BBCH about 80-89), 24 Aug. 1999 (BBCH 91-94), 31 Aug. 1999 (BBCH: 95-97)
test substance	Cymoxanil, batch 89800028, chem. purity 98.8 % (w/w) + [cyanoacetamide-2- ^{14}C]-cymoxanil ^{a)} , batch 3304.265, radiochem. purity 99.8 % (HPLC), specific activity 22.49 mCi/mmol	harvest time	10 Sept.1999 (BBCH: 97)
plant	potato, variety Bild Star	intervals	7-16 days
testing facility; test site	NOTOX B.V., 's-Hertogenbosch; Wageningen (field phase), 's-Hertogenbosch (analytical phase),	total rate	1.920 kg as/ha (1.488 kg unlabelled + 0.432 kg labelled as/ha)

	NL		
soil type	peat mould	PHI	10 days

- a) Position of radiolabel in ^{14}C -cymoxanil: * Denotes position of ^{14}C radiolabel



CAS: 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide, E isomer
IUPAC: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea
CAS reg. no: 57966-95-7

- b) Corresponding 8x 15 MBq total nominally applied radioactivity.
c) Calculated by RMS: (240 g as/ha) : (0.160 g as/L) = 1500 L/ha

Material and Methods

Study design: Three potato tubers (variety Bild Star) were planted (19 May 1999) and grown outdoor in peat mould in a polyethylene container (surface area approx. 0.54 m², approx. 0.18 m² per potato plant) at the test site. The potato plants were sprayed (using handsprayer operated with air pump to foliar application) eight times with an aqueous mixture of blank 50% WP-formulation, cymoxanil and ^{14}C -cymoxanil at 7-16 days spraying intervals and at a rate equivalent to 0.240 kg as/ha.

Sample collection: 10 days after the last application the potato plants (BBCH: 97) were harvested. All plant parts above the soil surface (dried tops) were taken away, the potato tubers were removed from the soil and rotten tubers (total 0.285 kg) were separated from the remaining tubers (total 5.708 kg). The potato tubers were stored at -20 °C in sealed plastic bags. Dried tops of potato or test soil were not analysed.

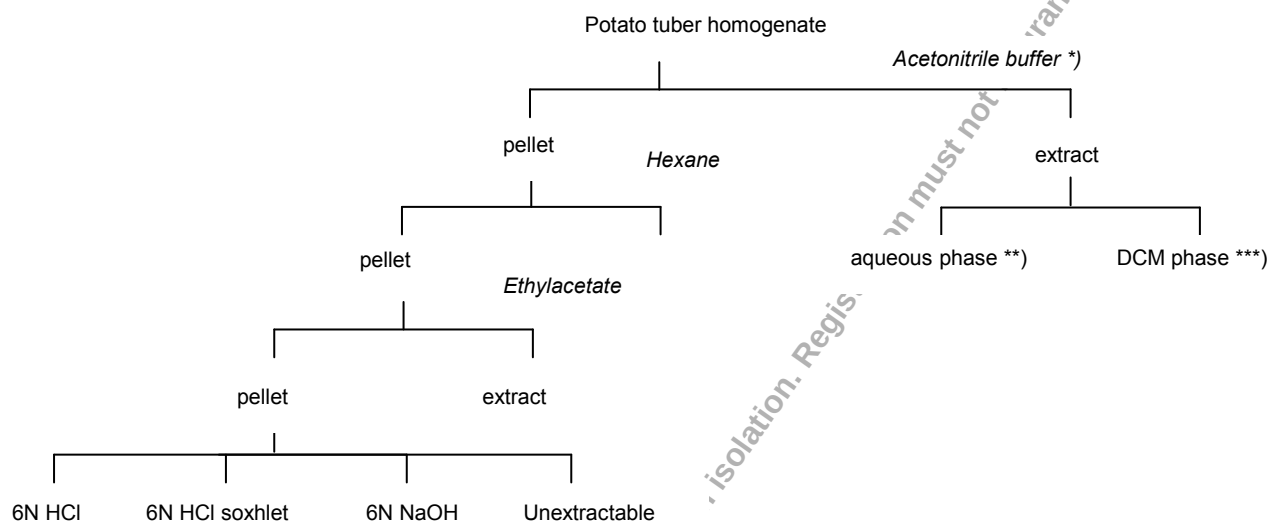
Sample preparation: Two months after the harvest a representative frozen tuber sample was thawed at room temperature, rinsed with water, dried with tissue, cut into pieces and complete macerated with dry ice in a food processor. After sublimation of dry ice the homogenized tuber matrix was divided in 4 samples and stored in plastic containers in the freezer.

Determination of TRR: The total radioactive residue (TRR) of homogenized mature potato tuber samples was determined by combustion/liquid scintillation counting (LSC) and calculated as mg equivalents of ^{14}C -cymoxanil per kg fresh weight basis. The limit of detection (LOD) was not reported in this study. According to the notifier a LOD of approx. 0.001 mg eq/kg was indicated.

Extraction of potato homogenate and identification of metabolites: A first extraction of potato tuber homogenate was conducted two months after harvest, but the radioactivity concentration in the aqueous fraction was too low to allow adequate identification of the radioactive residue. The extraction procedure was repeated after one year using a larger amount of potato homogenate and only two extraction steps with acetonitrile/buffer solution. All quantitative results were based on the detected radioactivity in the original (first) aqueous extract, as the fractionation pattern (Nov. 1999/Nov.2000) during the extraction steps and the TLC-chromatograms of the aqueous fraction

matched with each other. Figure B.7.1.2.2-1 gives an overview about the extraction scheme for potato tuber homogenate.

Figure B.7.1.2.2-1 Extraction scheme of (^{14}C)cymoxanil and its metabolites for potato tuber matrix (Oxon)



- *) acetonitrile/sodium acetate buffer (1.0 M, pH 4.0)
 **) the aqueous fraction was incubated with β -glucosidase
 ***) DCM ... dichloromethane

Notes: The storage stability of the frozen potato tuber extract was shown stable for at least 18 months with representative TLC data. As 102.2 % of TRR was achieved in the mass balance, the extraction procedure was considered suitable by the notifier.

For analysis of the potato tuber extracts various TLC- and HPLC-methods (cochromatographed with proposed metabolites as reference standards) were used. A specific analytical method for ^{14}C -glycine in aqueous extract was developed using ortho-phthaldialdehyde and N-acetylcysteine under alkaline conditions to form a fluorescent isoindole derivate determined by UV- or radiodetection on RP-HPLC.

Findings

Distribution/fractionation of residues: 4.7 % of the total applied radioactivity on the potato plants was detected in the mature potato tubers at harvest, equivalent to 1.045 mg ^{14}C -cymoxanil/kg fresh weight. Fractionation of the TRR revealed that 56.2 % of TRR (0.585 mg eq/kg) in mature potato tubers was extractable with acetonitrile/sodium acetate buffer. Partitioning of acetonitrile/buffer extract with dichloromethane (DCM) showed 53.2 % of TRR (0.556 mg eq/kg) in the aqueous fraction as polar compounds and 0.3 % of TRR (0.003 mg eq/kg) in the organic fraction (DCM). Further extraction steps of the residual solids (remaining after acetonitrile/buffer fractionation) with hexane or extraction of the hexane solid with ethyl acetate released ≤ 0.1 % of TRR (≤ 0.001 mg eq/kg) in the organic phase by each single solvent. Treatment of the PES (post extraction solid) with strong acid and base released radioactivity, which accounted for 9.4 % of TRR (0.098 mg eq/kg) and 2.7 % of TRR (0.029 mg eq/kg), respectively. The unextracted radioactivity after strong

acid and following strong alkaline treatment represented 36.4 % of TRR (0.380 mg eq/kg). In table B.7.1.2.2-1 the distribution/fractionation of radioactive residues is summarised.

Table B.7.1.2.2-1 Distribution/fractionation of ^{14}C -residues in mature potato tubers treated equivalent to 8x 0.240 kg ^{14}C -cymoxanil and radioactive unlabelled cymoxanil per hectare, PHI: 10 days

	Radioactive concentration	
	[% of TRR]	[mg eq/kg] *)
Extractable residues		
acetonitrile/aqueous buffer	56.2	0.585
aqueous phase	53.2	0.556
dichloromethane(DCM) phase ^{a)}	0.3	0.003
hexane ^{a)}	0.1	< 0.001
ethylacetate ^{a)}	< 0.1	0.001
Unextractable residues	50.5	0.527
6 M hydrochloric acid	9.4	0.098
6 M sodium hydroxide	2.7	0.029
remaining unextractables ^{b)}	36.4	0.380
Total residues ^{c)} (sum of extractable and unextractable residues)	102.2	1.068

*) mg eq/kg ... mg equivalents of ^{14}C -cymoxanil based on kg fresh weight

^{a)} organic soluble fraction; not further identified

^{b)} unextractable fraction

^{c)} Standardisation to 100 % of TRR results in 1.045 mg ^{14}C -cymoxanil equivalents/kg.

Characterisation of residues and identification of metabolites: Analysis of the aqueous phase extract done by HPLC (see table B.7.1.2.2-2) revealed up to five radioactive zones, one large unretained zone (34.1% of TRR; 0.356 mg eq/kg) and four smaller zones (≤ 5.9 % TRR, ≤ 0.062 mg eq/kg) were obtained. After comparison with reference standards (postulated metabolites) the zones (compounds) were not advanced identified, only characterised as very polar. Glycine analysis of the aqueous fraction confirmed the absence of free ^{14}C -glycine. After acid hydrolyses of the aqueous fraction a response for ^{14}C -glycine derivate equivalent to about 27 % of the TRR (about 0.282 mg eq/kg) was obtained. Neither (^{14}C -)cymoxanil nor any other metabolites were released after β -glucosidase treatment (analysed with TLC). TLC separated the radioactive residue of the aqueous fraction into three zones (see table B.7.1.2.2-2), which were not identified in this study. No analysis were performed from the rinse water of mature potato tubers. In table 7.1.2.2-2 the characterisation/partially identification of radioactive residue in the aqueous fraction from mature potato tuber is shown.

Table B.7.1.2.2-2 Characterisation/partly identification of ^{14}C -residues (53.2 % of TRR, 0.556 mg eq/kg) in aqueous extract from mature potato tuber *)

Analysis method (best examples)	compounds	Radioactive concentration	
		[% of TRR]	[mg eq/kg] **)
TLC ***)	unidentified	4.2	0.043
	conjugated ^{14}C -glycine proposed ^{a)}	27.0	0.282
	unidentified	22.0	0.230
HPLC ****)	identified as conjugated ^{14}C -glycine ^{b)}	27.0	0.282
	unidentified	4.1	0.043
	unidentified	5.6	0.059
	unidentified	3.4	0.036
	unidentified	5.9	0.062

*) The potato plants in field container were treated equivalent to 8x 0.240 kg ^{14}C -cymoxanil + unlabelled cymoxanil per hectare, PHI (10 days)

**) mg eq/kg ... mg equivalents of ^{14}C -cymoxanil based on kg fresh weight

***) Plate: RP 18, 20 x 20 cm; mobile phase: methanol/0.2% formic acid, 7/3 (v/v)

****) Column: Lichrospher 60 RP-select B, 250 x 4 mm, dp 5 μm ; mobile phase: methanol/0.01 M TBABr in water, 37.5/62.5 (v/v)

^{a)} Spot was proposed as conjugated ^{14}C -glycine, but not suitable for identification.

^{b)} Approximately values. The whole region (peak) contains 34.1 % of TRR and 0.356 mg eq/kg, whereas 7.1 % of TRR or 0.074 mg eq/kg were not identified.

Note: No further identification was reported.

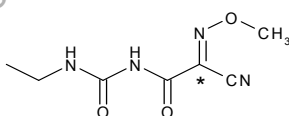
Conclusion

No parent compound was detected in mature potato tubers (variety Bild Star). Metabolism of cymoxanil resulted in conjugated glycine (about 27% TRR or 0.282 mg eq/kg) as the main identified metabolite in mature potato tuber. The majority of the unidentified radioactive residue was characterised as polar metabolites or material incorporated in natural compounds.

B.7.1.3 Overall conclusion on the metabolic fate on crops (DuPont + Oxon)

With respect to the intended uses the plant metabolism of cymoxanil was demonstrated on two crop groups, i.e. leaf vegetables (lettuce, lactuca sativa) and root and tuber vegetables (potato), respectively. From each notifier (DuPont and Oxon) one metabolism study per crop group was submitted. All four studies were conducted under outdoor conditions using pots or containers.

In all four submitted plant metabolism studies ^{14}C -cymoxanil was radioactive labelled at the same position:



* Denotes position of ^{14}C radiolabel

CAS: 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide, E-isomer

IUPAC: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea

CAS reg. no: 57966-95-7

Lettuce

Only the lettuce foliage was analysed in both studies. A small amount of parent compound was identified in both studies (DuPont: 2.1 % of TRR or 0.23 mg eq/kg in aqueous fraction; Oxon: 1.4 % of TRR or 0.015 mg eq/kg in organosoluble fraction). Conjugated glycine was identified as a main metabolite in both studies, with range of approx. 13.0 % of TRR or 0.139 mg eq/kg (Oxon) to 30.6 % of TRR or 3.30 mg eq/kg (DuPont). Only in the study submitted by DuPont a further main metabolite, i.e. glucose, was detected with 21.2 % of TRR or 2.28 mg eq/kg in the aqueous fraction. Another main metabolite, i.e. IN-W3595 up to 18.1 % of TRR or 0.193 mg eq/kg, was identified in the study submitted by Oxon, only. As minor metabolites IN-KQ960 (7.4 % of TRR, 0.80 mg eq/kg) and IN-KP533 (2.8 % of TRR, 0.31 mg eq/kg) were detected in the aqueous fraction of lettuce foliage only in the DuPont presented study (with significant higher amount of total applied ¹⁴C-cymoxanil and lower PHI than in the Oxon study). The two submitted studies provide supplementary and feasible information on the metabolism pathway of cymoxanil in lettuce foliage. An extensive metabolism of cymoxanil on/in lettuce can be considered with intermediate metabolites (like IN-W3595, IN-KQ960 or IN-KP533), glycine as main and relevant metabolite, which can be form conjugates or be incorporated in natural plant products (like carbohydrates, peptides or proteins).

Potato

In both submitted studies only the potato tuber was analysed. The parent compound was not detected in relevant concentrations in/on mature potato tuber of both studies. The main metabolite was glycine identified after acid hydrolysis of mature potato tuber homogenate in both studies. The released glycine was detected in a concentration range from 27.5 % of TRR or 0.280 mg eq/kg (Oxon) to 78.5 % of TRR or 0.54 mg eq/kg (DuPont). As minor metabolite glucose (originating from starch) was detected at a concentration level of 8.1 % of TRR (0.06 mg eq/kg) after acid hydrolysis of mature potato tuber pellet only in the DuPont submitted study (with significant higher applied amount of total ¹⁴C-cymoxanil and lower PHI). No further metabolites were identified in the study presented by Oxon. The both studies submitted show an extensive metabolism of cymoxanil resulting in glycine (conjugated/incorporated with/in natural substances) as main detected metabolite and glucose (primarily originating from starch). Intermediate metabolites were not identified in the two presented studies.

Lettuce and potato

The submitted metabolism studies on lettuce (leaf vegetable) and potato (roots and tuber vegetable) indicated a rapid and extensive degradation of parent compound. Cymoxanil was metabolised over intermediates (like IN-W3595, IN-KQ960 or IN-KP533) to glycine, which was further conjugated or incorporated with or in natural substances (e.g. carbohydrates, peptides or proteins). The proposed plant metabolic pathway based on the results of the metabolism studies on lettuce and potato submitted by DuPont and Oxon is presented in figure B.7.1.3.-1.

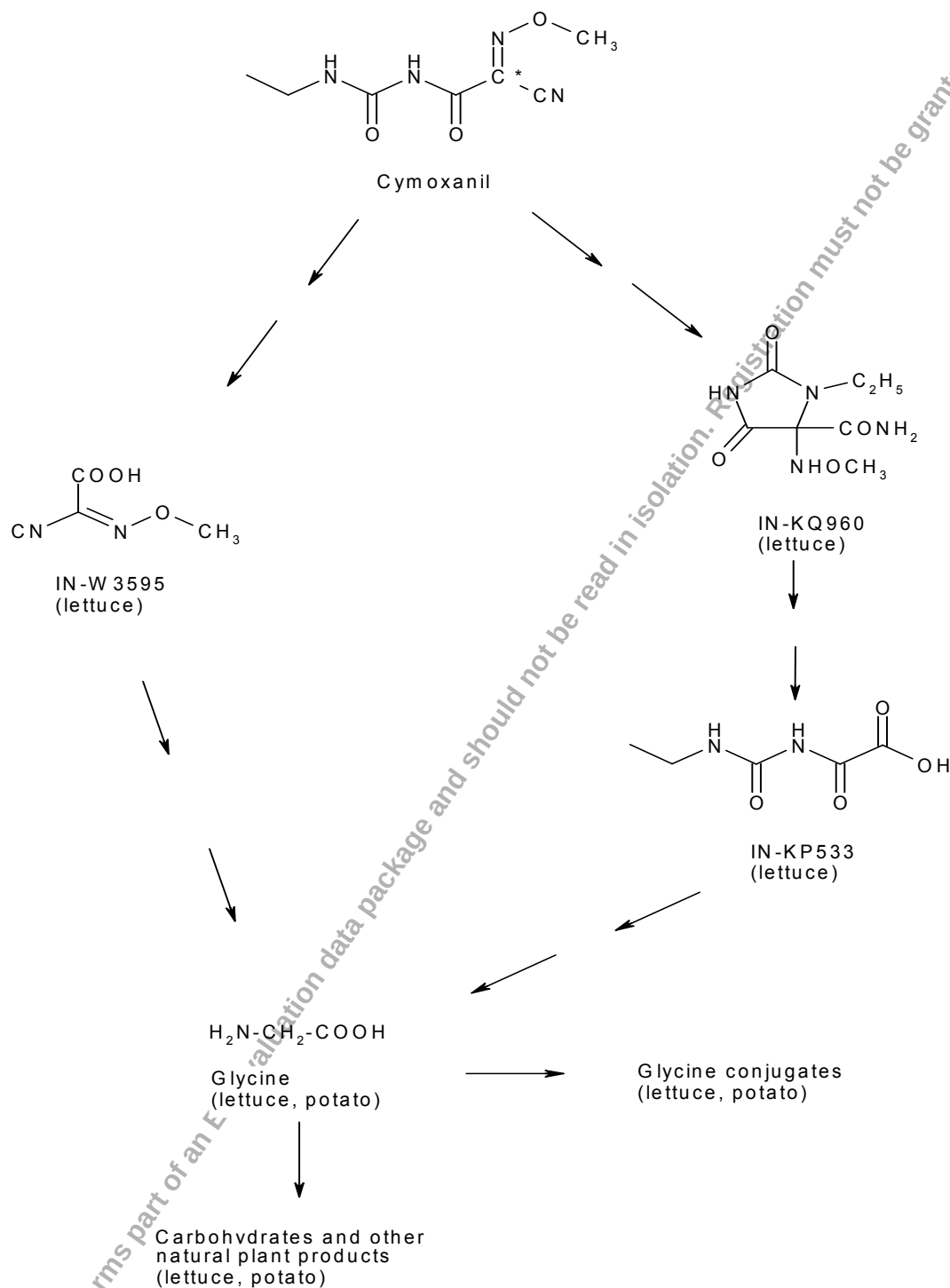
As shown in the rat metabolism studies several intermediate metabolites (like IN-W3595 or IN-KQ960) were obtained, which finally led to glycine and its further conjugation and re-incorporation

with/in natural substances. Nevertheless the metabolite IN-KP533, which was identified in one of the two lettuce metabolism studies at a low concentration of 2.8 % TRR in surface wash and foliage extract, was not examined in the rat model system. To get a complete picture of the toxicological relevance there should be an investigation additionally on IN-KP533 in the rat.

As a proposal cymoxanil is considered as toxicological relevant compound and therefore recommended for the residue definition of risk assessment and monitoring, respectively.

Figure B.7.1.3-1

Proposed metabolic pathway of cymoxanil in plant matrixes (lettuce leaves and potato tubers)



* indicates radioactive label site

Cymoxanil ... 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide, E-isomer, CAS reg. no: 57966-95-7

IN-KQ960 3-Ethyl-4-(methoxyamino)-2,5-dioxo-4-imidazolidinecarboxamide

IN-KP533 ... [[[(Ethylamino)carbonyl]amino]oxoacetic acid, CAS reg. no. 105919-00-4

IN-W3595 ... 2-cyano-2-methoxyiminoacetic acid, CAS reg. no. 78325-17-4

B.7.2 Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2; Annex IIIA, 8.1)

The metabolism of cymoxanil in livestock has been investigated in mature lactating goat. Additionally, an in vitro test with bovine rumen (micro)organisms was supplementary submitted by DuPont only (data sharing with Oxon).

Note: Since there is evidence of a "non residue" situation of cymoxanil (< LOQ, < 0.05 mg/kg) in the plant raw agriculture commodities as shown in the supervised residue trials (lettuce and potato), rotational crop studies (lettuce, sugar beets and spring wheat) and plant metabolism studies (lettuce and potato), no metabolism study in livestock is regarded necessary.

B.7.2.1 Anderson J. J., Gaddamidi V., Moghaddam M. F., Swain R. S., Hashinger B.M., Lilly D.E., 1996, The distribution of [2-14C]-DPX-T3217 (cymoxanil) in the lactating goat (nature of residue study to EPA guideline)

Report No.: AMR 2084-91

Guideline: US EPA guideline part 171-4a, nature of the residue in livestock.

Note from notifier: No major variations to the recommended DG Sanco doc. 7030/VI/95-rev 3, 22/7/1997, metabolism and distribution in domestic animals

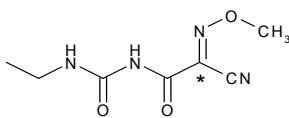
GLP: Yes

Study is considered acceptable for evaluation.

Study characteristics

reference	Anderson J.J, et al., 1996 (IIA 6.2/01)	group size	for goat: 1 treated ^{b)} , 1 control
study title	The distribution of [2-14C]-DPX-T3217 (cymoxanil) in the lactating goat (nature of residue study to EPA guideline)	exposure	for goat: 3 consecutive days
type of study	distribution and metabolism	dose rate	for goat: 15 mg ¹⁴ C-cymoxanil/day, total: 45 mg ¹⁴ C-cymoxanil/3 consecutive days
year of execution	1992-1996	route	for goat: oral, by gelatine capsule
test substance	for goat: [2- ¹⁴ C]-cymoxanil ^{a)} , NEN lot 2732-155, radiochem. purity 98.2 % (HPLC), specific activity 14.09 mCi/g for in vitro rumen fluid test: [2- ¹⁴ C]-cymoxanil ^{a)} , no NEN lot no. reported, radiochem. purity 99.5 % (HPLC), specific activity 76.35 mCi/g	testing facility; test site	[REDACTED] [REDACTED] [REDACTED]
animal and other test system	goat (British Saanen); in vitro rumen fluid test system	origin of bovine rumen fluid	rumen-fistulated, 7-8 year old Holstein steer; [REDACTED]

- a) Position of radiolabel in ^{14}C -cymoxanil: * Denotes position of ^{14}C radiolabel



CAS: 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide, E isomer

IUPAC: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea

CAS reg. no.: 57966-95-7

Notes: Z-isomer was co-chromatographed by HPLC and separated from E-isomer.

- b) equivalent to 10 mg ^{14}C -cymoxanil/kg diet and day and to 0.34 mg ^{14}C -cymoxanil/kg bw and day, assuming a body weight of 44 kg and a feed intake of 1.5 kg/day.

Material and Methods

Study design for lactating goat

Dosing: Two mature lactating goats (British Saanen, *capra hircus*) were used, one as control and the second were treated with radioactive labelled cymoxanil. ^{14}C -cymoxanil was administered to lactating goat by gavage in a gelatine capsule just after morning milking on each of three consecutive days at a dose equivalent to a dietary concentration of 10 mg/kg diet and day. The control goat received the gelatine capsules by gavage with no added ^{14}C -cymoxanil.

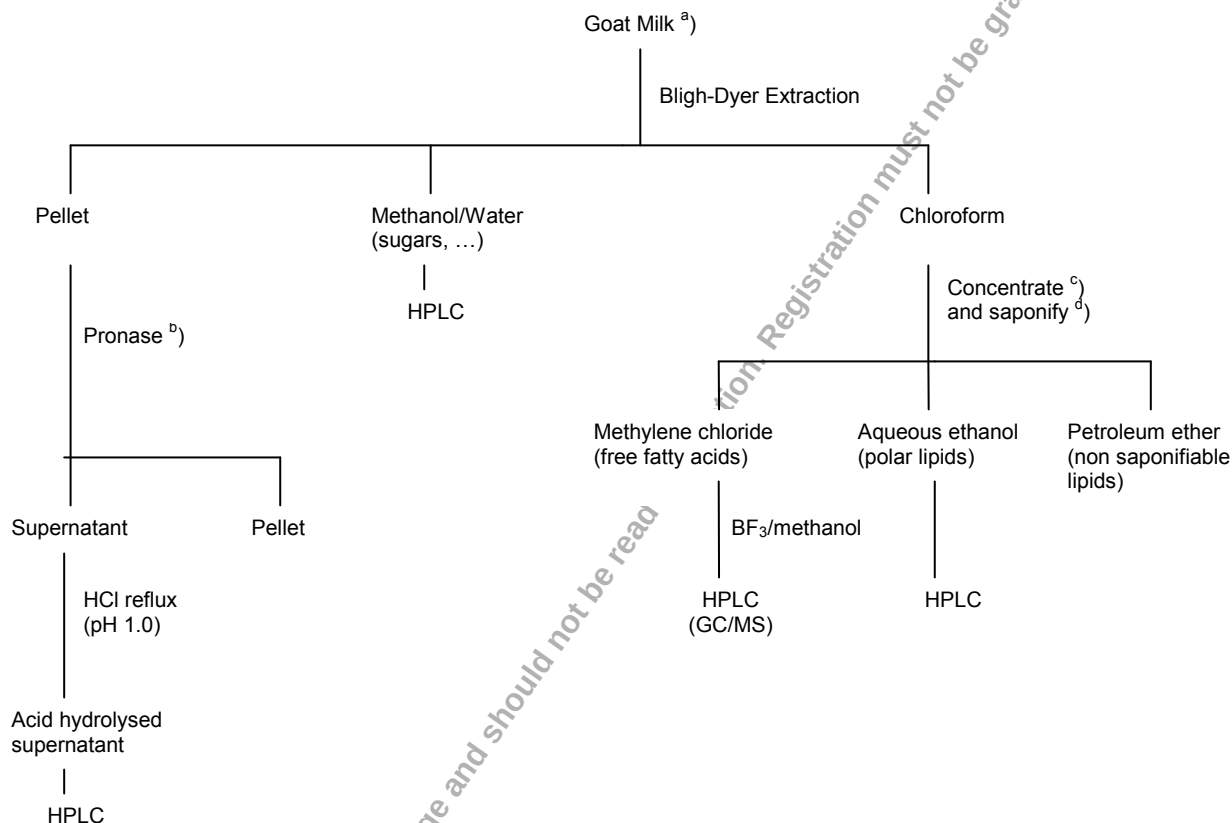
Sample collection: Urine, feces and milk (twice per day: immediately prior to each dose and in the late afternoon) from each goat were collected separately. In addition, expired air was collected from the treated goat. The goats were sacrificed 24 hours following the third dose. A blood sample was collected just prior to sacrifice. After sacrifice the whole liver, whole kidneys, a sample of renal and omental fat, samples of skeletal muscle (like rump, leg and shoulder), the entire gastro-intestinal tract and content as well as the remaining carcass were sampled. Equal weights (ca. 50 g each) of renal and omental fat were combined to give a single fat sample. Likewise, equal weight (ca. 100 g each) of rump, leg and shoulder were combined for a single composite muscle sample. All samples, except cage wash and expired air solvents, were stored deep frozen (-20°C) and analysed for TRR within 14 days after collection.

Determination of TRR: The TRR (total radioactive residue) was measured by LSC (liquid scintillation counting) after combustion of the collected, combined samples, including carcass. Samples from the control goat provided background radioactivity data. Limits of detections (LODs) as mg eq/kg or mg eq/L animal matrix were not stated.

Sample extraction, analysis and characterisation: Milk, liver, kidney, muscle and fat were extracted according to the principles proposed by Bligh and Dyer (1959), which yield a lipid and neutral molecule containing non-polar fraction (in chloroform), a polar fraction (in methanol/water) and a pellet. The pellet was further hydrolysed with proteolytic enzymes, and/or if necessary with mild to harsh acid and base hydrolysis to release solvent unextractable residues. A **small scale extraction (SSE)** of milk, liver and kidney were performed to examine particularly ^{14}C -cymoxanil, which was expected in the chloroform phase (including also dissolved lipids). Prior for further analysis, the original chloroform soluble radioactivity was partitioned between acetonitrile (containing any cymoxanil) and hexane. A **large scale extraction (LSE)** of milk, liver and kidney were conducted to characterise and identify the radioactive components in the respective extracts and pellets. Only single level extractions of fat and muscle sample were executed.

The following figures present the principles of large scale Bligh-Dyer extraction and characterisation scheme of ^{14}C -cymoxanil and related metabolites on goat milk (figure B.7.2.1-1), goat liver (figure B.7.2.1-2) and goat kidney (figure B.7.2.1-3).

Figure 7.2.1-1 Extraction scheme for large scale Bligh-Dyer extraction and characterisation of cymoxanil and metabolites from goat milk (DuPont)



^{a)} total 400 ml milk volume, combined by 4x 100 ml milk from the milking sampling dates (i.e. 24-30, 30-48, 48-54 and 54-72 hours after dosing)

^{b)} protease digestion (37 °C, pH 7.5, 18 and 41 hours)

^{c)} by closed cell and nitrogen evaporation

^{d)} with ethanolic KOH under reflux for 1 hour with N₂ purge

Notes: Various HPLC methods were used to determine a broad range of components with different polarities.

Stability: A freezer storage stability of goat extracts was not performed, because ^{14}C -cymoxanil was rapidly metabolised and the radioactivity occurred in stable natural products (like fatty acids, lactose or hydrolysable formic acid groups).

Extraction process: Recovery of the added radioactivity accounted for 100.4 %.

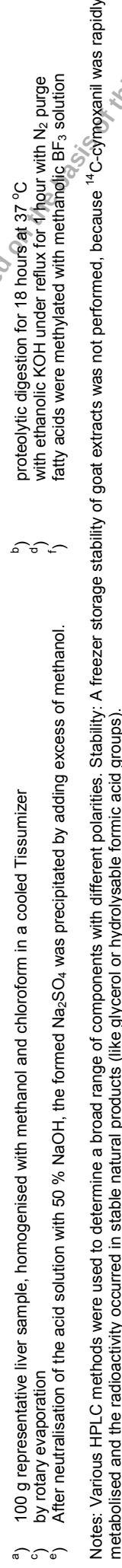
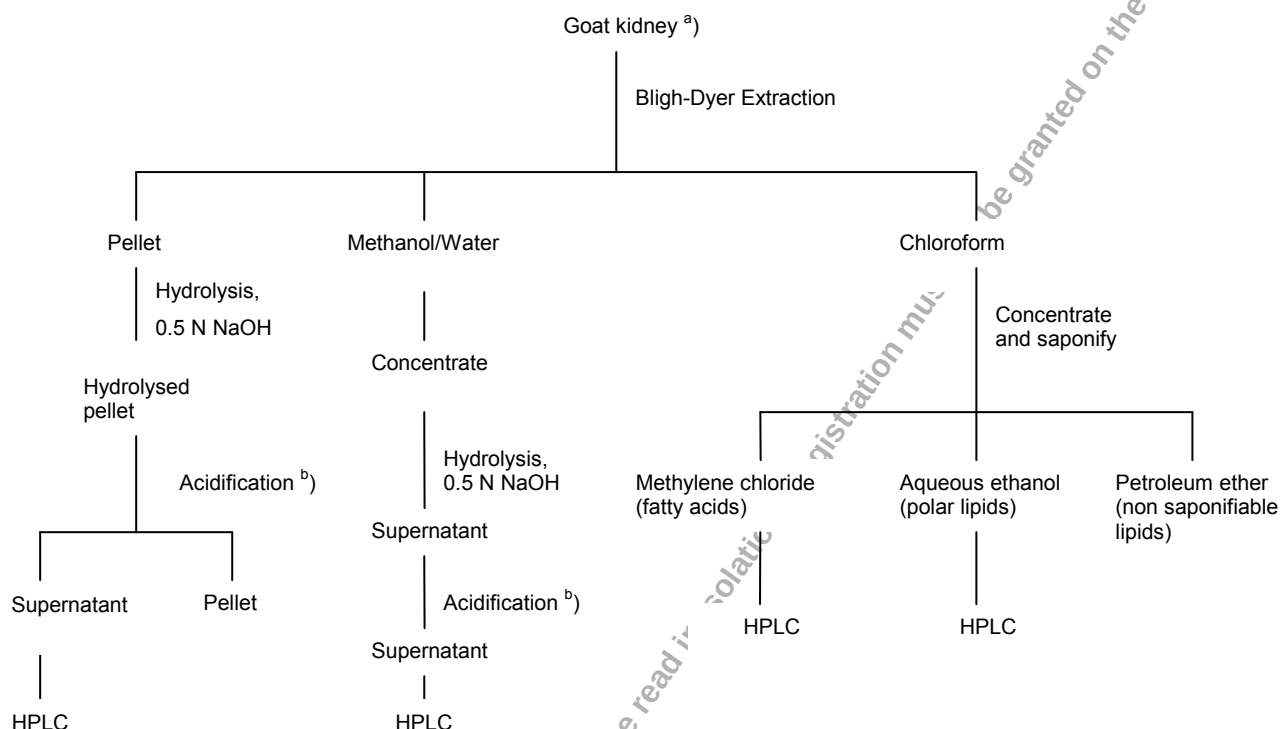


Figure 7.2.1-3 Extraction scheme for large scale Bligh-Dyer extraction and characterisation of cymoxanil and metabolites from goat kidney (DuPont)



^{a)} 50 g representative kidney sample, homogenised with methanol and chloroform in a cooled Tissumizer

^{b)} using 36 - 38 % HCl until ca. pH 3.0 - 3.5 was reached

Notes: Various HPLC methods were used to determine a broad range of components with different polarities.

Stability: A freezer storage stability of goat extracts was not performed, because ¹⁴C-cymoxanil was rapidly metabolised and the radioactivity occurred in stable natural products (like glycerol or hydrolysable formic acid groups).

Composite goat muscle or fat sample was extracted in the same manner as performed for the goat liver. Combined goat urine samples (0-72 hours after dosing) were mixed with methanol (attempts to precipitate urine salts were unsuccessful, such as solid phase extraction). Aliquots of the remaining partially purified urine were treated with β -glucuronidase or acid hydrolysed (6 N HCl) and derivatised with phenylisothiocyanate (PITC) to form phenylthiocarbamate (PTC) derivatives of amino acids (remained in the acid ethyl ether fraction) or directly derivatised (without acid hydrolysis). The combined goat feces samples were determined for TRR only.

Chromatographic analysis: HPLC and GC analyses were carried out with various methods to determine ¹⁴C-cymoxanil and its proposed metabolites in different goat matrices. Limits of quantifications (LOQs) regarding different goat matrices were not stated in the report.

Study design of in vitro bovine rumen fluid test system

Test system: Bovine rumen fluid was obtained from a rumen-fistulated 7-8 year old Holstein steer after hand squeezing of the rumen content and filtration of the fluid. About 100 ml active rumen fluid was incubated with ¹⁴C-cymoxanil treatment solution (resulting to ca. 0.947 mg labelled cymoxanil/100 mL fluid) in a 250 ml glass Erlenmeyer flask gas purging bottle under N₂ protection. Immediately, the contents of the incubation chamber were mixed using a magnetic stirrer. As soon

as feasible (20 - 40 sec after addition of ^{14}C -cymoxanil) 2 aliquotes of incubation solution were taken. The biological activity was quenched with absolute ethanol or 6 % trifluoroacetic acid (TFA). The quenched samples were stored in a freezer (ethanol quenching) or on wet ice (TFA quenching). Thereafter, in a similar way aliquots of samples were taken after 7.5, 15 and 30 min and after 1, 2, 4, 6 and 22 hours incubation time, biological activity quenched and stored. The remainder of the incubation solution after 22 hours was frozen until analysis. For control purpose, a heat treated rumen fluid (at boiling bath for about 25 min) was incubated with ^{14}C -cymoxanil solution and mixed. Samples from the control were taken after 15 and 30 min and after 1, 2, 4 hours of incubation and quenched with equal volume of either 6% TFA or ethanol. Any emerged $^{14}\text{CO}_2$ and other volatile organic acids from the incubation chamber were collected in a 1 N NaOH containing trap.

Analysis: Aliquotes of the TFA quenched samples only were analysed by LSC and HPLC.

Findings

Lactating goat

Both animals remained in good health throughout the study, no abnormalities were observed in the treated animal. After three consecutive daily single oral dose with a target dose level of 15 mg ^{14}C -cymoxanil/day (equivalent to 10 mg ^{14}C -cymoxanil/kg diet and day), the moiety of the administered dose was mainly excreted via the urine (23.6 % of recovered dose) and feces (18.3 % of recovered dose). In milk 2.6 % of total administrated dose was determined. In expired air (using an ethanolamine trap for e.g. $^{14}\text{CO}_2$) 2.2 % of total applied dose could be found. 22.1 % of applied radioactivity remained in the organs, carcass and intestinal tract. Overall, a low recovery (68.8 %) of the administered dose was found from the treated goat. The methane formed from rumen metabolism was not collected. An overview about the distribution of recovered total administered dose in matrices of one lactating goat is given in table B.7.2.1-1.

Table B.7.2.1-1 Recovery of ^{14}C -residues in matrices of one lactating goat after single daily oral dose of 15 mg ^{14}C -cymoxanil/day during three consecutive days equivalent to 10 mg ^{14}C -cymoxanil/kg diet and day

Matrix/Tissue	Radioactive concentration [% recovery of total administered dose] *)
Urine	23.57
Feces	18.29
Milk	2.64
Cage wash	0.51
Expired air	2.21
Liver	3.53
Kidney	0.13
Intestinal contents	9.16
Intestinal wall	2.24
Carcass	6.54
Total (sum of matrix + tissue)	68.82

*) total 45 mg ¹⁴C-cymoxanil was dosed for the one treated goat over 3 consecutive days

Goat milk: The concentration of radiolabelled residues in the milk during the course of dosing ranged from 0.15 to 0.33 mg eq/kg. After the second dose indications of a steady state of eliminating radioactive residues could be assessed (refer to table B.7.2.1-2).

Table B.7.2.1-2 Distribution of mg ¹⁴C-cymoxanil equivalents/liter goat milk at various sampling times after single oral dose of 15 mg ¹⁴C-cymoxanil/day during three consecutive days equivalent to 10 mg ¹⁴C-cymoxanil/kg diet and day

Goat milk *)	Total radioactive residue (TRR) [mg eq/L] **)
hours after first dose administration	
0-6	0.15
6-24	0.19
24-30	0.29
30-48	0.25
48-54	0.33
54-72	0.27
Sum of dose from 0-72 hours	1.48

*) One lactating goat was multiple dosed with ¹⁴C-cymoxanil, total 45 mg ¹⁴C-cymoxanil

**) mg eq/L ... mg equivalents of ¹⁴C-cymoxanil based on liter fresh weight

The characterisation of radioactivity in goat milk after small and large scale Bligh-Dyer extraction (SSE and LSE) show a similar result (summarised in table B.7.2.1-3). No ¹⁴C-cymoxanil (expected in chloroform phase with milk fat or separated from milk fat in acetonitrile phase) could be detected in both scaling extractions. The major extractable radioactive residue was found in the polar fraction (methanol/water system) with 67.6 % of TRR (0.19 mg eq/L) by SSE and 63.5 % of TRR (0.18 mg eq/L) by LSE, respectively. The major amount of radioactivity in the concentrated, saponified and new partitioned chloroform phase was detected in the methylene chloride phase with 7.0 % of TRR (0.02 mg eq/L). In the aqueous ethanol or petroleum ether fraction <0.01 mg eq/L each were obtained (see table B.7.2.1-3).

Table B.7.2.1-3 Characterisation of ^{14}C -residues in goat milk after small and large scale Bligh-Dyer extraction

	Small scale extraction (SSE)		Large scale extraction (LSE)	
Fractionation of combined goat whole milk samples *)	Radioactive concentration			
	[% of TRR] **)	[mg eq/L] ***)	[% of TRR] **)	[mg eq/L] ***)
Extractable residues				
Chloroform ^{a), b)}	10.3	0.03	11.1	0.03
Methylene chloride ^{c)}	---	---	7.0	0.02
Aqueous ethanol ^{c)}	---	---	1.3	<0.01
Petroleum ether ^{c)}	---	---	0.1	<0.01
Methanol/water	67.6	0.19	63.5	0.18
Unextractable residue (pellet)	22.1	0.06	23.8	0.07
Lost of residues	---	---	1.6	<0.01
Total residues (= sum of extractable and unextractable residues)	100.0	0.28	100.0	0.28

*) SSE: composed of equal amounts of the 24-30, 30-48, 48-54 and 54-72 hour whole milk samples of one multiplied dosed lactating goat
LSE: composite milk from 24 to 72 hours

**) % TRR = (dpm in fraction : total dpm) x 100

***) mg eq/L ... mg equivalents of ^{14}C -cymoxanil based on liter goat milk

a) SSE: The partition behaviour of ^{14}C -cymoxanil was investigated between hexane and acetonitrile. The concentrated chloroform phase was partitioned between hexane and acetonitrile to separate any residual cymoxanil (acetonitrile) from radioactivity in milk fat (hexane). No ^{14}C -cymoxanil was detected in the unspiked milk sample.

b) LSE: Drying of the chloroform phase resulted in some loss of radioactivity (like volatile acids), which was equivalent to <0.01 mg eq/L from the original milk.

c) LSE, after concentration and saponification of the chloroform fraction: The methylene chloride phase corresponded to free fatty acids. The aqueous ethanol phase was associated with more polar components, such as glycerol. The petroleum ether phase was associated with neutral, non-saponifiable lipids such as cholesterol.

The aqueous ethanol and methylene chloride phase were investigated further to prove the incorporation of ^{14}C -cymoxanil derived radioactivity in natural products. In the aqueous ethanol phase, ^{14}C -glycerol originating from ^{14}C -cymoxanil was present at a concentration <0.01 (0.003) mg eq/L milk. The methylene chloride phase contained fatty acids, after acidification of the aqueous ethanol phase. After derivatisation with BF_3 /methanol and partitioning with hexane the fatty acids were obtained as methyl esters. The major amount of TRR, 5.7 % (0.02 mg eq/L) were detected in the hexane fraction. Both the aqueous fraction (ethanol/water) and dissolved precipitate contained < 0.01 mg eq/L milk each. See table B.7.2.1-4.

Table B.7.2.1-4 Characterisation of extractable ^{14}C -residues in methylene chloride fraction after methylation of fatty acids from goat milk

Fractionation of methylene chloride phase *)	Radioactive concentration	
	[% of TRR] **)	[mg eq/L] ***)
Hexane ^{a)}	5.7	0.02
Ethanol/water ^{b)}	0.9	<0.01
Dissolved precipitate ^{b)}	0.1	<0.01

*) LSE: After saponification of the chloroform fraction of whole goat milk the acidified methylene chloride fraction containing free fatty acids, which were methylated with BF_3 /methanol.

**) % TRR = (dpm in fraction : total dpm) x 100

- ***) mg eq/L ... mg equivalents of ¹⁴C-cymoxanil based on liter milk
a) Further characterised.
b) No further characterisation was done.

At least 9 distinct components of the radioactive residue in the hexane fraction were identified by TLC, HPLC or GC/MS as methyl ester of caproic, caprylic, capric, lauric, arachidonic, myristic, linoleic, oleic acids and one unidentified peak. Each component were detected at a radioactive level <0.01 mg eq/L.

The predominantly radioactivity in the methanol/water fraction, which represents 63.5 % of milk TRR (0.18 mg eq/L) by large scale extraction, was identified as ¹⁴C-lactose by HPLC with an amount of 46.4 % of milk TRR (0.13 mg eq/L) in this fraction. 2 peaks, ≤0.03 mg eq/L each, remained unidentified. Unresolved radioactivity accounted for 0.01 mg eq/L.

The radioactivity in the goat milk pellet, representing 23.8 % of TRR (0.07 mg eq/L) by large scale extraction, was further characterised by protease digestion and following acid hydrolysis. About half of the radioactivity associated with the milk pellet, 10.7 % of TRR (0.03 mg eq/L), was released after treatments. Further final identification was not performed.

Goat liver: 3.5 % of administered dose was detected in goat liver. The large scale extraction (LSE) of goat liver showed that 4.5 % of TRR (0.10 mg eq/kg) and 29.7 % of TRR (0.63 mg eq/kg) were extractable with chloroform and methanol/water, respectively. The major part of radioactivity, 70.0 % of liver TRR or 1.49 mg eq/kg, was located in the liver pellet. A further characterisation of the chloroform fraction after saponification results to 2.4 % of TRR (0.05 mg eq/kg) in aqueous/ethanol phase (containing polar lipids), 2.0 % of TRR (0.04 mg eq/kg) in methylene chloride phase (covering fatty acids) and 0.1 % of TRR (<0.01 mg eq/kg) in petroleum ether phase (corresponds to non-saponifiable lipids). See summarised results in table B.7.2.1-5.

Table B.7.2.1-5 Characterisation of mg ¹⁴C-residues in goat liver after small and large scale Bligh-Dyer extraction

	Small scale extraction (SSE)		Large scale extraction (LSE)	
Fractionation of combined goat liver samples *)	Radioactive concentration			
	[% of TRR] **)	[mg eq/kg] ***)	[% of TRR] **)	[mg eq/kg] ***)
Extractable residues				
Chloroform ^{a)}	4.7	0.10	4.5(4)	0.10
Methylene chloride	---	---	2.0	0.04
Aqueous ethanol	---	---	2.4	0.05
Petroleum ether	---	---	0.1	<0.01
Methanol/water	30.5	0.65	29.7	0.63
Unextractable residue (pellet)	not analysed	not analysed	70.0	1.49
Lost of residues	---	---	---	---
Total residues (= sum of extractable and unextractable residues)	not calculable	not calculable	104.2 ^{b)}	2.22 ^{b)}

- *) SSE: ca. 5 g goat liver used for extraction; LSE: 100 g goat liver tested
 **) % TRR = (dpm in fraction : total dpm) x 100
 ***) mg eq/kg ... mg equivalents of ¹⁴C-cymoxanil based on kg fresh weight
 a) SSE: The partition behaviour of ¹⁴C-cymoxanil was investigated between hexane and acetonitrile. The concentrated chloroform phase was partitioned between hexane and acetonitrile to separate any residual cymoxanil (acetonitrile). No ¹⁴C-cymoxanil was detected in the unspiked liver sample.
 b) Standardisation to 100.0 % of TRR results to 2.13 mg eq/kg goat liver.
 Note: Further characterisation and identification of ¹⁴C-residue were performed on the LSE. No ¹⁴C-cymoxanil or structurally related metabolites were detected in an LSE fraction of goat liver.

Fatty acids of the methylene chloride fraction were methylated with BF₃/methanol and partitioned in the hexane phase. Less of half of the TRR in the methylene chloride fraction, 0.7 % of TRR or 0.02 mg eq/kg (hexane: 0.3 % of TRR or 0.01 mg eq/kg; dissolved precipitate: 0.4 % of TRR or 0.01 mg eq/kg) were recovered after methylation. The loss of radioactivity (about 0.02 mg eq/kg) was justified to volatile short fatty acids during heating process of methylation.

HPLC analyses of the concentrated, acidified and desalted aqueous ethanol fraction showed 2 peaks. The major single peak (1.4 % of liver TRR) could be identified as ¹⁴C-glycerol, which accounted for 0.03 mg eq/kg. The minor peak remained unidentified.

19.2 % of liver TRR (0.41 mg eq/kg) was identified by HPLC as hydrolysable formic acid group in the water/methanol fraction. The remaining radioactivity in this fraction was not identified.

57.5 % of TRR (1.23 mg eq/kg) of radioactivity associate with goat liver pellet was released after Pronase digestion in the supernatant, which was further hydrolysed (6 N HCl), neutralised, desalted and analysed by HPLC. Formic and acetic acid at concentration of 39.4 % of liver TRR (0.84 mg eq/kg) and 14.0 % of liver TRR (0.30 mg eq/kg) were identified in the acid hydrolysed Pronase supernatant. Thereafter, the remaining Pronase pellet was further hydrolysed with 0.1 N HCl and 1% (NH₄)₂CO₃. HPLC analyses of acidic and base hydrolysate detected ¹⁴C-formic acid as the major component. Finally the remaining acid and base hydrolysed Pronase pellet was hydrolysed with 6 N HCl, but no further analysis was performed. For details see summarised results in table B.7.2.1-6.

Table B.7.2.1-6 Characterisation and partially identification of extractable ¹⁴C-residues from goat liver pellet

Fractionation of goat liver pellet*)	Radioactive concentration	
	[% of TRR] **)	[mg eq/kg] ***)
Pronase supernatant	57.5	1.23
Hydrolysed Pronase supernatant	53.3	1.14
Formic acid	39.4	0.84
Acetic acid a)	14.0	0.30
Pronase pellet	19.7	0.42
0.1 N HCl hydrolysate	13.8	0.29
Formic acid	9.4	0.20

Fractionation of goat liver pellet*)	Radioactive concentration	
	[% of TRR] **)	[mg eq/kg] ***)
Combined (NH ₄) ₂ CO ₃	1.4	0.03
Formic acid	0.9	0.02
Glycerol	0.4	0.01
Total (sum of Pronase supernatant + pellet)	77.2	1.65

*) Sample size: 100 g goat liver for large scale extraction.

**) % TRR = (dpm in fraction : total dpm) x 100; goat liver: 100 % of TRR = 2.13 mg eq/kg

***) mg eq/kg ... mg equivalents of ¹⁴C-cymoxanil based on kg fresh weight

a) Acetic acid was not confirmed in a second HPLC system.

To minimise the losses of goat liver radioactivity (i.e. hydrolysable formic acid group) in the water/methanol phase and pellet, a direct extraction method was performed: 1.0 N NaOH reflux extraction of liver, which resulted in 96.6 % of TRR (2.06 mg eq/kg) in the caustic reflux liver extract. The majority of the radioactivity was identified by HPLC as hydrolysable formic acid moiety.

Goat kidney: Much lower levels of radioactivity in comparison with milk and liver were detected in kidney with 0.13 % of administered radioactivity. In the large scale extraction (LSE) of goat kidney 5.8 % of TRR (0.03 mg eq/kg) were extractable with chloroform and 33.2 % of TRR (0.15 mg eq/kg) with methanol/water, respectively. The major part of radioactivity in kidney, 69.2 % of TRR (0.32 mg eq/kg), was associated with kidney pellet. A further characterisation of the chloroform fraction from LSE of kidney was not conducted due to low concentration of radioactivity and no evidence of ¹⁴C-cymoxanil from SSE investigations.

An aliquot of concentrated methanol/water extract was hydrolysed by 0.5 N NaOH in order to avoid the loss of volatile organic components. HPLC analyses of the acidified caustic hydrolysate identified ¹⁴C-formic acid, ¹⁴C-acetic acid (not confirmed) and ¹⁴C-glycerol, which accounted for 10.9 % of TRR (0.05 mg eq/kg), 2.2 % of TRR (0.01 mg eq/kg) and 4.3 % of TRR (0.02 mg eq/kg) in kidney, respectively.

With caustic hydrolysis (0.5 N NaOH) the majority of kidney pellet associated radioactivity was released and ¹⁴C-formic acid (17.4 % of TRR, 0.08 mg eq/kg), ¹⁴C-glycerol (4.3 % of TRR, 0.02 mg eq/kg) and tentatively acidic acid (8.7 % of TRR, 0.04 mg eq/kg) could be identified by HPLC.

The findings are summarised in table B.7.2.1-7.

Table B.7.2.1-7 Characterisation of ¹⁴C-residues in goat kidney after small and large scale Bligh-Dyer extraction

	Small scale extraction (SSE)		Large scale extraction (LSE)	
Fractionation of combined goat kidney samples *)	Radioactive concentration			
	[% of TRR] **)	[mg eq/kg] ***)	[% of TRR] **)	[mg eq/kg] ***)
Extractable residues				
Chloroform ^{a)}	4.6	0.02	5.8	0.03
Methanol/water ^{b)}	44.6	0.20	33.2	0.15

	Small scale extraction (SSE)		Large scale extraction (LSE)	
Fractionation of combined goat kidney samples *)	Radioactive concentration			
	[% of TRR] **)	[mg eq/kg] ***)	[% of TRR] **)	[mg eq/kg] ***)
0.5 N NaOH hydrolysate ^{c)} acidified caustic hydrolysate ^{d)}	---	---	31.1 32.2	0.14 0.15
Unextractable residue (pellet)	not analysed	not analysed	69.2	0.32
0.5 N NaOH hydrolysate	---	---	62.6	0.29
Lost of residues	---	---	---	---
Total residues (= sum of extractable and unextractable residues)	not calculable	not calculable	108.2 ^{e)}	0.50 ^{e)}

*) SSE: ca. 5 g goat kidney used for extraction, LSE: 50 g goat kidney tested

**) % TRR = (dpm in fraction : total dpm) x 100

***) mg eq/kg ... mg equivalents of ¹⁴C-cymoxanil based on kg fresh weight

a) SSE: The partition behaviour of ¹⁴C-cymoxanil was investigated between hexane and acetonitrile. The concentrated chloroform phase was partitioned between hexane and acetonitrile to separate any residual cymoxanil (acetonitrile). No ¹⁴C-cymoxanil was detected in the unspiked kidney sample.

LSE: No further characterisation was performed.

b) SSE: No cymoxanil was detected in the methanol/water extract of goat kidney by HPLC.

c) LSE: A concentrate aliquot of the methanol/water extract was hydrolysed with 0.5 N NaOH.

d) LSE: Acidified caustic hydrolysate with pH 3.5.

e) Standardisation to 100.0 % of TRR results to 0.46 mg eq/kg goat kidney.

Note: Further characterisation and identification of ¹⁴C-residue were performed on the LSE. No ¹⁴C-cymoxanil or structurally related metabolite were detected in any LSE fraction of goat kidney.

A single, direct caustic hydrolysis of goat kidney tissue using 1.0 N NaOH (without Bligh-Dyer extraction) was conducted in attempt to minimise fractionation and therefore losses of radioactivity. The HPLC analysis of the acidified caustic hydrolysate (pH 3.5) confirmed the presence and identity of the major extractable component, i.e. ¹⁴C-formic acid (75.1 % of chromatographic TRR, 0.34 mg eq/kg).

Goat muscle, goat fat: A low radioactivity was detected in both goat samples, composite muscle with ca. 0.09 mg eq/kg and composite fat with ca. 0.06 mg eq/kg, respectively. The characterisation of Bligh-Dyer extraction of composite goat muscle and fat is given in table B.7.2.1-8.

Table B.7.2.1-8 Characterisation of ¹⁴C-residues in composite samples of goat muscles and fat after Bligh-Dyer extraction

	Goat muscle		Goat fate	
Fractionation of combined goat samples *)	Radioactive concentration			
	[% of TRR] **)	[mg eq/kg] ***)	[% of TRR] **)	[mg eq/kg] ***)
Extractable residues				
Chloroform ^{a)}	2.1	<0.01	3.7	<0.01
Methanol/water ^{b)}	52.5	0.04	24.3	0.02
Unextractable residue (pellet) ^{a)}	61.3	0.05	58.5	0.04
Total residues (= sum of extractable and unextractable residues)	115.9	0.09	86.5	0.06

*) Ca. 20 g combined representative goat muscle (or fat) tested
 **) % TRR = (dpm in fraction : total dpm) x 100
 ***) mg eq/kg ... mg equivalents of ¹⁴C-cymoxanil based on kg fresh weight
 Note: No further characterisations were reported.

Only the methanol/water fractions of goat muscle and fat were analysed by HPLC, whereas no ¹⁴C-cymoxanil was determined in any fractions.

Goat urine: Hydrolysis (6 N HCl), PITC derivatisation (to form PTC amino acids) and ethyl ether partitioning of hydrolysed raw urine (samples from 0-72 hours) resulted in 14.1 % of TRR in basic ethyl ether phase, 15.8 % of TRR in acidic ethyl ether phase and 23.4 % of TRR remaining in the acidic aqueous phase (overall recovery 53.3 % of TRR in urine). 52 % of radioactivity in the acidic ether phase (8.0 % of TRR in urine) was identified as PTC ¹⁴C-glycine. Hydrolysis (6 N HCl) of urine and volume reduction of hydrolysate led to a significant loss of volatile radioactivity. A majority of the volatile radioactivity was determined as ¹⁴C-formic acid by HPLC.

Unhydrolysed goat urine was derivatised with PITC to form PTC derivatives of any free amino acids that were present before acid (6 N HCl) hydrolysis. PITC derivatisation and ethyl ether partitioning of unhydrolysed raw urine resulted in 13.6 % TRR in basic ethyl ether phase, 13.8 % of TRR in acidic ethyl ether phase and 85.6 % of TRR remaining in the acidic aqueous phase (overall recovery 113.0 % of TRR in urine). Regarding the acidic ether phase, free ¹⁴C-glycine accounted for about 2 % of TRR in urine.

No ¹⁴C-cymoxanil or related metabolites were detected in hydrolysed or unhydrolysed goat urine.

In vitro fluid bovine rumen test system

¹⁴C-cymoxanil was metabolised rapidly (within 7.5 minutes from incubation start) in the bovine rumen test system, to form multiple polar components unrelated to any ¹⁴C-cymoxanil hydrolysis products. After 1 hour incubation significant incorporation of radiolabel into valeric acid had occurred (indicating that initial metabolites of ¹⁴C-cymoxanil fed into existing rumen microbial pathways). At later sampling points formic acid and acetic acid formation was more prominent (likely a result of death microbes that could not utilise these components).

Conclusion

Lactating goat

After three consecutive single daily doses, equivalent to 10 mg ¹⁴C-cymoxanil per kg diet and day, a majority of the recovered administered radioactivity 23.6 % and 18.3 % of applied dose on one lactating goat was detected in excreta and urine, respectively. Goat milk contained 2.6 %, liver 3.5 % and kidney 0.1 % of administered dose. The total recovery of dosed radioactivity on the goat amounted to 68.8 %. The following summarised results are based on the small and large scale Bligh-Dyer extraction procedures:

The major ¹⁴C-cymoxanil derived residue detected in goat milk was lactose, which represents 46.4 % of milk TRR (0.13 mg eq/L). Fatty acids (i.e. caproic, caprylic, capric, lauric, arachidonic, myristic, lioleic, oleic acids) accounted for 5.7 % of milk TRR (0.02 mg eq/L). About half of the radioactivity

associated with the milk pellet was released (10.7 % of milk TRR, 0.03 mg eq/L) after protease digestion and acid hydrolysis, but remained unidentified.

Formic acid (i.e hydrolysable formyl group) was identified as the primary metabolite in goat liver. The total formic acid (after acid hydrolysis of extracts or protolytic digestion of pellet) represents 68.9 % of liver TRR (1.47 mg eq/kg). Acetic acid was released after protolytic and acid hydrolysis at a concentration level of 14.0 % of liver TRR (0.30 mg eq/kg) from the pellet. 1.8 % of liver TRR (0.04 mg eq/kg) was identified as ^{14}C -glycerol, whereas 1.4 % of TRR (0.03 mg eq/kg) was solvent extractable.

In the goat kidney a hydrolysable formic acid group, accounted for 28.3 % of kidney TRR (0.13 mg eq/kg) was primarily identified. Radioactive glycerol and acetic acid were detected at 8.6 % and 10.9 % of kidney TRR (0.04 and 0.05 mg eq/kg), respectively.

Residues in composite goat muscle or fat fractions were low and remained unidentified. Radioactivity in goat urine was very polar and the conclusively identified metabolites after acid hydrolysis were glycine and formic acid. No significant characterisation of residues in feces was attempted.

No ^{14}C -cymoxanil or structurally related metabolites were detected in any goat sample (i.e. milk, liver, kidney, muscles, fat or urine).

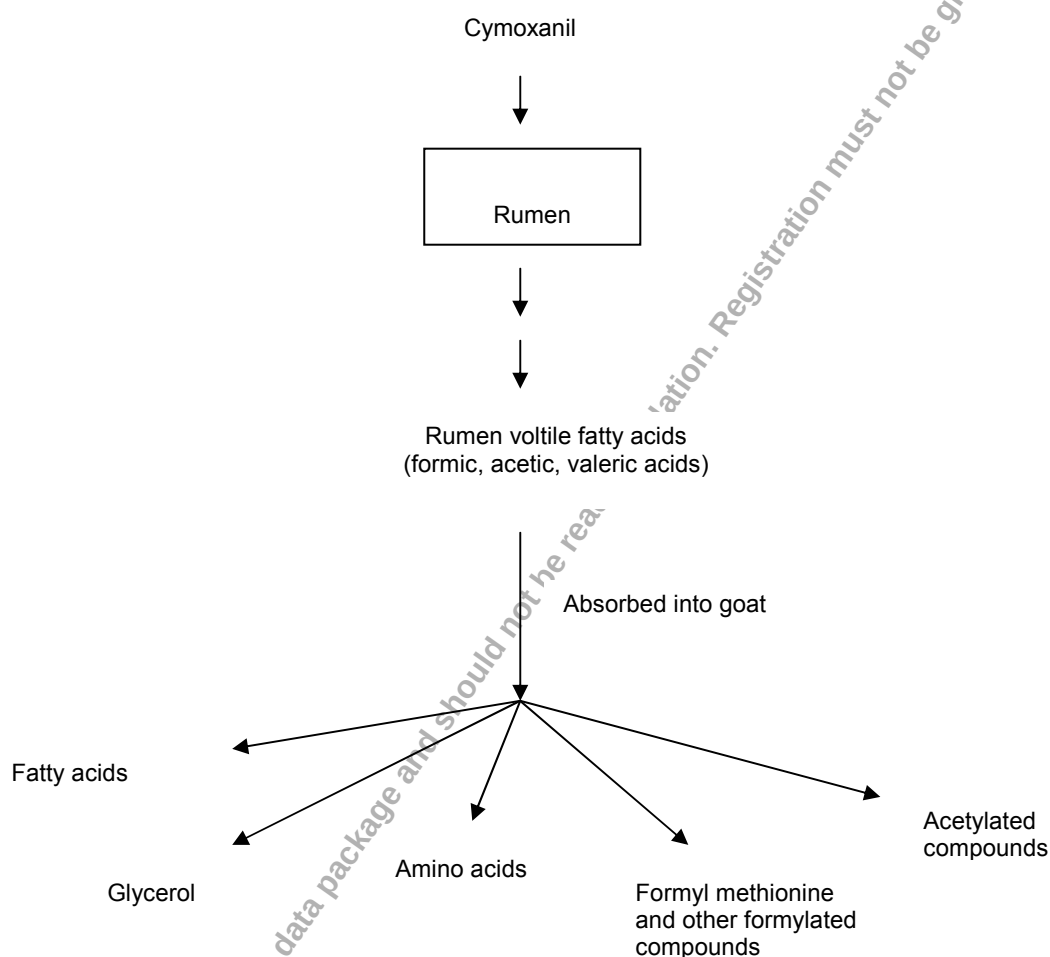
In vitro fluid bovine rumen test system

The applied test system shows a fast degradation of ^{14}C -cymoxanil to polar components including formic, acetic and valeric acid, which were incorporated into natural products. Initial metabolism of ^{14}C -cymoxanil was mediated by rumen (micro)organisms. No ^{14}C -cymoxanil or structurally related metabolites were detected.

Overall Conclusion

^{14}C -cymoxanil dosed over 3 consecutive days, equivalent to 10 mg ^{14}C -cymoxanil per kg diet and day, was metabolised by one tested lactating goat essentially to natural products including fatty acids, glycerol, glycine and other amino acids, lactose and hydrolysable formyl and acetyl group. ^{14}C -Methan was hypothesised as a metabolite. Neither ^{14}C -cymoxanil nor structurally related metabolites were detected in any tissue, milk or in the urine (radioactivity in feces was not further determined) of the goat. Additionally, an in vitro bovine rumen fluid test was performed. An initial metabolism of ^{14}C -cymoxanil was mediated by rumen (micro)organisms, which metabolised ^{14}C -cymoxanil primarily to polar natural products like formic, acetic and valeric acid and incorporated the resulted metabolites into natural products (e.g. lipids, sugars, proteins or others). In figure B.7.2.1-4 the proposed metabolic pathway of cymoxanil in lactating goat is presented.

Figure B.7.2.1-4 Proposed metabolic pathway of cymoxanil in lactating goat



B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)

For definition of relevant residues of cymoxanil in raw agricultural commodities (RAC) the following facts were taken into account:

Studies on the uptake and metabolism of cymoxanil in lettuce (leaf vegetable) and potato (root and tuber vegetable) were evaluated. After spray application (intended growth stage for lettuce BBCH 40-49 and potato BBCH 21-95) cymoxanil was metabolised rapidly and resulted essentially to polar

substances (like glycine), which were further incorporated or conjugated in or with natural products (like carbohydrates and others). Intermediates were detected in lettuce only: IN-W3595 only was found up to 18.1 % of TRR in the lettuce study submitted by Oxon. IN-KQ960 and IN-KP533 with 7.4 % of TRR and 2.8 % of TRR, respectively, were detected in lettuce samples according to the DuPont study only. Based on the results of toxicological studies provided, no toxicological relevance with respect to metabolites IN-W3595 and IN-KQ960 is evident. Metabolite IN-KP533 was found in one of two lettuce metabolism studies only at low amounts and was not tested in the rat model system (reaching a whole picture of the toxicological relevance investigations of IN-KP533 in the rat model system should be supplementary performed). Nevertheless, cymoxanil only is proposed as residue of concern.

The supervised residue trials on lettuce and potatoes showed no cymoxanil at or above the LOQ (= 0.05 mg/kg per each crop) or structurally related metabolites at commercial harvest time. Similarly, the tested rotational crops, i.e. lettuce (head), sugar beets (tops or roots) and spring wheat (forage, straw or grain) show no evidence of cymoxanil or structurally related metabolites. The trigger value of 0.1 mg residue/kg dry feed regarding intake by livestock is formally open for potato use (i.e. pig and beef cattle diet). Note, the respective trigger value is not explicit based on dry weight in for the data submitter available and in forced DG Sanco doc. 7031/VI/95, rev. 4, 22 July 1996, livestock feeding studies. Nevertheless, a lactating goat metabolism study (including an in vitro bovine rumen test) has been submitted, which showed "no residue" of cymoxanil or structurally related metabolites in the goat organs, tissues or milk. Cymoxanil was rapidly metabolised in the goat model system and the radioactivity determined in natural polar products (like fatty acids, glycerol, lactose, amino acids) or formylated or acetylated compounds.

In consideration of the above presented arguments, the proposed residue definition for plant products (essentially based on the lettuce and potato metabolism studies) for risk assessment and monitoring purpose is cymoxanil (parent compound).

A residue definition for food of animal origin (primarily based on "non residue" situation in potato tubers and on the supplementary lactating goat metabolism study) is not regarded necessary.

B.7.4 Use pattern

The use patterns of existing authorized uses of cymoxanil in the EU Member States for potato and lettuce are summarised exemplarily in table B.7.4-1 (reported by DuPont for potato) and table B.7.4-2 (reported by Oxon for potato and lettuce).

Table B.7.4-1 Use pattern of existing authorized uses of cymoxanil on potatoes in the EU Member States reported by DuPont

Country	Application rate per treatment [kg cymoxanil/ha]	Maximal number of applications	Total amount [max. kg cymoxanil/ha]	Pests controlled	time of application, growth stage [BBCH]	PHI [days]
Austria	0.175	6	1.050	Phytophthora, Alternaria	29-79	14
Belgium	0.150	6	0.900	Phytophthora infestans	not specified	14
Cyprus	0.125	Not specified	1.000	Phytophthora infestans, Alternaria solani	not specified	8
Czech Republic	0.175	4	0.700	Phytophthora infestans	10-92	14
Estonia	0.175	6	1.050	Phytophthora infestans, Alternaria solani	21-35	14
Finland	0.175	3	0.525	Phytophthora infestans, Alternaria solani	not specified	14
France	0.120	4	0.480	Mildew GS not specified	not specified	14
Germany	0.175	8	1.400	Phytophthora infestans	29-79	14
Greece	0.160	8	1.280	Phytophthora infestans, Alternaria solani	14-89	15
Hungary	0.160	9	1.440	Phytophthora infestans, Alternaria	post plant, pre-emergence	7
Ireland	0.175	6	1.050	Phytophthora infestans	not specified	14
Italy	0.120	8	0.960	Peronospora, Phytophthora infestans, Alternaria solani, botrytis, A. licopersyci	not specified	14
Latvia	0.175	6	1.050	Phytophthora infestans, Alternaria solani	not specified	14
Lithuania	0.175	6	1.050	Phytophthora, Alternaria	not specified	14
The Netherlands	0.150	3	0.450	Phytophthora infestans	10-60	14
Poland	0.175	3	0.525	Phytophthora infestans, Alternaria tenuis	31-90	11
Portugal	0.120	9	1.080	Phytophthora infestans	n/a	7
Slovakia	0.150	6	0.900	Phytophthora infestans	10-92	14
Slovenia	0.120	4	0.480	Phytophthora infestans	n/a	14
Spain	0.120	5	0.600	Phytophthora infestans, Alternaria solani, Septoria, Mildew	n/a	15
Sweden	0.175	3	0.525	Phytophthora, Alternaria	not specified	14
United Kingdom	0.175	6	1.050	Phytophthora infestans	not specified	14

Notes: No information about the intervals between the applications was given by the notifier.

Table B.7.4-2 Use pattern of existing authorized uses of cymoxanil on potato and lettuce in the EU Member States reported by Oxon

Country	crop	Product *) [% of Cymoxanil]	Formulation type	Application rate per treatment **) [kg cymoxanil/ha]	Maximal number of applications (intervals in days)	Maximal total amount [max. kg cymoxanil/ha]	Pests controlled	PHI **) [days]
Belgium	potato	mix. with MZ 4.5	WP	up to 0.112	not reported	not calculable	Phytophthora infestans	21
		mix. with CTN 50		0.100	not reported	not calculable	Phytophthora infestans	21
France	potato	mix. with MZ 6	WP	0.100	6 (not reported)	0.600	Phytophthora infestans	not rep.
		mix. with CTN 50	SC	0.150	2 (not reported)	0.300	Phytophthora infestans	not rep.
Greece	potato	mix. with MZ 4	WP	0.120	4 (7-14)	0.480	Phytophthora infestans	15
Italy	potato	only CYM 45	WG	up to 0.240	not reported (8-10)	not calculable	Phytophthora infestans	not rep.
		mix. with CO 4	WP	up to 0.140	not reported (8-10)	not calculable	Phytophthora infestans, Alternaria porri	not rep.
		mix. with CH 4	WP	up to 0.140	not reported (8-10)	not calculable	Phytophthora infestans, Alternaria porri	not rep.
		mix. with CTN 4	WG	up to 0.150	2 (not reported)	0.300	Phytophthora infestans, Botrytis cinerea	not rep.
Portugal	potato	mix. with CO 4	WP	300g fp/hl	not reported (7-10)	not calculable	Phytophthora infestans	not rep.
		mix. with MZ 4	WP	300g fp/hl	not reported (7-10)	not calculable	Phytophthora infestans	not rep.
Spain	potato	mix. with BEN+FOL 3.2	WP	up to 325 g fp/hl	not reported (14)	not calculable	Phytophthora infestans	not rep.
		mix with CS 3	WP	400g fp/hl	not reported (10-15)	not calculable	Phytophthora infestans, Alternaria solani	not rep.
		mix. with MZ 4	WP	300g fp/hl	not reported (10-15)	not calculable	Phytophthora infestans, Alternaria solani	not rep.
		mix. with BEN+MZ 3.2	WP	350g fp/hl	not reported (10-20)	not calculable	Phytophthora infestans, Alternaria solani	not rep.
		mix. with FOL 4	WP	300g fp/hl	not reported (10-15)	not calculable	Phytophthora infestans	not rep.
Sweden	potato	only CYM 50	WP	0.120	4 (7)	0.480	Phytophthora infestans	7
		mix. with MZ 6	WP	0.090	6 (7)	0.540	Phytophthora infestans	7
Italy	lettuce	only CYM 45	WG	0.240	not reported (8-10)	not calculable	Bremia lactucae	not rep.
		mix. with CO 4	WP	0.120	not reported (8-10)	not calculable	Bremia lactucae	not rep.
		mix. with CH 2	WP	0.120	not reported (8-10)	not calculable	Bremia lactucae	not rep.
Spain	lettuce	mix. with FOL 4	WP	0.300 g fp/hl	not reported (10-15)	not calculable	Bremia lactucae, Botrytis cinerea	not rep.

Notes: The time of application (growth stage) is not reported.

*) abbreviations: BEN = Benalaxyl, CTN = Chlorthalonil, CH = Copper Hydroxide, CO = Copper Oxychloride, CS = Copper sulphate, CYM = Cymoxanil, FOL = Folpet, FA = Fosetyl Aluminium, mix = mixture, MZ = Mancozeb

**) abbreviation: fp ... formulated product; the amounts of hl/ha are not reported

*) abbreviation: not rep. ... not reported

Details of the intended uses on cymoxanil in Europe by DuPont and Oxon are listed in table 7.4-3.

Table 7.4-3 Intended uses of cymoxanil in Europe, i.e. supported uses for evaluation under Council Directive 91/414/EEC

Crop and/or situation	Member state or Country (a)	Product Name(b)	F, G or I (c)	Pests or Groups of pests controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks (e)
					Type	Conc. of as	method kind	growth stage (d)	Number min max	Interval between application	kg as/ha min max	water l/ha min max	kg as/ha min max		
Lettuce	South	CYM 50	F	<i>Bremia lactucae</i>	WP	500 g/kg	Spray	40-49	3-4	7	0.240	500-800	0.03-0.048	10	Oxon
Potato	North	CYM 50	F	<i>Phytophthora infestans</i>	WP	500 g/kg	Spray	21-95	4	7-10	0.120	200-450	0.026-0.060-	7	Oxon
	South	CYM 50			WP	500 g/kg	Spray	21-95	5	7	0.120	500-1000	0.012-0.024	7	Oxon
	North	Tanos	F	<i>Phytophthora infestans</i>	WG	250g/kg	Spray	21-95	6-8	7-10	0.175	300-600	0.029-0.058	14	DuPont
	South														

(a) South ... Southern Europe, North ... Northern Europe

(b) CYM 50 = cymoxanil 50 %WP; Tanos = cymoxanil/famoxadone 250/250 g/kg.

(c) Outdoor or field use (F), glasshouse application (G) or indoor application (I).

(d) Growth stage at the first application according to BBCH (Biologische Bundesanstalt Bundessortenamt and Chemical industry) system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species.

(e) GAP for products supported by Oxon or DuPont.

WARNING: This document forms part of an EC evaluation data package and should not be relied on for regulatory purposes. Registration must not be granted on the basis of this document.

B.7.5 Identification of critical GAPs

Regarding the critical GAPs see list of intended uses for DuPont and Oxon under chapter B.7.4, table B.7.4-3.

B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2)

According to the intended uses representative supervised residue trials (SRTs) from European locations for leaf vegetable (lettuce, *lactuca sativa*) or root and tuber vegetable (potato, *solanum* spp) were submitted by DuPont and Oxon.

Lettuce:

SRTs from DuPont: none submitted

SRTs from Oxon: 6 trials from Southern Europe, thereof 4 trials from 2000 and 2 trials from 2001; not supported for Northern Europe

Potato:

SRTs from DuPont: 6 trials from Northern Europe, all from 1996

6 trials from Southern Europe, all from 1996

SRTs from Oxon: 4 trials from Northern Europe, all from 2002

15 trials from Southern Europe, thereof 6 trials from 1996, 6 trials from 1997 and 3 trials from 2001

In the following subchapters the evaluated single residue trials were presented in a consolidated form.

B.7.6.1 Cymoxanil residues in lettuce - southern Europe, Oxon

References:

Soler, J.R., 2001, Generation of lettuce specimens suitable for residue analysis of cymoxanil following applications of Cimoxnilo 50 (Cymoxanil 50% WP), field phase, report no. CY2/LE.

Freschi, G., 2001a, Residue analysis of Cymoxanil in lettuce samples (plant), analytical phase, report no. SIP1264

Domenichini, P., 2001, Generation of lettuce specimens suitable for residue analysis following applications of Cymoxanil 50% WP, field phase, report no. CY1/LA.

Freschi, G., 2001a, Residue analysis of Cymoxanil in lettuce samples (plant), analytical phase, report no. SIP1264

Domenichini, P., 2003a, Generation of lettuce plant samples, suitable for residue analysis (RAC and decline curve) following applications of Cymoxanil 50% WP; field phase, report no. CY2.

Freschi, G., 2001b, Residue analysis of Cymoxanil in lettuce samples (plant), analytical phase, report no. SIP1297

Guideline: DG Sanco 7029/VI/95, rev. 5, 22/7/97, General recommendations for the design, preparation and realization of residue trials; others

GLP: Yes

Studies are considered acceptable for evaluation.

6 supervised residue trials of lettuce conducted in Southern Europe (2x Spain and 4x Italy) were submitted by Oxon. In figure B.7.6.1-1 the locations of trials to determine the residues of cymoxanil in/on lettuce were shown. There are no residue trial data for lettuce submitted by DuPont.

Figure 7.6.1-1 Locations of supervised residue trials on lettuce - Oxon



Location (southern Europe)

- | | | |
|------------------------------------|----------------------------------|-------------------------------------|
| 1 Cartagena, Murcia, Spain | 2 Torrepacheco, Murcia, Spain | 3 Cavazzana Di Lusia, Rovigo, Italy |
| 4 Salerano sul Lambro, Lodi, Italy | 5 Canneto sull'Oglio (MN), Italy | 6 Gazzo Veronese (VR), Italy |

Material and Methods

Criteria for the lettuce trials:

Lettuce plants were treated with CYM 50 (WP formulation containing 50.0 % cymoxanil as active ingredient) at the growth stages from BBCH 12 to BBCH 49 with a nominal rate of 240 g cymoxanil per hectare, 4 applications per season, an application interval of 7 days and a PHI of 9-10 days for harvest.

Critical GAP for lettuce (Oxon):

The use of cymoxanil on lettuce under outdoor condition in southern Europe is supported by Oxon only:

Spray application, growth stage: 40-49, up to 4 foliar treatments with a rate of 0.240 kg as/ha each, maximal up to 0.960 kg as/ha, 7 days spray intervals and a PHI of 10 days.

Sampling, storage and analysis of lettuce leaves:

After separated hand sampling of treated and untreated lettuce plants (removing the roots and any leaves in touch with the soil according to the usual GAP) at maturity of the crop (harvest studies)

and before (decline curve studies) from the field site the lettuce specimen were kept frozen at -18 °C or at lower temperature until the analysis were carried out. The extraction of the lettuce samples was started at least within 6 months after sampling. The analysis was finished at least 12 months after sampling. A representative subsample of lettuce was extracted with ethyl acetate using ultra-turrax. The evaporated aqueous extract was initially washed with hexane, then extracted with dichloromethane, purified on silica gel column and analysed by gas chromatography with a nitrogen specific detector (NPD). The residue level in the sample was determined with the external standard method. The limit of quantification of cymoxanil was 0.05 mg/kg in lettuce matrix.

Findings

9 - 10 days after last treatment of lettuce plants with CYM 50 the residue concentration was <0.05 mg cymoxanil/kg lettuce based on fresh weight. Significant amount of cymoxanil were found in lettuce leaves from 0.48 - 4.29 mg as/kg at PHI of 0 day, 0.45 - 2.72 mg as/kg at PHI of 1 day and < 0.05 - 0.95 mg as/ha at PHI of 3 days. Table B.7.6.1.1 gives an overview of the residue data for cymoxanil in lettuce from 6 supervised residue trials (3 harvest + 3 decline curve studies) on open fields in Southern Europe for the growing seasons 2000 and 2001 reported by Oxon, only.

Table B.7.6.1-1 Overview of residue data for cymoxanil on/in lettuce (Oxon)

Crop, trial site country, year	Application					Residues		Reference
	Formulation, content of as [% (w/w)]	Rate [kg as/ha]	Number of applications	Interval between applications [days]	Total amount of cymoxanil per season [kg as/ha]	DAT [days]	Cymoxanil [mg/kg]	
Lettuce Cartagena Spain 2000	WP 50 %	0.240 - 0.247	4	7	0.977	10	<u><0.05</u>	Soler J.R., 2001, field phase, report no.: CY2/LE
Lettuce Torrepacheco Spain 2000	WP 50 %	0.246 - 0.252	4	7	0.992	0 1 3 10	0.48 0.45 <u><0.05</u> <u><0.05</u>	Freschi G., 2001a, analytical phase, report no.: SIP 1264
Lettuce Cavazzana di Lusia Italy 2000	WP 50 %	0.238 - 0.242	4	7	0.959	10	<u><0.05</u>	Domenichini P., 2001, field phase, report no. CY1/LA,
Lettuce Salerno sul lambro Italy 2000	WP 50 %	0.235 - 0.245	4	7	0.959	0 1 3 10	4.29 2.72 0.95 <u><0.05</u>	Freschi G., 2001a, analytical phase, report no. SIP 1264
Lettuce Canneto sull'Oglio Italy 2001	WP 50 %	0.225 - 0.245	4	7	0.941	10	<u><0.05</u>	Domenichini P., 2003a, field phase, report no. CY2,
Lettuce Gazzo Veronese Italy 2001	WP 50 %	0.242 - 0.248	4	7	0.976	0 1 3 9	3.63 0.55 <u><0.05</u> <u><0.05</u>	Freschi G., 2001b, analytical phase, report no. SIP 1267

Note: The underlined residue values were used for MRL calculation.

A more detailed presentation of the residue data from the 6 supervised residue trials of lettuce submitted by Oxon is given in Annex Ia.

Conclusion

In all submitted 6 supervised residue trials (SRTs) on lettuce by Oxon (3 harvest and 3 decline curve studies, performed outdoor at two vegetation periods in Southern Europe, in accordance with supported GAP) no residue at or above the LOQ of 0.05 mg/kg could be detected.

Note: The nominal required number of 8 supervised residue trials per main crop (see guideline doc. DG Sanco 7029/VI/95, rev. 5, 22/7/97) is not fulfilled formally, but in a cases where no residue could be quantified smaller but sufficient numbers of SRTs are acceptable.

B.7.6.2 Cymoxanil residues in potato - northern and southern Europe, DuPont

References:

Dubey, L., Jernberg, K.M, 1997b, Magnitude of DPX-JE874 and cymoxanil residues in/on soil and potatoes grown in the northern European region - 1996 season, field and analytical phase, report no. AMR 3788-96

Dubey, L., Jernberg, K.M, 1997a, Magnitude of DPX-JE874 and cymoxanil residues in/on soil and potatoes grown in the southern European region - 1996 season, field and analytical phase, report no. AMR 3767-96

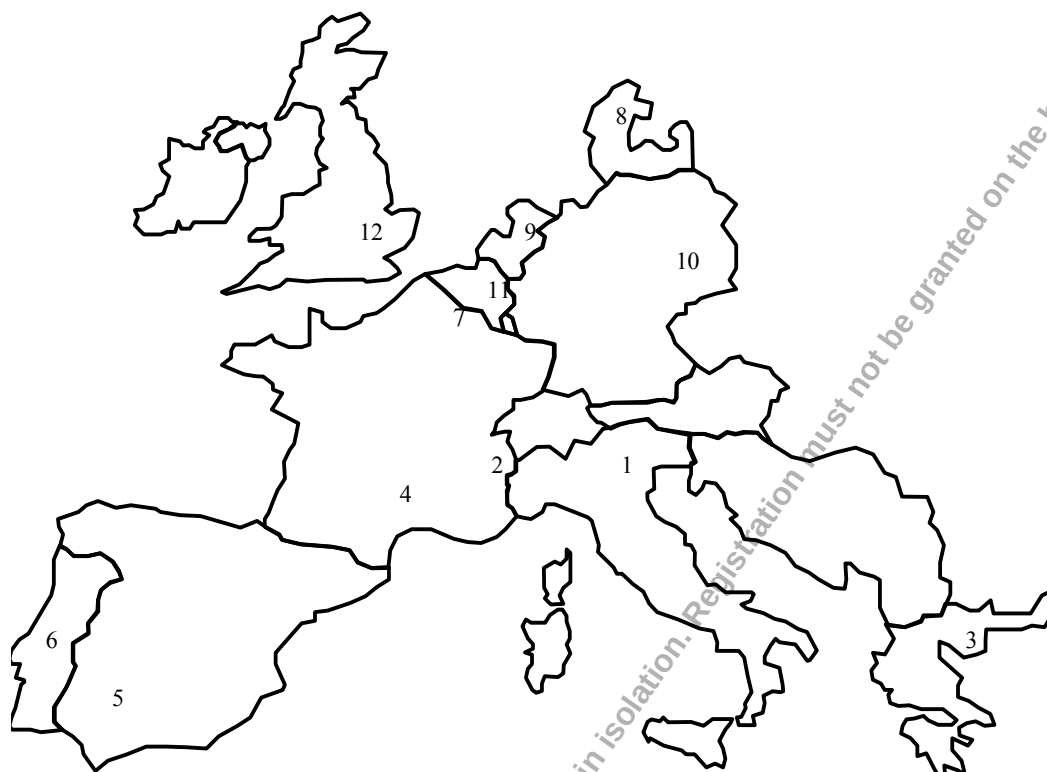
Guideline: DG Sanco 7029/VI/95, rev. 5, 22/7/97, General recommendations for the design, preparation and realization of residue trials; others

GLP: Yes

Studies are considered acceptable for evaluation.

12 supervised outdoor residue trials (harvest trials) on potato conducted 1996 in representative European locations were submitted by DuPont. 6 field tests were performed each in Southern and Northern Europe, respectively. In figure B.7.6.2-1 the locations of the outdoor residue trials were indicated.

Figure B.7.6.2-1 Locations of supervised residue trials on potato - DuPont



Location (southern Europe)

- 1 Italy, Caleppio di Settala, Lombardia
- 2 S-France, Reyrieux, Ain
- 3 Greece, Kalikratia, Chalkidiki
- 4 S-France, Sernhac, Gard
- 5 Spain, Utrera, Sevilla
- 6 Portugal, Ermegeira, Torres Vedras

Location (northern Europe)

- 7 N-France, Rainecourt, Somme
- 8 Denmark, Middelfart
- 9 Netherlands, Bemmel, Gelderland
- 10 Germany, Gnaschwitz, Sachsen
- 11 Belgium, Saint-Amand, Hainaut
- 12 United Kingdom, Melbourne, Derbyshire

Material and methods

Criteria for potato trials in northern Europe:

Before and at the flowering stage potato plants were treated with DPX-KP481-25 (WG formulation containing 25.0 % cymoxanil + 25.0 % famoxadone as active ingredients) at a nominal rate of 200 g cymoxanil and 200 g famoxadone per hectare, 11 to 12 number of applications per season, total up to 2.400 kg as/ha nominally, an application interval of 5-13 days and a PHI of 14 days. Mature potato tuber were harvested.

Criteria for potato trials in southern Europe:

Potato plants about the flowering stage were treated up to 8 times with DPX-KX007-9 (WG formulation: 30.0 % cymoxanil + 22.5 % famoxadone) at a nominal rate of 240 g cymoxanil and 180 g formoxadone per hectare, total up to 1.920 kg as/ha nominally, a 6 - 11 days interval between the applications. The mature potato tubers were harvested 14 days after last treatment.

Critical GAP for potato (DuPont):

The critical GAP on use of cymoxanil supported by DuPont is identical for N-EU and S-EU:

Spray application, growth stage: BBCH 21-95, up to 8 foliar treatments with a rate of 0.175 kg as/ha each, maximal up to 1.400 kg as/ha, a 7-10 days spray interval and a PHI of 14 days.

Sampling, storage and analysis of potato tubers:

Potato tubers from N-EU or S-EU outdoor trial sites were harvested at maturity (random field samples, minimum ca. 1 kg each) 14 days after the last application. Control and treated potato tubers and homogenised tuber samples (plus soil from limited test sites: only determined for famoxadone) were stored under frozen conditions at about -18 °C from sample collection to analysis. The maximum length of freezer storage of potato tuber test samples for the cymoxanil analyses were about 3 months on samples from N-EU and 6 months on samples from S-EU, respectively.

A representative potato tuber sample was homogenized, extracted with ethyl acetate, concentrated by evaporation of the solvent after addition of distilled water, the aqueous residue was washed with hexane and further cleaned up with silica cartridge. Cymoxanil residues were analysed in the potato tuber matrix by GC using a nitrogen-selective detector (NPD). The LOQ of cymoxanil residues in potato tuber is 0.05 mg/kg.

Findings

There are no residues of cymoxanil determined at or above the LOQ (= 0.05 mg/kg) in/on potato tuber samples from representative northern and southern European test site locations (each 6 trials, 1 growing season for all trials: April - Sept. 1996 in N-EU and April - Oct. 1996 in S-EU) and harvested 14 days after last treatment under critical GAP conditions (DuPont, N-EU identical with S-EU). All 12 outdoor trials on potato in northern and southern regions of Europe submitted by DuPont are covered nearly by the critical GAP (DuPont). The following table B.7.6.2-1 gives an overview of the residue data for cymoxanil in/on potato tuber from 12 supervised field residue trials (each 6 harvest studies from northern and southern EU were performed at 1 growing season) reported by DuPont.

Table B.7.6.2-1 Overview of residue data for cymoxanil on/in potato (DuPont)

Crop, trial site country, year	Application					Residues		Reference
	Formulation, content of as [% (w/w)]	Rate [kg as/ha]	Number of appli- cations	Interval between applications [days]	Total amount of cymoxanil per season [kg as/ha]	DAT [days]	Cymoxanil [mg/kg]	
Northern Europe								
Potato Rainecourt N-France, 1996	DPX-KP481- 25 WG 25 % cymoxanil + 25% famoxadone	0.176- 0.211	11	6-8	2.175	14	<0.05	Dubey L, Jernberg K.M, 1997b, field and analytical phase, report no.: AMR 3788-96
Potato Middlefart Denmark, 1996		0.200 ^{a)}	12	6-8	2.400 ^{a)}	14	<0.05	
Potato Bemmel Netherlands, 1996		0.183- 0.209	12	7-8	2.387	14	<0.05	
Potato Gnaschwitz Germany, 1996		0.200 ^{a)}	12	5-13 ^{c)}	2.400 ^{a)}	14	<0.05	
Potato Saint-Amand Belgium, 1996		0.203- 0.235	12	6-10	2.617	14	<0.05	
Potato Melbourne (Derbyshire) United Kingdom, 1996		0.204- 0.225	12	6-8	2.570	14	<0.05	
Southern Europe								
Potato Caleppio di Settala Italy, 1996	DPX-KX007-9 WG 30 % cymoxanil + 22.5% famoxadone	0.234- 0.251	8	6-8	1.962	14	<0.05	Dubey L, Jernberg K.M, 1997a, field and analytical phase, report no.: AMR 3767-96
Potato Reyrieux S-France, 1996		0.267- 0.294	6	6-8	1.655	14	<0.05	
Potato Kalikratia Greece, 1996		0.235- 0.265	8	7	1.997	14	<0.05	
Potato Sernhac S-France, 1996		0.243- 0.265	7	7	1.809	14	<0.05	
Potato Utrera Spain, 1996		0.221- 0.259	8	6-8	1.987	14	<0.05	
Potato Ermegeira Portugal, 1996		0.244- 0.258	8	7-11 ^{b)}	2.010	14	<0.05	

^{a)} nominal values

^{b)} a 11-days interval occurred once on the application intervals

^{c)} a 5-days and 13-days interval occurred once on the application intervals

Note: The underlined residue values were used for MRL calculation.

A more detailed presentation of the residue data from the supervised outdoor residue trials on potato from northern and southern Europe submitted by DuPont is given in Annex Ib and Ic, respectively.

Conclusion

A limited number of 12 supervised outdoor residue trials on potato (6 trials each carried out in representative northern and southern Europe locations) for only one growing season (1996) were submitted by DuPont. All trials were accepted for the evaluation process. A residue of cymoxanil in/on mature potato tuber samples from all 12 residue trials was not determined at or above the LOQ (= 0.05 mg/kg).

B.7.6.3 Cymoxanil residues in potato - northern and southern Europe, Oxon

References:

Röser K., 2003, Determination of residues of cymoxanil after application of Cymoxanil 50% WP in potatoes at 4 sites in Europe, 2002; field phase, report no. 20021200/E1-FPPO.
Freschi G., 2003, Determination of cymoxanil residues in potato specimens (tuber) after application of Cymoxanil 50% WP; analytical phase, report no. SIP1353.

Domenichini P., 1997, Generation of crop samples suitable for residue analysis following applications of cymoxanil + mancozeb, cymoxanil + Cu-oxychloride, cymoxanil + chlorothalonil; field phase, report no. COF/1.

Wasser C., 1997, Determination of the residues of cymoxanil in grapes, potatoes and tomatoes in Italy, 1996; analytical phase, report no. R 6124.

Domenichini P., 1998, Generation of crop samples suitable for residue analysis following applications of cymoxanil + mancozeb, cymoxanil + Cu-oxychloride, cymoxanil + chlorothalonil (2nd year of trial); field phase, report no. COF/2.

Wasser C., 1999, Magnitude of residues of cymoxanil in samples of grapes, tomatoes and potatoes; analytical phase, report no. R 7180

Domenichini P., 2003b, Generation of potato tuber samples, suitable for residue analysis (decline curve) following applications of (cymoxanil 6% + mancozeb 70%) WP; field phase, report no. CY2/I/02PA.

Freschi G., 2001c, Residue analysis of cymoxanil in potato samples (tuber); analytical phase, report no. SIP1298.

Soler J.R., 2002, Generation of potato specimens, suitable for residue analysis of cymoxanil following applications of Micene Plus (cymoxanil 4% + mancozeb 40% WP); field phase, report no. CY2/PO.

Freschi G., 2001d, Residue analysis of cymoxanil in potato samples (tuber); analytical phase, report no. SIP1265.

Guideline: DG Sanco 7029/VI/95, rev. 5, 22/7/97, General recommendations for the design, preparation and realization of residue trials; others

GLP: Yes

Studies are considered acceptable for evaluation.

19 supervised outdoor residue trials on potato plants performed in representative European regions were submitted by Oxon.

Northern-Europe: 4 trials from growing season 2002 including 2 decline curve studies.

Southern-Europe: 15 trials, composed of each 6 trials from growing season 1996 and 1997, and 3 trials from growing season 2001 including two decline curve studies.

The locations of the supervised residue trials in Europe are denoted in figure B.7.6.3-1.

Figure B.7.6.3-1 Locations of supervised residue trials on potato - Oxon



Location (northern Europe)

- 1 N-France, Innenheim, Alsace
- 2 Germany, Dollern, Lower Saxony
- 3 Germany, Perouse, Baden-Württemberg
- 4 Netherlands, Slochteren, Groningen

Location (southern Europe)

- 5 Italy, Salerano sul Lambro, Lodi
- 6 Italy, S. Agostino (FE), Emilia Romagna
- 7 Italy, Cavazzana Di Lusio, Rovigo
- 8 Italy, S. Giovanni Persiceto, Bologna
- 9 Italy, Salerano sul Lambro, Lodi
- 10 Spain, Chipiona, Cádiz
- 11 Spain, Sanlúcar la Mayor, Sevilla

Material and methods

Criteria for the trials on potato in northern Europe:

- 4 residue trials on potato were performed in the growing season 2002 in N-EU:
- 2 harvest studies: N-France (Apr.-Aug. 2002) and Netherlands (Apr.-Sep. 2002)
- 2 decline curve studies: Germany (Apr.-Aug. 2002)

At the flowering stage (up from BBCH 69) potato plants were treated with CYM 50 (WP formulation containing 50.0 % cymoxanil as active substance) at a nominal rate of 0.120 kg as/ha, 4 numbers of applications per season, total 0.480 kg as/ha, an application interval of 6-8 days and a PHI of 7 days (except decline curve studies and 1 harvest study).

Criteria for the trials on potato in southern Europe:

- 15 field residue trials on potato were carried out between growing seasons 1996 and 2001 in S-EU:
- Each 6 harvest trials in Italy from two growing seasons, Apr.-Aug. 1996 and Feb.-Jul. 1997.

3 decline curve studies were performed in one growing season, one study from Italy (Apr.-Aug. 2001) and two studies from Spain (growing periods: Dec. 2000-Apr. 2001 and Feb.-May 2001).

At the flowering stage (in general up from BBCH 60-65) potato plants were treated with different cymoxanil containing products (see formulation) at a nominal rate of 0.120 kg as/ha, 5 numbers of applications per season, total 0.600 kg as/ha nominally, an application interval of 7-8 days and a PHI of 7 days (except decline curve studies).

Critical GAP for potato (Oxon):

The critical GAP on the use of cymoxanil on potato supported by Oxon is:

Northern-Europe: 4 times spray application each with 0.120 kg cymoxanil/ha, total application: 0.480 kg as/ha, growth stage: BBCH 21-95 and a PHI of 7 days.

Southern-Europe: 5 times spray application each with 0.120 kg cymoxanil/ha, total application: 0.600 kg as/ha, growth stage: BBCH 21-95 and a PHI of 7 days.

Formulations:

For N-EU: Only CYM 50 (WP formulation, containing 50.0 % cymoxanil) was used in the residue trials.

For S-EU: The potato plants were treated with different representative formulations (like WP 60 g cymoxanil/kg + mancozeb, WP 40 g cymoxanil/kg + mancozeb, WP 40 g cymoxanil/kg + Cu-oxychloride or SC 50 g cymoxanil/L + chlorothalonil).

Sampling, storage and analysis of potato tubers:

Potato tubers were harvested at maturity (random field samples, minimum 2.0 kg potato tubers per each sample) in general 7 days (harvest studies) or 0, 1, 3, 7, 14 days (decline curve studies) after the last application.

Control and treated potato tubers and homogenised tuber samples were stored under frozen conditions at about -18 °C from sample collection to analysis. The maximum length of freezer storage of potato tuber test samples before cymoxanil analyses were about 1 month (samples from N-EU) and 1 to 11.5 months (samples from S-EU).

A representative potato tuber sample was homogenized, extracted with ethyl acetate, concentrated by evaporation of the solvent, after addition of distilled water the aqueous extract was washed with hexane and extracted with dichloromethane, finally cleaned up on silica gel column. Cymoxanil residues were analysed by GC equipped with a nitrogen-selective detector (NPD) - cymoxanil method 513 (DFG Manual of pesticide residue analysis, Volume II, 1992, p. 157-161). The limit of quantification (LOQ) of cymoxanil residues in potato tuber matrix is <0.05 mg/kg.

Findings

Potato trials from northern Europe:

There were no residues of cymoxanil detected (< LOQ, i.e. <0.05 mg/kg potato tuber) from the 4 potato residue trials (2 harvest and 2 decline curve studies) performed in northern Europe (4 treatments each with 0.120 kg as/ha nominally, product CYM 50, growing season 2002) and

submitted by Oxon. The 2 decline curve studies show a none residue situation at the sampling stage of 0, 1, 3 or 7 days after the last application. See table B.7.6.3-1.

Potato trials from southern Europe:

Oxon submitted 15 field residue trials on potato (12 harvest and 3 decline curve studies) carried out in southern Europe. No residues of cymoxanil were determined in/on potato in all 15 trials, which were performed 5 times with 0.120 kg as/ha nominally. Concerning harvest trials 3 formulations (WP 6% cymoxanil + mancozeb, WP 4% cymoxanil + Cu-oxychloride and SC ca. 5% cymoxanil + chlorothalonil) were proved on two different locations (1x Italy, 1x Spain) and 2 growing seasons (1996 and 1997). At the stage of 0, 1, 3, 7 (14) days after the last application no residue of cymoxanil was detected in the 3 decline curve studies (1 trial from Italy with WP 6% cymoxanil + mancozeb and 2 trials from Spain with WP 4% cymoxanil + mancozeb) from the growing season 2001.

However some "deficiency" in the study design concerning the single and total dose of active substance (in most cases, i.e. harvest studies, fallen below the intended kg as/ha) is identifiable. Nevertheless, the deviations of kg cymoxanil/ha at single or total amount basis (for total amount up to 18%) are within the 25 % tolerance range.

Table B.7.6.3-1 gives an overview of the residue data for cymoxanil in potato tuber from 19 supervised outdoor residue trials reported by Oxon.

Table B.7.6.3-1 Overview of residue data for cymoxanil on/in potato (Oxon)

Crop, trial site, country, year	Application					Residues		Reference
	Formulation, content of as [% (w/w)]	Rate [kg as/ha]	Number of applica tions	Interval between applications [days]	Total amount of cymoxanil per season [kg as/ha]	DAT [days]	Cymoxanil [mg/kg]	
Northern Europe								
Potato Innenheim N-France, 2000	WP 50 % cymoxanil	0.122- 0.125	4	6-7	0.495	6	<u><0.05</u>	Röser K., 2003, field phase, report no.: 20021200/E1- FPPO, GLP: Yes
Potato Dollern Germany, 2002		0.118- 0.128	4	6-8	0.492	0 1 3 7	<0.05 <0.05 <0.05 <u><0.05</u>	
Potato Perouse Germany, 2002		0.120- 0.124	4	6-8	0.487	0 1 3 7	<0.05 <0.05 <0.05 <u><0.05</u>	
Potato Slochteren Netherlands, 2002		0.119- 0.127	4	7-8	0.449	7	<u><0.05</u>	Freschi G., 2003, analytical phase, report no. SIP 1353, GLP: Yes
Southern Europe								
Potato Saleranno sul Lambro Italy, 1996	WP 6% cymoxanil + mancozeb	0.098- 0.099	5	7-8	0.492	7	<u><0.05</u>	Domenichini P., 1997, field phase, report no.: COF/1, GLP: Yes
Potato S. Agostino Italy, 1996		0.095- 0.113	5	7	0.516	7	<u><0.05</u>	Wasser C., 1997, Analytical phase,

Crop, trial site, country, year	Application					Residues		Reference
	Formulation, content of as [% (w/w)]	Rate [kg as/ha]	Number of applicat ions	Interval between applications [days]	Total amount of cymoxanil per season [kg as/ha]	DAT [days]	Cymoxanil [mg/kg]	
Potato Saleranno sul Lambro Italy, 1996	WP 4% cymoxanil + Cu-oxychloride	0.099- 0.102	5	7-8	0.502	7	<u><0.05</u>	report no.: R 6124, GLP: Yes
Potato S. Agostino Italy, 1996		0.095- 0.115	5	7	0.519	7	<u><0.05</u>	Domenichini P., 1997, field phase, report no.: COF/1, GLP: Yes
Potato Saleranno sul Lambro Italy, 1996	SC ca. 5% cymoxanil + chlorothalonil	0.097- 0.103	5	7-8	0.505	7	<u><0.05</u>	Wasser C., 1997, Analytical phase, report no.: R 6124, GLP: Yes
Potato S. Agostino Italy, 1996		0.103- 0.111	5	7	0.538	7	<u><0.05</u>	
Potato Cavazzano di Luisa Italy, 1997	WP 6% cymoxanil + mancozeb	0.091- 0.108	5	7	0.508	7	<u><0.05</u>	Domenichini P., 1998, field phase, report no.: COF/2, GLP: Yes
Potato S. Giovanni Persiceto Italy, 1997		0.096- 0.105	5	7	0.511	7	<u><0.05</u>	
Potato Cavazzano di Luisa Italy, 1997	WP 4% cymoxanil + Cu-oxychloride	0.105- 0.113	5	7	0.541	7	<u><0.05</u>	Wasser C., 1999, analytical phase, report no.: R 7180, GLP: Yes
Potato S. Giovanni Persiceto Italy, 1997		0.096- 0.102	5	7	0.496	7	<u><0.05</u>	
Potato Cavazzano di Luisa Italy, 1997	WP ca 5% cymoxanil + chlorothalonil	0.098- 0.111	5	7	0.516	7	<u><0.05</u>	Domenichini P., 2003b, field phase, report no.: CY2, GLP: Yes
Potato S. Giovanni Persiceto Italy, 1997		0.097- 0.100	5	7	0.496	7	<u><0.05</u>	
Potato Salerno sul Lambro Italy, 2001	WP 6% cymoxanil + mancozeb	0.116- 0.121	5	7	0.589	0 (+3 h) 1 3 7	<0.05 < 0.05 <0.05 <u><0.05</u>	Freschi G., 2001c analytical phase, report no.: SIP 1298, GLP: Yes
Potato Chiponia Spain, 2001	WP 4% cymoxanil + mancozeb	0.115- 0.122	5	7	0.598	7 14	<u><0.05</u> <0.05	Soler J.R., 2002 field phase, report no.: CY2/P0 GLP: Yes
Potato Sanlúcar la Mayor Spain, 2001		0.119- 0.125	5	7	0.611	0 1 3 7 14	<0.05 < 0.05 <0.05 <u><0.05</u> <0.05	Freschi G., 2001d analytical phase, report no.: SIP 1265, GLP: Yes

Note: The underlined residue values were used for MRL calculation

A more detailed presentation of the residue data from the supervised outdoor residue trials of potato from northern and southern Europe submitted by Oxon is given in Annexes Id to Ih.

Conclusion

A number of 19 supervised outdoor residue trials on potato carried out in representative locations in northern (4 trials, each 4 times spray application, nominally each with 0.120 kg as/ha and total 0.480 kg as/ha, GS: 69-93, 6-8 days interval, PHI: 6-7 days - except decline curve studies) over 1 growing season (2002) and southern Europe (15 trials, each 5 times spray application, nominally with 0.120 kg as/ha and total 0.600 kg as/ha each, GS: in general 60-93, 7-8 days interval, PHI: 7 days - except decline curve studies) over 3 growing seasons (1996, 1997 and 2001) were submitted by Oxon. A residue of cymoxanil on/in mature potato tuber samples from all trials (N-EU: 2 harvest + 2 decline curve studies; S-EU: 12 harvest + 3 decline curve studies) was not determined at or above the LOQ (< 0.05 mg/kg).

Regarding intended GAP of Oxon for potato in N-EU:

Residues of cymoxanil at or above the LOQ (= 0.05 mg/kg) could not be determined on/in potato tuber samples from 4 residue trials (2 harvest and 2 decline curve studies) performed on N-EU at 2002.

Concerning intended GAP of Oxon for potato in S-EU (= critical GAP):

A single evaluation of the residue trial data on potato from S-EU submitted by Oxon shows a none residue situation. Enough numbers of trials over more than one growing season (each 6 harvest trials from 1996 and 1997, respectively; 3 decline curve studies from 2001) were submitted.

B.7.6.4 Overall conclusion on supervised residue trials, lettuce, potato - DuPont + Oxon

According to the intended uses supervised residue field trials were performed on two crop groups, i.e. leaf vegetables (lettuce, only S-EU) and root and tuber vegetables (potato, N-EU and S-EU). Regarding lettuce respective trials were submitted by Oxon only. Concerning potato respective trials were submitted by DuPont and Oxon.

Lettuce

A limited number of 6 supervised residue trials on lettuce (3 harvest + 3 decline curve studies, growing periods 2000 and 2001, 4 foliar applications per trial each with 0.225 - 0.252 kg as/ha, total up to 0.992 kg as/ha and 9-10 days PHI) were performed in Southern Europe in accordance with the intended GAP by Oxon only (DuPont does not support lettuce). In accordance to the supported use on lettuce the trials show no quantifiable (at or above the LOQ, i.e. <0.05 mg/kg) residue of cymoxanil in/on lettuce leaves at maturity (10 days after last treatment). In contrast, residues of cymoxanil could be found in all decline curve studies in a range from 0.48 to 4.29 mg/kg (at 0 days after treatment, DAT), 0.45 to 2.72 mg/kg (at 1 DAT) and < 0.05 to 0.95 mg/kg (at 3 DAT).

Potato

With regard to Northern Europe 6 harvest residue field trials from growing season 1996 and 4 field residue trials (2 harvest + 2 decline curve studies) from growing season 2002 at different representative locations in Europe according with the supported GAPs were submitted by DuPont

and Oxon, respectively. In the DuPont trials up to 12 foliar applications, each with 0.176 - 0.235 kg as/ha, total up to 2.617 kg as/ha and 14 days PHI were proved. In the Oxon trials 4 foliar applications, each with 0.118 - 0.128 kg as/ha, total up to 0.495 kg as/ha and 6-7 days PHI were tested on potato plants. Cymoxanil was not detected at or above the limit of quantification (LOQ <0.05 mg/kg) in any of the 10 potato residue trials conducted at representative locations in Northern Europe. Even in the 2 decline curve studies from Oxon (potato tuber sampling: 0, 1, 3 and 7 days after last treatment) no cymoxanil was quantified at or above the LOQ at any sampling date. According to the intended uses of cymoxanil on potato for Northern Europe there could be considered a non residue situation with 14 and 7 days PHI by DuPont and Oxon, respectively.

With respect of Southern Europe 6 harvest field residue trials from different locations on the growing season 1996 were received from DuPont in consistence with the intended use. Oxon submitted 15 field residue trials (each 6 harvest trials for growing seasons 1996 and 1997, 3 decline curve studies from growing season 2001) from representative locations according to the respective GAP. In the DuPont trials up to 8 foliar applications, each with 0.221 - 0.294 kg as/ha, total up to 2.010 kg as/ha and 14 days PHI were proved. In the Oxon trials 5 foliar applications, each with 0.091 - 0.125 kg as/ha, total up to 0.611 kg as/ha and 7 days PHI were tested. Cymoxanil was not detected at or above the LOQ (<0.05 mg/kg) in any of the 18 submitted harvest studies or 3 decline curve studies (Oxon: sampling dates are 0, 1, 3, 7, 14 days after last treatment). Regarding the intended uses of cymoxanil on potato for Southern Europe there could be considered a non residue situation with 14 and 7 days PHI by DuPont and Oxon, respectively.

Lettuce and potato

A residue level of cymoxanil at or above the LOQ (< 0.05 mg/kg) in mature lettuce leaves or potato tuber matrix could not be detected in any of the 6 and 31 submitted residue trial studies on lettuce and potato, respectively. Nevertheless, cymoxanil was quantified only in the lettuce decline studies in a range from 0.48 to 4.29 mg/kg at 0 days after treatment (DAT), 0.45 to 2.72 mg/kg at 1 DAT and < 0.05 to 0.95 mg/kg at 3 DAT. The field trials were performed in accordance to the intended uses by DuPont (potato: N-EU and S-EU) and Oxon (lettuce: S-EU; potato: N-EU and S-EU). The submitted residue data from the outdoor residue trials are considered as sufficient and valid to propose a non residue situation under the intended GAP of DuPont and Oxon, respectively. A proposal of MRLs for lettuce and potato tuber is possible.

B.7.6.5 Stability of residues prior to analysis (DuPont + Oxon)

To demonstrate a sufficient storage stability (freezer conditions: about -20 °C and darkness) of lettuce and potato tuber samples respective studies were submitted by the notifiers.

Oxon: 1 storage stability study on lettuce (whole lettuce and homogenate).

DuPont: 1 storage stability study on potato (whole potato tuber homogenate).

B.7.6.5.1 Freschi G., 2004, Freezer storage stability of cymoxanil residue in lettuce plants - (Oxon)

Report No.: SIP 1379

Guideline: DG Sanco doc. 7032/VI/95, rev. 5, 2277/97, Storage stability of residue samples; others
Note from the notifier: There are no major variations to DG Sanco doc. 7032/VI/1995 rev. 5 (22.June 1997), storage stability of residue samples.

GLP: Yes

Study is considered acceptable for evaluation.

Study characteristics

reference	Freschi G., 2004, (IIA 6.0.1/02)	crop/commodity	lettuce (lactuca sativa), variety not recorded whole lettuce and homogenate
study title	Freezer storage of cymoxanil residue in lettuce plants	testing facility; test site	SIPCAM S.p.A. (analytical phase), Salerano sul Lambro, Italy
type of study	storage stability	fortification level (nominal)	whole lettuce: 0.515 mg/kg lettuce homogenate: 0.515 mg/kg
time of execution	Sep. 2003 - Sep. 2004	storage period	whole lettuce: 0-7-15-30 days, 3-7-12 months lettuce homogenate: 0-7-15-30 days
test substance	1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea *), batch no. 90126, purity 98 % (HPLC)	LOQ (Cymoxanil)	0.05 mg/kg in lettuce matrix (GC-NPD)

*) Cymoxanil, CAS reg. no.: 57966-95-7

Material and Methods

Sample preparation, storage:

The lettuce specimen, at least 5 kg lettuce was obtained from untreated plots of residue trials on Italian area, where lettuce is a main crop. Representative amounts of whole lettuce and lettuce homogenates were transferred in glass containers (with hermetic screw stopper) and spiked with cymoxanil at a nominal concentration of 0.515 mg/kg. The lettuce samples were stored up to 30 days (homogenized lettuce) and 12 months (whole lettuce plants) at about -20 °C in darkness (freezer).

Analysis:

At different time intervals (homogenized lettuce: 0, 7, 15 and 30 days; whole lettuce plant: 0, 7, 15 and 30 days, further 3, 7 and 12 months) the amounts of cymoxanil in fortified lettuce samples (triplicate samples per interval point) and untreated control samples (duplicate sample per interval point) were determined. Additionally the procedural recoveries (duplicate freshly fortified untreated lettuce matrices before extraction phase at each interval point on a level of 0.515 mg cymoxanil/kg) were established.

The lettuce matrix (whole or homogenised lettuce) was extracted with ethyl acetate using an ultra-turrax. The filtrate was evaporated under vacuum condition to dryness and the extract transferred in a separator funnel with distilled water. The aqueous extract was initially washed with hexane (the organic phase was discarded). The aqueous phase was then extracted with dichloromethane and the filtrates were combined and evaporated to dryness. Finally the extract (dissolved in ethyl

acetate) was purified on silica gel column. Cymoxanil was analysed by gas chromatography (GC) using a nitrogen specific detector (NPD). The limit of quantification (LOQ) is 0.05 mg/kg lettuce matrix.

Findings

Procedural recovery:

Table B.7.6.3.1 presents an overview on the procedural recoveries for cymoxanil freshly spiked in lettuce matrix.

Table B.7.6.5.1-1 Overview on procedural recovery data for cymoxanil in lettuce matrices from stability samples fortified with 0.515 mg cymoxanil/kg

Matrix	Range of recoveries [%]	No of samples	Mean recovery [%]	Standard deviation [%]	Coefficient of variations [%]
Whole lettuce	80.9 - 107.9	14 ^{a)}	91.79	8.527	9.28
Homogenized lettuce	82.8 - 97.3	8 ^{b)}	90.58	5.463	6.03

^{a)} At 7 time points (0, 7, 15 and 30 days, 3, 7 and 12 months) each 2 fortified samples were analysed.

^{b)} At 4 time points (0, 7, 15 and 30 days) each 2 fortified samples were analysed.

Note: The mean recovery, standard deviation and coefficient of variations were calculated by RMS

Based on the data shown in table B.7.6.5.1-1 it can be concluded that the analytical method applied on cymoxanil is acceptable (procedural recoveries are in the range of 70 to 110 %).

Storage stability of lettuce:

The whole lettuce plant samples stored over 1 year show a decrease of cymoxanil residue level (range: 2.7 - 9.5 % decline), except the sample from 7 months storage point (16.9 % increase of residue).

In the homogenised lettuce samples stored over a period of 30 days no substantial decrease (maximum 2.0 % at 7 days storage interval) of cymoxanil level was obtained, contrary, however a distinct increase of cymoxanil residue level was determined (15 days storage: + 8.9 %; 30 days storage: + 15.0 %). Additionally a high variation in the storage residue data at 30 days interval for homogenised lettuce samples was obtained.

Table B.7.6.5.1-2 presents an overview about the percentage of degradation for cymoxanil in frozen stored lettuce samples (whole plant and homogenised specimens) based on uncorrected data.

Table B.7.6.5.1-2 Overview on uncorrected degradation data for cymoxanil in lettuce matrices from storage samples fortified with 0.515 mg cymoxanil/kg

Crop commodity	Storage interval [days]	Single values on residue of cymoxanil [mg/kg]			Mean values on residue of cymoxanil [mg/kg] *)	Degradation [%] **)
Whole	0	0.419	0.527	0.482	0.4760	---
	7	0.448	0.498	0.443	0.4630	2.73

Crop commodity	Storage interval [days]	Single values on residue of cymoxanil [mg/kg]			Mean values on residue of cymoxanil [mg/kg] *)	Degradation [%] **)
lettuce	15	0.424	0.458	0.484	0.4553	4.35
	30	0.440	0.407	0.445	0.4307	9.52
	90	0.466	0.460	0.481	0.4690	1.47
	210	0.548	0.556	0.565	0.5563	increase 16.87
	365	0.427	0.443	0.451	0.4403	7.50
Homogenised lettuce	0	0.449	0.437	0.482	0.4560	---
	7	0.424	0.486	0.431	0.4470	1.97
	15	0.490	0.490	0.510	0.4967	increase 8.93
	30	0.566	0.422	0.585	0.5243	increase 14.98

*) RMS: Calculated from 3 single uncorrected residue values at each interval point.

**) Calculated by RMS:

% degradation = $100 - [(mg/kg \text{ residue at defined interval} : mg/kg \text{ residue at 0 day}) \times 100]$.

Note: There are very inhomogeneous single storage stability data notably at 0 day interval (whole lettuce) and at 30 days interval (homogenised lettuce). A lower residue level at 0 day interval is identifiable for homogenised lettuce samples in comparison with whole lettuce samples.

Conclusion

A sufficient content of cymoxanil (nominally 0.515 mg/kg lettuce head whole or homogenised; LOQ of cymoxanil in lettuce matrix is 0.05 mg/kg) was proved under storage conditions at about -20 °C and darkness. A significant degradation (more than 30%) of cymoxanil level in frozen stored whole lettuce samples (12 months storage) or lettuce homogenates (30 days storage) could not be obtained. Because of the high residue variation at a certain storage check point or over the total storage period there could be found the opposite an increase of cymoxanil determined in homogenised lettuce samples up to 15.0 % at 30 days after storage start and on the whole lettuce samples at 7 months storage with 16.9 % could be observed.

Based on the submitted storage stability report on lettuce from Oxon it can be concluded that residues of cymoxanil are stable under frozen condition (about -20 °C and darkness) at least for 30 days in homogenised lettuce matrix and 12 months in whole lettuce plant matrix.

Note: The analyses of the lettuce samples from the supervised residue trials on lettuce (see chapter B.7.6.1) was finished within 12 months after sampling and frozen storage of lettuce head.

B.7.6.5.2 Nathan E.C., 1996, Magnitude of residues of cymoxanil in potatoes following application of Curzate M-8 fungicide at maximum label rates and at five times maximum use rates to investigate the need for magnitude of residue data in processed fractions - (DuPont)

Report No.: AMR 3296-95

Guideline: US EPA Pesticide Assessment Guidelines, subdivision O, 171-4(e), 171-4((k); others
Note from the notifier: There are no major variations to DG Sanco doc. 7032/VI/1995 rev. 5 (22.June 1997), storage stability of residue samples.

GLP: Yes

Study is considered acceptable for evaluation.

Study characteristics

reference	Nathan E.C., 1996, (IIA 6.0.1/01)	crop/commodity	potato tubers, varieties not addressed
study title	Magnitude of residues of cymoxanil in potatoes following application of Curzate M-8 fungicide at maximum label rates and at five times maximum use rates to investigate the need for magnitude of residue data in processed fractions *)	testing facility; test site	DuPont Experimental station, Wilmington, Delaware (field phase), USA Morse Laboratories Inc., Sacramento (analytical phase), California, USA
type of study	field trial study including storage stability	fortification level (cymoxanil, nominal)	potato tuber homogenate: 0.250 mg/kg
time of execution	May 1999 - June 1996	storage period	potato tuber homogenate: 0 day, 10.5 and 12 months
test substance	1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea **), batch no. T3217-101, purity 99,9 % ***)	LOQ (cymoxanil)	0.056 mg/kg in potato tuber matrix (HPLC, UV detection)

*) The evaluated storage stability data of frozen stored potato tubers samples were part of this residue field trial study (performed in USA).

**) Cymoxanil, CAS reg. no.: 57966-95-7

***) Method is not reported.

Material and MethodsSample preparation, storage:

Representative amounts of control potato tuber samples obtained from a field trial study performed in the USA 1995 were fortified with cymoxanil at a nominal concentration level of 0.250 mg/kg. Potatoes were not peeled but homogenised in dry ice before fortification. The potato samples were frozen stored up to 12.5 months at about -20 °C in darkness.

Analysis:

After 0 day, 10.5 and 12.5 months, one control, two fortified potato samples and two controls freshly fortified at 0.250 mg cymoxanil/kg potato tuber nominally were analysed at each sampling interval point.

The homogenised potato tubers were extracted with ethyl acetate in a centrifuge bottle. Ethyl acetate was allowed to evaporate. Hexane-saturated acetonitrile and hexane were added, the sample was grounded and centrifugated. Resulting extracts were combined, hexane was removed and an aliquot of the acetonitrile solution was concentrated and finally purified on a silica gel cartridge. Cymoxanil was analysed by reversed HPLC using UV detection (absorption at 245 nm). The limit of quantification (LOQ) of cymoxanil is 0.056 mg/kg in potato matrix.

FindingsProcedural recovery:

Table B.7.6.5.2-1 outlines the range of the procedural (freshly spiked) recovery data of cymoxanil in potato matrix.

Table B.7.6.5.2-1 Overview on procedural recovery data for cymoxanil in potato matrices from stability samples fortified with 0.250 mg cymoxanil/kg

Matrix	Range of recoveries [%]	No of samples	Mean recovery [%]	Standard deviation [%]	Coefficient of variations [%]
Whole potato tuber homogenate	72 - 96	6 ^{a)}	82.7	9.69	11.7

^{a)} At 3 time points (0 day, 10.5 and 12.5 months) 2 fortified samples each were analysed.

Note: The mean recovery, standard deviation and coefficient of variations were calculated by RMS.

The analytical method (HPLC-UV) used on cymoxanil in potato matrix is acceptable (procedural recoveries are in the range of 70 to 110 %). However, the amount of proved cymoxanil concentration was 4.5 times higher than the LOQ (= 0.056 mg/kg).

Storage stability of potato tuber:

The actual amount of single cymoxanil fortification (0.250 mg/kg nominally) on potato tuber samples does not fulfil the formal requirements of DG Sanco doc. 7032/VI/1995 rev. 5 (22.June 1997), storage stability of residue samples (fortification of storage samples: at least 10 times higher than LOQ, which is 0.056 mg/kg potato matrix used HPLC-UV). Nevertheless, a tendency of residue stability of the tested fortification level (about 4.5 times higher than LOQ) under frozen storage condition (about -20 °C, darkness) for 12.5 months was shown for potato tuber homogenate. Table B.7.6.5.2-2 presents an overview about the percentage of degradation for cymoxanil in frozen stored potato homogenate based on uncorrected data.

Table B.7.6.5.2-2 Overview on uncorrected degradation data for cymoxanil in potato matrices from storage samples fortified with 0.250 mg cymoxanil/kg

Crop commodity	Storage interval [months]	Single values on residue of cymoxanil [mg/kg]		Mean values on residue of cymoxanil [mg/kg] *)	Degradation [%] **)
Whole potato tuber homogenate	0 ^{a)}	0.22	0.21	0.215	---
	10.5 ^{b)}	0.23	0.27	0.250	increase 16.3
	12.5 ^{c)}	0.20	0.22	0.210	2.3

^{a)} 0 day

^{b)} ca. 318 days

^{c)} ca. 377 days

*) RMS: Calculated from 2 single uncorrected residue values at each interval point.

**) Calculated by RMS:

% degradation = $100 - [(mg/kg \text{ residue at defined interval} : mg/kg \text{ residue at 0 day}) \times 100]$.

Note: There are inhomogeneous single storage residues at 10.5 months interval.

Conclusion

Despite a formal deficiency in the storage stability design (tested fortification level of 0.250 mg cymoxanil/kg potato sample was about 4.5 times higher and not 10 times higher than the LOQ = 0.056 mg/kg as regarded by DG Sanco doc. 7032/VI/95, rev. 5, 22/7/97, Storage stability of residue samples) there was demonstrated a storage stability on frozen potato tuber homogenate (stored at about -20 °C and darkness) at least for 12.5 months.

Remark:

The storage periods of frozen potato tuber samples prior to analyses in all evaluated supervised residue trials submitted by DuPont and Oxon were shorter than 12 months.

Report No.: AMR 4375-97

Guideline: US EPA OPPTS 860.1300, nature of the residue, plant, livestock

GLP: Yes

Study is considered acceptable for evaluation.

A field test study on rotational crops with cymoxanil was not submitted either by DuPont or Oxon. It is considered by RMS as not necessary for evaluation, as a model study on rotational crops (lettuce, sugar beet and spring wheat) is available and valid (see B.7.9.1).

B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4)

Not relevant, because the residue level on/in lettuce or potato tubers is lower than the LOQ (= 0.05 mg/kg).

B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3)

Not relevant, since "no residue" of cymoxanil at or above the LOQ (= 0.05 mg/kg) was detected on/in the supported crops (see metabolism studies and supervised residue trials of lettuce and potatoes). Additionally, evidence of a "non residue" situation was identified in lactating goat (see animal metabolism study).

B.7.9 Residues in succeeding crops or rotational crops (Annex IIA 6.6; Annex IIIA 8.5)

To determine the nature and amount of residue uptake in representative rotational crops a model test study was submitted by DuPont only (data sharing with Oxon). The test was performed with confined succeeding crops (i.e. lettuce, sugar beet and spring wheat grown in plastic pots under greenhouse conditions at 2 different rotational crop intervals) after single soil treatment with radioactive labelled cymoxanil. A respective field test study with rotational crops after cymoxanil application on soil was not submitted either by DuPont or Oxon.

B.7.9.1 Singles S.K., Strek H.J., Sheftic G.D., 1996, Accumulation of residues in confined rotational crops: Lettuce, wheat, and beets after treatment with [¹⁴C]cymoxanil - DuPont

Report No.: AMR 3575-95

Guideline: US EPA Pesticide Assessment Guidelines, subdivision N, 165-1 and OPPTS 860.1900.

WARNING: This document forms part of an Evaluation data package and should not be released for public use. Registration must not be granted on the basis of this document.

Statement from data submitter: There are no major variations to the recommended EU guideline DG Sanco doc. 7524/VI/95, testing of plant protection products in rotational crops.

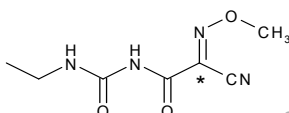
GLP: Yes

Study is considered acceptable for evaluation.

Study characteristics

reference	Singles S.K., Streck H.J., Sheftic G.D, 1996, (IIA 6.6/01)	testing facility; test site	DuPont Agricultural Products; Newark (field + greenhouse phase) plus Wilmington (analytical phase), Delaware, USA
study title	Accumulation of residues in confined rotational crops: Lettuce, wheat, and beets after treatment with [¹⁴ C]cymoxanil	soil type	Sassafras sandy loam soil
type of study	rotational crop (model test)	area	USA, pots in greenhouse
period of execution (soil treatment - end of analytical phase)	Aug. 1995 - Sep. 1996	soil treatment, total	single, equivalent to 1.212 kg as/ha (1.08 lb as/acre), ^{b)}
test substance	[cyanoacetamide-2- ¹⁴ C]-cymoxanil ^{a)} , NEN lot no. 2732-155, radiochem. purity 95.3 % (HPLC), specific activity 14.09 mCi/g	soil aging (rotational crop interval, RCI)	30 or 120 days RCI under field condition
plants	lettuce (lactuca sativa), variety Prizehead sugar beet, variety Monogerm Hybrid spring wheat, variety Butte 86	sampled RAC ^{c)} (days after single soil treatment)	lettuce head (76/167) ^{d)} sugar beet foliage (132/210) ^{d)} sugar beet roots (132/210) ^{d)} spring wheat forage (57/152) ^{d)} spring wheat straw (132/210) ^{d)} spring wheat grain (132/210) ^{d)}

^{a)} Positions of radiolabel in ¹⁴C-cymoxanil: * Denotes position of ¹⁴C radiolabel



CAS: 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide, E isomer
IUPAC: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea
CAS reg. no.: 57966-95-7

^{b)} See intended EU GAP from DuPont for Europe (N-EU = S-EU) with a total rate up to 1.400 kg as/ha (up to 8x 0.175 kg as/ha) with a PHI of 14 days.

^{c)} RAC ... raw agricultural commodities

^{d)} The first and second value relate to a rotational crop interval of 30 and 120 days, respectively.

Materials and Methods

Study design: A single application of radiolabelled cymoxanil test solution (formulated in acetone and diluted with distilled water immediately prior to application) was applied directly onto the levelled bare Sassafras sandy loam soil (soil drench technique) contained in plastic pots (about 27,9 cm diameter and a volume of 18.9 liter per each pot) at a concentration level equivalent to 1.212 kg ¹⁴C-cymoxanil/ha. A total of 18 pots were treated with radioactive labelled cymoxanil and 9 pots were reserved as controls. The soil in the pots was aged under field conditions for 30 or 120 days rotational crop intervals (RCIs). Thereafter, the pots were placed in the greenhouse and planted with confined rotational crops (lettuce, sugar beets and spring wheat). Three ¹⁴C-cymoxanil-treated pots for each crop were planted at two different rotational crop intervals. One control (untreated) pot for each crop was planted at the end of soil aging periods.

Soil core and crop sampling:

At the time of ^{14}C -cymoxanil application three representative soil cores (about 2.54 cm diameter) were taken randomly from the treated pots. Immediately prior to crop planting 1 representative soil core was taken for each crop and aging period. In addition soil samples were taken at each crop sampling. The soil cores were placed in plastic sleeves and stored in freezer until analysis.

Plant samples at each RCI were taken from the control and treated pots. Immature wheat e.g. was harvested to represent forage, all other crop fractions were harvested at maturity to represent wheat straw, wheat grain, sugar beet tops, sugar beet roots and lettuce heads. The samples of raw agricultural commodities were placed in plastic bags and stored under freezer condition until analysis.

Preparation of soil core samples: Soil cores sampled immediately after treatment with ^{14}C -cymoxanil and taken before planting at the 30 days rotational interval were quickly dried by washing with acetone and vacuum filtration. Soil samples of wheat forage from 30 days rotational crop interval were extracted using methanol/acetate buffer (pH 4.6). For all remaining soil samples from the 30 and 120 days rotational intervals, the soil cores were dried and manually homogenised (without extraction process).

Homogenisation of crop samples: The raw agricultural commodities were frozen by mixing with dry ice or liquid nitrogen and then homogenised with a blender or food processor.

Determination of TRR:

Soil core: The total radioactive residue (TRR) of soil core samples (extracted/unextracted soils and extracts with organic solvents) were determined by combustion and liquid scintillation counting (LSC). The TRR of soil samples was calculated on a dry weight basis. The limit of determination (LOD) for soil combustion analysis was calculated as 0.002 mg ^{14}C -cymoxanil equivalents/kg dried soil.

Rotational Crops: A representative subsample of the homogenized plant material was air-dried and the TRR in each homogenized sample was determined with LSC after combustion. The TRR of plant samples was calculated on a fresh weight basis. The limit of determination (LOD) for crop combustion analysis was calculated as 0.003 mg ^{14}C -cymoxanil equivalents/kg. Crop fractions containing more than 0.01 mg/kg ^{14}C -cymoxanil equivalents were extracted and analysed by radio-HPLC.

Extraction of soil core and crop samples, analyses and characterisation:

Soil core: A part of the soil core samples (i.e. from 30 days rotational crop interval) were extracted with methanol/acetate buffer (pH 4.6). A further characterisation (or distribution over the length of the core) of the TRR was not reported in this study.

Rotational crops:

Representative aliquots of each homogenised raw agriculture commodity were extracted separately. The homogenised, mixed plant material was centrifugated (extracted) with acetate

buffer (20 mM, pH 4.6). The supernatant was decanted, filtered and the extraction procedure repeated. Finally the plant material was extracted with acetonitrile/acetate buffer (pH 4.6) and centrifugated. All supernatants were combined, concentrated and aliquots were analysed by LSC. The remaining extracts were stored in a freezer until radio-HPLC analysis (2 different columns used: Zorbax Rx-C8 with LOQ of 0.001 mg eq/kg and Hamilton PRP with LOQ of 0.002 mg eq/kg).

Findings

Soil core samples:

Immediately after soil treatment with radioactive labelled cymoxanil the TRR determined was 1.04 mg ¹⁴C-cymoxanil equivalents/kg dried soil. The starting value of TRR declined rather rapidly to 0.31 mg and 0.20 mg ¹⁴C-cymoxanil equivalents/kg dried soil after 30 and 120 days outdoor aging of the soil, respectively. On average the TRR values in the soil core samples decreases slowly after planting of the rotational crop and harvest of the raw agricultural commodities. A further characterisation of TRRs in soil core samples was not reported.

The TRRs in soil core samples are summarised in table B.7.9.1-1.

Table B.7.9.1-1 Total radioactive residue in soil core samples from test pots after single application of ¹⁴C-cymoxanil equivalent to 1.211 kg as/ha at different sampling dates

		Total radioactive residue [mg ¹⁴ C-cymoxanil equivalents/kg soil] *)							
	0 DASST ^{a)}	30 DASST ^{a)} (rotational crop interval)				120 DASST ^{a)} (rotational crop interval)			
Total soil residue at application ^{**)}	1.04	---				---			
Total soil residues at planting ^{***)}	---	0.31				0.20			
Soil residue at harvest: ^{a)}		76 DASST	132 DASST	57 DASST	132 DASST	167 DASST	210 DASST	152 DASST	210 DASST
Mature lettuce	---	0.22	---	---	---	0.15	---	---	---
Mature sugar beets	---	---	0.29	---	---	---	0.20	---	---
Spring wheat forage	---	---	---	0.33	---	---	---	0.20	---
Spring wheat grain and straw	---	---	---	---	0.20	---	---	---	0.16
Average over all RAC	---	0.26				0.18			

*) based on dry weight of the soil samples

**) one composite soil sample, immediately sampled and analysed after soil application

***) averaged for the 3 tested crops, sampled immediately before crop planting

^{a)} DASST ... days after single soil treatment

Notes: The radioactive soil core samples were analysed within 6 months from sampling.

Rotational crop samples:

In mature lettuce samples the detected TRR was less than 0.01 mg ¹⁴C-cymoxanil equivalents/kg at 30 and 120 days rotational crop intervals (RCIs) between single ¹⁴C-cymoxanil soil treatment and

crop harvesting. The foliage and root samples of mature sugar beet from the pots with 30 and 120 days RCIs containing TRRs > 0.01 mg ¹⁴C-cymoxanil equivalent/kg were further characterised by HPLC, as well as the spring wheat commodities (forage, straw and grain). In all analysed raw agriculture commodities of lettuce, sugar beet and spring wheat samples no ¹⁴C-cymoxanil could be identified.

In table B.7.9.1-2 there is presented a summary of the observed TRRs in the rotational crops.

Table B.7.9.1-2 Distribution of TRR in rotational crop samples from test pots after single soil application of ¹⁴C-cymoxanil equivalent to 1.212 kg as/ha at different rotational crop intervals and sampling dates

Raw agricultural commodities		30 days RCI *)		120 days RCI *)	
Rotational crop	Stage of crop	DASST **)	TRR ***) [mg eq/kg]	DASST **)	TRR ***) [mg eq/kg]
lettuce head	mature	76	<0.01	167	<0.01
sugar beet foliage	mature	132	0.02	210	<0.01
sugar beet roots	mature	132	0.01	210	<0.01
spring wheat forage	immature	57	0.07	152	0.01
spring wheat straw	mature	132	0.14	210	0.12
spring wheat grain	mature	132	0.04	210	0.05

*) RCI ... rotational crop interval, time between single soil application of ¹⁴C-cymoxanil and crop planting
 **) DASST ... days after single soil treatment

***) total radioactive residue (TRR) expressed as mg ¹⁴C-cymoxanil equivalents/kg (mg eq/kg) fresh weight of raw agricultural commodity (RAC)

Raw agricultural commodities containing more than 0.01 mg ¹⁴C-cymoxanil equivalents/kg were extracted and analysed by radio-HPLC. In mature lettuce heads the TRR was less than 0.01 mg eq/kg for both RCIs. Regarding sugar beets (i.e. foliage and roots) only at 30 days RCI a TRR more than 0.01 mg eq/kg was obtained (i.e. foliage: total 0.02 mg eq/kg, all extractable; roots: total 0.01 mg eq/kg, all extractable). In spring wheat forage TRRs of 0.07 mg eq/kg (thereof 0.06 mg eq/kg were extractable) and 0.01 mg eq/kg (thereof < 0.01 mg eq/kg were extractable) were detected at 30 and 120 days RCIs, respectively. Regarding spring wheat grain TRR values of 0.04 mg eq/kg (thereof 0.02 mg eq/kg were extractable) at 30 days RCI and 0.05 mg eq/kg (thereof 0.02 mg eq/kg were extractable) at 120 days RCI were obtained. The highest TRR values were detected in spring wheat straw, namely 0.14 mg eq/kg (thereof 0.10 mg eq/kg extractable) at 30 days RCI and 0.12 mg eq/kg (thereof 0.08 mg eq/kg were extractable) at 120 days RCI. In all RAC extracts (i.e. lettuce, sugar beet and spring wheat) containing ≥0.01 mg eq/kg and for both RCIs the majority of cymoxanil derived radioactivity was extractable. ¹⁴C-cymoxanil or structurally related metabolites were not identified in any analysed RAC (i.e. lettuce, sugar beet and spring wheat) at any RCI. No individual components detected in any RACs accounted for more than 0.02 mg eq/kg.

In table B.7.9.1-3 an overview is given concerning a further characterisation of the TRR in the rotational crops after single soil treatment.

Table B.7.9.1-3 Characterisation of TRR (extractable/unextractable) and distribution in rotational crop samples from test pots after single soil application of ^{14}C -cymoxanil equivalent to 1.212 kg as/ha at different rotational crop intervals and sampling dates

RAC **)	Radioactive residue [mg ^{14}C -cymoxanil equivalents/kg]									
	30 days RCI *)					120 days RCI *)				
	DASST ***)	extr. residue	unextr. residue	lost residue	total residue ****)	DASST ***)	extr. residue	unextr. residue	lost residue	total residue ****)
lettuce heads	76	---	---	---	<0.01	167	---	---	---	<0.01
sugar beet foliage	132	0.02 ^{a)}	<0.01	---	0.02	210	---	---	---	<0.01
sugar beet roots	132	0.01 ^{a)}	<0.01	---	0.01	210	---	---	---	<0.01
spring wheat forage	57	0.06 ^{a)}	0.01	---	0.07	152	<0.01 ^{c)}	<0.01	---	0.01
spring wheat straw	132	0.10 ^{b)}	0.03	0.01	0.14	210	0.08 ^{b)}	0.02	0.02	0.12
spring wheat grain	132	0.02 ^{a)}	0.01	0.01	0.04	210	0.02 ^{a)}	<0.01	0.02	0.05

*) RCI ... Rotational crop interval, time between single soil application of ^{14}C -cymoxanil and crop planting

**) RAC ... Raw agricultural commodities of rotational crops

***) DASST ... Days after single soil treatment

****) Total residue: Sum of extr. (extractable), unextr. (unextractable) and lost residue.

^{a)} No single component was > 0.01 mg ^{14}C -cymoxanil equivalent/kg.

^{b)} No single component was > 0.02 mg ^{14}C -cymoxanil equivalent/kg.

^{c)} No further characterisation was performed.

Note: The longest interval between crop sampling and initial extraction was 174 days (< 6 months).

Immature crop samples were used to validate the extraction method, showing that ^{14}C -cymoxanil could be extracted completely.

Conclusions

A rotational crop model study was submitted by DuPont only (data sharing with Oxon). The study was performed with lettuce, sugar beet and spring wheat in plastic pots under greenhouse conditions at 30 and 120 days rotational crop intervals (RCIs) using a single soil treatment equivalent to 1.212 kg ^{14}C -cymoxanil/ha.

In soil core samples taken 0, 30 and 120 days after single soil treatment with ^{14}C -cymoxanil there was detected an average TRR concentration of 1.04, 0.31 and 0.20 mg ^{14}C -cymoxanil equivalents/kg soil dry matter, respectively. ^{14}C -cymoxanil was not detected in soil core samples at 30 or 120 days RCIs or at any sampling dates for the rotational crops.

In/on raw agricultural commodities like lettuce head, sugar beet tops or roots and spring wheat forage, straw or grain there were no ^{14}C -cymoxanil or structurally related metabolites detected. The cymoxanil derived radioactivity were not significant (TRR < 0.01 mg eq/kg) in lettuce heads at 30 and 120 days RCIs, in sugar beet foliage or roots and wheat forage at 120 days RCI. Significant

amounts of TRR (the major parts were extractable) without definite decrease of radioactivity from 30 to 120 days RCI were detected in wheat grain (≤ 0.05 mg eq/kg) and with the highest level in wheat straw (≤ 0.14 mg eq/kg).

Note by RMS:

The current submitted rotational model crop study (lettuce, sugar beet and spring wheat) by DuPont (intended GAP for potato) supports also the intended Oxon GAP for lettuce and potato. On the results of the model rotational crop study a supplementary rotational field crop study performed in Europe is not required. There is no evidence of possible residues in the tested rotational crops after application of cymoxanil according to intended uses.

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post harvest uses (Annex IIA 6.8; Annex IIIA 8.7)

B.7.10.1 Proposed pre-harvest intervals for each relevant crop

Based on the information on the intended uses by the notifiers (DuPont/Oxon), the following pre harvest intervals are proposed:

Table B.7.10-1 PHI proposed by DuPont after application of cymoxanil

Crop	Food/feed item	PHI [days]	Remarks
potatoes	tubers	14 days	outdoor or field use, for N-EU and S-EU

Table B.7.10-2 PHI proposed by Oxon after application of cymoxanil

Crop	Food/feed item	PHI [days]	Remarks
potatoes	tubers	7 days	outdoor or field use, for N-EU and S-EU
lettuce	heads	10 days	outdoor or field use, for S-EU

B.7.10.2 Re-entry period for livestock to areas to be grazed

Not relevant, because the intended, treated crops are not used for grazing by livestock.

B.7.10.3 Re-entry period for man to crops, buildings or spaces treated

Based on the results of corresponding toxicological studies, a re-entry period for man (workers) is considered not necessary shortly after spraying (refer to section B.6, mammalian toxicology).

B.7.10.4 Withholding period for animal feeding stuffs

In consideration of the evidence of "non residue" situation (especially for potato tubers, which are addressed as feed item) for intended crops, a setting of withholding period for animal feeding stuffs is not necessary.

B.7.10.5 Waiting period between application and handling treated product

Based on the results of corresponding toxicological studies, including the pre-harvest intervals of the intended crops (see table B.7.10-1 and B.7.10-2) and the non necessary re-entry shortly after spraying, a setting of a waiting period between application and handling treated product is considered not required (refer to section B.6, mammalian toxicology).

B.7.11 Community MRL and MRLs in EU Member States (Annex IIIA 12.2)

Actual there are no harmonised EU-MRLs regarding cymoxanil established.

B.7.12 Proposed EU MRLs and justification for the acceptability of those MRLs (Annex IIA 6.7; Annex IIIA 8.6)

B.7.12.1 Food of plant origin

B.7.12.1.1 Lettuce

The results of 6 supervised residue trials (3 harvest + 3 decline curve studies, covering 2 growing years) performed in Southern Europe are regarded as relevant according to the intended uses (Oxon only). Since at harvest no residue at or above the LOQ could be detected in/on any lettuce head samples analysed, a **MRL of 0.05 mg/kg** (= LOQ) expressed as parent compound is proposed for lettuce.

B.7.12.1.2 Potatoes

According to the intended uses of DuPont and Oxon the results of 10 supervised residue trials (DuPont: 6 harvest studies covering 1 growing year, Oxon: 2 harvest + 2 decline curve studies covering 1 growing year) performed in Northern Europe and 21 supervised residue trials (DuPont: 6 harvest studies covering 1 growing year, Oxon: 12 harvest studies covering 2 growing years + 3 decline curve studies covering 1 growing year) performed in Southern Europe are considered as relevant. Since at harvest no residue at or above the LOQ could be detected in/on any potato tuber samples analysed, a **MRL of 0.05 mg/kg** (= LOQ) expressed as parent compound is proposed for potatoes.

B.7.12.2 Food of animal origin

Based on the intended uses, the residue data provided (according to the provisional residue definition) and the fact that no accumulation of residues was observed in the additional submitted livestock metabolism study on lactating goat, **no MRL for food of animal origin is proposed.**

B.7.13 Proposed EU import tolerances and justification for the acceptability of those residues

No import tolerances has been intended by the notifiers (DuPont or Oxon).

B.7.14 Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs

There are no CAC MRLs regarding cymoxanil established.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

B.7.15.1 Estimation of the potential and actual exposure through diet

B.7.15.1.1 Estimation of chronic exposure through diet

The TMDI (theoretical maximum daily intake) of cymoxanil was calculated based on the maximum residue level (MRL) derived from valid and relevant supervised residue trials. The proposed MRL regarding lettuce (heads) is at LOQ (= 0.05 mg cymoxanil/kg). Concerning potato (tubers) a MRL at 0.05 mg cymoxanil/kg (= LOQ) is proposed. Based on the results of a 1-year study in dog a **proposed ADI** (acceptable daily intake) value of **0.03 mg/kg bw/d** is established.

The diet consumption data for TMDI calculation were based on:

WHO diet model: from the European diet compiled in the WHO Publication on Cultural and Global Diets, 1994 (adult, 60 kg bw).

German diet model (VELS model): published in Bundesgesundheitsblatt 2005, 48, p. 84-98 (for a child, 16.15 kg bw); electronic calculation sheet is published on BfR homepage, version not addressed, 10 April 2006.

UK (PSD's) ten consumer model: published on PSD homepage, NEDI model, excel workbook, version 1.1, released 23 January 2006.

The calculated TMDIs for lettuce and potatoes with WHO, German VELS model or UK (PSD's) ten consumer model do not exceed the proposed ADI value (see summarised results in tables B.7.15.1.1-1 to B.7.15.1.1-3).

Table B.7.15.1.1-1 Theoretical Maximum Daily Intake (TMDI) of cymoxanil residues based on WHO diet model (adult, 60 kg bw)

Food/intake	Average daily consumption of food item [g/day]	MRL *) [mg/kg]	Chronic intake(TMDI) [mg/person/day]
lettuce (lactuca sativa)	22.5	0.05	0.001125
potatoes	240.8	0.05	0.012040
TMDI [mg/person/day]			0.013465
TMDI [mg/kg bw/day]			0.000224
ADI [%]			0.7

*) Residues expressed as mg cymoxanil/kg. Values represent the limit of quantification (LOQ) of cymoxanil in the respective matrix.

Table B.7.15.1.1-2 Theoretical Maximum Daily Intake (TMDI) of cymoxanil residues based on German VELS model (child, 16.15 kg bw)

Food/intake	Average daily consumption of food item [g/day]	MRL *) [mg/kg]	Chronic intake(TMDI) [mg/person/day]
lettuce (lactuca sativa)	1.4	0.05	0.000070
potatoes	41.4	0.05	0.002070
TMDI [mg/person/day]			0.002140
TMDI [mg/kg bw/day]			0.0001325
ADI [%]			0.4

*) Residues expressed as mg cymoxanil/kg. Values represent the limit of quantification (LOQ) of cymoxanil in the respective matrix.

Table B.7.15.1.1-3 Theoretical Maximum Daily Intake (TMDI) of cymoxanil residues based on UK consumer chronic intake model for 10 consumer groups

		adult		infant		toddler		4-6 year old child		7-10 year old child	
commodity	MRL ^{a)} [mg/kg]	TMDI ^{b)} [mg/kg bw/d]	% ADI	TMDI ^{b)} [mg/kg bw/d]	% ADI	TMDI ^{b)} [mg/kg bw/d]	% ADI	TMDI ^{b)} [mg/kg bw/d]	% ADI	TMDI ^{b)} [mg/kg bw/d]	% ADI
lettuce	0.05	0.00003	0.1	0.00002	<0.1	0.00004	0.1	0.00003	0.1	0.00004	0.1
potatoes	0.05	0.00016	0.5	0.00056	1.9	0.00046	1.5	0.00041	1.4	0.00035	1.2
sum of commodities		0.00019	0.6	0.00058	1.9	0.00050	1.6	0.00044	1.5	0.00039	1.3
		11-14 year old child		15-18 year old child		vegetarian		elderly - own home		elderly - residential	
commodity	MRL ^{a)} [mg/kg]	TMDI ^{b)} [mg/kg bw/d]	% ADI	TMDI ^{b)} [mg/kg bw/d]	% ADI	TMDI ^{b)} [mg/kg bw/d]	% ADI	TMDI ^{b)} [mg/kg bw/d]	% ADI	TMDI ^{b)} [mg/kg bw/d]	% ADI
lettuce	0.05	0.00002	<0.1	0.00002	<0.1	0.00004	0.1	0.00003	0.1	0.00001	<0.1
potatoes	0.05	0.00027	0.9	0.00023	0.8	0.00018	0.6	0.00016	0.5	0.00016	0.5
sum of commodities		0.00029	0.9	0.00025	0.8	0.00022	0.7	0.00019	0.6	0.00017	0.5

^{a)} Residues expressed as mg cymoxanil/kg. Values represent the limit of quantification (LOQ) of cymoxanil in the respective matrix.

^{b)} The chronic commodity intakes are based on the 97.5th percentiles. Processing factor = 1.

Calculation data for lettuce:

47 g/day (adult, bw: 76.0 kg), 3 g/d (infant, bw: 8.7 kg), 12 g/d (toddler, bw: 14.5), 14 g/d (4-6 year old child, bw: 20.5), 22 g/d (7-10 year old child, bw: 30.9), 19 g/d (11-14 year old child, bw: 48.0 kg), 28 g/d (15-18 year old child, bw: 63.8), 47 g/d (vegetarian, bw: 66.7 kg), 38 g/d (elderly - own home, bw: 70.8 kg), 17 g/d (elderly - residential, bw: 61.6 kg)

Calculation data for potatoes:

247 g/day (adult, bw: 76.0 kg), 97 g/d (infant, bw: 8.7 kg), 133 g/d (toddler, bw: 14.5), 169 g/d (4-6 year old child, bw: 20.5), 217 g/d (7-10 year old child, bw: 30.9), 256 g/d (11-14 year old child, bw: 48.0 kg), 290 g/d (15-18 year old child, bw: 63.8), 236 g/d (vegetarian, bw: 66.7 kg), 232 g/d (elderly - own home, bw: 70.8 kg), 196 g/d (elderly - residential, bw: 61.6 kg)

B.7.15.1.2 Estimation of acute exposure through diet

The proposed ARfD (Acute Reference Dose) is established at **0.08 mg/kg bw**, basing on the results of a rabbit teratogenicity study.

The calculations of short term intake are based on the German diet model (VELS) and the UK (PSDs) intake model for 10 consumer groups:

German diet model (VELS model): published in Bundesgesundheitsblatt 2005, 48, p. 84-98 (for a child, 16.15 kg bw); BfR homepage, electronic version 1.6, released 10 April 2006.

UK (PSD's) ten consumer model: published on PSD homepage, NESTI model, excel workbook, version 1.1, released 5 January 2006.

The acute intakes for lettuce or potatoes by toddler, adults and other consumer groups do not exceed the AfRD. See summarised results in table B.7.15.1.2-1 (NESTI, German VELS-model, child) and B.7.15.1.2-2 (NESTI, UK 10 consumer group model).

Table B.7.15.1.2-1 National Estimate of Short Term Intake (NESTI) of cymoxanil residues based on German VELS-model (child, 16.15 kg bw)

Commodity	MRL ^{a)} [mg/kg]	Proc. factor ^{b)} [---]	LP ^{c)} [g/day/ person]	U ^{d)} [g]	v ^{e)} [---]	Case ^{f)} 2b	Acute intake (NESTI) ^{g)} [mg/kg bw/day]	% AfRD
lettuce	0.05	1	86.9	535	3	2b	0.0008071	1.0
potatoes	0.05	1	218.8	90	7	2a	0.0023492	2.9

^{a)} Residues expressed as mg cymoxanil/kg. Values represent the limit of quantification (LOQ) of cymoxanil in the respective matrix.

^{b)} Proc. factor ... Processing factor

^{c)} LP ... food Large Portion

^{d)} U ... Unit weight of edible portion

^{e)} v ... Variability factor

^{f)} Case 2a: U < LP; case 2b U > LP

^{g)} IESTI-calculation:

Case 2a: NESTI = [U x (HR or HR-P) x v + (LP-U) x (HR or HR-P)] : bw

Case 2b: NESTI = [LP x (HR or HR-P) x v] : bw

Note: Values of STMR or STMR-P, HR or HR-P are identical with MRL.

The acute commodity intakes are based on the 97.5th percentiles.

Table B.7.15.1.2-2 National Estimate of Short Term Intake (NESTI) of cymoxanil residues based on UK consumer acute intake model for 10 consumer groups

		adult		infant		toddler		4-6 year old child		7-10 year old child	
commodity	HR [mg/kg]	NESTI [mg/kg bw/d]	% AfRD	NESTI [mg/kg bw/d]	% AfRD	NESTI [mg/kg bw/d]	% AfRD	NESTI [mg/kg bw/d]	% AfRD	NESTI [mg/kg bw/d]	% AfRD
lettuce	0.05	0.00049	0.6	0.00063	0.8	0.00060	0.8	0.00089	1.1	0.00067	0.8
potatoes	0.05	0.00120	1.5	0.00769	9.6	0.00532	6.6	0.00400	5.0	0.00275	3.4
		11-14 year old child		15-18 year old child		vegetarian		elderly - own home		elderly - residential	
commodity	HR [mg/kg]	NESTI [mg/kg bw/d]	% AfRD	NESTI [mg/kg bw/d]	% AfRD	NESTI [mg/kg bw/d]	% AfRD	NESTI [mg/kg bw/d]	% AfRD	NESTI [mg/kg bw/d]	% AfRD
lettuce	0.05	0.00042	0.5	0.00040	0.5	0.00055	0.7	0.00035	0.4	0.00020	0.2
potatoes	0.05	0.00195	2.4	0.00145	1.8	0.00149	1.9	0.00119	1.5	0.00130	1.6

Notes:

HR (highest residue) = MRL = LOQ (= 0.05 mg/kg). Residues are expressed as mg cymoxanil/kg.

Processing factor = 1. HR-P (highest residue processed)

The acute commodity intakes are based on the 97.5th percentiles.

NESTI = [U x HR-P x v] + [(F - U) x HR-P] : bw; if U > F, then F is applied in the first term and the second term drops out.

Calculation data for lettuce:

Unit weight (U): 588 g, variability factor (v): 5, full portions (F): 150 g/day (adult), 22 g/d (infant), 35 g/d (toddler), 73 g/d (4-6 year old child), 83.1 g/d (7-10 year old child), 80 g/d (11-14 year old child), 102.4 g/d (15-18 year old child), 146.6 g/d (vegetarian), 99.8 g/d (elderly - own home), 48.6 g/d (elderly - residential)

Calculation data for potatoes: Unit weight (U): 0.216 kg, variability factor (v): 7, full portions (F): 530.5 g/day (adult), 191.1 g/d (infant), 245.6 g/d (toddler), 345.8 g/d (4-6 year old child), 405.2 g/d (7-10 year old child), 573.4 g/d (11-14 year old child), 558.7 g/d (15-18 year old child), 694.4 g/d (vegetarian), 385.9 g/d (elderly - own home), 304.3 g/d (elderly - residential)

B.7.15.2 Estimation of the potential and actual exposure through other means

No cymoxanil residues above 0.1 µg/L are expected to occur in drinking water. Therefore cymoxanil residue intake through drinking water can be assessed as theoretical maximum with 0.2 µg cymoxanil/person/day assuming a daily intake of 2 liter drinking water.

B.7.16 Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9)

Storage stability:

Without significant decrease in residue level the storage stability of cymoxanil under frozen conditions (about -20 °C and darkness) was demonstrated at least for 30 days in homogenised lettuce and 12 months in whole lettuce plant (Oxon). On the above described conditions potato tuber homogenate were stable at least for 12.5 months (DuPont). A storage stability for whole potato tuber was not performed.

Metabolism of cymoxanil in plants:

Extend and nature of residues in plant material after application of cymoxanil were tested at single application rates of 0.036 - 0.840 (total: 0.108 - 3.360) kg labelled as/ha in lettuce and 0.186 - 0.404 (total: 0.432 - 1.212) kg labelled as/ha in potatoes, respectively. A small amount of cymoxanil up to 0.23 mg eq/kg was detected in lettuce only. Cymoxanil was rapidly and extensively metabolised in both crops to glycine and incorporated/conjugated in/with other natural products (like carbohydrates, peptides).

In the two submitted lettuce studies (DuPont and Oxon each) only intermediates were identified. In one study (Oxon) only IN-W3595 (18.1 % of TRR) and in the second study (DuPont) IN-KQ960 (7.4 % of TRR) and IN-KP533 (2.8 % of TRR) only were detected.

Regarding the two submitted potato studies (DuPont and Oxon each) no intermediates could be determined.

Metabolism of cymoxanil in livestock:

Supplementary, since there is evidence of a "non residue" situation on cymoxanil (< LOQ, < 0.5 mg /kg) in lettuce and potato (metabolism and supervised residue trials) and the tested rotational crops (lettuce, sugar beets and spring wheat), an animal metabolism study on lactating goat was submitted by DuPont only (data sharing with Oxon). One lactating goat was dosed over three days, equivalent to 10 mg labelled cymoxanil per kg diet and day. 23.6 % and 18.3 % of dosed radioactivity were determined in excreta and urine. The administered dose was contained in liver (3.5 %), milk (2.6%) and kidney (0.1 %). Low residue level was determined in goat muscle and fat, which remained unidentified. The recovery of administered radioactivity was low (68.8 %). The methane formed by rumen metabolism was not collected.

In the goat milk a steady state of eliminating radioactive residue could be assessed after 24 hours of first dosing. Lactose (46.4 % of milk TRR) was detected as major cymoxanil derived residue in goat milk. Fatty acids were identified for 5.7 % of milk TRR. In goat liver the formic acid group represents 68.9 % of liver TRR, acetic acid and glycerol were detected at 14.0 % and 1.8 % of liver TRR, respectively. In goat kidney hydrolysable formic acid group was determined at 28.3 % of kidney TRR, acetic acid and glycerol were identified at 10.9 % and 8.6 % of kidney TRR,

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

respectively. Overall, no radioactive labelled cymoxanil or structurally related metabolites were determined in any goat sample (i.e. milk, liver, kidney, muscles, fat or urine). The metabolism of cymoxanil in lactating goat leads to natural compounds (like fatty acids, glycerol, lactose, amino acids, formylated or acetylated compounds).

Supervised residue field trials:

No detectable residue of cymoxanil at or above the LOQ of 0.05 mg /kg have been determined in lettuce head or potato tuber at harvest time in all field trials of the two crops (DuPont and Oxon each). These findings are consistent with the respective results obtained in the plant metabolism studies (lettuce and potato) from DuPont and Oxon.

Rotational crops:

In the study submitted by DuPont only (data sharing with Oxon) no accumulation of cymoxanil or related metabolites was observed in the tested rotational crops (lettuce, sugar beet and spring wheat) at 30 and 120 days rotational crop intervals (RCIs) harvested at mature. Significant amounts of TRR (the major parts were extractable) without definite decrease of radioactivity from 30 to 120 days RCI were detected in wheat grain (≤ 0.05 mg eq/kg) and wheat straw (≤ 0.14 mg eq/kg).

Livestock feeding studies:

With regard to the results of metabolism studies and residue trials on potato (cymoxanil residues are $< \text{LOQ}$, < 0.05 mg/kg), livestock feeding studies are regarded as not necessary for cymoxanil. Additionally, the metabolism study of lactating goat indicates an extensive metabolism and no accumulation of cymoxanil in goat tissues or milk.

Processing studies:

These studies (industrial processing and/or household preparation) are considered as not necessary, due to the evidence of a "non residue" situation (no residues above the LOQ, i.e. 0.05 mg/kg, are expected based on the results of residue trials on lettuce and potatoes and the intended GAP by the applicants).

Proposed residue definition:

Considering the results of the evaluation on plant metabolism studies (lettuce and potato) primarily, further the supervised field residue trials (lettuce and potato) and rotational crop study (lettuce, sugar beet and spring wheat), the **proposed residue definition** with regard to **plant products (lettuce heads and potato tubers) for risk assessment and monitoring** purpose is **cymoxanil (parent compound only)**.

A residue definition for **food of animal origin** (based on the "non residue" situation in/on lettuce and potato primarily and the results of supplementary lactating goat metabolism) is **regarded as not necessary**.

Proposed maximum residue level (MRL):

Food of plant origin: Based on the “non residue” situation in/on lettuce heads and potato tubers with respect to the intended application scenarios (lettuce: Oxon only; potato: DuPont and Oxon each), the following MRLs at LOQ levels of cymoxanil are proposed:

Lettuce heads: MRL of 0.05* mg cymoxanil/kg

Potato tubers: MRL of 0.05* mg cymoxanil/kg

* ... = LOQ

Food of animal origin: No MRL is regarded necessary.

Risk assessment:

Long term daily intake, chronic risk assessment: On the basis of the MRL values above, the TMDI (theoretical maximum daily intake) calculation results in a “non exceeding” situation of the ADI (acceptable daily intake) value:

ADI: 0.03 mg/kg bw/day
WHO diet model (adult, 60 kg bw): 0.7 % of ADI
German diet model (VELS model, child, 16.15 kg bw): 0.4 % of ADI
UK diet model (10 consumer groups): 0.5 to 1.9 % of ADI (elderly-residential to infant)

Short term intake, acute risk assessment: On the basis of the LOQ (= 0.05 mg cymoxanil/kg) the NESTI (national estimation of short term intake) calculation results in a “non exceeding” situation of the AfRD (acute reference dose) value:

AfRD: 0.08 mg/kg bw
German diet model (VELS model, child, 16.15 kg bw): 1.0 % of AfRD for lettuce
 2.9 % of AfRD for potato
UK diet model (10 consumer groups), highest values: 1.1 % of AfRD for lettuce (4-6 year old child)
 9.6 % of AfRD for potato (infant)

Therefore, the chronic or acute consumer risk due to uptake of cymoxanil residues in raw agriculture commodities of plant (lettuce heads and potato tubers) and animal origin can be regarded as negligible.

Table B.7.16.1 Key results of crop residue trials conducted with cymoxanil (with relevance to MRL-estimation)

Crop	Application [kg as/ha]	Region *)	No. of trials	PHI [days]	Lowest residue at harvest [mg/kg] **)	Highest residue at harvest [mg/kg] **)	Remarks
lettuce	4 x 0.225-0.248	S-EU	6	0-1-3-(9)-10	<0.05	<0.05	Oxon
potato	4 x 0.118-0.128	N-EU	4	0-1-3-(6)-7	<0.05	<0.05	Oxon
potato	5 x 0.095-0.125	S-EU	15	0-1-3-7-14	<0.05	<0.05	Oxon
potato	11-12 x 0.176-0.235	N-EU	6	14	<0.05	<0.05	DuPont
potato	6-8 x 0.221-0.294	S-EU	6	14	<0.05	<0.05	DuPont

*) N-EU: Northern Region of Europe; S-EU: Southern Region of Europe

**) Residues expressed as cymoxanil

Note: LOQ for cymoxanil in lettuce or potato is 0.05 mg/kg by GC-NPD.

Table B.7.16-2 MRL proposals for cymoxanil (based on the intended uses)

Commodity	Proposed EU-MRLs according to the intended uses [mg/kg] *)	Comments
Lettuce (heads)	0.05*	---
Potato (tubers)	0.05*	---

*) Residues expressed in mg cymoxanil/kg. Asterisk on figure indicates that the proposed MRL is at LOQ level.

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.7.17 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, 6.0.1/01	Nathan, E.C.	1996	Magnitude of residues of cymoxanil in potatoes following application of Curzate M-8 fungicide at maximum label rates and at five times maximum use rates to investigate the need for magnitude of residue data in processed fractions Morse Laboratories Inc; DuPont Experimental Station AMR 3296-95 GLP: Yes Published: No	N	DuPont
IIA, 6.0.1/02	Freschi G.	2004	Freezer storage stability of cymoxanil residue in lettuce plants Sipcam Research Centre; SIP1379 GLP: Yes Published: No	Y	Oxon
IIA, 6.1/01	Fox, G.C.	1999	Metabolism of [2- ¹⁴ C]cymoxanil in lettuce DuPont Experimental Station; Wilmington, Delaware, USA AMR 4375-97 GLP: Yes Published: No	Y	DuPont
IIA, 6.1/02	Melkebeke, T., van Noorloos, B.	2003a	Metabolism, distribution and expression of cymoxanil residues in lettuce NOTOX B.V., 's-Hertogenbosch, The Netherlands 257794 GLP: Yes Published: No	Y	Oxon
IIA, 6.1/03	Li, Y., Hausmann, S.M.	1996	Plant metabolism of [2- ¹⁴ C]cymoxanil in potatoes DuPont Experimental Station; Wilmington, Delaware, USA AMR 3408-95 GLP: Yes Published: No	Y	DuPont
IIA, 6.1/04	Melkebeke, T., van Noorloos, B.	2003b	Metabolism, distribution and expression of cymoxanil residues in potatoes NOTOX B.V., 's-Hertogenbosch, The Netherlands 257772 GLP: Yes Published: No	Y	Oxon
IIA, 6.2/01	Anderson, J.J., Gaddamidi, V., Moghaddam, M.F., Swain, R.S., Hashinger, B.M., Lilly, D.E.	1996	The distribution of [2- ¹⁴ C]-DPX-T3217 (cymoxanil) in the lactating goat (nature of residue study to EPA guidelines) [REDACTED] AMR 2084-91 GLP: Yes Published: No	Y	DuPont

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, 6.3/01	Soler, J.R.	2001	Generation of lettuce specimens suitable for residue analysis of cymoxanil following applications of Cimoxnilo 50 (Cymoxanil 50% WP) (field phase) Sipcam Inagra, Spain CY2/LE GLP: Yes Published: No	Y	Oxon
	Freschi, G.	2001a	Residue Analysis of Cymoxanil in Lettuce Samples (Plant) (Analytical Phase). Sipcam Residues Analysis Unit, Italy SIP1264 GLP: Yes Published: No	Y	
IIA, 6.3/02	Domenichini, P.	2001	Generation of lettuce specimens suitable for residue analysis following applications of Cymoxanil 50% WP (field phase) Sipcam Experimental Service, Italy CY1/LA GLP: Yes Published: No	Y	Oxon
	Freschi, G.	2001a	Residue analysis of cymoxanil in lettuce samples (plant) (analytical phase). Sipcam Residues Analysis Unit, Italy SIP1264 GLP: Yes Published: No	Y	
IIA, 6.3/03	Domenichini, P.	2003a	Generation of lettuce plant samples, suitable for residue analysis (RAC and decline curve) following applications of Cymoxanil 50% WP (field phase) Sipcam Experimental Service, Italy CY2 GLP: Yes Published: No	Y	Oxon
	Freschi, G.	2001b	Residue analysis of cymoxanil in lettuce samples (plant) (analytical phase). Sipcam Residues Analysis Unit, Italy SIP1297 GLP: Yes Published: No	Y	
IIA, 6.3/04	Dubey, L., Jernberg, K.M.	1997a	Magnitude of DPX-JE874 and cymoxanil residues in/on soil and potatoes grown in the southern European region-1996 season Battelle Europe-Centre de Recherche de Geneve AMR 3767-96 GLP: Yes Published: No	N	DuPont
IIA, 6.3/05	Dubey, L., Jernberg, K.M.	1997b	Magnitude of DPX-JE874 and cymoxanil residues in/on soil and potatoes grown in the northern European region-1996 season Battelle Europe-Centre de Recherche de Geneve AMR 3788-96 GLP: Yes Published: No	N	DuPont

WARNING: This document forms part of a data package for which no patent is claimed in isolation. Registration must not be granted on the basis of this document.

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, 6.3./06	Röser, K.	2003	Determination of residues of cymoxanil after application of Cymoxanil 50% WP in potatoes at 4 sites in Europe, 2002 (field phase) Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Germany 20021200/E1-FPPO GLP: Yes Published: No	Y	Oxon
	Freschi, G.	2003	Determination of cymoxanil residues in potato specimens (tuber) after application of Cymoxanil 50% WP (analytical phase). Sipcam Residues Analysis Unit, Italy SIP1353 GLP: Yes Published: No	Y	
IIA, 6.3/07	Domenichini, P.	1997	Generation of crop samples suitable for residue analysis following applications of cymoxanil + mancozeb, cymoxanil + Cu-oxychloride, cymoxanil + chlorothalonil (field phase) Sipcam Experimental Service, Italy COF/1 GLP: Yes Published: No	N	Oxon
	Wasser, C.	1997	Determination of the residues of cymoxanil in grapes, potatoes and tomatoes in Italy, 1996 (analytical phase). Anadiag S.A., France R 6124 GLP: Yes Published: No	N	
IIA, 6.3/08	Domenichini, P.	1998	Generation of crop samples suitable for residue analysis following applications of cymoxanil + mancozeb, cymoxanil + Cu-oxychloride, cymoxanil + chlorothalonil (2 nd year of trial) (field phase) Sipcam Experimental Service, Italy COF/2 GLP: Yes Published: No	Y	Oxon
	Wasser, C.	1999	Magnitude of residues of cymoxanil in samples of grapes, tomatoes and potatoes (analytical phase). Anadiag S.A., France R 7180 GLP: Yes Published: No	Y	
IIA, 6.3/09	Domenichini, P.	2003b	Generation of potato tuber samples, suitable for residue analysis (decline curve) following applications of (cymoxanil 6% + mancozeb 70%) WP (field phase) Sipcam Experimental Service, Italy CY2/I/02PA GLP: Yes Published: No	Y	Oxon
	Freschi, G.	2001c	Residue analysis of cymoxanil in potato samples (tuber) (analytical phase). Sipcam Residues Analysis Unit, Italy SIP1298 GLP: Yes Published: No	Y	

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, 6.3/10	Soler, J.R.	2002	Generation of potato specimens, suitable for residue analysis of cymoxanil following applications of Micene Plus (cymoxanil 4% + mancozeb 40% WP) (field phase) Sipcam Experimental Service, Italy CY2/PO GLP: Yes Published: No	Y	Oxon
	Freschi, G.	2001d	Residue analysis of cymoxanil in potato samples (tuber) (analytical phase). Sipcam Residues Analysis Unit, Italy SIP1265 GLP: Yes Published: No	Y	
IIA, 6.6/01	Singles, S.K., Strek, H.J., Sheftic, G.D.	1996	Accumulation of residues in confined rotational crops: Lettuce, wheat, and beets after treatment with [¹⁴ C]cymoxanil DuPont Experimental Station AMR 3575-95 GLP: Yes Published: No	N	DuPont
Annex III Data and Information					
---	---	---	---	---	---

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.

Annex Ia: Summary of supervised residue trials of lettuce from Southern-Europe - Oxon

Active substance (common name):	Cymoxanil	Method of treatment:	Spray
Crop/crop group:	Lettuce/Leaf vegetables	Indoor/Glasshouse/Outdoor:	Outdoor
Content of active substance (g/kg or g/L):	500 g/kg	Other active substance in the formulation (common name and content):	none
Formulation (e.g. WP):	WP	*) Residues calculated as:	Cymoxanil (mg/kg)
Commercial Product (name):	---		

Report No.	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment		Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues *) (mg/kg)	PHI (days)	Remarks
				kg as/ha	Water (L/ha)	kg as/hL					
Oxon report no.: CY2/LE											
CY2/LE, S-01	Lettuce Iceberg	1. 29/09/2000 2. n.i. 3. 20/11/2000	Spray air-compress 4 nozzles, 50 cm, 3 bar, 56-59 ml/s	1 st : 0.245 2 nd : 0.240 3 rd : 0.245 4 th : 0.247	1 st : 800 2 nd : 783 3 rd : 800 4 th : 807	1 st : 0.0306 2 nd : 0.0307 3 rd : 0.0306 4 th : 0.0306	1 st : 20/10/2000 2 nd : 27/10/2000 3 rd : 03/11/2000 4 th : 10/11/2000	BBCH 40 BBCH 42 BBCH 46 BBCH 47	Lettuce head	10	HS (Harvest Study) Field code: CY2/LE/S-01 Analytical report: SIP 1264 Clay soil, trickle irrigation Oct: 10-31, mean 24.3°C Nov: 4-26.5, mean 19.3°C GC-NPD; LOQ=0.05 mg/kg
CY2/LE, S-02	Lettuce Iceberg	1. 26/09/2000 2. n.i. 3. 10+11+13+ 20/11/2000	Spray air-compress 4 nozzles, 50 cm, 3 bar, 56-59 ml/s	1 st : 0.247 2 nd : 0.247 3 rd : 0.246 4 th : 0.252	1 st : 807 2 nd : 807 3 rd : 803 4 th : 820	1 st : 0.0306 2 nd : 0.0306 3 rd : 0.0306 4 th : 0.0307	1 st : 20/10/2000 2 nd : 27/10/2000 3 rd : 03/11/2000 4 th : 10/11/2000	BBCH 40 BBCH 42 BBCH 46 BBCH 47-48	Lettuce head	0 1 3 10	DCS (Decline Curve Study) Field code: CY2/LE/S-02 Analytical report: SIP 1264 Clay soil, trickle irrigation Oct: 10-31, mean 24.3°C Nov: 4-26.5, mean 19.3°C GC-NPD; LOQ=0.05 mg/kg
Oxon report no.: CY1/LA											
CY1/LA, I-02	Lettuce Gentile	1. 04/08/2000 2. n.i. 3. 18/09/2000	Spray backpack boom spray 6 nozzles, 50 cm, 4 bar, 6.4-8.9 ml/s	1 st : 0.238 2 nd : 0.241 3 rd : 0.238 4 th : 0.242	1 st : 495 2 nd : 502 3 rd : 497 4 th : 505	1 st : 0.0481 2 nd : 0.0480 3 rd : 0.0479 4 th : 0.0479	1 st : 18/08/2000 2 nd : 25/08/2000 3 rd : 01/09/2000 4 th : 08/09/2000	BBCH 19 BBCH 19 BBCH 41 BBCH 49	Lettuce head	10	HS (Harvest Study) Field code: CY1/I-02LA Analytical report: SIP 1264 Sandy soil, sprinkler irrigation Aug: 12.7-37.1°C Sept: 7.9-30.5°C GC-NPD; LOQ=0.05 mg/kg

Report No. Location (region)	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues *) (mg/kg)	PHI (days)	Remarks
				kg as/ha	Water (L/ha)	kg as/hL						
Annex la continued												
Oxon report no.: CY1/LA continued												
CY1/LA, I-03 Salerano sul Lambro (Lodi), Italy 2000	Lettuce Carioca	1. 17/05/2000 2. n.i. 3. 20-21-23- 30/06/2000	Spray backpack boom spray 4 nozzles, 50 cm, 4 bar, 11-12.5 ml/s	1 st : 0.235 2 nd : 0.238 3 rd : 0.245 4 th : 0.241	1 st : 490 2 nd : 495 3 rd : 510 4 th : 503	1 st : 0.0480 2 nd : 0.0481 3 rd : 0.0480 4 th : 0.0479	1 st : 31/05/2000 2 nd : 06/06/2000 3 rd : 13/06/2000 4 th : 20/06/2000	BBCH 17 BBCH 19 BBCH 43-46 BBCH 46-49	Lettuce head	4.29 2.72 0.95 ≤ 0.05	0 (+3h) 1 3 10	DCS (Decline Curve Study) Field code: CY1/I03LA Analytical report: SIP 1264 Medium loam sandy soil, no irrigation May: 13-31°C; June: 15-34°C GC-NPD: LOQ=0.05 mg/kg
Oxon report no.: CY2												
CY2/LA, I-03 Canneto sull'Oglio (MN), Italy 2001	Lettuce Justine	1. 30/05/2001 2. n.i. 3. 06/07/2001	Spray air-compress 5 nozzles, 50 cm, 4 bar, 9.6-9.9 ml/s	1 st : 0.242 2 nd : 0.245 3 rd : 0.229 4 th : 0.225	1 st : 606 2 nd : 611 3 rd : 571 4 th : 563	1 st : 0.0399 2 nd : 0.0401 3 rd : 0.0401 4 th : 0.0400	1 st : 05/06/2001 2 nd : 12/06/2001 3 rd : 19/06/2001 4 th : 26/06/2001	BBCH 12 BBCH 19 BBCH 41 BBCH 45	Lettuce head	≤ 0.05	10	HS (Harvest Study) Field code: CY2/I03LA Analytical report: SIP 1297 Sandy soil, sprinkler irrigation June: 9-33°C July: 14-34°C GC-NPD: LOQ=0.05 mg/kg
CY2/LA, I-04 Gazzo Veronese (VR), Italy 2001	Lettuce Wendi	1. 02/04/2001 2. n.i. 3. 23+24+26/05 +01/06/2001	Spray air-compress 6 nozzles, 50 cm, 4 bar, 8.6-9.1 ml/s	1 st : 0.243 2 nd : 0.248 3 rd : 0.243 4 th : 0.242	1 st : 507 2 nd : 517 3 rd : 507 4 th : 503	1 st : 0.0479 2 nd : 0.0480 3 rd : 0.0479 4 th : 0.0481	1 st : 02/05/2001 2 nd : 09/05/2001 3 rd : 16/05/2001 4 th : 23/05/2001	BBCH 19 BBCH 41 BBCH 43 BBCH 45	Lettuce head	3.63 0.55 < 0.05 ≤ 0.05	0 (+3h) 1 3 9	DCS (Decline Curve Study) Field code: CY2/I04LA Analytical report: SIP 1297 Loam soil, sprinkler irrigation May: 11.1-34.4°C June: 8.4-32.7°C GC-NPD: LOQ=0.05 mg/kg

n.i. ... not indicated; underlined residue values were used for MRL calculation

*) residues calculated as mg Cymoxanil/kg

WARNING: This document is a draft and should not be used for regulatory purposes. Registration must not be granted on the basis of this document.

Annex Ib: Summary of supervised residue trials of potato from Northern Europe - DuPont

Active ingredient (a.s.)(common name): : Cymoxanil
 Crop/crop group: : Potatoes/Root and Tuber Vegetables
 Content of active substance: (g/kg of : 250 g/kg
 g/L):
 Formulation (e.g., WP): : WG
 Commercial product (name): : Tanos (DPX-KP481-25)

Method of treatment:
 Indoor/Outdoor:
 Other active substance in the formulation (common name):
 Other active substance content (g/kg or g/l):
 *) Residues calculated as:

: Ground foliar spraying
 : Outdoor
 : Famoxadone
 : 250 g/kg
 : Cymoxanil

Report No. Location including Postal Code	Commodity/ Variety	Date of: 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or sampling date	Portion Analysed	Residues *) (mg/kg)	PHI (days)	Remarks
				kg as/ha	water (L/ha)	kg as/hL **)						
DuPont report no.: AMR 3788-96												
AMR 3788-96, test no. 1 Raineourt 80 Somme, Northern France 1996	Potato Binje	1. 12/04/1996 2. n.i. 3. 16/09/1996	Plot sprayer (propane) 110015VS Teeljet XR, 2.8 bar, 8 ml/s	1 st : 0.190 2 nd : 0.202 3 rd : 0.199 4 th : 0.205 5 th : 0.192 6 th : 0.203 7 th : 0.211 8 th : 0.202 9 th : 0.176 10 th : 0.197 11 th : 0.198	1 st : 233 2 nd : 248 3 rd : 245 4 th : 252 5 th : 237 6 th : 250 7 th : 260 8 th : 248 9 th : 217 10 th : 243 11 th : 243	1 st : 0.0815 2 nd : 0.0815 3 rd : 0.0812 4 th : 0.0813 5 th : 0.0810 6 th : 0.0812 7 th : 0.0812 8 th : 0.0815 9 th : 0.0811 10 th : 0.0811 11 th : 0.0815	1 st : 24/06/1996 2 nd : 01/07/1996 3 rd : 08/07/1996 4 th : 15/07/1996 5 th : 22/07/1996 6 th : 29/07/1996 7 th : 05/08/1996 8 th : 12/08/1996 9 th : 20/08/1996 10 th : 26/08/1996 11 th : 02/09/1996	BBCH 501 BBCH 509 BBCH 521 BBCH 609 BBCH 609 BBCH 6N9 BBCH 6N9 BBCH 405 BBCH 405 BBCH 407 BBCH 408	potato tuber	≤ 0.05	14 HS (Harvest Study) Field code: AMR 3788-96, test no. 1 Analytical report: Battelle study A-11-96-16 Loamy clay soil, no irrigation June: 10.6-21.7 °C mean July: 11.2-23.3 °C mean Aug: 12.0-23.6 °C mean Sep: 7.7-18.1 °C mean GC-NPD, LOQ = 0.05 mg/kg	
AMR 3788-96, test no. 2 5500 Middelfart, Denmark, 1996	Potato Sava	1. 25/04/1996 2. n.i. 3. 10/09/1996	AZO Knapsack precision sprayer (CO ₂), 4110-12 Handi, 3 bar, 8.3 ml/s	1 st : 0.200 ^a 2 nd : 0.200 ^a 3 rd : 0.200 ^a 4 th : 0.200 ^a 5 th : 0.200 ^a 6 th : 0.200 ^a 7 th : 0.200 ^a 8 th : 0.200 ^a 9 th : 0.200 ^a 10 th : 0.200 ^a 11 th : 0.200 ^a 12 th : 0.200 ^a	1 st : 260 2 nd : 249 3 rd : 247 4 th : 249 5 th : 242 6 th : 263 7 th : 255 8 th : 250 9 th : 249 10 th : 254 11 th : 254 12 th : 249	1 st : 0.0769 2 nd : 0.0803 3 rd : 0.0810 4 th : 0.0803 5 th : 0.0826 6 th : 0.0760 7 th : 0.0784 8 th : 0.0800 9 th : 0.0803 10 th : 0.0787 11 th : 0.0787 12 th : 0.0803	1 st : 10/06/1996 2 nd : 17/06/1996 3 rd : 24/06/1996 4 th : 01/07/1996 5 th : 08/07/1996 6 th : 16/07/1996 7 th : 22/07/1996 8 th : 29/07/1996 9 th : 05/08/1996 10 th : 12/08/1996 11 th : 19/08/1996 12 th : 27/08/1996	BBCH 25 BBCH 30-35 BBCH 35 BBCH 38 BBCH 50 BBCH 52 BBCH 61 BBCH 69 BBCH 70 BBCH 81 BBCH 81-82 BBCH 85-89	potato tuber	≤ 0.05	14 HS (Harvest Study) Field code: AMR 3788-96, test no. 2 Analytical report: Battelle study A-11-96-16 Sandy laom soil, irrigation June: 5-31 °C mean July: 5-30 °C mean Aug: 8-35 °C mean Sep: 2-25 °C mean GC-NPD, LOQ = 0.05 mg/kg	
AMR 3788-96, test no. 3 6681 Bemmel, The Netherlands 1996	Potato Agria	1. 05/04/1996 2. n.i. 3. 17/09/1996	Sprayer (com- pressed air), flat nozzle XR11003US , 3 bar, 75.67 ml/s	1 st : 0.204 2 nd : 0.196 3 rd : 0.183 4 th : 0.196 5 th : 0.200 6 th : 0.204 7 th : 0.200 8 th : 0.209 9 th : 0.196 10 th : 0.204 11 th : 0.191 12 th : 0.204	1 st : 307 2 nd : 293 3 rd : 273 4 th : 293 5 th : 300 6 th : 307 7 th : 300 8 th : 313 9 th : 293 10 th : 307 11 th : 287 12 th : 307	1 st : 0.0664 2 nd : 0.0669 3 rd : 0.0670 4 th : 0.0669 5 th : 0.0667 6 th : 0.0664 7 th : 0.0667 8 th : 0.0668 9 th : 0.0669 10 th : 0.0664 11 th : 0.0666 12 th : 0.0664	1 st : 18/06/1996 2 nd : 25/06/1996 3 rd : 01/07/1996 4 th : 09/07/1996 5 th : 16/07/1996 6 th : 23/07/1996 7 th : 30/07/1996 8 th : 06/08/1996 9 th : 13/08/1996 10 th : 20/08/1996 11 th : 27/08/1996 12 th : 03/09/1996	BBCH 50 BBCH 55 BBCH 55 BBCH 60 BBCH 65 BBCH 69 BBCH 75 BBCH 91 BBCH 91 BBCH 93 BBCH 95	potato tuber	≤ 0.05	14 HS (Harvest Study) Field code: AMR 3788-96, test no. 3 Analytical report: Battelle study A-11-96-16 Sandy clay soil, sprinkler irrigation June: 10.0-20.4 °C mean July: 10.9-20.8 °C mean Aug: 12.1-22.5 °C mean Sep: 7.4-17.1 °C mean GC-NPD, LOQ = 0.05 mg/kg	

Report No. Location including Postal Code	Commodity/ Variety	Date of: 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or sampling date	Portion Analysed	Residues *) (mg/kg)	PHI (days)	Remarks
				kg as/ha	water (L/ha)	kg as/hL **)						
Annex Ib continued DuPont report no.: AMR 3788-96 continued												
AMR 3788-96, test no. 4 02692 Gnaschwitz, Germany 1996	Potato Granola	1. 30/04/1996 2. n.i. 3. 24/09/1996	Agrotop PL 1 Sprayer mobile plot w/2.5 m boom, Lurmark SD 015/110 nozzles, 2.5 bar	1 st : 0.200 ^{a)} 2 nd : 0.200 ^{a)} 3 rd : 0.200 ^{a)} 4 th : 0.200 ^{a)} 5 th : 0.200 ^{a)} 6 th : 0.200 ^{a)} 7 th : 0.200 ^{a)} 8 th : 0.200 ^{a)} 9 th : 0.200 ^{a)} 10 th : 0.200 ^{a)} 11 th : 0.200 ^{a)} 12 th : 0.200 ^{a)}	1 st : 300 ^{a)} 2 nd : 300 ^{a)} 3 rd : 300 ^{a)} 4 th : 300 ^{a)} 5 th : 300 ^{a)} 6 th : 300 ^{a)} 7 th : 300 ^{a)} 8 th : 300 ^{a)} 9 th : 300 ^{a)} 10 th : 300 ^{a)} 11 th : 300 ^{a)} 12 th : 300 ^{a)}	1 st : 0.0667 2 nd : 0.0667 3 rd : 0.0667 4 th : 0.0667 5 th : 0.0667 6 th : 0.0667 7 th : 0.0667 8 th : 0.0667 9 th : 0.0667 10 th : 0.0667 11 th : 0.0667 12 th : 0.0667	1 st : 21/06/1996 2 nd : 27/06/1996 3 rd : 04/07/1996 4 th : 11/07/1996 5 th : 18/07/1996 6 th : 31/07/1996 7 th : 09/08/1996 8 th : 16/08/1996 9 th : 22/08/1996 10 th : 29/08/1996 11 th : 03/09/1996 12 th : 10/09/1996	BBCH 29 BBCH 29 BBCH 31 BBCH 61 BBCH 71 BBCH 81 BBCH 82 BBCH 85 BBCH 87 BBCH 89 BBCH 90 BBCH 91	potato tuber	< 0.05	14	HS (Harvest Study) Field code: AMR 3788-96, test no. 4 Analytical report: Battelle study A-11-96-16 Loamy soil, no irrigation June: 16.0 °C mean July: 16.2 °C mean Aug: 17.7 °C mean Sep: 10.4 °C mean GC-NPD, LOQ = 0.05 mg/kg
AMR 3788-96, test no. 5 6221 Saint-Amand, Belgium 1996	Potato Bintje	1. 10/05/1996 2. n.i. 3. 19/09/1996	Boom sprayer (N2) 1.6-1.8 kg/cm2, DG8003VS, 6 nozzles	1 st : 0.225 2 nd : 0.211 3 rd : 0.223 4 th : 0.203 5 th : 0.218 6 th : 0.210 7 th : 0.222 8 th : 0.214 9 th : 0.235 10 th : 0.212 11 th : 0.219 12 th : 0.225	1 st : 217 2 nd : 207 3 rd : 216 4 th : 185 5 th : 199 6 th : 207 7 th : 214 8 th : 205 9 th : 224 10 th : 192 11 th : 205 12 th : 211	1 st : 0.1037 2 nd : 0.1019 3 rd : 0.1032 4 th : 0.1097 5 th : 0.1095 6 th : 0.1014 7 th : 0.1037 8 th : 0.1044 9 th : 0.1049 10 th : 0.1104 11 th : 0.1068 12 th : 0.1066	1 st : 19/06/1996 2 nd : 26/06/1996 3 rd : 03/07/1996 4 th : 10/07/1996 5 th : 17/07/1996 6 th : 25/07/1996 7 th : 31/07/1996 8 th : 07/08/1996 9 th : 14/08/1996 10 th : 20/08/1996 11 th : 30/08/1996 12 th : 05/09/1996	maturity maturity maturity maturity maturity maturity maturity maturity maturity maturity maturity maturity	potato tuber	< 0.05	14	HS (Harvest Study) Field code: AMR 3788-96, test no. 5 Analytical report: Battelle study A-11-96-16 Loamy soil, no irrigation June: 9.4-16.8 °C mean July: 12.0-21.5 °C mean Aug: 12.4-22.4 °C mean Sep: 7.4-17.0 °C mean GC-NPD, LOQ = 0.05 mg/kg
AMR 3788-96, test no. 6 Melbourne, Derbyshire, United Kingdom 1996	Potato Marfona	1. 14/05/1996 2. n.i. 3. 27/09/1996	AUK Plot Sprayer, Lurmark SD015- F110 nozzle, 3.0 bar	1 st : 0.218 2 nd : 0.213 3 rd : 0.219 4 th : 0.214 5 th : 0.209 6 th : 0.216 7 th : 0.205 8 th : 0.215 9 th : 0.204 10 th : 0.216 11 th : 0.216 12 th : 0.225	1 st : 307 2 nd : 300 3 rd : 309 4 th : 301 5 th : 295 6 th : 305 7 th : 289 8 th : 304 9 th : 288 10 th : 305 11 th : 304 12 th : 317	1 st : 0.0710 2 nd : 0.0710 3 rd : 0.0709 4 th : 0.0711 5 th : 0.0708 6 th : 0.0708 7 th : 0.0709 8 th : 0.0707 9 th : 0.0708 10 th : 0.0708 11 th : 0.0711 12 th : 0.0710	1 st : 27/06/1996 2 nd : 05/07/1996 3 rd : 12/07/1996 4 th : 18/07/1996 5 th : 25/07/1996 6 th : 02/08/1996 7 th : 09/08/1996 8 th : 17/08/1996 9 th : 24/08/1996 10 th : 30/08/1996 11 th : 07/09/1996 12 th : 13/09/1996	BBCH 22-23 BBCH 23-24 BBCH 31-33 BBCH 61 BBCH 69 BBCH 70 BBCH 75 BBCH 625/705 BBCH 629 BBCH 89 BBCH 89 BBCH 92	potato tuber	< 0.05	14	HS (Harvest Study) Field code: AMR 3788-96, test no. 6 Analytical report: Battelle study A-11-96-16 Sandy clay loam soil, no irrigation June: 8.1-20.1 °C mean July: 11.0-22.2 °C mean Aug: 11.8-22.1 °C mean Sep: 9.3-17.9 °C mean GC-NPD, LOQ = 0.05 mg/kg

n.i. ... not indicated; underlined residue values were used for MRL calculation

*) residues calculated as mg Cymoxanil/kg

**) calculated by RMS

a) nominal values; the actual data were not submitted

b) not reported

This document is a confidential part of an EC evaluation data. Reproduction must not be granted on the basis of this document.

Annex Ic: Summary of supervised residue trials of potato from Southern Europe - DuPont

Active ingredient (a.s.)(common name): : Cymoxanil
 Crop/crop group: : Potatoes/Root and Tuber Vegetables
 Content of active substance: (g/kg or : 300 g/kg
 g/L):
 Formulation (e.g., WP): : WG
 Commercial product (name): : Cymoxanil

Method of treatment:
 Indoor/Outdoor: : Ground foliar spraying
 Other active substance in the formulation (common name): : Outdoor
 Other active substance content (g/kg or g/l): : Famoxadone
 *) Residues calculated as: : 225 g/kg
 : Cymoxanil

Report No. Location including Postal Code	Commodity/ Variety	Date of: 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or sampling date	Portion Analysed	Residues) (mg/kg)	PHI (days)	Remarks										
				kg as/ha	water (L/ha)	kg as/hL)																
DuPont report no.: AMR 3767-96																						
AMR 3767-96, test no. 1 Caleppio Di Settala (Mi), Italy 1996	Potato Lady Rosetta	1. 25/04/1996 2. n.i. 3. 05/08/1996	ECHO SHRU100- AM3, 11001 TeeJet, 4 atm, 6.6 ml/s	1 st : 0.240	1 st : 300	1 st : 0.0800	1 st : 04/06/1996 2 nd : 10/06/1996 3 rd : 17/06/1996 4 th : 24/06/1996 5 th : 01/07/1996 6 th : 09/07/1996 7 th : 15/07/1996 8 th : 22/07/1996	start flowering flowering end flowering dev. of fruits growing tubers growing tubers growing tubers maturity of tubers	potato tuber	≤ 0.05	14	HS (Harvest Study) Field code: AMR 3767-96, test no. 1 Analytical report: Battelle study A-11-96-17 Loam + sandy soil, sprinkler irrigation June: 16.75-22.85 °C mean July: 17.13-27.55 °C mean Aug: 16.35-25.55 °C mean GC-NPD, LOQ = 0.05 mg/kg										
				2 nd : 0.248	2 nd : 310	2 nd : 0.0800																
				3 rd : 0.243	3 rd : 304	3 rd : 0.0799																
				4 th : 0.248	4 th : 310	4 th : 0.0800																
				5 th : 0.251	5 th : 314	5 th : 0.0799																
				6 th : 0.250	6 th : 312	6 th : 0.0801																
				7 th : 0.234	7 th : 292	7 th : 0.0801																
				8 th : 0.248	8 th : 310	8 th : 0.0800																
AMR 3767-96, test no. 2 Hameau de Pouilleux 01600 (Reynieux), Southern France 1996	Potato José	1. 04/05/1996 2 n.i. 3. 27/08/1996	AZO sprayer (propane) 11002 TeeJet VS, 2.5 bar, 11.3 ml/s	1 st : 0.281	1 st : 278	1 st : 0.1011	1 st : 09/07/1996 2 nd : 15/07/1996 3 rd : 22/07/1996 4 th : 29/07/1996 5 th : 05/08/1996 6 th : 13/08/1996	BBCH 605 BBCH 609 BBCH 700 BBCH 701 BBCH 705 BBCH 709	potato tuber	≤ 0.05	14	HS (Harvest Study) Field code: AMR 3767-96, test no. 2 Analytical report: Battelle study A-11-96-17 Loamy soil, sprinkler irrigation July: 11.3-24.9 °C mean Aug: 13.3-24.2 °C mean GC-NPD, LOQ = 0.05 mg/kg										
				2 nd : 0.267	2 nd : 264	2 nd : 0.1011																
				3 rd : 0.269	3 rd : 266	3 rd : 0.1011																
				4 th : 0.272	4 th : 269	4 th : 0.1011																
				5 th : 0.272	5 th : 269	5 th : 0.1011																
				6 th : 0.294	6 th : 290	6 th : 0.1014																
				AMR 3767-96, test no. 3 Kalikratia, Chalkidiki, Greece 1996	Potato Spunda	1. 10/04/1996 2. n.i. 3. 23/07/1996							Fox Motori F-320, 5 atm, hollow cone TeeJet, 21 ml/s	1 st : 0.245	1 st : 485	1 st : 0.0505	1 st : 21/05/1996 2 nd : 28/05/1996 3 rd : 04/06/1996 4 th : 11/06/1996 5 th : 18/06/1996 6 th : 25/06/1996 7 th : 02/07/1996 8 th : 09/07/1996	BBCH 51-59 BBCH 59-61 BBCH 65 BBCH 70 BBCH 81 BBCH 81-89 BBCH 85-89 BBCH 89	potato tuber	≤ 0.05	14	HS (Harvest Study) Field code: AMR 3767-96, test no. 3 Analytical report: Battelle study A-11-96-17 Loamy soil, sprinkler irrigation May: 10.1-31.6 °C mean June: 11.3-37.1 °C mean July: 13.1-36.8 °C mean GC-NPD, LOQ = 0.05 mg/kg
														2 nd : 0.235	2 nd : 465	2 nd : 0.0505						
3 rd : 0.244	3 rd : 483	3 rd : 0.0505																				
4 th : 0.262	4 th : 518	4 th : 0.0506																				
5 th : 0.265	5 th : 525	5 th : 0.0505																				
6 th : 0.253	6 th : 501	6 th : 0.0505																				
7 th : 0.250	7 th : 495	7 th : 0.0505																				
8 th : 0.243	8 th : 481	8 th : 0.0505																				

Report No. Location including Postal Code	Commodity/ Variety	Date of: 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or sampling date	Portion Analysed	Residues *) (mg/kg)	PHI (days)	Remarks
				kg as/ha	water (L/ha)	kg as/hL (**)						
Annex Ic continued DuPont report no.: AMR 3767-96 continued												
AMR 3767-96, test no. 4 30210 Semhac, Southern France 1996	Potato Agatha	1. 16/07/1996 2. n.i. 3. 25/10/1996	AZO (compre ssee d air) XR TeelJet 1002VS, 1.8 bar, 8.7 ml/s	1 st : 0.265 2 nd : 0.265 3 rd : 0.259 4 th : 0.256 5 th : 0.243 6 th : 0.262 7 th : 0.259	1 st : 263 2 nd : 263 3 rd : 256 4 th : 253 5 th : 241 6 th : 259 7 th : 256	1 st : 0.1008 2 nd : 0.1008 3 rd : 0.1012 4 th : 0.1012 5 th : 0.1008 6 th : 0.1012 7 th : 0.1012	1 st : 30/08/1996 2 nd : 06/09/1996 3 rd : 13/09/1996 4 th : 20/09/1996 5 th : 27/09/1996 6 th : 04/10/1996 7 th : 11/10/1996	BBCH 625 BBCH 65 BBCH 675 BBCH 73 BBCH 85 BBCH 87 BBCH 90	potato tuber	≤ 0.05	14	HS (Harvest Study) Field code: AMR 3767-96, test no. 4 Analytical report: Battelle study A-11-96-17 Sandy loam soil, sprinkler irrigation Aug: 17.4-27.8 °C mean Sep: 12.6-22.6 °C mean Oct: 11.2-19.6 °C mean GC-NPD, LOQ = 0.05 mg/kg
AMR 3767-96, test no. 5 Utrera (Sevilla), Spain 1996	Potato Claustar	1. 02/02/1996 2. n.i. 3. 10/06/1996	Sprayer (compre ssee d air) 4 nozzles (XR110015 VS), 2.4 bar, 36.5 ml/s	1 st : 0.259 2 nd : 0.243 3 rd : 0.253 4 th : 0.221 5 th : 0.245 6 th : 0.258 7 th : 0.255 8 th : 0.253	1 st : 320 2 nd : 300 3 rd : 313 4 th : 273 5 th : 303 6 th : 318 7 th : 315 8 th : 313	1 st : 0.0809 2 nd : 0.0810 3 rd : 0.0808 4 th : 0.0810 5 th : 0.0809 6 th : 0.0811 7 th : 0.0810 8 th : 0.0808	1 st : 08/04/1996 2 nd : 15/04/1996 3 rd : 23/04/1996 4 th : 29/04/1996 5 th : 07/05/1996 6 th : 13/05/1996 7 th : 20/05/1996 8 th : 27/05/1996	BBCH 19-21 BBCH 21-23 BBCH 53-61 BBCH 61-63 BBCH 65-67 BBCH 71 BBCH 75-77 BBCH 85	potato tuber	≤ 0.05	14	HS (Harvest Study) Field code: AMR 3767-96, test no. 5 Analytical report: Battelle study A-11-96-17 Sandy loam soil, sprinkler irrigation Apr: 10.2-23.6 °C mean May: 12.7-24.8 °C mean Jun: 15.7-32.4 °C mean GC-NPD, LOQ = 0.05 mg/kg
AMR 3767-96, test no. 6 Ermegeira, Torres Vedras, Portugal 1996	Potato Desiree	1. 05/03/1996 2. n.i. 3. 09/07/1996	Atomizer	1 st : 0.245 2 nd : 0.252 3 rd : 0.258 4 th : 0.258 5 th : 0.244 6 th : 0.256 7 th : 0.250 8 th : 0.247	1 st : 456 2 nd : 498 3 rd : 490 4 th : 475 5 th : 501 6 th : 502 7 th : 450 8 th : 430	1 st : 0.0537 2 nd : 0.0506 3 rd : 0.0527 4 th : 0.0543 5 th : 0.0487 6 th : 0.0510 7 th : 0.0556 8 th : 0.0574	1 st : 26/04/1996 2 nd : 03/05/1996 3 rd : 10/05/1996 4 th : 20/05/1996 5 th : 30/05/1996 6 th : 10/06/1996 7 th : 18/06/1996 8 th : 25/06/1996	mid flowering mid flowering end flowering dev. of tubers dev. of tubers dev. of tubers leaf yellowing leaf yellowing	potato tuber	≤ 0.05	14	HS (Harvest Study) Field code: AMR 3767-96, test no. 6 Analytical report: Battelle study A-11-96-17 Sandy soil, no irrigation Apr: 11.7-20.9 °C mean May: 12.7-21.5 °C mean Jun: 17.0-28.4 °C mean Jul: 18.2-29.1 °C mean GC-NPD, LOQ = 0.05 mg/kg

n.i. not indicated, dev. ... develop

undefined residue values were used for MRL calculation

*) residues calculated as mg Cymoxanil/kg

**) calculated by RMS

a) nominal values; the actual data were not submitted

Annex Id: Summary of supervised residue trials of potato from northern Europe - Oxon

Active substance (common name):
Cymoxanil
Crop/crop group: Potato/Potatoes
Content of active substance (g/kg or g/L): 500 g/kg
Formulation (e.g. WP): WP

Method of treatment:
Producer of commercial product:
Indoor/Glasshouse/Outdoor:
Other active substance in the formulation
(common name and content):
(*) Residues calculated as:

Spray:
Oxon
Outdoor
none
Cymoxanil

Report No. Location (region)	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues *) (mg/kg)	PHI (days)	Remarks
				kg as/ha	Water (L/ha)	kg as/hL						
F02N028R Innheim (Alsace), Northern France 2002	Potato Spunta	1. 03/04/2002 2. n.i. 3. 27/08/2002	Spray portable boom spray 6 nozzles, 50 cm, 2 bar 100 mL/s	1 st : 0.124	1 st : 467	1 st : 0.0266	1 st : 02/08/2002	BBCH 89-91	potato tuber	< 0.05	6	HS (Harvest Study) Field code: F02N028R Analytical report: SIP 1353 Silty loam, no irrigation Aug: mean 19.9 °C GC-NPD: LOQ=0.05mg/kg
				2 nd : 0.122	2 nd : 458	2 nd : 0.0266	2 nd : 08/08/2002	BBCH 91				
				3 rd : 0.125	3 rd : 469	3 rd : 0.0267	3 rd : 15/08/2002	BBCH 91-93				
				4 th : 0.124	4 th : 464	4 th : 0.0267	4 th : 21/08/2002	BBCH 95				
G02N008R Dollern (Lower Saxony), Germany 2002	Potato Cilena	1. 05/04/2002 2. n.i. 3. 29+30/07+01+05/08/2002	Spray portable boom spray 6 nozzles, 50 cm, 2-3 bar 54-71 mL/s	1 st : 0.128	1 st : 427	1 st : 0.0300	1 st : 08/07/2002	BBCH 69	potato tuber	< 0.05	0	DCS (Decline Curve Study) Field code: G02N008R Analytical report: SIP 1353 Loamy sand, no irrigation July: mean 18.2 °C Aug: mean 20.2 °C GC-NPD: LOQ=0.05mg/kg
				2 nd : 0.118	2 nd : 392	2 nd : 0.0301	2 nd : 15/07/2002	BBCH 71				
				3 rd : 0.122	3 rd : 405	3 rd : 0.0301	3 rd : 23/07/2002	BBCH 79				
				4 th : 0.124	4 th : 413	4 th : 0.0300	4 th : 29/07/2002	BBCH 91				
G02N009R Perouse (Baden-Württemberg), Germany 2002	Potato Granola	1. 04/04/2002 2. n.i. 3. 19+20+22+26/08/2002	Spray portable boom spray 6 nozzles, 50 cm, 2.2 bar 124.2 mL/s	1 st : 0.124	1 st : 412	1 st : 0.0301	1 st : 29/07/2002	BBCH 81	potato tuber	< 0.05	0	DCS (Decline Curve Study) Field code: G02N009R Analytical report: SIP 1353 Sandy clayey loam, no irrigation July: mean 19.3 °C Aug: mean 20.0 °C GC-NPD: LOQ=0.05mg/kg
				2 nd : 0.120	2 nd : 398	2 nd : 0.0302	2 nd : 05/08/2002	BBCH 91				
				3 rd : 0.120	3 rd : 400	3 rd : 0.0300	3 rd : 13/08/2002	BBCH 93				
				4 th : 0.123	4 th : 408	4 th : 0.0301	4 th : 19/08/2002	BBCH 93				
NL02N001R Slochteren (Groningen), The Netherlands 2002	Potato Elkana	1. 08/04/2002 2. n.i. 3. 02/09/2002	Spray portable boom spray 6 nozzles, 50 cm, 2 bar 58-71 mL/s	1 st : 0.126	1 st : 420	1 st : 0.0300	1 st : 04/08/2002	BBCH 73	potato tuber	< 0.05	7	HS (Harvest Study) Field code: NL02N001R Analytical report: SIP 1353 Humous sand, no irrigation Aug: mean 20.1 °C Sept: mean 16.1 °C GC-NPD: LOQ=0.05mg/kg
				2 nd : 0.125	2 nd : 418	2 nd : 0.0299	2 nd : 12/08/2002	BBCH 75				
				3 rd : 0.127	3 rd : 422	3 rd : 0.0301	3 rd : 19/08/2002	BBCH 76-78				
				4 th : 0.119	4 th : 398	4 th : 0.0299	4 th : 26/08/2002	BBCH 90				

n.i. not indicated

Annex Ie: Summary of supervised residue trials of potato from southern Europe - Oxon

Active substance (common name): **Cymoxanil**
 Crop/crop group: **Potato/Potatoes**
 Content of active substance (g/kg or g/L): **60 g/kg**
 Formulation (e.g. WP): **WP**
 Commercial product (name): **---**
 Method of treatment: **Spray**
 Producer of commercial product: **Oxon**
 Indoor/Glasshouse/Outdoor: **Outdoor**
 Other active substance in the formulation: **Mancozeb**
 (common name and content): **700 g/kg**
 *) Residues calculated as: **Cymoxanil**

Report No. Location (region)	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment		Dates of treatment(s) or treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues *) (mg/kg)	PHI (days)	Remarks
				kg as/ha	Water (L/ha) kg as/hL						
COF/1/3/P Salerano sul Lambro (Lodi), Lombardia region Italy 1996	Potato Kennebec	1. 19/03/1996 2. n.i. 3. 08/08/1996	Spray air pressure knapsack (PULVAL model CRISTAL)	1 st : 0.099	1 st : 989	1 st : 0.0100	BBCH 60-65	potato tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/3/P Analytical report: R 6124 Sandy soil, flooding irrigation July: mean 17.0-28.3 °C Aug: mean 17.2-28.3 °C GC-NPD; LOQ=0.05mg/kg
				2 nd : 0.098	2 nd : 979	2 nd : 0.0100	BBCH 60-65				
				3 rd : 0.098	3 rd : 976	3 rd : 0.0100	BBCH 69				
				4 th : 0.098	4 th : 981	4 th : 0.0100	BBCH 92				
				5 th : 0.099	5 th : 987	5 th : 0.0100	BBCH 93				
COF/1/4/P S. Agostino (FE), Emilia Romagna Region Italy 1996	Potato Agata	1. 14/03/1996 2. n.i. 3. 04/07/1996	Spray air pressure knapsack (PULVAL model CRISTAL)	1 st : 0.102	1 st : 511	1 st : 0.0200	BBCH 60-65	potato tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/4/P Analytical report: R 6124 Clay soil, rain irrigation May: 10.4-30.3 °C June: 13-36.2 °C July: 12.7-34.9 °C GC-NPD; LOQ=0.05mg/kg
				2 nd : 0.095	2 nd : 475	2 nd : 0.0200	BBCH 69				
				3 rd : 0.113	3 rd : 568	3 rd : 0.0199	BBCH 69				
				4 th : 0.103	4 th : 514	4 th : 0.0200	BBCH 80-82				
				5 th : 0.103	5 th : 513	5 th : 0.0201	BBCH 91-93				
COF/2/3/P Cavazzana di Lusla (Rovigo), Veneto region Italy 1997	Potato Jaerla	1. 03/02/1997 2. n.i. 3. 20/06/1997	Spray air pressure knapsack (PULVAL model CRISTAL)	1 st : 0.108	1 st : 542	1 st : 0.0199	BBCH 60-65	potato tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/3/P Analytical report: R 7180 Sandy soil, sprinkler irrigation May: 5-32, mean 11.7-24.5 °C June 9-31, mean 15.6-25.9 °C GC-NPD; LOQ=0.05mg/kg
				2 nd : 0.091	2 nd : 454	2 nd : 0.0200	BBCH 69				
				3 rd : 0.103	3 rd : 515	3 rd : 0.0200	BBCH 69				
				4 th : 0.104	4 th : 521	4 th : 0.0200	BBCH 80-82				
				5 th : 0.102	5 th : 508	5 th : 0.0201	BBCH 91-93				
COF/2/4/P S. Giovanni Persiceto (Bologna), Emilia Romagna Region Italy 1997	Potato Primura	1. 28/02/1997 2. n.i. 3. 09/07/1997	Spray air pressure knapsack (PULVAL model CRISTAL)	1 st : 0.096	1st: 480	1 st : 0.0200	BBCH 60-65	potato tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/4/P Analytical report: R 7180 Sandy loam, rain irrigation Jun: 10-34, mean 14.9-28.5 °C July: 9-35, mean 15.9-32.2 °C GC-NPD; LOQ=0.05mg/kg
				2 nd : 0.105	2nd: 525	2 nd : 0.0200	BBCH 69				
				3 rd : 0.103	3rd: 515	3 rd : 0.0200	BBCH 69				
				4 th : 0.104	4th: 520	4 th : 0.0200	BBCH 80-82				
				5 th : 0.103	5th: 515	5 th : 0.0200	BBCH 91-93				

Report No. Location (region)	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate per treatment					Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues *) (mg/kg)	PHI (days)	Remarks	
				kg as/ha	Water (L/ha)	kg as/hL									
Annex le continued															
CY2/I/02PA Salerano sul Lambro (Lodi), Lombardia region Italy 2001	Potato Kennebec	1. 26/04/2001 2. 12/06/2001 3. 06+07+09+ 13/08/2001	Spray air-compress 4 nozzles, 50 cm, 4 bar, 9.9-30.3 ml/s	1st: 0.118 2nd: 0.117 3rd: 0.121 4th: 0.116 5th: 0.117	1st: 490 2nd: 487 3rd: 506 4th: 483 5th: 488	1st: 0.0241 2nd: 0.0240 3rd: 0.0239 4th: 0.0240 5th: 0.0240	1st: 09/07/2001 2nd: 16/07/2001 3rd: 23/07/2001 4th: 30/07/2001 5th: 06/08/2001	BBCH 69 BBCH 71 BBCH 75 BBCH 83 BBCH 89	potato tuber	< 0.05 < 0.05 < 0.05 <u>< 0.05</u>	0 (+3h) 1 3 7	DCS (Decline Curve Study) Field code: CY2/I/02PA Analytical report: SIP 1298 Sandy clay loam, flowing irrigation July: 12-36, mean 17.9-31.9°C Aug: 14-36, mean 18.8-32.7°C GC-NPD; LOQ=0.05 mg/kg			

n.i. not indicated

underlined residue values were used for MRL calculation

*) residues calculated as mg Cymoxanil/kg

WARNING: This document is part of an EU Pesticide Data Package and should not be read in isolation. Registration must not be granted on the basis of this document.

Annex If: Summary of supervised residue trials of potato from southern Europe - Oxon

Active substance (common name): **Cymoxanil**
 Crop/crop group: **Potato/Potatoes**
 Content of active substance (g/kg or g/L): **40 g/kg**
 Formulation (e.g. WP): **WP**
 Commercial product (name): **---**
 Method of treatment: **Spray**
 Producer of commercial product: **Oxon**
 Indoor/Glasshouse/Outdoor: **Outdoor**
 Other active substance in the formulation: **Mancozeb**
 (common name and content): **400 g/kg**
 *) Residues calculated as: **Cymoxanil**

Report No. Location (region)	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues *) (mg/kg)	PHI (days)	Remarks
				kg as/ha	Water (L/ha)	kg as/hL						
CY2/PO/S-01 Chipiona (Cádiz), Spain 2001	Potato Espunta	1. 17/12/2000 2. n.i. 3. 09+16/04/ 2001	Spray air-compress 4 nozzles, 50 cm, 3 bar, 111-115 ml/s	1 st : 0.122	1 st : 1020	1 st : 0.0120	1 st : 05/03/2001	BBCH 43	potato tuber	≤ 0.05	7	Decline Curve Study (DCS) Field code: CY2/PO/S-01 Analytical report: SIP 1265 Sandy soil, no irrigation March: 8.3-26.0°C April: 9.0-29.5°C GC-NPD; LOQ=0.05mg/kg
				2 nd : 0.121	2 nd : 1012	2 nd : 0.0120	2 nd : 12/03/2001	BBCH 44		< 0.05	14	
				3 rd : 0.115	3 rd : 960	3 rd : 0.0120	3 rd : 19/03/2001	BBCH 45				
				4 th : 0.118	4 th : 980	4 th : 0.0120	4 th : 26/03/2001	BBCH 46				
				5 th : 0.122	5 th : 1020	5 th : 0.0120	5 th : 02/04/2001	BBCH 47				
CY2/PO/S-02 Sanlúcar la Mayor (Sevilla) Spain 2001	Potato Espunta	1. 07/02/2001 2. n.i. 3. 14+15+17+ 21+28/05/2001	Spray air-compress 4 nozzles, 50 cm, 3 bar, 112-116 ml/s	1 st : 0.119	1 st : 994	1 st : 0.0120	1 st : 16/04/2001	BBCH 43	potato tuber	< 0.05	0	Decline Curve Study (DCS) Field code: CY2/PO/S-02 Analytical report: SIP 1265 Loamy sand soil, trickle irrigation April: 7.0-30.5°C May: 7.0-37.0°C GC-NPD; LOQ=0.05mg/kg
				2 nd : 0.121	2 nd : 1006	2 nd : 0.0120	2 nd : 23/04/2001	BBCH 44		< 0.05	1	
				3 rd : 0.123	3 rd : 1024	3 rd : 0.0120	3 rd : 30/04/2001	BBCH 45		< 0.05	3	
				4 th : 0.123	4 th : 1022	4 th : 0.0120	4 th : 07/05/2001	BBCH 46		< 0.05	7	
				5 th : 0.125	5 th : 1040	5 th : 0.0120	5 th : 14/05/2001	BBCH 48		< 0.05	14	

underlined residue values were used for MRL calculation

Annex Iq: Summary of supervised residue trials of potato from southern Europe - Oxon

Active substance (common name): **Cymoxanil**
 Crop/crop group: **Potato/Potatoes**
 Content of active substance (g/kg or g/L): **40 g/kg**
 Formulation (e.g. WP): **WP**
 Commercial product (name): **---**
 Method of treatment: **Spray**
 Producer of commercial product: **Oxon**
 Indoor/Glasshouse/Outdoor: **Outdoor**
 Other active substance in the formulation (common name and content): **Cu-oxychloride 400 g/kg**
 *) Residues calculated as: **Cymoxanil**

Report No. Location (region)	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues *) (mg/kg)	PHI (days)	Remarks
				kg as/ha	Water (L/ha)	kg as/hL						
COF/1/3/P Salerano sul Lambro (Lodi), Lombardia region, Italy 1996	Potato Kennebec	1. 19/03/1996 2. n.i. 3. 08/08/1996	Spray air pressure knapsack (PULVAL model CRISTAL)	1st: 0.100	1st: 1003	1st: 0.0997	1st: 03/07/1996	BBCH 60-65	potato tuber	≤ 0.05	7	HS (Harvest Study) Field code: COF/1/3/P Analytical report: R 6124 Sandy soil, flooding irrigation July: mean 17.0-28.3 °C Aug: mean 17.2-28.3 °C GC-NPD; LOQ=0.05mg/kg
				2nd: 0.100	2nd: 997	2nd: 0.100	2nd: 10/07/1996	BBCH 60-65				
				3rd: 0.101	3rd: 1011	3rd: 0.0100	3rd: 18/07/1996	BBCH 69				
				4th: 0.099	4th: 991	4th: 0.0100	4th: 25/07/1996	BBCH 92				
				5th: 0.102	5th: 1021	5th: 0.0100	5th: 01/08/1996	BBCH 93				
COF/1/4/P S. Agostino (FE), Emilia Romagna Region, Italy 1996	Potato Agata	1. 14/03/1996 2. n.i. 3. 04/07/1996	Spray air pressure knapsack (PULVAL model CRISTAL)	1st: 0.115	1st: 579	1st: 0.0199	1st: 30/05/1996	BBCH 60-65	potato tuber	≤ 0.05	7	HS (Harvest Study) Field code: COF/1/4/P Analytical report: R 6124 Clay soil, rain irrigation May: 10.4-30.3 °C June: 13-36.2 °C July: 12.7-34.9 °C GC-NPD; LOQ=0.05mg/kg
				2nd: 0.095	2nd: 475	2nd: 0.0200	2nd: 06/06/1996	BBCH 69				
				3rd: 0.102	3rd: 511	3rd: 0.0200	3rd: 13/06/1996	BBCH 69				
				4th: 0.103	4th: 514	4th: 0.0200	4th: 20/06/1996	BBCH 80-82				
				5th: 0.104	5th: 520	5th: 0.0200	5th: 27/06/1996	BBCH 91-93				
COF/2/3/P Cavazzana di Lusia (Rovigo), Veneto region, Italy 1997	Potato Jaerla	1. 03/02/1997 2. n.i. 3. 20/06/1997	Spray air pressure knapsack (PULVAL model CRISTAL)	1st: 0.110	1st: 550	1st: 0.0200	1st: 16/05/1997	BBCH 60-65	potato tuber	≤ 0.05	7	HS (Harvest Study) Field code: COF/2/3/P Analytical report: R 7180 Sandy soil, sprinkler irrigation May: 5-32, mean 11.7-24.5 °C June 9-31, mean 15.6-25.9 °C GC-NPD; LOQ=0.05mg/kg
				2nd: 0.113	2nd: 567	2nd: 0.0199	2nd: 23/05/1997	BBCH 69				
				3rd: 0.105	3rd: 521	3rd: 0.0202	3rd: 30/05/1997	BBCH 69				
				4th: 0.105	4th: 525	4th: 0.0200	4th: 06/06/1997	BBCH 80-82				
				5th: 0.108	5th: 542	5th: 0.0199	5th: 13/06/1997	BBCH 91-93				
COF/2/4/P S. Giovanni Persiceto (Bologna), Emilia Romagna Region, Italy 1997	Potato Primura	1. 28/02/1997 2. n.i. 3. 09/07/1997	Spray air pressure knapsack (PULVAL model CRISTAL)	1st: 0.102	1st: 510	1st: 0.0200	1st: 04/06/1997	BBCH 60-65	potato tuber	≤ 0.05	7	HS (Harvest Study) Field code: COF/2/4/P Analytical report: R 7180 Sandy/loam, rain irrigation Jun: 10-34, mean 14.9-28.5 °C July: 9-35, mean 15.9-32.2 °C GC-NPD; LOQ=0.05mg/kg
				2nd: 0.099	2nd: 495	2nd: 0.0200	2nd: 11/06/1997	BBCH 69				
				3rd: 0.100	3rd: 500	3rd: 0.0200	3rd: 18/06/1997	BBCH 69				
				4th: 0.096	4th: 480	4th: 0.0200	4th: 25/06/1997	BBCH 80-82				
				5th: 0.099	5th: 495	5th: 0.0200	5th: 02/07/1997	BBCH 91-93				

n.i. not indicated, underlined residue values were used for MRL calculation

Annex Ih: Summary of supervised residue trials of potato from southern Europe - Oxon

Active substance (common name): **Cymoxanil**
 Crop/crop group: **Potato/Potatoes**
 Content of active substance (g/kg or g/L): **50 g/L**
 Formulation (e.g. WP): **SC**

Method of treatment:
 Producer of commercial product: **Spray Oxon**
 Indoor/Glasshouse/Outdoor: **Outdoor**
 Other active substance in the formulation (common name and content): **Chlorothalonil 375 g/L**
 *) Residues calculated as: **Cymoxanil**

Commercial product (name): ---

Report No. Location (region)	Commodity/ Variety	Date of 1. Sowing or Planting 2. Flowering 3. Harvest	Method of treatment	Application rate Per treatment			Dates of treatment(s) or no. of treatment(s) and last date	Growth stage at last treatment or date	Portion analyzed	Residues *) (mg/kg)	PHI (days)	Remarks:
				kg as/ha	Water (L/ha)	kg as/hL						
COF/1/3/P Salerano sul Lambro (Lodi), Lombardia region Italy 1996	Potato Kennebec	1. 19/03/1996 2. n.i. 3. 08/08/1996	Spray air pressure knapsack (PULVAL model CRISTAL)	1st: 0.102	1st: 1021	1st: 0.0100	1st: 03/07/1996	BBCH 60-65	potato tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/3/P Analytical report: R 6124 Sandy soil, flooding irrigation July: mean 17.0-28.3 °C Aug: mean 17.2-28.3 °C GC-NPD; LOQ=0.05mg/kg
				2nd: 0.103	2nd: 1035	2nd: 0.0100	2nd: 10/07/1996	BBCH 60-65				
				3rd: 0.103	3rd: 1031	3rd: 0.0100	3rd: 18/07/1996	BBCH 69				
				4th: 0.100	4th: 1000	4th: 0.0100	4th: 25/07/1996	BBCH 92				
				5th: 0.097	5th: 971	5th: 0.0100	5th: 01/08/1996	BBCH 93				
COF/1/4/P S. Agostino (FE), Emilia Romagna Region, Italy 1996	Potato Agata	1. 14/03/1996 2. n.i. 3. 04/07/1996	Spray air pressure knapsack (PULVAL model CRISTAL)	1st: 0.108	1st: 543	1st: 0.0199	1st: 30/05/1996	BBCH 60-65	potato tuber	< 0.05	7	HS (Harvest Study) Field code: COF/1/4/P Analytical report: R 6124 Clay soil, rain irrigation May: 10.4-30.3 °C June: 13-36.2 °C July: 12.7-34.9 °C GC-NPD; LOQ=0.05mg/kg
				2nd: 0.109	2nd: 543	2nd: 0.0201	2nd: 06/06/1996	BBCH 69				
				3rd: 0.107	3rd: 536	3rd: 0.0200	3rd: 13/06/1996	BBCH 69				
				4th: 0.103	4th: 514	4th: 0.0200	4th: 20/06/1996	BBCH 80-82				
				5th: 0.111	5th: 555	5th: 0.0200	5th: 27/06/1996	BBCH 91-93				
COF/2/3/P Cavazzana di Lusita (Rovigo) Veneto region, Italy 1997	Potato Jaerla	1. 03/02/1997 2. n.i. 3. 20/06/1997	Spray air pressure knapsack (PULVAL model CRISTAL)	1st: 0.098	1st: 488	1st: 0.0201	1st: 16/05/1997	BBCH 60-65	potato tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/3/P Analytical report: R 7180 Sandy soil, sprinkler irrigation May: 5-32, mean 11.7-24.5 °C June 9-31, mean 15.6-25.9 °C GC-NPD; LOQ=0.05mg/kg
				2nd: 0.104	2nd: 521	2nd: 0.0200	2nd: 23/05/1997	BBCH 69				
				3rd: 0.100	3rd: 500	3rd: 0.0200	3rd: 30/05/1997	BBCH 69				
				4th: 0.103	4th: 513	4th: 0.0201	4th: 06/06/1997	BBCH 80-82				
				5th: 0.111	5th: 554	5th: 0.0200	5th: 13/06/1997	BBCH 91-93				
COF/2/4/P S. Giovanni Persiceto (Bologna), Emilia Romagna Region, Italy 1997	Potato Primura	1. 28/02/1997 2. n.i. 3. 09/07/1997	Spray air pressure knapsack (PULVAL model CRISTAL)	1st: 0.100	1st: 500	1st: 0.0200	1st: 04/06/1997	BBCH 60-65	potato tuber	< 0.05	7	HS (Harvest Study) Field code: COF/2/4/P Analytical report: R 7180 Sandy loam, rain irrigation Jun: 10-34, mean 14.9-28.5 °C July: 9-35, mean 15.9-32.2 °C GC-NPD; LOQ=0.05mg/kg
				2nd: 0.100	2nd: 500	2nd: 0.0200	2nd: 11/06/1997	BBCH 69				
				3rd: 0.097	3rd: 485	3rd: 0.0200	3rd: 18/06/1997	BBCH 69				
				4th: 0.100	4th: 500	4th: 0.0200	4th: 25/06/1997	BBCH 80-82				
				5th: 0.099	5th: 495	5th: 0.0200	5th: 02/07/1997	BBCH 91-93				

n.i. ... not indicated
 underlined residue values were used for MRL calculation

Appendix 2: Specific terms and abbreviations

RCI rotational crop interval

DASST days after single soil treatment

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.