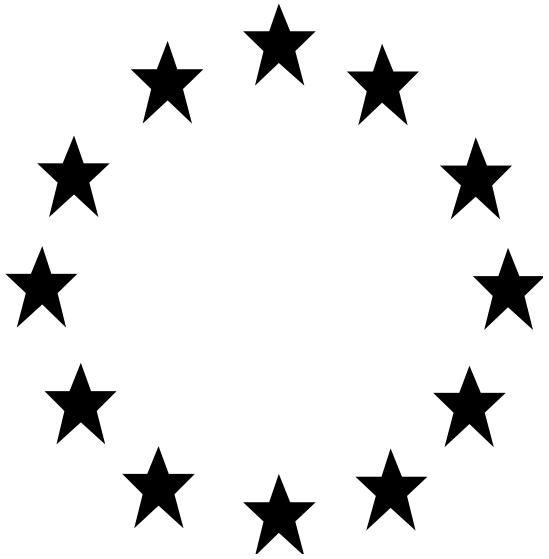

Final addendum to the

Additional Report

- public version -

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

CARBOSULFAN

according to the Accelerated Resubmission Procedure laid down in Commission Regulation (EC) No. 33/2008

October 2009

Table of contents

ANNEX B

Addendum July 2009

Carbosulfan

B.7 Residue data

Point 3(4) in the reporting tables: Vol. B.7.3.2 Residue definition in animal products

-Identification of ^{14}C-Residues in Tissues and Eggs from Poultry Administered (Ring-^{14}C) Carbosulfan (Markle J.C.; 1982a)
-Identification of ^{14}C-Residues in Tissues and Eggs from Poultry Administered (Dibutylamino-^{14}C) Carbosulfan (Markle J.C.; 1982b)

Table B.7.2.1-2: Material balance and metabolites distribution of the residues of carbosulfan in thigh muscle and liver of the laying hens after oral administration of (Phenyl ring -UL-^{14}C)-Carbosulfan and (Dibutylamino-^{14}C)-Carbosulfan (Nominal feeding level: 5.0 mg/kg feed) – (Residues expressed in percent of the total radioactive residues and in mg ^{14}C carbosulfan equiv./kg)

Labelling forms	**(Phenyl ring -UL-^{14}C) -Carbosulfan**		**(Dibutylamino-^{14}C)-Carbosulfan**						
Tissues	**Thigh muscle (0-day)***	**Liver (0-day)***	**Thigh muscle (0-day)***	**Liver (0-day)***	**Fat (0-day)***	**Egg white (0-day)***	**Egg yolk (0-day)***	**Egg white (9-12-day)***	**Egg yolk (9-12 -day)***
Total radioactive residues	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**
	100 (0.115)	100 (0.282)	100 (0.162)	100 (1.352)	100 (0.304)	100 (0.0793)	100 (1.513)	100 (0.088)	100 (1.542)
Extractability of radioactive residues									
Organosoluble partitioned phase	70.1 (0.08)	37.9 (0.106)	59.1 (0.095)	62.5 (0.845)	89.8 (0.272)			13.7 (0.012)	90.1 (1.389)
Water soluble partitioned phase	14.6 (0.016)	44.9 (0.126)	15.5 (0.025)	23.4 (0.316)	1.2 (0.0036)			6.50 (0.005)	1.9 (0.029)
Elucidation of radioactive residues									
Carbosulfan	Nd	nd			0.2 (<0.002)				
Carbofuran	1.1 (<0.002)	nd							
Dibutylamine			22.5 (0.036)	36.9 (0.498)	3.1 (0.009)			3.8 (0.0033)	4.3 (0.066)
3-hydroxy-carbofuran	36.9 (0.042)	1.1 (0.003)							
3-hydroxy-N-hydroxymethyl carbofuran	9.3 (0.01)	2.1 (0.005)							
3-keto-carbofuran	1.7 (<0.002)	4.2 (0.011)							
7-phenol	1.2 (<0.002)	3.3 (0.009)							
3-keto-7-phenol	7.9 (0.908)	2.5 (0.007)							
3-hydroxy-7-phenol	7.1 (0.816)	16.0 (0.045)							
Unidentified metabolites	13.0 (0.014)	15.7 (0.044)	36.6 (0.059)	25.6 (0.346)	86.7 (0.263)			9.9 (0.008)	85.8[(2)] (1.323)
Total identified metabolites	64.1 (0.073)	29.2 (0.082)	22.5 (0.036)	36.9 (0.498)	3.3 (0.011)			3.8 (0.0033)	4.3 (0.066)

Labelling forms	(Phenyl ring -UL-^{14}C) -Carbosulfan		(Dibutylamino-^{14}C)-Carbosulfan						
Tissues	Thigh muscle (0-day)*	Liver (0-day)*	Thigh muscle (0-day)*	Liver (0-day)*	Fat (0-day)*	Egg white (0-day)*	Egg yolk (0-day)*	Egg white (9-12-day)*	Egg yolk (9-12 -day)*
Total radioactive residues	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)
Residual radioactive residues (RRR)									
	1.6 (<0.002)	18.5 (0.052)	23.4[(1)] (0.037)	10.9[(1)] (0.147)	-			66.0[(1)] (0.058)	5.6 (0.086)
Recovery : partitioned phases + RRR									
	86.3 (0.099)	101.3 (0.285)	98.0 (0.157)	96.8 (1.308)	91.0 (0.275)			86.2 (0.075)	97.6 (1.504)

Nd : not radiodetected
*: 0-day withdrawal or within 6 hours after the last dosing.
[(1)]: Post extraction solids submitted to acid hydrolysis.
[(2)] : Saponified in alcoholic KOH and analysed for fatty acids.
na : not analysed.

Table B.7.2.1-2': Hydrolysis step (0.25N HCl) of the different fractions of liver and thigh muscle within 6 hours after the last dosing

Fractions	(Phenyl ring -UL-^{14}C) -Carbosulfan					
	Thigh muscle, 0-day Polar aqueous fraction		Liver, 0-day Post extraction solids fraction		Liver, 0-day Polar aqueous fraction	
	% TRR	Mg/kg	% TRR	Mg/kg	% TRR	Mg/kg
Initial	14.6	0.016	18.6	0.052	44.9	0.126
Polar (aqueous soluble phase)	6.1	0.00097	4.3	0.0022	21.5	0.027
Bound solids	----	----	1.7	0.00088	----	-----
Non polar (organosoluble phase)	7.1	0.00113	5.0	0.0026	11.0	0.0138
3-keto carbofuran	ND	<0.002	ND	<0.002	2.1	0.0026
3-keto-7-phenol	1.5	<0.002	ND	<0.002	ND	<0.002
7-phenol	1.2	<0.002	ND	<0.002	0.9	<0.002
Unknowns	4.4	0.0007	5.0	0.0026	4.5	0.0056

The post extraction solids from the liver (18.6 % of TRR) and the polar aqueous fraction from the liver (44.9 % of TRR) and thigh muscle (14.6 % of TRR) were hydrolysed with 0.25 N HCl to check for additional release of conjugated metabolites. Hydrolysis of the aqueous phases from the 0-day thigh muscle resulted in the release of an additional 7.1 % of TRR in the organo soluble phase. Of this, low levels of 3-keto-7-phenol (1.5% of TRR) and 7-phenol (1.2 % of TRR) were recovered.

Hydrolysis of the liver post extraction solids resulted in an additional release of 9.3 % of TRR. Of this, 4.3 % of TRR remained in the acidic aqueous fraction and 5 % of TRR was organo soluble. None of the carbosulfan metabolite was detected.

Hydrolysis of the polar aqueous phase from the 0-day liver (44.9% of TRR) resulted in an additional release of 11 % of TRR into the organo soluble fraction. Low levels of 3-keto carbofuran (2.1% of TRR) and 7-phenol (0.9 % of TRR) were identified.

Point 3(10) in the reporting tables: Supervised residue trials – Analytical methods

The following methods were reported in chapter B.5.2.1 – Carbosulfan additional report, revised April 2009.

- Determination of residues of carbosulfan and its metabolites carbofuran and 3-hydroxy carbofuran by HPLC-MS-MS in maize and sugar beet samples – Validation of the method. (Enriquez, 2006, Report BATTELLE A-17-05-13)
- Trials ***FA-17-04-02/01-02, FA-17-05-02/01-02 and FA-17-06-07/01-02***

GLP:
GLP-compliance stated
Principle of the method:
Carbosulfan and Carbofuran (CS-CF) is extracted from 5 g sample with a mixture of hexane – acetone (4:1, v/v) and filtered through Celite and sodium sulphate anhydrous.

The metabolite 3-hydroxy carbofuran (3-OHCF) is extracted from the remaining filter cake by refluxing with 0.25 M hydrochloric acid. After filtration the 3-hydroxy carbofuran is cleaned-up through a C18 SPE cartridge using methanol 1% in dichloromethane.

The combined organic extract (CS-CF and 3-OHCF) is evaporated (at temperatures below 35°C and after addition of 'keeper' 1-decanol, in order to avoid losses of carbosulfan), re-constituted and kept in acetonitrile. Then the re-constituted extract is diluted with acetonitrile and water (to have the same composition of the mobile phase) and analysed by HPLC (column: Aqua C18, 50mm x 2mm ID, 5µm particles) with MS-MS detection (ESI, positive mode).

Findings:

Specificity – interferences :
- Following ion transitions were monitored (MRM): m/z 381.1 → 118.1 (carbosulfan); m/z 222.1 → 123.0 (carbofuran); m/z 237.9 → 163.0 (3-OH-carbofuran);
  LC-MS/MS is highly specific → no need for separate confirmatory method.
- No significant interferences (>30% of LOQ) were observed at the retention times of carbosulfan, carbofuran or 3-hydroxy carbofuran in any blank or control sample.

Linearity : The detector response for each compound was linear over the concentration range 1 ng/mL to 25 ng/mL (corresponding to a residue conc. range of 2 to 50 ppb). Correlation coefficients > 0.99.

Recovery – precision : see Table B.5.2.1-6b

Validation by an independent laboratory : First validation of method by Battelle; ILV described in study by Zietz (2008) was conducted by SGS Institut Fresenius.

Limit of quantification (LOQ) : 0.005 mg/kg (= 5 ppb) for each analyte in maize and sugar beet

Table B.5.2.1-6b: Lab validation of LC-MS/MS method for residues of Carbofuran and 3-OH Carbofuran in maize and sugar beet (Enriquez, 2006) (FMC)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
Maize grain	Carbosulfan	0.005	5	77-82	80	3
		0.050	5	70-79	75	5
		Overall	*10*	*70-82*	*78*	*5*
	Carbofuran	0.005	5	87-94	89	3
		0.050	5	96-102	99	2
		Overall	*10*	*87-102*	*94*	*6*
	3-OH carbofuran	0.005	5	94-107	97	5
		0.050	5	100-104	101	2
		Overall	*10*	*94-107*	*100*	*4*
Sugar beet	Carbosulfan	0.005	5	67-77	73	5
		0.050	5	82-91	87	4
		Overall	*10*	*67-91*	*80*	*10*
	Carbofuran	0.005	5	82-94	86	6
		0.050	5	84-104	97	8
		Overall	*10*	*82-104*	*92*	*9*
	3-OH carbofuran	0.005	5	102-115	107	5
		0.050	5	75-100	92	11
		Overall	*10*	*75-115*	*100*	*11*

Conclusion:
The analytical method is suitable for the determination of carbosulfan and its metabolites carbofuran and 3-hydroxy carbofuran in maize and sugar beet samples with a LOQ of 5 ppb for each analyte.

- In trials ***FA-17-04-07,FA-17-04-04, FA-17-06-03, FA-17-04-06***, the determination of Carbosulfan and its metabolites Carbofuran and 3-OH-carbofuran residues in sugar/fodder beet leaves and roots was achieved by HPLC /UV-PCD (post-column derivatisation using fluorescence detection) according to the reported analytical method in the residue study N°A-17-03-25.
This analytical method was based on the procedure of the method entitled "Determination of residues of Carbosulfan and its metabolites Carbofuran and 3-Hydroxy Carbofuran in leafy cabbage, cauliflower and Brussels sprouts – Validation of the method (Ginzburg, 2001a)".
This analytical method was validated for a Limit of Quantification of 0.05 mg/kg for each analyte.
This analytical method is described as follows:

- Determination of residues of Carbosulfan and its metabolites Carbofuran and 3-Hydroxy Carbofuran in leafy cabbage, cauliflower and brussels sprouts – Validation of the method (Ginzburg, 2001a)
- Carbosulfan EU dossier DAR : Additional requirements on the methods of analysis (chapter B.5) (Oz, 2004)

GLP :
GLP-compliance stated
Principle of the method :
Carbosulfan and Carbofuran are extracted twice from the sample with hexane/acetone 4+1 (v+v). After centrifugation and filtration, Carbosulfan and Carbofuran are cleaned up on an Envi Carb SPE cartridge followed by an Amino-propyl SPE cartridge.
Metabolite *3-Hydroxy Carbofuran* is extracted from the filter cake by refluxing with 0.25 M HCl. After filtration, 3-OH Carbofuran is cleaned up on a C18-SPE cartridge followed by the same Amino-propyl SPE cartridge used for Carbosulfan and Carbofuran (elution with methanol 1% in dichloromethane).
After evaporation to dryness, the combined residues are reconstituted in a mixture of acetonitrile/water 30+70 (v+v) and analyzed by HPLC (Zorbax Bonus-RP (C_{14}), 5 µm, 25 cm x 4.6 mm i.d.) with post-column derivatization (PCD) and fluorescence detection. The post-column reaction includes acidic hydrolysis of carbosulfan to carbofuran, followed by alkaline hydrolysis after which the methyl amine formed is derivatized with o-phtalaldehyde and 2-mercaptoethanol to the corresponding fluorescent substituted isoindol. Quantification by external standardization.
Findings :
Specificity – interferences :
- HPLC-PCD with fluorescence detection is highly specific to N-methyl carbamates and their precursors → no need for confirmatory method
- no significant matrix interferences (control values < 30% of LOQ)

Linearity : response of fluorescence detector to resp. analytes (peak area vs. conc.) was demonstrated to be

linear within a concentration range of 0.025 to 0.25 mg/L; r > 0.99
Recovery – precision : see Table B.5.2.1-9
Validation by an independent laboratory : not addressed
Limit of quantification (LOQ) : 0.05 mg/kg
for each analyte

Table B.5.2.1-9 : Validation of HPLC-PCD method for residues in crops (Ginzburg, 2001a)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
leafy cabbage	Carbosulfan	0.05 0.5	5 5	75 – 107 73 – 81	97 76	13.2 4.3
	Carbofuran	0.05 0.5	5 5	97 – 104 81 – 102	100 94	2.6 9.4
	3-OH Carbofuran	0.05 0.5	5 5	79 – 103 84 – 112	89 103	11.7 11.8
cauliflower	Carbosulfan	0.05 0.5	5 7	82 – 112 76 – 96	99 89	12.3 7.4
	Carbofuran	0.05 0.5	5 7	104 – 108 62 – 94	106 82	1.9 16.1
	3-OH Carbofuran	0.05 0.5	5 7	92 – 109 77 – 91	101 87	6.4 5.5
brussels sprouts	Carbosulfan	0.05 0.5	5 5	89 – 106 62 – 74	96 70	8.4 6.7
	Carbofuran	0.05 0.5	5 5	74 – 87 92 – 107	78 99	6.6 6.5
	3-OH Carbofuran	0.05 0.5	5 5	70 – 86 95 – 106	77 102	7.8 4.1

Conclusion :
HPLC-PCD method with fluorescence detection is suitable for the determination of residues of Carbosulfan, Carbofuran and 3-Hydroxy Carbofuran in brassica crops with a LOQ for each analyte of 0.05 mg/kg.

ANNEX B

Addendum July 2009

Carbosulfan

B.9 Ecotoxicology

B.9.2.16 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

During Peer Review the notifier provided updated PECsw and PECsed calculations which were evaluated in the section on fate and behaviour.

In the table below, the PECsw and PECsed values for FOCUS step 3 are presented for the parent compound carbosulfan and its metabolites carbofuran, dibutylamine, carbofuran-7-phenol, 3-hydroxy-carbofuran and 3-keto-carbofuran.

Table B.9.2.16-1 : Calculated PEC values for carbosulfan and its metabolites (FOCUS Step 3) in surface water, application of 750 g carbosulfan/ha in sugar beet

Scenario	Compound	Max PECsw (µg/L)	Date of max PECsw	Max PECsed (µg/kg)	Date of max PECsed
D3 ((Ditch)	Carbosulfan	0.00E+00	1-janv-92	0.00E+00	1-janv-92
D4 ((Pond)	Carbosulfan	0.00E+00	9-déc-85	0.00E+00	31-déc-85
D4 ((Stream)	Carbosulfan	0.00E+00	9-déc-85	0.00E+00	9-déc-85
R1 ((Pond)	Carbosulfan	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	Carbosulfan	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	Carbosulfan	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	Carbofuran	1.11E-02	29-janv-93	2.00E-02	15-avr-93
D4 ((Pond)	Carbofuran	6.58E-02	30-janv-86	9.03E-02	6-mars-86
D4 ((Stream)	Carbofuran	4.62E-02	16-déc-85	4.10E-02	28-janv-86
R1 ((Pond)	Carbofuran	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	Carbofuran	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	Carbofuran	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	DBA	0.00E+00	1-janv-92	0.00E+00	1-janv-92
D4 ((Pond)	DBA	0.00E+00	9-déc-85	0.00E+00	1-janv-85
D4 ((Stream)	DBA	0.00E+00	5-déc-85	0.00E+00	9-déc-85
R1 ((Pond)	DBA	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	DBA	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	DBA	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	7-P-C	1.30E-05	29-janv-93	4.07E-04	4-avr-93
D4 ((Pond)	7-P-C	8.50E-05	31-janv-86	1.85E-03	1-mai-86
D4 ((Stream)	7-P-C	7.50E-05	1-janv-85	9.67E-04	1-févr-86
R1 ((Pond)	7-P-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	7-P-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	7-P-C	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	3-H-C	2.11E-04	29-janv-93	4.28E-04	16-avr-93
D4 ((Pond)	3-H-C	1.39E-03	30-janv-86	2.39E-03	20-mars-86
D4 ((Stream)	3-H-C	8.85E-04	17-déc-85	8.12E-04	28-janv-86
R1 ((Pond)	3-H-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	3-H-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	3-H-C	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	3-K-C	6.57E-04	30-janv-93	4.70E-03	1-mai-93
D4 ((Pond)	3-K-C	0.00E+00	3-sept-85	1.07E-06	1-mai-86
D4 ((Stream)	3-K-C	0.00E+00	6-juin-85	0.00E+00	25-avr-86
R1 ((Pond)	3-K-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	3-K-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84

R3 ((Stream)	3-K-C	0.00E+00	1-oct-80	0.00E+00	1-oct-80

Following the calculations performed above, the major-sediment metabolite carbofuran-phenol was modelled as a soil metabolite using a formation fraction of 1, which leads to almost no entry in the water system due to its high Koc and low DT50soil. In order to obtain an estimate of maximum concentrations of carbofuran-phenol in surface water and sediment, the maximum FOCUS Step 3 PECsw and PECsed for carbofuran are multiplied using a MW correction factor (164.2/221.3 = 0.74) and the maximum % occurrence of carbofuran-phenol in water-sediment (total AR of 30% following the Yeomans (1995 and 1996) study). It results in more critical values for PECsw (0.0146 µg/L carbofuran-phenol) and PECsed (0.02 µg/kg carbofuran-phenol).
For the TER calculations for carbofuran-7-phenol, these latter values were used in stead of the values presented by the notifier.

B.9.2.16.1 Risk assessment for the active substance

Table B.9.2.16.1-1 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to carbosulfan in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Lepomis macrochirus*	96 h	0.015	0.00001	1500000	100
D4	Pond				0.00001	1500000	100
D4	Stream				0.00001	1500000	100
R1	Pond				0.00001	1500000	100
R1	Stream				0.00001	1500000	100
R3	Stream				0.00001	1500000	100
D3	Ditch	*Oncorhynchus mykiss*	14 d	0.004	0.00001	400000	10
D4	Pond				0.00001	400000	10
D4	Stream				0.00001	400000	10
R1	Pond				0.00001	400000	10
R1	Stream				0.00001	400000	10
R3	Stream				0.00001	400000	10
D3	Ditch	*Daphnia magna*	48 h	0.0015	0.00001	150000	100
D4	Pond				0.00001	150000	100
D4	Stream				0.00001	150000	100
R1	Pond				0.00001	150000	100
R1	Stream				0.00001	150000	100
R3	Stream				0.00001	150000	100
D3	Ditch	*Daphnia magna*	48 h	0.00105	0.00001	150000	100
D4	Pond				0.00001	150000	100

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D4	Stream	(Marshal 10G)			0.00001	150000	100
R1	Pond				0.00001	150000	100
R1	Stream				0.00001	150000	100
R3	Stream				0.00001	150000	100
D3	Ditch	*Daphnia magna*	21 d	0.0032	0.00001	320000	100
D4	Pond				0.00001	320000	10
D4	Stream				0.00001	320000	10
R1	Pond				0.00001	320000	10
R1	Stream				0.00001	320000	10
R3	Stream				0.00001	320000	10

The risk of carbosulfan to aquatic organisms is acceptable for all FOCUS step 3 scenarios.

B.9.2.16.2 Risk assessment for the metabolites

Table B.9.2.16.2-1 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to carbofuran in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Lepomis macrochirus*	96 h	0.18	0.0111	16216	100
D4	Pond				0.0658	2736	100
D4	Stream				0.0462	3896	100
R1	Pond				0.00001	18000000	100
R1	Stream				0.00001	18000000	100
R3	Stream				0.00001	18000000	100
D3	Ditch	*Cyprinodon variegatus*	35 d	0.006	0.0111	541	10
D4	Pond				0.0658	91	10
D4	Stream				0.0462	130	10
R1	Pond				0.00001	600000	10
R1	Stream				0.00001	600000	10

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
R3	Stream				0.00001	600000	10
D3	Ditch	*Daphnia magna*	48 h	0.00205	0.0111	185	100
D4	Pond				0.0658	**31**	100
D4	Stream				0.0462	**44**	100
R1	Pond				0.00001	205000	100
R1	Stream				0.00001	205000	100
R3	Stream				0.00001	205000	100
D3	Ditch	*Ceriodaphnia dubia*	7 d	0.00016	0.0111	14	10
D4	Pond				0.0658	**2**	10
D4	Stream				0.0462	**3**	10
R1	Pond				0.00001	16000	10
R1	Stream				0.00001	16000	10
R3	Stream				0.00001	16000	10
D3	Ditch	*Chironomus riparius*	28 d	0.004	0.0111	360	10
D4	Pond				0.0658	61	10
D4	Stream				0.0462	87	10
R1	Pond				0.00001	400000	10
R1	Stream				0.00001	400000	10
R3	Stream				0.00001	400000	10

Table B.9.2.16.2-2 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to carbofuran in sediment for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/kg)	PEC_{sed} (µg/kg)	TER	Annex VI trigger
D3	Ditch	*Chironomus riparius*	28 d	0.0022	0.0200	110	10
D4	Pond				0.0903	24	10
D4	Stream				0.0410	54	10
R1	Pond				0.00001	220000	10
R1	Stream				0.00001	220000	10

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/kg)	PEC_{sed} (µg/kg)	TER	Annex VI trigger
R3	Stream				0.00001	220000	10

The risk of the metabolite carbofuran to fish and sediment dwelling organisms is acceptable for all FOCUS step 3 scenarios. The acute and chronic risk to aquatic invertebrates is acceptable for the D3 ditch scenario and for all the run-off scenarios (R1 pond, R1 stream, R3 stream). However, for the scenarios D4 pond and D4 stream, the TER values are below the triggers.

Table B.9.2.16.2-3 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 3-keto-carbofuran in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Ceriodaphnia dubia*	48 h	0.049	0.000657	74581	100
D4	Pond				0.00001	4900000	100
D4	Stream				0.00001	4900000	100
R1	Pond				0.00001	4900000	100
R1	Stream				0.00001	4900000	100
R3	Stream				0.00001	4900000	100

The risk of the metabolite 3-keto-carbofuran to aquatic invertebrates is acceptable for all FOCUS step 3 scenarios.

Table B.9.2.16.2-4 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 3-hydroxy-carbofuran in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Ceriodaphnia dubia*	48 h	0.023	0.000211	232227	100
D4	Pond				0.00139	35252	100
D4	Stream				0.000885	55367	100
R1	Pond				0.00001	4900000	100
R1	Stream				0.00001	4900000	100
R3	Stream				0.00001	4900000	100

The risk of the metabolite 3-hydroxy-carbofuran to aquatic invertebrates is acceptable for all FOCUS step 3 scenarios.

Table B.9.2.16.2-5 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 7-phenol in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Test substance	Organism	Toxicity end point (mg/L)	Time scale	PEC_{sw} (µg/L)	PEC_{twa} (µg/L)	TER	Annex VI Trigger
7-phenol	*Oncorhynchus mykiss*	32.3	96 h	0.0146	-	2212329	100

Test substance	Organism	Toxicity end point (mg/L)	Time scale	PEC_{sw} (µg/L)	PEC_{twa} (µg/L)	TER	Annex VI Trigger
7-phenol	*Daphnia magna*	25	48 h	0.0146	-	1712329	100
7-phenol	*Pseudokirchneriella subcapitata*	47	72 h	0.0146	-	3219178	10
7-phenol	*Chironomus riparius*	0.004	25 d	0.0146	-	684932	10

Table B.9.2.16.2-6 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 7-phenol in sediment for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Test substance	Organism	Toxicity end point (mg/kg)	Time scale	PEC_{sed} (µg/kg)	PEC_{twa} (µg/kg)	TER	Annex VI Trigger
7-phenol	*Chironomus riparius*	1.36	25 d	0.02	-	68000	10

As mentioned before, the TER calculations are based on the more critical values for PECsw (0.0146 µg/L 7-carbofuran-phenol) and PECsed (0.02 µg/kg 7-carbofuran-phenol) calculated by RMS.
The risk of the metabolite 7-carbofuran-phenol to aquatic invertebrates is acceptable for all FOCUS step 3 scenarios.

The risk of the metabolite dibutylamine is acceptable based on FOCUS step 1 for fish and algae and on FOCUS step 2 for aquatic invertebrates (see DAR). Therefore, no further TER calculations based on FOCUS step 3 are necessary.

B.9.6.5 Field tests – residue content of earthworms (Annex IIIA 10.6.1.3)

Comparison of Two Methods for Assessing the Effects of Carbofuran on Soil Animal Decomposers in Cornfields. (Broadbent A.B., Tomlin, A.D., 1982).

Abstract :
The effects of spring treatments of an insecticide, carbofuran, on the soil animal decomposer community of an Ontario cornfield were assessed by measuring fluctuations in soil animal populations and by measuring changes in rates of leaf litter decomposition from bags of different mesh sizes. For several weeks after treatment, reductions in soil microfauna and reductions in the rate of corn leaf decomposition could be observed, but by autumn, the total number of soil micro-arthropods and litter decomposition rates were similar to those in untreated control plots. Earthworm populations seemed unaffected by in-row treatments of carbofuran, but broadcast treatments reduced worm populations significantly.

Guidelines :
Not applicable.
GLP:
No
Materials and Methods :
Test substance : carbofuran
Test species : soil fauna, earthworms
Test design :
The experimental site was a plot (120 m x 40 m) in a 12-ha field of continuous corn under regular tillage and management programs in Ontario. The soil was classified as a Burford loam, pH 6.9 with an organic matter content of 4.3 %.
On 10 May 1977, granular carbofuran was applied in-furrow (1.5 kg a.s./ha, in a 10-cm wide band along the corn rows). An untreated control plot of similar dimensions (60 m long, 10 rows wide, rows on 90-cm centers) was also made. On 23 May 1978 (1.1 kg a.s./ha) and on 22 May 1979 (11.2 kg a.s./ha), granular carbofuran was applied in-furrow and as broadcast treatment. An untreated control plot was also made (similar design but only 30 m long).
For 6, 9 and 6 dates, respectively, during the summers of 1977, 1978 and 1979, 50 soil cores (2.5 cm in diameter by 15 cm deep) were taken from each plot to determine residues of carbofuran, 3-keto-carbofuran and 3-OH-carbofuran. Carbofuran was extracted with chloroform from soil and analysed by gas-liquid chromatography as described by Miles and Harris (1979).
Sampling of earthworm populations :
Earthworm populations were estimated using the formaldehyde expellent method of Raw (1959). Nine liters of a 0.55 % formaldehyde solution was applied to the soil surface within a wooden "quadrat" (1.8 by 0.2 m) placed over the seed row. In 1977, earthworm populations and biomass were estimated by using 18 quadrats from each of the treated and control plots on 28 October (24 weeks after treatment). In 1978, populations and biomass were estimated from 12 quadrats from each of the control, row-treated and broadcast-treated plots, and from the untreated zone between rows in the row-treated plot on 3 May (3 weeks before treatment), 23 June (4 weeks after treatment), and 25 October (22 weeks after treatment).
Findings :
Residue determination :
Carbofuran residues disappeared rapidly from the cornfield soil in each of the 3 years of the study. 3-keto-carbofuran and 3-OH-carbofuran were not detected in excess of 0.15 and 0.02 ppm, respectively. The slower rate of carbofuran disappearance in 1978 varied with lower precipitation and soil moisture levels during the 10 weeks after treatment. However, by 10 and 8 weeks, respectively, after treatment in 1978 and 1979, soil residue levels in the row treatments had dropped to levels near the corresponding broadcast treatments.
Populations of soil fauna :
In 1977, 24 weeks after treatment there were 44.2 earthworms per m^2 in row-treated plots and 35.0 earthworms per m^2 in the control plots; at least 90 % of the worms were *Aporrectodea tuberculata* (Eisen). There was no significant difference in mean biomass of earthworms between carbofuran-treated (13.6 g/m^2) and control plots (12.1 g/m^2).
Samples taken in 1978, 3 weeks before treatment and 4 weeks after treatment, contained only three earthworms from 12 quadrats and seven from 18 quadrats, respectively. The small number of earthworms extracted was due to unusually dry soil conditions. In 1978, 22 weeks after treatment, there was no significant difference in

numbers of biomass of earthworms among "between row-treated" (50.8/m², 9.0 g/m²), row-treated (44.7/m², 9.0 g/m²), and control plots (50.3/m², 9.8 g/m²); significantly fewer earthworms were found in the broadcast treatment (28.9/m²), but the earthworm biomass, although smaller in this plot (7.2 g/m²), was not significantly different from the other plots. Broadcast treatments of carbofuran are more harmful to earthworms than row treatments, possibly because there is no untreated refugial soil to which earthworms can escape after a broadcast treatment.
Carbofuran treatment at 11.2 kg a.s./ha caused swellings and abnormal pigmentation of the earthworms similar to that described by Stenersen *et al*. (1973), and 2 weeks after treatment, dead earthworms exhibiting these symptoms were observed on the soil surface.

Conclusion of the RMS :
The RMS has reservations towards this study due to several shortcomings.
Very low numbers of earthworms were collected in 1978, 3 weeks before and 4 weeks after treatment due to unusual dry soil conditions. However, the sampling at 4 weeks after treatment is important to assess the effects of carbofuran on earthworm populations. The sampling at 22 weeks after treatment demonstrated that significantly fewer earthworms were found in the broadcast treatment compared to the control plots, row-treated and "between row-treated" plots. However, carbofuran residues disappeared rapidly from the cornfield, in 1978, 10 weeks after treatment, soil residue levels in the row treatments had dropped to levels near the corresponding broadcast treatments. From the graph of 1978, the soil residue levels at 22 weeks after treatment had fallen to 0 mg/kg. Therefore, the sampling at 22 weeks is not appropriate to estimate effects of carbofuran nor to compare the in-furrow treatment with the broadcast treatment.

ANNEX B

Addendum July 2009-Before TC
(Updated version-August 2009)

Carbosulfan

B.7 Residue data

Point 3(4) in the reporting tables: Vol. B.7.3.2 Residue definition in animal products

-Identification of ^{14}C-Residues in Tissues and Eggs from Poultry Administered (Ring-^{14}C) Carbosulfan (Markle J.C.; 1982a)
-Identification of ^{14}C-Residues in Tissues and Eggs from Poultry Administered (Dibutylamino-^{14}C) Carbosulfan (Markle J.C.; 1982b)

Table B.7.2.1-2: Material balance and metabolites distribution of the residues of carbosulfan in thigh muscle and liver of the laying hens after oral administration of (Phenyl ring -UL-^{14}C)-Carbosulfan and (Dibutylamino-^{14}C)-Carbosulfan (Nominal feeding level: 5.0 mg/kg feed) – (Residues expressed in percent of the total radioactive residues and in mg ^{14}C carbosulfan equiv./kg)

Labelling forms	**(Phenyl ring -UL-^{14}C) -Carbosulfan**		**(Dibutylamino-^{14}C)-Carbosulfan**						
Tissues	**Thigh muscle (0-day)***	**Liver (0-day)***	**Thigh muscle (0-day)***	**Liver (0-day)***	**Fat (0-day)***	**Egg white (0-day)***	**Egg yolk (0-day)***	**Egg white (9-12-day)***	**Egg yolk (9-12 -day)***
Total radioactive residues	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**
	100 (0.115)	100 (0.282)	100 (0.162)	100 (1.352)	100 (0.304)	100 (0.0793)	100 (1.513)	100 (0.088)	100 (1.542)
Extractability of radioactive residues									
Organosoluble partitioned phase	70.1 (0.08)	37.9 (0.106)	59.1 (0.095)	62.5 (0.845)	89.8 (0.272)			13.7 (0.012)	90.1 (1.389)
Water soluble partitioned phase	14.6 (0.016)	44.9 (0.126)	15.5 (0.025)	23.4 (0.316)	1.2 (0.0036)			6.50 (0.005)	1.9 (0.029)
Elucidation of radioactive residues									
Carbosulfan	Nd	nd			0.2 (<0.002)				
Carbofuran	1.1 (<0.002)	nd							
Dibutylamine			22.5 (0.036)	36.9 (0.498)	3.1 (0.009)			3.8 (0.0033)	4.3 (0.066)
3-hydroxy-carbofuran	36.9 (0.042)	1.1 (0.003)							
3-hydroxy-N-hydroxymethyl carbofuran	9.3 (0.01)	2.1 (0.005)							
3-keto-carbofuran	1.7 (<0.002)	4.2 (0.011)							
7-phenol	1.2 (<0.002)	3.3 (0.009)							
3-keto-7-phenol	7.9 (0.908)	2.5 (0.007)							
3-hydroxy-7-phenol	7.1 (0.816)	16.0 (0.045)							
Unidentified metabolites	13.0 (0.014)	15.7 (0.044)	36.6 (0.059)	25.6 (0.346)	86.7 (0.263)			9.9 (0.008)	85.8[(2)] (1.323)

Labelling forms	(Phenyl ring -UL-^{14}C) -Carbosulfan		(Dibutylamino-^{14}C)-Carbosulfan						
Tissues	Thigh muscle (0-day)*	Liver (0-day)*	Thigh muscle (0-day)*	Liver (0-day)*	Fat (0-day)*	Egg white (0-day)*	Egg yolk (0-day)*	Egg white (9-12-day)*	Egg yolk (9-12 - day)*
Total radioactive residues	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)
Total identified metabolites	64.1 (0.073)	29.2 (0.082)	22.5 (0.036)	36.9 (0.498)	3.3 (0.011)			3.8 (0.0033)	4.3 (0.066)
Residual radioactive residues (RRR)									
	1.6 (<0.002)	18.5 (0.052)	23.4[(1)] (0.037)	10.9[(1)] (0.147)	-			66.0[(1)] (0.058)	5.6 (0.086)
Recovery : partitioned phases + RRR									
	86.3 (0.099)	101.3 (0.285)	98.0 (0.157)	96.8 (1.308)	91.0 (0.275)			86.2 (0.075)	97.6 (1.504)

Nd : not radiodetected
*: 0-day withdrawal or within 6 hours after the last dosing.
(1): Post extraction solids submitted to acid hydrolysis.
(2) : Saponified in alcoholic KOH and analysed for fatty acids.
na : not analysed.

Table B.7.2.1-2': Hydrolysis step (0.25N HCl) of the different fractions of liver and thigh muscle within 6 hours after the last dosing

	(Phenyl ring -UL-^{14}C) -Carbosulfan					
Fractions	**Thigh muscle, 0-day Polar aqueous fraction**		**Liver, 0-day Post extraction solids fraction**		**Liver, 0-day Polar aqueous fraction**	
	% TRR	**Mg/kg**	**% TRR**	**Mg/kg**	**% TRR**	**Mg/kg**
Initial	14.6	0.016	18.6	0.052	44.9	0.126
Polar (aqueous soluble phase)	6.1	0.00097	4.3	0.0022	21.5	0.027
Bound solids	----	----	1.7	0.00088	----	-----
Non polar (organosoluble phase)	7.1	0.00113	5.0	0.0026	11.0	0.0138
3-keto carbofuran	ND	<0.002	ND	<0.002	2.1	0.0026
3-keto-7-phenol	1.5	<0.002	ND	<0.002	ND	<0.002
7-phenol	1.2	<0.002	ND	<0.002	0.9	<0.002
Unknowns	4.4	0.0007	5.0	0.0026	4.5	0.0056

The post extraction solids from the liver (18.6 % of TRR) and the polar aqueous fraction from the liver (44.9 % of TRR) and thigh muscle (14.6 % of TRR) were hydrolysed with 0.25 N HCl to check for additional release of conjugated metabolites. Hydrolysis of the aqueous phases from the 0-day thigh muscle resulted in the release of an additional 7.1 % of TRR in the organo soluble phase. Of this, low levels of 3-keto-7-phenol (1.5% of TRR) and 7-phenol (1.2 % of TRR) were recovered.

Hydrolysis of the liver post extraction solids resulted in an additional release of 9.3 % of TRR. Of this, 4.3 % of TRR remained in the acidic aqueous fraction and 5 % of TRR was organo soluble. None of the carbosulfan metabolite was detected.

Hydrolysis of the polar aqueous phase from the 0-day liver (44.9% of TRR) resulted in an additional release of 11 % of TRR into the organo soluble fraction. Low levels of 3-keto carbofuran (2.1% of TRR) and 7-phenol (0.9 % of TRR) were identified.

Open point 3.1 in the Evaluation tables: *"RMS to check the raw data in the goat metabolism study in terms of the respective ratio between free and conjugated carbofuran and 3-OH-carbofuran".*

-Nature of the Residue in Livestock: Metabolism of Carbosulfan in Lactating Goats (Curry S.J., Weintraub R.A.; 1996)

See Tables B.7.2.2-3 and 4 in the revised DAR, April 2009

In the extraction procedure, the residual aqueous samples from the phenyl-label liver and kidney were subjected to acid hydrolysis to release additional conjugated radioactive residues.
Enzymatic digestion and acid hydrolysis of the post extraction solids of liver, kidney and lumbar muscle was applied in order to hydrolyse the peptide linkages and to release additional radioactivity from the bound residues, respectively.

- *Phenyl label treated milk*:
Almost all the radioactive residues were extractable with acetonitrile (98.6 % TRR). Following partitioning against hexane, enzymatic digestion and solid phase extraction of the hydrolysate, only 1% TRR partitioned into hexane while hydrolysis of glucuronide and sulfate conjugates released most of the radioactivity in the methanol eluate (97.5 % TRR as conjugate metabolites). Metabolites identification occurred on the eluate fraction only.
3-OH-carbofuran was recovered at a level of 34.2 % of TRR
No Carbosulfan and Carbofuran were recovered.

-*Phenyl label treated liver*:
37.5 % of the TRR was extractable with methanol followed by partitioning against hexane and methylene chloride to provide an organo soluble phase (12.2 % of TRR) and an aqueous soluble phase (25.1 % of TRR) further submitted to enzymatic digestion and solid phase extraction chromatography.
The non-extractable residues fraction (62.7 % of TRR) was subjected to enzyme digestion to provide an aqueous phase and the residual radioactive residues. The aqueous phase was further partitioned against methylene chloride and acid hydrolysis to further release an aqueous soluble fraction (22.6 % of TRR) and several organosoluble fractions accounting for 27.4 % of the TRR.
Metabolites identification was performed on the organosoluble fractions only.
3-OH-carbofuran occurred in this fraction at a level of 9.5 % of TRR along with other phenolic and carbamate metabolites (see table B.7.2.2-4 in the revised DAR).
Carbosulfan and Carbofuran were detected at a trace level (0.1-0.2 % TRR).

The radioactivity in the non organo soluble phase was characterized as polar metabolites (10.4 % TRR), protein-associated metabolites (22.6 % TRR) and fat-associated metabolites (0.2 % TRR) without any further investigation.

-Phenyl label treated kidney:

61.8 % of TRR were extractable with methanol followed by successive methylene chloride (MEC) partitioning, enzymatic digestion and HCl hydrolysis on the aqueous soluble fractions.
The aqueous soluble and MEC organosoluble fractions amounted 44 % of TRR and 17.9 % of TRR, respectively.
The non-extractable residues fraction (38.2 % TRR) was submitted to enzymatic digestion followed by MEC partitioning to provide an aqueous soluble phase (19.1 % TRR) further submitted to several acid hydrolysis steps giving the successive correspondent organosoluble fractions (18.8 % TRR).
3-OH-carbofuran was recovered in the organo soluble phase at a rate of 21.5 % TRR
Carbosulfan and Carbofuran were detected at a trace level (0.1-0.8 % TRR).
The radioactivity in the non organo soluble phase was characterized as polar metabolites (18.4 % TRR) and protein-associated metabolites (2.5 % TRR) without any further investigation.

JMPR 1997 report:

Carbofuran livestock metabolism:

Metabolism of Carbofuran in laying hens (Hoffman and Robinson, 1994b):

Test substance: Carbofuran was uniformly labelled in the phenyl ring.
Experimental design:
15 laying hens (3 groups of 5 hens-bw: 1.34-1.68 kg) each received a capsule containing 3 mg of the test substance for 7 consecutive days (corresponding to 1.98 mg/kg bw and equivalent to 25 mg/kg in feed). Eggs were collected each day, separated into white and yolk and pooled by group. Excreta were collected daily. Within 22 hours after the final dose, the hens were killed and samples of breast, thigh muscle, fat with skin, liver and kidney were collected from each hen.
The tissues samples were extracted sequentially with acetonitrile and methanol/water. Egg white was extracted with acetonitrile and egg yolk with a mixture of acetonitrile/hexane.
The released radioactivity by solvent extraction, enzymatic digestion and acid/base hydrolysis was carried out using normal phase TLC and reverse-phase HPLC.
The post-extraction solids from liver and kidney were treated sequentially by protease digestion, acid and base hydrolysis.

Total radioactive residues as cumulative percentage of administered dose and as carbofuran equivalents

Sample	**Day**	**% of applied dose**	**Total ^{14}C Carbofuran (mg/kg)**
Excreta	**1**	70.6	
	3	75.2	
	7	82.8	

Egg white	**1**	0.18	0.032
	3	0.21	0.069
	7	0.27	0.059
Egg yolk	**1**	0.07	0.027
	3	0.09	0.078
	7	0.21	0.141
Liver	**7**	0.11	0.137
Kidney	**7**	0.01	0.034
Breast muscle	**7**	0.02	<0.01
Thigh muscle	**7**	<0.01	<0.01
Skin and fat	**7**	<0.01	<0.01
Total recovery		*83.4*	

Characterization and identification of the total radio labelled residue from the administration of ^{14}C-carbofuran to laying hens.

	Liver		**Kidney**		**Egg white**		**Egg yolk**	
TRR (mg/kg)	0.137		0.034		0.069		0.141	
Extracted phase (%TRR)	16		41		91		91	
Metabolite identification								
	%TRR	**Mg/kg**	**%TRR**	**Mg/kg**	**%TRR**	**Mg/kg**	**%TRR**	**Mg/kg**
3-OH-carbofuran	-	-	-	-	-	-	12	0.019
7-phenol	5.7[1]	0.008	4.9[1]	0.001	-	-	16	0.026
3-OH-7-phenol	-	-	-	-	-	-	39	0.062
3-keto-7-phenol	-	-	-	-	-	-	8.5	0.014
Phenolic conjugates	-	-	-	-	90	0.060	-	-
Enzyme digestion aqueous fraction	7.3	0.010	4.6	0.002	-	-	4.6	0.007
Acid hydrolysis aqueous fraction - Mild	3.1	0.004	5.8	0.002	-	-	-	-
Acid hydrolysis aqueous fraction – Strong	12	0.016	8.2	0.003	-	-	-	-
Mild base hydrolysis aqueous fraction	4.0	0.005	3.6	0.001	-	-	-	-
Polar residues from initial extractions	12	0.016	8.2	0.003	-	-	-	-
Post extraction solids	84		59		9		9	
Protease digestion	25		19		-	-	-	-
Acid/base hydrolysis	48		28		-	-	-	-
Residual radioactive residues								
	11		12		9		9	

[1]: Conjugated, released by enzyme treatment.
Remark: There were some discrepancies between the level of total radioactive residues recovered in each matrix and the % of the TRR related to each of the metabolite.

Conclusion:
Carbofuran was not detected in any of the poultry matrix which is consistent with the results observed in the poultry metabolism study reported in the revised DAR, April 2009.
3-OH-carbofuran was detected only in egg yolk while in the revised DAR, it was recovered also in muscle and liver.
No information on the ratio 3-OH-carbofuran free and this metabolite under its conjugated form could be derived based on the data here above.

Metabolism of Carbofuran in lactating goats (Hoffman and Robinson, 1994a):

Test substance: Carbofuran was uniformly labelled in the phenyl ring.
Experimental design:
The test substance was administered orally to lactating goats for 7 consecutive days.
The dose was equivalent to 25 mg/kg carbofuran in the feed. Urine, feces and milk were collected twice daily. The goats were slaughtered within 24 hours of the final dose and samples of muscle (leg and loin), liver, kidney, omental fat and blood were taken.
Milk was extracted with acetone. Muscle, liver and kidney were sequentially extracted with chloroform and methanol/water.
The post extraction solids of liver and kidney were sequentially treated with protease, mild acid extraction and strong acid hydrolysis.
The released radioactive residues were identified by normal phase TLC and reverse-phase HPLC.

Sample	Day	% of applied dose	TRR as ^{14}C carbofuran (mg/kg)
Milk	**1**	0.32	0.010
	3	0.29	0.14
	7	0.30	0.098
Urine	**1**	95	
	3	90	
	7	88	
Faeces	**1**	4.1	
	3	5.1	
	7	5	
Liver	**7**	0.025	0.11
Kidney	**7**	<0.01	0.18
Leg muscle	**7**	<0.01	<0.01
Loin muscle	**7**	<0.01	0.01
Omental fat	**7**	<0.01	<0.01
Total recovery		95	

Characterization and identification of the total radio labelled residue from the administration of ^{14}C-carbofuran to lactating goats.

	Milk		Muscle		Liver		kidney	
TRR (mg/kg)	0.32		0.01		0.11		0.18	
Extracted phase (%TRR)	99		30		27		20	
Metabolite identification								
	%TRR	**Mg/kg**	**%TRR**	**Mg/kg**	**%TRR**	**Mg/kg**	**%TRR**	**Mg/kg**
Carbofuran	0.41	0.001	-	-	-	-	-	-
3-OH-carbofuran	10	0.032	-	-	4.03[3]	0.005	11[6]	0.029
7-phenol	15	0.048	-	-	2.4[4]	0.003	-	-
3-OH-7-phenol	6.8[1]	0.021	-	-	12[5]	0.017	16[7]	0.042
3-keto-7-phenol	32[2]	0.10	-	-	-	-	-	-
Aqueous fraction from initial extraction	6.3	0.020	28	0.003	5.0	0.007	3.5	0.009
Aqueous fraction from enzymatic digestion	-	-	-	-	16	0.022	13	0.035
Aqueous fraction from mild acid hydrolysis	-	-	-	-	4.5	0.007	5.1	0.014
Aqueous fraction from strong acid hydrolysis	-	-	-	-	6.3	0.009	6.7	0.018
Polar residues	22	0.070	-	-	6.9	0.010	17	0.044
Post extraction solids	1		70		73		80	
Protease digestion					41		49	
Mild acid hydrolysis					12		12	-

Residual radioactive residues								
	1		70		20		19	

[1]including 2% conjugated, released by sulfatase treatment

[2] including 29% conjugated, released by sulfatase treatment

[3] including 2.2% conjugated, released by protease treatment

[4] conjugated, released by protease treatment

[5] including 11% conjugated, released by protease treatment

[6] including 8.2% conjugated, released by protease treatment

[7]conjugated, released by protease treatment.

Remark: There were some discrepancies between the level of total radioactive residues recovered in each matrix and the % of the TRR related to each of the metabolite.

Conclusion:
The parent compound was detected at a trace level in milk only.
3-OH-carbofuran was recovered at a level around 10 % of TRR in milk and kidney and at a level of 4% TRR in liver.
The information was given that in milk, 10 % of the TRR occurred as free 3-OH-carbofuran.
In liver, the 4 % of the total residues included 2.2% conjugated 3-OH-carbofuran, released by protease treatment.
While in kidney, 11 % of the total residues included 8.2% conjugated 3-OH-carbofuran, released by protease treatment.
There is no indication whether the aqueous fractions resulting from the mild/strong acid hydrolysis were further characterized.

Point 3(10) in the reporting tables: Supervised residue trials – Analytical methods

The following methods were reported in chapter B.5.2.1 – Carbosulfan additional report, revised April 2009.

- Determination of residues of carbosulfan and its metabolites carbofuran and 3-hydroxy carbofuran by HPLC-MS-MS in maize and sugar beet samples – Validation of the method. (Enriquez, 2006, Report BATTELLE A-17-05-13) - Trials ***FA-17-04-02/01-02, FA-17-05-02/01-02 and FA-17-06-07/01-02***

GLP:
GLP-compliance stated
Principle of the method:
Carbosulfan and Carbofuran (CS-CF) is extracted from 5 g sample with a mixture of hexane – acetone (4:1, v/v) and filtered through Celite and sodium sulphate anhydrous.

The metabolite 3-hydroxy carbofuran (3-OHCF) is extracted from the remaining filter cake by refluxing with 0.25 M hydrochloric acid. After filtration the 3-hydroxy carbofuran is cleaned-up through a C18 SPE cartridge using methanol 1% in dichloromethane.

The combined organic extract (CS-CF and 3-OHCF) is evaporated (at temperatures below 35°C and after addition of 'keeper' 1-decanol, in order to avoid losses of carbosulfan), re-constituted and kept in acetonitrile. Then the re-constituted extract is diluted with acetonitrile and water (to have the same composition of the mobile phase) and analysed by HPLC (column: Aqua C18, 50mm x 2mm ID, 5µm particles) with MS-MS detection (ESI, positive mode).

Findings:

Specificity – interferences : - Following ion transitions were monitored (MRM): m/z 381.1 → 118.1 (carbosulfan); m/z 222.1 → 123.0 (carbofuran); m/z 237.9 → 163.0 (3-OH-carbofuran);
LC-MS/MS is highly specific → no need for separate confirmatory method.
- No significant interferences (>30% of LOQ) were observed at the retention times of carbosulfan, carbofuran or 3-hydroxy carbofuran in any blank or control sample.

Linearity : The detector response for each compound was linear over the concentration range 1 ng/mL to 25 ng/mL (corresponding to a residue conc. range of 2 to 50 ppb). Correlation coefficients > 0.99.

Recovery – precision : see Table B.5.2.1-6b

Validation by an independent laboratory : First validation of method by Battelle; ILV described in study by Zietz (2008) was conducted by SGS Institut Fresenius.

Limit of quantification (LOQ) : 0.005 mg/kg (= 5 ppb) for each analyte in maize and sugar beet

Table B.5.2.1-6b: Lab validation of LC-MS/MS method for residues of Carbofuran and 3-OH Carbofuran in maize and sugar beet (Enriquez, 2006) (FMC)

Matrix	**Analyte**	**Fortification level (mg/kg commodity)**	**Recovery**			
			Number of samples	**Range (%)**	**Mean (%)**	**RSD (%)**
Maize grain	Carbosulfan	0.005 0.050	5 5	77-82 70-79	80 75	3 5
		Overall	*10*	*70-82*	*78*	*5*
	Carbofuran	0.005 0.050	5 5	87-94 96-102	89 99	3 2
		Overall	*10*	*87-102*	*94*	*6*

	3-OH carbofuran	0.005 0.050	5 5	94-107 100-104	97 101	5 2
		Overall	*10*	*94-107*	*100*	*4*
Sugar beet	Carbosulfan	0.005 0.050	5 5	67-77 82-91	73 87	5 4
		Overall	*10*	*67-91*	*80*	*10*
	Carbofuran	0.005 0.050	5 5	82-94 84-104	86 97	6 8
		Overall	*10*	*82-104*	*92*	*9*
	3-OH carbofuran	0.005 0.050	5 5	102-115 75-100	107 92	5 11
		Overall	*10*	*75-115*	*100*	*11*

Conclusion:
The analytical method is suitable for the determination of carbosulfan and its metabolites carbofuran and 3-hydroxy carbofuran in maize and sugar beet samples with a LOQ of 5 ppb for each analyte.

- In trials ***FA-17-04-07,FA-17-04-04, FA-17-06-03, FA-17-04-06***, the determination of Carbosulfan and its metabolites Carbofuran and 3-OH-carbofuran residues in sugar/fodder beet leaves and roots was achieved by HPLC /UV-PCD (post-column derivatisation using fluorescence detection) according to the reported analytical method in the residue study N°A-17-03-25.
This analytical method was based on the procedure of the method entitled "Determination of residues of Carbosulfan and its metabolites Carbofuran and 3-Hydroxy Carbofuran in leafy cabbage, cauliflower and Brussels sprouts – Validation of the method (Ginzburg, 2001a)".
This analytical method was validated for a Limit of Quantification of 0.05 mg/kg for each analyte.
This analytical method is described as follows:

- Determination of residues of Carbosulfan and its metabolites Carbofuran and 3-Hydroxy Carbofuran in leafy cabbage, cauliflower and brussels sprouts – Validation of the method (Ginzburg, 2001a)
- Carbosulfan EU dossier DAR : Additional requirements on the methods of analysis (chapter B.5) (Oz, 2004)

GLP :
GLP-compliance stated
Principle of the method :
Carbosulfan and Carbofuran are extracted twice from the sample with hexane/acetone 4+1 (v+v). After centrifugation and filtration, Carbosulfan and Carbofuran are cleaned up on an Envi Carb SPE cartridge followed by an Amino-propyl SPE cartridge.
Metabolite *3-Hydroxy Carbofuran* is extracted from the filter cake by refluxing with 0.25 M HCl. After filtration, 3-OH Carbofuran is cleaned up on a C18-SPE cartridge followed by the same Amino-propyl SPE cartridge used for Carbosulfan and Carbofuran (elution with methanol 1% in dichloromethane).
After evaporation to dryness, the combined residues are reconstituted in a mixture of acetonitrile/water 30+70 (v+v) and analyzed by HPLC (Zorbax Bonus-RP (C_{14}), 5 µm, 25 cm x 4.6 mm i.d.) with post-column derivatization (PCD) and fluorescence detection. The post-column reaction includes acidic hydrolysis of carbosulfan to carbofuran, followed by alkaline hydrolysis after which the methyl amine formed is derivatized with o-phtalaldehyde and 2-mercaptoethanol to the corresponding fluorescent substituted isoindol. Quantification by external standardization.
Findings :
Specificity – interferences : - HPLC-PCD with fluorescence detection is highly specific to N-methyl carbamates and their precursors → no need for confirmatory method
- no significant matrix interferences (control values < 30% of LOQ)
Linearity : response of fluorescence detector to resp. analytes (peak area vs. conc.) was demonstrated to be linear within a concentration range of 0.025 to 0.25 mg/L; r > 0.99
Recovery – precision : see Table B.5.2.1-9
Validation by an independent laboratory : not addressed
Limit of quantification (LOQ) : 0.05 mg/kg for each analyte

Table B.5.2.1-9 : Validation of HPLC-PCD method for residues in crops (Ginzburg, 2001a)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
leafy cabbage	Carbosulfan	0.05 0.5	5 5	75 – 107 73 – 81	97 76	13.2 4.3
	Carbofuran	0.05 0.5	5 5	97 – 104 81 – 102	100 94	2.6 9.4
	3-OH Carbofuran	0.05 0.5	5 5	79 – 103 84 – 112	89 103	11.7 11.8
cauliflower	Carbosulfan	0.05 0.5	5 7	82 – 112 76 – 96	99 89	12.3 7.4
	Carbofuran	0.05 0.5	5 7	104 – 108 62 – 94	106 82	1.9 16.1
	3-OH Carbofuran	0.05 0.5	5 7	92 – 109 77 – 91	101 87	6.4 5.5
brussels sprouts	Carbosulfan	0.05 0.5	5 5	89 – 106 62 – 74	96 70	8.4 6.7
	Carbofuran	0.05 0.5	5 5	74 – 87 92 – 107	78 99	6.6 6.5
	3-OH Carbofuran	0.05 0.5	5 5	70 – 86 95 – 106	77 102	7.8 4.1

Conclusion :
HPLC-PCD method with fluorescence detection is suitable for the determination of residues of Carbosulfan, Carbofuran and 3-Hydroxy Carbofuran in brassica crops with a LOQ for each analyte of 0.05 mg/kg.

Data gap: 3.3 in the Evaluation Tables:
"Data to address residues in rotational crops, in particular further metabolite identification in the edible parts of the rotational crops is required".

Open point: 3.5 in the Evaluation Tables:
"Experts may consider whether the approach as suggested by the applicant is justified to consider 10% TRR in rotational crops in the consumer risk assessment"

JMPR report 1997

Rotational crops:
Experimental design:
In a confined crop rotation study (Phenyl)-14C-Carbofuran was applied directly to a silt loam soil at an application rate of 3.4 kg as/ha. Wheat, soya beans and sugar beet were seeded into the treated soil 4 and 12 months after treatment and grown to maturity. Wheat forage, straw and grain, soya bean silage, stems, pods and beans and sugar beet tops and roots were assayed for the determination of the total radioactive residues.
Extraction procedure:
Each sample was extracted with Methanol/water and separated into non polar and polar fractions for further metabolites identification. Conjugated metabolites were hydrolysed with 0.25 N HCl. Metabolites were identified by TLC, by co-chromatography with reference standards.
Findings:

Crop	Sample	Total radioactive residues (mg/kg)	
		4 months	12 months
Wheat	Forage	-	1.40

	Straw	54.0	0.30
	Grain	0.60	0.04
Soya bean	**Silage**	16.0	0.50
	Stem	18.0	0.70
	Pod	5.0	0.10
	Beans	1.0	0.08
Sugar beet	**Top**	0.40	0.05
	Root	0.20	0.05

The phenolic metabolites (3-OH-7-phenol, 3-keto-7-phenol, 7-phenol) were the main degradation products recovered in the rotated crops. The carbamates (carbofuran, 3-OH-carbofuran and 3-keto-carbofuran) constituted a small proportion of the total radioactive residues (<10 % of the TRR in any crop sown at 4 and 12 months).

ANNEX B

Addendum August 2009

Carbosulfan

B.9 Ecotoxicology

B.9.2.16 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

During Peer Review the notifier provided updated PECsw and PECsed calculations which were evaluated in the section on fate and behaviour.

In the table below, the PECsw and PECsed values for FOCUS step 3 are presented for the parent compound carbosulfan and its metabolites carbofuran, dibutylamine, carbofuran-7-phenol, 3-hydroxy-carbofuran and 3-keto-carbofuran.

Table B.9.2.16-1 : Calculated PEC values for carbosulfan and its metabolites (FOCUS Step 3) in surface water, application of 750 g carbosulfan/ha in sugar beet

Scenario	Compound	Max PECsw (µg/L)	Date of max PECsw	Max PECsed (µg/kg)	Date of max PECsed
D3 ((Ditch)	Carbosulfan	0.00E+00	1-janv-92	0.00E+00	1-janv-92
D4 ((Pond)	Carbosulfan	0.00E+00	9-déc-85	0.00E+00	31-déc-85
D4 ((Stream)	Carbosulfan	0.00E+00	9-déc-85	0.00E+00	9-déc-85
R1 ((Pond)	Carbosulfan	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	Carbosulfan	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	Carbosulfan	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	Carbofuran	1.11E-02	29-janv-93	2.00E-02	15-avr-93
D4 ((Pond)	Carbofuran	6.58E-02	30-janv-86	9.03E-02	6-mars-86
D4 ((Stream)	Carbofuran	4.62E-02	16-déc-85	4.10E-02	28-janv-86
R1 ((Pond)	Carbofuran	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	Carbofuran	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	Carbofuran	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	DBA	0.00E+00	1-janv-92	0.00E+00	1-janv-92
D4 ((Pond)	DBA	0.00E+00	9-déc-85	0.00E+00	1-janv-85
D4 ((Stream)	DBA	0.00E+00	5-déc-85	0.00E+00	9-déc-85
R1 ((Pond)	DBA	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	DBA	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	DBA	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	7-P-C	1.30E-05	29-janv-93	4.07E-04	4-avr-93
D4 ((Pond)	7-P-C	8.50E-05	31-janv-86	1.85E-03	1-mai-86
D4 ((Stream)	7-P-C	7.50E-05	1-janv-85	9.67E-04	1-févr-86
R1 ((Pond)	7-P-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	7-P-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	7-P-C	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	3-H-C	2.11E-04	29-janv-93	4.28E-04	16-avr-93
D4 ((Pond)	3-H-C	1.39E-03	30-janv-86	2.39E-03	20-mars-86
D4 ((Stream)	3-H-C	8.85E-04	17-déc-85	8.12E-04	28-janv-86
R1 ((Pond)	3-H-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	3-H-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	3-H-C	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	3-K-C	6.57E-04	30-janv-93	4.70E-03	1-mai-93
D4 ((Pond)	3-K-C	0.00E+00	3-sept-85	1.07E-06	1-mai-86
D4 ((Stream)	3-K-C	0.00E+00	6-juin-85	0.00E+00	25-avr-86
R1 ((Pond)	3-K-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84

R1 ((Stream)	3-K-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	3-K-C	0.00E+00	1-oct-80	0.00E+00	1-oct-80

Following the calculations performed above, the major-sediment metabolite carbofuran-phenol was modelled as a soil metabolite using a formation fraction of 1, which leads to almost no entry in the water system due to its high Koc and low DT50soil. In order to obtain an estimate of maximum concentrations of carbofuran-phenol in surface water and sediment, the maximum FOCUS Step 3 PECsw and PECsed for carbofuran are multiplied using a MW correction factor (164.2/221.3 = 0.74) and the maximum % occurrence of carbofuran-phenol in water-sediment (total AR of 30% following the Yeomans (1995 and 1996) study). It results in more critical values for PECsw (0.0146 µg/L carbofuran-phenol) and PECsed (0.02 µg/kg carbofuran-phenol).
For the TER calculations for carbofuran-7-phenol, these latter values were used in stead of the values presented by the notifier.

B.9.2.16.1 Risk assessment for the active substance

Table B.9.2.16.1-1 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to carbosulfan in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Lepomis macrochirus*	96 h	0.015	0.00001	1500000	100
D4	Pond				0.00001	1500000	100
D4	Stream				0.00001	1500000	100
R1	Pond				0.00001	1500000	100
R1	Stream				0.00001	1500000	100
R3	Stream				0.00001	1500000	100
D3	Ditch	*Oncorhynchus mykiss*	14 d	0.004	0.00001	400000	10
D4	Pond				0.00001	400000	10
D4	Stream				0.00001	400000	10
R1	Pond				0.00001	400000	10
R1	Stream				0.00001	400000	10
R3	Stream				0.00001	400000	10
D3	Ditch	*Daphnia magna*	48 h	0.0015	0.00001	150000	100
D4	Pond				0.00001	150000	100
D4	Stream				0.00001	150000	100
R1	Pond				0.00001	150000	100
R1	Stream				0.00001	150000	100
R3	Stream				0.00001	150000	100

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Daphnia magna* (Marshal 10G)	48 h	0.00105	0.00001	150000	100
D4	Pond				0.00001	150000	100
D4	Stream				0.00001	150000	100
R1	Pond				0.00001	150000	100
R1	Stream				0.00001	150000	100
R3	Stream				0.00001	150000	100
D3	Ditch	*Daphnia magna*	21 d	0.0032	0.00001	320000	100
D4	Pond				0.00001	320000	10
D4	Stream				0.00001	320000	10
R1	Pond				0.00001	320000	10
R1	Stream				0.00001	320000	10
R3	Stream				0.00001	320000	10

The risk of carbosulfan to aquatic organisms is acceptable for all FOCUS step 3 scenarios.

B.9.2.16.2 Risk assessment for the metabolites

Table B.9.2.16.2-1 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to carbofuran in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Lepomis macrochirus*	96 h	0.18	0.0111	16216	100
D4	Pond				0.0658	2736	100
D4	Stream				0.0462	3896	100
R1	Pond				0.00001	18000000	100
R1	Stream				0.00001	18000000	100
R3	Stream				0.00001	18000000	100
D3	Ditch	*Cyprinodon variegatus*	35 d	0.006	0.0111	541	10
D4	Pond				0.0658	91	10

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D4	Stream				0.0462	130	10
R1	Pond				0.00001	600000	10
R1	Stream				0.00001	600000	10
R3	Stream				0.00001	600000	10
D3	Ditch	*Daphnia magna*	48 h	0.00205	0.0111	185	100
D4	Pond				0.0658	**31**	100
D4	Stream				0.0462	**44**	100
R1	Pond				0.00001	205000	100
R1	Stream				0.00001	205000	100
R3	Stream				0.00001	205000	100
D3	Ditch	*Ceriodaphnia dubia*	7 d	0.00016	0.0111	14	10
D4	Pond				0.0658	**2**	10
D4	Stream				0.0462	**3**	10
R1	Pond				0.00001	16000	10
R1	Stream				0.00001	16000	10
R3	Stream				0.00001	16000	10
D3	Ditch	*Chironomus riparius*	28 d	0.004	0.0111	360	10
D4	Pond				0.0658	61	10
D4	Stream				0.0462	87	10
R1	Pond				0.00001	400000	10
R1	Stream				0.00001	400000	10
R3	Stream				0.00001	400000	10

Table B.9.2.16.2-2 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to carbofuran in sediment for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/kg)	PEC_{sed} (µg/kg)	TER	Annex VI trigger
D3	Ditch	*Chironomus riparius*	28 d	0.0022	0.0200	110	10
D4	Pond				0.0903	24	10

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/kg)	PEC_{sed} (µg/kg)	TER	Annex VI trigger
D4	Stream				0.0410	54	10
R1	Pond				0.00001	220000	10
R1	Stream				0.00001	220000	10
R3	Stream				0.00001	220000	10

The risk of the metabolite carbofuran to fish and sediment dwelling organisms is acceptable for all FOCUS step 3 scenarios. The acute and chronic risk to aquatic invertebrates is acceptable for the D3 ditch scenario and for all the run-off scenarios (R1 pond, R1 stream, R3 stream). However, for the scenarios D4 pond and D4 stream, the TER values are below the triggers.

Table B.9.2.16.2-3 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 3-keto-carbofuran in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Ceriodaphnia dubia*	48 h	0.049	0.000657	74581	100
D4	Pond				0.00001	4900000	100
D4	Stream				0.00001	4900000	100
R1	Pond				0.00001	4900000	100
R1	Stream				0.00001	4900000	100
R3	Stream				0.00001	4900000	100

The risk of the metabolite 3-keto-carbofuran to aquatic invertebrates is acceptable for all FOCUS step 3 scenarios.

Table B.9.2.16.2-4 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 3-hydroxy-carbofuran in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Ceriodaphnia dubia*	48 h	0.023	0.000211	232227	100
D4	Pond				0.00139	35252	100
D4	Stream				0.000885	55367	100
R1	Pond				0.00001	4900000	100
R1	Stream				0.00001	4900000	100
R3	Stream				0.00001	4900000	100

The risk of the metabolite 3-hydroxy-carbofuran to aquatic invertebrates is acceptable for all FOCUS step 3 scenarios.

Table B.9.2.16.2-5 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 7-phenol in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Test substance	Organism	Toxicity end point (mg/L)	Time scale	PEC_{sw} (µg/L)	PEC_{twa} (µg/L)	TER	Annex VI Trigger

Test substance	Organism	Toxicity end point (mg/L)	Time scale	PEC_{sw} (µg/L)	PEC_{twa} (µg/L)	TER	Annex VI Trigger
7-phenol	*Oncorhynchus mykiss*	32.3	96 h	0.0146	-	2212329	100
7-phenol	*Daphnia magna*	25	48 h	0.0146	-	1712329	100
7-phenol	*Pseudokirchneriella subcapitata*	47	72 h	0.0146	-	3219178	10
7-phenol	*Chironomus riparius*	0.004	25 d	0.0146	-	684932	10

Table B.9.2.16.2-6 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 7-phenol in sediment for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Test substance	Organism	Toxicity end point (mg/kg)	Time scale	PEC_{sed} (µg/kg)	PEC_{twa} (µg/kg)	TER	Annex VI Trigger
7-phenol	*Chironomus riparius*	1.36	25 d	0.02	-	68000	10

As mentioned before, the TER calculations are based on the more critical values for PECsw (0.0146 µg/L 7-carbofuran-phenol) and PECsed (0.02 µg/kg 7-carbofuran-phenol) calculated by RMS.
The risk of the metabolite 7-carbofuran-phenol to aquatic invertebrates is acceptable for all FOCUS step 3 scenarios.

The risk of the metabolite dibutylamine is acceptable based on FOCUS step 1 for fish and algae and on FOCUS step 2 for aquatic invertebrates (see DAR). Therefore, no further TER calculations based on FOCUS step 3 are necessary.

Open point 5.10 in the evaluation table:

From the mesocosm study with Marshal 25CS (Foekema E.M. *et al*., 2002) a NOAEC of 0.4 µg carbosulfan/L was derived. With an assessment factor of 4, this leads to an EAC = 0.1 µg carbosulfan/L.
Based on the laboratory toxicity endpoints for *Daphnia magna* (EC_{50} = 0.00205 mg carbofuran/L) and *Ceriodaphnia dubia* (NOEC = 0.00016 mg carbofuran/L) and the FOCUS step 3 PECsw values (750 g a.s./ha), TER values did not pass the trigger for 2 out of 6 scenarios, namely D4 pond and D4 stream (see above).

The EAC of 0.1 µg carbosulfan/L is equivalent with 0.058 µg carbofuran/L, based on molecular weight ratio carbofuran/carbosulfan of 221.3/380.5. With this EAC = 0.058 µg carbofuran/L, the scenario D4 stream (0.0462 µg carbofuran/L) would show acceptable risk, whereas scenario D4 pond (0.0658 µg carbofuran/L) is little above the EAC.

In conclusion, the endpoint of the mesocosm study could be applied to refine the risk assessment for aquatic invertebrates. At the application rate of 750 g a.s./ha, 5 out of 6 scenarios show acceptable risk (only scenario D4 pond does not pass the trigger of 4).

B.9.6.5 Field tests – residue content of earthworms (Annex IIIA 10.6.1.3)

Comparison of Two Methods for Assessing the Effects of Carbofuran on Soil Animal Decomposers in Cornfields. (Broadbent A.B., Tomlin, A.D., 1982).

Abstract :
The effects of spring treatments of an insecticide, carbofuran, on the soil animal decomposer community of an Ontario cornfield were assessed by measuring fluctuations in soil animal populations and by measuring changes in rates of leaf litter decomposition from bags of different mesh sizes. For several weeks after treatment, reductions in soil microfauna and reductions in the rate of corn leaf decomposition could be observed, but by autumn, the total number of soil micro-arthropods and litter decomposition rates were similar to those in untreated control plots. Earthworm populations seemed unaffected by in-row treatments of carbofuran, but broadcast treatments reduced worm populations significantly.

Guidelines :
Not applicable.
GLP:
No
Materials and Methods :
Test substance : carbofuran
Test species : soil fauna, earthworms
Test design :
The experimental site was a plot (120 m x 40 m) in a 12-ha field of continuous corn under regular tillage and management programs in Ontario. The soil was classified as a Burford loam, pH 6.9 with an organic matter content of 4.3 %.
On 10 May 1977, granular carbofuran was applied in-furrow (1.5 kg a.s./ha, in a 10-cm wide band along the corn rows). An untreated control plot of similar dimensions (60 m long, 10 rows wide, rows on 90-cm centers) was also made. On 23 May 1978 (1.1 kg a.s./ha) and on 22 May 1979 (11.2 kg a.s./ha), granular carbofuran was applied in-furrow and as broadcast treatment. An untreated control plot was also made (similar design but only 30 m long).
For 6, 9 and 6 dates, respectively, during the summers of 1977, 1978 and 1979, 50 soil cores (2.5 cm in diameter by 15 cm deep) were taken from each plot to determine residues of carbofuran, 3-keto-carbofuran and 3-OH-carbofuran. Carbofuran was extracted with chloroform from soil and analysed by gas-liquid chromatography as described by Miles and Harris (1979).
Sampling of earthworm populations :
Earthworm populations were estimated using the formaldehyde expellent method of Raw (1959). Nine liters of a 0.55 % formaldehyde solution was applied to the soil surface within a wooden "quadrat" (1.8 by 0.2 m) placed over the seed row. In 1977, earthworm populations and biomass were estimated by using 18 quadrats from each of the treated and control plots on 28 October (24 weeks after treatment). In 1978, populations and biomass were estimated from 12 quadrats from each of the control, row-treated and broadcast-treated plots, and from the untreated zone between rows in the row-treated plot on 3 May (3 weeks before treatment), 23 June (4 weeks after treatment), and 25 October (22 weeks after treatment).
Findings :
Residue determination :
Carbofuran residues disappeared rapidly from the cornfield soil in each of the 3 years of the study. 3-keto-carbofuran and 3-OH-carbofuran were not detected in excess of 0.15 and 0.02 ppm, respectively. The slower rate of carbofuran disappearance in 1978 varied with lower precipitation and soil moisture levels during the 10 weeks after treatment. However, by 10 and 8 weeks, respectively, after treatment in 1978 and 1979, soil residue levels in the row treatments had dropped to levels near the corresponding broadcast treatments.
Populations of soil fauna :
In 1977, 24 weeks after treatment there were 44.2 earthworms per m^2 in row-treated plots and 35.0 earthworms per m^2 in the control plots; at least 90 % of the worms were *Aporrectodea tuberculata* (Eisen). There was no significant difference in mean biomass of earthworms between carbofuran-treated (13.6 g/m^2) and control plots (12.1 g/m^2).

Samples taken in 1978, 3 weeks before treatment and 4 weeks after treatment, contained only three earthworms from 12 quadrats and seven from 18 quadrats, respectively. The small number of earthworms extracted was due to unusually dry soil conditions. In 1978, 22 weeks after treatment, there was no significant difference in numbers of biomass of earthworms among "between row-treated" (50.8/m², 9.0 g/m²), row-treated (44.7/m², 9.0 g/m²), and control plots (50.3/m², 9.8 g/m²); significantly fewer earthworms were found in the broadcast treatment (28.9/m²), but the earthworm biomass, although smaller in this plot (7.2 g/m²), was not significantly different from the other plots. Broadcast treatments of carbofuran are more harmful to earthworms than row treatments, possibly because there is no untreated refugial soil to which earthworms can escape after a broadcast treatment.
Carbofuran treatment at 11.2 kg a.s./ha caused swellings and abnormal pigmentation of the earthworms similar to that described by Stenersen *et al*. (1973), and 2 weeks after treatment, dead earthworms exhibiting these symptoms were observed on the soil surface.

Conclusion of the RMS :
The RMS has reservations towards this study due to several shortcomings.
Very low numbers of earthworms were collected in 1978, 3 weeks before and 4 weeks after treatment due to unusual dry soil conditions. However, the sampling at 4 weeks after treatment is important to assess the effects of carbofuran on earthworm populations. The sampling at 22 weeks after treatment demonstrated that significantly fewer earthworms were found in the broadcast treatment compared to the control plots, row-treated and "between row-treated" plots. However, carbofuran residues disappeared rapidly from the cornfield, in 1978, 10 weeks after treatment, soil residue levels in the row treatments had dropped to levels near the corresponding broadcast treatments. From the graph of 1978, the soil residue levels at 22 weeks after treatment had fallen to 0 mg/kg. Therefore, the sampling at 22 weeks is not appropriate to estimate effects of carbofuran nor to compare the in-furrow treatment with the broadcast treatment.

During the Peer Review of Carbosulfan the notifier submitted following statement in August 2009.

Updated and Comparative Risk Assessment of the carbosulfan use on sugar beet at 100 g ai/ha versus 750 g ai/ha. (2009).

Introduction :
FMC re-applied for Annex I inclusion of the active ingredient carbosulfan under the rules laid down in Regulation 33/2008/EC – Chapter 3 (accelerated procedure). Article 15(1b) of this Regulation states that:

> "*The supported uses are the same as those that were the subject of the non-inclusion Decision. They may only be changed insofar as this is necessary, in the light of the reasons which gave rise to the non-inclusion Decision, to permit inclusion of that substance in Annex I to Directive 91/414/EEC*".

Whilst we still support the use of carbosulfan on sugar beet at 750 g ai/ha[1] – and welcome the efforts to evaluate the risk assessment at this dose rate - we also appreciate that interpretation of endpoints and acceptability of refinement route may differ from the notifier to the evaluator's view. Therefore, we introduced additional risk assessments at lower dose rates, in particular at 100 g ai/ha, in order to increase the chances to identify a safe use scenario.

The RA conducted by the RMS shows that while the risk to granular intake at 750 g ai/ha is acceptable according to the EPPO scheme; the risk to secondary poisoning via ingestion of treated seedlings, earthworms and/or arthropods needs further refinement. This suggests that a lower application rate should be considered for the risk assessments, as wisely foreseen by Article 15b of the Regulation.

Should the EC decide that registration of carbosulfan is possible only with limitation on its maximum applied dose rate, this issue would be dealt by FMC at national level. Indeed, we are confident that certain technologies are efficient at dose rate equal or lower to 100 g carbosulfan/ha.

The aim of the present document is to compare the critical outcome of the risk assessment for application of 100 g carbosulfan/ha versus 750 g carbosulfan/ha. It will shortly investigate Operator exposure, consumer exposure, PEC calculation, Risk to non-target organisms and in particular birds and mammals.

Risk to birds and mammals :

Granule intake
The RA conducted by the RMS shows that the risk to granular intake at 750 g ai/ha is acceptable according to the EPPO sub scheme. A similar conclusion can be drawn from the Probabilistic Risk assessment submitted by FMC (Bastiansen F. and Wang M., 2008). Reducing the dose rate to 100 g ai/ha can only further increase the confidence that the risk to birds and mammals via granule intake is acceptable.

Secondary poisoning risk assessment for birds and mammals

Residue of carbofuran in seedlings
Several residue trials and metabolism studies are evaluated in the carbosulfan and carbofuran DAR in order to investigate the residue in seedling. The most valuable information in order to assess the carbosulfan residue in seedlings comes from the residue trials performed by Zietz (2008):

[1] At planting – in furrow – granule buried at 7 cm from the surface.

Residues of carbosulfan, carbofuran and 3-OH-carbofuran in beet seedlings

Trial No.	Specimen material	Appl. Rate kg/ha	Timing (BBCH)	DALA	Carbosulfan (mg/kg)	Carbofuran (mg/kg)	3-OH-carbofuran (mg/kg)
07-UK-042	Sugar beet seedling	control	12	33	< 0.005 nd	< 0.005 nd	< 0.005 nd
07-UK-042		7.5	12	33	**< 0.005**	**0.30**	**0.92**
07-UK-042		1.0	12	33	< 0.005	0.026	0.087
07-UK-043	Sugar beet seedling	control	12	32	< 0.005 nd	< 0.005 nd	< 0.005 nd
07-UK-043		7.5	12	32	**< 0.005**	**0.16**	**0.58**
07-UK-043		1.0	12	32	< 0.005	0.014	0.064
07-NF-044	Sugar beet seedling	control	12	28	< 0.005 nd	< 0.005 nd	< 0.005 nd
07-NF-044		7.5	12	28	**< 0.005**	**< 0.005**	**0.016**
07-NF-044		1.0	12	28	< 0.005 nd	< 0.005	0.008
07-NF-045	Sugar beet seedling	control	12	28	< 0.005 nd	< 0.005 nd	< 0.005 nd
07-NF-045		7.5	12	28	**< 0.005**	**< 0.005**	**0.023**
07-NF-045		1.0	12	28	< 0.005	< 0.005	0.006

DALA = days after last treatment

< 0.005 nd = no peak was detected

On the basis of these trials, the following residue should be considered for the risk assessment:

Seedling residue values to be used for the birds and mammals RA

Risk assessment	750 g carbosulfan/ha		100 g carbosulfan / ha	
	Maximum concentrations	Mean of the maximum concentrations	Maximum concentrations	Mean of the maximum concentrations
	acute	short-term and long-term	acute	short-term and long-term
carbosulfan	< 0.005	< 0.005	< 0.005	< 0.005
carbofuran (sum of carbofuran + 3-OH-carbofuran)	1.22	0.50	0.113	0.051

It should be noted that these experimental results confirm that the RUD rule applies to the carbamate soil application. Indeed, the residue results are linear with the applied dose rate.

Residue of carbofuran in earthworms and insects

Residue in earthworms and beetles should only consider carbosulfan and carbofuran. Indeed, 3-OH-carbofuran is a minor metabolite in soil (<5%: see B.8.1.1.1 of original DAR) and will therefore not contaminate insect and soil dwelling arthropods in any significant concentrations. This is confirmed in the DAR of benfuracarb were the notifier Otsuka analysed both carbofuran and 3-OH-carbofuran in earthworm. These data confirm the modest contribution of 3-OH-carbofuran to the carbofuran residue.

Proposed Residue values are derived by normalizing measured residue obtained from Brown *et al*. (2007) at an application rate of 750 g as/ha. The RUD rules apply well to these values since residue in earthworms and arthropods is driven by the PEC soil, which is a linear function of the applied dose rate. As a worst case assumption, the residue of carbosulfan and carbofuran can be summed and compared to the carbofuran endpoints for TER calculation.

Earthworms and arthropods residue values to be used for the birds and mammals RA at 750 g ai/ha

		Maximum concentrations	Mean of the maximum concentrations	Time weighted average mean concentrations on the interval 1-9 days
Risk assessment at 750 g carbosulfan/ha		acute	short-term	long-term
Beetles	carbosulfan + carbofuran	1.60	1.22	0.51
Earthworms	carbosulfan + carbofuran	0.29	0.23	0.10

Earthworms and arthropods residue values to be used for the birds and mammals RA at 100 g ai/ha

		Maximum concentrations	Mean of the maximum concentrations	Time weighted average mean concentrations on the interval 1-9 days
Risk assessment at 100 g carbosulfan/ha		acute	short-term	long-term
Beetles	carbosulfan + carbofuran	0.21	0.16	0.07
Earthworms	carbosulfan + carbofuran	0.04	0.03	0.01

Birds and mammals TER summaries

The TER values calculated by the RMS took into account the above residue in seedling, earthworms and beetle at 750 g ai/ha. The TER values obtained by RMS can be summarized as follows:

Birds and mammals Risk assessment at 750 g ai/ha – summary table

Species	TERacute	TERst	TERlt
Woodpigeon	2.83	15.56	6.22
Yellow wagtail	0.73	2.15	2.05
Blackbird	2.24	6.36	5.85
Skylark	0.85	3.62	2.01
Hare	41	nd	13

Taking into account the same refinements and parameters used by RMS for calculating the TERs at 750 g ai/ha, then the risk assessment at 100 g ai/ha – on the basis of the corresponding residue values - will lead to the following TERs:

Birds and mammals Risk assessment at 100 g ai/ha – summary table

Species	TERacute	TERst	TERlt
Woodpigeon	30.6	152.5	61.0
Yellow wagtail	5.6	16.4	14.9
Blackbird	16.24	48.76	58.5
Skylark	8.0	31.1	18.0
Hare	440.2	nd	139.2

These TER values show that reducing the dose rate to 100 g ai/ha allows finding an acceptable risk to mammals and significantly improves the risk assessment to birds. TERacute values for yellow wagtail and skylark are still respectively 5.6 and 8.0, but they could be further refined if taking into account:

- The use of a PT value on top of a PD value;
- Consideration of the reversibility of the AChE inhibition and rapid metabolisation/excretion of carbofuran;
- Or any other refinement step the evaluator judges appropriate in order to reflect the risk to birds and mammals in a more realistic manner.

It is also interesting to note that the RA at 100 g ai/ha still present :

- TERlt for birds above 10, if taking into account the experts opinion that safety factor around the birds LC10 should be raised to 10 (instead of 5);
- TERa of 62 and TER lt of 19.6 for hares when considering a long-term NOEL of 0.1 mg/kg bw/day (instead of 0.71).

Conclusion of the RMS :
The RMS maintains its position that the proposal of the notifier for an additional risk assessment at a reduced granular dose rate of 100 g a.s./ha, corresponding to the doses used for seed treatment, is not acceptable. It is indeed very questionable whether such use can be considered as a representative use :

- It is not representative for the use of a granular formulation as the dosage of 100 g carbosulfan/ha is much lower than the authorized dosages. The GAPs for granule formulations that were authorized in 2002 in EU MS consisted in applications at sowing or transplant time, with incorporation in the furrow at maximum rate of 750-1200 g a.s./ha. (Broadcast applications were performed at even higher dosages).
- It is not representative for the use of a seed treatment formulation at similar rates of 100 g carbosulfan/ha because the exposure routes and risk assessments are not equivalent; for example, it is obvious that the exposures of the consumer, of the operator, of the birds and mammals will be significantly different if we compare a granular application to a seed treatment.
- The resubmitted dossier does not contain sufficient trials performed at 100 g carbosulfan/ha in order to determine the residue level in bird and mammal feed items (sugar beet seedlings, earthworms, arthropods).

The RMS concluded on the **granule** intake by birds, in addition to the notifiers statement :
The risk for birds from ingestion of Marshal 10G granules is acceptable for the intended use (750 g a.s./ha) based on the EPPO risk assessment scheme. However, RMS did not accept the probabilistic risk assessment presented by the notifier due to several shortcomings.

The RMS cannot agree with the notifiers statement that residues in earthworms and beetles should only consider carbofuran (3-OH-carbofuran residues are negligible) based on the benfuracarb dossier. If data from benfuracarb dossier are used, this should be accompanied by a letter of access.

The RMS disagrees with the statement of the notifier that the **residue values** at 100 g a.s./ha show a linear response compared to 750 g a.s./ha.
The residue values for sugar beet seedlings were derived from 4 residue trials conducted at both 750 and 100 g a.s./ha. However, too much uncertainty remains on the residue values. The trials were conducted under protected conditions and residues were measured at only one time point (at stage BBCH 12).
The residue values for earthworms and beetles were derived from 2 adjacent field trials conducted only a 750 g a.s./ha. Also uncertainty remains on these residue values since the contribution of the metabolite 3-OH-carbofuran was not measured.

RMS also raises questions about the **reduced dose rate** :

- If carbosulfan is applied at 100 g a.s./ha, the product will be applied in the plant hole, closer to the plant, to be as effective as the higher dose rate. No extrapolation of the residues is possible from a residue trial conducted at 750 g a.s./ha in the furrow. Only a residue trial conducted at 100 g a.s./ha in the plant hole will give the residue level in the field situation.
- If 100 g a.s./ha is efficient, why is the use at 750 g a.s./ha supported?

The RMS concluded that the risk of carbosulfan to birds and mammals consuming **sugar beet seedlings, earthworms and arthropods** is not acceptable for the intended use based on insufficient information on the actual residue level in feed items. In order to refine the risk to birds and mammals, more information is needed on the actual residue levels in feed items (sugar beet seedlings, earthworms, arthropods). The information should allow to perform statistical evaluations (enough residue trials, N and S European conditions, sampling over time, enough sampling material, ….). Also, the residue trials should be relevant for the intended use (crop, application rate, granular or seed treatment use).

Risk to aquatic organisms :
Risk assessment for aquatic organisms was re-calculated using the PEC surface water recently re-calculated and the ecotoxicological endpoints agreed in the DAR. TERs are provided in the tables in the document of the notifier (for application rate of 750 g carbosulfan/ha and 100 g carbosulfan/ha). Failing scenarios are highlighted in bold.
TERs of carbosulfan are not presented since no contamination occurs in surface water or in sediment in any of the FOCUS step 3 scenarios.
TERs of DBA and 7-phenol- carbofuran are not presented since these metabolites pass already at FOCUS step 2 and their PECsw and PECsed are minimum.

Conclusion of the notifier :
Whilst all run-off scenarios show acceptable risk to the aquatic organisms for applications at 750 or 100 g carbosulfan/ha, the low dose rate application at 100 g carbosulfan/ha also presents acceptable risk to *Dapnia magna* and *Cerodapnia dubia* in the drainage scenarios.

Calculations of the RMS :
The risk assessment for the metabolite carbofuran and aquatic invertebrates at lowered dose rate are presented below. RMS agrees with the PECsw calculations at 100 g a.s./ha for carbofuran presented by the notifier.

Table B.9.2.16.2-1bis : Toxicity Exposure Ratio's (TERs) for aquatic organisms exposed to carbofuran in surface water for the intended use in sugar beet (1 x 100 g a.s./ha) based on FOCUS Step 3 calculations

Scenario	**Water body type**	**Test organism**	**Time scale**	**Toxicity end point (mg/L)**	**PECsw (µg/L)**	**TER**	**Annex VI trigger**
D3	Ditch	*Daphnia magna*	48 h	0.00205	0.00118	1737	100
D4	Pond				0.00798	257	100
D4	Stream				0.00577	355	100
R1	Pond				0.00001	205000	100
R1	Stream				0.00001	205000	100
R3	Stream				0.00001	205000	100
D3	Ditch	*Ceriodaphnia dubia*	7 d	0.00016	0.00118	136	10
D4	Pond				0.00798	20	10
D4	Stream				0.00577	28	10
R1	Pond				0.00001	16000	10
R1	Stream				0.00001	16000	10
R3	Stream				0.00001	16000	10

Conclusion of the RMS :
The calculations of the notifier and RMS for the metabolite carbofuran and aquatic invertebrates give the same TER values. The scenarios that did not pass the trigger at the higher application rate of 750 g a.s./ha (D4 pond and D4 stream) for *Daphnia magna* (acute) and *Ceriodaphnia dubia* (chronic), do pass the trigger at the lower application rate of 100 g a.s./ha.

Risk to earthworms, bees, non-target arthropods, soil macro-organisms, soil micro-organisms and non-target plants :

The use of 750 g carbosulfan/ha to these organisms is acceptable. Reducing the dose rate can only offer more level of confidence.

Conclusion of the RMS :
RMS agrees with the notifier.

ANNEX B

Addendum July 2009-Before TC
(Updated version-September 2009)

Carbosulfan

B.7 Residue data

Point 3(4) in the reporting tables: Vol. B.7.3.2 Residue definition in animal products

-Identification of ^{14}C-Residues in Tissues and Eggs from Poultry Administered (Ring-^{14}C) Carbosulfan (Markle J.C.; 1982a)
-Identification of ^{14}C-Residues in Tissues and Eggs from Poultry Administered (Dibutylamino-^{14}C) Carbosulfan (Markle J.C.; 1982b)

Table B.7.2.1-2: Material balance and metabolites distribution of the residues of carbosulfan in thigh muscle and liver of the laying hens after oral administration of (Phenyl ring -UL-^{14}C)-Carbosulfan and (Dibutylamino-^{14}C)-Carbosulfan (Nominal feeding level: 5.0 mg/kg feed) – (Residues expressed in percent of the total radioactive residues and in mg ^{14}C carbosulfan equiv./kg)

Labelling forms	**(Phenyl ring -UL-^{14}C) -Carbosulfan**		**(Dibutylamino-^{14}C)-Carbosulfan**						
Tissues	**Thigh muscle (0-day)***	**Liver (0-day)***	**Thigh muscle (0-day)***	**Liver (0-day)***	**Fat (0-day)***	**Egg white (0-day)***	**Egg yolk (0-day)***	**Egg white (9-12-day)***	**Egg yolk (9-12 -day)***
Total radioactive residues	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**	**% TRR (mg/kg)**
	100 (0.115)	100 (0.282)	100 (0.162)	100 (1.352)	100 (0.304)	100 (0.0793)	100 (1.513)	100 (0.088)	100 (1.542)
Extractability of radioactive residues									
Organosoluble partitioned phase	70.1 (0.08)	37.9 (0.106)	59.1 (0.095)	62.5 (0.845)	89.8 (0.272)			13.7 (0.012)	90.1 (1.389)
Water soluble partitioned phase	14.6 (0.016)	44.9 (0.126)	15.5 (0.025)	23.4 (0.316)	1.2 (0.0036)			6.50 (0.005)	1.9 (0.029)
Elucidation of radioactive residues									
Carbosulfan	Nd	nd			0.2 (<0.002)				
Carbofuran	1.1 (<0.002)	nd							
Dibutylamine			22.5 (0.036)	36.9 (0.498)	3.1 (0.009)			3.8 (0.0033)	4.3 (0.066)
3-hydroxy-carbofuran	36.9 (0.042)	1.1 (0.003)							
3-hydroxy-N-hydroxymethyl carbofuran	9.3 (0.01)	2.1 (0.005)							
3-keto-carbofuran	1.7 (<0.002)	4.2 (0.011)							
7-phenol	1.2 (<0.002)	3.3 (0.009)							
3-keto-7-phenol	7.9 (0.908)	2.5 (0.007)							
3-hydroxy-7-phenol	7.1 (0.816)	16.0 (0.045)							
Unidentified metabolites	13.0 (0.014)	15.7 (0.044)	36.6 (0.059)	25.6 (0.346)	86.7 (0.263)			9.9 (0.008)	85.8[(2)] (1.323)

Labelling forms	(Phenyl ring -UL-^{14}C) -Carbosulfan		(Dibutylamino-^{14}C)-Carbosulfan						
Tissues	Thigh muscle (0-day)*	Liver (0-day)*	Thigh muscle (0-day)*	Liver (0-day)*	Fat (0-day)*	Egg white (0-day)*	Egg yolk (0-day)*	Egg white (9-12-day)*	Egg yolk (9-12 - day)*
Total radioactive residues	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)	% TRR (mg/kg)
Total identified metabolites	64.1 (0.073)	29.2 (0.082)	22.5 (0.036)	36.9 (0.498)	3.3 (0.011)			3.8 (0.0033)	4.3 (0.066)
Residual radioactive residues (RRR)									
	1.6 (<0.002)	18.5 (0.052)	23.4[(1)] (0.037)	10.9[(1)] (0.147)	-			66.0[(1)] (0.058)	5.6 (0.086)
Recovery : partitioned phases + RRR									
	86.3 (0.099)	101.3 (0.285)	98.0 (0.157)	96.8 (1.308)	91.0 (0.275)			86.2 (0.075)	97.6 (1.504)

Nd : not radiodetected
*: 0-day withdrawal or within 6 hours after the last dosing.
(1): Post extraction solids submitted to acid hydrolysis.
(2) : Saponified in alcoholic KOH and analysed for fatty acids.
na : not analysed.

Table B.7.2.1-2': Hydrolysis step (0.25N HCl) of the different fractions of liver and thigh muscle within 6 hours after the last dosing

	(Phenyl ring -UL-^{14}C) -Carbosulfan					
Fractions	**Thigh muscle, 0-day Polar aqueous fraction**		**Liver, 0-day Post extraction solids fraction**		**Liver, 0-day Polar aqueous fraction**	
	% TRR	**Mg/kg**	**% TRR**	**Mg/kg**	**% TRR**	**Mg/kg**
Initial	14.6	0.016	18.6	0.052	44.9	0.126
Polar (aqueous soluble phase)	6.1	0.00097	4.3	0.0022	21.5	0.027
Bound solids	----	----	1.7	0.00088	----	-----
Non polar (organosoluble phase)	7.1	0.00113	5.0	0.0026	11.0	0.0138
3-keto carbofuran	ND	<0.002	ND	<0.002	2.1	0.0026
3-keto-7-phenol	1.5	<0.002	ND	<0.002	ND	<0.002
7-phenol	1.2	<0.002	ND	<0.002	0.9	<0.002
Unknowns	4.4	0.0007	5.0	0.0026	4.5	0.0056

The post extraction solids from the liver (18.6 % of TRR) and the polar aqueous fraction from the liver (44.9 % of TRR) and thigh muscle (14.6 % of TRR) were hydrolysed with 0.25 N HCl to check for additional release of conjugated metabolites. Hydrolysis of the aqueous phases from the 0-day thigh muscle resulted in the release of an additional 7.1 % of TRR in the organo soluble phase. Of this, low levels of 3-keto-7-phenol (1.5% of TRR) and 7-phenol (1.2 % of TRR) were recovered.

Hydrolysis of the liver post extraction solids resulted in an additional release of 9.3 % of TRR. Of this, 4.3 % of TRR remained in the acidic aqueous fraction and 5 % of TRR was organo soluble. None of the carbosulfan metabolite was detected.

Hydrolysis of the polar aqueous phase from the 0-day liver (44.9% of TRR) resulted in an additional release of 11 % of TRR into the organo soluble fraction. Low levels of 3-keto carbofuran (2.1% of TRR) and 7-phenol (0.9 % of TRR) were identified.

Open point 3.1 in the Evaluation tables: *"RMS to check the raw data in the goat metabolism study in terms of the respective ratio between free and conjugated carbofuran and 3-OH-carbofuran".*

-Nature of the Residue in Livestock: Metabolism of Carbosulfan in Lactating Goats (Curry S.J., Weintraub R.A.; 1996)

See Tables B.7.2.2-3 and 4 in the revised DAR, April 2009

In the extraction procedure, the residual aqueous samples from the phenyl-label liver and kidney were subjected to acid hydrolysis to release additional conjugated radioactive residues.
Enzymatic digestion and acid hydrolysis of the post extraction solids of liver, kidney and lumbar muscle was applied in order to hydrolyse the peptide linkages and to release additional radioactivity from the bound residues, respectively.

- *Phenyl label treated milk*:
Almost all the radioactive residues were extractable with acetonitrile (98.6 % TRR). Following partitioning against hexane, enzymatic digestion and solid phase extraction of the hydrolysate, only 1% TRR partitioned into hexane while hydrolysis of glucuronide and sulfate conjugates released most of the radioactivity in the methanol eluate (97.5 % TRR as conjugate metabolites). Metabolites identification occurred on the eluate fraction only.
3-OH-carbofuran was recovered at a level of 34.2 % of TRR
No Carbosulfan and Carbofuran were recovered.

-*Phenyl label treated liver*:
37.5 % of the TRR was extractable with methanol followed by partitioning against hexane and methylene chloride to provide an organo soluble phase (12.2 % of TRR) and an aqueous soluble phase (25.1 % of TRR) further submitted to enzymatic digestion and solid phase extraction chromatography.
The non-extractable residues fraction (62.7 % of TRR) was subjected to enzyme digestion to provide an aqueous phase and the residual radioactive residues. The aqueous phase was further partitioned against methylene chloride and acid hydrolysis to further release an aqueous soluble fraction (22.6 % of TRR) and several organosoluble fractions accounting for 27.4 % of the TRR.
Metabolites identification was performed on the organosoluble fractions only.
3-OH-carbofuran occurred in this fraction at a level of 9.5 % of TRR along with other phenolic and carbamate metabolites (see table B.7.2.2-4 in the revised DAR).
Carbosulfan and Carbofuran were detected at a trace level (0.1-0.2 % TRR).

The radioactivity in the non organo soluble phase was characterized as polar metabolites (10.4 % TRR), protein-associated metabolites (22.6 % TRR) and fat-associated metabolites (0.2 % TRR) without any further investigation.

-*Phenyl label treated kidney*:

61.8 % of TRR were extractable with methanol followed by successive methylene chloride (MEC) partitioning, enzymatic digestion and HCl hydrolysis on the aqueous soluble fractions.
The aqueous soluble and MEC organosoluble fractions amounted 44 % of TRR and 17.9 % of TRR, respectively.
The non-extractable residues fraction (38.2 % TRR) was submitted to enzymatic digestion followed by MEC partitioning to provide an aqueous soluble phase (19.1 % TRR) further submitted to several acid hydrolysis steps giving the successive correspondent organosoluble fractions (18.8 % TRR).
3-OH-carbofuran was recovered in the organo soluble phase at a rate of 21.5 % TRR
Carbosulfan and Carbofuran were detected at a trace level (0.1-0.8 % TRR).
The radioactivity in the non organo soluble phase was characterized as polar metabolites (18.4 % TRR) and protein-associated metabolites (2.5 % TRR) without any further investigation.

JMPR 1997 report:

Carbofuran livestock metabolism:

Metabolism of Carbofuran in laying hens (Hoffman and Robinson, 1994b):

Test substance: Carbofuran was uniformly labelled in the phenyl ring.
Experimental design:
15 laying hens (3 groups of 5 hens-bw: 1.34-1.68 kg) each received a capsule containing 3 mg of the test substance for 7 consecutive days (corresponding to 1.98 mg/kg bw and equivalent to 25 mg/kg in feed). Eggs were collected each day, separated into white and yolk and pooled by group. Excreta were collected daily. Within 22 hours after the final dose, the hens were killed and samples of breast, thigh muscle, fat with skin, liver and kidney were collected from each hen.
The tissues samples were extracted sequentially with acetonitrile and methanol/water. Egg white was extracted with acetonitrile and egg yolk with a mixture of acetonitrile/hexane.
The released radioactivity by solvent extraction, enzymatic digestion and acid/base hydrolysis was carried out using normal phase TLC and reverse-phase HPLC.
The post-extraction solids from liver and kidney were treated sequentially by protease digestion, acid and base hydrolysis.

Total radioactive residues as cumulative percentage of administered dose and as carbofuran equivalents

Sample	Day	% of applied dose	Total ^{14}C Carbofuran (mg/kg)
Excreta	**1**	70.6	
	3	75.2	
	7	82.8	

Egg white	**1**	0.18	0.032
	3	0.21	0.069
	7	0.27	0.059
Egg yolk	**1**	0.07	0.027
	3	0.09	0.078
	7	0.21	0.141
Liver	**7**	0.11	0.137
Kidney	**7**	0.01	0.034
Breast muscle	**7**	0.02	<0.01
Thigh muscle	**7**	<0.01	<0.01
Skin and fat	**7**	<0.01	<0.01
Total recovery		*83.4*	

Characterization and identification of the total radio labelled residue from the administration of ^{14}C-carbofuran to laying hens.

	Liver		**Kidney**		**Egg white**		**Egg yolk**	
TRR (mg/kg)	0.137		0.034		0.069		0.141	
Extracted phase (%TRR)	16		41		91		91	
Metabolite identification								
	%TRR	**Mg/kg**	**%TRR**	**Mg/kg**	**%TRR**	**Mg/kg**	**%TRR**	**Mg/kg**
3-OH-carbofuran	-	-	-	-	-	-	12	0.019
7-phenol	5.7[1]	0.008	4.9[1]	0.001	-	-	16	0.026
3-OH-7-phenol	-	-	-	-	-	-	39	0.062
3-keto-7-phenol	-	-	-	-	-	-	8.5	0.014
Phenolic conjugates	-	-	-	-	90	0.060	-	-
Enzyme digestion aqueous fraction	7.3	0.010	4.6	0.002	-	-	4.6	0.007
Acid hydrolysis aqueous fraction - Mild	3.1	0.004	5.8	0.002	-	-	-	-
Acid hydrolysis aqueous fraction – Strong	12	0.016	8.2	0.003	-	-	-	-
Mild base hydrolysis aqueous fraction	4.0	0.005	3.6	0.001	-	-	-	-
Polar residues from initial extractions	12	0.016	8.2	0.003	-	-	-	-
Post extraction solids	84		59		9		9	
Protease digestion	25		19		-	-	-	-
Acid/base hydrolysis	48		28		-	-	-	-
Residual radioactive residues								
	11		12		9		9	

[1]: Conjugated, released by enzyme treatment.
Remark: There were some discrepancies between the level of total radioactive residues recovered in each matrix and the % of the TRR related to each of the metabolite.

Conclusion:
Carbofuran was not detected in any of the poultry matrix which is consistent with the results observed in the poultry metabolism study reported in the revised DAR, April 2009.
3-OH-carbofuran was detected only in egg yolk while in the revised DAR, it was recovered also in muscle and liver.
No information on the ratio 3-OH-carbofuran free and this metabolite under its conjugated form could be derived based on the data here above.

Metabolism of Carbofuran in lactating goats (Hoffman and Robinson, 1994a):

Test substance: Carbofuran was uniformly labelled in the phenyl ring.
Experimental design:
The test substance was administered orally to lactating goats for 7 consecutive days.
The dose was equivalent to 25 mg/kg carbofuran in the feed. Urine, feces and milk were collected twice daily. The goats were slaughtered within 24 hours of the final dose and samples of muscle (leg and loin), liver, kidney, omental fat and blood were taken.
Milk was extracted with acetone. Muscle, liver and kidney were sequentially extracted with chloroform and methanol/water.
The post extraction solids of liver and kidney were sequentially treated with protease, mild acid extraction and strong acid hydrolysis.
The released radioactive residues were identified by normal phase TLC and reverse-phase HPLC.

Sample	Day	% of applied dose	TRR as ^{14}C carbofuran (mg/kg)
Milk	**1**	0.32	0.010
	3	0.29	0.14
	7	0.30	0.098
Urine	**1**	95	
	3	90	
	7	88	
Faeces	**1**	4.1	
	3	5.1	
	7	5	
Liver	**7**	0.025	0.11
Kidney	**7**	<0.01	0.18
Leg muscle	**7**	<0.01	<0.01
Loin muscle	**7**	<0.01	0.01
Omental fat	**7**	<0.01	<0.01
Total recovery		95	

Characterization and identification of the total radio labelled residue from the administration of ^{14}C-carbofuran to lactating goats.

	Milk		Muscle		Liver		kidney	
TRR (mg/kg)	0.32		0.01		0.11		0.18	
Extracted phase (%TRR)	99		30		27		20	
Metabolite identification								
	%TRR	**Mg/kg**	**%TRR**	**Mg/kg**	**%TRR**	**Mg/kg**	**%TRR**	**Mg/kg**
Carbofuran	0.41	0.001	-	-	-	-	-	-
3-OH-carbofuran	10	0.032	-	-	4.03[3]	0.005	11[6]	0.029
7-phenol	15	0.048	-	-	2.4[4]	0.003	-	-
3-OH-7-phenol	6.8[1]	0.021	-	-	12[5]	0.017	16[7]	0.042
3-keto-7-phenol	32[2]	0.10	-	-	-	-	-	-
Aqueous fraction from initial extraction	6.3	0.020	28	0.003	5.0	0.007	3.5	0.009
Aqueous fraction from enzymatic digestion	-	-	-	-	16	0.022	13	0.035
Aqueous fraction from mild acid hydrolysis	-	-	-	-	4.5	0.007	5.1	0.014
Aqueous fraction from strong acid hydrolysis	-	-	-	-	6.3	0.009	6.7	0.018
Polar residues	22	0.070	-	-	6.9	0.010	17	0.044
Post extraction solids	1		70		73		80	
Protease digestion					41		49	
Mild acid hydrolysis					12		12	-

Residual radioactive residues								
	1		70		20		19	

[1]including 2% conjugated, released by sulfatase treatment

[2] including 29% conjugated, released by sulfatase treatment

[3] including 2.2% conjugated, released by protease treatment

[4] conjugated, released by protease treatment

[5] including 11% conjugated, released by protease treatment

[6] including 8.2% conjugated, released by protease treatment

[7]conjugated, released by protease treatment.

Remark: There were some discrepancies between the level of total radioactive residues recovered in each matrix and the % of the TRR related to each of the metabolite.

Conclusion:
The parent compound was detected at a trace level in milk only.
3-OH-carbofuran was recovered at a level around 10 % of TRR in milk and kidney and at a level of 4% TRR in liver.
The information was given that in milk, 10 % of the TRR occurred as free 3-OH-carbofuran.
In liver, the 4 % of the total residues included 2.2% conjugated 3-OH-carbofuran, released by protease treatment.
While in kidney, 11 % of the total residues included 8.2% conjugated 3-OH-carbofuran, released by protease treatment.
There is no indication whether the aqueous fractions resulting from the mild/strong acid hydrolysis were further characterized.

Point 3(10) in the reporting tables: Supervised residue trials – Analytical methods

The following methods were reported in chapter B.5.2.1 – Carbosulfan additional report, revised April 2009.

- Determination of residues of carbosulfan and its metabolites carbofuran and 3-hydroxy carbofuran by HPLC-MS-MS in maize and sugar beet samples – Validation of the method. (Enriquez, 2006, Report BATTELLE A-17-05-13) - Trials ***FA-17-04-02/01-02, FA-17-05-02/01-02 and FA-17-06-07/01-02***

GLP:
GLP-compliance stated
Principle of the method:
Carbosulfan and Carbofuran (CS-CF) is extracted from 5 g sample with a mixture of hexane – acetone (4:1, v/v) and filtered through Celite and sodium sulphate anhydrous.

The metabolite 3-hydroxy carbofuran (3-OHCF) is extracted from the remaining filter cake by refluxing with 0.25 M hydrochloric acid. After filtration the 3-hydroxy carbofuran is cleaned-up through a C18 SPE cartridge using methanol 1% in dichloromethane.

The combined organic extract (CS-CF and 3-OHCF) is evaporated (at temperatures below 35°C and after addition of 'keeper' 1-decanol, in order to avoid losses of carbosulfan), re-constituted and kept in acetonitrile. Then the re-constituted extract is diluted with acetonitrile and water (to have the same composition of the mobile phase) and analysed by HPLC (column: Aqua C18, 50mm x 2mm ID, 5µm particles) with MS-MS detection (ESI, positive mode).

Findings:

Specificity – interferences : - Following ion transitions were monitored (MRM): m/z 381.1 → 118.1 (carbosulfan); m/z 222.1 → 123.0 (carbofuran); m/z 237.9 → 163.0 (3-OH-carbofuran);
LC-MS/MS is highly specific → no need for separate confirmatory method.
- No significant interferences (>30% of LOQ) were observed at the retention times of carbosulfan, carbofuran or 3-hydroxy carbofuran in any blank or control sample.

Linearity : The detector response for each compound was linear over the concentration range 1 ng/mL to 25 ng/mL (corresponding to a residue conc. range of 2 to 50 ppb). Correlation coefficients > 0.99.

Recovery – precision : see Table B.5.2.1-6b

Validation by an independent laboratory : First validation of method by Battelle; ILV described in study by Zietz (2008) was conducted by SGS Institut Fresenius.

Limit of quantification (LOQ) : 0.005 mg/kg (= 5 ppb) for each analyte in maize and sugar beet

Table B.5.2.1-6b: Lab validation of LC-MS/MS method for residues of Carbofuran and 3-OH Carbofuran in maize and sugar beet (Enriquez, 2006) (FMC)

Matrix	**Analyte**	**Fortification level (mg/kg commodity)**	**Recovery**			
			Number of samples	**Range (%)**	**Mean (%)**	**RSD (%)**
Maize grain	Carbosulfan	0.005 0.050	5 5	77-82 70-79	80 75	3 5
		Overall	*10*	*70-82*	*78*	*5*
	Carbofuran	0.005 0.050	5 5	87-94 96-102	89 99	3 2
		Overall	*10*	*87-102*	*94*	*6*

	3-OH carbofuran	0.005 0.050	5 5	94-107 100-104	97 101	5 2
		Overall	*10*	*94-107*	*100*	*4*
Sugar beet	Carbosulfan	0.005 0.050	5 5	67-77 82-91	73 87	5 4
		Overall	*10*	*67-91*	*80*	*10*
	Carbofuran	0.005 0.050	5 5	82-94 84-104	86 97	6 8
		Overall	*10*	*82-104*	*92*	*9*
	3-OH carbofuran	0.005 0.050	5 5	102-115 75-100	107 92	5 11
		Overall	*10*	*75-115*	*100*	*11*

Conclusion:
The analytical method is suitable for the determination of carbosulfan and its metabolites carbofuran and 3-hydroxy carbofuran in maize and sugar beet samples with a LOQ of 5 ppb for each analyte.

- In trials ***FA-17-04-07,FA-17-04-04, FA-17-06-03, FA-17-04-06***, the determination of Carbosulfan and its metabolites Carbofuran and 3-OH-carbofuran residues in sugar/fodder beet leaves and roots was achieved by HPLC /UV-PCD (post-column derivatisation using fluorescence detection) according to the reported analytical method in the residue study N°A-17-03-25.
This analytical method was based on the procedure of the method entitled "Determination of residues of Carbosulfan and its metabolites Carbofuran and 3-Hydroxy Carbofuran in leafy cabbage, cauliflower and Brussels sprouts – Validation of the method (Ginzburg, 2001a)".
This analytical method was validated for a Limit of Quantification of 0.05 mg/kg for each analyte.
This analytical method is described as follows:

- Determination of residues of Carbosulfan and its metabolites Carbofuran and 3-Hydroxy Carbofuran in leafy cabbage, cauliflower and brussels sprouts – Validation of the method (Ginzburg, 2001a)
- Carbosulfan EU dossier DAR : Additional requirements on the methods of analysis (chapter B.5) (Oz, 2004)

GLP :
GLP-compliance stated
Principle of the method :
Carbosulfan and Carbofuran are extracted twice from the sample with hexane/acetone 4+1 (v+v). After centrifugation and filtration, Carbosulfan and Carbofuran are cleaned up on an Envi Carb SPE cartridge followed by an Amino-propyl SPE cartridge.
Metabolite *3-Hydroxy Carbofuran* is extracted from the filter cake by refluxing with 0.25 M HCl. After filtration, 3-OH Carbofuran is cleaned up on a C18-SPE cartridge followed by the same Amino-propyl SPE cartridge used for Carbosulfan and Carbofuran (elution with methanol 1% in dichloromethane).
After evaporation to dryness, the combined residues are reconstituted in a mixture of acetonitrile/water 30+70 (v+v) and analyzed by HPLC (Zorbax Bonus-RP (C_{14}), 5 µm, 25 cm x 4.6 mm i.d.) with post-column derivatization (PCD) and fluorescence detection. The post-column reaction includes acidic hydrolysis of carbosulfan to carbofuran, followed by alkaline hydrolysis after which the methyl amine formed is derivatized with o-phtalaldehyde and 2-mercaptoethanol to the corresponding fluorescent substituted isoindol. Quantification by external standardization.
Findings :
Specificity – interferences : - HPLC-PCD with fluorescence detection is highly specific to N-methyl carbamates and their precursors → no need for confirmatory method
- no significant matrix interferences (control values < 30% of LOQ)
Linearity : response of fluorescence detector to resp. analytes (peak area vs. conc.) was demonstrated to be linear within a concentration range of 0.025 to 0.25 mg/L; r > 0.99
Recovery – precision : see Table B.5.2.1-9
Validation by an independent laboratory : not addressed
Limit of quantification (LOQ) : 0.05 mg/kg for each analyte

Table B.5.2.1-9 : Validation of HPLC-PCD method for residues in crops (Ginzburg, 2001a)

Matrix	Analyte	Fortification level (mg/kg commodity)	Recovery			
			Number of samples	Range (%)	Mean (%)	RSD (%)
leafy cabbage	Carbosulfan	0.05 0.5	5 5	75 – 107 73 – 81	97 76	13.2 4.3
	Carbofuran	0.05 0.5	5 5	97 – 104 81 – 102	100 94	2.6 9.4
	3-OH Carbofuran	0.05 0.5	5 5	79 – 103 84 – 112	89 103	11.7 11.8
cauliflower	Carbosulfan	0.05 0.5	5 7	82 – 112 76 – 96	99 89	12.3 7.4
	Carbofuran	0.05 0.5	5 7	104 – 108 62 – 94	106 82	1.9 16.1
	3-OH Carbofuran	0.05 0.5	5 7	92 – 109 77 – 91	101 87	6.4 5.5
brussels sprouts	Carbosulfan	0.05 0.5	5 5	89 – 106 62 – 74	96 70	8.4 6.7
	Carbofuran	0.05 0.5	5 5	74 – 87 92 – 107	78 99	6.6 6.5
	3-OH Carbofuran	0.05 0.5	5 5	70 – 86 95 – 106	77 102	7.8 4.1

Conclusion :
HPLC-PCD method with fluorescence detection is suitable for the determination of residues of Carbosulfan, Carbofuran and 3-Hydroxy Carbofuran in brassica crops with a LOQ for each analyte of 0.05 mg/kg.

Data gap: 3.3 in the Evaluation Tables:
"Data to address residues in rotational crops, in particular further metabolite identification in the edible parts of the rotational crops is required".

Open point: 3.5 in the Evaluation Tables:
"Experts may consider whether the approach as suggested by the applicant is justified to consider 10% TRR in rotational crops in the consumer risk assessment"

JMPR report 1997

Rotational crops:
Experimental design:
In a confined crop rotation study (Phenyl)-14C-Carbofuran was applied directly to a silt loam soil at an application rate of 3.4 kg as/ha. Wheat, soya beans and sugar beet were seeded into the treated soil 4 and 12 months after treatment and grown to maturity. Wheat forage, straw and grain, soya bean silage, stems, pods and beans and sugar beet tops and roots were assayed for the determination of the total radioactive residues.
Extraction procedure:
Each sample was extracted with Methanol/water and separated into non polar and polar fractions for further metabolites identification. Conjugated metabolites were hydrolysed with 0.25 N HCl. Metabolites were identified by TLC, by co-chromatography with reference standards.
Findings:

Crop	Sample	Total radioactive residues (mg/kg)	
		4 months	12 months
Wheat	**Forage**	-	1.40

	Straw	54.0	0.30
	Grain	0.60	0.04
Soya bean	**Silage**	16.0	0.50
	Stem	18.0	0.70
	Pod	5.0	0.10
	Beans	1.0	0.08
Sugar beet	**Top**	0.40	0.05
	Root	0.20	0.05

The phenolic metabolites (3-OH-7-phenol, 3-keto-7-phenol, 7-phenol) were the main degradation products recovered in the rotated crops. The carbamates (carbofuran, 3-OH-carbofuran and 3-keto-carbofuran) constituted a small proportion of the total radioactive residues (<10 % of the TRR in any crop sown at 4 and 12 months).

Open point 3.3 in the discussion table (TC 21):

It was agreed in the first peer review on carbofuran that all metabolites with carbamate moiety (including the 3-keto) are toxicologically relevant. The 3-keto-carbofuran is classified with T, R25. Tox data for the metabolite are very limited. However it is very likely that the reference values of carbofuran will cover the toxicity of the metabolite.

RMS to provide an overview table with the recovered residue levels of 3-keto-carbofuran in plant and animal matrices.

ANNEX B

Addendum August 2009
Update September 2009

Carbosulfan

B.9 Ecotoxicology

B.9.2.16 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

During Peer Review the notifier provided updated PECsw and PECsed calculations which were evaluated in the section on fate and behaviour.

In the table below, the PECsw and PECsed values for FOCUS step 3 are presented for the parent compound carbosulfan and its metabolites carbofuran, dibutylamine, carbofuran-7-phenol, 3-hydroxy-carbofuran and 3-keto-carbofuran.

Table B.9.2.16-1 : Calculated PEC values for carbosulfan and its metabolites (FOCUS Step 3) in surface water, application of 750 g carbosulfan/ha in sugar beet

Scenario	Compound	Max PECsw (µg/L)	Date of max PECsw	Max PECsed (µg/kg)	Date of max PECsed
D3 ((Ditch)	Carbosulfan	0.00E+00	1-janv-92	0.00E+00	1-janv-92
D4 ((Pond)	Carbosulfan	0.00E+00	9-déc-85	0.00E+00	31-déc-85
D4 ((Stream)	Carbosulfan	0.00E+00	9-déc-85	0.00E+00	9-déc-85
R1 ((Pond)	Carbosulfan	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	Carbosulfan	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	Carbosulfan	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	Carbofuran	1.11E-02	29-janv-93	2.00E-02	15-avr-93
D4 ((Pond)	Carbofuran	6.58E-02	30-janv-86	9.03E-02	6-mars-86
D4 ((Stream)	Carbofuran	4.62E-02	16-déc-85	4.10E-02	28-janv-86
R1 ((Pond)	Carbofuran	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	Carbofuran	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	Carbofuran	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	DBA	0.00E+00	1-janv-92	0.00E+00	1-janv-92
D4 ((Pond)	DBA	0.00E+00	9-déc-85	0.00E+00	1-janv-85
D4 ((Stream)	DBA	0.00E+00	5-déc-85	0.00E+00	9-déc-85
R1 ((Pond)	DBA	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	DBA	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	DBA	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	7-P-C	1.30E-05	29-janv-93	4.07E-04	4-avr-93
D4 ((Pond)	7-P-C	8.50E-05	31-janv-86	1.85E-03	1-mai-86
D4 ((Stream)	7-P-C	7.50E-05	1-janv-85	9.67E-04	1-févr-86
R1 ((Pond)	7-P-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	7-P-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	7-P-C	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	3-H-C	2.11E-04	29-janv-93	4.28E-04	16-avr-93
D4 ((Pond)	3-H-C	1.39E-03	30-janv-86	2.39E-03	20-mars-86
D4 ((Stream)	3-H-C	8.85E-04	17-déc-85	8.12E-04	28-janv-86
R1 ((Pond)	3-H-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R1 ((Stream)	3-H-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	3-H-C	0.00E+00	1-oct-80	0.00E+00	1-oct-80
D3 ((Ditch)	3-K-C	6.57E-04	30-janv-93	4.70E-03	1-mai-93
D4 ((Pond)	3-K-C	0.00E+00	3-sept-85	1.07E-06	1-mai-86
D4 ((Stream)	3-K-C	0.00E+00	6-juin-85	0.00E+00	25-avr-86
R1 ((Pond)	3-K-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84

R1 ((Stream)	3-K-C	0.00E+00	1-mars-84	0.00E+00	1-mars-84
R3 ((Stream)	3-K-C	0.00E+00	1-oct-80	0.00E+00	1-oct-80

Following the calculations performed above, the major-sediment metabolite carbofuran-phenol was modelled as a soil metabolite using a formation fraction of 1, which leads to almost no entry in the water system due to its high Koc and low DT50soil. In order to obtain an estimate of maximum concentrations of carbofuran-phenol in surface water and sediment, the maximum FOCUS Step 3 PECsw and PECsed for carbofuran are multiplied using a MW correction factor (164.2/221.3 = 0.74) and the maximum % occurrence of carbofuran-phenol in water-sediment (total AR of 30% following the Yeomans (1995 and 1996) study). It results in more critical values for PECsw (0.0146 µg/L carbofuran-phenol) and PECsed (0.02 µg/kg carbofuran-phenol).
For the TER calculations for carbofuran-7-phenol, these latter values were used in stead of the values presented by the notifier.

B.9.2.16.1 Risk assessment for the active substance

Table B.9.2.16.1-1 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to carbosulfan in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Lepomis macrochirus*	96 h	0.015	0.00001	1500000	100
D4	Pond				0.00001	1500000	100
D4	Stream				0.00001	1500000	100
R1	Pond				0.00001	1500000	100
R1	Stream				0.00001	1500000	100
R3	Stream				0.00001	1500000	100
D3	Ditch	*Oncorhynchus mykiss*	14 d	0.004	0.00001	400000	10
D4	Pond				0.00001	400000	10
D4	Stream				0.00001	400000	10
R1	Pond				0.00001	400000	10
R1	Stream				0.00001	400000	10
R3	Stream				0.00001	400000	10
D3	Ditch	*Daphnia magna*	48 h	0.0015	0.00001	150000	100
D4	Pond				0.00001	150000	100
D4	Stream				0.00001	150000	100
R1	Pond				0.00001	150000	100
R1	Stream				0.00001	150000	100
R3	Stream				0.00001	150000	100

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Daphnia magna* (Marshal 10G)	48 h	0.00105	0.00001	150000	100
D4	Pond				0.00001	150000	100
D4	Stream				0.00001	150000	100
R1	Pond				0.00001	150000	100
R1	Stream				0.00001	150000	100
R3	Stream				0.00001	150000	100
D3	Ditch	*Daphnia magna*	21 d	0.0032	0.00001	320000	100
D4	Pond				0.00001	320000	10
D4	Stream				0.00001	320000	10
R1	Pond				0.00001	320000	10
R1	Stream				0.00001	320000	10
R3	Stream				0.00001	320000	10

The risk of carbosulfan to aquatic organisms is acceptable for all FOCUS step 3 scenarios.

B.9.2.16.2 Risk assessment for the metabolites

Table B.9.2.16.2-1 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to carbofuran in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Lepomis macrochirus*	96 h	0.18	0.0111	16216	100
D4	Pond				0.0658	2736	100
D4	Stream				0.0462	3896	100
R1	Pond				0.00001	18000000	100
R1	Stream				0.00001	18000000	100
R3	Stream				0.00001	18000000	100
D3	Ditch	*Cyprinodon variegatus*	35 d	0.006	0.0111	541	10
D4	Pond				0.0658	91	10

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D4	Stream				0.0462	130	10
R1	Pond				0.00001	600000	10
R1	Stream				0.00001	600000	10
R3	Stream				0.00001	600000	10
D3	Ditch	*Daphnia magna*	48 h	0.00205	0.0111	185	100
D4	Pond				0.0658	**31**	100
D4	Stream				0.0462	**44**	100
R1	Pond				0.00001	205000	100
R1	Stream				0.00001	205000	100
R3	Stream				0.00001	205000	100
D3	Ditch	*Ceriodaphnia dubia*	7 d	0.00016	0.0111	14	10
D4	Pond				0.0658	**2**	10
D4	Stream				0.0462	**3**	10
R1	Pond				0.00001	16000	10
R1	Stream				0.00001	16000	10
R3	Stream				0.00001	16000	10
D3	Ditch	*Chironomus riparius*	28 d	0.0032	0.0111	288	10
D4	Pond				0.0658	49	10
D4	Stream				0.0462	69	10
R1	Pond				0.00001	320000	10
R1	Stream				0.00001	320000	10
R3	Stream				0.00001	320000	10

Table B.9.2.16.2-2 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to carbofuran in sediment for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/kg)	PEC_{sed} (µg/kg)	TER	Annex VI trigger
D3	Ditch	*Chironomus riparius*	28 d	0.0022	0.0200	110	10
D4	Pond				0.0903	24	10

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/kg)	PEC_{sed} (µg/kg)	TER	Annex VI trigger
D4	Stream				0.0410	54	10
R1	Pond				0.00001	220000	10
R1	Stream				0.00001	220000	10
R3	Stream				0.00001	220000	10

The risk of the metabolite carbofuran to fish and sediment dwelling organisms is acceptable for all FOCUS step 3 scenarios. The acute and chronic risk to aquatic invertebrates is acceptable for the D3 ditch scenario and for all the run-off scenarios (R1 pond, R1 stream, R3 stream). However, for the scenarios D4 pond and D4 stream, the TER values are below the triggers.

Table B.9.2.16.2-3 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 3-keto-carbofuran in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Ceriodaphnia dubia*	48 h	0.049	0.000657	74581	100
D4	Pond				0.00001	4900000	100
D4	Stream				0.00001	4900000	100
R1	Pond				0.00001	4900000	100
R1	Stream				0.00001	4900000	100
R3	Stream				0.00001	4900000	100

The risk of the metabolite 3-keto-carbofuran to aquatic invertebrates is acceptable for all FOCUS step 3 scenarios.

Table B.9.2.16.2-4 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 3-hydroxy-carbofuran in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Scenario	Water body type	Test organism	Time scale	Toxicity end point (mg/L)	PECsw (µg/L)	TER	Annex VI trigger
D3	Ditch	*Ceriodaphnia dubia*	48 h	0.023	0.000211	232227	100
D4	Pond				0.00139	35252	100
D4	Stream				0.000885	55367	100
R1	Pond				0.00001	4900000	100
R1	Stream				0.00001	4900000	100
R3	Stream				0.00001	4900000	100

The risk of the metabolite 3-hydroxy-carbofuran to aquatic invertebrates is acceptable for all FOCUS step 3 scenarios.

Table B.9.2.16.2-5 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 7-phenol in surface water for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Test substance	Organism	Toxicity end point (mg/L)	Time scale	PEC_{sw} (µg/L)	PEC_{twa} (µg/L)	TER	Annex VI Trigger

Test substance	Organism	Toxicity end point (mg/L)	Time scale	PEC_{sw} (µg/L)	PEC_{twa} (µg/L)	TER	Annex VI Trigger
7-phenol	*Oncorhynchus mykiss*	32.3	96 h	0.0146	-	2212329	100
7-phenol	*Daphnia magna*	25	48 h	0.0146	-	1712329	100
7-phenol	*Pseudokirchneriella subcapitata*	47	72 h	0.0146	-	3219178	10
7-phenol	*Chironomus riparius*	0.004	25 d	0.0146	-	684932	10

Table B.9.2.16.2-6 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to 7-phenol in sediment for the intended use in sugar beet (1 x 0.750 kg a.s./ha) based on FOCUS Step 3 calculations

Test substance	Organism	Toxicity end point (mg/kg)	Time scale	PEC_{sed} (µg/kg)	PEC_{twa} (µg/kg)	TER	Annex VI Trigger
7-phenol	*Chironomus riparius*	1.36	25 d	0.02	-	68000	10

As mentioned before, the TER calculations are based on the more critical values for PECsw (0.0146 µg/L 7-carbofuran-phenol) and PECsed (0.02 µg/kg 7-carbofuran-phenol) calculated by RMS.
The risk of the metabolite 7-carbofuran-phenol to aquatic invertebrates is acceptable for all FOCUS step 3 scenarios.

The risk of the metabolite dibutylamine is acceptable based on FOCUS step 1 for fish and algae and on FOCUS step 2 for aquatic invertebrates (see DAR). Therefore, no further TER calculations based on FOCUS step 3 are necessary.

Open point 5.10 in the evaluation table:

From the mesocosm study with Marshal 25CS (Foekema E.M. *et al*., 2002) a NOAEC of 0.4 µg carbosulfan/L was derived. With an assessment factor of 4, this leads to an EAC = 0.1 µg carbosulfan/L. Also, a NOEC of 0.1 µg carbosulfan/L could be derived with an assessment factor of 1. In conclusion, a RAC (regulatory acceptable concentration) of 0.1 µg carbosulfan/L was agreed by Member States during Peer Review.
Based on the laboratory toxicity endpoints for *Daphnia magna* (EC_{50} = 0.00205 mg carbofuran/L) and *Ceriodaphnia dubia* (NOEC = 0.00016 mg carbofuran/L) and the FOCUS step 3 PECsw values (750 g a.s./ha), TER values did not pass the trigger for 2 out of 6 scenarios, namely D4 pond and D4 stream (see above).

The RAC of 0.1 µg carbosulfan/L is equivalent with 0.058 µg carbofuran/L, based on molecular weight ratio carbofuran/carbosulfan of 221.3/380.5. With this RAC = 0.058 µg carbofuran/L, the scenario D4 stream (0.0462 µg carbofuran/L) would show acceptable risk, whereas scenario D4 pond (0.0658 µg carbofuran/L) is little above the EAC.

In conclusion, the endpoint of the mesocosm study could be applied to refine the risk assessment for aquatic invertebrates. At the application rate of 750 g a.s./ha, 5 out of 6 scenarios show acceptable risk (only scenario D4 pond does not pass the trigger of 4).

B.9.6.5 Field tests – residue content of earthworms (Annex IIIA 10.6.1.3)

Comparison of Two Methods for Assessing the Effects of Carbofuran on Soil Animal Decomposers in Cornfields. (Broadbent A.B., Tomlin, A.D., 1982).

Abstract :
The effects of spring treatments of an insecticide, carbofuran, on the soil animal decomposer community of an Ontario cornfield were assessed by measuring fluctuations in soil animal populations and by measuring changes in rates of leaf litter decomposition from bags of different mesh sizes. For several weeks after treatment, reductions in soil microfauna and reductions in the rate of corn leaf decomposition could be observed, but by autumn, the total number of soil micro-arthropods and litter decomposition rates were similar to those in untreated control plots. Earthworm populations seemed unaffected by in-row treatments of carbofuran, but broadcast treatments reduced worm populations significantly.

Guidelines :
Not applicable.
GLP:
No
Materials and Methods :
Test substance : carbofuran
Test species : soil fauna, earthworms
Test design :
The experimental site was a plot (120 m x 40 m) in a 12-ha field of continuous corn under regular tillage and management programs in Ontario. The soil was classified as a Burford loam, pH 6.9 with an organic matter content of 4.3 %.
On 10 May 1977, granular carbofuran was applied in-furrow (1.5 kg a.s./ha, in a 10-cm wide band along the corn rows). An untreated control plot of similar dimensions (60 m long, 10 rows wide, rows on 90-cm centers) was also made. On 23 May 1978 (1.1 kg a.s./ha) and on 22 May 1979 (11.2 kg a.s./ha), granular carbofuran was applied in-furrow and as broadcast treatment. An untreated control plot was also made (similar design but only 30 m long).
For 6, 9 and 6 dates, respectively, during the summers of 1977, 1978 and 1979, 50 soil cores (2.5 cm in diameter by 15 cm deep) were taken from each plot to determine residues of carbofuran, 3-keto-carbofuran and 3-OH-carbofuran. Carbofuran was extracted with chloroform from soil and analysed by gas-liquid chromatography as described by Miles and Harris (1979).
Sampling of earthworm populations :
Earthworm populations were estimated using the formaldehyde expellent method of Raw (1959). Nine liters of a 0.55 % formaldehyde solution was applied to the soil surface within a wooden "quadrat" (1.8 by 0.2 m) placed over the seed row. In 1977, earthworm populations and biomass were estimated by using 18 quadrats from each of the treated and control plots on 28 October (24 weeks after treatment). In 1978, populations and biomass were estimated from 12 quadrats from each of the control, row-treated and broadcast-treated plots, and from the untreated zone between rows in the row-treated plot on 3 May (3 weeks before treatment), 23 June (4 weeks after treatment), and 25 October (22 weeks after treatment).
Findings :
Residue determination :
Carbofuran residues disappeared rapidly from the cornfield soil in each of the 3 years of the study. 3-keto-carbofuran and 3-OH-carbofuran were not detected in excess of 0.15 and 0.02 ppm, respectively. The slower rate of carbofuran disappearance in 1978 varied with lower precipitation and soil moisture levels during the 10 weeks after treatment. However, by 10 and 8 weeks, respectively, after treatment in 1978 and 1979, soil residue levels in the row treatments had dropped to levels near the corresponding broadcast treatments.
Populations of soil fauna :
In 1977, 24 weeks after treatment there were 44.2 earthworms per m^2 in row-treated plots and 35.0 earthworms per m^2 in the control plots; at least 90 % of the worms were *Aporrectodea tuberculata* (Eisen). There was no significant difference in mean biomass of earthworms between carbofuran-treated (13.6 g/m^2) and control plots (12.1 g/m^2).

Samples taken in 1978, 3 weeks before treatment and 4 weeks after treatment, contained only three earthworms from 12 quadrats and seven from 18 quadrats, respectively. The small number of earthworms extracted was due to unusually dry soil conditions. In 1978, 22 weeks after treatment, there was no significant difference in numbers of biomass of earthworms among "between row-treated" (50.8/m², 9.0 g/m²), row-treated (44.7/m², 9.0 g/m²), and control plots (50.3/m², 9.8 g/m²); significantly fewer earthworms were found in the broadcast treatment (28.9/m²), but the earthworm biomass, although smaller in this plot (7.2 g/m²), was not significantly different from the other plots. Broadcast treatments of carbofuran are more harmful to earthworms than row treatments, possibly because there is no untreated refugial soil to which earthworms can escape after a broadcast treatment.
Carbofuran treatment at 11.2 kg a.s./ha caused swellings and abnormal pigmentation of the earthworms similar to that described by Stenersen *et al*. (1973), and 2 weeks after treatment, dead earthworms exhibiting these symptoms were observed on the soil surface.

Conclusion of the RMS :
The RMS has reservations towards this study due to several shortcomings.
Very low numbers of earthworms were collected in 1978, 3 weeks before and 4 weeks after treatment due to unusual dry soil conditions. However, the sampling at 4 weeks after treatment is important to assess the effects of carbofuran on earthworm populations. The sampling at 22 weeks after treatment demonstrated that significantly fewer earthworms were found in the broadcast treatment compared to the control plots, row-treated and "between row-treated" plots. However, carbofuran residues disappeared rapidly from the cornfield, in 1978, 10 weeks after treatment, soil residue levels in the row treatments had dropped to levels near the corresponding broadcast treatments. From the graph of 1978, the soil residue levels at 22 weeks after treatment had fallen to 0 mg/kg. Therefore, the sampling at 22 weeks is not appropriate to estimate effects of carbofuran nor to compare the in-furrow treatment with the broadcast treatment.

During the Peer Review of Carbosulfan the notifier submitted following statement in August 2009.

Updated and Comparative Risk Assessment of the carbosulfan use on sugar beet at 100 g ai/ha versus 750 g ai/ha. (2009).

Introduction :

FMC re-applied for Annex I inclusion of the active ingredient carbosulfan under the rules laid down in Regulation 33/2008/EC – Chapter 3 (accelerated procedure). Article 15(1b) of this Regulation states that:

> "*The supported uses are the same as those that were the subject of the non-inclusion Decision. They may only be changed insofar as this is necessary, in the light of the reasons which gave rise to the non-inclusion Decision, to permit inclusion of that substance in Annex I to Directive 91/414/EEC*".

Whilst we still support the use of carbosulfan on sugar beet at 750 g ai/ha[2] – and welcome the efforts to evaluate the risk assessment at this dose rate - we also appreciate that interpretation of endpoints and acceptability of refinement route may differ from the notifier to the evaluator's view. Therefore, we introduced additional risk assessments at lower dose rates, in particular at 100 g ai/ha, in order to increase the chances to identify a safe use scenario.

The RA conducted by the RMS shows that while the risk to granular intake at 750 g ai/ha is acceptable according to the EPPO scheme; the risk to secondary poisoning via ingestion of treated seedlings, earthworms and/or arthropods needs further refinement. This suggests that a lower application rate should be considered for the risk assessments, as wisely foreseen by Article 15b of the Regulation.

Should the EC decide that registration of carbosulfan is possible only with limitation on its maximum applied dose rate, this issue would be dealt by FMC at national level. Indeed, we are confident that certain technologies are efficient at dose rate equal or lower to 100 g carbosulfan/ha.

The aim of the present document is to compare the critical outcome of the risk assessment for application of 100 g carbosulfan/ha versus 750 g carbosulfan/ha. It will shortly investigate Operator exposure, consumer exposure, PEC calculation, Risk to non-target organisms and in particular birds and mammals.

Risk to birds and mammals :

Granule intake

The RA conducted by the RMS shows that the risk to granular intake at 750 g ai/ha is acceptable according to the EPPO sub scheme. A similar conclusion can be drawn from the Probabilistic Risk assessment submitted by FMC (Bastiansen F. and Wang M., 2008). Reducing the dose rate to 100 g ai/ha can only further increase the confidence that the risk to birds and mammals via granule intake is acceptable.

Secondary poisoning risk assessment for birds and mammals

Residue of carbofuran in seedlings

Several residue trials and metabolism studies are evaluated in the carbosulfan and carbofuran DAR in order to investigate the residue in seedling. The most valuable information in order to assess the carbosulfan residue in seedlings comes from the residue trials performed by Zietz (2008):

[2] At planting – in furrow – granule buried at 7 cm from the surface.

Residues of carbosulfan, carbofuran and 3-OH-carbofuran in beet seedlings

Trial No.	Specimen material	Appl. Rate kg/ha	Timing (BBCH)	DALA	Carbosulfan (mg/kg)	Carbofuran (mg/kg)	3-OH-carbofuran (mg/kg)
07-UK-042	Sugar beet seedling	control	12	33	< 0.005 nd	< 0.005 nd	< 0.005 nd
07-UK-042		7.5	12	33	**< 0.005**	**0.30**	**0.92**
07-UK-042		1.0	12	33	< 0.005	0.026	0.087
07-UK-043	Sugar beet seedling	control	12	32	< 0.005 nd	< 0.005 nd	< 0.005 nd
07-UK-043		7.5	12	32	**< 0.005**	**0.16**	**0.58**
07-UK-043		1.0	12	32	< 0.005	0.014	0.064
07-NF-044	Sugar beet seedling	control	12	28	< 0.005 nd	< 0.005 nd	< 0.005 nd
07-NF-044		7.5	12	28	**< 0.005**	**< 0.005**	**0.016**
07-NF-044		1.0	12	28	< 0.005 nd	< 0.005	0.008
07-NF-045	Sugar beet seedling	control	12	28	< 0.005 nd	< 0.005 nd	< 0.005 nd
07-NF-045		7.5	12	28	**< 0.005**	**< 0.005**	**0.023**
07-NF-045		1.0	12	28	< 0.005	< 0.005	0.006

DALA = days after last treatment

< 0.005 nd = no peak was detected

On the basis of these trials, the following residue should be considered for the risk assessment:

Seedling residue values to be used for the birds and mammals RA

Risk assessment	750 g carbosulfan/ha		100 g carbosulfan / ha	
	Maximum concentrations	Mean of the maximum concentrations	Maximum concentrations	Mean of the maximum concentrations
	acute	short-term and long-term	acute	short-term and long-term
carbosulfan	< 0.005	< 0.005	< 0.005	< 0.005
carbofuran (sum of carbofuran + 3-OH-carbofuran)	1.22	0.50	0.113	0.051

It should be noted that these experimental results confirm that the RUD rule applies to the carbamate soil application. Indeed, the residue results are linear with the applied dose rate.

Residue of carbofuran in earthworms and insects

Residue in earthworms and beetles should only consider carbosulfan and carbofuran. Indeed, 3-OH-carbofuran is a minor metabolite in soil (<5%: see B.8.1.1.1 of original DAR) and will therefore not contaminate insect and soil dwelling arthropods in any significant concentrations. This is confirmed in the DAR of benfuracarb were the notifier Otsuka analysed both carbofuran and 3-OH-carbofuran in earthworm. These data confirm the modest contribution of 3-OH-carbofuran to the carbofuran residue.

Proposed Residue values are derived by normalizing measured residue obtained from Brown *et al*. (2007) at an application rate of 750 g as/ha. The RUD rules apply well to these values since residue in earthworms and arthropods is driven by the PEC soil, which is a linear function of the applied dose rate. As a worst case assumption, the residue of carbosulfan and carbofuran can be summed and compared to the carbofuran endpoints for TER calculation.

Earthworms and arthropods residue values to be used for the birds and mammals RA at 750 g ai/ha

		Maximum concentrations	Mean of the maximum concentrations	Time weighted average mean concentrations on the interval 1-9 days
Risk assessment at 750 g carbosulfan/ha		acute	short-term	long-term
Beetles	carbosulfan + carbofuran	1.60	1.22	0.51
Earthworms	carbosulfan + carbofuran	0.29	0.23	0.10

Earthworms and arthropods residue values to be used for the birds and mammals RA at 100 g ai/ha

		Maximum concentrations	Mean of the maximum concentrations	Time weighted average mean concentrations on the interval 1-9 days
Risk assessment at 100 g carbosulfan/ha		acute	short-term	long-term
Beetles	carbosulfan + carbofuran	0.21	0.16	0.07
Earthworms	carbosulfan + carbofuran	0.04	0.03	0.01

Birds and mammals TER summaries

The TER values calculated by the RMS took into account the above residue in seedling, earthworms and beetle at 750 g ai/ha. The TER values obtained by RMS can be summarized as follows:

Birds and mammals Risk assessment at 750 g ai/ha – summary table

Species	TERacute	TERst	TERlt
Woodpigeon	2.83	15.56	6.22
Yellow wagtail	0.73	2.15	2.05
Blackbird	2.24	6.36	5.85
Skylark	0.85	3.62	2.01
Hare	41	nd	13

Taking into account the same refinements and parameters used by RMS for calculating the TERs at 750 g ai/ha, then the risk assessment at 100 g ai/ha – on the basis of the corresponding residue values - will lead to the following TERs:

Birds and mammals Risk assessment at 100 g ai/ha – summary table

Species	TERacute	TERst	TERlt
Woodpigeon	30.6	152.5	61.0
Yellow wagtail	5.6	16.4	14.9
Blackbird	16.24	48.76	58.5
Skylark	8.0	31.1	18.0
Hare	440.2	nd	139.2

These TER values show that reducing the dose rate to 100 g ai/ha allows finding an acceptable risk to mammals and significantly improves the risk assessment to birds. TERacute values for yellow wagtail and skylark are still respectively 5.6 and 8.0, but they could be further refined if taking into account:

- The use of a PT value on top of a PD value;
- Consideration of the reversibility of the AChE inhibition and rapid metabolisation/excretion of carbofuran;
- Or any other refinement step the evaluator judges appropriate in order to reflect the risk to birds and mammals in a more realistic manner.

It is also interesting to note that the RA at 100 g ai/ha still present :

- TERlt for birds above 10, if taking into account the experts opinion that safety factor around the birds LC10 should be raised to 10 (instead of 5);
- TERa of 62 and TER lt of 19.6 for hares when considering a long-term NOEL of 0.1 mg/kg bw/day (instead of 0.71).

Conclusion of the RMS :
The RMS maintains its position that the proposal of the notifier for an additional risk assessment at a reduced granular dose rate of 100 g a.s./ha, corresponding to the doses used for seed treatment, is not acceptable. It is indeed very questionable whether such use can be considered as a representative use :

- It is not representative for the use of a granular formulation as the dosage of 100 g carbosulfan/ha is much lower than the authorized dosages. The GAPs for granule formulations that were authorized in 2002 in EU MS consisted in applications at sowing or transplant time, with incorporation in the furrow at maximum rate of 750-1200 g a.s./ha. (Broadcast applications were performed at even higher dosages).
- It is not representative for the use of a seed treatment formulation at similar rates of 100 g carbosulfan/ha because the exposure routes and risk assessments are not equivalent; for example, it is obvious that the exposures of the consumer, of the operator, of the birds and mammals will be significantly different if we compare a granular application to a seed treatment.
- The resubmitted dossier does not contain sufficient trials performed at 100 g carbosulfan/ha in order to determine the residue level in bird and mammal feed items (sugar beet seedlings, earthworms, arthropods).

The RMS concluded on the **granule** intake by birds, in addition to the notifiers statement :
The risk for birds from ingestion of Marshal 10G granules is acceptable for the intended use (750 g a.s./ha) based on the EPPO risk assessment scheme. However, RMS did not accept the probabilistic risk assessment presented by the notifier due to several shortcomings.

The RMS cannot agree with the notifiers statement that residues in earthworms and beetles should only consider carbofuran (3-OH-carbofuran residues are negligible) based on the benfuracarb dossier. If data from benfuracarb dossier are used, this should be accompanied by a letter of access.

The RMS disagrees with the statement of the notifier that the **residue values** at 100 g a.s./ha show a linear response compared to 750 g a.s./ha.
The residue values for sugar beet seedlings were derived from 4 residue trials conducted at both 750 and 100 g a.s./ha. However, too much uncertainty remains on the residue values. The trials were conducted under protected conditions and residues were measured at only one time point (at stage BBCH 12).
The residue values for earthworms and beetles were derived from 2 adjacent field trials conducted only a 750 g a.s./ha. Also uncertainty remains on these residue values since the contribution of the metabolite 3-OH-carbofuran was not measured.

RMS also raises questions about the **reduced dose rate** :

- If carbosulfan is applied at 100 g a.s./ha, the product will be applied in the plant hole, closer to the plant, to be as effective as the higher dose rate. No extrapolation of the residues is possible from a residue trial conducted at 750 g a.s./ha in the furrow. Only a residue trial conducted at 100 g a.s./ha in the plant hole will give the residue level in the field situation.
- If 100 g a.s./ha is efficient, why is the use at 750 g a.s./ha supported?

The RMS concluded that the risk of carbosulfan to birds and mammals consuming **sugar beet seedlings, earthworms and arthropods** is not acceptable for the intended use based on insufficient information on the actual residue level in feed items. In order to refine the risk to birds and mammals, more information is needed on the actual residue levels in feed items (sugar beet seedlings, earthworms, arthropods). The information should allow to perform statistical evaluations (enough residue trials, N and S European conditions, sampling over time, enough sampling material, ….). Also, the residue trials should be relevant for the intended use (crop, application rate, granular or seed treatment use).

Risk to aquatic organisms :
Risk assessment for aquatic organisms was re-calculated using the PEC surface water recently re-calculated and the ecotoxicological endpoints agreed in the DAR. TERs are provided in the tables in the document of the notifier (for application rate of 750 g carbosulfan/ha and 100 g carbosulfan/ha). Failing scenarios are highlighted in bold.
TERs of carbosulfan are not presented since no contamination occurs in surface water or in sediment in any of the FOCUS step 3 scenarios.
TERs of DBA and 7-phenol- carbofuran are not presented since these metabolites pass already at FOCUS step 2 and their PECsw and PECsed are minimum.

Conclusion of the notifier :
Whilst all run-off scenarios show acceptable risk to the aquatic organisms for applications at 750 or 100 g carbosulfan/ha, the low dose rate application at 100 g carbosulfan/ha also presents acceptable risk to *Dapnia magna* and *Cerodapnia dubia* in the drainage scenarios.

Calculations of the RMS :
The risk assessment for the metabolite carbofuran and aquatic invertebrates at lowered dose rate are presented below. RMS agrees with the PECsw calculations at 100 g a.s./ha for carbofuran presented by the notifier.

Table B.9.2.16.2-1bis : Toxicity Exposure Ratio's (TERs) for aquatic organisms exposed to carbofuran in surface water for the intended use in sugar beet (1 x 100 g a.s./ha) based on FOCUS Step 3 calculations

Scenario	**Water body type**	**Test organism**	**Time scale**	**Toxicity end point (mg/L)**	**PECsw (µg/L)**	**TER**	**Annex VI trigger**
D3	Ditch	*Daphnia magna*	48 h	0.00205	0.00118	1737	100
D4	Pond				0.00798	257	100
D4	Stream				0.00577	355	100
R1	Pond				0.00001	205000	100
R1	Stream				0.00001	205000	100
R3	Stream				0.00001	205000	100
D3	Ditch	*Ceriodaphnia dubia*	7 d	0.00016	0.00118	136	10
D4	Pond				0.00798	20	10
D4	Stream				0.00577	28	10
R1	Pond				0.00001	16000	10
R1	Stream				0.00001	16000	10
R3	Stream				0.00001	16000	10

Conclusion of the RMS :
The calculations of the notifier and RMS for the metabolite carbofuran and aquatic invertebrates give the same TER values. The scenarios that did not pass the trigger at the higher application rate of 750 g a.s./ha (D4 pond and D4 stream) for *Daphnia magna* (acute) and *Ceriodaphnia dubia* (chronic), do pass the trigger at the lower application rate of 100 g a.s./ha.

Risk to earthworms, bees, non-target arthropods, soil macro-organisms, soil micro-organisms and non-target plants :

The use of 750 g carbosulfan/ha to these organisms is acceptable. Reducing the dose rate can only offer more level of confidence.

Conclusion of the RMS :
RMS agrees with the notifier.