MINISTERIO DE AGRICULTURA, PESCA Y ALIMENTACIÓN

INSTITUTO NACIONAL DE INVESTIGACIÓN Y TECNOLOGÍA AGRARIA Y ALIMENTARIA (I.N.I.A.)

Ctra. De La Coruña, km. 7

28040 – Madrid

ESPAÑA

Monograph prepared in the context of the inclusion of the following active substance in Annex I of the Council Directive 91/414/EEC

ENDOSULFAN

Addendum Volume III

October 2001

THE ADDENDUM WAS PREPARED UNDER THE RESPONSIBILITY OF:

Dr. García-Baudín, J.Mª (Co-ordinatior)
Dr. Alonso-Prados, J.L. (Co-ordinator)
Instituto Nacional de Investigaciones Agrarias y Alimentarias (INIA) / Ministerio de Ciencia y Tecnología.
Departamento de Protección Vegetal. Ctra. de la Coruña, Km. 7,500. 28040 – Madrid. Spain.

CHAPTER B-1: Identity

Dr. Alonso-Prados, J.L.
Dr. Magrans-Soria, J.Oriol
Instituto Nacional de Investigaciones Agrarias y Alimentarias (INIA) / Ministerio de Ciencia y Tecnología.
Departamento de Protección Vegetal. Ctra. de la Coruña, Km. 7,500. 28040 – Madrid. Spain.

CHAPTERS B-2: Physical and chemical properties and B-3: Data on application and further information

Dr. Magrans-Soria, J. Oriol
Dr. Alonso-Prados, J.L.
Instituto Nacional de Investigaciones Agrarias y Alimentarias (INIA) / Ministerio de Ciencia y Tecnología.
Departamento de Protección Vegetal. Ctra. de la Coruña, Km. 7,500. 28040 – Madrid. Spain.

CHAPTER B-5: Methods of analysis

Dr. Magrans-Soria, J. Oriol
Instituto Nacional de Investigaciones Agrarias y Alimentarias (INIA) / Ministerio de Ciencia y Tecnología.
Departamento de Protección Vegetal. Ctra. de la Coruña, Km. 7,500. 28040 – Madrid. Spain.

CHAPTER B-6: Toxicology and metabolism

Dr. Marqués Marqués, F.
Dr. Medina Alonso, J.
Instituto Nacional de Medicina y Seguridad del Trabajo / Escuela Nacional de Medicina del Trabajo.
Instituto de Salud Carlos III. Pabellón 8. Ciudad Universitaria. ES-28040-Madrid. Spain.
Dra. Barrueco Fernández – Cuervo, C.
Instituto de Salud Carlos III, Ctra. Majadahonda - Pozuelo, km. 2. ES-28220 - Majadahonda (Madrid).
Dra. Fernández Cruz, Mª L.
Instituto Nacional de Investigaciones Agrarias y Alimentarias (INIA) / Ministerio de Ciencia y Tecnología.
Departamento de Protección Vegetal. Ctra. de la Coruña, Km. 7,500. 28040 – Madrid. Spain.
Dr. Sánchez-Fortún Rodríguez, S.
Dpto. de Toxicología y Farmacología, Universidad Complutense de Madrid, Avda. Puerta de Hierro, s/n, ES-28040 - Madrid.

CHAPTER B-7: Residue data

Dr. Alonso-Prados, J.L.
Dr. Magrans-Soria, J. Oriol
Instituto Nacional de Investigaciones Agrarias y Alimentarias (INIA) / Ministerio de Ciencia y Tecnología.
Departamento de Protección Vegetal. Ctra. de la Coruña, Km. 7,500. 28040 – Madrid. Spain.

CHAPTER B-9: Ecotoxicology

Dr. Tarazona-Lafarga, J.V.
Dra. Pablos-Chi, Mª V.
Instituto Nacional de Investigaciones Agrarias y Alimentarias (INIA) / Ministerio de Ciencia y Tecnología.
Departamento de Medio Ambiente. Ctra. de la Coruña, Km. 7,500. 28040 – Madrid. Spain.

ADDENDUM TO ANNEX B

ENDOSULFAN

B - 1: IDENTITY

B.1 Identity

The main notifier (Task Force Aventis/ Makhteshim) proposed a new list of GAP at the ECCO 106. **Cotton and tomatoes have been selected as representative uses for Annex I inclusion**. Although based on the annotation made by Aventis/Makhteshim, the task force supports all the uses listed in the previous list of GAP and intends to seek registrations in some Member States after Annex I listing of the active substance. **The previous list of GAP included citrus, hazelnut, pome fruits, stone fruits, grapes, sugar beet, pepper, potatoes and the following imported crops tea, soyabean, citrus, coffee. The RMS took into account all these uses in the risk assessment that was discussed during the ECCO Peer Review and several data GAPs were identified; a safe use was not identified in the ECCO Peer Review.**

The new risk assessment is made for **COTTON and TOMATO**, all the other uses are not supported by the available information.

The evaluation of the new information received from the main notifier have been included in the evaluation table.

LIST OF USES SUPPORTED BY AVAILABLE DATA – REPRESENTATIVE USES (DATE: 28.09.2001)

Active substance: **Endosulfan**

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI** (days) (l)	**Remarks:** (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applicat-ions (min)	kg as/hl min max	water l/ha min max	kg as/ha min max		
Cotton	Southern Europe	Thiodan 35 EC	F	I, A	EC	350 g/l	Medium /high volume spray	Last applicat ion when balls are partly open	3	14 – 21 days	0.105	800	0.84	21	Short PHI of 21 days is required, if chem. agent is used for desiccation of foliage.
Tomatoes	Southern Europe	Thiodan 35 EC	F	I, A	EC	350 g/l	Medium /high volume spray	At any stage	2	14 days	0.053 – 0.105	500 - 1000	max. 0.53	3	
			G						2	14 days	0.053	1500	0.8	3	

Remarks:

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.1.3 References relied on

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	N.N. 2001a Description of beginning materials and manufacturing process Certificate of analysis (Addendum to Doc. A48048) Endosulfan technical Code: AE F002671 Aventis CropScience GmbH, DEU; Regulatory Affairs Europe, Frankfurt. Doc. No. C013031 Confidential Business Information acc. to article 14 of Dir. 91/414/EEC	Y	N	Aventis	Y
	Rexer, K. 2001b Quality control data of ten recent batches Endosulfan emulsifiable concentrate 352 g/L Code: AE F002671 00 EC33 B3 Aventis CropScience GmbH, DEU; Regulatory Affairs Europe, Frankfurt. Doc. No. C014749 Confidential Business Information acc. to article 14 of Dir. 91/414/EEC	Y	N	Aventis	Y

ADDENDUM TO ANNEX B

ENDOSULFAN

B - 2: PHYSICAL AND CHEMICAL PROPERTIES

B.2 Physical and chemical properties

The evaluation of the new information received from the main notifier have been included in the evaluation table.

B.2.3 References relied on

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	Franke, J. 2001a Flammability (solids) Endosulfan substance, technical Code: AE F002671 00 1D98 0012 Aventis CropScience GmbH, DEU; Produktanalytik, Frankfurt. Doc. No. C015668	Y	N	Aventis	Y
	Franke, J. 2001b Explosive properties Endosulfan substance, technical Code: AE F002671 00 1D98 0012 Aventis CropScience GmbH, DEU; Produktanalytik, Frankfurt. Doc. No. C015667	Y	N	Aventis	Y
	Rexer, K. 2001a Determination of the storage stability (Accelerated storage test 14 days at 54 degrees C) Endosulfan emulsifiable concentrate 352 g/L. Code: AE F002671 00 EC33 B3 Formulierung Forschung & Entwicklung, Frankfurt. Doc. No.: C014750	Y	N	Aventis	Y
	Buerkle, L.W. 2001 Endosulfan Summary of the Photolytic degradation in the Atmosphere. Endosulfan Code: AE F002671 Aventis CropScience GmbH, DEU; Frankfurt. Doc. No. C013028			Aventis	N
	Buerkle, L.W. 2001 Estimation of the reaction with photochemically produced hydroxyl radicals in the atmosphere. Endosulfan sulfate. AE F051327 Aventis CropScience GmbH, DEU; Frankfurt. Doc. No. C012732			Aventis	N

ADDENDUM TO ANNEX B

ENDOSULFAN

B – 3: DATA ON APPLICATION AND FURTHER INFORMATION

B.3 Data on application and further information

New information on data requirement included and evaluated in the Evaluation Tables.

B.3.6 References relied on

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA 3.8.1	Butterworth 2001 Endosulfan Substance, Technical. Pyrolitic Behaviour Safe Disposal. 2001. (Report No JLB 01-01) Doc. No. C014450	N	N	Aventis	N

ADDENDUM TO ANNEX B

ENDOSULFAN

B - 4: PROPOSAL FOR CLASSIFICATION AND LABELLING

B.4 Proposal for classification and labelling

Classification and proposed labelling (Annex IIA, point 10)

With regard to physical/chemical data	None
With regard to toxicological data	T+ Very toxic R21 Harmful in contact with skin R28 Very toxic if swallowed R26 Very toxic by inhalation
With regard to fate and behaviour data	N Dangerous for the environment
With regard to ecotoxicological data	R50/53 Highly toxic to aquatic organism, may cause long-term adverse effects in the aquatic environment.

ADDENDUM TO ANNEX B

ENDOSULFAN

B - 5: METHODS OF ANALYSIS

B.5 Methods of analysis (IIA, 4; IIIA, 5)

Foreword: During ECCO 106 main notifier for endosulfan (Task Force Aventis formely AgrEvo/Makhteshim) introduced a new list of intended uses limiting the use of endosulfan to only two crops: tomato and cotton. Therefore, the following evaluation is made in order to ensure that these uses are actually supported with the available information. Any other use that may be required in the future for endosulfan will need further assessment.

B.5.1 Analytical methods for formulation analysis (IIA, 4.1; IIIA, 5.1)

B.5.1.3 Plant Protection Product

Aventis

Method originally submitted in the dossier was considered acceptable. Essentially it was the CIPAC method that was demonstrated to be acceptable for the Plant Protection Product supported by the main notifier. **Data requirement fulfilled (ECCO 106).**

Calliope

There is no data on the analytical methods for the determination of the formulants.

A study on the applicability of the CIPAC method will be provided as soon as completed (November 2001). **Data requirement maintained**.

B.5.2 Analytical methods (residues) for food and feed (IIA 4.2.1, IIIA, 5.2.1)

B.5.2.1 Animal products

No fully validated methods were submitted with the original dossier. A method validation report was submitted in JUNE 2001. Since the uses have been limited to tomato and cotton no methods for animal products are needed. In case new uses are applied in the future, methods of analysis for animal products must be evaluated and required if necessary.

B.5.2.2 Plant material

Aventis

Plant matrices (rape seed and peach)

Method: DGM F01/97-0. Method adapted from Dutch multiresidue method MRM-1. α-Endosulfan, β-Endosulfan and Endosulfan-sulphate are analysed with this method. Also Deltamethrin may be analysed simultaneously.

All compounds are extracted from sample matrix (5 g) with acetone (30 mL) followed by dichloromethane / petroleum ether (1/1 v/v) (20 mL). Later, for the analysis of samples from field trials,

this extraction step was simplified by taking a mixture of acetone / dichloromethane / petroleum ether (1/1/1 v/v/v) (50 mL) (Method DGM F01/97-1, see below). Sample is macerated in the extracting solvent. If the upper organic layer shows suspended matter, tube is centrifuged at 4000 rpm. An aliquot of 10 mL of the organic layer is taken out and it is filtered through sodium sulphate, sodium sulphate is washed with acetone. Dodecane (200 µL) are added and sample is evaporated until only dodecane is left using a vacuum rotatory evaporator (40 – 50 ºC). Two clean up steps are performed, first with a GPC column and the second with a silica gel column. Sample is dissolved in toluene and analysed by GC/ECD.

Validation: basic validation is given for the original method (two steps extraction acetone and dichloromethane / petroleum ether). Matrices employed on the fortification experiments are: potato, peach (fruit), onion (bulb), and rape (seed). Method was validated with five samples at LOQ and five samples at 10 x LOQ with two blank controls. Recoveries and RSD are within the acceptable limits for all the matrices but data are given here only for rape seed that accounts for a matrix with high fat content and supports the analytical method requirements for cotton. Further validation data are given from analysis of field trial samples. These data account for the improved method with a single extraction step (acetone / dichloromethane / petroleum ether). Matrices included are mandarin, grapevine, orange, sugar beet, melon and peach. Since matrices with high water content are included tomato may be considered to be covered by the method.

Table 5.2.2-1 Validation data for rape seed matrix

Compound	Fortification level (mg / kg)	Mean recovery	Mean recovery corrected (%)	RSD (%)	n
α-Endosulfan	0.02	68	78[1]	6.4	5
	0.2	94	94	1.7	5
	overall mean recovery	81	86	10.5	10
β-Endosulfan	0.02	76	87[2]	2.9	5
	0.2	91	91	3.1	5
	overall mean recovery	83	89	3.8	10
Endosulfan sulphate	0.02	53	81[3]	12.2	5
	0.2	95	95	3.0	5
	overall mean recovery	74	88	11.4	10

[1]Values at this fortification level were corrected for GC-response (mean: 87 %)
[2]Values at this fortification level were corrected for GC response (mean: 87 %)
[3]Values at this fortification level were corrected for GC response (mean: 65 %)

Linearity: data are near linear, however calibration curves are adjusted to parabolic equations for the evaluation of results from residue trials. For basic validation one point calibration was employed.

Limit of Quantification: the limit of quantification has been established at 0.02 mg / kg for each of teh residue components. LOQ for the complete residue definition is therefore 0.06 mg / kg.

Repeativility: RSD < 20 %.

Reproducibility: See ILV below.

GLP: Yes

Reference: Analytical method and validation for the determination of residues of Endosulfan and Deltramethrin by GC. R. Martens, 1998 (Method DGM F01/97-0, Doc C000413, Study 97/028).

Evaluation and conclusion: The method is acceptable for monitoring. However it seems that GC-ECD response is not enough stable to ensure the limit of quantification and data obtained for the 0.02 mg / kg fortification level need always to be corrected. An alternative detection method will be desirable. Confirmatory method is required.

Plant matrices (tomato)

Method: DGM F01/97-0. Same as above.

Validation: basic validation is given for the original method (two steps extraction acetone and dichloromethane / petroleum ether). Matrices employed on the fortification experiments are: cucumber, orange, melon and tomato. Method was validated with five samples at LOQ and five samples at 10 x LOQ with two blank controls. Recoveries and RSD are within the acceptable limits for all the matrices but data are given here only for tomato included in the new GAPs table.

Table 5.2.2-1 Validation data for tomato matrix

Compound	Fortification level (mg / kg)	Mean recovery	Mean recovery corrected (%)	RSD (%)	n
α-Endosulfan	0.02	87	85[1]	0.7	3[2]
	0.2	91	91	2.3	5
	overall mean recovery	89	88	4.5	10
β-Endosulfan	0.02	89	88[3]	2.6	5
	0.2	94	94	3.8	5
	overall mean recovery	91	91	4.9	10
Endosulfan sulphate	0.02	85	79[4]	5.3	5
	0.2	95	98	2.2	5
	overall mean recovery	90	89	11.7	10

[1]Values at this fortification level were corrected for GC-response (mean: 103 %)
[2]Ficve sample were fortified but test substance was lost in two of them due to too long evaporation under high vacuum.
[3]Values at this fortification level were corrected for GC response (mean: 102 %)
[4]Values at this fortification level were corrected for GC response (mean: 108 %)

Linearity: For basic validation one point calibration was employed. Linearity may not be assessed from the data provided in this study. This calibration method is not considered acceptable by Guidance document on residue analytical methods (SANCO/825/00 rev 6). Either duplicate determinations at three or more concentrations or single determinations at 5 or more concentrations must be made.

Limit of Quantification: the limit of quantification has been established at 0.02 mg / kg for each of the residue components. LOQ for the complete residue definition is therefore 0.06 mg / kg.

Repeativility: RSD < 20 %.

Reproducibility: See ILV below.

GLP: Yes

Reference: Validation of analytical method DGM F01797-0 for residues of Endosulfan and Deltamethrin in cucumber, orange, melon and tomato. 1998 (Doc C001152, Study CR 97/027).

Evaluation and conclusion: The method is acceptable for monitoring. However it seems that GC-ECD response is not enough stable to ensure the limit of quantification and data obtained for the 0.02 mg / kg fortification level need always to be corrected. An alternative detection method will be desirable.

Independent Laboratory Validation

Method: Method DGM F01/97-1 is a modification of method DGM F01/97-0 for which validation data are provided in the previously summarised reports. Both are derived from the multiresidue method MRM-1. Method DGM F01/97-1 is what was previously called DGM F01/97-0 optimised. Main difference with method DGM F01/97-0 is that a single extraction step is performed employing a mixture of acetone/dichloromethane/petroleum ether (1/1/1). Some validation data for various matrices were reported in the previously summarised reports in which these modifications were already introduced. This modified method is the one actually employed in new residue trials. Some minor modifications were made in this ILV in order to adapt the method to equipment available at the performing laboratory. As in the original method quantitation was performed by GCD / ECD.

Validation: For each matrix type, five replicates fortified at the LOQ and five replicates fortified at 10 x LOQ were analysed. Two blank samples were also analysed for each matrix.

Lettuce:

Two sets were anlysed.

First set. Mean recoveries were acceptable for β-endosulfan and endosulfan sulphate but no for α-endosulfan (122 %).

Second set. Carried out by a second analyst after changing the column. Mean recovery values for α-, β-endosulfan and endosulfan sulphate were 99.6 ± 8.02 %, 108 ± 8.45 %, 105 ± 12.2 %.

Orange:

Mean recovery values for α-, β-endosulfan and endosulfan sulphate were 95.2 ± 12.0 %, 104 ± 12.8 %, 91.4 ± 6.28 %.

Linearity: Peak area of analytes was computed for a series of five calibration standard levels. Data points were adjusted to second order polynomial regression.

GLP: Yes

Reference: Independent Laboratory Validation for the Determination of Residues of Deltamethrin in Lettuce, Oranges, Milk and Fat and Endosulfan in Lettuce and Oranges Using Method DGM F01/97-1. B. K. Haines (Xenos Laboratories), 2001. (Doc B003259, Xenos Project Number XEN00-31).

Evaluation and conclusions: Method DGM F01/97-1 has been successfully validated for lettuce and orange matrices by an independent laboratory. Since lettuce is a matrix with high water content method for tomato may also be considered to be validated by an independent laboratory. Confirmatory method has not been provided neither with the original validation nor with the ILV.

Confirmatory method: Expert Statement. A review of the available reports indicates that there is a confirmatory endosulfan plant method in addition to the primary method and the validations. However, it seems that Aventis CropScience had overlooked submission of the document earlier and is submitting it with this explanation. Details are given below.

Aventis report C000413 contains the basic endosulfan plant method of analysis (DGM F01/97-0) that was patterned after the Dutch MRM-1. In this method the compounds (alpha-endosulfan, beta-endosulfan, and endosulfan sulfate) were extracted from the matrix with acetone/dichloromethane/petroleum ether. After centrifugation and cleanup via GPC (gel permeation chromatography) and mini silica-gel column, the analytes are determined by GC with ECD detection. For this method the compounds are quantified by GC-ECD using a 30 meter 0.25 µm ALLTECH EC-1 column (i.d. 0.32 mm).

This report (C000413) includes validation of the method for potato (tuber), peach (fruit), onion (bulb) and rape (seed). Acceptable recoveries were obtained at levels of 0.02 mg/kg and 0.2 mg/kg for each compound in each matrix.

Aventis report C001152 is further validation of the method (DGM F01/97-0) for cucumber, orange, melon and tomato. It uses the same extraction and analysis techniques. Again, acceptable recoveries were obtained.

Aventis report C006935 contains validation of the method for dry crops (grain). Again, it is the same extraction and quantification techniques as C000413 and C001152. Therefore, the basic method of

analysis for endosulfan residues (alpha-endosulfan, beta-endosulfan and endosulfan sulfate) in plants has been developed and validated.

The European Commission Guidance document on residue analytical methods indicates that one acceptable approach for a confirmatory method is to use a column with a "… different stationary phase and/or mobile phase of different selectivity." Aventis report C006935 contains a confirmatory method for analysis of endosulfan residues in plants. This confirmatory method uses an alternate GC column with different polarity than the one used in the original method. The original method, as well as the validations, used an EC-1 column (or its equivalent DB-1). This is a non-polar column containing 100% dimethylpolysiloxane column. The confirmatory method contained in Aventis report C006935 uses a DB-17 column which is a medium-polarity column containing (50% phenyl)-50% methylpolysiloxane.

The data in Aventis report C006935 demonstrate that endosulfan residues can be quantified using either the DB-1 or the DB-17 column. Analysis using the DB-17 column would serve as the confirmatory method for plants.

GLP: No

Reference: Confirmatory Method Analysis for Plant Material Statement to Questions Raised by the Rapporteur Member State Sapin During the Reviw for Annex I Inclusion. Richard Heintzeman. 2001. (Report Nr. Ks-01.10.12).

Evaluation and conlusions: The employ of a different stationary phase is acceptable as confirmatory method for plants. **Data requirement fulfilled.**

B.5.3 Analytical methods (residues) soil, water, air (IIA, 4.2.2 to 4.2.4; IIIA, 5.2.2)

Aventis

Soil

Method: Method AL 60/86. The active ingredient and the metabolite endosulfan sulphate were extracted from the soil with acetone. After dilution of the extract with saline and clean-up by liquid-liquid partition with dichloromethane and silica gel column, determination was carried out by gas chromatography using ECD.

Validation. Seven untreated soil samples were fortified at 0.01 mg / kg and 0.1 mg / kg levels with alpha-, beta and endosulfan sulphate. Average recoveries and standard deviations are given in Table 5.3-1.

Table 5.3-1

Compound	Fortification level (mg / kg)	Mean recovery	RSD (%)	n
α-Endosulfan	0.01	91	16.7	7
	0.1	89.5	15.9	10
β-Endosulfan	0.01	99	19.7	7
	0.1	87	15.0	10
Endosulfan sulphate	0.01	100	19.1	7
	0.1	85.7	14.6	10

Limit of quantitation: A LOQ of 0.01 mg / kg has been probed.

Specificity: Confirmatory method available. See below.

Linearity: linearity has been demonstrated with a five point calibration curve for each residue component.

Repeatibility: RSD < 20 %.

GLP: Yes

Reference: Analysis of endosulfan residues in soil. F. Seefeld. 1990 (Doc. C008891, translation of Doc A46890).

Evaluation and conclusion: The method is acceptable and successfully validated to a LOQ of 0.01 mg/kg.

Confirmatory method: The parent compound and metabolites (endosulfan sulphate, endosulfan lactone, and endosulfan diol) are extracted from soil (50 g) with acetone (3 x 70 mL) and the extracts mixed with 600 mL NaCl solution (4g / 200 mL, Type I water). Analytes are partitioned with dichloromethane (100 mL + 2 x 50 mL). Organic phase is evaporated near dryness and reconstituted with hexane. Evaporation and reconstitution procedure is repeated till no all dichloromethane has been removed. Finally, sample is reconstituted to 1 mL in hexane and transferred to a GC vial. 0.100 mL of MSTFA are added to sylilate diol metabolite. Analytes are quantified by GC/MS.
Limit of quantification: 0.01 μg / g in 50 g soil.

Specifity: this method is proposed as confirmatory method and it is highly specific.

Linearity. linear calibration curves are provided.

GLP: no

Reference: Confirmatory Method of Analysis for Endosulfan-alpha, Endosulfan-beta, Endosulfan-diol, Endosulfan-lactone and Endosulfan-sulphate in soil. A. Callison and C. Simons. 2001. Exygen Research. 01M-019-062.

Evaluation and conclusions: The confirmatory method is acceptable and sufficient validation data have been provided.

Water (including drinking water)

Method: The analytes are extracted from water (700 mL) with hexane (50 mL) in a separatory funnel. An aliquot of 10 mL is taken out of the organic phase and 200 µL of dodecane are added. The aliquot is reduced until only dodecane remains 1 mL of toluene is added and anlytes are determined by GC with EC-detection.

Linearity: linearity is demonstrated by a calibrations curve with five concentration levels.

Specificity: no confirmatory method is provided. Detection method may not be considered highly specific.

LOQ = 0.05 µg / L both for drinking water and surface water. Recovery ranges between 77 % and 108 % and RSD is < 10 % in all cases.

GLP: Yes

Reference: Enforcement method and validation for water by GC Deltamethrin and Endosulfan. R. Martens. 1999 (C005528).

Evaluation and conclusions: The method is acceptable for alpha and beta endoslufan and endosulfan sulfate. Since, there is a validated confirmatory method for soil that constitutes a much more difficult matrix than water, and this method employs MS detector, it may be assumed that this GC-MS step could also be applied to water in other to ensure specificity. However, residue defintion in water includes also hidroxycarboxilic acid endosulfan that is not analysed by this method. **Data requirement maintained for endosulfan hidroxycarboxilic acid metabolite.**

Air

Method summarised in the monograph was considered acceptable and only a confirmatory method was required.

Confirmatory method: Endosulfan-alpha and Endosulfan-beta are collected from air onto ORBO™ tubes with Tenax® packing using an airflow pump. The analytes are extracted off the Tenax with ethyl acetate. Detection of endosulfan alpha and beta is accomplished by gas chromatography / mass spectrometry (GC/MS) analysis using selected ion monitoring (SIM).

The proposed limit of quantitation (LOQ) for this method is 2.4 µg each Endosulfan-alpha and Endosulfan-beta in 480 L air. This is equivalen to 10 µg of both standards in 1 m^3 of air. Quantification is performed using calibration standards prepared in ethyl acetate for GC/MS analysis.

Specifity: The method is a GC/MS method and therefore highly specific.

Method was validated under GLP.

GLP: Yes for the method validation.

References: Confirmatory Method for the Detection of Endosulfan-alpha and Endosulfan-beta in Air. A. Callison and C. Simon. 2001 (Exygen Research. Method Nr. 01M-019-063); Validation of confirmatory Method of Analysis for Endosulfan-alpha and Endosulfan-beta in Air. C. Simons and A. Callison. 2001. (Exygen study #019-063; Aventis Study #01BJ33040A).

Evaluation and conclusion: The method is acceptable.

B.5.4 Analytical methods (residues) wildlife and for use in support of diagnostic and therapeutic regimes (IIA, 4.2.5; IIIA 5.2)

B.5.4.1 Body Tissues

Method: The tissue sample was suspended with hexane; the volume of filtered organic extract was reduced, but not evaporated to dryness, and cleaned up using silica gel. The volume of eluate (toluene/acetone, 95:5) was reduced to exactly one millilitre and split into two portions. One portion was used to analyse the extract for α-endosulfan, β-endosulfan and the metabolites endosulfan sulphate, endosulfan lactone and hydroxyendosulfan ether by gas cromatography. The other portion of the eluate was derivatized with MSTFA and analysed for endosulfan alcohol using the same equipment.

The analytes were detected by gas cromatography with electron capture detector (GC-ECD) and for confirmation by gas cromatography with a mass spectrometer as detection system (GC-MS) operated in the negative chemical ioization mode (NCI).

The method was validated for small sample amounts of human tissue. The verified lower limit of working range was set as limit of quantification corresponding to 0.05 mg/kg for each analyte taking into account approximately 200 mg of sample material.

The following recovery data were obtained for 10 samples fortified with nominal 0.05 to 0.5 mg/kg of each analyte. The higher number of determinations for the data generated with GC-MS (NCI) are explained by replicate injections of the same extracts.

Average Recoveries and Coefficients of Variation (cv)		
Analyte	**GC-ECD**	**GC-MS (NCI)**
α-endosulfan	99 % (cv = 14, n = 10)	93 % (cv = 14, n = 12)
β-endosulfan	93 % (cv = 8, n = 10)	99 % (cv = 18, n = 12)
endosulfan sulphate	94 % (cv = 5, n = 10)	99 % (cv = 20, n = 14)
endosufan lactone	95 % (cv = 9, n = 10)	99 % (cv = 13, n = 12)
endosulfan diol	84 % (cv = 15, n = 10)	99 % (cv = 9, n = 7)
hydroxyendosulfan ether	not performed	99 % (cv = 20, n = 6)

Linearity: was demonstrated by calibration curves. The least square fit curves were calculated according to the first or second order.

GLP: Yes

Reference: Validation of a method to determine α-endosulfan, β-endosulfan, endosulfan sulphate, endosulfan alcohol, endosulfan lactone and hydroxyendosulfan ether (endosulfan aldehyde) in human tissue by GC-MS. E. Zietz, T. Egert. 1999 (C003907).

Evaluation and conclusion: The method is acceptable.

Method: EM F-05/98-0. Whole blood is hemolysed and then deproteinised. After extraction of the supernatant, blood levels are determined by GC-MS. The method can be performed in 120 minutes: Azinphos-methyl, Bendiocarb, β-Cyfluthrin, Deltamethrin, Endosulfan, Fenamiphos, Fenthion, Fluquinconazole, Heptenophos, Methaminophos, Methiocarb, Parthion-methyl, Pyrazophos, Tralomethrin, Triazophos.

These compounds can be identified down to concentrations between 100 to 100 ng / mL by comparison of their mass-spectra to those in a commercial pesticide mass –spectra library. Using the standard addition method, they can be quantified down to concentrations between 30 to 200 ng / mL. For α-endosulfan and β-endosulfan acceptable mean recoveries (105 % and 107 % respectively) and relative standard deviations (12 %) are obtained at levels of 100 ng / mL. The method has been successfully validated by an independent laboratory.

GLP: No

References: Rapid Multimethod for Verification and Determination of Toxic Pesticides in Whole Blood by Means of Capillary GC-MS. T. Frenzel, H. Sochor , K. Speer, M. Uihlein. 1998.

Independent Laboratory Validation of Method EM F-05/98-0 “Rapid Multimethod for Verification and Determination of Toxic Pesticides in Whole Blood by Means of Capillary GC-MS” According European Guidelines.

Evaluation and conclusion: the multiresidue method is acceptable to determine endosulfan in human blood samples. Confirmatory method is not necessary since MS detection is employed.

B.5.4.2 Wildlife

No methods provided. **A method for the determination of endosulfan and relevant metabolites in fish is required.** Main notifier Aventis has communicated to the rapporteur that a new method will be submitted in November 2001.

B.5.6 References relied on

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	Brennecke, R. 1998 Independent laboratory validation of method EM F-05/98-0 “Rapid Multimethod for verification and determination of Toxic Pesticides in Whole Blood by means of capillary GC-MS” According to European Guidelines Bayer AG. Report No.: MR-918/98 - Doc. No. C002476	N	N	Aventis	N
	Callison, A.; Simons, Ch. 2001 Confirmatory method of analysis for Endosulfan-alpha, Endosulfan-beta, Endosulfan-diol, Endosulfan-lactone and Endosulfan-sulfate in soil Centre method No. 01M-019-062	N	N	Qventis	N
	Frenzel, T.; Sochor, H.; Speer, K.; Uihlein, M. Rapid multimethod for verification and determination of toxic pesticides in whole blood by means of capillary GC-MS Hoechst Schering AgrEvo GmbH - Doc. No. A67646	N	N	Aventis	N
	Haines, B.; Tauber, R. 2001a Independent Laboratory validation for the determination of residues of Deltametrhin in lettuce, oranges, milk and fat and Endosulfan in lettuce and oranges using method DGM F01/97-1 Xenos Laboratories Inc. Doc. No. B003259	Y	N	Aventis	Y
	Martens, R. 1998a Validation of analytical method DGM F01/97-0 for residues of endosulfan and deltamethrin in cucumber, orange, melon and tomato Deltamethrin, endosulfan Code: AE F032640, AE F002671 Hoescht Schering AgrEvo GmbH; Rueckstaende und Verbrauchers, Frankfurt. Doc. No. C001152	Y	N	Aventis	Y
	Martens, R. 1998b Analytical method and validation for the determination of residues of endosulfan and deltamethrin by GC (1st addendum) Deltamethrin, endosulfan Code: AE F032640, AE F002671 Hoescht Schering AgrEvo GmbH; Entw. Rueckstaende und Verbrauchers, Frankfurt. Doc. No. C001652	Y	N	Aventis	Y

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	Martens, R. 1998c Analytical method and validation for the determination of residues of endosulfan and deltamethrin by GC Deltamethrin, endosulfan Code: AE F032640, AE F002671 Hoescht Schering AgrEvo GmbH; Entw. Rueckstaende und Verbrauchers, Frankfurt. Doc. No. C000413	Y	N	Aventis	Y
	Martens, M. 1999 Enforcement method and validation for water by GC Deltamethrin Endosulfan Codes AE F032640, AE F002671 Hoechst Schering AgrEvo GmbH. Study Identification CR 99/023; Doc. No. C005528	Y	N	Aventis	N
	Seefeld, F. 1990a Validation report Analysis of endosulfan residues in soil Biolog. Zentralanstalt Berlin, Kleinmachnow; Institut fuer Toxikologie und Oekotoxikologie Hoechst AG. Doc. No. C008891	Y	N	Aventis	Y
	Simons, Ch.; Callison, A. 2001 Validation on confirmatory method of analysis for Endosulfan-alpha and Endosulfan-beta in air. Doc. No. B003459	Y	N	Aventis	N
	Zietz, E.; Egert, T. 1999a Validation of a method to determine alpha-endosulfan, beta-endosulfan, endosulfan sulfate, endosulfan alcohol, endosulfan lactone and hydroxyendosulfan ether (endosulfan aldehyde) in human tissue by GC-MS Hoechst Schering AgrEvo GmbH; Residues and Consumer Safety, Frankfurt. Doc. No. C003907	Y	N	Aventis	Y

ADDENDUM TO ANNEX B

ENDOSULFAN

B - 6: TOXICOLOGY AND METABOLISM

This second addendum, corresponding to Mammalian Toxicology (Section 6) has been prepared by the Toxicology Evaluation Group of the Instituto de Salud Carlos III after the ECCO 106 Overview meeting held in York in July, 2001. It addresses the points of concern raised at that meeting and intends to clarify the position of the RMS with respect to the main open issues.

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics)

Open Point 4.2 of the Evaluation Tables

Although a value of 60% oral absorption for Endosulfan was set at the ECCO 102 (Mammalian Toxicology Meeting), the main notifier submitted in May 2001 new toxicokinetic studies, supporting higher values. Additionally, the main notifier has very recently submitted and expert report addressing (among others) this issue, which is currently undergoing evaluation by the RMS. The evaluation by the RMS of the studies submitted in May is presented below.

Rat oral single dose/ toxicokinetic study			
Autor(s):	Needham D & Gutierrez Giulianotti L	Study design:	Assessment of health condition. Acclimatisation period: 3 days. Rats housed single in a metabolism cage. Urine and faeces were collected at 6-, 12-, 24-, 48-, 72- and 96h. The animals were sacrificed 96 h after treatment (killed by aortic bleeding under deep isoflurane anesthesia), and the tssues/organs were removed for analysis.The samples were analysed by LSC. Metabolite profiling and quantification by TLC, HPLC and MS.
Study Title:	Endosulfan – [^{14}C] Code AE F002671: Distribution, metabolism and excretion in the rat following a single oral dose of 1 or 6 mg/kg body weight		
Testing facility:	AgrEvo		
Report Number:	A59694		
Study duration:	From August 15 1997 to December 19 1997	Dose:	1 or 6 mg/kg b.w.
Date of report:	1997	Vehicle/Solvent:	Corn oil
Test Substance:	^{14}C labelled endosulfan	Route:	Oral by gavage
Batch Nº.:	Z27040-0 001B99 0007	Statistics/ Measurements:	
Radiochemical purity:	98.1 %		
Test Animals:	Male and female Wistar rats	GLP:	Yes
Origin:	[redacted]	Guideline:	OECD 1981
Bodyweight:	150-180 g	Deviation:	
Groups:	4 animals/sex/group	Acceptability:	The study is acceptable

Findings

The results are summarised in Tables 6.1-1, 6.1-2, 6.1-3 and 6.1-4.

Table 6.1-1: Excretion and cumulative excretion of radiolabelled dose from rats following a single oral administration of 1 or 6 mg endosulfan/kg b.w.

SAMPLE	**TIME AFTER DOSING (h)**	**EXCRETION mean±SD (%)**			
		1 mg/kg b.w.		**6 mg/kg b.w.**	
		MALES	**FEMALES**	**MALES**	**FEMALES**
URINE	6	4.53±0.24	5.15±0.98	3.39±0.59	2.55±1.08
	12	1.85±1.22	5.40±0.83	1.63±0.60	4.00±1.34
	24	1.82±1.22	4.76±1.79	1.85±0.57	5.48±0.44
	48	0.76±0.24	2.62±0.73	0.87±0.20	5.06±1.50
	72	0.44±0.12	1.01±0.24	0.48±0.14	1.31±0.32
	96	0.30±0.10	0.54±0.13	0.30±0.09	0.72±0.23
subtotal		**9.7±2.88**	**19.49±1.62**	**8.51±1.68**	**19.12±1.42**
FAECES	24	84.91±2.28	49.56±13.69	71.52±5.29	35.75±6.19
	48	6.26±1.90	19.22±12.66	8.09±2.65	22.69±3.80
	72	1.11±0.37	3.67±1.49	1.41±0.72	5.96±1.84
	96	0.51±0.16	1.67±0.77	0.50±0.08	3.10±1.32
subtotal		**93.03±2.42**	**74.12±1.26**	**81.52±3.12**	**67.50±1.16**
CAGE WASH	6	0.45±0.09	0.63±0.31	0.57±0.27	0.38±0.30
	12	0.19±0.10	0.69±0.37	0.17±0.06	0.45±0.10
	24	0.20±0.22	0.58±0.34	0.34±0.12	0.82±0.16
	48	0.09±0.03	0.35±0.23	0.15±0.04	0.50±0.18
	72	0.05±0.02	0.17±0.08	0.06±0.01	0.13±0.02
	96	0.06±0.02	0.36±0.16	0.05±0.01	0.42±0.41
subtotal		**1.04±0.29**	**2.78±1.35**	**1.35±0.43**	**2.71±0.47**
TOTAL		**103.54±4.81**	**96.32±1.37**	**91.38±1.15**	**89.33±1.61**
CARCASS		0.93±0.34	1.93±0.72	0.79±0.26	2.70±1.15
GRAND TOTAL		**104.47±5.14**	**98.32±1.72**	**92.18±0.98**	**92.03±1.28**

Table 6.1-2: Quantification of endosulfan metabolites following hexane extraction from faeces.

METABOLITES (Time point, hr)	MALE		FEMALE	
	high dose (% dose)	low dose (% dose)	high dose (% dose)	low dose (% dose)
endosulfan α y β				
24	16.58	29.30	1.24	7.00
48	0.07	0.10	0.19	0.23
72	0.15	0.01	0.01	ND
96	Trace	Trace	Trace	ND
Total	**16.795**	**29.41**	**1.44**	**7.24**
hydroxy endosulfan ether				
24	0.85	0.42	0.91	0.74
48	0.05	0.08	0.14	0.16
72	Trace	0.02	0.025	0.01
96	Trace	Trace	0.02	0.01
Total	**0.91**	**0.51**	**1.09**	**0.94**
endosulfan sulphate				
24	0.72	2.22	0.22	1.05
48	ND	0.01	0.075	0.07
72	Trace	ND	0.03	0.077
96	ND	ND	0.02	0.01
Total	**0.72**	**2.23**	**0.34**	**1.21**
endosulfan lactone				
24	0.46	0.95	0.23	ND
48	0.04	0.06	0.06	0.1
72	Trace	ND	0.04	0.07
96	ND	ND	0.01	0.01
Total	**0.51**	**1.01**	**0.34**	**0.18**
endosulfan diol				
24	0.25	0.52	0.25	ND
48	0.01	0.03	0.05	0.07
72	Trace	0.01	0.04	0.05
96	Trace	0.01	0.018	0.01
Total	**0.26**	**0.57**	**0.36**	**0.13**
endosulfan ether				
24	ND	ND	ND	ND
48	ND	ND	ND	ND
72	ND	ND	Trace	ND
96	ND	ND	Trace	ND
Total	**--**	**--**	**--**	**--**
unknown (RT=9.01 min)				
24	ND	ND	ND	ND
48	ND	ND	ND	ND
72	ND	ND	ND	ND
96	ND	Trace	ND	ND
Total	**--**	**--**	**--**	**--**
unknown (RT=9.63 min)				
24	ND	ND	ND	ND
48	ND	0.01	ND	ND
72	ND	ND	0.01	ND
96	ND	ND	Trace	ND
Total	**--**	**0.01**	**0.01**	**--**
unknown (RT=16.13 min)				
24	ND	0.21	ND	ND
48	ND	ND	ND	ND
72	ND	ND	ND	ND
96	ND	ND	ND	ND
Total	**--**	**0.21**	**--**	**--**

Table 6.1-3: Quantification of endosulfan metabolites in urine, at high dose, following incubation with glucuronidase/sulfatase.

Time (hr)	MALE (% dose)						
	Endosulfan diol	**Hydroxy endosulfan ether**	**Endosulfan lactone**	**Unknown 1RT=8 min**	**Unknown 2RT=10.40 min**	**Polar**	**Mean % dose in urine**
6	ND	ND	ND	ND	0.11	3.28	3.37
12	ND	ND	ND	ND	0.16	1.47	1.63
24	0.13	ND	ND	ND	ND	1.72	1.85
48	ND	ND	ND	ND	ND	0.87	0.87
72	ND	ND	ND	ND	ND	0.48	0.48
96	ND	ND	ND	ND	ND	0.30	0.30
Total	**0.13**	**ND**	**ND**	**ND**	**0.27**	**8.12**	**8.52**
	FEMALE (% dose)						
	Endosulfan diol	**Hydroxy endosulfan ether**	**Endosulfan lactone**	**Unknown 1RT=8 min**	**Unknown 2RT=10.40 min**	**Polar**	**Mean % dose in urine**
6	0.13	0.15	ND	ND	0.10	2.17	2.55
12	0.13	0.12	0.18	ND	0.23	3.34	4.00
24	0.12	0.20	ND	0.57	0.24	4.16	5.48
48	0.27	0.32	ND	0.09	0.34	2.53	5.06
72	ND	ND	ND	ND	ND	1.31	1.31
96	ND	ND	ND	ND	ND	0.72	0.72
Total	**0.65**	**0.79**	**0.18**	**0.66**	**0.91**	**14.23**	**19.12**

Table 6.1-4: Quantification of endosulfan metabolites in urine, at low dose, following incubation with glucuronidase/sulfatase.

Time (hr)	MALE (% dose)		FEMALE (% dose)	
	Mean % dose in urine	**Polar**	**Mean % dose in urine**	**Polar**
6	4.53	4.53	5.15	5.15
12	1.85	1.85	5.40	5.40
24	1.82	1.82	4.76	4.76
48	0.77	0.77	2.62	2.62
72	0.44	0.44	1.01	1.01
96	0.30	0.30	0.55	0.55
Total	**9.71**	**9.71**	**19.49**	**19.49**

Conclusions

In summary, total excretion percentages (96 hours) of 100% (males) and 96.32% (females) in rats dosed at 1 mg/kg b.w., and 91.38% (males) and 89.33% (females) in rats dosed at 6 mg/kg b.w., were obtained. Moreover, in the quantification studies of endosulfan metabolites, α- and β-endosulfan were found in faeces at percentages of 16.8 (male)-1.44 (female)% at the high dose, and 29.41(male)-7.24(female)% at the low dose. Assuming these percentages, as well as the radioactivity percentages found in carcass, the rate of absorption estimated on the basis of total excretion and parent compound unchanged in faeces, was 70% in males, and 87% in females, within 96 h in rats. **Based on the reported data, the rate of absorption was estimated to be 70% in males, and 87% in females, within 96 h in rats.**

These oral absorption values are provisional, pending evaluation of the expert statement recently submitted by Aventis.

Open Point 4.3. of the Evaluation Tables

At the ECCO 102 Mammalian Toxicology Meeting, the question whether endosulfan might accumulate in the body was raised, based on data indicating relatively long t½ values for the liver and the kidney. This question could not be addressed at the ECCO 106 Overview Meeting, and therefore a clarification is provided in this Addendum. The main notifier has provided a new study with recently generated data. The evaluation by the RMS is presented below:

Rat oral repeated daily dose/ toxicokinetic studies			
Autor(s):	Needham D & Creedy CL & Hemming PA	**Study design:**	Assessment of health condition. Acclimatisation period: 3 days. Rats housed single in a metabolism cage. Groups of 4+4 animals were killed 24 h after receiving 1,10,16,22 and 28 doses and blood and tissues were removed. A further group (4+4) were placed into metabowls after receiving 28 doses and urine and faeces were collected over the next 4 days and some samples were collected. The final group (4+4) also received 28 doses and blood samples were collected at 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hr after termination, and some samples were collected. Samples were analysed by LSC, and metabolites were identified by HPLC.
Study Title:	Endosulfan – [^{14}C] Code AE F002671 00 1E: Toxicokinetics in the rat following repeated daily oral administration of 1 mg/kg bodyweight for up to 28 days		
Testing facility:	AgrEvo		
Report Number:	A67138		
Study duration:	From June 18 1997 to April 9 1998	**Dose:**	1 mg/kg b.w. (28 days)
Date of report:	1998	**Vehicle/Solvent:**	Corn oil
Test Substance:	^{14}C labelled endosulfan	**Route:**	Oral by gavage
Batch Nº.:	Z27052-0	**Statistics/ Measurements:**	
Radiochemical purity:	98.6 %		
Test Animals:	Male and female Wistar rats	**GLP:**	Yes
Origin:	[redacted]	**Guideline:**	OECD 1981
Bodyweight:	135-160 g	**Deviation:**	
Groups:	64 animals, 8 groups, 4 animals/sex/group	**Acceptability:**	**The study is acceptable**

Findings

The results show that there was an increase in the concentration of residues in the tissues of male and female rats following repeated daily oral dosing of 1 mg/kg b.w.. In most tissues, the residues reached a maximum value by days 23 (greatest concentration in kidneys, 42.7 mg/kg b.w. males and 31.6 mg/kg

b.w. females. Examination of the residues in the fat and kidney showed that endosulfan sulphate was the major component in the fat, and that all of the residue in the kidney was associated with polar compounds.

The overall recovery of radioactivity, administered over 28 days, was 9.253±0.486 % (males) and 9.794±0.352 % (females). 12.7±1.7 % of the recovered radioactivity was found in the urine, and 65.5±3.5 % in the faeces.

Residues in the blood of rats after receiving the last od 28 daily doses (1 mg/kg b.w.) indicated that there was a sex difference seen with the maximum residue concentration found in the blood of male rats being higher than in the case of female rats (1.48-2.05 mg/kg b.w. for males, and 0.649-0.748 mg/kg b.w. for females), and this is supported by a slower elimination half life in the male than in the female rats (128.2-184.8 h compared with 91.49-111.9 h).

Conclusions

Following repeated daily oral dosing of 1 mg endosulfan/kg b.w., the concentration of radioactive residues in all tissues increased with increasing dosing and reached a maximum value within 22 doses in the case of most of the tissues examined. Apart from the liver and kidney, the concentration of endosulfan residues peaked at 0.244-1.211 mg/kg b.w. in the case of the male rats, and 0.298-3.044 mg/kg b.w. in the case of the female rats. The reproductive organs did not contain residue levels greater than general tissues, neither did they display a greater degree of accumulation of endosulfan residues.

Following cessation of dosing, the concentration of radioactive residues in all of the tissues fell significantly over the next 5 days to levels that for most tissues were similar to those seen 24 h after a single oral dose.

The mean maximum concentration of endosulfan residues in the blood were found to be 1.64 and 0.685 mg/kg for male and female rats respectively 6-8 h after receiving the last dose. The terminal half-life in the blood to be 97.75 h for female and 146.6 h for males.

The profile of excretion of dosed radioactivity did not appear to be significantly affected by repeated daily administration of endosulfan.

Data requirement 4.2 of the Evaluation Tables

At the ECCO 102 Mammalian Toxicology Meeting, during the discussion on endosulfan metabolism, the amounts of unchanged endosulfan present in the urine in the rat metabolism study (Dorough et al, 1978; IIA, 5.1.1/01) were queried. This question could not be addressed at the ECCO 106 Overview Meeting, and therefore a clarification is provided in this Addendum. Since the original reports of the above mentioned study were not available, the main notifier has provided new studies with recently generated data to address this question. The evaluation by the RMS is presented below:

The following new toxicokinetics and metabolism studies with endosulfan have been included in this revaluation:

- Needham & Gutierrez-Giulianotti, 1997 (Doc. Nº A59694). The evaluation of this study is included above, in the section dealing with oral absorption of the present addendum (see above, reply to open point 4.2 of the evaluation tables).

- Needham, Creedy & Hemmings, 1998 (Doc. Nº A67138). The evaluation of this study is included above, in the section dealing with accumulation of the present addendum (see above, reply to open point 4.3 of the evaluation tables).

- Needham, 2001 (Doc. Nº C010989).

- Buerkle, 2001 (Doc. Nº C013032)

In **summary**, in the quantification studies of endosulfan metabolites 96 h after oral administration of a single dose of 1 or 6 mg/kg bw, percentages of α- and β-endosulfan in faeces of 16.8 (male)-1.44 (female)% at the high dose, and 29.41 (male) and 7.24 (female)% at the low dose were obtained. These data are more reliable than those reported in the above-mentioned original study by Dorough *et al*., because the design of the study was more robust, it was performed with a more sophisticated technology, and the individual metabolites were quantified by TLC, HPLC and MS (as opposed to simple extraction of fractions, in the original study by Dorough *et al*.).

Rat oral single dose/ toxicokinetic study	
Autor(s):	Needham D & Gutierrez Giulianotti L
Study Title:	Endosulfan – [^{14}C] Code AE F002671: Distribution, metabolism and excretion in the rat following a single oral dose of 1 or 6 mg/kg body weight
Report Number:	A59694
Date of report:	1997
Other study details:	See study design, additional details above (reply to Open Point 4.1 of the Evaluation Tables).

Findings

See results above (reply to Open Point 4.1 of the Evaluation Tables).

Conclusions (re. accumulation of endosulfan)

Following the administration of either 1 or 6 mg endosulfan/kg b.w., the dose was well absorbed by both male and female rats and excreted mainly in the faeces, There was a sex-related difference in both the level of faecal excretion and the amount of unchanged endosulfan present in faeces with both figures being higher in male rats than in females at both dose levels.

Tissue residue levels, 4 days after dosing, were low in both male and female rats, with the highest concentration being found in the kidneys. There was also a sex-related difference in the residue levels in fat with the concentration found in female rats being up to 1 order of magnitude greater than those found in male rats.

Apart from unchanged α- and β-endosulfan, the hydroxyendosulfan ether, endosulfan sulphate, lactone, diol and ether (high dosed females only) were found to be excreted in the faeces, and the glucuronide or sulphate conjugates of hydroxyendosulfan ether and endosulfan diol were excreted in the urine. The main portion of the dose was excreted as polar metabolites in both urine and faeces.

Rat oral single dose/ metabolite studies			
Autor(s):	Needham D	**Study design:**	Assessment of health condition. Acclimatisation period: 3 days. Rats housed single in a metabolism cage. Urine and faeces were collected at 6-, 12-, 24-, 48-, 72- and 96h. The animals were sacrificed 96 h after treatment (killed by cervical dislocation), and the carcass was retained and digested. The samples were analysed by LSC.
Study Title:	Endosulfan – [^{14}C]: Rat-Analysis of polar metabolites following a single oral dose of 6 mg/kg bodyweight		
Testing facility:	Aventis		
Report Number:	C010989		
Study duration:	From November 26 1998 to April 23 1999	**Dose:**	6 mg/kg b.w.
Date of report:	2001	**Vehicle/Solvent:**	Corn oil
Test Substance:	^{14}C labelled endosulfan	**Route:**	Oral by gavage
Batch Nº.:	Z27052-0 001B99 0007	**Statistics/ Measurements:**	
Radiochemical purity:	95.19 %		
Test Animals:	Male and female Wistar rats	**GLP:**	Yes
Origin:	[redacted]	**Guideline:**	OECD 1997
Bodyweight:	174-187 g	**Deviation:**	
Groups:	4 animals/sex	**Acceptability:**	**The study is acceptable**

Findings

The excretion of endosulfan, following oral dosing, are summarised in Table 6.1-5. The apolar metabolites of endosulfan had been identified by HPLC, and 6 metabolic peaks were isolated by GC/MS (α- and β-endosulfan as the main metabolites). A new metabolite of endosulfan has been identified in this study (by further oxidation of endosulfan ether). The metabolites identified in purified extracts of faeces and urine from male and female rats, by GC-MS, are summarised in Table 6.1-6 and 6.1-7, respectively. The metabolites identified in purified extracts of faeces and urine from male and female rats, by LC-MS (detection of sulphate conjugates and possible polymers), are summarised in Table 6.1-8 and 6.1-9, respectively.

Table 6.1-5: Final mean totals for excretion of radio labelled dose from rats following single oral administration of 6 mg/kg b.w.

SEX	TOTAL EXCRETION OF ENDOSULFAN (% DOSE)				
	URINE	FAECES	CAGE WASH	CARCASS	TOTAL
MALE	12.09	85.63	2.18	4.63	104.54
FEMALE	18.67	76.62	3.00	4.85	103.15

Table 6.1-6: Metabolites identified in purified extracts of faeces, by GC-MS.

SAMPLE	RETENTION TIME (min)	IDENTITY
TOX97098A-1120	12.36	Endosulfan diol TMS
TOX97098A-1121	11.14 11.37 12.22	Hydroxyendosulfan ether Hydroxyendosulfan ether TMS Endosulfan lactone
TOX97098A-1122	11.13 11.37 12.21	Hydroxyendosulfan ether Hydroxyendosulfan ether TMS Endosulfan lactone
TOX97098A-1123	10.68 11.13 12.21 14.08	Endosulfan ether Hydroxyendosulfan ether (weak) Endosulfan lactone Endosulfan sulphate
TOX97098A-1124	10.68 12.22 13.57	Endosulfan ether Endosulfan lactone β-endosulfan
TOX97098A-1125	10.68 12.70	Endosulfan ether α-endosulfan

Table 6.1-7: Metabolites identified in purified extracts of urine, by GC-MS..

SAMPLE	RETENTION TIME (min)	IDENTITY
TOX97098A-731	11.12 11.38	Hydroxyendosulfan ether Hydroxyendosulfan ether TMS
TOX97098A-733	12.04	Hydroxyendosulfan ether TMS Dihydroxyendosulfan ether TMS

Table 6.1-8: Metabolites identified in purified extracts of faeces, by LC-MS..

SAMPLE	RETENTION TIME (min)	IDENTITY
TOX97098A-1078	27.89/29.01 28.66 29.64	Dihydroxyendosulfan ether sulphate Endosulfan diol sulphate Hydroxyendosulfan ether sulphate
TOX97098A-1178	28.76/30.09 29.29/29.60 30.62	Dihydroxyendosulfan ether sulphate Endosulfan diol sulphate Hydroxyendosulfan ether sulphate

Table 6.1-9: Metabolites identified in purified extracts of urine, by LC-MS..

SAMPLE	RETENTION TIME (min)	IDENTITY
TOX97098A-724	3.21	Dihydroxyendosulfan ether sulphate
TOX97098A-731	3.21 3.49	Dihydroxyendosulfan ether sulphate Endosulfan diol sulphate
TOX97098A-732	3.18 3.81/4.82	Dihydroxyendosulfan ether sulphate Dihydroxyendosulfan ether
TOX97098A-733	4.82	Dihydroxyendosulfan ether
TOX97098A-726	3.81 3.67	Dihydroxyendosulfan ether Dihydroxyendosulfan ether sulphate
TOX97098A-727	3.77	Dihydroxyendosulfan ether sulphate

Conclusions

A number of polar metabolites have been identified. These are mainly derived from dihydroxyendosulfan ether (parent compound), 2 isomeric sulphate conjugates and 1 disulphate conjugate. These metabolites accounted for approximately 2.1-8.6 % of the dose in the urine, and further 5.5-8.6 % of the dose in the acetonitrile extract of the 0-24 h faeces.

The remaining polar metabolites remain unidentified. The behaviour of these metabolites on HPLC, the probability that they were not protein conjugates, and the failure to detect any endosulfan-derived molecules in by HPLC/MS suggests that they may be polymers of the dialdehyde tautomer of dihydroxyendosulfan ether.

Author(s):	Buerkle LW , 2001	**This report is a summary of the three previous reports:** **Needham & Gutierrez Giulanotti, (1997; A59694)** **Needham *et al.* (1998; A67138)** **Needham (2001; C010989)**
Study Title:	Summary of New ADME Studies with Rats and Comparison of Rat and Plant Metabolism	
Testing facility:	Aventis	
Report Number:	C013032	

B.6.8.1 Toxicity of metabolites

B.6.8.1.1 Endosulfan lactone

Summary

The main notifier had been requested to address the toxicity of endosulfan-lactone. This data requirement was confirmed at the ECCO 106 Overview Meeting (data requirement 4.5 and open point 4.6).

Previous acute oral toxicity studies of endosulfan-lactone in rats had been performed (see evaluation in the addendum to Annex B of the Endosulfan Monograph of may 2001). These studies were not considered acceptable because there were some deficiencies in their performance and the purity of the test substance was not reported in any of them. From the results of these studies, the lower oral LD50 in

male rats was considered to be 105 mg/kg bw. According to Commission Directive 2001/59/EC, endosulfan-lactone should be classified as T, R25 “Toxic if swallowed”.

A new study has been submitted and evaluated (Griffon, B. and Guillaumat, P.O., 2001 (Aventis Crop Science, C 013506). This study showed that males were more sensitive to the test substance than females as 4/5 males died with the dose level of 200 mg/kg b.w. In this sense the LD50 should be calculated for males (< 200 mg/kg bw) instead of been expressed as male and female combined LD50 (273 mg/kg b.w).

In conclusion, from the information given by these studies it can be postulated that LD50 for males is < 200 mg/kg b.w. Therefore endosulfan-lactone should be considered a toxicologically significant metabolite.

The metabolite endosulfan lactone has not been included in the plant residue definition for the proposed uses, that only covers FRUITS. Nevertheless this metabolite is present in equilibrium with endosulfan hydroxycarboxylic acid, metabolite included in the water residue definition and present in tomato and cucumber leaves. It is necessary to point out that tea was proposed as imported crop, tea is classified as leafy crop and there is not available a metabolism study on leafy crops, with the available information the residue profile in leaves may be different than the proposed residue definition for fruit crops. **There are no toxicological data about endosulfan hydroxycarboxylic acid. Therefore toxicity studies or information should be submitted in order to determine the full toxicological profile of this metabolite**.

As endosulfan lactone is in equilibrium with endosulfan hydroxy carboxylic acid and the detection of endosulfan hidroxicarboxilic acid does not demostrate the absence of endosulfan lactone, further subchronic and genotoxic studies are required for endosulfan lactone.

Endosulfan-lactone (AE F051328) acute oral toxicity in rats
Griffon, B. and Guillaumat, P.O., 2001 (Aventis Crop Science, C 013506)

Dates of experimental work: 31 July 2000- 2 August 2000

Date of report: 24 April 2001

Objectives: The study was conducted to evaluate the toxicity of the test substance endosulfan-lactone (AE F051328) following a single oral administration in rats.

Guidelines: OECD Guideline No. 401 (1987); EC Directive 92/69/EEC, Method B.1. Some deviations of the protocol have been observed, the relative humidity recorded in the animal room was sometimes outside of the target ranges 30-70% and the females given the test substance at the dose level of 200 mg/kg were not weighed at the end of the observation period (day 15). These deviations were

considered not to have compromised the validity or integrity of the study. However, the dose levels used to determine the LD_{50} in males have not been the most appropriate.

GLP: Yes.

The study was not validated. It does not allow establishing the LD_{50} for male rats, the most sensitive sex.

Materials and Methods

The acute oral toxicity of the test substance endosulfan-lactone (AE F051328) (batch No. 0161X: white powder containing AE F051328 (purity: 96.7%) was evaluated in rats. The test substance was administered by oral route (gavage) to groups of five male and five female fasted Wistar rats. The test substance was prepared in 0.5% carboxymethylcellulose and administered to the animals under a volume of 10 ml/kg, at three dose levels, 200, 600 and 1000 mg/kg b.w.

The animals were checked for clinical signs, mortality and body weight gain for a period of up to 14 days following the single administration of the test substance. A necropsy was performed on each animal.

The LD_{50} was calculated according to Probit's method (Weber, 1972; Bliss, 1938). The 70 to 95% confidence interval limits were calculated statistically according to Fieller's method (1944).

Findings

At the 200 mg/kg b.w. dose level, 4/5 males were found dead on day 2. Piloerection was observed prior to death in these animals as well as in the surviving male on day 1. Hypoactivity and piloerection were recorded in one female on day 2.

At the 600 mg/kg b.w. dose level, 5/5 males and 2/5 females were found dead on day 2 or 4. Hypoactivity, piloerection and dyspnea were observed in these animals prior to death as well as in the surviving animals up to day 3.

At the 1000 mg/kg b.w. dose level, 4/5 males and 5/5 females died within the hours following the treatment; no clinical signs were observed prior to death. In the remaining animal, sedation, piloerection, tremors and dyspnea were recorded on day 1; it was found dead on day 2.

The overall body weight gain of the surviving animals was not affected by treatment with the test substance.

Macroscopic examination of all animals revealed no apparent abnormalities.

Table 6.8.1.1-1: Mortality data after administration of three dose levels of endosulfan-lactone to male and female rats

Dose (mg/kg b.w.)	No. of deaths		Day of death			
			Males		Females	
	Males	Females	d.1	d.2	d.1	d.2
200	4/5	0/0	0	4	0	0
600	5/5	2/5	0	4	0	2
1000	5/5	5/5	4	1	5	-

Conclusions

The notifier concluded that the acute median lethal oral dose (LD_{50}) of the test substance endosulfan-lactone (AE F051328) is 273 (81-436) mg/kg b.w. for males and females combined with 95% confidence interval limits. The RMS disagree with this conclusion and concludes that males were more sensitive to the test substance than females as 4/5 males died with the dose level of 200 mg/kg b.w. In this sense the LD_{50} should be calculated for males (< 200 mg/kg bw) instead of been expressed as male and female combined LD_{50} (273 mg/kg b.w).

In conclusion, from the information given by these studies it can be postulated that LD_{50} for males is < 200 mg/kg b.w. Therefore endosulfan-lactone should be considered a toxicologically significant metabolite.

From the results it can be concluded that males are more sensitive to the test substance than females. Therefore, separate LD_{50} values for males and females should be calculated and reported by the notifier. This has important implications, because the use of separate LD_{50} values for males and females might lead to a different classification than the use of a combined LD_{50} value.

B.6.15 References relied on

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	Buerkle LW 2001 Summary of New ADME Sutdies with Rats and Comparison of Rat and Plant Metabolism Aventis Study No. C013032	N	N	Aventis	Y
	Griffon, B. and Guillaumat, P.O. 2001e Endosulfan-lactone (AE F051328) acute oral toxicity in rats Aventis Crop Science, C 013506	Y	N	Aventis	Y
	Needham D 2001a Endosulfan – [^{14}C]: Rat-Analysis of polar metabolites following a single oral dose of 6 mg/kg bodyweight Aventis Study No. C010989	Y	N	Aventis	Y

ADDENDUM TO ANNEX B

ENDOSULFAN

B - 7: RESIDUE DATA

B.7 Residue data

This addenda has been prepared by the RMS (Spain) after the overview meeting ECCO 106 held in York (UK) on 13-17 July 2001. At the Overview meeting the main notifier, (Task Force Aventis/Makhteshim), submitted a new list of supported uses. This new list of supported uses is included in the table 7-1. The re-assessment and the new consumer risk assessment have been made based on the data requirements of the evaluation table (Doc. SANCO/4326/2001 rev.0-2 (18.07.01) after the ECCO 106 and the new list of GAPs.

After the ECCO 104 the RMS received a full residue data package of studies finalised before the monograph was been prepared. These studies had been required by the RMS in several contacts with the notifier. Those studies considered esential to support the actual GAP have been taken into consideration and have been evaluated, the rest of studies have not been evaluated by the RMS.

Table 7.1: Summary of intended uses

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hl min max	water l/ha min max	kg as/ha min max		
Cotton	Southern Europe		F	Chew+suck.insects, mites	EC	350 g/l	Medium/high volume spraying	Last application when balls are partly open	3	14-21	0.0105	800	0.84	21	
Tomatoes	Southern Europe		F	Chew+suck.insects, mites	EC	350 g/l	Medium/high volume spraying	At any stage	2	14	0.053-0.105	500-1000	Maz 0.53	3	
			G						2	14	0.053	1500	0.8	3	

Remarks:

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

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

The new GAP includes two uses, cotton and tomato. A metabolism study on tomato (Buerkle and Würz, 1990; Doc. No.:A44894), a metabolism study on cucumber (Buerkle, 1995. Doc No.: A56011) and a metabolism study on apple (Schwab, W., 1995. Doc. No.: A53662) were included in the draft monograph. The three studies were considered acceptable by the RMS and the ECCO 104 and it was agreed that metabolites were sufficiently identified as α-endosulfan, β-endosulfan and endosulfan sulphate.

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

On July 2001 the RMS received the following studies

- Reynolds C.M.M. 1996a. A56354. Endosulfan Distribution, elimination and the nature of the metabolite residues in the eggs and edible tissues of the laying hen.
- Leah J.M., Reynolds C.M:M. 1996a. A57041. Endosulfan. Distribution, elimination and the nature of the metabolite residues in the milk and the edible tissues of a lactating cow.
- Indranignsih, McSweeney C.S., Ladds P.W. 1992a. A51447. Residues of endosulfan in the tissues of lactating goats.

The submission of the original dossier to the RMS was made on 1996 and in this submission the mentioned studies were not included, the draft monograph was finalised on 1999, after several contacts and meetings with the notifier, in the draft monograph the information concerning the metabolism in livestock were considering insufficient and a data requirement was proposed. The ECCO 104 confirmed this data requirement.

Actually the endosulfan uses in EU are cotton and tomato, therefore the ingestion of feed containing endosulfan residues by domestic animals is not expected, and obviously residues in products of animal origin are not expected. Therefore the data requirement 5.5 should not be considered for Annex I inclusion and the evaluation and assessment of the mentioned studies are not necessary for Annex I inclusion and should be made at MS level.

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

The definition of the residue for both risk assessment and GAP monitoring purposes should be considered as the **parent compound (α and β isomers)** and its main and most toxic metabolite **endosulfan sulphate** but this residue definition only cover **FRUITS**.

The ECCO 102 (Toxicology) considered the endosulfan lactone as a toxicologically significant metabolite, based on the results from acute toxicity studies, although its acute toxicity (LD_{50} = 105 mg/kg be) was lower than that of the parent compound, endosulfan (LD_{50} = 10 mg/kg bw). The Overview meeting ECCO 106, required further toxicological studies on endosulfan-lactone. The notifier

announced at the ECCO 106 that the LD_{50} of endosulfan lactone is 273 mg/kg bw, but this study was not validated by the RMS and the results of this study does not allow to calculate the LD_{50} for each sex. ($LD_{50}(♂) < 200$ mg/kg bw).

With the available plant metabolism studies the covered crop category is **fruits**. A plant metabolism study on oil seeds should not be required since cotton seed is not used for human consumption. The residue definition for **FRUIT CROPS** is α-endosulfan, β-endosulfan and endosulfan sulphate. The residue on other category of crops (root and tuber crops, leafy crops, oilseed crops, pulses and legume crops) are not covered by this residue definition. The notifier included soyabean and tea as imported crops, the residue in soyabean is not covered by the actual residue definition, because there is not a plant metabolism study on oilseeds, this data requirement was classified to be dealt with at Member State level (Data requirement 5.2) in the Overview Meeting. The data requirement 5.4 is related to the residue definition on leafy crops, specially on tea. The available information does not allow proposing a residue definition on leafy crops, for imported tea, but the different metabolite profile in tomato and cucumber leaves allow suspecting that the residue in leaves could differ from the residue in fruits, based on that reason the data requirement 5.4 was proposed in the ECCO 104. The notifier concluded that different metabolic profiles in the leaves are a result of rate differences in the individual reaction steps. However, they do not have an influence on the metabolic pattern in the edible fruits, because endosulfan and its metabolites are not systemic. The RMS agrees with this conclusion, but it is clear that the metabolic profile in leaves could be different than the residue in fruits and other metabolites, not included in the actual residue definition, might be included in the residue definition for other crops categories. **This issue should be discussed in the evaluation group.**

The endosulfan lactone metabolite was classified as a toxicological relevant based on its acute toxicity. This metabolite does not appear in fruit but could appear in leaves since the available information demonstrated that a 24% TRR was hydroxy endosulfan carboxylic acid that it is in equilibrium with the lactone metabolite. **The residue definition for leafy crops, as tea (imported crop), must be reviewed and the lactone metabolite may be included in the residue definition.**

For Annex I inclusion only the use on tomato and cotton is supported by the available data and the residue definition for fruits is Endosulfan (α+β) and endosulfan sulphate.

B.7.4 Use pattern

This addenda has been prepared by the RMS (Spain) after the overview meeting ECCO 106 celebrated at the PSD on 13-17 July 2001. At the Overview meeting the main notifier, Aventis, submitted a new list of supported uses. This new list of supported uses is included in the table 7.4-1. The re-assessment and the new consumer risk assessment have been made based on the data requirements of the evaluation table (Doc. SANCO/4326/2001 rev.0-2 (18.07.01) after the ECCO 106 and the new list of GAPs.

Table 7.4-1: Summary of intended uses

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hl min max	water l/ha min max	kg as/ha min max		
Cotton	Southern Europe		F	Chew+suck.insects, mites	EC	350 g/l	Medium/high volume spraying	Last application when balls are partly open	3	14-21	0.0105	800	0.84	21	
Tomatoes	Southern Europe		F	Chew+suck.insects, mites	EC	350 g/l	Medium/high volume spraying	At any stage	2	14	0.053-0.105	500-1000	Maz 0.53	3	
			G						2	14	0.053	1500	0.8	3	

Remarks:

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.7.5 Identification of critical GAPs

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks: (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hl min max	water l/ha min max	kg as/ha min max		
Cotton	Southern Europe		F	Chew+suck.insects, mites	EC	350 g/l	Medium/high volume spraying	Last application when balls are partly open	3	14-21	0.0105	800	0.84	21	
Tomatoes	Southern Europe		F	Chew+suck.insects, mites	EC	350 g/l	Medium/high volume spraying	At any stage	2	14	0.053-0.105	500-1000	Maz 0.53	3	
			G						2	14	0.053	1500	0.8	3	

Remarks:

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.7.6 Residue resulting from supervised trials (IIA, 6.3; IIIA, 8.2)

A re-assessment of all the residue trials submitted by the main notifier has been made taking into account the new GAP submitted by the main notifier on August 2001.

B.7.6.1 Fruiting vegetables

B.7.6.1.1 Tomato

The use on tomato is summarised in table 7.6.1.1-1.

Table 7.6.1.1-1: Critical GAP on tomato

Crop and/ or situation (a)	Member State or Country	F G or I (b)	Formulation		Application				Application rate per treatment			PHI (days) (l)
			Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hl min max	water l/ha min max	kg as/ha min max	
Tomatoes	Southern Europe	F	EC	350 g/l	Medium/high volume spraying	At any stage	2	14	0.053-0.105	500-1000	Maz 0.53	3
		G					2	14	0.053	1500	0.8	3

Table 7.6.7-2: Summary of supervised trials for fruiting vegetables according the critical GAP

Crop/ Variety	Country/ Year	F or G	Form.	Application rate		Nº	Growth Stage	Portion analysed	Residue (mg/kg)	PHI (days)	Ref.
				kg a.s/ha	conc % a.s						
Tomato Prieto	Spain (S) 1993	G	EC 352 g/l	0.5376 0.5376	0.0528 0.0528	2		fruit fruit fruit fruit	0.2 0.1 0.05 0.03	0 **-> 3** 7 14	A54361
Tomato Prieto	Spain (S) 1993	G	EC 352 g/l	1.0752 1.0752	0.1056 0.1056	2		fruit fruit fruit fruit	0.38 0.2 0.13 0.09	0 **->3** 7 14	A54361
Tomato Maiorca	Italy (S) 1993	G	EC 352 g/l	0.8975 0.8975	0.0528 0.0528	2	11-19 11-19	fruit fruit fruit fruit	0.31 0.08 0.32 0.07	0 **-> 3** 7 14	A54361
Tomato Maiorca	Italy (S) 1993	G	EC 352 g/l	1.7954 1.7954	0.1056 0.1056	2	11-19 11-19	fruit fruit fruit fruit	0.8 0.37 0.08 0.01	0 **-> 3** 7 14	A54361

Crop/ Variety	Country/ Year	For G	Form.	Application rate		Nº	Growth Stage	Portion analysed	Residue (mg/kg)	PHI (days)	Ref.
				kg a.s/ha	conc % a.s						
Tomato Presto	Spain (S) 1994	G	EC 352 g/l	1.074 0.809	0.0528 0.0528	2	22 23	fruit fruit fruit fruit fruit fruit	0.22 0.11 0.1 0.05 < 0.03 < 0.03	0 **-> 3** 7 14 21 29	A54360
Tomato Presto	Spain (S) 1994	G	EC 352 g/l	1.919 1.655	0.1056 0.1056	2	22 23	fruit fruit fruit fruit fruit fruit	0.32 0.29 0.23 0.15 0.13 0.05	0 **->3** 7 14 21 29	A54360
Tomato Caruso	Spain (S) 1994	G	EC 352 g/l	0.616 0.720	0.0528 0.0528	2	22 23	fruit fruit fruit fruit fruit fruit	0.14 0.06 0.04 0.04 < 0.03 < 0.03	0 **-> 3** 7 14 21 29	A54360
Tomato Caruso	Spain (S) 1994	G	EC 352 g/l	1.168 1.121	0.1056 0.1056	2	22 23	fruit fruit fruit fruit fruit fruit	0.17 0.21 0.13 0.07 0.04 < 0.03	0 **->3** 7 14 21 29	A54360
Tomato Vemone	Italy (S) 1994	G	EC 352 g/l	0.898 0.898	0.0528 0.0528	2	11-17 11-21	fruit fruit fruit fruit fruit fruit	0.38 0.27 0.14 0.05 < 0.03 < 0.03	0 **->3** 7 14 21 28	A54360
Tomato Vemone	Italy (S) 1994	G	EC 352 g/l	1.795 1.795	0.1056 0.1056	2	11-17 11-21	fruit fruit fruit fruit fruit fruit	0.86 0.72 0.48 0.21 0.07 0.05	0 **->3** 7 14 21 28	A54360
Tomato San Marzano (Italdor)	Italy (S) 1994	G	EC 352 g/l	1.056 1.056	0.0528 0.0528	2	15-17 15-21	fruit fruit fruit fruit fruit fruit	0.31 0.12 0.08 0.11 0.06 < 0.03	0 **->3** 7 14 21 27	A54360
Tomato San Marzano (Italdor)	Italy (S) 1994	G	EC 352 g/l	2.112 2.112	0.1056 0.1056	2	15-17 15-21	fruit fruit fruit fruit fruit fruit	0.72 0.6 0.13 0.25 0.11 0.06	0 **-> 3** 7 14 21 27	A54360
Tomato Genaro	Spain (S) 1998	G	CS 330 g/l	0.798 0.886	0.207 0.207	2	72 74	fruit	0.3 0.27 **0.23** 0.23	0 1 **3** 7	C00445
Tomato Arleta	Greece (S) 1998	G	CS 330 g/l	0.798 0.798	0.207 0.207	2	72 74	fruit	0.30 0.19 **0.17** 0.20	0 1 **3** 7	C00445

Crop/ Variety	Country/ Year	For G	Form.	Application rate		Nº	Growth Stage	Portion analysed	Residue (mg/kg)	PHI (days)	Ref.
				kg a.s/ha	conc % a.s						
Tomato Arleta	Greece (S) 1998	G	CS 330 g/l	0.798 0.798	0.207 0.207	2	87 87	fruit	0.24 0.31 **0.24** 0.10	0 1 **3** 7	C00445
Tomato Vemone	Italy (S) 1998	G	CS 330 g/l	0.798 0.798	0.207 0.207	2	75 77	fruit	0.83 0.69 **0.65** 0.41	0 1 **3** 7	C00445
Tomato Zapata	Portugal (S) 1998	G	CS 330 g/l	0.798 0.798	0.207 0.207	2	73 79	fruit	0.30 0.26 **0.28** 0.11	0 1 **3** 7	C00445
Tomato Ipanema	Spain (S) 1993	F	EC 352 g/l	0.2642 0.2642	0.0528 0.0528	2	17 19	fruit fruit fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice (steril.) tomato paste (steril.) pomace wash water	0.19 0.08 0.05 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 0.1 < 0.03	0 **3** -> 7 14 14 14 14 14 14 14 14 14	A54363
Tomato Ipanema	Spain (S) 1993	F	EC 352 g/l	0.528 0.528	0.1056 0.1056	2	17 19	fruit fruit fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice (steril.) tomato paste (steril.) pomace wash water	0.26 0.2 0.06 0.05 < 0.03 0.07 0.04 0.03 < 0.03 < 0.03 0.2 < 0.03	0 **3** -> 7 14 14 14 14 14 14 14 14 14	A54363
Tomato Justar	Spain (S) 1993	F	EC 352 g/l	0.2642 0.2642	0.0528 0.0528	2	21 21	fruit fruit fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice (steril.) tomato paste (steril.) pomace wash water	0.19 0.07 0.07 0.05 < 0.03 0.06 0.09 0.03 < 0.03 < 0.03 0.19 < 0.03	0 **3** -> 7 14 14 14 14 14 14 14 14 14	A54363

Crop/ Variety	Country/ Year	For G	Form.	Application rate		Nº	Growth Stage	Portion analysed	Residue (mg/kg)	PHI (days)	Ref.
				kg a.s/ha	conc % a.s						
Tomato Justar	Spain (S) 1993	F	EC 352 g/l	0.528 0.528	0.1056 0.1056	2	21 21	fruit fruit fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice (steril.) tomato paste (steril.) pomace wash water	0.43 0.2 0.1 0.08 < 0.03 0.07 0.06 0.04 < 0.03 0.03 0.35 < 0.03	0 **3** -> 7 14 14 14 14 14 14 14 14 14	A54363
Tomato Marcoro	Italy (S) 1993	F	EC 352 g/l	0.2642 0.2642	0.0377 0.0377	2	11-17 17-19	fruit fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice (steril.) tomato paste (steril.) pomace wash water fruit	0.1 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 0.07 < 0.03 < 0.03	0 **3** -> 7 14 14 14 14 14 14 14 14 14	A54363
Tomato Marcoro	Italy (S) 1993	F	EC 352 g/l	0.528 0.528	0.0754 0.0754	2	11-17 17-19	fruit fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice (steril.) tomato paste (steril.) pomace wash water fruit	0.21 0.04 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 0.15 < 0.03 < 0.03	0 **3** -> 7 14 14 14 14 14 14 14 14 14	A54363
Tomato V.C. 82 B.	Italy (S) 1993	F	EC 352 g/l	0.2642 0.2642	0.0264 0.0264	2	17-19 19-21	fruit fruit fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice (steril.) tomato paste (steril.) pomace wash water	0.22 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03 0.07 < 0.03	0 **3** -> 7 14 14 14 14 14 14 14 14 14	A54363

Crop/ Variety	Country/ Year	For G	Form.	Application rate		Nº	Growth Stage	Portion analysed	Residue (mg/kg)	PHI (days)	Ref.
				kg a.s/ha	conc % a.s						
Tomato V.C. 82 B.	Italy (S) 1993	F	EC 352 g/l	0.528 0.528	0.0528 0.0528	2	17-19 19-21	fruit fruit fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice (steril.) tomato paste (steril.) pomace wash water	0.24 0.04 0.06 0.03 < 0.03 < 0.03 0.03 0.03 < 0.03 < 0.03 0.14 < 0.03	0 **3** -> 7 14 14 14 14 14 14 14 14 14	A54363
Tomato Red Zetor	Spain (S) 1994	F	EC 352 g/l	0.264 0.264	0.0755 0.0755	2	17 19	fruit fruit fruit fruit fruit	0.1 0.07 0.08 < 0.03 < 0.03	0 **3** -> 7 14 20 27	A54362
Tomato Red Zetor	Spain (S) 1994	F	EC 352 g/l	0.528 0.528	0.1509 0.1509	2	17 19	fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice pomace wash water fruit fruit fruit fruit	0.28 0.12 < 0.03 0.09 0.09 0.09 < 0.03 0.61 < 0.03 0.09 0.05 < 0.03 < 0.03	0 **3** 6 6 6 6 6 6 6 -> 7 14 20 27	A54362
Tomato Pluton	Spain (S) 1994	F	EC 352 g/l	0.264 0.264	0.0755 0.0755	2	17-19 21	fruit fruit fruit fruit	0.09 < 0.03 0.03 < 0.03	0 **3** -> 7 14	A54362
Tomato Pluton	Spain (S) 1994	F	EC 352 g/l	0.528 0.528	0.1509 0.1509	2	17-19 21	fruit fruit fruit fruit	0.37 0.06 0.05 0.04	0 **3** -> 7 14	A54362
Tomato Petto 95	Spain (S) 1994	F	EC 352 g/l	0.264 0.264	0.0755 0.0755	2	17-19 19	fruit fruit fruit fruit fruit fruit	0.14 0.04 < 0.03 < 0.03 < 0.03 < 0.03	0 **3** -> 8 14 21 28	A54362
Tomato Petto 95	Spain (S) 1994	F	EC 352 g/l	0.528 0.528	0.1509 0.1509	2	17-19 19	fruit fruit fruit fruit fruit fruit	0.18 0.08 0.04 < 0.03 < 0.03 < 0.03	0 **3** -> 8 14 21 28	A54362
Tomato Loni	Italy (S) 1994	F	EC 352 g/l	0.264 0.264	0.0264 0.0264	2	17-19 17-19	fruit fruit fruit fruit fruit fruit	0.04 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03	0 **3** -> 7 14 21 29	A54362

Crop/ Variety	Country/ Year	For G	Form.	Application rate		Nº	Growth Stage	Portion analysed	Residue (mg/kg)	PHI (days)	Ref.
				kg a.s/ha	conc % a.s						
Tomato Loni	Italy (S) 1994	F	EC 352 g/l	0.528 0.528	0.0528 0.0528	2	17-19 17-19	fruit fruit fruit fruit fruit fruit	0.13 <u>0.06</u> 0.03 0.03 < 0.03 < 0.03	0 **<u>3</u>** -> 7 14 21 29	<u>A54362</u>
Tomato U. C. 82	Italy (S) 1994	F	EC 352 g/l	0.264 0.264	0.022 0.022	2	15-17 15-19	fruit fruit fruit fruit fruit fruit	0.07 0.07 0.07 0.04 < 0.03 < 0.03	0 **3** -> 7 14 21 28	A54362
Tomato U. C. 82	Italy (S) 1994	F	EC 352 g/l	0.528 0.528	0.044 0.044	2	15-17 15-19	fruit fruit fruit canning liquid fruit, unwashed fruit, washed fruit, preserved juice pomace wash water fruit fruit fruit	0.3 <u>0.1</u> 0.08 < 0.03 0.07 0.07 0.07 < 0.03 0.29 < 0.03 0.08 0.05 0.04	0 **<u>3</u>** -> 7 -> 7 -> 7 -> 7 -> 7 -> 7 -> 7 -> 7 14 21 28	<u>A54362</u>

Under greenhouse conditions 17 trials were carried out in Spain and Italy during 1993, 1994 and 1998. Spraying solutions with concentrations between 0.053% and 0.207% were applied twice separated 14 days, resulting in rates of up 2.11 kg as/ha. Those trials with an application rate higher or lower than 25% of the critical GAP (0.053 kg as/hl and 0.8 kg as/ha) were considered not acceptable for MRL calculation. Therefore only 11 trials were considered acceptable for MRL calculation. The results indicated residues 3 days after the last treatment ranged from 0.06 to 0.65 mg/kg. The reference of theses acceptable trials and the result relevant for MRL calculation appear underlined in table 7.6.7-2. **There are sufficient trials to calculate the MRL.**

Under field conditions 18 trials were carried out in Spain and Italy during 1993 and 1994. Spraying solutions with concentrations between 0.02% and 0.105% were applied twice separated 14 days, resulting in rates of up 0.53 kg as/ha. Those trials with an application rate higher or lower than 25% of the critical GAP (0.053-0.105 kg as/hl and 0.53 kg as/ha) were considered not acceptable for MRL calculation. Therefore only 14 trials were considered acceptable for MRL calculation. The results indicated residues 3 days after the last treatment ranged from <0.03 to 0.2 mg/kg. The reference of theses acceptable trials and the result relevant for MRL calculation appear underlined in table 7.6.7-2. There are sufficient trials to calculate the MRL.

B.7.6.2 Oilseed

B.7.6.2.1 Cotton

The use on cotton is summarised in table 7.6.7.2-1.

Table 7.6.7.2-1: Critical GAP on cotton

Crop and/ or situation (a)	Member State or Country	F G or I (b)	Formulation		Application				Application rate per treatment			PHI (days) (l)
			Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	kg as/hl min max	water l/ha min max	kg as/ha min max	
Cotton	Southern Europe	F	EC	350 g/l	Medium/ high volume spraying	Last application when balls are partly open	3	14-21	0.105	800	0.84	21

On July 2001 after the ECCO 104 the RMS received from the main notifier a full residue data package 8 residue trials on cotton were included in this package.

The notifier has submitted on July 2001 the same residue data submitted on 1996, these residue trials are summarised in Table 7.6.2.1-1, these residue trials were included in the Draft Monograph and were considered non acceptable for MRL calculation, because were not carried out according the critical GAP (3 appl.). **8 Residue trials are required.**

Table 7.6.2.1-1: Residue trials on cotton

Crop/ Variety	Country/ Year	Form.	Application rate		Nº	Growth Stage	Portion analysed	Residue (mg/kg)	PHI (days)	Ref.
			kg a.s/ha	conc % a.s						
Cotton Crema 111	Spain (S) 1992	EC 350 g/l	0.63	0.105	1	60 % bolls open	seeds	2.99 0.78 0.27 **0.05**	0 3 7 **15**	A49593 (A53965)
Cotton Stoneville 506	Spain (S) 1992	EC 350 g/l	0.63	0.105	1	75 % bolls open	seeds	2.96 0.35 0.3 **0.05**	0 3 7 **15**	A49594 (A53965)
Cotton Crema 111	Spain (S) 1992	EC 350 g/l	1.00	0.105	1	75 % bolls open	seeds	0.91 0.2 0.17 **0.02**	0 3 7 **15**	A49595 (A53965)
Cotton Cocker 310	Spain (S) 1992	EC 350 g/l	1.00	0.105	1	70 % bolls open	seeds	0.86 0.22 0.22 **0.25**	0 3 7 **15**	A49596 (A53965)
Cotton Stoneville 443	Spain (S) 1992	EC 350 g/l	1.00	0.105	1	75 % bolls open	seeds	0.79 0.62 0.25 0	0 3 7 15	A49597 (A53965)
Cotton Crema 111	Spain (S) 1992	EC 350 g/l	1.00	0.105	1	80 % bolls open	seeds	0.68 0.1 0.1 **0.12**	0 3 7 **15**	A49598 (A53965)
Cotton Max 9	Spain (S) 1992	EC 350 g/l	1.11	0.105	1	20 % bolls open	seeds	1.39 0.24 0.11 **0.07**	0 3 7 **15**	A49599 (A53965)

B.7.6.3 Residue Storage stability

Storage stability studies for animal tissue and dairy matrices and for raw agricultural commodities and processed commodities were made available to RMS on July 2001. The storage stability studies were required in the Draft monograph. The RMS has evaluated those storage stability studies needed to support the actual GAP (cotton and tomato). The storage stability studies for animal tissue and dairy matrices are not relevant for Annex I inclusion.

B.7.6.3.1 Storage stability of residues on crop raw agricultural commodities and processed commodities (grape, potato, tomato, melon and lettuce)

Endosulfan-fre RAC matrices (grape, potato, tomato, melon and lettuce) and processed commodities (grape juice, potato flakes, potato wet peel, tomato paste and tomato puree) were fortified at 0.25 ppm with endosulfan (alpha, beta and sulphate) and stored frozen at approximately < -10ºC. Unfortified control samples were stored frozen under the same conditions. One unfortified control and two freshly fortified controls were analysed concurrently with stored fortification samples at each analysis interval to determine procedural recovery. At the end of the study, recovery results from the stored fortification samples were corrected for the average recovery of the corresponding fresh fortification samples, if the concurrent average was < 100%.

The anlysis results indicated that endosulfan was stable for 18 months in RAC matrices (grape, potato, tomato, melon and lettuce) and PC matrices (grape juice, potato flakes, potato wer peel, tomato paste and tomato puree). The overall fresh procedural recoveries for all matrices ranged from 71% to 136% for endosulfan (alpha, beta and sulphate). The recovery ranges for the stored fortifications are shown in the tables 7.6.3.1-1 and 7.6.3.1-2, corrected and uncorrected for the average fresh fortification recovery.

Table 7.6.3.1-1: % Recovery Range for 18-Month Stored Fortifications (Uncorrected)

Matrix	% Recovery Range for 18-Month Stored Fortifications (Uncorrected)		
	α -endosulfan	β - endosulfan	endosulfan sulphate
Grape	93, 91	100, 93	102, 94
Potato	54, 57	59, 61	62, 63
Tomato	79, 88	81, 91	80, 95
Cantaloupe	81, 102	81, 103	78, 98
Lettuce	86, 104	86, 109	84, 112
Grape Juice	92, 89	92, 98	96, 99
Potato Flakes	68, 69	75, 74	80, 80
Potato Wet Peel	97, 112	97, 117	109, 92
Tomato Paste	95, 102	97, 106	99, 108
Tomato Puree	81, 105	85, 113	81, 114

Table 7.6.3.1-2: % Recovery Range for 18-Month Stored Fortifications (Corrected)

Matrix	% Recovery Range for 18-Month Stored Fortifications (Corrected)		
	α -endosulfan	β - endosulfan	endosulfan sulphate
Grape	99, 97	109, 101	111, 102
Potato	73, 77	80, 82	78, 80
Tomato	101, 113	101, 114	100, 119
Cantaloupe	95, 120	100, 127	101, 127
Lettuce	86, 104	86, 109	84, 112
Grape Juice	93, 90	99, 105	98, 101
Potato Flakes	74, 75	84, 83	92, 92
Potato Wet Peel	97, 112	97, 117	109, 92
Tomato Paste	96, 103	100, 109	98, 111
Tomato Puree	91, 118	96, 127	93, 131

The study is considered acceptable. The analysis results indicated that endosulfan was stable for 18 months in RAC matrices (grape, potato, tomato, cantaloupe and lettuce) and PC matrices (grape juice, potato flakes, potato wet peel, tomato paste and tomato puree).

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

Further data required addressing the effect of processing on the nature of the residue

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

Livestock feeding studies are not required, since the endosulfan uses in EU are cotton and tomato, therefore the ingestion of feed containing endosulfan residues by domestic animals is not expected, and obviously residues in products of animal origin are not expected. Therefore this data requirement should not be considered for Annex I.

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

B.7.12.1 Tomato

Field Trials

0.03	0.04	0.04	0.04	0.06	0.06	0.07	0.07	0.08	0.08	0.1	0.12	0.2	0.2

Method I:

R = Mean residue	0.085
SD	0.054
K	2.614
R max = R + SD x K	**0.227**

Supervised Trial Median Residues (STMR)	0.07
Number (n)	14
P=T/100	0.75
T=Percentil value	75
J=integer of (n+1) x P	11
G=modulus of (n+1) x P	0.25
R(J) = Residue in place J	0.1
R(J+1) = Residue in place J+1	0.12
R(0.75)	0.105
R(ber) = 2 x R(0.75) in mg/kg	**0.21**

Proposed MRL : 0.3 mg/kg

Proposed PHI: 3 days

Greenhouse Trials

0.06	0.08	0.1	0.11	0.12	0.17	0.23	0.24	0.27	0.28	0.65

The data 0.65 was considered as an outlier based on DIXON test

Method I:

R = Mean residue	0.166
SD	0.082
K	2.911
R max = R + SD x K	**0.40**

Supervised Trial Median Residues (STMR)	0.145
Number (n)	10
P=T/100	0.75
T=Percentil value	75
J=integer of (n+1) x P	8
G=modulus of (n+1) x P	0.25
R(J) = Residue in place J	0.24
R(J+1) = Residue in place J+1	0.27
R(0.75)	0.247
R(ber) = 2 x R(0.75) in mg/kg	**0.495**

Proposed MRL : 0.5 mg/kg

Proposed PHI: 3 days

The greenhouse conditions must be considered as a worst case, therefore for tomato the MRL proposed is 0.5 mg/kg

B.7.12.2 Cotton

Trials according the GAP are required

B.7.14 Estimation of potential and actual dietary exposure through diet and other means (IIA, 6.9; IIIA, 8.8)

B.7.14.1 TMDI

The use of endosulfan in tomato represent a 10% of the proposed ADI, therefore there is no risk for consumers.

Active Ingredient	Endosulfan			
ADI [mg/kg bw/d]		0.006		
Consumption data		**European diet. WHO 1995**		
Body weight [kg]		60		
Crop/food	**MRL [mg/kg]**	**Consumption [g/d]**	**TMDI [µg/kg bw/d]**	**TMDI [% ADI]**
Citrus	-	49	-	-
Tree nuts	-	3.8	-	-
Pome fruits	-	22.8	-	-
Stone fruits	-	51.3	-	-
Grapes	-	13.8	-	-
Sugarbeet	-	2	-	-
Sugar refined	-	96.8	-	-
Tomatoes	0.5	66	0.55	9.17
Pepper	-	10.4		
Melon	-	18.3	-	-
Watermelons	-	7.8	-	-
Squash	-	7.5	-	-
Cotton	-	0	-	-
Potatoes	-	240.8	-	-
Tea	-	2.3	-	-
Coffee	-	5.8	-	-
Cacao	-	3.1	-	-
Pinapple	-	15.8	-	-
Sum of crops to be registered			**0.55**	**9.17**
Chicken meat	-	63.3	-	-
Other meat	-	155.5	-	-
Milk	-	340.8	-	-
Eggs	-	37.5	-	-
Sum of products			**0.00**	**0.00**
Sum of total diet			**0.55**	**9.17**

Considering all the uses not supported for Annex I inclusion as an open position and using the limit of determination for consumer risk assessment a 30.8% of the ADI is achieved, no risk for consumer is expected.

Active Ingredient	**Endosulfan**			
ADI [mg/kg bw/d]	0.006			
Consumption data	**European diet. WHO 1995**			
Body weight [kg]	60			
Crop/food	**MRL [mg/kg]**	**Consumption [g/d]**	**TMDI [µg/kg bw/d]**	**TMDI [% ADI]**
Citrus	0.06	49	0.05	0.82
Tree nuts	0.06	3.8	0.00	0.06
Pome fruits	0.06	22.8	0.02	0.38
Stone fruits	0.06	51.3	0.05	0.86
Grapes	0.06	13.8	0.01	0.23
Sugarbeet	0.06	2	0.00	0.03
Sugar refineed	0.06	96.8	0.10	1.61
Tomatoes	**0.5**	66	0.55	9.17
Pepper	0.06	10.4	0.01	0.17
Melon	0.06	18.3	0.02	0.31
Watermelons	0.06	7.8	0.01	0.13
Squash	0.06	7.5	0.01	0.13
Cotton	0.06	0	0.00	0.00
Potatoes	0.06	240.8	0.24	4.01
Tea	0.06	2.3	0.00	0.04
Coffee	0.06	5.8	0.01	0.10
Cacao	0.06	3.1	0.00	0.05
Pinapple	0.06	15.8	0.02	0.26
Sum of crops to be registered			**1.10**	**18.36**
Chicken meat	0.075	63.3	0.08	1.32
Other meat	0.075	155.5	0.19	3.24
Milk	0.075	340.8	0.43	7.10
Eggs	0.075	37.5	0.05	0.78
Sum of products			**0.75**	**12.44**
Sum of total diet			**1.85**	**30.79**

B.7.14.2 Acute exposure

The NESTI calculation was made for the use on tomato and using a 97.5th percentile consumption. This represent a 16.8% of the ArfD for adult consumers and a 77.1% of the ArfD for toddler consumers, therefore there is no acute risk expected due to consumption of tomatoes treated with endosulfan.

B.7.14.2 Acute exposure

Active substance **Endosulfan**
ARfD 0,015 mg/kg

Commodity	residue/MRL-P (mg/kg)	STMR-P (mg/kg)	U (wt of 1st unit) (kg)	*v* factor	ADULT		TODDLER		Uadult (kg)	Utoddler (kg)
					F (daily portion) (kg/day)	NESTI (mg/kg bw/day)	F (daily portion) (kg/day)	NESTI (mg/kg bw/day)		
Tomatoes	0.28	0.14	0.085	7	0.157	0.0025	0.093	0.0116	0.085	0.085

B.7.15 References relied on

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	David A. Winkler 1997a Freezer storage stability of Endosulfan (α, β and Sulphate) on crop raw agricultural commodities and processed commodities BJ-95R-11 – A57831	Y	N	Aventis	Y
	David A. Winkler 1998b Freezer storage stability of Endosulfan (α, β and Sulphate) on crop raw agricultural commodities and processed commodities. Amendment No. 1 to Final Report BJ-95R-11 – A67528	Y	N	Aventis	Y
	Berthold Krebs, Helmut Bürstell, Gerald Huth 1996 Residue data summary from supervised trials and processing studies in Fruiting Vegetables PSR96/052 - 57133	Y		Agrevo	N
	H. Welcker, R. Martens 1999a Decline of residues in protected tomatoes European Union [southern zone] 1998 – Endosulfan, AE F002671 (suspension of microcapsules (CS)) 25.78% w/w (= 330 g/L) ER 98 ECS 753 – C004455	Y	N	Aventis	Y

ADDENDUM TO ANNEX B

ENDOSULFAN

B - 8: ENVIRONMENTAL FATE AND BEHAVIOUR

B.8 Environmental fate and behaviour

There is not new data to be assessed and included in this adenda. In august 2002 new data will be available.

ADDENDUM TO ANNEX B

ENDOSULFAN

B - 9: ECOTOXICOLOGY

B.9 Ecotoxicology

This addendum presents the new studies submitted by the notifier or the ecotoxicological assessment of endosulfan. In the mean time, the notifier has modied the GAP, therefore a new risk assessment, according to the new intended uses has been included.

B.9.1 Effects on birds (IIA, 8.1; IIIA, 10.1)

No additional information on the toxicity of endosulfan to birds has been presented. The new GAPs limit the proposed uses to cotton and tomatoes, therefore, the use in orchards is not longer considered. However, exposures through contaminated insects and secondary poisoning followed the consumption of contaminated aquatic organisms are still relevant for the proposed uses. The initial tier assessment for these risks, as evidenced in the monograph and in the list of endpoints suggest a potential risk. The ongoing studies on residues in insects or the mesocosm study are key elements for refining the risk. These studies are not available yet. Therefore, the refined risk assessment for birds cannot be conducted with the current information.

B.9.2.1 Acute toxicity to aquatic organisms

B.9.2.1.1 Acute toxicity to fish

- **Isomers of the active substance**

Gries and vand der Kolk, 2001a

The test was developed in order to investigate the acute toxicity of ^{14}C-α-endosulfan (isomer of ^{14}C-endosulfan; 99.2 % radiopurity) with carp (*Cyprinus carpio*) under semi-static conditions. The test was based on the OECD guidelines and EC methods for the determination of ecotoxicity; and was in compliance with GLP (excepting the range finding test, the routine water and food contaminant screening and the maintenance of records on the test).

Animals were exposed to six concentrations (control and 0.1, 0.22, 0.48, 1.1, 2.3 and 5.4 µg/l) during 96 hours. Test solutions were renewed at 24, 48 and 72 hours. The concentrations were measured and were given as time weight mean measured concentrations (control, 0.17, 0.34, 0.81, 1.58 and 4.03 µg/l). No samples of the nominal 0.1 test concentrations were analysed since this concentrations was not used for the calculations of the biological endpoints. Thus, no mean measured test concentration is given for this concentration. During the analytical confirmation, the metabolites endosulfan sulfate and endosulfandiol were identified.

Sublethal effects were observed in the test concentrations ranging from 0.3 to 4.03 µg/l ^{14}C-α-endosulfan. The 96 hour LC_{50} was 0.75 µg/l (0.53-1 95% CI). The NOEC was 0.17 µg/l ^{14}C-α-endosulfan.

Gries and vand der Kolk, 2001b

The test was developed in order to investigate the acute toxicity of ^{14}C-β-endosulfan (isomer of ^{14}C-endosulfan; 99% radiopurity) with carp (*Cyprinus carpio*) under semi-static conditions. The test was based on the OECD guidelines and EC methods for the determination of ecotoxicity; and was in compliance with GLP (excepting the range finding test, the routine water and food contaminant screening and the maintenance of records on the test).

The test design is similar to the described above. Nominal test concentrations were: control, 0.1, 0.22, 0.48, 1.1, 2.3, 5.2 µg/l of ^{14}C-β-endosulfan. Based on the biological results, only the treatment levels with nominal concentrations of 1.1, 2.3 and 5.2 µg/l were important for the interpretation of the results. For these concentrations, the time weighted mean were determined (control, 0.78, 2.23 and 3.11 µg/l of ^{14}C-β-endosulfan).

Sublethal effects were observed in the test solutions with mean measured concentrations of 2.23 and 3.11 µg/l of ^{14}C-β-endosulfan. The LC_{50} 96 hours was higher than 3.11 µg/l and the NOEC was 0.78 µg/l.

- **Metabolites**

Madsen and Leak, 2001b

The study was conducted to determine the acute toxicity of endosulfan ether (metabolite of endosulfan), to the common carp, *Cyprinus carpio,* under flow-through test conditions. The test was performed under GLP and was in compliance with EPA and OECD guidelines.

The mean recoveries ranged from 76% to 88% of the nominal concentrations. No mortality was observed in the controls or any of the treatments. Based on mean measured concentrations, the 96 hours LC50 was estimated to be > 1.65 mg/l. The NOEC proposed by the study authors' was 1.65 mg/l, however, some animals at this concentration exhibited loss of equilibrium. This effect was also observed at the measured concentration of 0.759 mg/l and presents a positive dose-response relationship. Therefore, the rapporteur considers that the validable acute NOEC is 0.38 mg/l.

Abedi and Young, 2001a

The study was conducted to determine the acute toxicity of endosulfan lactone (metabolite of endosulfan), to the common carp, *Cyprinus carpio,* under flow-through test conditions. The test was performed under GLP and was in compliance with EPA and OECD guidelines.

The mean measured concentrations ranged from 15 to 19% of nominal concentrations. All toxicity values were based on these mean measured concentrations. The EC_{50} 96 hours was estimated to be 0.57 mg/l (0.51-0.63 95% i.c) and the NOEC was 0.33 mg/l.

Abedi and Young, 2001b

The study was conducted to determine the acute toxicity of endosulfan hydroxyether, to the common carp, *Cyprinus carpio,* under static renewal system. The test was performed under GLP and was in compliance with EPA and OECD guidelines.

The test concentration samples were renewed every 24 hours, and test substance was measured. The mean measured concentrations in the old treated samples ranged from 98 to 104%, and from 106 to 116% in the new treated samples. Thus, the toxicity values are based on nominal concentrations. The dose/response curve moves from 0% mortality at 1.8 mg/l to 100% mortality at the next concentration of 3 mg/l.

The LC_{50} 96 hours was estimated on 2.32 mg/l (1.8 to 3 mg/l, 95% i.c). The NOEC was 0.65 mg/l.

Gries and vand der Kolk, 2000

The test was developed in order to investigate the acute toxicity of ^{14}C-endosulfan sulphate (metabolite of ^{14}C-endosulfan, 99.9 % radiopurity) with carp (*Cyprinus carpio*) under semi-static conditions. The test was based on the OECD guidelines and EC methods for the determination of ecotoxicity; and was in compliance with GLP (excepting the range finding test, the routine water and food contaminant screening and the maintenance of records on the test).

Animals were exposed for 96 hours to six concentrations of ^{14}C-endosulfan sulphate (control and 0.75, 1.5, 2.7, 4.9, 8.9 and 16 µg/l). The test solutions were renewed at 24, 48 and 72 hours, and concentrations were measured (control, 0.92, 1.93, 3.44, 6.03, 9.37 and 21.21 µg/l). The two highest concentrations were initial measured concentrations and the others are time weight mean measured concentrations, based on the total radioactivity.

Sublethal effects were observed in the test concentrations ranging from 1.93 to 21 µg/l ^{14}C-endosulfan sulphate. No sublethal effects were observed in the control and the test concentration of 0.92 µg/l ^{14}C-endosulfan sulphate. Based on the results, the LC_{50} 96 hours was calculated to be 2.2 µg/l ^{14}C-endosulfan sulphate (0.92-3.4 µg/l 95% confidence intervals) and the NOEC was 0.92 µg/l ^{14}C-endosulfan sulphate.

B.9.2.1.2 Acute toxicity to aquatic invertebrates

- **Isomers of the active substance**

Gries, 2001a

The study was developed to investigate the acute toxicity of ^{14}Cβ-endosulfan (99% radiopurity) on Daphnia magna under semi-static conditions. The test was based on OECD and EC guidelines, and was in agreement with GLP (excepting the preliminary range finding test, the maintenance of records on the test item and the routine water and food contaminant screening analyses).

Daphnids were exposed to control, control solvent and five nominal concentrations of β-endosulfan (4, 16, 63, 250 and 1000 µg/l).

Test solutions were renewed every 24 hours and concentrations were measured; the mean measured test concentrations were calculated as time weight mean (2.9, 13.7, 51.1, 241.3 and 641 µg/l).

The results showed a 48 hours-EC_{50} of 528 ug/l (95% IC of 214.3->641 µg/l).

Gries 2001b

The study estimated the acute toxicity of $^{14}C\alpha$-endosulfan (99.2% radiopurity) to Daphnia magna under semi-static test conditions. The test was based on OECD and EC guidelines, and was in agreement with GLP (excepting the preliminary range finding test, the maintenance of records on the test item and the routine water and food contaminant screening analyses).

The test species were exposed for 48 hours to different concentrations of the test item (control, control solvent, 4, 16, 63, 250 and 1000 ug/l). Test solutions were renewed each 24 hours. The mean measured concentrations were calculated as time weight mean measured concentrations (2.7, 10.8, 48.4, 155, 545.6 ug/l).

The 48-hours EC_{50} was estimated to be 224 µg/l (95% CI 155 to 339 µg/l). The 48 hour-EC_0 was 10.8 µg/l and the NOEC was 2.7 ug/l.

- **Metabolites**

Gries, 2000c

The test was conducted to investigate the acute toxicity of ^{14} C endosulfan sulphate (99.9 % radiopurity) on daphnids under static conditions. The test was based on OECD guidelines and EC methods, and was in agreement with GLP (excepting the preliminary range finding test, the maintenance of records on the test item and the routine water and food contaminant screening analyses).

Daphnids were exposed to six nominal concentrations (0.13, 0.25, 0.5, 1, 2 and 4 mg/l) plus a control and a control solvent. Concentrations were measured and a large reduction was observed at 48 hours for some concentrations. Therefore, exposure concentrations were calculated as time weight mean measured. The dose/response curve was very sloppy, moving from 5% mortality at a measured concentration of 0.22 mg/l to 100% mortality at 0.45 mg/l.

The 48-hour EC_{50} for endosulfan sulphate was 0.3 mg/l (95% CI 0.22 to 0.45 mg/l), and the NOEC based on sublethal effects was <0.12 mg/l.

Madsen and Leak, 2001a

The study was conducted to determine the acute toxicity of endosulfan ether (metabolite of endosulfan), to the water flea, *Daphnia magna,* under flow-through test conditions. The test was performed under GLP and was in compliance with EPA and OECD guidelines.

Mean measured concentrations ranged from 73% to 97% of nominal concentrations at 0 hours and from 70 to 125 % of nominal concentrations at 48 hours. Based on mean measured concentrations, the 48 hours EC50 was estimated to be 0.577 mg/l (95% confidence limits of 0.403 and 1.04 mg/l). The proposed NOEC is 0.207 mg/l, however, quiescence was observed for concentrations of 0.1 mg/l and above and showed positive relationships with the concentration and the exposure time. Therefore, the rapporteur considers that an acute NOEC of 0.049 g/l is more appropriate.

Abedi and Young, 2001a

The study assessed the acute toxicity of endosulfan lactone to the water flea, *Daphnia magna,* in a flow-through system. The test was conducted based on EPA and OECD guidelines and was in agreement with GLP.

The mean measured concentrations (0.11, 0.17, 0.37, 0.59 and 1.3 mg/l) of endosulfan lactone ranged from 8 to 12% of the nominal concentrations over the course of the study. All toxicity values are based on these mean measured concentrations.

No mortality or sub-lethal effects occurred in any of the treatment samples, excepting one mortality in the 0.37 mg/l treatment sample. All daphnids at the highest mean measured concentration of 1.3 mg/l exhibited signs of lethargy. Due to the rapid flow rate of the diluter, between 10 and 35% of the daphnids in all treatments were observed trapped on the surface. These daphnids appeared normal after resubmerging.

The EC_{50} 48 hours of endosulfan lactone was > 1.3 mg/l, and the NOEC was 0.59 mg/l.

Abedi and Young, 2001b

The acute toxicity of endosulfan hydroxyether to the water flea, *Daphnia magna,* was assessed in a static renewal system. The test was conducted based on EPA and OECD guidelines and was in agreement with GLP.

Five concentrations of the endosulfan hydroxyether were tested; a negative control and a solvent control were used. The test samples were renewed at 24 hours. The mean measured concentrations ranged from 115 to 119 % of nominal concentrations in the freshly prepared treated samples, and from 115 to 121% in the old samples. Based on these results, the toxicity values were calculated based on nominal concentrations. The EC_{50} 48 hours was calculated as 1.6 mg/l (1.4 to 1.7 mg/l). The NOEC was 0.65 mg/l.

B.9.2.2 Chronic toxicity to aquatic organisms

B.9.2.2.1 Chronic toxicity to fish

Williams and Caunter, 1999

The report describes a 21 day flow-through test which forms the basis of a short term in vivo screen for detecting endocrine disruption in fish. The approach is based on the OECD Test Guideline 204, adapted for sublethal exposure to the test substance. The study was conducted in compliance with the GLP, excepting the apparatus for measuring the vitellogenin.

Daily observation of mortality, behaviour and appearance was made. After 21 days, the animals were sacrificed and measures of weight and length were made. Measures of vitellogenin were also made.

All toxicity values are based on mean measured concentrations. The NOEC for survival was 0.28 μg/l; and the NOEC for length, weight was 0.62 μg/l.

The study suggests a similar NOEC of 0.62 μg/l for vitellogenin concentrations, based on the lack of statistically significant differences, however, the results are not so clear. Vitellogenin concentrations in the control group ranged within three orders of magnitude (from <2 to >2000 ng/l) and therefore, only large differences, as those observed for the group treated with EE2 become statistically significant. It must be considered that both, the mean values and the number of fish with concentrations above the control mean raised by a factor of about 2 for all endosulfan treated concentrations. No dose-response relationships are evident but this lack of relationship has also been observed for endocrine disrupters and is explained by the co-occurrence of several mechanisms of action, not of all them related to endocrine disruption. The study does not include histopathological or even anatomo-pathological observations, or sex determination. Therefore, the rapporteur does not accept the proposed NOEC for vitellogenin concentrations.

Heusel 1999. Endocrine effects on fish

This report presents a review on the evaluation of possible endocrine effects of endosulfan in fish. The references cited in the report have not been fully submitted, therefore the rapporteur cannot check the exactitude of some descriptions.

The first part of the report focuses on lack of vitellogenin induction described in the study by **Williams and Caunter, 1999**, conclusion which, as mentioned above, is not supported by the rapporteur. The second part present brief summaries of some published literature data. The report indicates the estrogenic activity of endosulfan obviously at concentrations much lower than estradiol, as well as some histopathological effects. In the rapporteur opinions, no conclusive evidence on the relevance or not of endocrine disruption in the mode of action of endosulfan can be achieved from the presented data.

B.9.2.6 Risk assessment for aquatic organisms

The risk of endosulfan applications for aquatic organisms can be initially addressed comparing laboratory data versus the Predicted Environmental Concentrations.

B.9.2.6.1 Risk assessment for fish

The fate and behaviour section does not present enough information to establish the maximum level of each isomer and metabolite under realistic conditions. Therefore, the risk assessment for the isomers and metabolites has considered the maximum endosulfan PEC for setting TER values. Due to the low relevance, no corrections for the molecular weight have been considered.

Table 9.2.6.1-1 summarises the acute toxicity and the estimated acute risk of different endosulfan isomers and metabolites.

Table 9.2.6.1-1: Acute TER estimations for fish

Isomer or metablite	Application rate	Nº	SI Days	Distance m	Max. Level %	Maximum PECsw µg/L	TOXICITY 96h LC_{50} µg/l	TER
α-endosulfan	0.84	3	14	1		11.20	0.75	0.067
				10		1.12		0.67
				30		0.28		2.68
β-endosulfan	0.84	3	14	1		11.20	>3.11	>0.28
				10		1.12		>2.77
				30		0.28		11
Endosulfan sulfate	0.84	3	14	1		11.20	2.2	0.19
				10		1.12		1.96
				30		0.28		7.86
Endosulfan ether	0.84	3	14	1		11.20	>1650	>147
				10		1.12		>1473
Endosulfan lactone	0.84	3	14	1		11.20	570	50.9
				10		1.12		509
Endosulfan hydroxiether	0.84	3	14	1		11.20	2320	207
				10		1.12		2071

The TER values confirm the high risk of endosulfan isomers and the metabolite endosulfan sulfate to fish. The other metabolites evaluated do not present a significant acute risk, as suggested by the TER values over the trigger of 100. However, it must be considered that the toxicity is clearly lower than for the parent, but still significant. In fact endosulfan lactone should be classified as highly toxic to aquatic organisms, and the others will fall in the category of toxic.

B.9.2.6.2 Risk assessment for aquatic invertebrates

The fate and behaviour section does not present enough information to establish the maximum level of each isomer and metabolite under realistic conditions. Therefore, the risk assessment for the isomers

and metabolites has considered the maximum endosulfan PEC for setting TER values. Due to the low relevance, no corrections for the molecular weight have been considered.

Table 9.2.6.1-2 summarises the acute toxicity and the estimated acute risk of different endosulfan isomers and metabolites.

Table 9.2.6.1-2: Acute TER estimations for daphnia

Isomer or metablite	Application rate	Nº	SI Days	Distance m	Max. Level %	Maximum PECsw µg/L	TOXICITY 96h LC50 µg/l	TER
α-endosulfan	0.84	3	14	1		11.20	224	20
				10		1.12		200
β-endosulfan	0.84	3	14	1		11.20	528	47
				10		1.12		471
Endosulfan sulfate	0.84	3	14	1		11.20	300	26.7
				10		1.12		267
Endosulfan ether	0.84	3	14	1		11.20	577	51
				10		1.12		515
Endosulfan lactone	0.84	3	14	1		11.20	>1300	>116
				10		1.12		>1160
Endosulfan hydroxiether	0.84	3	14	1		11.20	1600	143
				10		1.12		1429

The TER values indicate that a buffer zone of 10 m is enough for getting the trigger of 100. However, it must be considered that the toxicity of the metabolites is in this case similar or only slightly lower than for the isomers. In this case endosulfan sulfate and endosulfan ether should be classified as highly toxic to aquatic organisms, and the others will fall in the category of toxic.

B.9.3 Effects on other terrestrial vertebrates (IIIA, 10.3)

Bremmer and Leist, 1998. Endocrine effects on mammals

This report summarises a set of studies on mammals. As for fish, there are indications of estrogenic effects, and a set of reported effects with no conclusive evidence on the implication of endocrine disruption in the reported effects.

B.9.4 Effects on bees (IIA, 8.3.1; IIA, 10.4)

Schur, 2000

The study describes the side effects of the endosulfan on honeybee *Apis mellifera,* in fields following application during bee-flight in Spain, according to BBA and EPPO guidelines.

Fields of flowering *Phacelia tanacetifolia* were treated with endosulfan (33% w/w) at a rate of 0.8 kg ai/ha. It must be noticed that the study includes a single application while the proposed GAPs include the possibility for two applications within the season. The effects of the application were examined on bee colonies used for honey production, placed near the test fields. Two trials were carried out at two

different test locations in Spain (one in Northern and one in Middle Spain). Mortality (in front of the hives and in the field), flight intensity in the field, behaviour in the bees in the entrance of the hives and development of the bee brood were recorded.

The results of the study are summarised for endpoints:

Mortality:

Northern Spain trial: The average post-application mortality was 5 dead bees/hive/day in the test substance, 0.8 dead bees/hive/day in the control and 122.2 dead bees/hive/day in the toxic standard. The increase observed for the endosulfan treated group is reported as non-statistically significant. The average daily pre and post-application mortality using Qm (average) was calculated as 0.7, 1.1 and 11.6 for test substance control and test reference, respectively.

Middle Spain trial: in this case, an increase of bee mortality was observed on the day of application. The average post-application mortality was 12.4 dead bees/hive/day in the test substance, 0.7 dead bees/hive/day in the control and 36.8 dead bees/hive/day in the toxic standard. The Qm (average) was 31, 2.3 and 92 for test substance, control and test reference.

Effects on honey bee flight intensity:

In both trials, a repellent effect occurred directly after application of the test substance and the foraging bees were observed returning to their hive.

In the first trial, the average daily post-application level of flight intensity was similar in the test substance and the control variant (6.9 bees/m^2/day and 7.1 bees/m^2/day respectively) compared to 2.7 bees/m^2/day in the toxic standard variant.

In the second trial, average daily post-application level of flight intensity was slightly decreased in the test substance (8.1 bees/m^2/day) in comparison to the control variant (12.3 bees/m^2/day); the toxic reference was 2.3 bees/m^2/day.

Effects on honey bee brood development:

In relation with colonies strength and bee brood development, no abnormal differences, which could be attributed to the test substance, were observed between test substance and control variant.

Risk assessment for bees

The submitted study indicates the possibility of some treated related effects at the selected dose which corresponds to the higher intended dose but using a single applications, The relevance of these effects for the new GAPs is not ver high, however, risk reduction methods should be presented by the notifier.

B.9.5 Other non-target arthropods

Knäbe, 2001

The study was developed to investigate the effects of endosulfan on the non-target arthropods fauna in a citrus orchard in Spain. The study was based on the Candolfi et al., (2000) and Anonymous, (1981) guidelines. With the exception of weather data, farmer's information and soil characterization, the study was conducted under GLPs. Test substance (endosulfan 34% analysed) was applied at two different doses (530 g ai/ha and 840 g ai/ha) three applications per plot. Dimethoate 40 was used as reference substance and water was used as control.

For the main test, arthropods were sampled using inventory sampling within each plot to determine the density and abundance of arthropods. The samplings were done frequently (every five weeks) with a shorter interval between samplings before and after applications. The numbers of pests and non-target arthropods on shoots were assessed at the same time than the inventory samplings took place.

Arthropods were examined for taxonomic determinations; the abundance of selected species and groups was plotted against time. The data were analysed by one-way analysis of variance and pair-wise comparison if they were normally distributed. For not normally distributed data the Kruskal Wallis test was used.

During the trial period, 11 samplings and visual assessments were done in the citrus orchard. The highest proportion of insects was from Hymenoptera (27.9%) in the control. Other dominant taxa were Stenorrhyncha (26.8%) and Diptera (17.15%); Auchenorrhyncha (3.85%), Coleoptera (4.5%) and spiders (7.98%) were subdominant. Separate groups have been considered if the abundance was more than 3% in the control samples.

For the low dosage of the test substance a reduction in the abundance of single taxons could be observed. The taxons and times were:

The order Diptera on the 5th day after application 2, the order Coleoptera on the 5th day after application 2, the Auchenorrhyncha on the 5th day after application 2 and the first day before application 3. Other effects were an increase in the number of ants and aphids and a reduction in the number of spiders. The reasons might be a disturbing effect of ants or maybe the lack of pray which might be caused by the activity of ants.

A significant decreasing effect of the high test substance dosage on the spider population could be calculated only for samples on the 5th day after treatment 1. The order Coleoptera was reduced on the 5th day after application 2, the order Auchenorrhynchaon the 5 th day after application 1, the 5th day after application 2 and 1 day before application 3. No further reductions were observed and it can be concluded that recovery occurred. These effects could only be registered for a short time and recovery occurred at the 12th day after treatment 3.

It can concluded that the test substance endosulfan will not have influence on a wide range of epigaeic arthropods when used under field conditions and following good agricultural practice. Even the highest dosage (840 g ai/ha) with multiple applications did not show long-term reduction in activity and abundance of dominant species. Effects on some eu- and subdominant taxa were found in a lesser extent and the reduction in numbers compensated by recovery through the present popoulations. Any long-lasting effects beyond one season from applications of endosulfan are not expected.

Risk assessment for non-target arthropods

The study indicates some effects related to the treatment with endosulfan, but only for certain specific groups and with recovery after treatment.

Although the study has been conducted in citrus, which is not included in the new GAPs, the rapporteur considers that due to the treatment conditions and the presence of a large number of species from different invertebrate taxa in the study, these results can be extrapolated to other crops. The need for risk management measures should be considered at MS level.

B.9.11 References relied on

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	Abedi, J.; Young, B. 2001a The 48 hour acute toxicity to the water Flea, Daphnia magna, in a Flow-Throuh System: AE F051328 (endosulfan lactone) sunbstance, pure 96.7% w/w Aventis CropScience USA LP. Doc. No.: B003206; Report No. BJ00W516	Y	N	Aventis	Y
	Abedi, A.; Young, B. 2001b The 96 hour acute toxicity to the common carp, Cyprinus carpio, in a Flow-Through System AE F051326 (endosulfan hydroxyether) substance, Pure 96.7% w/w Aventis CropScience USA LP. Doc. No.: B003208; Report No. BJ00W519	Y	N	Aventis	Y
	Abedi, A.; Young, B. 2001c The 96 hour acute toxicity to the common carp, Cyprinus carpio, in a Static Renewal System AE F051326 (endosulfan hydroxyether) substance, Pure 95.7% w/w Aventis CropScience USA LP. Doc. No.: B003209; Report No. BJ00W521	Y	N	Aventis	Y
	Bremmer, J.N.; Leist, K.H. 1998 Evaluation of possible endocrine effects in mammalian species Endosulfan Hoechst Schering AgrEvo GmbH Doc. No. C001570; Report No. TOX98/046			Aventis	N
	Gries, T. 2000a Acute toxicity test with carp (Cyprinus carpio) under semistatic conditions ^{14}C-endosulfansulfat (Metabolite of ^{14}C-endosulfan) [redacted] Doc. No. C010869; Report No. 1049.014.170	Y	N	Aventis	Y
	Gries, Th.; van der Kolk, J. 2001a Acute toxicity test with carp (Cyprinus carpio) under semistatic conditions ^{14}C-Beta-Endosulfan [redacted] C012039; Report No. 1049.018.170	Y	N	Aventis	Y
	Gries, Th.; van der Kolk, J. 2001b Acute toxicity test with carp (Cyprinus carpio) under semistatic conditions ^{14}C-Alpha-Endosulfan [redacted] C012038; Report No. 1049.017.170	Y	N	Aventis	Y

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	Gries, Th. 2001a Acute immobilisation test with daphnids (Daphnia magna) under semi-static conditions ^{14}C-alfa-endosulfan Code: AE F052618 Springborn Laboratories (Europe) AG. Doc. No. C012048; Report No. 1049.017.110	Y	N	Aventis	Y
	Gries, Th. 2001b Acute immobilisation test with daphnids (Daphnia magna) under semi-static conditions ^{14}C-Beta-endosulfan Code: AE F052619 Springborn Laboratories (Europe) AG. Doc. No. C012047; Report No. 1049.018.110	Y	N	Aventis	Y
	Gries, Th. 2001a Acute immobilisation test with daphnids (Daphnia magna) under static conditions ^{14}C-endosulfansulfat (metabolite of ^{14}C-endosulfan) Springborn Laboratories (Europe) AG. Doc. No. C012413; Report No. 1049.014.110	Y	N	Aventis	Y
	Heusel, R. 1999 Evaluation of possible endocrine effects in fish Endosulfan Code: AE F002671 Hoechst Schering AgrEvo GmbH Doc. No. C004471; Report No. OE99/010			Aventis	N
	Knaebe, S. 2001 An evaluation of the effects of endosulfan (AE F002671 00 EC33 C703) on the non-target arthropod fauna in a citrus orchard in Sapin Arbeitsgemeinsch. GAB GmbH & IFU GmbH. Doc. No. C012888; Report No. 20001050/S1-FNTO			Aventis	N
	Madsen, T.; Leak, T. 2001a Metabolite of pesticide Endosulfan, to the common carp, Cyprinus carpio, determined under Flow-Through Test conditions [redacted]: B003100; Report No. 46289	Y	N	Aventis	Y
	Madsen, T.; Leak, T. 2001b Acute toxicity of AE F051330 (Endosulfan ether), a metabolite of the pesticide Endosulfan, to the Water Flea, Daphnia magna, determined under Flow-Through Test conditions ABC Laboratories, Inc. Doc. No.: B003099; Report No. 46288	Y	N	Aventis	Y

Annex IIA or Annex IIIA point	Author(s) Year Title Reference	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	Schur, A. 2000 Assessment of side effects of AE F002671 00 EC33 C703 on the honey bee (Apis mellifera L.) in the field following application during bee-flight in Spain Arbeitsgemeinsch GAB GmbH & IFU GmbH. Doc. No. C010572; Report No. 20001050/S1-BFEU			Aventis	N
	Williams, T.D.; Caunter, J.E. 1999 Effect on the survival, weight, length and vitellogenin concentration of juvenile fathead minnow (Pimephales promelas) Endosulfan substance technical Code: AE F002671 00 1D99 0008 [redacted] Doc. No. C006997; Report No. BL6664/B	Y	N	Aventis	Y

TABLE OF CONTENTS