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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

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CHAPTER B-7: Residue data

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ADDENDUM TO ANNEX B

ENDOSULFAN

B - 7: RESIDUE DATA

B.7 Residue data

This addenda has been prepared by the RMS (Spain) after the submission of all the data required. The deadline for submission of these data was established by the Decision of the Commission 2001/810/CE on 31 May 2003.

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.2-2 (01.04.02).

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 essential to support the current 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.7.1.1 Metabolism of ^{14}C-Endosulfan in Soybeans

A new metabolism study in soybeans was submitted on May 2003. This study was required to support the use of endoulfan on oilseeds crops (cotton and soybean as imported crop).

Title: Metabolism of 14C-Endosulfan in Soybeans
Author: Suresh Mislankar, Patricia J. Tull
Date: May 16, 2003.
Report No: B004326
Study identification: 601BJ
GLP: Yes

The study was conducted to determine the extent and nature of the metabolic breakdown of [^{14}C]-endosulfan formulated as 35 EC in soybeans treated under a typical field application pattern.

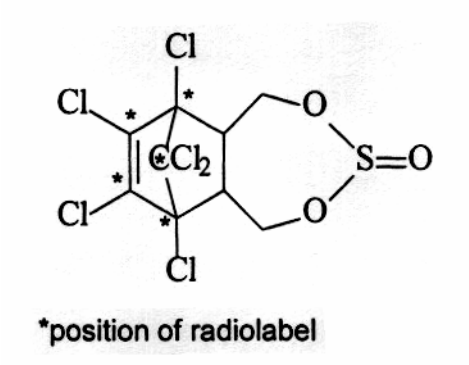
The study was conducted in accordance with EU guidelines, European Council Directive 91/414/EEC, 7028/VI/95 rev. 3 Appendix A, United States Environmental Protection Agency (EPA), Pesticide Assessment Guidelines, OPPTS 860.1300 and Good Laboratory Practice Standards set forth in 40 CFR part 160.

Materials and methods:

The soybean were treated with two applications of 530 g a.i./ha each, equivalent to the annual maximum of 1060 g a.i./ha. Applications were made at R5 (forage stage) and R6.5 (hay stage). Endosulfan used in the commercial formulation is composed of two stereoisomers: alpha-endosulfan (64-67% of the technical grade) and beta-endosulfan (29-32%). A similar isomeric mixture was used in this study.

Test Substance:

Label : [6,7,8,9.10-^{14}C]-endosulfan
Supplier: Aventis Pharma Deutschland GmbH Frankfurt, Germany
Batch No: Z-32024-0
Original specific activity: 73.19 μCi/mg
Radiochemical Purity Structure: 98.8%
Structure:



The radiochemical purity of the dosing solution was determined by HPLC prior to both applications and it was >99.9% radiochemicaly pure

The specific activity of the radiochemical used for treatment was 50.1μCi/mg which is sufficient to determine residues of 0.002 ppm.

Isomer composition in Dosing Solution: Alpha-lsomer (AE FO52618) 69-76% and Beta-lsomer (AE FO52619) 31-33%.

The maximum proposed seasonal field use of endosulfan applied to soybeans is 1.06 kg ai/ha. Each plot received two applications of 0.53 kg ai/ha. Plants were treated twice using maximum rate, at 61 and 38 days before the final harvest.

Soybean (seed) samples were analysed in duplicate at maturity to determine the metabolic profile of the endosulfan. Soybeans (forage and hay) were also analysed at intermediate time points to establish the trend in metabolism. These time points included Day 0 (immediately post-treatment at R5, forage stage) and day 23 (prior to the second application at R6.5, hay stage).

The residue in the day zero soybean forage was recovered by acetonitrile surface wash and acetonitrile extraction. Residue remaining in the extracted fibber was determined by combustion. Residues in hay and beans were recovered extraction with by acetonitrile followed by acetonitrile:water (80:20) and soxhlet extraction with acetonitrile:water (80:20). The fiber was combusted to determine non-extractable residue. Metabolites in the extractable residue were identified by retention time comparison with authentic standards. The identities were confirmed by mass spectral analysis.

Results:

The residue level and the extraction profiles of the duplicate samples are summarized in table7.1.1-1:

Table 7.1.1-1: Summary of Total Residues in treated soybeans and their distribution on extraction

Harvest time	Replicate	Total residue	Surface Wash Residue	Extractable Residue	Non extractable residue

			% Total	ppm	% Total	ppm	% Total	ppm
Day 0 Forage	1	27.742	76.0	21.097	22.5	6.246	1.4	0.399
	2	15.356	74.8	11.480	23.4	3.595	1.8	0.280
	Mean	**21.549**	**75.4**	**16.289**	**23.0**	**4.921**	**1.6**	**0.340**
Day 23 Hay	1	0.924	NA	NA	87.4	0.808	12.6	0.116[b]
	2	0.151	NA	NA	86.5	0.131	13.5	0.02
	Mean	**0.538**	**NA**	**NA**	**87.0**	**0.470**	**13.1**	**0.068**
Day 61 Beans	1	0.503	NA	NA	93.9	0.473	6.1	0.031
	2	0.445	NA	NA	95.0	0.423	5.0	0.022
	Mean	**0.474**	**NA**	**NA**	**94.5**	**0.448**	**5.6**	**0.026**

a) Total Residue determined from sum of residue in extracts and fiber

b) This was further extracted with strong acid and strong base.

The total residue in forage samples at day 0 was 21.549 ppm, but after 23 days (prior to second application) this had declined to 0.538 ppm. The residue at final harvest in beans (Day 61) was 0.474 ppm.

The majority of residue at all time points was solvent extractable. In forage at day 0, the residue remained principally on the leaf surface and 75.4% of the TRR was recovered in an acetonitrile wash. The rest (23.0%) was extractable with acetonitrile, for a total of 98.4% of the TRR.

In hay samples 87.0% of the TRR was extractable with acetonitrile and acetonitrile:water (80:20).

Similarly, in beans 95.0% of the TRR was extractable with acetonitrile, acetonitrile:water (80:20), and acetonitrile:water (80:20) soxhlet extraction.

HPLC results of the extracts at each sampling point are shown in Table 7.1.1-2.

Table 7.1.1-2: Summary of metabolite identification by HPLC

Crop Part	Replicate	Extract type	Extractable		α-Endosulfan		β-Endosulfan		Endosulfan sulfate		Total % of TRR Identified
			%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	
Forage (Day 0)	1	AcCNWash Extract	76.0	21.097	47.1	13.078	28.9	8.019	ND	ND	
			22.5	6.626	14.3	4.209	8.0	2.349	0.2	0.067	
		Combined	98.5	27.723	61.4	17.287	36.8	10.368	0.2	0.067	98.5
	2	AcCNWash Extract	74.8	11.480	46.2	7.096	28.6	4.384	ND	ND	
			23.4	3.595	14.8	2.271	8.3	1.272	0.3	0.052	
		Combined	98.2	15.075	61.0		36.9		0.3		98.2
	Mean		**98.4**		**61.2**		**36.8**		**0.3**		**98.4**
Hay	1	Combined Extract	87.4	0.808	2.5	0.023	4.5	0.042	57.9	0.535	64.9
	2	Combined Extract	86.5	0.131	ND	ND	3.4	0.005	44.5	0.067	47.9
	Mean		**87.0**		**1.3**		**4.0**		**51.2**		**56.4***
Beans	1	Combined Extract	93.9	0.473	3.0	0.014	5.9	0.030	78.3	0.394	87.3
	2	Combined Extract	95.0	0.423	ND	ND	4.7	0.021	78.5	0.349	83.3
	Mean		**94.5**		**1.5**		**5.3**		**78.4**		**85.3**

ND= Not Detected

* Remainder of the extracted material consists of multiple water soluble peaks, none of which are more than 0.05 ppm or 10% of the TRR

The residue immediately after application (forage samples, day O) was, as would be expected, primarily comprised of endosulfan and the isomeric ratio of alpha- and beta-endosulfan was comparable to that in the original radioactive test substance. A trace amount of endosulfan sulfate (0.3%) of the TRR was also detected.

In hay samples the major metabolite was endosulfan sulfate (AE F051327) and it accounted for 51.2% of the TRR. Alpha endosulfan and beta-endosulfan accounted for 1.3 and 4.0% of the TRR respectively. The rest of the extractable material consisted of multiple water soluble peaks, none of which accounted for more than 0.05 ppm or 10% of the TRR

In beans 85.3% of the TRR was identified. The major component consisted of endosulfan sulfate and it accounted for 78.4% of the TRR. Alpha- and beta- endosulfan accounted for 1.5 and 5.4 % of the TRR respectively

Identity of parent endosulfan and metabolite endosulfan sulfate (AE F051327) was confirmed by mass spectral analysis

Non-extractable residue was very low in forage and beans and it accounted for a maximum of 2.0% in forage and 6.0% in beans. In one hay sample non- extractable residue accounted for 13.1 % of the TRR. This was further subjected to hydrolysis with strong acid and base. The non extractable residue in hay after acid and base hydrolysis accounted for 2% of the TRR.

In summary, over 85.3% of the total residue in beans and 98.4% of the TRR in forage was identified. The metabolic profile of endosulfan in soybeans was identical to that in the other raw agricultural commodities. The primary metabolites being alpha-endosulfan, beta-endosulfan and endosulfan sulfate.

Conclusion and assessment: The study is considered valid. The metabolic profile of endosulfan in soybeans was identical to that in other raw agricultural commodities. The primary route of metabolism was oxidation of the endosulfan to endosulfan sulfate (AE FO51327), establishing the primary residue targets as alpha-endosulfan, beta-endosulfan and endosulfan sulfate.

B.7.1.2 Evaluation of plant metabolism studies

Investigations on the metabolism and distribution of endosulfan and its relevant metabolites in plants have been carried out with the ^{14}C-labelled active substance on relevant crops like tomato and cucumber plants, apple trees and soybean

The metabolic profile of endosulfan in the tested crops was identical. The primary route of metabolism was oxidation of the endosulfan to endosulfan sulfate (AE FO51327), establishing the primary residue targets as alpha-endosulfan, beta-endosulfan and endosulfan sulfate.

The relevant residue of endosulfan in plant material consists of the total of the two stereoisomers α-endosulfan and β-endosulfan, as well as of their transformation product endosulfan sulphate. Whereas shortly after the first application the residue consists only of the two stereoisomers, the metabolite endosulfan sulphate is formed later and accounts for a considerable part of the total residue in plant material.

Two categories of crops have been covered by the available plant metabolism studies: Fruits and oilseeds. The two uses proposed in the current list of GAP are covered by these studies.

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.

B.7.2.1 Distribution, elimination and nature of the metabolite residues in milk and edible tissues of lactating cow

Title: Distribution, elimination and nature of the metabolite residues in milk and edible tissues of lactating cow
Authors: Leah J.M., Reynolds C.M.M.
Date: May 21, 1996.
Report No: A57041
GLP: Yes

The study was designed to investigate the distribution, elimination , magnitude and nature of endosulfan residues in the edible tissues and milk of a dairy cow following oral administration according to current international regulatory guidelines.

Material and methods:

Label : [6,7,8,9.10-^{14}C]-endosulfan

Supplier: Hoechst, Kinetics and metabolism, Hoechst AG, Frankfurt

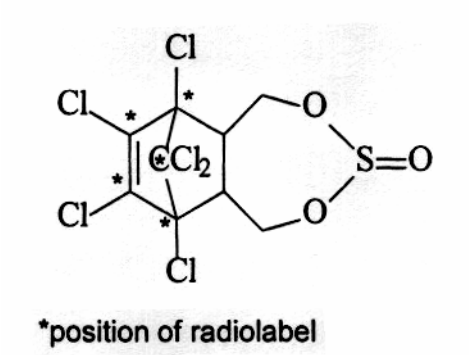
Batch No:24 057 II

Original specific activity: 10.71 μCi/mg; 392.10 MBq/g

Radiochemical Purity Structure: 98.6%

Isomeric ratio: α-endosulfan; β-endosulfan 70:30

Structure:



A dairy cow was orally dosed with [^{14}C] -endosulfan with a mean daily dose of 288.25 mg, equivalent to 0.641 mg/kg body weight/day, for five consecutive days. The dose was equivalent to approximately twice the maximum expected exposure following dietary ingestion and equivalent to approximately 22 ppm in the diet.

Urine and faeces were collected daily, milk was collected twice daily. At necropsy, 120 hours after initial dose and approximately 22 hours after final dose, liver, kidney, renal fat, subcutaneous fat, muscle (psoas and hindquarter), heart, lungs, rumen and abdominal fluid and bile were sampled and quantified. Identification of the metabolite residues was carried out in all edible tissues namely liver, kidney, heart, muscle, fat and milk, and the metabolic profile of urine was also determined.

Results:

The overall mean daily dose of [^{14}C]-endosulfan administered orally to the cow was 288.33 ± 2.22 kg, therefore the actual mean dietary intake of [^{14}C]-endosulfan was 21.51 mg/kg diet or 21.51 ppm in the diet.

During the period of dosing, the temperature range in the animal room varied from 16-22ºC. Relative humidity ranged from 50-54%. Milk yields were consistent throughout the study, at 24.55 ± 0.5 kg per dose day.

Residue levels:

Milk: The residue levels reached a plateau with a maximum concentration of 0.171 mg eq endosulfan/kg following the fourth daily dose.

Urine: The maximum residue level of 2.571 mg equivalents endosulfan/kg urine was reached 96 hours after administration of the initial dose.

Tissues: Residue levels were highest in liver (3.572 mg eq endosulfan/kg). Kidney (0.785 mg eq endosulfan/kg and in omental and renal fat (1.278 and 0.840 mg/kg respectively). The relatively high levels found in fatty tissues was indicative of a lipophilic compound. The residue in the muscle was an order of magnitude lower at 0.031 mg eq/kg.

Blood and plasma: Residues of endosulfan and metabolites in whole blood and plasma remained relatively low during the first 24 h following the initial dose administration, reaching a maximum level of 0.052 mg endosulfan eq/kg in plasma at 12 hours after dosing. Following the repeated daily doses the residue levels rose slowly, reaching a maximum of 0.207 and 0.248 mg eq/kg in blood and plasma 6 hours after the fifth and final dose.

Faecal excretion was the major route of elimination of endosulfan residues, representing 39.7% of the administered dose. Urinary excretion accounted for only 3.95% of the administered dose. The recovery of endosulfan in excreta was therefore low, possibly due to problems encountered with the solubility of endosulfan in faeces and thereby in obtaining an accurate quantification of faecal residue.

Identification of metabolites:

Muscle: The residue level of endosulfan and its metabolites in muscle (psoas) was 0.052 mg endosulfan equivalents/kg. Since muscle from the hindquarter was 0.031 mg/kg, the muscle tissue with the higher residue level was used for extraction and analysis. Overall 69.24% of the total ^{14}C residue was organo-extractable. The major metabolite identified as endosulfan sulfate accounted for 50.73% of the TRR (0.026 ppm), with a further 15.14% (0.008 ppm) identified as α-endosulfan.

Heart: The residue level of endosulfan and its metabolites in the heart was found to be 0.161 mg endosulfan eq/kg. Overall, 53.22% of the total ^{14}C residue in heart was organo extractable with a further 2.02% extractable in water, making a total of 55.24%. Endosulfan sulfate accounted for 14.20% of the TRR (0.023 ppm) with a further 26.83% (0.048 ppm) identified as endosulfan lactone and 2.17% (0.004 ppm) as α-endosulfan. A further 33.18% was realised following enzymatic digestions. Although each extract was below the limit of analysis (< 10 ppb), attempts were made to pool the fractions for chromatographic analysis, but were unsuccessful.

Renal fat: The residue level of endosulfan and its metabolites in renal fat was 0.840 mg endosulfan eq/kg. Overall, 96.46% of the total radioactivity was organo-extractable. A single major metabolite, endosulfan sulfate was identified, accounting for 83.83% of the total ^{14}C residue in renal fat. A further 1.86% was extracted in fractions below the levels requiring analysis in accordance with the Schmitt memorandum.

Omental fat: The residue level of endosulfan and its metabolites in omental fat was 1.278 mg endosulfan equ/kg. Overall, 95.7% of the total omental fat residue was organo- extractable and no further extraction was performed. Endosulfan sulfate was the single major metabolite identified,

containing 82.08% of the total ^{14}C residue in omental fat (1.049 pprn) with only a small amount of polar material seen, 1.56% (0.020 pprn).

Subcutaneous fat: The residue level of endosulfan and its metabolites in subcutaneous fat was 0.305 mg endosulfan eq/kg. Overall, 81.34% of the total radioactivity was organo-extractable and no further extraction was performed. Endosulfan sulfate was the single major metabolite detected, accounting for 64.09% of the total residue (0.195 ppm) .

Kidney: The residue level of endosulfan and its metabolites residues in kidney was 0.785 mg endosulfan equivalents/kg. An aliquot of kidney was fractionated into cellular components to determine the distribution of radioactivity through the cells.

Of the total radioactive residue in the kidney, 38.43% was associated with the PCA (perchloric acid) fraction, 23.26% with the lipid fraction and 25.11% with the protein fraction, making up 86.8% of the total radioactive residue. This indicates that a major proportion of the total radioactive residue contains peptides, amino acids, low molecular weight proteins and water soluble metabolites such as glutathione conjugates which are often readily formed from chlorinated xenobiotics such as endosulfan.

Overall, 43.49% of the total ^{14}C residue in kidney was extractable, with a further 52.24% released by enzyme and mild acid/base incubations. The majority of the residue consisted of polar residues resistant to solvent extraction. The residue remaining after organic and aqueous extraction of the kidney was air-dried and quantified. Over 55% of the TRR was unextractable (0.444 ppm) and was therefore subjected to a battery of enzymatic treatments in order to release the activity.

Collagenase extract contained 8.12% TRR in kidney (0.064 ppm) with a further 17.70% (0.139 ppm) recovered in extract following incubation with pancreatin. After incubation with γ-glutamyl tranpeptidase the extract contained 6.62% (0.052 ppm) of the TRR, whilst only 2.84% (0.022 ppm) was recovered in the extract following incubation with carboxy-peptidase A.

A second pancreatin incubation was performed on the unextracted residue with the successful release of a further 8.01% of the TRR (0.063 ppm) in extract. The strong proteolytic action of proteinase K resulted in the recovery of 5.24% of the residue in the extract representing 0.041 ppm.

Following enzymatic digestion of the unextractable residue the remaining residue was refluxed with 1 N HCl to release a further 1.47% of the residue (0.012 ppm) in the extract followed by base hydrolysis releasing 2.25% (0.018 ppm) in extract.

In an attempt to analyse the extracts obtained from the enzymatic digestion (8.12%) and acid/base hydrolysis (2.25%), this extracts plus water extract (10.68% - 0.084 ppm) were pooled and freeze-dried and resuspended in a small volume of water. The recovery of activity was greater than 100% of that expected, with 62.92% of the total ^{14}C residue in kidney (0.494 ppm) recovered in the extract. Both TLC and HPLC analysis showed this extract to consist of polar material.

An aliquot of the last extract was refluxed with 6 N HCl overnight then extracted into ethyl acetate extract. Following reduction extract represented 55.83% of the total ^{14}C residue in kidney (0.438 ppm). Analysis by TLC was inconclusive with polar material still evident. HPLC analysis showed the presence of several metabolites summarised as follows: endosulfan diol (4.96%, 0.039 ppm), hydroxyendosulfan ether (4.77%, 0.037 pprn), endosulfan lactone (6.86%, 0.054 ppm), endosulfan sulfate (4.32%, 0.034 ppm), endosulfan ether (2.13%, 0.017 ppm) and βendosulfan (3.08%, 0.024 ppm)

Overall, 43.49% of the total ^{14}C residue in kidney was extractable (including 10.68% from a final water rinse). A further 52.24% of the total ^{14}C residue was released by enzymic and mild acid/base incubations. 88.30% of the total ^{14}C residue was identified/characterised as follows: endosulfan sulfate (12.58%, 0.099 ppm), endosulfan lactone (6.86%, 0.054 ppm), endosulfan diol (4.96%, 0.039 ppm), hydroxyendosulfan ether (4.77%, 0.037 ppm), β-endosulfan (3.08%, 0.024 ppm), endosulfan ether (2.13%, 0.017 ppm) and polar material (51.65%, 0.405 ppm) inclusive of metabolites released by acid hydrolysis. With the exception of endosulfan sulfate, all other metabolites were hydrolysis products originating from polar material. The remaining polar material (51.65%, 0.405 ppm) which was resistant to acid hydrolysis was probably comprised of very stable water soluble metabolites such as glutathione conjugates.

Liver: The residue level of endosulfan and its metabolites in liver was 3.572 mg endosulfan equivalents/kg. A fractionation of liver into cellular components was undertaken in order to determine distribution of radioactivity through the cells.

Fractionation indicated that only 30.18% of the total liver residue was associated with lipids and that the majority of the residue was associated with more polar fractions such as proteins (23.93%) and the perchloric acid fraction (32.26%). This suggests that a large proportion of the residue is hydrophilic in nature. There was little incorporation of radioactivity into RNA and DNA

Overall, 37.98% of the total ^{14}C residue in liver was extractable and a further 53.87% released by enzymic and mild acid/base incubations, making a total of 91.85%. A large proportion of the residue was unextractable using sol vents and required enzymic and mild acid/base hydrolysis to release this residue, which was polar in nature. The polar material could only be identified following 6 N hydrochloric acid hydrolysis to yield known metabolites . However, a proportion of the residue remained resistant to severe acid hydrolysis, demonstrating the inert nature of this residue.

A bioavailability study described in which cattle liver containing polar residues of endosulfan was fed to rats, showed that the non-extractable residues do not accumu1ate in rat tissues and are readily excreted unchanged.

Milk: The residue level of endosulfan and its metabolites in milk was 0.147mg endosulfan equivalents/kg. Overall, 95.32% of the total milk residue was organo-extractable, with identification of 88.57% of the total ^{14}C residue as a single metabolite, namely endosulfan sulfate.

Urine: The urine extract was analysed by HPLC and found to contain polar material and endosulfan lactone. The polar residues were probably glutathione conjugates. Enzymatic deconjugation of the polar residue was attempted. However, the polar residue was resistant to deconjugation by glucuronidase and sulfatase indicating that conjugates of glucuronides and/or sulfates were not a major component of this residue.

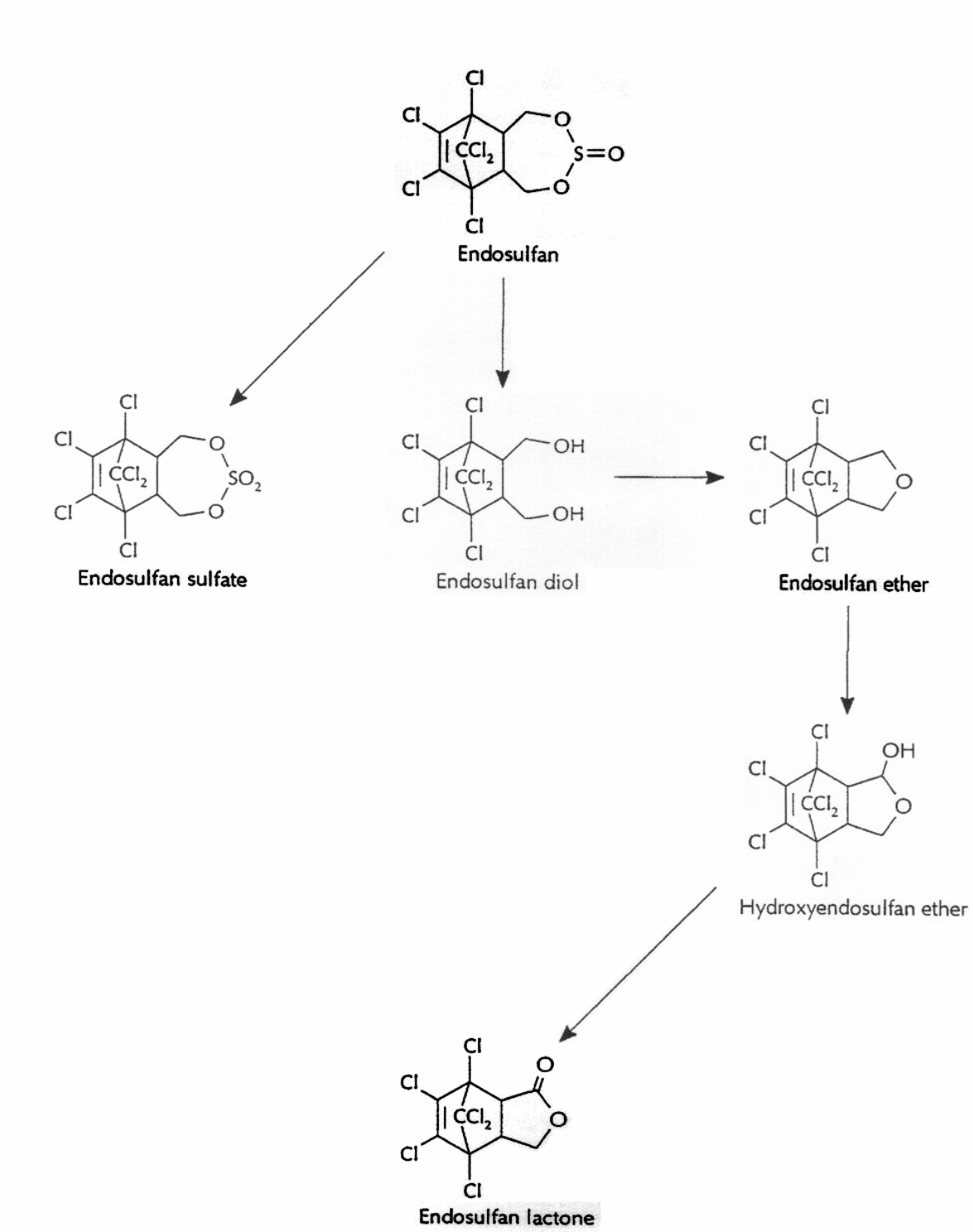
Table 7.2.1-1 shows the isolation and identification of the residues in tissues

Table 7.2.1-1: Endosulfan residues in tissues of lactating cow

	Tissue							
	Liver	**Kidney**	**Heart**	**Muscle**	**Omental fat**	**Renal fat**	**Subcutaneous fat**	**Milk (54 hrs)**
Residue level (ppm)	3.572	0.785	0.161	0.052	1.278	0.840	0.305	0.147
% Extracted	37.98	43.49	55.24	69.24	95.70	96.46	81.34	95.32
% released [(1)]	53.87	52.24	33.18	13.86	-	-	-	-
% Identified/characterised	87.80	86.03	49.22	65.87	83.64	83.83	67.79	88.57
α-Endosulfan	2.72	-	2.17	15.14	-	-	-	-
β-Endosulfan	-	3.08	-	-	-	-	-	-
Endosulfan sulfate	27.16	12.58	14.21	50.73	82.08	83.83	67.79	88.57
Endosulfan lactone	9.16	6.86	29.53	-	-	-	-	-
Endosulfan diol	6.48	4.96	-	-	-	-	-	-
Hydroxyendouslfan ether	-	4.77	-	-	-	-	-	-
Endosulfan ether	6.76	2.13	-	-	-	-	-	-
Polar	35.49	51.65	3.32	-	1.56	-	-	-
% Below limit of analysis	0	0	39.19	15.65	0.32	1.86	0	3.22

Metabolism of endosulfan in dairy cow: From the results obtained in the isolation and identification of metabolites from tissues, a metabolic pathway can be proposed for the dairy cow (figure 7.2.1-1), and is consistent with previous findings.

Figure 7.2.1-1: Proposed metabolic pathway for endosulfan in the lactating cow



A major metabolite produced in all tissues was endosulfan sulfate formed as a result of oxidation of endosulfan. However, there was no evidence of further metabolism to endosulfan diol sulfuric acid and dicarboxylic acid as proposed by Stumpf and Asshauer. Parent endosulfan was present in liver, kidney, heart and muscle. Extractable endosulfan lactone was found in heart tissue and liver and kidney tissue following hydrolysis in 6 N acid. The relatively large proportion of polar material present in liver and kidney tissue is likely to be composed of conjugated endosulfan metabolites, probably glutathione

conjugates. Acid hydrolysis of increasing concentration resulted in the following hydrolysis products: endosulfan diol, endosulfan lactone, endosulfan ether and hydroxyendosulfan ether being released from the polar material.

Conclusions and assessment:

The study is considered valid. Following dosing of [^{14}C] -endosulfan at a dose rate equivalent to 21.59 ppm in the diet for 5 consecutive days the mean combined daily recovery in urine and faeces was 43.65%. The major route of elimination was via the faeces, accounting for 39.7% of the total dose compared to 3.95% in urine.

Tissue residues of endosulfan and/or its metabolites were generally low except for the liver residue which was 3.572 ppm and the omental fat (1.278 ppm), with all other tissue residues below 1.0 ppm.

In milk, residue levels were detectable after 6 hours, reaching a maximum of 0.171 mg equivalents/kg 102 hours after the first dose.

Tissue residue levels of endosulfan and/or its metabolites 120 hours after initial dose and approximately 22 hours after final dose were generally low with the exception of the liver where the residue levels were found to be 3.572 mg equivalents/kg tissue. Residue levels in fat were as follows: omental fat (1.278 mg equivalents/kg), renal fat (0.840 mg equivalents/kg) and subcutaneous fat (0.840 mg equivalents/kg). Residue levels in kidney (0.785 mg equivalents/kg) and lungs (0.673 mg equivalents/kg) were comparable with levels in fat, residue levels in heart (0.161 mg equivalents/kg) and muscle (0.052 mg equivalents/kg) were an order of magnitude lower.

Following the first dose of [^{14}C] -endosulfan, 29.58% of the administered dose was recovered within the first twenty-four hours of dosing in urine and faeces. The mean daily recovery in faeces was 39.7 ± 7.31% and 3.95 ± 2.01% in urine giving a total daily recovery in excreta of 43.65%.

Metabolism of endosulfan in the dairy cow followed two pathways, one route producing the major metabolite, endosulfan sulfate and the second route producing lactone (heart tissue only). Endosulfan lactone, diol, hydroxyether and ether metabolites could be derived from polar residues indicating that whilst these metabolites did not accumulate to detectable levels, they were intermediates formed along the metabolic pathway to polar material. Polar material was very resistant to breakdown and was assumed to consist of stable conjugated metabolites. This was confirmed in a rat bioavailability study which showed that polar material fed to rats was readily excreted unchanged in the faeces.

The major metabolite identified in all tissues was endosulfan sulfate with endosulfan lactone being found in heart, kidney and liver tissue indicating that the compound was readily cleaved following dosing of endosulfan to a dairy cow.

B.7.2.2 Distribution, elimination and nature of the metabolite residues in the eggs and edible tissues of laying hen

Title: Distribution, elimination and nature of the metabolite residues in the eggs and edible tissues of laying hen

Authors: Reynolds C.M.M.

Date: March 26, 1996.

Report No: A56354

GLP: Yes

The study was designed to investigate the distribution, elimination , magnitude and nature of endosulfan residues in the edible tissues and eggs of a laying hen following oral administration according to current international regulatory guidelines.

Material and methods:

Label : [6,7,8,9.10-^{14}C]-endosulfan

Supplier: Hoechst, Kinetics and metabolism, Hoechst AG, Frankfurt

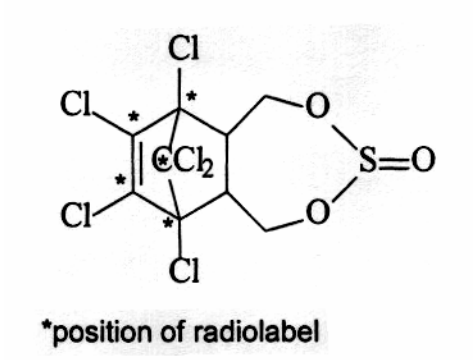
Batch No:24 057 I

Original specific activity: 56.20 μCi/mg; 2058 MBq/g

Radiochemical Purity Structure: > 97%

Isomeric ratio: α-endosulfan; β-endosulfan 68:32

Structure:



Six laying hens were orally dosed with [^{14}C] -endosulfan at 1.36 mg per bird per day for twelve consecutive days. The dose was equivalent to 6 times the maximum expected exposure following dietary ingestion and equivalent to approx. 11 ppm in the diet.

Excreta, cage washings and eggs were collected daily. At necropsy, liver, abdominal and subcutaneous fat skin, skeletal muscle and undeveloped eggs were removed for determination of the distribution and magnitude of [^{14}C]-endosulfan. All samples collected were analysed to determine the residues of [14^{C}] -endosulfan and its metabolites.

Isolation and identification of the metabolite residues has been performed in all edible tissues in accordance with the current regulatory guidelines.

In brief where a total radioactive residue was 0.01 ppm or less no identification was performed. For residues above this trigger value organic extraction was performed and each extract quantified, where the extract was below 0.01 ppm, again no identification was performed, between 0.01 and 0.05 ppm the extract was characterised. Attempts were made to identify the nature of each of the residues present in the extract at levels greater than 0.05 ppm. Unextractable metabolic residues of greater than 0.1 ppm or 10% of the total radioactive residues were analysed to attempt to elucidate their nature.

Results:

Residue levels :

The highest residues of endosulfan and its metabolites were seen in the adipose tissue, namely the abdominal and subcutaneous fat at 0.974 ± 0.084 and 0.875 ± 0.134 mg equivalent/kg, respectively. The residue in the skin and liver was 0.689 ± 0.133 and 0.466 ± 0.119 mg equivalent/kg, respectively. The residue in the muscle was an order of magnitude lower at 0.028 ± 0.004 mg equivalent/kg.

Eggs: The residue in egg yolks rose rapidly within 48 hours of administration of the initial dose, and continued to rise steadily to reach a plateau by day 10 of dosing at 0.853 ± 0.115 mg equivalent/kg. The residue levels in egg whites were an order of magnitude lower than those seen in the egg yolks and reached a maximum of 0.013 ± 0.008 mg equivalent by day 6 of dosing. The residue in undeveloped eggs was 0.768 ± 0.145 mg equivalent/kg reflecting the residue detected in egg yolks.

Table 7.2.2-1: Residue (mg eq endosulfan/kg) in eggs after administration of [^{14}C]Endosulfan

Day	Egg Yolk		Egg White	
	Mean	**SD**	**Mean**	**SD**
1	ND	ND	ND	ND
2	0.078	0.063	0.007	0.004
3	0.242	0.115	0.008	0.004
4	0.371	0.060	0.010	0.003
5	0.553	0.119	0.011	0.004
6	0.671	0.155	0.013	0.008
7	0.769	0.127	0.008	0.003
8	0.785	0.081	0.011	0.003
9	0.880	0.066	0.009	0.005
10	0.853	0.115	0.011	0.004
11	0.853	0.137	0.008	0.003
12	0.899	0.138	0.010	0.003

Elimination of radioactivity: The excreta collected was combined with cage washings and quantified as one, recovered on a daily basis. The excretion of radioactivity after administration of the first dose of

[^{14}C]-endosulfan was fairly rapid with 50.70 ±. 21.88% of the administered dose recovered in the first twenty-four hours following dosing.

The mean daily recovery of the radioactive dose (mean for six birds) was 86.81 ± 18.68% indicating that the majority of the dose was excreted with a proportion of the daily administered dose available for incorporation into tissues.

Table 7.2.2-2: Isolation and identification/characterisation of the residue in the tissues of laying hens after administration of ^{14}C-endosulfan

Tissue	Residue level (ppm)	% Extracted	% Identified/Characterised						
			α Endosulfan	β endosulfan	Endosulfan sulfate	Endosulfan lactone	Endosulfan diol	Polar	BLA *
Egg Yolk	0.853	92.17[a]	4.74	1.32	46.38	1.82	-	20.18[b]	0.89
Egg White	0.013	64.91	-	-	-	-	-	-	64.91
Skin	0.651	94.43	11.66	4.79	51.32	4.50	-	17.55 [b]	0.52
Subcut fat	0.875	98.21	16.15	8.90	61.10	5.03	-	4.71	0.51
Abdom. Fat	0.974	97.04	16.77	7.80	65.45	4.99	-	-	0.56
Liver	0.466	91.41	0.99	1.60	45.62	6.27	4.25	23.27[c]	-
Muscle	0.028	64.40	6.52	4.40	35.83	3.51	-	4.71	8.15
Excreta	NA	60.62	4.90	6.25	0.79	-	1.43	46.61	-

[a] Each polar component was >0.03 ppm

[b] Hydrolysed to identified metabolites

[c] Unknown metabolite less polar than parent compound 6.03% total

NA: Not applicable

BLA: Below level of analysis

Muscle: Overall 64.40% of the total ^{14}C residue in muscle was organo-extractable. The major metabolite identified as endosulfan sulfate accounted for 35.83% of the total residue (0.010 ppm). Other minor metabolites seen were endosulfan lactone, α and β-endosulfan accounting for 3.51% (<0.001 ppm) , 6.52% (0.002 ppm) and 4.40% (0.001 ppm) , respectively.

Some 35.60% of the total [^{14}C] -residue in muscle was unextractable but this was below the limits requiring further analysis at 0.010 ppm.

Egg white: The residue level of endosulfan and its metabolites in egg whites was low reaching a plateau by day 6 of dosing of 0.013 mg equivalent/kg. The 64.91% of the total residue (0.008 ppm) was organoextractable. No further analysis was performed.

Abdominal fat: Over 97% of the total ^{14}C residue in the abdominal fat was organo-extractable. The major metabolite was identified as endosulfan sulfate, representing 65.45% (0.638 ppm) of the total residue, with unchanged parent compound α and β-endosulfan representing 14.07 and 7.80%, respectively (0.137 and 0.076 ppm), and a smaller amount 4.99% (0.049 ppm) of endosulfan lactone

was also seen. In addition, up to 2.70% of the total residue was composed of unchanged α-endosulfan in an extract analysed by TLC only.

Overall identification and characterisation of 95.01% of the total (14C]-residue in abdominal fat was made (0.926 ppm).

Subcutaneous fat: Over 98% of the total .^{14}C residue in the subcutaneous fat was organo-extractable. The major metabolite identified was endosulfan sulfate which represented 61.10% of the total ^{14}C residue in subcutaneous fat (0.535 ppm) , unchanged α and β-endosulfan representing 16.15 and 8.90%, respectively (0.141 and 0.078 ppm) , and 5.03% of the residue was identified as endosulfan lactone (0.044 ppm). A further 4.71% of the residue was polar in nature (0.041 ppm).

Overall 95.89% of the total residue in subcutaneous fat was identified and characterised (0.839 pprn).

Skin: Over 94% of the total ^{14}C residue in skin was organo-extractable. The major metabolite identified was endosulfan sulfate which represented 51.32% of the total ^{14}C residue in skin (0.354 ppm), unchanged α and β-endosulfan representing 11.66 and 4.79% of the residue, respectively (0.080 and 0.033 ppm), and 4.50% (0.031 ppm) as endosulfan lactone. Some 17.55% of the ^{14}C residue in skin was characterised as polar in nature, accounting for 0.121 ppm, however on treatment with 1 N HCl the polar residue was completely identified as endosulfan sulfate, endosulfan diol, hydroxyether and endosulfan lactone.

Overall approximately 90.0% of the total residue in skin was identified and characterised (0.620 ppm).

Liver: Over 91% of the total [^{14}C] -residue in the liver was extractable following an extensive series of organic and aqueous extractions followed by enzymatic digestions.

Successful identification of 58.73% of the residue as endosulfan and its metabolites was made with the major metabolite identified as endosulfan sulfate (45.62%), with endosulfan lactone representing 6.27%, α- and β-endosulfan representing 0.99 and 1.60% respectively and endosulfan diol representing 4.25%.

Some 23.27% of the total ^{14}C residue was found to consist of components which were polar in nature but each represented less than 0.03 ppm. A further 8.85% was accounted for in procedural losses due partly to the particulate nature of some of the samples.

Overall 91.41% of the ^{14}C residue was extractable with 82% identified and characterised (0.382 ppm).

Egg yolks: Overall 92.17% of the total ^{14}C residue in egg yolks was extractable following organic and aqueous extraction and enzyme digestion.

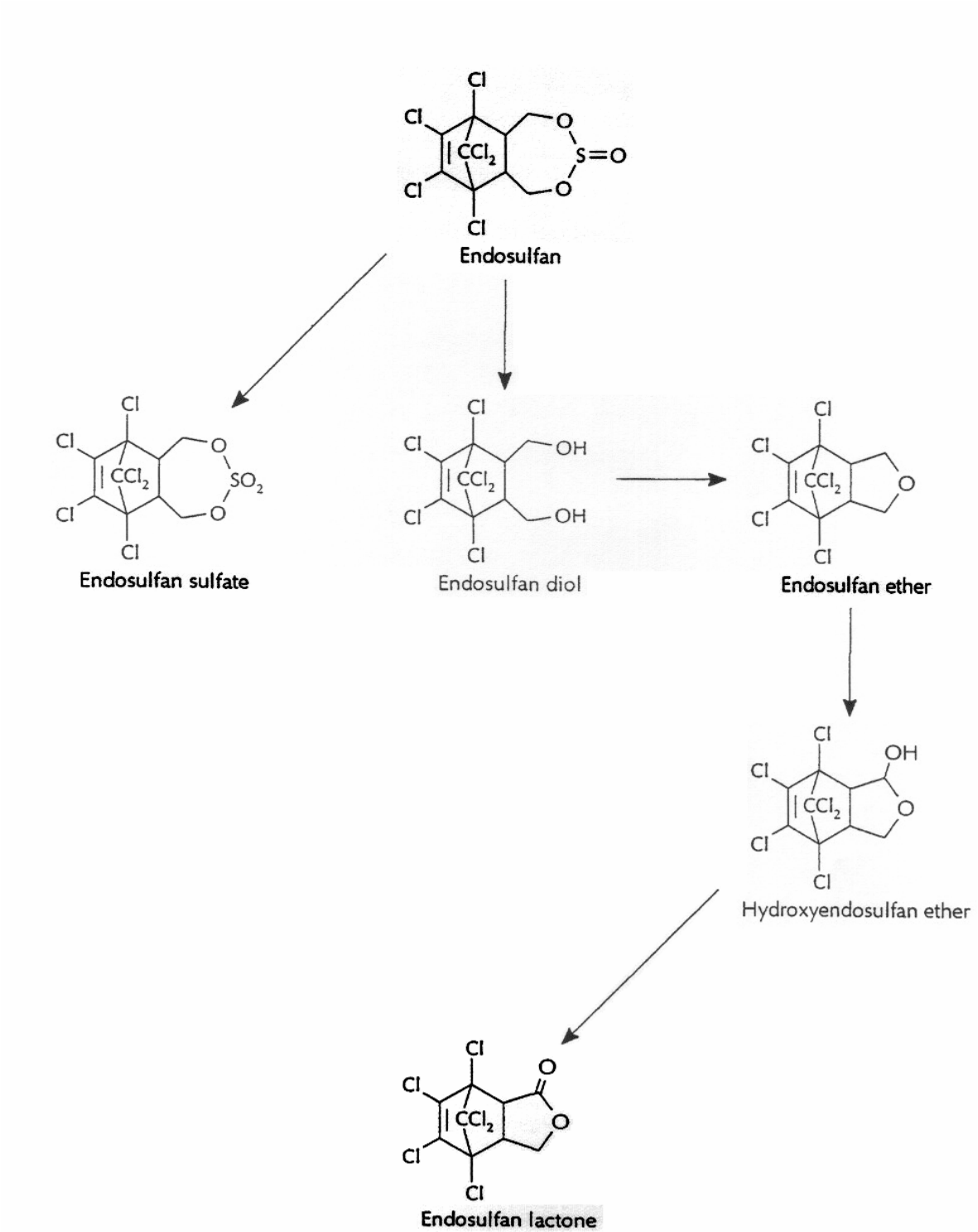
Successful identification/characterisation of 80.47% of the residue as endosulfan and its metabolites was completed with the major metabolite identified as endosulfan sulfate accounting for 46.38% of the residue, endosulfan lactone for 1.82% and unchanged α- and β- endosulfan representing 4.74 and 1.32% respectively, with 20.18% of the residue polar in nature. On treatment with 1 N HCl 7.85% of the polar residue was broken clown to identifiable metabolites. A further 9.81% of the extractable activity was acid hydrolysed and extracted into ethyl acetate. Analysis indicated the presence of an unknown metabolite accounting for 6.03% of the total residue in egg yolks (0.051 ppm).

Metabolism of endosulfan un laying hens: From the results obtained in the isolation and identification of metabolites from the tissues of the laying hen following dosing with [^{14}C]-endosulfan it was possible to propase a metabolic pathway for endosulfan in the hen.

The parent compound α and β-endosulfan remains present unchanged in a small quantity in most tissues, but the major metabolite seen in all tissues was endosulfan sulfate formed from oxidation of endosulfan. There was no evidence of further metabolism of endosulfan sulfate to endosulfan diol sulfuric acid ester and dicarbonic acid. In addition, hydrolysis and oxidation of endosulfan α and β to the sulfur-free metabolites endosulfan diol, endosulfan ether and endosulfan lactone, was also seen.

Polar material seen in the tissues and excreta was likely to be composed of conjugates of endosulfan which when subject to acid hydrolysis of increasing concentration formed the products hydrolysis namely endosulfan diol and lactone, respectively.

Figure 7.2.2-1: Proposed metabolic pathway for endosulfan in the laying hens



Conclusions and assessment:

The study is considered valid.

In egg yolks and whites residue levels of endosulfan were detectable within 48 hours of the initial dose administration, with residue levels in egg yolks continuing to rise to reach a plateau by day 10 of dosing at a concentration of 0.853 ± 0.115 mg equivalent/kg tissue. The residue in egg whites were an order of magnitude lower with a maximum concentration of 0.013 ± 0.008 mg equivalent/kg tissue seen on day 6 of dosing.

In undeveloped eggs the mean concentration of endosulfan-derived residues was 0.768 ± 0.145 mg equivalent/kg.

Tissue residue levels of endosulfan and/or its metabolites were generally low with the highest residues seen in the subcutaneous and abdominal fat at 0.875 ± 0.134 and 0.974 ± 0.084 mg equivalent/kg, respectively. The residues in skin and liver were slightly lower at 0.689 ± 0.133 and 0.466 ± 0.119 mg equivalent/kg, respectively, and in the muscle were much lower at 0.028 ± 0.004 mg equivalent/kg.

Following the first dose of [^{14}C] -endosulfan, elimination was fairly rapid with 50.70 ± 21.88% of the administered dose recovered within the first twenty-four hours of dosing. The overall mean daily recovery was 86.81 ± 18.68% over the twelve day study period indicating that the majority of the dose was excreted.

Following dosing of [^{14}C] -endosulfan at a dose rate equivalent to 10 ppm in the diet for 12 consecutive days, residue levels were detectable in all edible tissues at between 0.013 and 0.974 mg equivalent/kg or ppm therefore indicative that the compound was readily cleaved following dosing of endosulfan to laying hens. The major metabolite identified in all tissues was endosulfan sulfate, with a small percentage of unchanged α and β endosulfan also seen plus the products of hydrolysis and oxidation namely endosulfan diol and endosulfan lactone.

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 and OILSEEDS CROPS**.

The definition of the residue for animal products, 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 metabolites **endosulfan sulphate and endosulfan lactone**.

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 categories of crops are **fruits and oilseeds crops**.. The residue definition for **FRUIT CROPS and OILSEEDS CROPS** is α-endosulfan, β-endosulfan and endosulfan sulphate. The residue on other category of crops (root and tuber crops, leafy crops, pulses and legume crops) are not covered by this residue definition.

The data requirement 5.4 is related to the residue definition on leafy crops, especially on tea. The available information does not allow to propose 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.

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 might be included in the residue definition.**

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

B.7.4 Use pattern

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	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 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	0.49 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	Spain (S) 2001	G	EC 352 g/l	0.8 0.8	0.162 0.162	2	76 86	fruit	0.079 **0.089**	0 **3**	C020750
Tomato	France (S) 2001	G	EC 352 g/l	0.88 0.8	0.162 0.162	2	81 81	fruit	0.129 **0.179**	0 **3**	C020750
Tomato	Italy (S) 2001	G	EC 352 g/l	0.8 0.8	0.162 0.162	2	81 87	fruit	0.319 **0.189**	0 **3**	C020750
Tomato	Greece (S) 2001	G	EC 352 g/l	0.8 0.8	0.162 0.162	2	77 82	fruit	0.229 **0.229**	0 **3**	C020750
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 0.06 0.03 0.03 < 0.03 < 0.03	0 **3** -> 7 14 21 29	A54362
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 0.1 0.08 < 0.03 0.07 0.07 0.07 < 0.03 0.29 < 0.03 0.08 0.05 0.04	0 **3** -> 7 -> 7 -> 7 -> 7 -> 7 -> 7 -> 7 -> 7 14 21 28	A54362

Under greenhouse conditions 21 trials were carried out in Spain, France, Greece and Italy during 1993, 1994, 1998 and 2001. Spraying solutions with concentrations between 0.053% and 0.207% were applied twice separated 14 days. 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 15 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 these 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 7.6.2.1-1.

Table 7.6.2.1-1: 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

Table 7.6.2.1-2: 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)
Cotton Sonia	Spain (S) 2001	CS 330 g/l	0.84	0.326	3	67 72 80	Bolls Bolls Bolls Lint Seeds	0.97 0.22 0.12 0.42 **0.03**	0 7 14 21-22 21-22	C022557
Cotton Sonia	Spain (S) 2001	CS 330 g/l	0.84	0.326	3	65 73 79	Bolls Bolls Bolls Lint Seeds	1.09 0.57 0.19 0.10 **0.01**	0 7 14 21-22 21-22	C022557

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 Bravada	Spain (S) 2001	CS 330 g/l	0.84	0.326	3	72 76 81	Bolls Bolls Bolls Lint Seeds	1.45 0.80 0.68 0.86 **0.05**	0 7 14 21-22 21-22	C022557
Cotton S.G. 125	Greece (S) 2001	CS 330 g/l	0.84	0.543	3	75 78 80	Bolls Bolls Bolls Lint Seeds	0.03 0.39 0.23 0.21 **0.11**	0 7 14 21-22 21-22	C022557
Cotton Selia	Italy (S) 2001	CS 330 g/l	0.84	0.543	3	75 78 82	Bolls Bolls Bolls Lint Seeds	0.23 3.42 3.44 3.05 **0.51**	0 7 14 21-22 21-22	C022557
Cotton Sonia	Spain (S) 2001	EC 352	0.84	0.255	3	67 72 80	Bolls Bolls Bolls Lint Seeds	0.35 0.10 0.02 0.14 **0.02**	0 7 14 21-22 21-22	C022557
Cotton Sonia	Spain (S) 2001	EC 352	0.84	0.255	3	65 73 79	Bolls Bolls Bolls Lint Seeds	1.95 0.35 0.43 0.09 **0.01**	0 7 14 21-22 21-22	C022557
Cotton Bravada	Spain (S) 2001	CS 330	0.48	0.19	3	72 76 81	Bolls Bolls Bolls Lint Seeds	0.86 0.19 0.26 0.81 0.03	0 7 14 21-22 21-22	C022557
Cotton S.G 125	Greece (S) 2001	EC 352	0.84	0.426	3	75 78 80	Bolls Bolls Bolls Lint Seeds	0.24 0.12 0.05 0.15 **0.03**	0 7 14 21-22 21-22	C022557
Cotton Selia	Italy (S) 2001	CS 330	0.48	0.317	3	75 78 82	Bolls Bolls Bolls Lint Seeds	0.08 2.16 1.51 1.51 0.14	0 7 14 21-22 21-22	C022557
Cotton Tabladilla	Spain (S) 2002	EC 352	0.84	0.464	3	75 80 82	Bolls Lint Seeds	0.50 0.61 **0.08**	0 20 20	C029815
Cotton Roca	Spain (S) 2002	EC 352	0.84	0.464	3	71 75 80	Bolls Lint Seeds	1.1 0.27 **<0.06**	0 20 20	C029815
Cotton SG 125	Greece (S) 2002	EC 352	0.84	0.426	3	77 79 83	Bolls Lint Seeds	2.51 0.33 **0.08**	0 28 28	C029815
Cotton. SG 125	Greece (S) 2002	EC 352	0.84	0.426	3	77 79 80	Bolls Lint Seeds	0.42 0.07 **<0.06**	0 28 28	C029815
Cotton Midas	Greece (S) 2002	EC 352	0.84	0.426	3	77 79 85	Bolls Lint Seeds	3.40 0.88 **<0.06**	0 28 28	C029815

Under field conditions 22 trials were carried out in Spain., Greece and Italy during 1993, 1994 and 2001. Those trials with an application rate higher or lower than 25% of the critical GAP (0.105 kg as/hl and 0.84 kg as/ha) were considered not acceptable for MRL calculation. Therefore only 12 trials were considered acceptable for MRL calculation. The results indicated residues 21 days after the last treatment ranged from 0.01 to 0.51 mg/kg. The reference of theses acceptable trials and the result

relevant for MRL calculation appear underlined in table 7.6.2.1-2. There are sufficient trials to calculate the MRL.

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).

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). **However, there is no data on stability of residues in cotton.**

B.7.6.3.2 Storage stability of residues on Animal Tissue and Dairy Matrices

Title: Freezer Storage stability of residues (alpha, beta and sulfate on Animal Tissue and Dairy Matrices)

Author: D.A Winkler

Date: June 22, 1998

Ref: A67512

GLP: Yes

The objective of this study was to determine the stability of endosulfan (alpha, beta and sulfate) in animal tissues and dairy matrices when stored frozen.

Control samples of animal tissuers (beef muscle and liver), eggs (whites and yolks) and milk were fortified at 0.25 ppm with endosulfan (alpha, beta and sulfate) and stored frozen at approximately < -10ºC. Unfortified control samples were stored frozen under the same conditions and one unfortified control and two freshly fortified controls were analyzed concurrently with stored fortification samples at each anaIysis 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.

The analysis results indicated that endosulfan was stable for 12 months in animal tissues (beef muscle and liver), egg (whites and yolks) and milk. The overall fresh procedural recoveries for all matrices ranged from 63 % to 104% for endosulfan (alpha, beta and sulfate), with the exception of 4 recoveries, ranging from 52% - 59%, shown as outliers. The recovery ranges for the stored fortifications are shown in the following tables, uncorrected and corrected for the average fresh fortification recovery .

Table 7.6.3.2-1: % Recovery Range for stored fortifications (Uncorrected) 3, 6, 9 and 12 month

Matrix	Alpha	Beta	Sulfate
Beef Muscle	76 – 95	79 – 101	82 –101
Beef Liver	57 – 82	71 – 94	69 – 118
Egg Whites	61 – 79	62 – 81	78 – 84
Egg Yolks	63 – 83	50 – 84	58 – 84
Milk	73 - 104	76 - 114	74 – 115

Table 7.6.3.2-2: % Recovery Range for stored fortifications (Corrected) 3, 6, 9 and 12 month

Matrix	Alpha	Beta	Sulfate
Beef Muscle	95 – 110	96 –113	92 –116
Beef Liver	90 –114	97 – 125	99 – 144
Egg Whites	85 – 97	89 –101	95 – 112
Egg Yolks	64 – 105	51-105	94 – 106
Milk	95 –112	99 – 119	95 – 116

The study is considered acceptable. The recovery ranges indicate that endosulfan (alpha, beta and sulfate) is stable in all matrices for 12 months. **However, the stability of the endosulfan lactone must be demonstrate.**

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

B.7.7.1 Nature of the residue

Title: Endosulfan: Investigation of the nature of the potential residue in the products of industrial processing or household preparation

Author: T. Maurer

Date: January 22, 2002

Ref: C018814

GLP: Yes

Endosulfan is applied to various crops, which may be processed prior to consumption. This study was designed to determine the nature and quantity of residues which might be formed during processing of raw agricultural commodities. Hydrolysis is the main degradation pathway in processing, since all enzymes present in the substrate are generally inactivated under processing. Thus, hydrolysis conditions

were applied simulating pasteurisation, brewing, baking, boiling and sterilisation. [6,7,8,9,1 0-U-^{14}C]-Endosulfan (each carbon which is linked with chlorine) at a specific radioactivity of 5 548 MBq/g with a radio-purity of >95% was used. The treatment was carried out at two incubation rates: 0.1 mg/L and 1.0 mg/L. Each experiment was conducted using replicate samples. Residues were analysed using radio-HPLC and radio- TLC.

The radiochemical balance ranged from 92.2 to 98.8 % of applied radioactivity (AR) at the 0.1 mg/L and from 96.1 to 104.0 % of applied radioactivity at the 1.0 mg/L level for each of the processes simulated.

The composition of residues following different types of food processing is given in the table 7.7.1-1

Table 7.7.1-1: Mean distribution of radioactivity (%, mg/l as eq.)

Process	**Rate**	**α-Endosulfan**		**β-Endosulfan**		**Endosulfan diol**		**Identified**		**Sum of n.i.**
	mg/l	**%**	**mg/l**	**%**	**mg/l**	**%**	**mg/l**	**%**	**mg/l**	**%**
Pasteurisation 90ºC, pH 4, 20 min	0.1	68.29	0.071	29.15	0.030			97.44	0.101	
	1.0	64.95	0.640	26.35	0.259	3.98	0.039	95.28	0.938	5.8
Baking 100ºC, pH 5, 60 min	0.1	34.28	0.036	13.04	0.014	41.97	0.044	89.29	0.094	2.9
	1.0	29.92	0.298	11.87	0.118	49.27	0.491	91.05	0.91	6.4
Sterilisation 120 ºC, pH 6, 20 min	0.1					71.68	0.075	72.48	0.075	23.0
	1.0					75.72	0.749	75.72	0.749	24.7

n.i.: non identified radioactivity (up to nine components, 0.5 to 5.1% of applied radioactivity one compound at 8.1%)

Alpha-endosulfan and beta-endosulfan were the main components following pasteurisation (90°C) at 0.1 mg/L as well as at 1.0 mg/L treatment level, beside small amounts of endosulfan-diol.

In the brewing, baking and boiling simulation (100°C) the hydrolysis product endosulfan diol was the major single compound at both incubation levels. The sum of alpha- and beta endosulfan represented nearly half of the applied radioacticity. Furthermore one degradation product at 0.1 mg/L and three degradation products at 1.0 mg/L treatment level were observed representing less than 3.4 % of applied radioactivity, each.

The sterilisation process (120°C) resulted in a complete degradation of endosulfan. Endosulfan diol was the major degradation product amounting to approximately 75 % of the radioactivity applied. With one exception, the compounds represent more polar compounds than Endosulfan diol. None of the other reference standards used in HPLC or TLC investigations in this study corresponded to one of the resulting radio-peaks in the chromatograms. However, each of these non identified components represent only 0.5 to 8.1 % of applied radioactivity (mean values).

Evaluation and conclusion: The study was considered acceptable.

The results of the study have shown that under simulated conditions of pasteurisation parent endosulfan remained as the major compound with 91.3 - 97.4 % of AR (mean values, sum of alpha- and beta-endosulfan) of applied radioactivity. Small quantities (4.0 % of applied radíoactivity) endosulfan diol were formed in the 1 mg/L treatment level, beside an unknown degradation product (5.81 %; mean value).

Under conditions that simulate brewing, baking and boiling process the degradation product endosulfan diol was formed as the major compound at both incubation levels (42.0 % - 49.3% of AR, 0.0442 mg/L - 0.4919 mg/L). The sum of alpha- and beta-endosulfan represented only half of the applied radioactivity.

The sterilisation process led to complete degradation of endosulfan. Endosulfan diol was the major degradation product with approximately 75 % of the radioactivity applied. With one exception the remaining radioactivity represents compounds that are more polar than Endosulfan diol. None of the other reference standards used in HPLC or TLC corresponded to one of the peaks in the chromatograms.

Summing up, this investigation showed that endosulfan remained stable under conditions of pasteurisation (90 °C), but hydrolysed by half to endosulfan diol under conditions of brewing, baking and boiling (100 °C). Under conditions of sterilisation (120 °C) the hydrolysis product endosulfan diol was formed as the major residue component.

The notifier gave the following reasonament regarding to endosulfan diol formation: The first step in the industrial preparation of juice, puree and paste is the so-called "Hot Break" (Food Industries Manual, 1993, p. 231-232; ref.14): tomato fruit are heated as rapid as possible to approx. 85°C to destroy the enzymes and so obtain a better retention of pectin, which is desirable because it prevents the separation of the suspended solids. The heated tomatoes are then passed to continous extractors in which fine screens are incorporated; seeds, skins and hard portions of the fruit are removed. This leads to a drastic reduction of endosulfan residues in the tomato juice. And even if there is a small amount of residues retained in the juice, transformation to endosulfan-diol should be negligible due to the following reasons:

- Tomato juice is sufficiently acid to be stable after pasteurisation (Food Industries Manual, 1993, p. 232; ref.14); as the model study shows, no significant diol formation occurs under conditions simulating pasteurisation. Puree and paste are concentrated under vacuum and pasteurised, conditions that are not relevant for diol formation, too.

- Tomato fruit might be preserved under more stringent conditions of temperature and pressure; however, normally the peel is removed before canning and together with that by far the biggest fraction of the residue which is therefore not available for transformation.

This was confirmed by the following study in which the residue level (α- β- endosulfan, endosulfan sulfate and endosulfan diol) was measured in into tomato juice, canned peeled tomatoes and canned unpeeled tomatoes processed from tomatoes treated with Endosulfan CS 330 g/l.

This study showed that endosulfan diol appeared in peel (0.18 mg/kg) and in wet tomato pomace (0.06 mg/kg). In all the other fraction the residue was < LOQ. Therefore endosulfan diol does not appear in processed tomatos for human consumption.

B.7.7.2 Level of residues

Title: Processing of tomatoes treated with Endosulfan CS (330 g/l) into tomato juice and canned tomatoes

Author: A. Erbel and K. Ertz

Date: May 20, 2003

Ref: C030836

GLP: Yes

Material and methods:

The purpose of this study was the processing of tomatoes treated with Endosulfan CS 330 g/l into tomato juice, canned peeled tomatoes and canned unpeeled tomatoes. The tomato fruits were harvested after two applications of Endosulfan CS 330 g/l, 3 days after the last application. The field study was conducted in Southern Europe: Trial Nº 02R643-1 in Greece and Trial Nº 02R643-2 in Italy. Table 7.7.2-1 shows the results of the field trial.

Table 7.7.2-1: Results of the field residue trials

Crop/ Variety Trial No	Country/ Year	For G	Form.	Application rate		Nº	GW	Portion analysed	Residue (mg/kg)					PHI (days)
				kg a.s/ha	conc. % a.s				α	β	sulfate	diol	TOTAL	
Tomato Titane R643-1	Greece (S) 2002	F	CS 330g/l	1.060	0.823	2	87 88	fruit	0.71	0.35	<0.02	<0.02	1.09	3
Tomato Locale Di Molfetta R643-2	Italy (S) 2002	F	CS 330 g/l	1.060	0.823	2	84 88	fruit	0.06	0.04	<0.02	nd	0.10	3

The washing and peeling of tomatoes was done using household practice. The pasteurisation of tomato juice and the canning of tomato fruits simulated the industrial practice at a laboratory scale. Flow charts and material balances of the processing are shown in the original report

Preparation of Washed Fruits

The tomatoes were weighed and then washed in standing water by moving them around slowly. After washing, one part of the washed tomatoes was taken, weighed and sto red deep-frozen for 2 - 9 days at -18°C or below.

After storage, the deep-frozen washed tomatoes were shredded with dry ice, transferred into polystyrene boxes (examination samples) and stored deep-frozen at -18°C or below until analysis.

The washing water sample was also weighed, transferred into glass bottles (examination samples) and stored deep-frozen at -18°C or below until analysis.

Preparation of Tomato Juice

The tomatoes were washed in standing lukewarm water by moving them around slowly. After washing, the tomatoes were cut into small pieces. The cut tomatoes were heated with the addition of 100 mL water/kg tomatoes to 98 – 100ºC for 10 minutes in order to prevent enzymatic reactions. After this blanching process, the tomato pulp was passed through a strainer to separate juice and tomato pomace. The whole amount of tomato pomace was stored for 2 - 9 days at -18°C or below. One part of the tomato raw juice was transferred into polystyrene boxes and stored deep-frozen for 2 - 9 days at -18°C or below.

Sodium chloride (0.5 - 0.7% (w/w) relative to the amount of the juice) was added to the remaining part of tomato raw juice and this was filled into 1/1 preserving cans. Then the juice was pasteurised up to 91ºC. After pasteurisation, the tomato juice was transferred into polystyrene boxes and stored deep-frozen for 2 - 9 days at -18 ºC or below.

After storage, the tomato raw juice, the tomato pomace and the tomato juice were shredded with dry ice, transferred into polystyrene boxes (examination samples) and stored deep-frozen at –18ºC or below until analysis.

Preparation of Canned peeled Tomatoes

The deep-frozen tomatoes were washed in lukewarm water by moving them around slowly. After a few minutes the peel could be taken off. Subsequently, one part of the peeled tomatoes was weighed and stored deep-frozen for 1 - 8 days at -18°C or below. The peel was weighed and sto red deep-frozen for 1 - 8 days at –18º C or below.

The peeling water was stored deep-frozen at –18º C or below until analysis.

The other part of the peeled tomatoes was filled into 1/1 preserving cans and tomato juice was added. Then the tomato preserves were pasteurised up to 95°C. After pasteurisation, the canned peeled tomatoes were transferred into polystyrene boxes and stored deep-frozen for 1 - 8 days at -18 ºC or below.

After storage, the deep-frozen tomatoes, the peel and the canned tomatoes were shredded with dry ice, transferred into polystyrene boxes (examination samples) and stored at -18°C or below until analysis.

Preparation of Canned unpeeled Tomatoes

The tomatoes were washed in standing lukewarm water by moving them around slowly. After washing, the tomatoes were filled into 1/1 preserving cans and tomato juice was added. Then the tomato preserves were sterilised instead of pasteurised. This means that they were heated for longer time at temperatures above 100 ºC (about 12 minutes at temperatures up to 111°C) in arder to achieve a sufficient pasteurisation value.

After pasteurisation, the tomato preserves were taken and stored at -18°C or below until preparation of the examination samples at the same day.

The deep-frozen canned unpeeled tomatoes were shredded with dry ice, transferred into polystyrene boxes (examination samples) and sto red at -18°C or below until analysis.

Results:

α-Endosulfan:

Sample material	**R643-1 (DAA 3) Balance study (mg/kg)**	**R643-1 (DAA 3) Follow-up study (mg/kg)**
Tomatoes before processing	0.71	
Washed tomatoes	0.44	
Washing water	0.03	
Tomato raw juice	0.12	
Wet tomato pomace	3.65	
Tomate juice (pasteurised)	0.12	0.18
Peeled tomatoes	<0.02	
Peel	11.4	
Peeling water	0.05	
Canned peeled tomatoes (pasteurised)	0.05	0.05
Canned unpeeled tomatoes (sterilised)	0.32	

n.d.: not detectable

Sample material	**R643-2 (DAA 3) Balance study (mg/kg)**	**R643-2 (DAA 3) Follow-up study (mg/kg)**

Tomatoes before processing	0.06	
Washed tomatoes	0.06	
Washing water	0.005	
Tomato raw juice	<0.02	
Wet tomato pomace	0.63	
Tomate juice (pasteurised)	<0.02	<0.02
Peeled tomatoes	n.d.	
Peel	0.62	
Peeling water	0.01	
Canned peeled tomatoes (pasteurised)	<0.02	<0.02
Canned unpeeled tomatoes (sterilised)	0.03	

n.d.: not detectable

β-Endosulfan:

Sample material	**R643-1 (DAA 3) Balance study (mg/kg)**	**R643-1 (DAA 3) Follow-up study (mg/kg)**
Tomatoes before processing	0.35	
Washed tomatoes	0.26	
Washing water	0.02	
Tomato raw juice	0.09	
Wet tomato pomace	2.36	
Tomate juice (pasteurised)	0.09	0.10
Peeled tomatoes	<0.02	
Peel	6.51	
Peeling water	0.03	
Canned peeled tomatoes (pasteurised)	0.03	0.04
Canned unpeeled tomatoes (sterilised)	0.15	

n.d.: not detectable

Sample material	**R643-2 (DAA 3) Balance study (mg/kg)**	**R643-2 (DAA 3) Follow-up study (mg/kg)**
Tomatoes before processing	0.04	
Washed tomatoes	0.04	
Washing water	0.004	
Tomato raw juice	<0.02	
Wet tomato pomace	0.45	
Tomate juice (pasteurised)	<0.02	<0.02
Peeled tomatoes	n.d.	
Peel	0.53	

Peeling water	0.007	
Canned peeled tomatoes (pasteurised)	n.d.	<0.02
Canned unpeeled tomatoes (sterilised)	0.02	

n.d.: not detectable

Endosulfan-sulfate:

Sample material	**R643-1 (DAA 3) Balance study (mg/kg)**	**R643-1 (DAA 3) Follow-up study (mg/kg)**
Tomatoes before processing	n.d.	
Washed tomatoes	n.d.	
Washing water	0.00007	
Tomato raw juice	n.d.	
Wet tomato pomace	<0.02	
Tomate juice (pasteurised)	n.d.	n.d
Peeled tomatoes	n.d.	
Peel	0.03	
Peeling water	0.00014	
Canned peeled tomatoes (pasteurised)	n.d	n.d
Canned unpeeled tomatoes (sterilised)	n.d	

n.d.: not detectable

Sample material	**R643-2 (DAA 3) Balance study (mg/kg)**	**R643-2 (DAA 3) Follow-up study (mg/kg)**
Tomatoes before processing	n.d	
Washed tomatoes	n.d	
Washing water	0.00014	
Tomato raw juice	n.d.	
Wet tomato pomace	0.07	
Tomate juice (pasteurised)	n.d.	n.d.
Peeled tomatoes	n.d.	
Peel	0.06	
Peeling water	0.0005	
Canned peeled tomatoes (pasteurised)	n.d.	n.d.
Canned unpeeled tomatoes (sterilised)	<0.02	

n.d.: not detectable

Endosulfan-diol:

Sample material	**R643-1 (DAA 3) Balance study (mg/kg)**	**R643-1 (DAA 3) Follow-up study (mg/kg)**
Tomatoes before processing	<0.02	
Washed tomatoes	<0.02	

Washing water	0.001	
Tomato raw juice	n.d.	
Wet tomato pomace	0.06	
Tomate juice (pasteurised)	n.d.	<0.02
Peeled tomatoes	n.d.	
Peel	0.18	
Peeling water	0.002	
Canned peeled tomatoes (pasteurised)	n.d.	0.02
Canned unpeeled tomatoes (sterilised)	<0.02	

n.d.: not detectable

Since there were very low residues in the sample of the residue trial R643-2 no Endosulfan Diol was analysed.

Total Endosulfan:

Sample material	**R643-1 (DAA 3) Balance study (mg/kg)**	**R643-1 (DAA 3) Follow-up study (mg/kg)**
Tomatoes before processing	1.09	
Washed tomatoes	0.72	
Washing water	0.05	
Tomato raw juice	0.21	
Wet tomato pomace	6.10	
Tomate juice (pasteurised)	0.21	0.30
Peeled tomatoes	<0.04	
Peel	18.18	
Peeling water	0.07	
Canned peeled tomatoes (pasteurised)	0.08	0.11
Canned unpeeled tomatoes (sterilised)	0.49	

n.d.: not detectable

Sample material	**R643-2 (DAA 3) Balance study (mg/kg)**	**R643-2 (DAA 3) Follow-up study (mg/kg)**
Tomatoes before processing	0.10	
Washed tomatoes	0.10	
Washing water	0.009	
Tomato raw juice	<0.04	
Wet tomato pomace	1.15	
Tomate juice (pasteurised)	<0.04	<0.04
Peeled tomatoes	<0.02	
Peel	1.21	
Peeling water	0.02	
Canned peeled tomatoes (pasteurised)	<0.02	<0.04
Canned unpeeled tomatoes (sterilised)	0.07	

n.d.: not detectable

Summary of transfer factors

Based on the residue levels in the treatead processing products, transfer factors were calculated.

Transfer factors for residues of α endosulfan

Sample material	**Transfer factor R643-1 (DAA 3)**	**Transfer factor R643-2 (DAA 3)**
Balance study:		
Fruits, washed	0.62	1
Washing water	0.04	0.08
Fruits, peeled	0.03	-
Peel	16.1	10.3
Peeling water	0.07	0.17
Tomato Raw Juice	0.17	0.33
Tomato Juice (pasteurized)	0.17	0.33
Wet Tomato pomace	5.14	10.5
Canned Peeled Tomatoes (pasteurised)	0.07	0.33
Canned Unpeeled Tomatoes (sterilised)	0.45	0.5
Follow up study	**Transfer factor R643-1 (DAA 3)**	**Transfer factor R643-2 (DAA 3)**
Tomato Juice (pasteurised)	0.25	0.33
Canned Peeled Tomatoes (pasteurised)	0.07	0.33

Transfer factors for residues of β endosulfan

Sample material	**Transfer factor R643-1 (DAA 3)**	**Transfer factor R643-2 (DAA 3)**
Balance study:		
Fruits, washed	0.74	1
Washing water	0.06	0.1
Fruits, peeled	0.05	-
Peel	18.6	13.3
Peeling water	0.09	0.2
Tomato Raw Juice	0.26	0.5
Tomato Juice (pasteurized)	0.26	0.5
Wet Tomato pomace	6.74	11.3
Canned Peeled Tomatoes (pasteurised)	0.09	-
Canned Unpeeled Tomatoes (sterilised)	0.43	0.5
Follow up study	**Transfer factor R643-1 (DAA 3)**	**Transfer factor R643-2 (DAA 3)**
Tomato Juice (pasteurised)	0.29	0.5
Canned Peeled Tomatoes (pasteurised)	0.11	0.5

Transfer factors for residues of endosulfan sulfate

For endosulfan sulfate residues were below the LOQ or not detectable. The transfer factors were not calculated

Transfer factors for residues of endosulfan diol

Sample material	**Transfer factor R643-1 (DAA 3)**
Balance study:	
Fruits, washed	1.0
Washing water	0.05
Peel	9.0
Peeling water	0.1
Wet Tomato pomace	3.0
Follow up study	**Transfer factor R643-1 (DAA 3)**
Tomato Juice (pasteurised)	1.0
Canned Peeled Tomatoes (pasteurised)	1.0

Transfer factors for residues of total endosulfan

Sample material	**Transfer factor R643-1 (DAA 3)**	**Transfer factor R643-2 (DAA 3)**
Balance study:		
Fruits, washed	0.66	1.0
Washing water	0.05	0.09
Fruits, peeled	0.04	0.2
Peel	16.7	12.1
Peeling water	0.06	0.2
Tomato Raw Juice	0.20	0.4
Tomato Juice (pasteurized)	0.20	0.4
Wet Tomato pomace	5.60	11.5
Canned Peeled Tomatoes (pasteurised)	0.07	0.2
Canned Unpeeled Tomatoes (sterilised)	0.45	0.7
Follow up study	**Transfer factor R643-1 (DAA 3)**	**Transfer factor R643-2 (DAA 3)**
Tomato Juice (pasteurised)	0.27	0.4
Canned Peeled Tomatoes (pasteurised)	0.10	0.4

Assessment and conclusions: The study is acceptable.

Tomato fruits harvested in Southern Europe after two applications of Endosulfan CS (330 g/L), 3 days after the last application, were processed into juice, canned peeled tomatoes, canned unpeeled tomatoes and the corresponding intermediate processing fractions.

Comparing the transfer factors found in trial R643-1 with those in trial R643-2 it can be seen that in trial R643-2 in most cases higher values were found. This is most probably due to the fact that in tríal R643-2 the residues ín the raw agricultural commodity were much lower (0.1 mg/kg total endosulfan) than the corresponding value in trial R643-1 (1.09 mg/kg). However, both trials show similar trends regarding reduction or concentration of residues upon processing.

For washing water, peeling water, juice, peeled fruits, canned peeled and canned unpeeled fruits of trial R643-1 for total endosulfan transfer factors ranging from 0.04 to 0.45 were calculated. In trial R643-2 the corresponding transfer factors ranged from 0.09 to 0.7. These results indicate that upon processing in these fractions a reduction of residue concentration occurs.

For washed fruits transfer factors for total endosulfan of 0.66 (trial R643-1) and 1 (trial R643-2) were found, indicating that upon washing a small reduction of residue concentration may occur.

For wet pomace the transfer factor for total endosulfan ranged from 5.6 (trial R643-1) to 11.5 (trial R643-2). For peel this value ranged from 12.1 (trial R643-2) to 16.7 (trial R643-1). These results indicate that upon processing residues in peel and wet pomace are concentrated, which can be explained by the fact that most of the residues are found on the exterior of the tomatoes. This also explains why the transfer factors for canned unpeeled tomatoes (0.45 in trial R643-1 and 0.7 in trial R643-2) were higher than the values found for canned peeled tomatoes (0.07 in trial R643-1 and 0.2 in trial R643-2), although the canned unpeeled tomatoes had undergone a more severe heat treatment.

The fact that most of the residues are located on the exterior of the tomatoes is also reflected by the high percentage of residues recovered on the peel and in the material balances calculated for both trials. In the final processing products juice and canned peeled tomatoes only a low percentage is recovered, whereas for canned unpeeled tomatoes an intermediate value is found.

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

The metabolism studies indicated that significant residues may occur in edible animal tissue. However, livestock feeding studies are not required, since the endosulfan uses in EU are cotton and tomato, the animal diet included in the Guidance document 7030/VI/95 rev.4 Appendix G Livestock feeding studies does not included tomato and cotton, 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.7.9 Residues in succeeding or rotational crops. (IIA 6.6/01)

Title: Decline Residues in Tomatoes and following rotational crop European Union (Southern Zone) 2002

Author: E. H-J Klein

Date: May 20, 2003

Ref: C032692

GLP: Yes

The product was applied to tomatoes (*Lycopersicon escul*entum) at the maximum recommended rate and shortest pre-harvest interval (PHI) in order to investigate the residue pattern. The trial locations were spread over main growing areas of the EU southern zone (one in Greece and two in Italy) in order to cover different soil and climatic conditions. For the following crop for trial -1 spinach (*Spinacia olearacea*), for trial -2 leaf lettuce (*Lactuca sativa*) and for the trial -3 durum wheat (*Triticum durum*) was sown/planted on the same plots.

There were 2 applications at growth stage 83 - 88 (30 % of fruits show typical fullripe colour - 80 % of fruits show typical fullripe colour). The application rate was 1.61 kg/ha (1.5 L/ha) of formulated product (per application), which is equivalent to 529.8 gai/ha per application.

Samples were taken for analysis on the day of last application and at harvest. Additional samples were taken 2 and 3 days after last application in arder to determine residue decline in tomatoes. In trial -1 (Greece) spinach shoot were sampled 97 days after last application, in trial -2 (Italy) leaf lettuce shoots were samples 141 days after last application and for trial -3 (Italy) durum wheat shoots were sampled 247 days after last application.

The samples were analysed for α-Endosulfan, β-Endosulfan and Endosulfan sulfate by GC using ECD detection (method: AGR/MOA/ENO-3) with a limit of quantification (LOQ) of 0.020 mg/kg for the three analysed substances and for total endosulfan 0.06 mg/kg and all matrices. The results were reported as α-Endosulfan, β-Endosulfan and Endosulfan sulfate and total Endosulfan (AE F002671) in mg/kg.

Residues of treated plots are summarised in the following table:

Analyte	Crop	Matrix	DAA	Residues (mg/kg)
α-Endosulfan	Spinach	Shoots	97	<0.02
β-Endosulfan	Spinach	Shoots	97	<0.02
Endosulfan sulfate	Spinach	Shoots	97	<0.02
Total	Spinach	Shoots	97	<0.06

Analyte	Crop	Matrix	DAA	Residues (mg/kg)
α-Endosulfan	Lettuce	Shoots	141	<0.02
β-Endosulfan	Lettuce	Shoots	141	<0.02
Endosulfan sulfate	Lettuce	Shoots	141	<0.02
Total	Lettuce	Shoots	141	<0.06
α-Endosulfan	Wheat	Shoots	247	<0.02
β-Endosulfan	Wheat	Shoots	247	<0.02
Endosulfan sulfate	Wheat	Shoots	247	<0.02
Total	Wheat	Shoots	247	<0.06

Coclusions and assessment: The study is considered acceptable and showed that there is no residue of endosulfan in the rotational crop.

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	0.09	0.10	0.11	0.12	0.18	0.19	0.2	0.23	0.23	0.24	0.27	0.28	0.32	0.65

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

Method I:

R = Mean residue	0.186
SD	0.08
K	2.614
R max = R + SD x K	**0.39**

Supervised Trial Median Residues (STMR)	0.2
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	0.24
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

0.01	0.01	0.02	0.03	0.03	0.05	0.06	0.06	0.06	0.08	0.08	0.11	0.51

Method I:

R = Mean residue	0.085
SD	0.13
K	2.670
R max = R + SD x K	**0.43**
Supervised Trial Median Residues (STMR)	0.055

Number (n)	13
P=T/100	0.75
T=Percentil value	75
J=integer of (n+1) x P	10
G=modulus of (n+1) x P	0.5
R(J) = Residue in place J	0.08
R(J+1) = Residue in place J+1	0.08
R(0.75)	0.08
R(ber) = 2 x R(0.75) in mg/kg	**0.16**

Proposed MRL : 0.5 mg/kg

Proposed PHI: 21 days

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	0.5	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	**0.5**	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**

Considering all the uses not supported for Annex I inclusion as an open position and using the limit of determination for consumer risk assessment and using the UK model an unacceptable risk is achieved for the 97.5th percentile figures of Infants and Toddler.

Commodity	MRL (mg/kg)	TMDI mg/kgbw/day ADULT Mean	TMDI mg/kgbw/day ADULT 97.5th percentile	TMDI mg/kgbw/day CHILD Mean	TMDI mg/kgbw/day CHILD 97.5th percentile	TMDI mg/kgbw/day INFANT Mean	TMDI mg/kgbw/day INFANT 97.5th percentile	TMDI mg/kgbw/day TODDLER Mean	TMDI mg/kgbw/day TODDLER 97.5th percentile
CITRUS TOTAL	0,06	0,000038	0,000228	0,000036	0,000220	0,000086	0,000666	0,000164	0,000930
TREE NUTS TOTAL	0,06	0,000002	0,000028	0,000002	0,000034	-	-	0,000002	0,000074
POME FRUIT TOTAL	0,06	0,000027	0,000146	0,000039	0,000187	0,000114	0,000603	0,000113	0,000924
STONE FRUIT TOTAL	0,06	0,000005	0,000083	0,000003	0,000064	0,000016	0,000166	0,000012	0,000439
GRAPE-TABLE	0,06	0,000001	0,000069	0,000000	0,000057	-	-	0,000014	0,000245
GRAPE-WINE	0,06	0,000001	0,000131	-	-	-	-	-	-
TOMATO	0,5	0,000163	0,000539	0,000099	0,000414	0,000075	0,000782	0,000210	0,001283
PEPPER	0,06	0,000001	0,000017	0,000000	0,000015	-	-	0,000002	0,000161
MELON	0,06	0,000002	0,000093	-	-	-	-	-	-
POTATOES-TOTAL ALL	0,06	0,000113	0,000270	0,000184	0,000413	0,000198	0,000692	0,000226	0,000597
TEA	0,06	0,000004	0,000013	0,000002	0,000010	0,000003	0,000043	0,000002	0,000016
MILK-COWS	0,075	0,000275	0,000712	0,000522	0,001160	0,002912	0,007516	0,001585	0,004147
MEAT (ex poultry)	0,075	0,000090	0,000219	0,000110	0,000230	0,000104	0,000332	0,000143	0,000449
MEAT FAT	0,075	-	-	0,000000	0,000009	-	-	-	-
KIDNEY	0,075	0,000001	0,000024	0,000001	0,000022	0,000002	0,000032	-	-
LIVER	0,075	0,000004	0,000040	0,000005	0,000041	0,000007	0,000159	0,000002	0,000166
EGG	0,075	0,000024	0,000077	0,000031	0,000100	0,000104	0,000395	0,000052	0,000272
POULTRY MEAT	0,075	0,000021	0,000088	0,000018	0,000082	0,000029	0,000134	0,000043	0,000253
Total TMDI (mg/kg bw/day)		**0,0007722**	**0,002776362**	**0,0010535**	**0,003057947**	**0,0036501**	**0,011518678**	**0,0025702**	**0,009956**
% ADI		**12,870542**	**46,27270566**	**17,558295**	**50,96578746**	**60,835728**	**191,9779693**	**42,836782**	**165,9275862**

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 19.47% of the ArfD for adult consumers and a 88.27% 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	0.32	0.20	0.085	7	0.157	0.0029	0.093	0.0132	0.085	0.085

B.7.15 References relied on

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA 6.0	1998	David A. Winkler Freezer Storage Stability of Endosulfan (alpha, beta and Sulfate) on Animal Tissue and Dairy Matrices. [redacted] Report No. BJ96R006 A67512	Y	N	Bayer	Y
IIA 6.1 IIIA 8.1	2003	Suresh Mislankar, Patricia J.Tull Metabolism of ^{14}C-Endosulfan in Soybeans US EPA OPPTS 860.1300 EU Council Directive 91/414/EEC 7028/VI/95 Rev. 3, Appendix A [redacted] Report No. B004326 Study Identification: 601BJ	Y	N	Bayer	Y
IIA 6.2 IIIA 8.1	1996	C.M.M. Reynolds Distribution, elimination and the nature of the metabolite residues in the eggs and edible tissues of the laying hen. [redacted] Report No. TOX/95/142-2 TOX/94306	Y	N	Bayer	Y
IIA 6.2 IIIA 8.1	1996	C.M.M. Reynolds, J.M. Leah Distribution, elimination and the nature of the metabolite residues in the milk and edible tisúes of a lactating cow. [redacted] Report No. TOX/95/142-3 TOX/94308 TOX/94308A	Y	N	Bayer	Y
IIA 6.2 IIIA 8.2	2002	K-H Sonder Residues at Harvest in Tomatoes (Indoor) European Union (Southern Zone) 2001 Endosulfan, AE F002671 Emulsifiable concentrate (EC) 32.9% w/w (=32 g/l) AE F002671 00 EC33 C703 EU Commission Working Document 7029/VI/95 rev.5-22/07/97 Aventis CropScience. Germany Report No. 01 R 642 C020750	Y	N	Bayer	Y
IIA 6.3 IIIA 8.2	1999	H. Welcker, R.Martens 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) EU Commission Working Document 7029/VI/95 rev.5-22/07/97 Hoechst Schering AgrEvo GmbH. Germany Report No. ER 98 ECS 753 C004455	Y	N	Bayer	Y

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
IIA 6.3 IIIA 8.2	2003	E H-J Klein Residues at harvest in cotton; European Union (Southern zone) 2002 Endosulfan, AE F002671 Emulsifiable concentrate (EC) 32.9% w/w (=352g/l) AE F002671 00 EC33 B333 EU Commission Working Document 7029/VI/95 rev.5-22/07/97 Bayer CropScience GmbH. Residues and Human Exponsure D-65926 Frankfurt Germany. Report No. 02R170 C029815	Y	N	Bayer	Y
IIA 6.3 IIIA 8.2	2002	H.Welcker Decline of residues in cotton; European Union southern zone [2001]. Endosulfan, AE F002671 (suspension of micro-capsules (CS)) 25.78% w/w (=330 g/L) and (emulsifiable concentrate (EC) 32.9% w/w (=352 g/L). AE F002671 00 CS26 E218 AE F002671 00 EC33 C702 EU Comisión Working Document 7029/VI/95 rev.5-22/07/97. Aventis CropScience. Germany Report No. 01R170 C022557	Y	N	Bayer	Y
IIA 6.5	2003	A. Erbel, K. Ertz. Processing of Tomatoes Treated with Endosulfan CS (330 g/L) into Tomato Juice and Canned Tomatoes. Bayer CropScience GmbH. Residues and Human Exponsure D-65926 Frankfurt Germany. Report No. MR-510/02 Agredoc No. C030836 Study Identification: P662022508	Y	N	Bayer	Y
IIA 6.5	2002	T. Maurer Endosulfan: Investigation of the Nature of the Potential Residue in the Products of Industrial Processing or Household Preparation. Aventis CropScience. Germany Report No. CP01/022	Y	N	Bayer	Y
IIA 6.6	2003	E H-J Klein Decline of Residues in Tomatoes and following rotational crop European Union (Southern Zone) 2002. Endosulfan, AE F002671. Emulsifiable concentrate (EC) 32.9% w/w (=352g/L) Bayer CropScience GmbH. Residues and Human Exponsure D-65926 Frankfurt Germany. AE F002671 00 EC33 B333 Report No. 02R 171 EU Commission Working Document 7029/VI/95 rev. 5-22/07/97	Y	N	Bayer	Y