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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

Volume I

Report and Proposed Decision

December 1999

TABLE OF CONTENTS

LEVEL 1

LEVEL 2

LEVEL 3

LEVEL 2

ENDOSULFAN

Reasoned statement of the overall conclusions

2 Reasoned statement of the overall conclusions drawn by the Rapporteur Member State

2.1.1 Identity

This monograph has been prepared considering the documentation provided by three applicants: Hoechst Schering AgrEVO & Makhteshim Agan International (as a Task Force), Calliope, S.A. and B.V. Luxan.

Calliope was required to submit the Endosulfan manufacturer address and the location plant, this information was submitted on July 24th, 1998.

Endosulfan, 6,7,8,9,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-dioxathiepin-3-oxide, an insecticide, is a sulphurous acid ester of a chlorinated cyclic diol. Endosulfan as manufactured consists of the two stereo isomers α+β -endosulfan.

The applicant B.V. Luxan (Excel Industries Limited) has not submitted any acceptable data concerning the method or methods of manufacture, the specifications of purity of the active substance, the identity of isomers, impurities and additives, the maximum content of isomers and impurities, analytical profile of batches. All of this data are essential to the knowledge of the similarity of the active substances manufactured by the three different applicants.

The analytical profile of batches submitted by applicant Calliope showed that the content of one of the impurities is in some cases slightly above the FAO specifications.

Endosulfan is successfully used for controlling numerous insect pests and some mites in a wide variety of different crops. It acts via the GABA receptor system (opening the chloride transport, increasing glutamate level). It penetrates into the insect via the tracheas, by ingestion, and has some contact activity. When applied to plants, endosulfan can penetrate into plant tissue without developing systemic action. The product is hydrolysed by aqueous alkalis and acids to produce endosulfan diol. The lethal effect on the insects may be seen only after several hours (12-24h), there is no “knock down effect” first symptom is mainly tremor.

The plant protection products submitted as example for the EU Review by each one of the three applicants are Emulsifiable concentrates (EC). Hoechst Schering AgrEVO & Makhteshim Agan International (as a Task Force) have submitted the Thiodan 35EC, an emulsifiable concentrate containing 352 g of active ingredient per litre. This product is used for controlling numerous insect pests and some mites in a wide variety of crops grown in temperate, subtropical and tropical climate zones. The field of use is arable crops and greenhouse use in agriculture, horticulture, orchards, forestry and nurseries.

There exist a wide range and variety of uses of Endosulfan in the EU countries, the applicant Hoechst Schering AgrEVO & Makhteshim Agan International (as a Task Force) have carried out a review of

this uses and the use in northern EU was not considered in the evaluation. In orchards the higher application rate is in citrus (1050 g a.s/ha) and in stone fruit (800 g a.s/ha) in southern zone. In grapes the high dose rate is in southern zone with 1050 g a.s/ha, in horticulture crops the higher dose rate correspond to the use in solanaceas in green house (800 g a.s/ha) and finally it is important the dose rate recommended for cotton (840 g a.s/ha).

The method of application is conventional foliar spray using handheld equipment or motor diven boom sprayers and airborne sprayers. Number and timing of applications and duration of protection Endosulfan is preferably recommended as an early season product. The number of application is limited to 1 or 2 per year. Only under heavy insect pressure more applications are requested. Endosulfan is presented in use in combination with dimethoato, parathion-methyl and thiometan.

The applicant Calliope has submitted the plant protection product Callistar, an emulsifiable concentrate (EC) that contains 350 grams of active ingredient per litre. It is an insecticide for use in agriculture, horticulture, forestry and viticulture and for field and greenhouse use. It is act by contact and ingestion and controls chewing, sucking and boring insects and mites on a very wide range of crops. the proposed GAPs are only in France, for legume vegetables, brassica vegetables, stem vegetables, oil seed, potatoes and ornamentals. The range of dose rate is 0.26 to 0.61 kg as/ha. There are no authorised uses of Callistar yet in any of the EU member states, and the registration procedure for Callistar has been initiated in France.

The applicant B.V. Luxan (Excel Industries Ltd.) has submitted the emulsifiable concentrate (EC), called Endosulfan 35EC, for the EU review. **The applicant should submit the proposed GAPs in the European Union separated in northern and southern zone,** because the submitted GAPs are not clear. **No data were submitted concerning to the information of authorisations in EU member states.**

2.1.2 Physical and chemical properties

Endosulfan is a non volatile solid. Technical compound is a mixture of two stereo-isomers named α and β–endosulfan with melting points of 106-110 ^{o}C and 208-212 ^{o}C respectively. The isomeric mixture melts in a wide range between 70 ^{o}C and 124 ^{o}C. It is very low soluble in water and highly soluble in most of the organic solvents. **Due to the high partition coefficient (Pow > 4) risk for bio-accumulation must be contemplated for Endosulfan.** Hydrolysis to endosulfan-diol at pH = 9 . It is stable to photolysis but photoxidizes in air to endosulfan-sulphate. It is not flammable or autofammable not explosible and do not have oxidising properties. Most of the degradation products of Endosulfan are organochlorides that may be persistent and of environmental concern. **For this compounds the different routes degradation kinetics should be studied.**

Thiodan 35 EC is a light to dark brown liquid with an aromatic odour, showing a flash point closed of 43 ± 2 ºC. The pH-value of 7.0 is within the range that naturally occurs. The physical chemical properties allow storage at moderate temperatures for at least two years without deviation from

specification. Its viscosity, surface tension, foaming and emulsification properties indicate are acceptable for the proposed uses. Neither the emulsificable concentrate nor its spraying mixture have oxidising or reducing properties. Physico-chemical properties have been determined for Thiodan 35 EC. No further requirements are made.

Makhteshim-Agan has not provided information on its formulated plant protection product Thionex 35-EC.

The emulsifiable concentrate Callistar is neither explosive nor oxidising. The pH is somewhat low compared to that which naturally occurs in soil, but not considered to be of concern. Its stability allows storage under practical and commercial conditions. The shelf-life test (storage stability for 2 years) has not been finished yet. Callistar is claimed to be compatible with most pesticides but incompatible with strongly alkaline materials. In order to asses compatibility, the label prescribed testing before mixing with other chemicals. **This assessment is not acceptable and the physico-chemical compatibility must be studied with the formulate Callistar**.

Luxan B.V (Excel) has not provided any available documentation (Doc K) on plant protection product Endocel 35EC, this information should be required.

2.1.3 Details of uses and further information

Endosulfan is used for controlling numerous insect pests and some mites in a wide variety of different crops. In addition to numerous insects Thiodan also controls gall mites (*Eriophyidae*) and soft or broad mites (*Tarsonemidae*) damaging crops.

Endosulfan acts via the GABA recptor system. It penetrates into the insect via the tracheas, by ingestion, and some contact activity. When applied to plants, endosulfan can penetrate into plant tissue without developing system action. The product is hydrolysed by aqueous alkalis and acids to produce endosulfan diol. The lethal effect on the insect may be seen only after several hours (12-24), there is no " knock down effect", first symptom is mainly tremor.

Endosulfan is for use in arable crops and greenhouse use in agriculture, horticulture, orchards, forestry and nurseries. It controls harmful organism belonging to the following families: Aphids, White flies, Thrips, Lepidoptera, Peach twig and tree borer, Bugs, Psyllids, Coleoptera, Gall midge, Mites, Bud mites, Seed midge. The main metabolite endosulfan-sulphate has partly similar and partly less good efficacy compared to endosulfan. Resistance was reported for aphids in cotton, diamond backmoth in cabbage and cotton bollworm in parts of Australia.

Synergistic effects is reported in combination with Bacillus thur. products, synthetic pyrethroids and Bauveria formulations.

The plant protection products containing Endosulfan and that were submitted as example for the evaluation of the active substance for its inclusion in the Annex I are insecticides for use in agriculture and horticulture, orchards, forestry and nurseries, arable crops and greenhouse crops. When applied endosulfan penetrates into the insect via the tracheas, by ingestion and has some contact activity. Endosulfan can penetrate into plants tissue without developing systemic action. They are used for controlling numerous insects pests and some mites in a wide variety of different crops. The dose rate in southern Europe zones varies from 320 g a.i/ha to 1050 g ai/ha and the use of endosulfan in northern Europe zones was removed from the dossier during the elaboration of this monograph.

Endosulfan is classified as "very toxic to water organisms" therefore a contamination of water has to be prevented. In case of an accident contaminated water has to be collected separately and should not be allowed to enter the drainage system. Collected water has to be treated as active susbstance.

The preferred method for disposal of endosulfan is controlled incineration by an approved industrial incineartion plant. Small volumes may also be disposed of by communal waste incineration.

The applicant B. V. Luxan (Excel Industries Ltd.) did not submit any data concerning the packaging and compatibility with packaging materials, this data are essential to calculate the operator exposure. Moreover the applicant had not take into account the endosulfan toxicity for aquatic organism for the procedures for cleaning application equipment proposed. No data concerning the procedures for destruction or decontamination of the plant protection product and its packaging were submitted.

2.1.4 Classification and labelling

Hazard symbol: T+, N

Indication of danger: Very Toxic.
Dangerous for the environment

Risk phrases: R28: Very toxic if swallowed
R 21: Harmful in contact with skin
R26: Very toxic by inhalation
R50/53; Very toxic to aquatic organisms may cause long-term adverse effects in the aquatic environment

Safety phrases: S1/2: Keep locked up and out of reach of children
S4; Keep away from living quarters
S13; Keep away from food, drink and animal stuffs
S20; When using do not eat or drink
S27; Take off immediately all contaminated clothing

S28; After contact with skin, wash immediately with plenty of water
S36/37/39; Wear suitable protective clothing and gloves and eye/face protection
S38; In case of insufficient ventilation, wear suitable respiratory equipment.
S45; In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
S60; This material and its container must be disposed of as hazardous waste.
S61; Avoid release to the environment.

2.2 Methods of analysis

AgrEvo

AgrEvo submitted fully validated analytical methods for the analysis of the technical active substance, impurities and active ingredient in plant protection product.

For animal products only an acceptable method for liver, kidney and blood of Wistar rats has been submitted. Validation by an independent laboratory is required for this method.

For plant material many old methods, poorly validated, have been submitted. Only the analytical method for melons and vines and the method for potatoes are fully validated. For the rest of the methods no validation data are provided; these data are required to support residue trials that use those methods. Validation by an independent laboratory is also required for plant methods.

Two acceptable multi-residue methods where endosulfan is analysed are provided. One of them covers many pesticides not in use nowadays but the other is an up-dated method.

For soil method validation data and an English translation of the original report is required.

For drinking water validation data are required.

For surface water no method is provided and it is required.

A fully validated method for the analysis of air samples has been submitted.

No specific method for human plasma and body fluids is submitted. The use of the method for animal tissues validated for rats is proposed instead.

For wildlife an analytical method to determine endosulfan an its metabolites in fish is required.

Calliope

Methods provided by Calliope for technical active ingredient, purity, impurities (except impurity 1) and plant protection product are not acceptable.

A method for the determination of technical active ingredient purity and a method for impurities is required for inclusion of Calliope product in Annex 1 of Directive 91/414/EEC because are necessary to establish technical specifications of Calliope product.

No methodology was provided by Calliope for the quantitative determination of endosulfan residues in animal and human body fluids and tissues.

Methods for analysis of residues in plants provided by Calliope are not sufficiently validated. Validation and validation by an independent laboratory is required for these methods**. It is pointed out that Data Protection is required for the only two fully validated methods submitted by AgrEvo.**

Validation data are required to support the method for analysis of soil submitted by Calliope.

A validated method for the determination of endosulfan and its metabolite endosulfan sulphate in surface and drinking water is required to Calliope since the method submitted is not acceptable.

A method for the determination of endosulfan in air is required since the method submitted is not acceptable and Data Protection has been claimed for the method submitted by AgrEvo. A method for the determination of endosulfan in fish tissues is required.

2.3 Impact on human and animal health

2.3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities contained in the active substance or to their transformation products

Following oral administration of endosulfan,, either via single dose or dietary administration, elimination of the parent compound and its metabolites is extensive and relatively rapid in a range of species of experimental animals. In rats and mice, recovery of radiolabelled test material was generally greater than 85% of the administered dose, with a majority of this excretion occurring within a few days of administration. Excretion in rodents was mainly in the faeces, with a smaller amount excreted in the urine. Similarly, elimination of endosulfan was extensive in goats (>90%), with about 50% recovered in the faeces and 40% in the urine.

In mice endosulfan and its sulphate and diol metabolites were the major faecal excretion products, with the diol metabolite excreted in the urine, while in rats, biliary excretion was extensive (up to 50%), and there was a little enterohepatic circulation form the bile. There does not appear to be appreciable bioaccumulation of endosulfan residues in body tissues, with only trace amounts of endosulfan residues found in most tissues, including the fat, of most species. In Wistar rats, kidney and lover residues were highest, although the half life for residues in these organs was only 7 days and 3 days, respectively, and kidneys residues were also higher than other tissues in goats. No residues of endosulfan or its metabolites in cow or sheep milk were detected.

The metabolites of endosulfan include endosulfan sulphate, diol, hydroxy-ether, ether, and lactone but of its metabolites are polar substances which have not yet been identified.

Dermal absorption studies *in vivo* (rats and monkeys) and *in vitro* (human:rats) were performed They suggest that initial absorption is dose related, movement through skin is low (occurring over 168 h in the rat in vivo study), endosulfan continues absorbed from skin reservoirs after skin washing and penetration as per cent rate is lower in human skin than rat skin. Dermal absorption was reported to be as high as 25% in rats, and about 20% in Rhesus monkeys.

Endosulfan has been tested for acute toxicity, primary irritation and sensitisation potential Three notifier have submitted studies. The results obtained in the studies considered acceptable are summarised in 2.3.1-1. Purity, when reported, range between 96 and 97.3% among all the studies. The followed procedures were in accordance or without significant deviation from USEPA and OECD Guidelines. Not all the studies were performed to GLP.

The acute oral median lethal dose LD_{50} of Endosulfan Technical in rats was calculated to have a range between 48 and 160 mg/kg for male and 10 and 22.7 mg/kg for female rats. These results would require an **EEC classification of “T+” (very toxic) for the technical active ingredient, if based on the more sensitive sex alone.**

The dermal LD_{50} value for Endosulfan Technical in rats was greater than 4000 mg/kg b.w for male and 500 mg/kg b.w. for female. These results would require an **EEC classification of "Xn" (harmful) for the technical active ingredient.**

For **Endosulfan technical** an acute inhalation LC_{50} of 0.0345 mg/l air in male Wistar rats, and of 0.0126 mg/l air in females was determined. These results may require an **EEC classification of "T+" (very toxic).**

Skin and eye irritation studies submitted were considered not acceptable because purity of the technical product was not reported and exposition period after instillation into the eyes was very short. The applicant assumed Endosulfan should be considered not irritating to skin and eyes.

Based on the skin sensitisation studies (Buehler test), there is **no evidence that Endosulfan is a contact allergen and it is not classified based on EU criteria.**

In conclusion, based on acute oral toxicity studies in rats, and in accordance with EU criteria for classification, packaging and labelling of dangerous substances, Endosulfan is classified as 'very toxic', assigned the symbol "T+" and the risk phrase 'R28 very Toxic if swallowed'. Based on the dermal LD50 value in rats, it also should be classified as "Harmful" and be associated with the risk phrase "Harmful in contact with skin". Based on results of the acute inhalation study in rat, Endosulfan should be classified as 'very toxic', assigned the symbol "T+" and the risk phrase 'R26 very Toxic by inhalation' in accord with EU Guidelines.

Table 2.3.1-1: Summary of Acute Toxicity, Primary Irritation and Dermal Sensitisation Studies with Endosulfan Technical.

Route/Species/ Sex	Dose range (mg/kg BW)	Vehicle	Result	Reference
Oral				
Rat, Sherman, m	20, 32, 50, 80	ground-nut oil	LD_{50} = 48 mg/kg (m)	Scholz 1971
Rat, Sherman, f	6.3, 8.0, 10.0, 12.5	ground-nut oil	LD_{50} = 10 mg/kg (f)	Scholz 1971
Rat, Wistar, m/f	50, 100, 160, 250, 315 (m) 12.5, 25, 50 (f)	starch mucilage	LD_{50} = 100-160 mg/kg (m) LD_{50} = 22.7 mg/kg (f)	Diehl 1988
Dermal				
Rat, Wistar, m/f	3150, 4000 (m) 400, 630, 1000 (f)	----------	LD_{50} > 4000 mg/kg (m) LD_{50} = 500 mg/kg (f)	Diehl 1988
Inhalation				
Rat, SPF Wistar m/f	0.0123, 0.0288, 0.040, 0.0658 mg/L (m) 0.0036, 0.0123, 0.0288, 0.040, 0.0658 mg/L (f)	Ethanol-polyethylene 50:50	LC_{50} = 0.0345 mg/L (m) LC_{50} = 0.0126 mg/L (f)	Hollander 1983
Skin Sensitisation				
Guinea pig, SPF Pirbright-White f	----------	Polyethylene glycol 40%	No Sensitiser	Jung 1983

Several short-term toxicity studies were provided: a subacute oral toxicity study in rats, suchronic oral studies on rats and mice and, finally, dermal and inhalation studies on rats. The results of the studies considered as acceptable are summarised in table 2.3.1-2.

Table 2.3.1-2: Summary of acceptable short-term toxicity studies.

Study	NOAEL (mg/kg bw/day)	Main adverse effect	LOAEL (mg/kg bw/day)	Reference and year
Subacute studies				
30-days oral rats. Dose levels: 360 and 720 ppm (equal to 34 and 67.8 mg/kg/day)				Leist & Mayer, 1987 AgrEvo: IIA, 5.1.2.2/1
Subchronic studies				
90-day, diet, rat. Concentrations: 0, 10, 30, 60 and 360 mg/kg feed.(equal to 0, 0.64, 1.9, 3.8 and 23 mg/kg/day for males and 0.75, 2.3, 4.6 and 27 mg7kg/day for females)	3.85 (m)	Haematological changes	23.41 (m)	Barnard *et al*., 1985. AgrEvo IIA, 5.3.2.1/2
90-day, diet, mouse CD-1 Concentration 0, 2, 6, 18, and 54 mg/kg feed. (equal to 0, 0.24, 0.74, 2.13 or 7.3 mg/kg/day for males and 0, 0.27, 0.80, 2.39,0r 7.5 mg/kg/day for females)	2.3 (m/f)	Lethality and neurological signs	7.4 (m/f)	Barnard *et al*., 1984. AgrEvo IIA, 5.3.2.4/1
42 day, diet, mouse NMRKf. Dose levels 0, 18 ppm				Donaubauer *et al* 1985 AgrEvo IIA, 5.3.2.5/1
Other routes				
28-day dermal, rat 0, 1, 3, 9, 27 and 81 mg/kg bw/day				Ebert *et al* 1985 AgrEvo IIA, 5.3.3.1/1
28-day dermal, rat (males 0, 18.75, 37.50, 62.50 mg/kg bw/day, females 0, 9.83, 19.66, 32.00 mg/kg).		A NOAEL was not determined. Transient clinical symptoms were observed in the treated groups.		Dikshith *et al*. 1988 AgrEvo IIA, 5.3.3.1/4
29- days, nose-only inhalation, rat 0.0005, 0.0010, 0.0020 mg /l		No symptoms up the highest dose tested were observed.		Hollander *et al* 1984 AgrEvo IIA, 5.3.3.2/1

The subchronic oral toxicity study in rat revealed a NOAEL of 3.85 mg/kg bw/day (m), and a NOAEL of 2.3 mg/kg bw/day (m/f) in mice A 90-days feeding study in dogs is required.

The endosulfan genotoxicity data base has been prepared using the documentation submitted by AgrEvo, Excel and Calliope in support of the application. Numerous genotoxicity tests have been conducted with endosulfan. However, evaluation of the mutagenicity is confined to tests using technical endosulfan of clearly defined specifications. Results of these tests together with the information, presented by AgrEvo, about the genotoxicity of endosulfan-diol, a endosulfan metabolite, are summarised in Table 2.3.1-3.

The conclusions about the mutagenicity of endosulfan, based in data from studies carried out with technical material of clearly defined specifications, are the following:

1. Endosulfan does not induce gene mutation in bacterial or mammalian cells; and it appears to be non-mutagenic for yeast, however, results from the acceptable study cannot be considered conclusive because of its conduct.

2. Endosulfan was not clastogenic in cultured human lymphocytes following a short treatment but a continuous treatment without metabolic activation was not carried out.

3. Endosulfan did not induce DNA damage in bacteria (rec-assay) or in cultured mammalian cell (UDS); however, negative results, from the acceptable *Saccharomyces cerevisiae* mitotic gene conversion assay, cannot be considered conclusive because of its conduct.

4. Endosulfan appears to be non-clastogenic in mammalian somatic cells *in vivo*. Nevertheless, in the only study, considered acceptable in evaluating the mutagenicity of endosulfan, a micronucleus test, a dose greater than 10 mg/kg should have been tested. On the other hand, Thiodan 35 induced chromosomal aberrations in hamster; although any mutagenic activity may have resulted from non active constituents included in the formulation, it could be advisable to performed one study on chromosomal aberration induction with technical endosulfan.

5. The information given by the two presented chromosome aberration studies precludes any conclusion on the endosulfan clastogenicity for rodent germ cells, because in both studies the purity of the test substance was not reported. On the other hand, it is unlikely that a single isolated increase in dominant lethal mutations at the high dose is related with endosulfan administration; the lack of detail in the published study makes the significance of the isolated finding questionable.

6. Endosulfan induced sperm abnormalities in rodents. Nevertheless, it is unclear whether this effect is biologically significant.

The overall weight of evidence from the *in vitro* and *in vivo* studies is that endosulfan does not induce gene mutation. Nevertheless, although it appears to be non-clastogenic, more studies are required in order to give a definitive conclusion.

Table 2.3.1-3: Genotoxicity studies

Type of study	Species	Result with most sensitive species
In vitro studies	Bacteria	Negative for gene mutation in *Salmonella typhimurium* & *Escherichia coli*. Negative for rec-assay with *Bacillus subtilis*.
	Yeast	Inconclusive negative for gene mutation in *Schizosaccharomyces pombe.* and for mitotic gene conversion in *Saccharomyces cerevisiae*.
	Mammalian cells	Negative for gene mutation in mouse lymphoma cells. Inconclusive negative for CA in human lymphocytes. Negative for UDS in both rat hepatocytes and a human cell line.
In vivo studies with somatic cells	Rodent	Inconclusive negative for MN in mouse.
In vivo studies with germ cells	Rodent	Inconclusive positive for mouse dominant lethal test. Positive for mouse sperm abnormalities test.

The Long-term effect of endosulfan on rats, mice and dogs were evaluated from eight studies provided by different applicants and using the additional information found in IPCS document and Australian monograph (ANRA).

Four chronic toxicity studies, were performed on rats . (Keller, 1959c), mice (Arai, 1981) and . dogs (Keller, 1959b and Brunk 1989; 1990).

Chronic toxicity study on rats was carried out prior to GLP regulations and is not considered acceptable because the purity of the test substance was not reported The second study performed on mice is only a review of the original paper, thus only can be considered as additional information .

Finally, two 1-year feeding toxicity studies on dogs were presented by AgrEvo. The first study carried out on Mongrel dogs (Keller, 1959b), was performed prior to GLP regulations and is not considered acceptable for many reasons: the purity of the test substance was not reported, the higher dose level used did not induced any toxic effect and the number of dogs used by group does not permit obtaining significant results . Only, the other study carried out on Beagle dogs was conducted according to OCDE guidelines and GLPs compliance.

The combined chronic /carcinogenic studies were carried out on Charles River rats (Ruckman *et al.*, 1989) and on NMRI mice (Donaubauer 1989a, 1989b).

In the first case, the study was performed according to OECD: “Short-term and Long-Term toxicology group guideline” and following the GLP regulations Progressive glomerulonephrosis and aneurysms among in male rats aneurysms were detected. and, both signs were studied with more detail by

histophatology techniques by Gopinath & Cannon, (1990). A second addendum was provided by Leist et al., (1989a): the residues of α-endosulfan, β-endosulfan, endosulfan-hydroxiether, endosulfan-sulphate, endosulfan-lactone and endosulfan-diol, were determined in the liver and kidneys of mice after a chronic (2-year) feeding. study.

In the second combined study was evaluated the chronic oral toxicity and carcinogenic potential of endosulfan in NMRI-mice during two years . The study was conducted according to OECD 451 guideline in compliance with EPA guideline and following the GLP regulations. In support of this study, the residues of α-endosulfan, β-endosulfan, endosulfan-hydroxiether, endosulfan-sulphate, endosulfan-lactone and endosulfan-diol, were determined in the liver and kidneys (Leist. 1989b).

Both combined chronic and carcinogenic studies were summarised by Hack and published in Fd. Chem. Toxic. Vol.33, nº 11, pp: 941-950 (1995)

On the overall of these studies, no carcinogenic effect was observed in rats and mice at any Endosulfan dose tested.

Table 2.3.1-4: Summary of Long-term and Carcinogenic acceptable studies

Study	NOAEL		LOAEL		Main Adverse Effect	Reference/year
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d		
Chronic toxicity study						
1-year toxicity study in Beagle dogs. Oral. 1 year. Dose levels: 0, 3, 10,30 ppm.(equivalent to 0. 0.23, 0.77 and 2.3 mg/kgbw/day).	10	0.65 m 0.57 f	30	2.3	LOAEL based on the clinical signs (violent muscular contractions of the abdominal muscles),and reductions in body weights-	Brunk (1989; 1990) (AgrEvo: 5.3.2.3/3)
Carcinogenic studies						
Osborne-Mendel rats Oral. (78 weeks) and average dose levels: 0,220, 410 or 950 ppm for males and 220 and 400 for females males/females;	Not identified				No tumours were found in females; and no valid conclusion can be drawn about carcinogenicty in males	Thomas, LW *et al* (1978) (AgrEvo: IIA, 5.5.1/2) (AgrEvo: ANRA) (Calliope: IIA, 5.5/01)
:B6C3F1mice (78 weeks Oral.)Average dose levels: 3.5 and 6.9 ppm for males and 2 and 3.9 ppm for females	3.9 (f)	0.58 (f)			Owing the high early mortality rates, no conclusion can be drawn about carcinogenicty in males No carcinogenic effects in females.	Thomas, LW *et al* (1978) (AgrEvo: IIA, 5.5.1/2) (AgrEvo: ANRA) (Calliope: IIA, 5.5/01)
Combined chronic/carcinogenic studies						

Study	NOAEL		LOAEL		Main Adverse Effect	Reference/year
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d		
Charles River rats Oral.104 weeks.. Dose levels: 0,3,7.5, 15 and 75 ppm (equivalent to 0, 0.1, 0.3, 0.6 and 2.9 for males and 0, 0.1, 0.4, 0.7 and 3.8 mg/kg/day for females)	15(m/f)	M 0.6 F: 0.7	75(m/f	M 2.9 F 3.8	LOAEL based on low body gain weigh (m/f), low food consumption in females and kidney alterations in both sexes No evidence of increased carcinogenicity findings at any dose tested.	Ruckman SA et al., (1989) (AgrEvo: IIA, 5.5.1/4) (AgrEvo: ANRA) Hack et al., (1995) (Published) (AgrEvo:IIA, 5.5.1/6)
Combined toxicity/carcinogenicity study, in NMRI mice. Oral, 24 months. Dose levels:0, 2, 6, 18 ppm (equivalent to 0.28, 0.84 and 2.51 for males and 0.32, 0.97,and .2.86 mg/kg/day for females)	6	0.84 (m) 0.97 (f)	18	2.51 m 2.86 f	LOAEL base on decreased body weight in males at 24 months and decreased weight in males at 24 months and decreased weights of the liver, ovaries and lung in males and females at 12 and/or 18 months. No carcinogenic properties in mice	Donaubauer, HH (1989a, 1989b, 1990) (AgrEvo: IIA, 5.5.2/1/2/3) (AgrEvo: ANRA) Hack et al., (1995) (Published) (AgrEvo:IIA, 5.5.1/6)

m =male
f = female

Eight studies have been conducted to evaluate endosulfan toxicity on reproductive system. They include three multigeneration studies on rats and five developmental studies, four on rats and only on rabbits- All these studies are sponsored mainly by AgrEvo company.(table 2.3.1-5)

Multigeneration toxicity

To establish, the maximum tolerated dosage of endosulfan for use in a multigenerational study in rats was performed a preliminary study by Edwards *et al*., (1982). This study does not claim adherence to specific guidelines and GLP compliance.. Under the conditions of this study, it was concluded that 75 ppm (equivalent to 8.26 mg (kg/day and 8.36 mg/kg/day in males and females respectively), would be suitable fur use as the highest dose level in the subsequent multigeneration studies.

Kennedy *et a*l., (1965) study was conducted prior to the requirement of GLP and did not claim adherence to a specific guideline besides, the purity of the endosulfan was not reported , thus this study is considered as not acceptable. In addition, the dosages employed are referred to mg/kg/diet , thus it has not been possible to relate diet concentration of endosulfan to mass of endosulfan/kg bw animal/day In the study carried out by Edwards et al (1984) and Offer (1985) was evaluate endosulfan effects on the reproductive performance and developmental of F0, F1B and F2B generation rats.

Both studies were conducted to GLP compliance. Endosulfan did not affect reproductive performance or the growth or developmental of the offspring of rat over the course of a two generation study. The NOAEL for maternotoxicity was 1 mg/kg bw/day and for reproduction toxicity was 6 mg/kg bw/day. Developmental NOAEL could not be stabilised.

Developmental toxicity studies:

Five studies on developmental toxicity were performed, four of them on rats and one on rabbits:

1.-The first teratology study submitted was performed prior to GLP regulations and no guideline method was available at the time of the study. The study was published in Acta Pharmacol. Toxicol. vol 42: 150-152 by Gupta *et al.*, (1978). The level reporting in this published paper is not adequate for the purposes of defining an NOAEL for developmental toxicity Besides, the paper can not be considered acceptable because the purity of the test substance as the stability of the test substance and strain and age of the animals are not provided.

2.-An other study to determine the potential teratogenic of thiodan upon gravid albino rats was performed prior to GLP regulations and without any guideline specification (Haley, 1972). On the other hand, the dosages used in this study were not sufficiently high to induce any toxicity.

3.-.The only study performed according to OECD guideline referent to Teratogenicity studies and following the GLPs ,was carried out by Albrech and Baeder (1993). The NOAEL for maternotoxicty and for developmental toxicity was 2 mg/kgbw/day.

4.- A last report provide by AgrEvo company to evaluate the embriofetotoxicity in rats was designed by McKenzie et al (1980).The study was performed prior to GLP regulation and no guideline method was available at the time of the study**.** This study is considered as acceptable with some reservation, mainly because the replacement of animals during the study made difficult to interpret the data .

5.- Finally, one year later, the same author studied the embrio-fetal and teratogenic method nor GLP compliance. Besides, the interpretation of data is not clear .because some animals were also replacement during the study .

On the overall of these studies, non critical effect was identified to reproduction after administration of endosulfan and the fetotoxicity effects appear at maternal toxic doses.

Table 2.3.1-5: Summary of acceptable reproduction toxicity studies

Study	NOAEL		LOAEL		Main Adverse Effect	Reference/year
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d		
Preliminary study to determine doses used in two generation study in rats .Dosages: 0, 50, 75, 100 ppm	Maternal.50	M 6.25 F 5.92	Maternal: 75	M 8.26 F 8.36	Maternal: decreased of food consumption and body weights. Litter weights of dams were significantly decreased	Edward et al (1982) AgrEvo: IIA, 5.6.1/2
Two generation reproduction toxicity in rats. Dose levels: 0, 3, 15, 75 ppm (0.2,1, 4.99 mg/kg bw/day for males and 0.24, 1.23, 6.18 mg/kg bw/day for females)	Maternal 15 Reprod 75:	Maternal 1 Reprod 6	Maternal:75	Maternal:1	Maternal: Increased relative liver and Kidney weights-	Edwards et al., (1984) AgrEvo: IIA, 5.6.1/1 Offer., (1985) AgrEvo, IIA: 5.61/4
Developmental toxicity in rats. Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal:2 Develop::2		Maternal:6 Develop:6	Maternal:. On the basis of the deaths, clinical signs and decreased body weight Develop: increase incidence of fragmented thoracic vertebral centra No teratogenic effects	Albrech & Baeder, 1993 AgrEvo: IIA, 5.6.2.1/4
Developmental toxicity in rats Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal.. 0.66 Develop:2		Maternal:2 Develop:6	Maternal: decreased body weight gain and clinical signs. Develop: delayed development and a low incidence of isolated skeletal variation No teratogenic effects	McKenzie (1980) AgrEvo: IIA, 5.6.2.1/3)
Developmental toxicity in rabbits. Dose levels: 0, 0.3, 0.7, 1.8 mg/kgbw/day	:	Maternal 0.7 Develop: 1.8	:	Maternal:1.8	Maternal: based on Clinical signs (noisy, rapid breathing, hyperactivity and convulsions) No teratogenic effects	McKenzie et al., 1981 AgrEvo: IIA, 5.6.2.2/1

Two studies were reported by AgrEvo and Excel companies to evaluate delayed neurotoxicity of endosulfan (Robert & Phillips, 1983 and Gupta, 1976) , nevertheless the second study was considered as not acceptable because any reference about the purity of the test substance was provided. (table 2.3.1-6)

Robert & Phillips,(1983) designated a study to determine LD_{50} and delayed neurotoxicity of endosulfan in hens 200. birds were used and allocated in three different treatment: LD_{50} determination, protection assessment and neurotoxicity assessment. To determine LD_{50} was developed a preliminary range finding study on 5 groups of 2 birds doses with different concentrations to endosulfan. On the basis of this results, 30 birds were allocated to 6 treatment groups of 5 birds each,, at doses to 0, 40, 60, 90,135 and 110 mg/kg of endosulfan.

A small study was carried out to determine the protective effects of phenobarbitone, diazepam, atropine and 2-PAM when administered prior to dosing with endosulfan.

For neurotoxicity determination were used six groups of 10 birds each (including positive and negative control),treated with 96 mg/kg endosulfan (LD_{50} calculated).Negative control birds were dose only with corn oil and positive control with 500 mg/kg TOCP in corn oil Under the conditions of this study, endosulfan did not produce any clinical signs of neurotoxicity at the LD_{50} calculated .

Table 2.3.1-6: Neurotoxicity studies

Study type/species/ dose levels	**Comments**	**Reference and years**
Acute Delayed Neurotoxicity in hens. Dose levels 0,40,60,90,110, 135mg/kg	Any clinical signs of neurotoxicity at the LD_{50} calculated (LD_{50} value of the 96 mg/Kg	Roberts & Phillipps (1983) AgrEvo: IIA, 5.7/1
Neurotoxicity in Rats and mice	Endosulfan produce toxic effects due to CNS stimulation and the death may be due to direct depressant effect on some vital organ oft he body.	Gupta P(1976) Excell: IIA, 5.7/02)

There are several supplemental studies about, enzyme induction (endosulfan not induce hepatic microsomal enzyme activities on mice and rats), tumour promotion (No inhibition to enhance the incidence of GGT-positive hepatocyte in NDEA initiated was found in male rats treated with endosulfan.),endocrine system (endosulfan alone and in combination, may bind to estrogen receptors and may perturb the endocrine system), sperm effect (endosulfan does not produced significant changes), immunotoxicity (endosulfan does not have any adverse effect on the immune function of laboratory animals) and neurobehaviour (at highest dose levels alterations in neurobehaviour were observed with signs of frank toxicity), which them the almost were provided by the applicants and . additional information to cover these items has been found from IPCS (1998). Nevertheless, this information is only a little summary of the original papers, thus they have been considered only as additional information within of summary of each item. Table 2.3.1-7.

Table 2.3.1-7 Summary of supplemental studies

Study	Dose levels	Main Effects	Reference
Enzyme induction			
3-days. Oral gavage in male mice.	5 mg/kg/day	Cytochrome P-450 group of enzymes is not significantly activated.	Robacker et al., (1981) (AgrEvo: IIA, 5.1.3.2/2):
Promotion study			
In vitro metabolic cooperation (V79 cells) and scrape loading/dye transfer (WB cells) assays *Invite* EAF incidence assay, Oral gavage10-weeks, rats(m),	Doses: 1 and 5 mg /Kg/ bw/day	*In vitro*: ENDOαβ, ENDOα , ENDOβ, technical Endosulfan and Endosulfan-sulphate metabolite were potent inhibitors of intracellular communication in both assays in vitro. In addition Endosulfan-ether inhibited transfer in WB cells. *In vivo*: Technical endosulfan produced congestion of the peritoneum and inner organs, and increased liver weights	Flodström et al, (1988) (AgrEvo IIA, 5.5.3/1)
Endocrine system			
In vitro and In vivo studies		Endosulfan does not meet the criteria of a endocrine disrupter	Bremmer & Leist (1998) AgrEvo review
Effects on sperm			
Oral short-term/chronic study in male rats	2.5, 5. 7.5.10 mg/kg	Possible deleterious effects on male reproductive organs (testis) and byiosynthesis and secretion of testosterone	Singh & Padney(1989) (Excell, IIA, 5.5/01
Oral subchronic study in male Wistar rats	0, 7.5, 10 mg/kg/day	Testicular testosterone levels remained significantly decreased.	Singh & Padney(1990) (Excell, IIA, 5.5/03
Immunotoxicity studies			
Oral, six week study in male Wistar rats	0,10,30,50 ppm	Humoral and cellular immunity was depressed at doses of 30 and 50 ppm	Banerjee & Hussain (1987) (AgrEvo: IIA, 5.8.2.1/3)
Oral study in albino rats for up to 22 weeks	0,5,10,20 ppm	Marked suppression of the humoral and CMI responses in rats. Cellular and humoral immune responses were decreased in a dose-time dependent pattern.	Banerjee & Hussain (1986) (AgrEvo: IIA, 5.8.2.1/2)
Oral Wistar rats study	0.5, 1.5, 4.5 mg/kgbw/day		Hack & Leist (1988) (IPCS 1998)
Oral study in Wistar rats (3-weeks)	20, 100, 250 ppm	At 100 ppm: reduction in body weight gain.	Vos et al, (1982) (IPCS 1998)

Study	Dose levels	Main Effects	Reference
Neurobehavioral studies			
Oral acute study in rats	25, 50, 100 mg/kg/day (males) 3,6,12 mg/kg/day (females	LOAEL: 50 and 6 mg/kg/bw/day male and female respectively, based on serious neuropharmalcological effects.	Bury (1997) (IPCS 1998)
Rats	10mmol/L	No inhibition of rat brain AChE activity was observed for up to 75 min treatment.	Müller (1989) (IPCS 1998)
30-daysdietary study in Wistar rats	0, 3 and 6 mg/kg/day	A significant dose–related increase in motor activity in both sexes at low and high dose.	Paul , V et al., (1995) (AgrEvo:ANRA)
90-Days oral study in male rats	2 mg/kg/day	Changes in central nervous system, but not impair motor responses	Paul , V et al., (1993) (AgrEvo:ANRA)
90-Days oral study in male rats	2 mg/kg/day		Paul , V et al., (1994) (AgrEvo:ANRA)

Subchronic toxicity data from two different Endosulfan metabolites were presented: the ones with Thiodan sulphate are done without GLP compliance, since the ones with Hoe 051329 fulfil the requirements of GLP. The results of these studies are summarised in Table 2.3.1-8.

Table 2.3.1-8 Summary of oral subchronic studies

Study	NOAEL (mg/kg bw/day)	Main adverse effect	LOAEL (mg/kg bw/day)	Reference and year
90-day, oral, dog. Thiodan Sulphate	0.75 (m/f)	Salivation, muscular tremors and tonic-clonic convulsions	2.5 (m/f)	Cervenka, Kay and Calandra, 1964
90-day, oral , rat. Thiodan Sulphate				Wolf and Calandra, 1965.
90-day, oral, dog. Hoe 051329	9.1 male 8.4 female	bile duct proliferated with fibrosis	89.4 male 82.9 female	Stammberger 1994.
90-day, oral, rat. Hoe 051329	7.8 male 8.0 female	haematotoxicity and liver toxicity.	40.2 male 40.7 female	Ebert and Hack, 1996

The sub-chronic oral toxicity study with Thiodan sulphate revealed a no observed adverse effect level for the dog of 0.75 mg/kg bw/day, and with the other metabolite Hoe 051329 (Endosulfan diol) of 8.7 mg/kg bw/day (9.1 mg/kg bw/day male and 8.4 mg/kgbw/day female).

The NOAEL of Hoe 051329 (Endosulfan-diol) in the 90-day study in the rat was determined to be 7.8 mg/kg bw/day in male rats and 8.0 mg/kg bw/day in female rats, on aggregate 7.9 mg/kg bw/day for male and female rats.

Three studies using endosulfan-diol, a endosulfan metabolite, were sponsored and presented by AgrEvo**.** They included *in vitro* (gene mutation and UDS) and *in vivo* (micronucleus) assays. These studies are summarised in Table 2.3.1-9.

All studies were performed according to specific test guidelines and were GLP compliant. They were reported over the period 1992 to 1993.

Negative results were obtained in all studies.

The available genotoxicity tests show that endosulfan-diol could be considered as non genotoxic.

Table 2.3.1-9: Genotoxicity tests of metabolites (endosulfan-diol)

In vitro studies	Bacteria	Negative for gene mutation in *Salmonella typhimurium & Escherichia coli.*
	Mammalian cells	Negative for UDS in a human cell line.
In vivo studies with somatic cells	Rodent	Negative for MN in mouse.

In summary, of case report of human poisoning incidents, the lowest reported dose that caused death was 35 mg/kgbw. Higher doses caused death within 1 h. The clinical signs in these patients were dominated by tonic-clonic convulsion, consistent with the observations in experimental animal.

Table 2.3.1-10: Overall Evaluation of Mammalian Toxicology

Study	NOAEL		LOAEL		Main Adverse Effect
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d	
Short-term toxicity studies					
28-days oral, rats. Dose levels:360 and 720 ppm (equal to 34 and 67.8 mg/kg/day)	Not identified		Not identified		
28-day dermal, rat 0, 1, 3, 9, 27 and 81 mg/kg bw/day	Not identified.		Not identified.		
28-day dermal, rat (males 0, 18.75, 37.50, 62.50 mg/kg bw/day, females 0, 9.83, 19.66, 32.00 mg/kg).	Not identified.		Not identified.		
42 day, diet, mouse NMRKf. Dose levels 0, 18 ppm	Not identified.		Not identified.		
29- days, nose-only inhalation, rat 0.0005, 0.0010, 0.0020 mg /l	Not identified.		Not identified.		
90-day, diet, rat. Concentrations: 0, 10, 30, 60 and 360 mg/kg feed. d (equivalent to 0, 0.64,1.9, 3.8 and 23 mg/kgbw/day for males and 0, 0.75, 2.3, 4.6 and 27 mg/kgbw/day for females	60	3.85 (m/f)	360	23.41 (m/f)	Haematological changes
90-day, diet, mouse CD-1 Concentration 0, 2, 6, 18, and 54 mg/kg feed (equal to 0, 0.24., 0.74, 2.13 or 7.3 mg/kg/day for males and 0, 0.27, 0.80, 2.39 or 7.5 mg/kg/day for females).	18	2.3 m/f	54	7.4 m/f	LOAEL: based on lethality and neurological signs
Genotoxicity studies					

Study	NOAEL		LOAEL		Main Adverse Effect
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d	
In vitro studies in bacteria					Negative for gene mutation in *Salmonella typhimurium & Escherichia coli*. Negative for rec-assay with *Bacillus subtilis.*
In vitro studies in Yeast					Inconclusive negative for gene mutation in *Schizosaccharomyces pombe.* and for mitotic gene conversion in *Saccharomyces cerevisiae.*
In vitro studies in Mammalian cells					Negative for gene mutation in mouse lymphoma cells. Inconclusive negative for CA in human lymphocytes. Negative for UDS in both rat hepatocytes and a human cell line.
In vivo studies with somatic cells in rodents					Inconclusive positive for MN mouse
In vivo studies with germ cells in rodents					Inconclusive positive for mouse dominant lethal test. Positive for mouse sperm abnormalities test
Long-term and carcinogenic studies					
1-year oral toxicity study in Beagle dogs. Oral. 1 year. Dose levels: 0, 3, 10,30 ppm.(equivalent to 0. 0.23, 0.77 and 2.3 mg/kgbw/day).	10	0.65 m 0.57 f	30	2.3	LOAEL based on clinical signs (violent contractions of the abdominal muscles) and reductions in body weight gain

Study	NOAEL		LOAEL		Main Adverse Effect
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d	
Carcinogenic study: Osborne-Mendel rats Oral. (78 weeks) and average dose levels: 0,220, 410 or 950 ppm for males and 220 and 400 for females males/females;	Not identified				No tumours were found in females; and no valid conclusion can be drawn about carcinogenicty in males
Carcinogenic study: in B6C3F1mice (78 weeks Oral.)Average dose levels: 3.5 and 6.9 ppm for males and 2 and 3.9ppm for females	3.9 (f)	0.58 (f)			Owing the high early mortality rates, no conclusion can be drawn about carcinogenicity in males No carcinogenic effects in females.
Combined toxicity/carcinogenic study. in Charles River rats Oral.104 weeks.. Dose levels: 0,3,7.5, 15 and 75 ppm (equivalent to 0, 0.1, 0.3, 0.6 and 2.9 for males and 0, 0.1, 0.4, 0.7 and 3.8 mg/kg/day for females)	15(m/f)	M 0.6 F: 0.7	75(m/f	M 2.9 F 3.8	LOAEL based on low body gain weigh (m/f), low food consumption in females and kidney alterations in both sexes No evidence of increased carcinogenicity findings at any dose tested.
Combined toxicity/carcinogenic study, in NMRI mice. Oral, 24 months. Dose levels:0, 2, 6, 18 ppm (equivalent to 0.28, 0.84 and 2.51 for males and 0.32, 0.97,and .2.86 mg/kg/day for females)	6	0.84 (m) 0.97 (f)	18	2.51 m 2.86 f	LOAEL based on decreased body weight in males at 24 months and decreased weight in males at 24 months and decreased weights of the liver, ovaries and lung in males and females at 12 and/or 18 months. No carcinogenic properties in mice

Reproduction and developmental studies

Study	NOAEL		LOAEL		Main Adverse Effect
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d	
Preliminary study to determine doses used in two generation study in rats .Dosages: 0, 50, 75, 100 ppm	Maternal.50	M 6.25 F 5.92	Maternal: 75	M 8.26 F 8.36	Maternal: decreased of food consumption and body weights. Litter weights of dams were significantly decreased
Two generation reproduction toxicity in rats. Dose levels: 0, 3, 15, 75 ppm (0.2,1, 4.99 mg/kg bw/day for males and 0.24, 1.23, 6.18 mg/kg bw/day for females)	Maternal 15 Reprod 75:	Maternal 1 Reprod 6	Maternal: 75	Maternal:1	Maternal: Increased relative liver and Kidney weights-
Developmental toxicity in rats. Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal:2 Develop::2		Maternal:6 Develop:6	Maternal:. On the basis of the deaths, clinical signs and decreased body weight Develop: increase incidence of fragmented thoracic vertebral centra No teratogenic effects
Developmental toxicity in rats Dose levels: 0. 0.66, 2 and 6 mg/kg bw/day		Maternal.. 0.66 Develop:2		Maternal:2 Develop:6	Maternal: decreased body weight gain and clinical signs. Develop: delayed development and a low incidence of isolated skeletal variation No teratogenic effects
Developmental toxicity in rabbits. Dose levels: 0, 0.3, 0.7, 1.8 mg/kgbw/day		Maternal 0.7 Develop: 1.8		Maternal: 1.8	Maternal: based on Clinical signs (noisy, rapid breathing, hyperactivity and convulsions) No teratogenic effects

Neurotoxicity studies

Study	NOAEL		LOAEL		Main Adverse Effect
	ppm	mg/kg bwt/d	ppm	mg/kg bwt/d	
Acute Delayed Neurotoxicity in hens. Dose levels 0,40,60,90,110, 135mg/kg					Any clinical signs of neurotoxicity at the LD_{50} calculated . the 96 mg/Kg

2.3.2 ADI

The calculation of an ADI is based on the more sensitive of the following studies, chronic, carcinogenic and reproduction toxicity in dogs, rats and mice.

ADI was established in 0.006 mg/kg/day based on the lowest NOAEL obtained in the most sensitive specie, rat , and using a safety factor of 100. (2 years dietary study in rats)

2.3.3 ARfD (acute reference dose)

2.3.4 AOEL

Systemic AOEL was 0.006 mg/kg bw/day based on the lower NOAEL obtained in subchronic, chronic and reproduction studies on the most sensitive specie and using a safety factor of 100. (104-weeks dietary study in rats). (Oral absorption > 90%, assessment factor =1)

2.3.5 Drinking water limit

On basis that exposure through drinking water should not account for more than 10% of the ADI and that the average consumption is 2 litres of water/day for a 60 kg person, we propose a **Parametric Value for Drinking Water =0.018 mg/l**

2.3.6 Impact on human or animal health arising from exposure to the active substance or to impurities contained in it

Thiodan (AgrEvo) has been thoroughly tested for acute toxicity(the inhalation study was performed with Endosulfan emulsifiable concentrate (500 g/l)), primary irritation and sensitisation potential. Results obtained in these studies are summarised in Table 2.3.6-1. All studies were performed according procedures of the OECD and EPA and in compliance with GLP.

The acute oral median lethal dose (LD_{50}) of Thiodan in rats was calculated to be 67 mg/kg for male and 17 mg/kg for female. According to the EU Criteria, Thiodan should be classified with the symbol T+ (very toxic) and the risk expression R28 in rats.

The acute oral median lethal dose (LD_{50}) of Thiodan in mice was calculated to be 39 mg/kg for both male and female. According to the EU Criteria, Thiodan should be classified with the symbol T (toxic) and the risk expression R25 in mice.

The acute oral median lethal dose (LD_{50}) of Thiodan in rabbit was determined to be 75 mg/kg for male. In the female rabbit, the oral LD_{50} was determined to be 34 mg/kg. In the sexes combined the oral LD_{50} was determined to be 50 mg/kg. According to the EU Criteria, Thiodan should be classified with the symbol T (toxic) and the risk expression R25 in rabbit. (table 2.3.6-2).

The acute dermal median lethal dose (LD_{50}) of Thiodan for male rat was determined to be 412 mg/kg. For the female rat, the LD_{50} was approximately 266 mg/kg. According to the EU Criteria, Thiodan should be classified with the symbol T (toxic) and the risk expression R24.

The acute dermal median lethal dose (LD_{50}) of Thiodan for rabbit was greater than 400 mg/kg. According to the EU Criteria, Thiodan should be classified with the symbol Xn (harmful) and the risk expression R21.

The inhalation study was performed with Endosulfan-emulsifiable concentrate (500 g/l). (Hoe 002671 OI EC43 A103). The acute inhalation median lethal concentration (LC_{50}) of Endosulfan-emulsifiable concentrate (500 g/l) was determined to be 0.263 mg/l for male rats and 0.0594 for female rats. According to the EU Criteria, Endosulfan-emulsifiable concentrate (500 g/l) should be classified with the symbol T+ (very toxic) and the risk expression R26.

Material test (Thiodan) was considered to be irritant to rabbit skin. According to the EU Criteria, Thiodan should be classified as skin irritant (Xi) and the risk expression R38.

The acute eye irritation/corrosion test with Thiodan were irritant to rabbit eye. According to the EU Criteria, Thiodan should be classified as eye irritant and the risk expression R41.

A skin sensitisation study in guinea pig using the Buehler method demonstrated that Thiodan is not considered to be a skin sensitizer. According to the EU Criteria, Thiodan should not be classified as skin sensitising.

In conclusion, Thiodan might be considered very toxic by oral route in rats, and toxic for mice and rabbit. By dermal route, material test is considered toxic for rat and harmful for rabbit. Endosulfan emulsifiable concentrate (500 g/l) is very toxic by inhalation. Thiodan is irritant to skin, irritant to eye and not a skin sensitizer.

Table 2.3.6-1: Summary of acute toxicity studies of Thiodan

Species/strain	Sex	Route/Method	Result	Reference
Rat/Wistar	Both	Oral	LD_{50}(male)=67 mg/kg LD_{50}(female)=17 mg/kg	Ebert. 1989a
Mice/NMRI	Both	Oral	LD_{50}=39 mg/kg	Ebert 1989b
Rabbit/NZ	Both	Oral	LD_{50}(male)=75 mg/kg LD_{50}(female)=34 mg/kg	Ebert 1989d
Rat/Wistar	Both	Dermal	LD_{50}(male)=412 mg/kg LD_{50}(female)=266 mg/kg	Ebert.1989c
Rabbit/NZ	Both	Dermal	LD_{50}>400 mg/kg	Ebert.1989d
Rat/Wistar	Both	*Inhalation	LC_{50}(male)=0.263 mg/l LC_{50}(female)=0.0594 mg/l	Hollander 1984
Rabbit/NZW	Both	Dermal	Skin Irritant	Ebert.1989d
Rabbit/NZW	Female	Eye	Eye Irritant	Ebert.1989e
Albino Guinea pig/Himalaya	Both	Sensitisation (Buehler)	Not Sensitising	Ullmann.1986

* Material test: Endosulfan emulsifiable concentrate (500 g/l). code: Hoe 002671 OI EC 43 A103

Callistar Endosulfan 35 EC (Calliope) has been thoroughly tested for acute toxicity (oral and dermal), primary irritation and sensitisation potential. Results obtained in these studies are summarised in Table 2.3.6-2. All studies were undertaken with a single lot (lot. 1 del 10.01.91), and were performed according procedures of the OECD (except skin sensitisation which are performed according to an adaptation of Magnusson Kligman method) and in compliance with GLP.

The acute oral median lethal dose (LD_{50}) of Callistar is approximately 50 mg/kg for male and female rats (the mortality rates indicate that the LD_{50} will be situated between 30 and 80 mg/kg). According to the EU Criteria, Callistar should be classified with the symbol T (toxic) and the risk expression R25.

The acute dermal median lethal dose (LD_{50}) of Callistar for female rats alone is situated below 2000 mg/kg. Therefore, because 60% mortality occurred in the female group, a complete study should be performed.

Material test, Callistar, was considered to be irritant and corrosive in rabbits. According to the EU Criteria, Callistar should be classified with the symbol C (corrosive) and the risk expression R34 and with the symbol Xi (irritant) and the risk expression R38.

The acute eye irritation/corrosion test with Callistar in rabbits were irritant and due of duration of effects and according to the EU Criteria, Callistar must be considered as causing irreversible eye damage.

A skin sensitisation study in guinea pig using a modified version of Magnusson Kligman method demonstrated that Callistar is not considered to be a skin sensitizer.

Table 2.3.6-2: Summary of acute toxicity of Callistar Endosulfan 35 EC

Species/strain	Sex	Route/Method	Result	Reference
Rat/S-D	Both	Oral	LD_{50} approx. = 50 mg/kg	Halaviat. 1991a
Rat/Wistar	Both	Dermal	LD_{50}(male)>2000 mg/kg LD_{50}(female)<200 0mg/kg	Pinon 1991a
		Inhalation	Test not conducted	
Rabbit/NZW	n.a.	Dermal	Irritant and corrosive to skin	Halaviat 1991b
Rabbit/NZW	n.a.	Eye	Causing irreversible eye damage	Halaviat 1991c
Albino Guinea pig/Hartley	Both	Sensitisation (modified Magnusson /Kligman)	Not Sensitising	Pinon 1991b

n.a: not available.

Endosulfan 35% EC has been tested for acute toxicity (oral and dermal) and skin irritation. Results obtained in these studies are summarised in Table 2.3.5-3. All studies were undertaken with a single batch of formulation (F94/-/113) and were performed according procedures of the OECD and EC and in compliance with GLP.

The acute oral median lethal dose (LD_{50}) of Endosulfan 35% EC in rats was 69 mg/kg for the sexes combined. Estimated oral LD_{50} values for the males alone were 96 mg/kg and for females alone 28 mg/kg. According to the EU Criteria, Endosulfan 35% EC should be classified with the symbol T (toxic) and the risk expression R25.

The acute dermal median lethal dose (LD_{50}) of Endosulfan 35% EC in rats was 1006 mg/kg for the sexes combined. Estimated dermal LD_{50} values for the males were 1450 mg/kg and for females 449 mg/kg. According to the EU Criteria, Endosulfan 35% EC should be classified with the symbol Xn (harmful) and the risk expression R21.

Material test (Endosulfan 35% EC) was considered to be irritant and corrosive to rabbit skin. According to the EU Criteria, Endosulfan 35% EC should be classified with the symbol C (corrosive) and the risk expression R34 and with the symbol Xi (irritant) and the risk expression R38.

In conclusion, Endosulfan 35% EC might be considered toxic by oral route, harmful by dermal route and irritant and corrosive to rabbit skin.

Table 2.3.6-3: Summary of acute toxicity of Endosulfan 35% EC

Species/strain	Sex	Route/Method	Result	Reference
Rat/Wistar	Both	Oral	LD_{50} combined = 69 mg/kg LD_{50} approx. (male)= 96 mg/kg LD_{50} approx.(female)=28 mg/kg	Pels Rijcken 1994a
Rat/Wistar	Both	Dermal	LD_{50} combined = 1006 mg/kg LD_{50} approx. (male)=1450 mg/kg LD_{50} approx.(female)=449 mg/kg	Pels Rijcken 1994b
		Inhalation	Test not conducted	
Rabbit/NZW	Male	Dermal	Irritant and corrosive to skin	Pels Rijcken 1994c
		Eye	Test not conducted	
		Sensitisation	Test not conducted	

2.4 Residues

2.4.1 Definition of the residues relevant to MRLs

The definition of the residue for both risk assessment and GAP monitoring purposes should provisionally be considered as the parent compound (α and β isomers) and its main and most toxic metabolite endosulfan sulphate. **This is subject to a confirmation of the validity of the proposed plant metabolic behaviour and the metabolism in animals, which must be carried out in additional experiments that will be required from the applicants.**

2.4.2 Residues relevant to consumer safety

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 and apple trees.

According to the assessment 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.

The sum of main residue components of endosulfan (i.e. α-endosulfan, β-endosulfan and endosulfan sulphate) vary a great deal depending upon the crop investigated. Thus, these main components reach around 95% in apple and tomato, while only reaching 50% in cucumber. Additional information should be provided dealing with the nature of metabolites found in cucumber, in particular about those present in the non-polar and polar fractions. Special attention should also be given to the lactone metabolite due to its high toxicity as it is shown in the toxicity studies.

Additional experiments on metabolism in plants are required for oils seeds and root and tuber vegetables.

Animal tissue residue studies have been conducted in sheep, lactating diary cows and lactating goats. From the results of these studies it can be stated that endosulfan residues in livestock organs, in fat and muscular tissues, and milk fat consisted mainly of endosulfan sulphate and α- and β-endosulfan and in urine of endosulfan diol. Muscular tissue contained generally lower residues than offal and fatty tissues. The highest residue levels were detected in kidney and/or kidney fat. The unchanged parent substance occurred mainly in the faeces.

Studies performed are clearly insufficient and additional experiments must be carried out. Moreover, the metabolic pathway in animals should be indicated

Only one study using radiolabelled chemicals has been carried out (Doc A14216). Moreover, this was performed using a too low dose (0.3 mg/kg). A dose around 10 mg/kg would have been adequate for this study.

There is a lack of data on recoveries of radioactivity with reference to the measured radioactivity in specific tissues, and also on the extraction schemes used. Data on the extractability of residues should be given.

Studies on laying poultry (chickens) must be carried out, including residue data in different tissues and in animal products (eggs).

Consequently, the applicants must perform additional experiments on metabolism in livestock, and these experiments should be carried out according to the objectives and recommendations of the EU Directive.

Many of the residue trials carried out did not follow the GAP conditions. Consequently, only those residue data generated according to the GAPs were considered in MRLs calculation. Further residue trials are required in the level 4 of this monograph.

The fate of endosulfan residues during processing of raw agricultural commodities was investigated in several major registered crops and for the important processing procedures.

Endosulfan residues are effectively reduced in various commodities by heating processes. The remaining residues are most often found in waste or feedingstuff fractions. Concurrently, the parts for human consumption contain considerably less residues than the raw crop material.

After solvent extraction of oil containing crop material the residue may concentrate in the crude oil, but is effectively removed during the refining process.

The high transfer factor found for pomace in tomatoes (10-20) makes it advisable to present residue data in pomace for citrus fruit and other crops. Besides, additional experiments in prunes and raisins would be necessary to demonstrate if a residue concentration takes place in these products. The same can be applied for essential oils in citrus.

Special attention should be given to the high concentration factor found in pomace, due to the important part that this product can play in animal feeding. Therefore, residue data on orange pomace should also be presented and results on livestock feeding must be considered carefully.

High deviations in the residue data for dried tea were found in the residue trials performed, which lead to excessive MRLs. Although data available seem to demonstrate a small transfer of residues to tea infusions, the high residue levels found in some of the trials together with the importance of the tea infusion in the diet make advisable to perform additional residue trials and processing studies in tea.

It is important to emphasised the high transfer factor found in soybean crude oil, which can reach a value up to 4.3 and would lead to high residue levels. Although experiments demonstrate that refined oil did not contain endosulfan residues, it is convenient to consider the unfavourable situation for crude oil.

Livestock feeding studies were performed in lactating dairy cows and lactating goats. In order to assess the residue situation in food of animal origin after feeding of fodder contaminated with endosulfan, a hypothetical feeding ratio was composed and the theoretical residue concentration in the daily diet was calculated to be 0.1 mg/kg. **However, because animal feeding diets vary enormously, and the composition of animal feed varies from one country to another, different diets should be considered by the applicant trying to construct a worst case diet in calculate the 1x dose for relevant domestic animals.**

The feeding trials should comprise a control group, a group treated with the expected residue level (1x dose), and groups treated with excess doses (3-5x dose and 10x dose). Accordingly, additional experiments on livestock feeding are required to compliance the EU Directive.

Studies on poultry (laying hens) are needed, including dosage groups of at least 9 animals. In this case, residue data on eggs should also be included.

The stepwise approach developed by the German BBA in their guideline Part IV, 3-10, May 1988, was followed for the theoretical estimate of the residues in rotational crops.

At harvest, the crops contained lower residue concentrations than the corresponding soil samples.

However, uptake factors (soil/plant) found for different crops show significative variations. Field tests which provide information on the actual residue situation in rotational crops are required for selected leafy vegetables in different types of soil and climatic conditions.

Based on the residue data obtained from those residue trials that were performed according to the GAPs, most of MRLs proposed by the applicant were not consistent. Consequently, most of MRLs have to be considered just as provisional until more data is made available from the additional residue trials that have been required to the applicant.

The provisional theoretical maximum daily intake (TMDI) of endosulfan residues for a 60 kg body weight person has been estimated in 0.004528 mg/kg bw. This value does not exceed the toxicologically determined Acceptable Daily Intake (ADI) of 0.006 mg/kg bw. The theoretical maximum daily intake (TMDI) of endosulfan residues has to be recalculated taking into account the new MRL resulting from the residue trials required in the Level 4 of this Monograph.

2.4.3 Residues relevant to worker safety

All the exposures are higher than the systemic AOEL proposed by the rapporteur, It was impossible to obtain an exposition < AOEL.

2.4.4 Proposed EU MRLs and compliance with existing MRLs

The current position concerning EU MRL legislation, based on Council Directive 96/32/CE and 96/33/CE and the proposed MRL calculated according to the residue trials submitted for the elaboration of this monograph are summarised in table 2.4.4-1.

Table 2.4.4-1: EU MRLs and MRL proposed by the rapporteur for endosulfan

CROP	EU MRL (ppm)	MRL proposed (ppm)
1. Fruit, fresh, dried or uncooked preserved by freezing not containing added sugar; nuts		
I) CITRUS FRUITS	1 (a)	-
II) TREE NUTS	0.1 (*)	-
III) POME FRUIT	1 (a)	0.5
IV) STONE FRUIT	1 (a)	1.0 (**)
VI) BERRIES & SMALL FRUIT		
a) Grapes (table & wine)	1 (a)	0.2
b) Strawberries (not wild)	(*)	
c) Cane fruit (not wild)		
- Black berry	(*)	
- Rasp berry	1 (a)	
- Others	0.05 (*)	
d) Other berries and small fruit (not wild)		
- Currants	(*)	
- Gooseberry	(*)	
- Others	0.05 (*)	

CROP	EU MRL (ppm)	MRL proposed (ppm)
e) Wild berries and wild fruit	0.05 (*)	
VI) MISCELLANEOUS FRUIT		
Kiwi	1 (a)	
Olives	1 (a)	
Other	0.05 (*)	
2. Vegetable, fresh and uncooked, frozen or dry		
I) ROOT AND TUBER VEG		
Beet root	0.2 (a)	-
Carrot	0.2 (a)	
Celeriac	0.2 (a)	
Radish	0.2 (a)	
Kolhrabi	0.2 (a)	
Turnip	0.2 (a)	
Other	0.05*	
II) BULB VEG		
Onions	1 (a)	
Other	0.05 (*)	
FRUITING VEG		
Solanaceae	1 (a)	0.5
Cucurbits (edible peel)	1 (a)	
Cucurbits (inedible peel))	1 (a)	0.5
Sweet corn	0.05 (*)	
IV) BRASSICA VEG		
Flowering brassica	1 (a)	
Head brassica	1 (a)	
Leafy brassica	1 (a)	
Horseradish	0.05 (*)	
LEAFY VEG & FRESH HERBS		
Lettuce and similar	1 (a)	
Spinach and similar	1 (a)	
Watercress	0.05 (*)	
Witloof (Endivias)	0.05 (*)	
Herbs	0.05 (*)	
VI) LEGUME VEG	1 (a)	
VII) STEM VEG		
Edible Thistles	1 (a)	
Celerys	1 (a)	
Artichokes	1 (a)	
Leeks	1 (a)	
Others	0.05 (*)	
VIII) FUNGI		
Mushroom	1 (a)	
Wild Mushroom	0.05 (*)	
3. Pulses	0.05 (*)	
4. Oil seeds		
Leenseed	(a)	
Sunflower	(a)	
Rape seed	(a)	
Soybean	(a)	1.0
Mushtard	(a)	
Cotton seed	0.3	-
Others	0.1 (*)	
5. Potatoes	(a)	0.05
6. Tea	30 (see Directive 93/58/CEE)	
7. Hops	(c)	
Cereals :		
Wheat, rye, triticale, barley, oat	0.1 (a)	

CROP	EU MRL (ppm)	MRL proposed (ppm)
Corn Other	0.2 (a) 0.05 (*)	
Animal products Fat - Poultry meat - Others Milk Eggs	 (a) 0.1 0.004 (a)	

(a) LOD
(b) See the article 1 and the point 2 of the article 2 of the 96/32/CE Directive.
(a) (b) (c) (d) In case other limit have been not establish on April 30th of 2000, the following MRL will be apply: (a) 0.05 (*); (b) 0.02 (*); (c) 0.1 (*); (d) 0.01
- Insufficient data to set up the MRL
(*) Provisional MRL, calculated based on an insufficient number of resiude trials. This value has to be confirmed by means of additional resiude trials
(**) Provisional MRL based on residue trials performed only in N Europe.

2.4.5 Proposed EU import tolerances and compliance with existing MRLs

Table 2.4.5-1: Proposed import tolerances limit

Crop/Commodity	Proposed MRL
Tea	-
Coffee	0.05 (*)
Cacao	0.05 (*)
Pinapple	-

(*) Provisional MRL, calculated based on an insufficient number of residue trials. This value has to be confirmed by means of additional residue trials
(**) Provisional MRL based on residue trials performed only in N Europe
- Insufficient data to set up the MRL

2.5 Fate and behaviour in the environment

2.5.1 Definition of the residues to the environment

In light of all data obtained on degradation of endosulfan in soil and water, residues can be provisionally defined as both isomers of the active substance (α endosulfan and β endosulfan) as well as their common metabolite endosulfan sulphate.

However this definition must be considered incomplete. The degradation of endosulfan did not show any alteration of the hexaclor norborene bicycle and showed a very low mineralization (<5%). These two facts suggest a high persistence of a soil residue constituted by a number of chlorinated metabolites, which may not account individually for more than 10% of applied dose but that all together may represent high amount of it. Based on their chemical structure it may be expected that the physico-chemical properties of these compound will be similar and generally persistent and bio-accumulable. Therefore, a wider investigation of the degradation routes of this compound must be done in order to establish a proper residue definition.

2.5.2 Fate and behaviour in soil

¡Error! Marcador no definido.Endosulfan is a labile bicyclic sulphite diester with an additional moiety containing a hexachloronorborene ring. It consists of two isomers (α endosulfan and β endosulfan) which differ in the configuration of the isomer SO_3 group and the respective ring.

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- ¡Error! Marcador no definido.**Aerobic degradation**

Endosulfan aerobic degradation route and rate has been studied by Stumpf *et al*, 1995 (A53618); Gildemeister and Jordan, 1984 (A29680) and Stumpf, 1988 (A39424) in a variety of different soils (predominantly sandy loam and loamy sand soils) at different temperatures (21, 22 and 28ºC) and application rates ≥ than those recommended by GAP.

Results showed that aerobic degradation occurred via oxidation. In all studies**,** α endosulfan degraded quickly than the isomer β endosulfan. The main metabolite formed was endosulfan sulphate at a rate higher than 10% of applied radioactivity (18-40% at 60 days (Gildemeister and Jordan, 1984 (A29680)) and 46.1% at 365 days (Stumpf et al, 1995 (A53618)). This compound was slowly degraded to the more polar metabolites endosulfan diol, endosulfan lacton, endosulfan ether and other unknown compounds which appeared at <10% of applied radioactivity in all studies. Non-extractable residues were lower than 50% of applied radioactivity during the assay time 60 days (Gildemeister and Jordan, 1984 (A29680)) and lower than 25% of applied radioactivity at 100 days (Stumpf *et al*, 1995 (A53618))).

The CO_2 production was not properly measured in any of the studies, in some studies all the volatiles were measured and with this results the mineralization of endosulfan is expected to be low (<5%).

The degradation rate of endosulfan in soil laboratory studies can be summarised as follows (table 2.5.2-1).

Table 2.5.2-1: Summary of DT_{50} values (days) in soil from laboratory studies

¡Error! Marcador no definido.**COMPOUND**	**TEMPERATURE**	**DT_{50}**	**DT_{90}**	**R^2**	**n**
α endosulfan	21-22ºC	12	39	0.89	6
		39	128	0.96	8
		19	63	0.89	8
		14	46	0.93	6
	28	23	78	0.80	4
β endosulfan	21-22ºC	158	523	0.92	11
		264	877	0.92	13
		132	440	0.91	13
		108	357	0.84	8
		115	383	0.92	11
	28	58	194	0.99	4
Parent compound	21-22ºC	98	326	0.77	12
		128	426	0.90	13
		90	299	0.90	13
		92	305	0.71	8
		80	265	0.84	11

¡Error! Marcador no definido.**COMPOUND**	**TEMPERATURE**	**DT_{50}**	**DT_{90}**	**R^2**	**n**
		27	85	0.96	8
		37.5	124.7	0.57	8
	28	37	123	0.92	4

The lowest DT_{50} and DT_{90} values were observed at the highest temperatures (28±2ºC) showing a direct relationship. **DT_{50} and DT_{90} values for endosulfan sulphate has not been established in any study due to linear equations could not be fit from the laboratory data at the assay time (365 days for the longest study). The DT_{50} and DT_{90} values of endosulfan sulphate are required since it is a relevant metabolite in soil.**

- **Anaerobic degradation**

Anaerobic degradation was studied by Gildemeister *et al*, 1988 (A37589). Results showed that it proceed slower and with no significant differences between the isomers than during the aerobic degradation. In consequence, endosulfan sulphate was the main degradation product formed (15-33% of the applied radioactivity at 53 anaerobic condition days). It was accompanied by the formation of other metabolites (endosulfan diol and endosulfan lactone at <10% of the applied radioactivity) and low rates of non-extractable residues (15-33% of the applied radioactivity at 53 anaerobic condition days).

- **Photolysis**

Under photolytic conditions, endosulfan has not shown to be substantially degraded, showing similar results than dark controls. Although its half live time could not be estimated, it was suggested as >200 days. Endosulfan diol was the only metabolite observed in amounts lower than 10% of the applied radioactivity. Unknown compounds and non-extractable residues were not observed.

- **Field studies**

Field degradation studies were conducted in Northern Europe, Southern Europe and in the United States (in climates comparable to Southern Europe). Three type of studies have been presented:

Soil dissipation studies
Soil residue studies
Soil accumulation studies

All of them have been carried out with the formulate substance Thiodan 35 EC.

- **Field dissipation studies**

Different studies under Northern conditions have been carried out by Baetel *et al*, (A53554 and A54025) on silty loam, sandy silty loam, loamy sand and sandy loam soils at single application rates higher than those recommended by GAP, and for more than one year. DT_{50} and DT_{90} values from these studies (table 2.5.2-2).

Total endosulfan residues were found in the upper soil layer (0-20 cm). A relevant metabolite (endosulfan sulphate) was identified in all soil tested. It was accounted for >10% of applied concentration one year after application in three of these studies.

Three field dissipation studies have been presented (Hacker, 1989 (A42193); Mester, 1990 (A42997) and Czarnecki *et al*., 1992 (A51819)). These studies were performed on different soil types at application rates higher than those established by GAP and covering multiple endosulfan applications (2 or 5 per year). DT_{50} values presented by Hacker (A42193) and Mester (A42997) were estimated from endosulfan concentrations before the last application, it is considered that these studies represented worst field conditions, regarding application rate and number of applications. In all the studies it can be observed that the concentration of α+β Endosulfan in soil before the last application was <0.05 mg/kg, therefore all the studies are considered valid . **The calculation of the DT_{50} of endosulfan sulphate was considered irrelevant in all the studies since both processes (formation and disappearance) were not considered together in the calculation.** DT_{50} (α+β Endosulfan) values were estimated after each application in cropped and bareground loamy sand soil (table 2.5.2-2).

Table 2.5.2-2: DT_{50} (α+β Endosulfan) values (days) in soils under Southern conditions from field studies

DT_{50} (days)	**DT_{90} (days)**	**R^2**	**n**	**Kinetic**	**pH**	**Reference**
91.6	304.2	0.90	10	1st order	7.1	A53554 Silty loam soil
35.9	395.9	0.64	8	Root 1st order	5.2	A53554 Sandy silty soil
167.1	555.2	0.41	8	1st order		
38.5	424.6	0.9	10	Root 1st order	5.7	A54025 Loamy sand soil
123.7	410.9	0.57	10	1st order		
16.5	181.8	0.76	10	Root 1st order	5.6	A54025 Sandy loam soil
130.6	433.8	0.45	10	1st order		
75.86	252.02	0.88	18	1st order		A42193 Sandy loam (Crop)
89.6	297.7	0.86	18	1st order		A42193 Sandy loam (Bareground)
92.9	308.8	0.89	13	1st order	6.7	A42997 Clay loam (Crop)
89.5	297.5	0.82	13	1st order		A42997 Clay loam (Bareground)
61.10	202.9	0.61	11	1st order	6.8	A51819 Loamy sand (crop)
46.2	153.5	0.72	11	1st order		A51819 Loamy sand (Bareground)

The correct calculation, with the data of the field studies, of the DT_{50} of endosulfan sulphate considering the formation and degradation process is required.

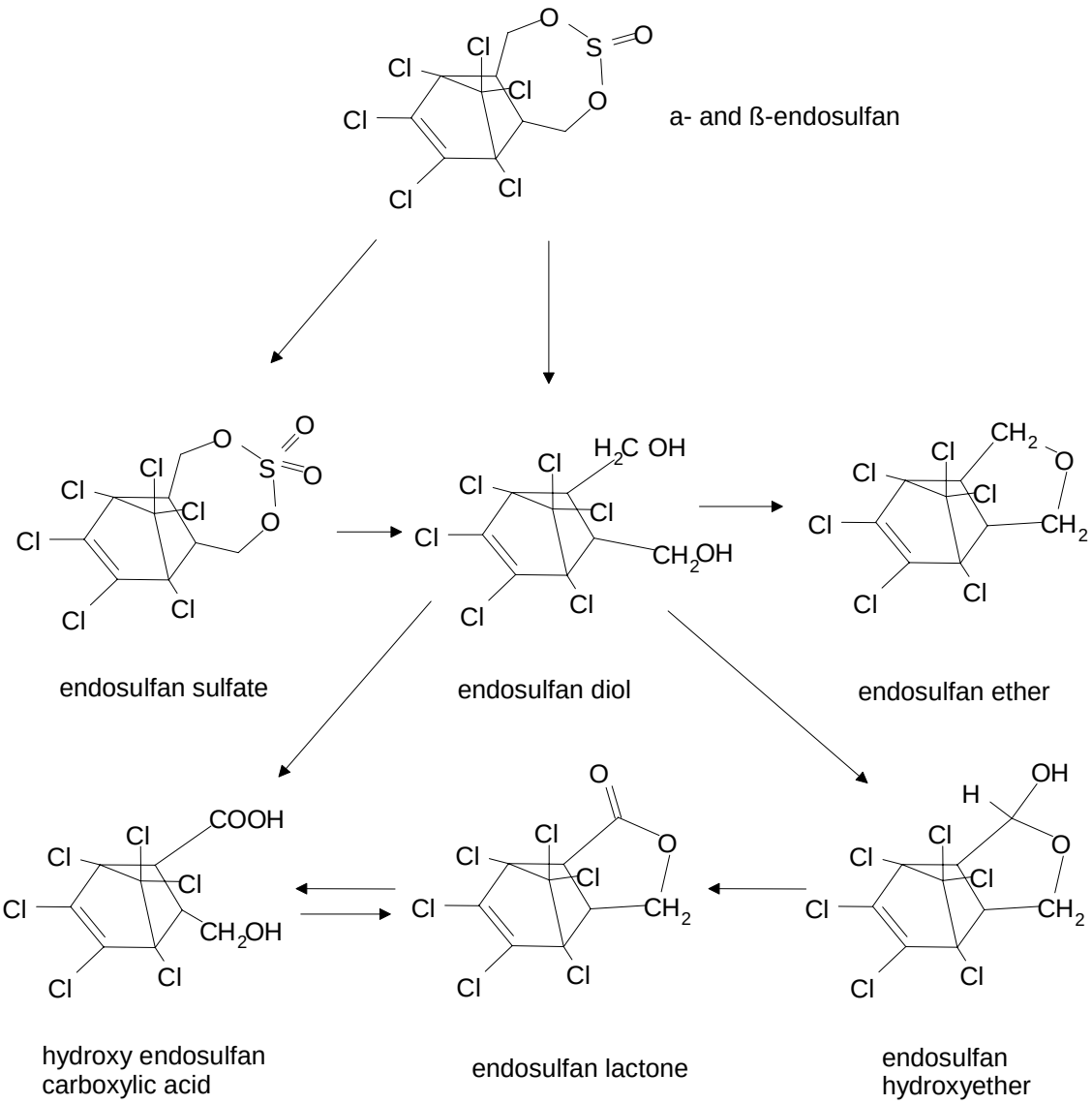
Soil residues were studied by Tiirma and Dorn, 1988 (A40218) in ten different soils after more than 3 years of use of formulated endosulfan. The maximum dosages per year were always higher than those proposed by GAP, from 0.5 to 3.2 kg as/ha. Monitoring was done 6 or 7 months after the last application. In all cases, even in areas where endosulfan was used intensively over several years, residues of parent endosulfan were lower than 10% of the applied concentration and there was no

evidence of leaching. The crop conditions do not seem to influence dissipation of endosulfan. However, **residues of endosulfan sulphate (>10% of the initial concentration) were observed in some cases.**

Soil accumulation was studied by Tiirmaa *et al*, 1993 (A53771). Eighth year old apple trees were treated in a loamy clay soil with 12 applications at 1.5 kg as/ha each in 4 consecutive years. Total residue (parent compound plus endosulfan sulphate) was always lower than 10% of the applied concentration at the end of each year of use. So, accumulation from one year to another should not be expected. Even though, should be taken into account, that the main metabolite endosulfan sulphate was observed at more than 10% of the initial concentrations up to 200 days after the 3rd application. Its plateau concentration rose 20-50 % of the initial concentration 5 months before the end of the study.

In summarising the results from all relevant degradation studies in soil, the following degradation scheme is proposed.

The degradation of endosulfan in soil did not show any alteration of the hexaclor norborene bicycle and showed a very low mineralisation (<5%). These two facts suggest a high persistence of a soil residue constituted by a number of chlorinated metabolites, which may not account individually for more than 10% of applied dose but that all together may represent high amount of it. Based on their chemical structure it may be expected that their physico chemical properties of these compound will be similar and generally persistent and bioaccumulable. Therefore, a wider investigation of the degradation routes of this compound must be done.



NO FURTHER DEGRADATION HAS BEEN DEMONSTRATED

- ¡Error! Marcador no definido.**Adsorption/desorption**

A range of different soils were used to determine Kd and Koc values (Goerlitz and Eyrich, 1988 (A37591 and A39353). α endosulfan, β endosulfan, endosulfan sulphate and endosulfan diol showed to be immobile in soil. All substances showed strong adsorption on soils related to organic carbon content, although this process was found to be almost completely reversible.

- ¡Error! Marcador no definido.**Leaching**

Laboratory leaching studies were performed with the active substance (Gildemeister and Grundschoettel, 1985 (A31700); Gildemeister and Jordan, 1982 (A49273) and Gildemeister and Remmert, 1983 (A27287)) and the formulated product (Thier, 1975 (A49270) in different soil types.

Results showed that endosulfan had not leaching potential but, on the contrary, to be nearly immobile under laboratory conditions. Even when irrigated with unrealistic high rates of water (200 mm/48 hours) and high application rates (1.4 kg a.s./ha) (Gildemeister and Remmert, 1983 (A27287)) no residues of endosulfan or its metabolites were detected in the leachates. These results showed to be confirmed by soil field studies where endosulfan was only detected in the upper soil layers. Therefore, a ground-water contamination by the total endosulfan residues is not expected.

As the degradation route in soil is not well defined and complete it may not be discarded the formation of more polar metabolites able to reach ground water.

2.5.2.1 Predicted environmental concentrations is soil (PECs) (IIIA, 9.1.3)

The calculated PEC_s was for α+β Endosulfan, the main metabolite endosulfan sulphate was not considered in this calculation since a good determination of its DT_{50} was not carried out. From the soil dissipation studies in field it can be considered that the higher amount of the endosulfan sulphate was 60% of the applied concentration (Initial PEC), multiplied by a factor of 0.9624. This estimation was confirmed by the soil accumulation study in which the plateau concentration of endosulfan sulphate rose 20-50% of the initial concentration 5 months before the end of the study, from this study it can be concluded that accumulation from one year to another would not be expected.

Table 2.5.2.1-1: DT_{50} of α+β endosulfan (days) in soils from filed studies

DT_{50} (days)	DT_{90} (days)	R^2	n	Kinetic	pH	Reference
91.6	304.2	0.90	10	1st order	7.1	A53554 Silty loam soil
35.9	395.9	0.64	8	Root 1st order	5.2	A53554 Sandy silty soil
167.1	555.2	0.41	8	1st order		
38.5	424.6	0.9	10	Root 1st order	5.7	A54025 Loamy sand soil
123.7	410.9	0.57	10	1st order		
16.5	181.8	0.76	10	Root 1st order	5.6	A54025 Sandy loam soil
130.6	433.8	0.45	10	1st order		
75.86	252.02	0.88	18	1st order	5.4	A42193 Sandy loam (Crop)
89.6	297.7	0.86	18	1st order		A42193 Sandy loam (Bareground)
92.9	308.8	0.89	13	1st order	6.7	A42997 Clay loam (Crop)
89.5	297.5	0.82	13	1st order		A42997 Clay loam (Bareground)
61.10	202.9	0.61	11	1st order	6.8	A51819 Loamy sand (crop)
46.2	153.5	0.72	11	1st order		A51819 Loamy sand (Bareground)

The higher value of the best fitted kinetics (R^2>0.8)was DT_{50} = 93 days, this DT_{50} represents a realistic worst case for all European condition

It was assumed to be 1.5 g/cm^3 dry weight. The depth of the penetrated soil layer was assumed to the immobility of endosulfan. This simulates a worst case scenario, since the active substance is concentrated in the top 5 cm which is considerably less than the plough layer. Adsorption/desorption and leaching studies summarised in point B.7.2.3. Confirm the immobility of endosulfan.

Based on these assumption, predicted environmental concentrations of endosulfan (PEC_{soil}) were calculated from the BBA draft guide based on:

The highest number of treatments, the shortest interval in between, and the single maximum application rates for each crop. This information was taken from data according to the GAP (July, 1998).

According to this scenario, the initial predicted environmental concentrations, PIEC values, have been calculated considering a crop intercept of 50% and 0%, this initial PEC are summarised in Table 2.5.2-2 and 2.5.2-3 respectively.

Table 2.5.2-2: Calculation of PIEC values for endosulfan assuming a crop intercept of 0%

¡Error! Marcador no definido.**Crops**	**Maximum Single Treatment Rate kg a.s./ha**	**Number of Applications**	**Spraying interval**	**PIEC mg sa/kg single application**	**PIEC mg sa/kg several applications**
Citrus , pome fruit and wine grapes	1.05	2	14	1.40	2.66
Cotton	0.84	3	14	1.12	3.03
Tomatoes	0.53	2	7	0.70	1.37
Potatoes	0.53	2	14	0.70	1.34
Stone fruits	0.8	3	14	1.06	2.89
Cucurbits	0.53	3	7	0.70	2.01
Sugar beet	0.5	2	14	0.66	1.26
Hazel nuts	0.8	2	14	1.06	2.02

Table 2.5.2-3: Calculation of PIEC values for endosulfan assuming a crop intercept of 50%

¡Error! Marcador no definido.**Crops**	**Maximum Single Treatment Rate kg a.s./ha**	**Number of Applications**	**Spraying interval**	**PIEC mg sa/kg single application**	**PIEC mg sa/kg several applications**
Citrus , pome fruit and wine grapes	1.05	2	14	0.70	1.33
Cotton	0.84	3	14	0.56	1.52
Tomatoes	0.53	2	7	0.35	0.69
Potatoes	0.53	2	14	0.35	0.67
Stone fruits	0.8	3	14	0.53	1.44
Cucurbits	0.53	3	7	0.35	1.00
Sugar beet	0.5	2	14	0.33	0.63
Hazel nuts	0.8	2	14	0.53	1.01

Based on these PIEC, the time weighted average predicted environmental concentration in soil (PEC_{TWA}) have been calculated, three cases have been considered as a worst case: citrus, cotton and cucurbit. They are summarised in tables 2.5.2-3, 2.5.2-4 and 2.5.2-5:

Table 2.5.2-3: Estimated PECs and TWA-PECs after last application in citrus fruit and assuming a crop intercept of 50%.

Days	PECs	TWA-PECs
0	1.33	1.33
1	1.32	1.32
2	1.31	1.32
4	1.29	1.31
7	1.26	1.29
14	1.18	1.25
21	1.13	1.23
28	1.08	1.20
42	0.97	11.14
86	0.70	0.98
156	0.41	0.78
286	0.16	0.55
351	0.09	0.47

Table 2.5.2-4: Estimated PECs and TWA-PECs after last application in cotton and assuming a crop intercept of 50%.

Days	PECs	TWA-PECs
0	1.52	1.52
1	1.51	1.51
2	1.49	1.50
4	1.45	1.49
7	1.44	1.48
14	1.36	1.44
21	1.29	1.40
28	1.23	1.37
42	1.11	1.30
72	0.88	1.17
152	0.48	0.90
272	0.20	0.65
337	0.12	0.55

Table 2.5.2-5: Estimated PECs and TWA-PECs after last application in cucurbit and assuming a crop intercept of 50%.

Days	PECs	TWA-PECs
0	1.00	1.00
1	0.99	1.00
2	0.99	0.99
4	0.97	0.99
7	0.95	0.98
14	0.90	0.95
21	0.86	0.93
28	0.81	0.90
42	0.73	0.86
136	0.36	0.63
286	0.11	0.41
351	0.07	0.35

No accumulation of parent endosulfan (α+β endosulfan) is expected due to continuous use of endosulfan, the highest PECs is 1.52 mg a.s/kg. **However, an accumulation of the endosulfan sulphate can be expected due to a continuous use during several years of endosulfan. Therefore the PEC and the plateau concentration for endosulfan sulphate should be estimated by the applicant, . So, its DT_{50} should be estimated. As a worst case estimation the highest expected concentration of endosulfan sulphate will be 0.88 mg/kg.**

2.5.3 Fate and behaviour in water

- ¡Error! Marcador no definido.**Hydrolysis**

The hydrolysis half live of endosulfan was studied by Goerlitz and Kloeckner, 1982 (A31069) and this study was considered unacceptable. A second study carried out by Goerlitz and Rutz, 1989 (A40003) was considered acceptable and studied the hydrolysis of endosulfan at different pH (5, 7 and 9). The rate of hydrolysis of α endosulfan and β endosulfan was extremely dependent of pH. Under acidic conditions no hydrolysis could be observed (>200 days), in a neutral medium the rate was moderate (10-19 days) and in an alkaline environment, it was very rapid (<1 day). In all cases, the only hydrolysis product identified was endosulfan diol, which occurred at >50% of the applied radioactivity.

- ¡Error! Marcador no definido.**Photolysis**

The photolytic degradation route of endosulfan at a wavelenght of <290 nm, was studied by Schumacher et al, 1973 (A25698); Dujera and Mukerjee, 1982 (A27138); Stumpf and Schink, 1988 (A37588) and Stumpf, 1988 (A37588). Results from these studies showed that photolysis can not be considered as an important degradation route due to the fact that both isomers are photolytically estable. In consequence, no relevant metabolites were detected.

- ¡Error! Marcador no definido.**Biological degradation**

None study was submitted concerning the biological degradation of endosulfan. The degradation in natural water (river and sea water) was studied in three trials, it is concluded that the main degradation route of endosulfan in water is the hydrolysis and that it is pH dependent.

- ¡Error! Marcador no definido. **Water /sediment studies**

Water /sediment studies have been provided by Gildemeister, 1985 (A31182); Stumpf, 1990b (A44231) and Cotham and Bidleman, 1989 (A41218), this last study was considered not valid since no data about degradation kinetics was submitted. All of them showed low DT_{50} values (Table 2.5.3-1).

Table 2.5.3-1: Summary of DT_{50} values from water/sediment studies

¡Error! Marcador no definido.**Study** ¡Error! Marcador no definido. ¡Error! Marcador no definido.	**System**	**Total system**						**Water phase**		
		Total endosulfan			**Parent endosulfan**			**Total endosulfan**		
		DT_{50} (days)	**R^2**	**n**	**DT_{50} (days)**	**R^2**	**n**	**DT_{50} (days)**	**R^2**	**n**
Gildemeister, 1985 (A31182)	River main	-	-	-	12	0.92	7	-	-	-
	Gravel pit	-	-	-	9.5	0.85	6	-	-	-
Stumpf, 1990b (A44231)*	River main	21	0.82	8	12	0.70	8	15	0.86	8
	Gravel pit	18	0.83	8	10	0.87	8	12	0.85	8

* = Data presented by Stumpf, 1990 (A44231) were based on results from Gildemeister, 1985 (A31182).

The route of degradation was studied by Gildemeister, 1985 (A31182). Under these conditions two relevant metabolites were identified, endosulfan sulphate and endosulfan hydrocarboxylic acid which were accounted for >10% of applied radioactivity. Other different metabolites as endosulfan lactone, endosulfan diol, endosulfan ether and an unidentified compound were individually accounted at <10% of the applied radioactivity. The $^{14}CO_2$ detected in the traps throughout the study was < 0.1%. Volatile compounds were always lower than 10% of the applied radioactivity (2-4%). Endosulfan and its metabolites showed a quick adsorption to sediment. **The DT values for the parent compound and the metabolites in sediment were not calculated, the residue is strongly absorbed to the sediment and this fact can affect to its bioavailability. Moreover the detected metabolites were the extractable an effort should be done to characterize the bound residues that they were 20% of the applied radioactivity and the plateu were not got.**

Additional information has been provided by a field study (Cornaby *et al,* 1989 (A41298). After three applications of endosulfan (1.12 kg as/ha) in a field cropped with tomatoes, the concentrations of α endosulfan, β endosulfan and endosulfan sulphate were determined in two experimental ponds after spray and runoff events. Immediately after spray drift events, 0.257-0.053 µg/L of total endosulfan were found in the water phase. Only after forced runoff events concentrations rose levels of 1.31-0.583 µg/L. They decreased to about 0.011 µg/L after 3-6 weeks. The concentrations were noticeably higher in the sediments. Thus, 49.2-99.1 µg/mg were determined 0-1 week after the runoff event. Based on these results, it can be stated that high endosulfan concentrations in water could mainly occur after runoff events. In all concentration ranges a relatively rapid degradation of endosulfan looked to occur.

However, concentrations in the sediment should be expected for longer periods of time (more than two months).

It can be concluded that the main degradation routes for endosulfan in water are hydrolysis since photolysis is not observed under environmental conditions. Its half life shows variability related to the water conditions, mainly pH. Under typical environmental conditions (pH = 7 and water/sediment systems) endosulfan DT_{50} can be expected to range from 10 to 12 days for parent endosulfan. **The DT values for the total residue in water, sediment and in the total system should be calculated correctly taking into account the process of formation and degradation a good kinetic should be proposed.**

Two main metabolites were identified under these conditions, endosulfan sulphate and endosulfan hydroxylic acid. Endosulfan diol, which was accounted for >10% of applied radioactivity in the hydrolysis degradation route, was only observed at lower rates in the water/sediment studies. However, poor information is available about fate and behaviour of endosulfan for this compartment. So, this process still need to be further investigated.

A correct determination of DT_{50} and DT_{90} values of parent endosulfan and its metabolites in water, sediment and total system should be required, a correct degradation kinetics (route and rates) should be proposed. The field studies submitted clearly showed the importance of the run-off in the endosulfan concentrations in water, therefore proper scenarios for the risk assessment of endosulfan in the crops and conditions included in the intended uses should be required.

2.5.3.1 Impact on water treatment procedures

Taking into account that conventional and natural water treatment procedures generally maintain alkalinic conditions in the medium, the endosulfan degradation rate is expected to be quick (4-7 hours) for the compound present in the medium. Therefore, endosulfan can be significatively degraded and diluted before arriving to the treatment system.

2.5.3.2 Predicted environmental concentrations in surface water and in ground water (PEC_{SW}, PEG_{GW})

- ¡Error! Marcador no definido. **Surface water (PEC_{SW})**

The environmental concentrations in surface water (PEC_{sw}) for endosulfan have been calculated from the BBA draft guide based on:

The maximum single application rates, the number of treatments and the intervals in between for each crop (SI).

A buffer zone from 0 to 50 m.

A deep water medium of 30 cm and 1 m.

DT_{50} = 15 days. This value has been estimated as the high value of the total endosulfan concentrations (α + β + endosulfan sulphate) in the water phase of two different sediment water systems (Stumpf, 1990 (A44231)).

According to this scenario, the initial PIEC values were estimated. Based on these results, actual concentrations (Ct) at different times and time weighted average concentrations were estimated as:

$$C_t = C_0 \times e^{-kt}$$

$$C_{TWA} = C_0 \times (1-e^{-kt})/kt$$

For crops with multiple applications, initial concentrations after each endosulfan use (PIECn) were estimated as:

PIECn = PIEC + concentration of endosulfan after Spray Interval ($C_{t=SI}$)

Additionally, actual concentrations (Ct) at different times and time weighted average concentrations after each application were also calculated.

Due to the high quantity of data, a summary of the most representative crops and conditions and their respective PIEC values and C_t, C_{TWA} concentrations after last application are expressed in tables 2.5.3.2-1 and 2.5.3.2-2.

Table 2.5.3.2-1: $PIEC_{sw}$ values for the selected crops after the last application

Crop	Application rate	Nº	SI	Distance	Drift	Initial PECsw (µg as/L)	
			days	m	%	0.3 m depth	1 m depth
Citrus	1.05	2	14	0	100.0	350.00	105
				3	15.5	54.25	16.275
				5	10.0	35.00	10.5
				10	4.5	15.75	4.725
				15	2.5	8.75	2.625
				20	1.5	5.25	1.575
				30	0.6	2.10	0.63
				40	0.4	1.40	0.42
				50	0.2	0.70	0.21
Vineyards	1.05	2	14	0	100.0	350.00	105
				3	7.5	26.25	7.875
				5	5.0	17.50	5.25
				10	1.5	5.25	1.575
				15	0.8	2.80	0.84
				20	0.4	1.40	0.42
				30	0.2	0.70	0.21
				40	0.2	0.70	0.21
				50	0.2	0.70	0.21
Arable crops (cotton)	0.84	3	14	0	100.0	280.00	84.00
				1	4.0	11.20	3.36
				3	1.0	2.80	0.84
				5	0.6	1.68	0.50
				10	0.4	1.12	0.34
				15	0.2	0.56	0.17
				20	0.1	0.28	0.08
				30	0.1	0.28	0.08
Arable crops (Cucumber)	0.53	3	7	0	100.0	176.67	53
				1	4.0	7.07	2.12
				3	1.0	1.77	0.53
				5	0.6	1.06	0.318
				10	0.4	0.71	0.212
				15	0.2	0.35	0.106
				20	0.1	0.18	0.053
				30	0.1	0.18	0.053

Table 2.5.3.2-2: TWA-PEC_{sw} values at 48h, 96 h and 21 days for the selected crops after the last application

Crop	Water distance (m)	TWA-PEC_{sw} (µg as/L) Days after last treatment								
		0	**1**	**2**	**4**	**7**	**14**	**21**	**28**	**42**
Citrus fruit	0	533.28	521.14	509.38	486.89	455.62	392.66	341.30	299.14	235.32
	3	82.66	80.78	78.95	75.47	70.62	60.86	52.90	46.37	36.47
	5	53.33	52.11	50.94	48.69	45.56	39.27	34.13	29.91	23.53
	10	24.00	23.45	22.92	21.91	20.50	17.67	15.36	13.46	10.59
	15	13.33	13.03	12.73	12.17	11.39	9.82	8.53	7.48	5.88
	20	8.00	7.82	7.64	7.30	6.83	5.89	5.12	4.49	3.53
	30	3.20	3.13	3.06	2.92	2.73	2.36	2.05	1.79	1.41
	40	2.13	2.08	2.04	1.95	1.82	1.57	1.37	1.20	0.94
	50	1.07	1.04	1.02	0.97	0.91	0.79	0.68	0.60	0.47
Vineyards	0	533.28	521.14	509.38	486.89	455.62	392.66	341.30	299.14	235.32
	3	40.00	39.09	38.20	36.52	34.17	29.45	25.60	22.44	17.65
	5	26.66	26.06	25.47	24.34	22.78	19.63	17.07	14.96	11.77
	10	8.00	7.82	7.64	7.30	6.83	5.89	5.12	4.49	3.53
	15	4.27	4.17	4.08	3.90	3.64	3.14	2.73	2.39	1.88
	20	2.13	2.08	2.04	1.95	1.82	1.57	1.37	1.20	0.94
	30	1.07	1.04	1.02	0.97	0.91	0.79	0.68	0.60	0.47
	40	1.07	1.04	1.02	0.97	0.91	0.79	0.68	0.60	0.47
	50	1.07	1.04	1.02	0.97	0.91	0.79	0.68	0.60	0.47
Cotton	0	503.4	491.9	480.8	459.6	430.1	370.7	322.2	282.4	222.1
	1	20.14	19.68	19.23	18.38	17.2	14.83	12.89	11.3	8.885
	3	5.034	4.919	4.808	4.596	4.301	3.707	3.222	2.824	2.221
	5	3.02	2.952	2.885	2.758	2.581	2.224	1.933	1.694	1.333
	10	2.014	1.968	1.923	1.838	1.72	1.483	1.289	1.13	0.889
	15	1.007	0.984	0.962	0.919	0.86	0.741	0.644	0.565	0.444
	20	0.503	0.492	0.481	0.46	0.43	0.371	0.322	0.282	0.222
	30	0.503	0.492	0.481	0.46	0.43	0.371	0.322	0.282	0.222
Cucumber	0	397	388	379.2	362.5	339.2	292.3	254.1	222.7	175.2
	1	15.88	15.52	15.17	14.5	13.57	11.69	10.16	8.908	7.008
	3	3.97	3.88	3.792	3.625	3.392	2.923	2.541	2.227	1.752
	5	2.382	2.328	2.275	2.175	2.035	1.754	1.525	1.336	1.051
	10	1.588	1.552	1.517	1.45	1.357	1.169	1.016	0.891	0.701
	15	0.794	0.776	0.758	0.725	0.678	0.585	0.508	0.445	0.35
	20	0.397	0.388	0.379	0.362	0.339	0.292	0.254	0.223	0.175
	30	0.397	0.388	0.379	0.362	0.339	0.292	0.254	0.223	0.175

As can be observed from the tables above, the higher concentrations of endosulfan in water should be expected for orchards and cotton. In fact, they are treated with the highest application rates and show the highest drift values.

Based on the results of the field study the main exposure route for endosulfan is the runoff, therefore proper scenarios for the risk assessment of endosulfan in the crops and conditions included in the intended uses should be required.

- **Ground water (PEC_{GW})**

As a result of laboratory studies on leaching and adsorption/desorption from soil, endosulfan and endosulfan sulphate endosulfan diol can be regarded as immobile in soil. A complete and rapid adsorption to the sediment is observed in water/sediment studies. So, a ground water contamination by parent endosulfan is not expected. **However, as the degradation route in soil is not well defined and complete, it may not be discarded the formation of more polar metabolites able to reach ground water.**

- **Sediment (PECs)**

Predicted environmental concentrations in sediment can not be estimated due to DT_{50} for parent or total endosulfan have not been studied by the applicant.

2.5.4 Fate and behaviour in air

Endosulfan is expected to be evaporated from soil. Atmospheric concentrations resulted in large summer-winter differences where the highest concentrations are always detectable close to the time of application. It is mainly due to after spraying endosulfan (α isomer > β isomer) is quickly evaporated (25 to 63.7%). Its half life in air (DT_{50} value) ranges from 8.5 to 27 days.

A high rates of endosulfan are expected to be evaporated from soil.

2.5.4.1 Predicted environmental concentrations in air (PEC_A)

Information about predicted environmental concentrations have not been submitted by the applicant. However, a high rate of evaporation should be expected.

2.6 Effects on non-target species

2.6.1 Effects on terrestrial vertebrates

The acute and chronic toxicity studies presented by the applicant indicate that technical endosulfan has a potential risk on birds. The applicant has not submitted studies on the plant protection product. The toxicity data in birds used for the risk assessment are summarised in the next table.

Table 2.6.1-1: Summary of toxicity data in birds.

Acute oral toxicity	**Route**	**Exposure**	**Chemical**	**LD_{50} mg/kg**		**Doc. No.**	**Study**	**Authors**	**Remark**
Bobwhite quail	Gavage	Single gavage	Technical grade 97.2%	42 (35-56)		A27035	GLP	Roberts & Phillips, 1983 a	
Mallard Duck	gavage	Single gavage	Technical 97.2%	28 (22-36)		A27036	GLP	Roberts & Phillips, 1983 b	
Short-term toxicity	**Route**	**Exposure**	**Chemical**	**LC_{50}**		**Doc no.**	**Study**	**Authors**	**Remark**
				ppm	**mg/kg/d**				
Japanese quail	dietary	5 days	Not specified	1250	250	A26820	No GLP or published	Hill et al., 1975	
Bobwhite quail	dietary	5 days		805	161				
Mallard duck	Dietary	5 days		1053	211				
Pheasant	dietary	5 days		1275	255				
Effectos on Reproduct	**Route**	**Exposure**	**Chemical**	**NOEC**		**Doc. No**	**Study**	**Authors**	**Remark**
				ppm	**mg/kg/d**				
Japanese quail	dietary	28 days	Active ingredient 97.1%	50	5	A18268	No GLP No publ.	Scholz & Weigand (1973)	
Bobwhite quail	dietary	>20 weeks	Technical 97.2%	60	6	A29572	GLP	Roberts and Phillipls, 1984	
Mallard duck	dietary	>20 weeks	Technical 97.2%	30	4	A 30678	GLP	Roberts and Phillips (1985)	
Mallard duck	dietary	>20 weeks	Technical (96%)	30	4	A 36310	GLP	Beavers et al. (1987)	
Bobwhite quail	dietary	>20 weeks	Technical (96%)	60	6	A 36311	GLP	Beavers et al. (1987b)	

The expected maximum and typical residue levels of endosulfan have been calculated using the method of Hoerger and Kenaga (1972). Considering the intended uses, leaves instead of grass have been considered as the most appropriated food for herbivorous vertebrates. TER acute calculations for both small and large birds have been estimated.

Table 2.6.1-2: TER estimations for acute oral toxicity studies of endosulfan in citrus, pome fruit and vineyards crops for large birds.

Feed	Application rate (kg a.s/ha)	Typical maximum residue (mg/kg)	Estimated initial residue (mg/kg)	Maximum daily intake (mg/kg bw)	Acute toxicity (mg/kg)	TERa
Leaves	1.05	31 X R	32.55	3.255	28	8.6
Insects	1.05	29 X R	30.45	3.045	28	9.2
Fruits	1.05	1.3 X R	1.365	0.1365	28	205.1

Table 2.6.1-3: TER estimations for acute oral toxicity studies of endosulfan in citrus, pome fruit and vineyards crops for small birds.

Feed	Application rate (kg a.s/ha)	Typical maximum residue (mg/kg)	Estimated initial residue (mg/kg)	Maximum daily intake (mg/kg bw)	Acute toxicity (mg/kg)	TERa
Leaves	1.05	31 X R	32.55	9.765	28	2.86
Insects	1.05	29 X R	30.45	9.13	28	3.06
Fruits	1.05	1.3 X R	1.365	0.4	28	70

Table 2.6.1-4: TER estimations for acute oral toxicity studies of endosulfan in Tomatoes, potatoes and cucurbits crops for large birds.

Feed	Application rate (kg a.s/ha)	Typical maximum residue (mg/kg)	Estimated initial residue (mg/kg)	Maximum daily intake (mg/kg bw)	Acute toxicity (mg/kg)	TERa
Leaves	0.53	31 XR	16.43	1.643	28	17.04
Insects	0.53	29 XR	15.37	1.537	28	18.21
Fruits	0.53	1.3 XR	0.68	0.068	28	411.7

Table 2.6.1-5: TER estimations for acute oral toxicity studies of endosulfan in Tomatoes, potatoes and cucurbits crops for small birds.

Feed	Application rate (kg a.s/ha)	Typical maximum residue (mg/kg)	Estimated initial residue (mg/kg)	Maximum daily intake (mg/kg bw)	Acute toxicity (mg/kg)	TERa
Leaves	0.53	31 XR	16.43	4.9	28	5.71
Insects	0.53	29 XR	15.37	4.61	28	6.07
Fruits	0.53	1.3 XR	0.68	0.20	28	140

Table 2.6.1-6: TER estimations for acute oral toxicity studies of endosulfan in stone fruits crops for large birds.

Feed	Application rate (kg a.s/ha)	Typical maximum residue (mg/kg)	Estimated initial residue (mg/kg)	Maximum daily intake (mg/kg bw)	Acute toxicity (mg/kg)	TERa
Leaves	0.8	31 XR	24.8	2.48	28	11.3
Insects	0.8	29 XR	23.2	2.32	28	12.06
Fruits	0.8	1.3 XR	1.04	0.104	28	269.2

Table 2.6.1-7: TER estimations for acute oral toxicity studies of endosulfan in stone fruits crops for small birds.

Feed	Application rate (kg a.s/ha)	Typical maximum residue (mg/kg)	Estimated initial residue (mg/kg)	Maximum daily intake (mg/kg bw)	Acute toxicity (mg/kg)	TERa
Leaves	0.8	31 XR	24.8	7.44	28	3.7
Insects	0.8	29 XR	23.2	6.96	28	4.02
Fruits	0.8	1.3 XR	1.04	0.312	28	89.74

Although there is a potential risk of endosulfan for large and small herbivorous and insectivorous birds in many crops, the rapporteur consider that the potential risk is higher for the insectivorous birds, taking into account the intended use of this substance.

The TER values for short-term dietary toxicity has been considered provisional due to the study presented by the applicant has to be validate at the ECCO level.

Table 2.6.1-8: TER estimations for acute dietary toxicity studies of endosulfan in citrus, pome fruit and vineyards crops.

Feed	Application rate (kg a.s/ha)	Estimated initial residue (mg/kg)	Acute dietary toxicity (ppm)	TERst
Leaves	1.05	32.55	805	24.73
Insects	1.05	30.45	805	26.4
Fruits	1.05	1.365	805	589.7

Table 2.6.1-10: TER estimations for acute dietary toxicity studies of endosulfan in tomatoes, potatoes and cucurbits crops.

Feed	Application rate (kg a.s/ha)	Estimated initial residue (mg/kg)	Acute dietary toxicity (ppm)	TERst
Leaves	0.53	16.43	805	49
Insects	0.53	15.37	805	52.37
Fruits	0.53	0.68	805	1183.8

Table 2.6.1-11: TER estimations for acute dietary toxicity studies of endosulfan in stone fruits crops.

Feed	Application rate (kg a.s/ha)	Estimated initial residue (mg/kg)	Acute dietary toxicity (ppm)	TERst
Leaves	0.8	24.8	805	32.45
Insects	0.8	23.2	805	34.7
Fruits	0.8	1.04	805	774.03

The calculations of TER lt show a potential long-term risk for birds; this risk has to be addressed by higher tier assays.

Table 2.6.1-12: TER estimations for reproduction toxicity studies of endosulfan in Citrus, pome fruits and vineyards.

Feed	Application rate (kg a.s/ha)	Estimated initial residue (mg/kg)	Reproductive toxicity (ppm)	TERlt
Leaves	1.05	32.55	30	0.92
Insects	1.05	30.45	30	0.98
Fruits	1.05	1.365	30	22

Table 2.6.1-13: TER estimations for reproduction toxicity studies of endosulfan in tomatoes, potatoes and cucurbits.

Feed	Application rate (kg a.s/ha)	Estimated initial residue (mg/kg)	Reproductive toxicity (ppm)	TERlt
Leaves	0.53	16.43	30	1.82
Insects	0.53	15.37	30	1.95
Fruits	0.53	0.68	30	44.11

Table 2.6.1-14: TER estimations for reproduction toxicity studies of endosulfan in stone fruits.

Feed	Application rate (kg a.s/ha)	Estimated initial residue (mg/kg)	Reproductive toxicity (ppm)	TERlt
Leaves	0.8	24.8	30	1.2
Insects	0.8	23.2	30	1.3
Fruits	0.8	1.04	30	28.8

The bioaccumulation potential of endosulfan has also been identified, and therefore the potential risk for fish eating birds must be estimated. Concentrations of endosulfan in water of about 1μg/l, supposes a concentrations of about 5 ppm in fish. The TER estimated for this concentration (30% daily food consumption) are:

TERa = 18
TER st = 161
TER lt = 6

Therefore it is concluded that water concentrations of endosulfan large enough to produce acute fish mortalities can also constitute a potential risk for fish-eating birds. However, those concentrations which are not expected to be lethal for fish species do not represent a significant risk for ictivorous birds.

The selected toxicity data for mammals are: acute LD50 of 10 mg/kg bw for female rat; and a NOEC of 1 mg/kg bw/day from the NOAEL obtained in the two generation study on rats, with is also at the same level that the NOEC for relevant effects observed for mice (combined toxicity/carcinogenicity) and rabbit (developmental toxicity). The value is lower than that observed in the subchronic oral studies, and therefore cover all long-term effects.

A daily food intake for small mammals of 25% their body weight have been used and the ETE values were estimated for leaves according to Hoeger and Kenaga. The values for leaves are similar to those expected in small insects, and therefore the assessment covers both herbivorous, insectivorous and omnivorous small mammals.

Table 2.6.1-15: TER acute estimation for terrestrial mammals

Application rate	Estimation initial residue	Maximum daily intake	TER
1.05 (citrus, pome fruits and vineyards)	32.55	8.1	1.2
0.53 (tomatoes, potatoes and cucrbits)	16.43	4.1	2.4
0.83 (stone fruits)	25.73	6.43	1.5

Table 2.6.1-16: TER estimation for long-term toxicity of endosulfan for terrestrial mammals.

Application rate	Estimation initial residue	Maximum daily intake	TER
1.05 (citrus, pome fruits and vineyards)	32.55	8.1	0.12
0.53 (tomatoes, potatoes and cucurbits)	16.43	4.1	0.24
0.83 (stone fruits)	25.73	6.43	0.15

The TERa and TERlt are lower than the trigger values and therefore a potential risk for small mammals has been identified.

As already commented for the bird assessment the use of initial ETE values instead of time-weighted average for the long-term assessment is justified by the intended uses covered by the GAPs and the lack of information for a most in depth assessment of expected long-term exposures.

2.6.2 Effects on aquatic organism

2.6.2.1 Effects on fish

All the validated data are summarised in the following tables:

Table 2.6.2.1-1: Acute toxicity of endosulfan (active substance) to fish.

Test organisms	Study type	Chemical	Test duration	LC_{50} and 95% CI (µg/l)	Study conditions	Doc, Authors	Remarks
Bluegill fish	Static	Technical (96.6%)	96 h	3..3	Published	Pickering & Henderson, 1966 A14124	Study with hard and soft water
Guppy fish	Static	Technical (96.6%)	96 h	3.7	Published	Pickering & Henderson, 1966 A14124	Study with hard and soft water
Rainbow trout	Static	Thiodan ®	96 h	1. 5	Published	Macek et al, 1969 A 23688	At 12º C
Rainbow trout	Static	Technical (96.4%)	96 h	0.3	Published	Schoettger (1970) A14253	At 10 º C
White sucker	Static	Technical (96.4%)	96 h	3.0	Published	Schoettger (1970) A14253	At 19 ºC
Fathead minnow	Intermitent flow-bioassay	Endosulfan (99%)	7 días	0.86	Published	Macek et al (1976)	
Golden orfe	Static	Active substance	96 h	2	No GLP. No publ.	Knauf (1977) A 167322	
Common carp	Static	Active substance	96 h	6.9	No GLP. No publ.	Knauf (1978) A 31512	
Mosquito fish	Static	Technical grade	96 h	8	Published	Joshi& rege (1980) A 29254	
Indian fish species	Flow through	Active ingredient	96 h	1.2 (1.1-1.3)	Published	Mohanaranga & Murty (1980) A 29255	
Labeo rohita Indian fish species	Flow through	Technical grade (96%)	96 h	1.1	Published	Rao et al (1980) A 22299	
Channa punctatus	Flow through	Technical grade (96%)	96 h	4.8	Published	Devi et al (1981) A 22297	
Walking catfish	Static	Technical grade (90%)	96 h	14 (14.5-13.4)	Published	Gopal et al (1981) A 23187	
Mystus vittatus	Dynamic	Not specified	96 h	1.9 (1.8-2.1)	Published	Rao &Murty 1982 A 26105	
M cavasius	Dynamic	Not specified	96 h	2.2 (2-2.4)	Published	Rao &Murty 1982 A 26105	
Heteropneustes fossilis	Dynamic	Not specified	96 h	1.1 (0.93-1.30)	Published	Rao &Murty 1982 A 26105	

Test organisms	Study type	Chemical	Test duration	LC_{50} and 95% CI (µg/l)	Study conditions	Doc, Authors	Remarks
Heteropneustes fossilis	Static	Not specified	96 h	9.7	Published	Singh & Narein, 1982 A 23196	
Heteropneustes fossilis	Static	No especificanque endosulfan	96 h	2 (1.8-2)	Published	Singh & Srivastava (1981) A 32901	
Rainbow trout	Static	Active ingredient (95.9%)	96 h	0.93 (0.81-1.08)	No GLP No published	Fischer (1983) A 26006	At 12ºC
Rainbow trout	Static	Technical grade	96 h	1.6	Published	Nebeker et al, 1983 A 27380	
Rainbow trout	Dynamic	Technical grade	96 h	0.3	Published	Nebeker et al, 1983 A 27380	
Fathead minnow	Static	Technical grade	96 h	0.8	Published	Nebeker et al, 1983 A 27380	
Fathead minnow	Dynamic	Technical grade	96 h	1	Published	Nebeker et al, 1983 A 27380	
Punctius ticto	Static	Technical grade (96.6%)	96 h	160	Published	Singh & Sahai (1984) A 36683	
Harlequin fish	Static	Technical grade (96.6%)	96 h	160	Published	Singh & Sahai (1984) A 36683	
Channa punctatus	Semi-static	Technical grade	96 h	5.78 (4.49-7.44)	Published	Haider & Moses (1986) A36292	
Saint Peter fish	Semi-static	Not specified	96 h	2.05-2.79	Published	Herzberg, 1986 A 36295	
Freshwater eel	Static	Endosulfan (96%)	96 h	20 (17-23)	Published	Ferrando & Moliner (1989) A 42966	At 29 ºC
Catla Catla	Dynamic	Technical grade (96%)	96 h	1.84 (1.78-1.91)	Published	Rao (1989) A 43108	
Freshwater eel	static	Technical grade (96%)	96 h	41 (33-50)	Published	Ferrando et al, (1991) A 47633	
Golden perch	Semi-static	Technical grade (96.2%)	96 h	0.3	Published	Sunderam (1992) A 49782	
Bony bream	Semi-static	Technical grade (96.2%)	96 h	0.2	Published	Sunderam (1992) A 49782	
Silver perch	Semi-static	Technical grade (96.2%)	96 h	2.3	Published	Sunderam (1992) A 49782	
Common carp	Semi-static	Technical grade	96 h	0.1	Published	Sunderam (1992) A	

Test organisms	Study type	Chemical	Test duration	LC_{50} and 95% CI (µg/l)	Study conditions	Doc, Authors	Remarks
		(96.2%)				49782	
Mosquito fish	Static	Technical grade (96.2%)	96 h	2.3	Published	Sunderam (1992) A 49782	
Rainbow trout	Static	Technical grade (96.2%)	96 h	0.7	Published	Sunderam (1992) A 49782	
Melanotaenia duboulayi	Flow-through	Technical grade (96.2%)	96 h	0.5	Published	Sunderam (1992) A 49782	At 25 º C
Harleqquin fish	Flow-through	Technical grade (96.2%)	96 h	0.2	Published	Sunderam (1992) A 49782	At 25 º C
Zebra fish	Semistatic	Technical grade (97%)	24 h	1.6	Published	Jonsson & Toledo (1993) A 51153	
Yellow tetra	Semistatic	Technical grade (97%)	24 h	2.6	Published	Jonsson & Toledo (1993) A 51153	
Lagodon rhomboides (pinfish)	Flow-through	Technical endosulfan	96 h	0.3	Published	Schimmel et al. (1977) A 22871	Filtered marine water at 23ºC
Striped bass	Flow-trhough	Technical grade (96%)	96 h	0.23	Published	Fujimura et al. 1991 A 47515	
Leiostomus xanthurus (spot)	Flow-through	Technical endosulfan	96 h	0.09	Published	Schimmel et al. (1977) A 22871	Filtered marine water at 23ºC
Mugil cephalus	Flow-through	Technical endosulfan	96 h	0.38	Published	Schimmel et al. (1977) A 22871	Filtered marine water at 23ºC

The studies suggest that endosulfan is highly toxic to fish. The rapporteur conclusion is an acute toxicity of endosulfan to fish in the range of 0.1-10 µg/l, with a value of about 1µg/l. Due to the large amount of information, a sensitivity distribution curve can be used. This distribution has been done using all the data excepting those obtained in static test and those data for species showing large differences between studies. Probabilistic curves are included.

Figure 2.6.2.1-1: Frequency distribution of LC_{50} values for acute toxicity in fish and log-normal distribution estimated by the rapporteur.

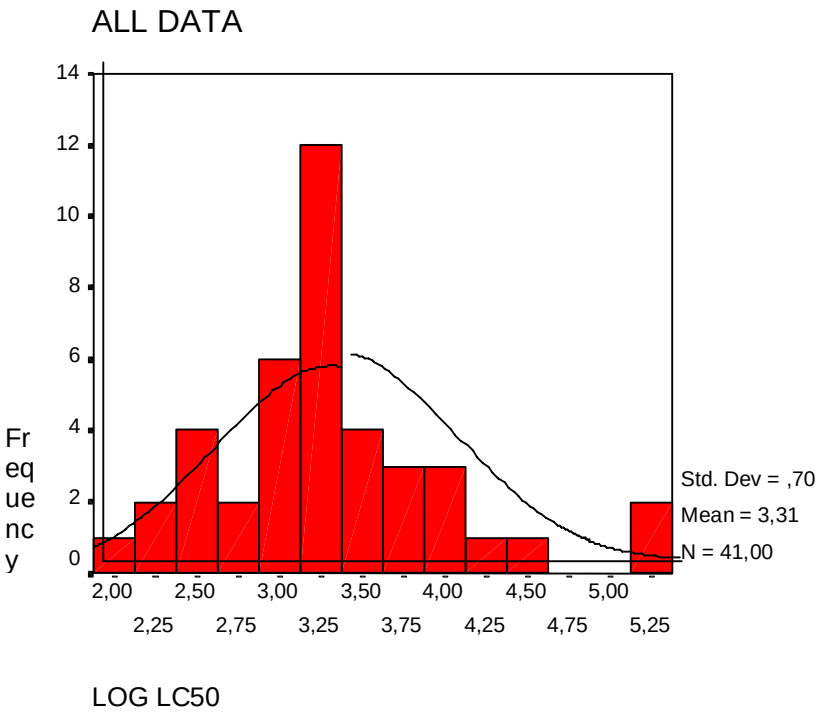
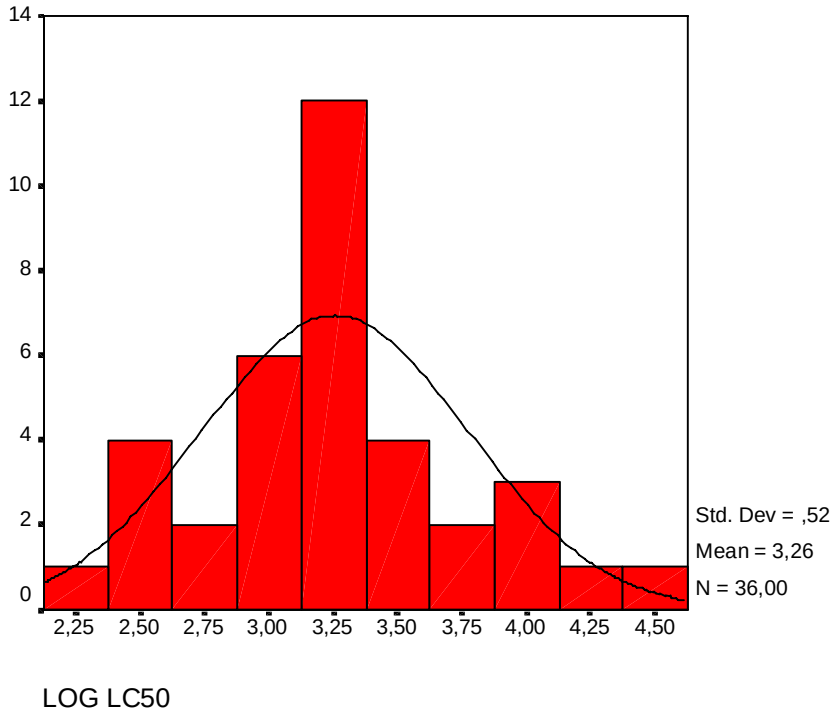

ALL DATA
Frequency
14
12
10
8
6
4
2
0
2,00
2,25
2,50
2,75
3,00
3,25
3,50
3,75
4,00
4,25
4,50
4,75
5,00
5,25
Std. Dev = ,70
Mean = 3,31
N = 41,00
LOG LC50

Figure 2.6.2.1-2: Frequency distribution of LC_{50} values for acute toxicity in fish and log-normal distribution estimated by the rapporteur excluding the values for carp and harlequin fish.



Technical endosulfan is a mixture of two isomers. The acute 96-h toxicity of these isomers has been studied on fish. The results are summarised in the following table.

Table 2.6.2.1-2: Acute toxicity of endosulfan isomers to fish.

Test organism	**96-h LC_{50} (µg/l) α-Endosulfan**	**96-h LC_{50} (µg/l) β-Endosulfan**	**96-h LC_{50} (µg/l) Technical endosulfan**	**Doc. No.:**	**Author**
Channa punctata	0.16	6.6	4.8	A22297	Devi et al. (1981)
Catla catla	0.36	7.67	1.84	A43108	Rao (1989)
Labeo rohita	0.33	7.1	1.1	A22299	Rao et al. (1980)

It seems that α-endosulfan is more toxic than β-endosulfan, but the results are not always congruent. Taking into account that the possible more toxic isomer is the one that shows a faster dissipation in the environment, the use of toxicity and exposure data for the technical product is considered a realistic worst case. Additional information could be considered.

The acute toxicity of the formulated product Thiodan to fish has been summarised in the following table.

Table 2.6.2.1-3: Acute toxicity of Thiodan to fish.

Test organisms	Study type	Chemical	Test duration	LC_{50} (µg/l)	Study conditions	Authors, Doc. Nº	Remarks
Puntius sophore	Static	Thiodan 35%	96 h	1.2	Published	Arora et al. 1971 A 25870	
Mystus vittatus	Static	Thiodan 35%	96 h	0.24	Published	Gopalakrishna Reddy & Gomathy (1977) A 259913	
Golden orfe	Static	Thiodan 35%	96 h	7	No GLP or Published	Knauf (1977b) A 16730	
Rainbow trout	Static	Thiodan (not specified)	96 h	4.7	No GLP or published	Knauf (1977 c) A 14970	
Cyprinus carpio	Static	Thiodan 35%	96 h	11	No GLP or published	Knauf (1977d) A 14970	
Channa gachua	Static	Thiodan 35%	96 h	10.6	Published	Dalela et al. (1978) A 25861	
Guppy fish	Static	Thiodan (not specified)	96 h	5.2	No GLP or published	Knauf (1978) A 18466	
Mosquito fish	Static	Thiodan 35%	96 h	3.2	Published	Joshi & Rege (1980) A 29254	Data referred to active ingredient
Labeo rohita	Continuous flow system	Thiodan 35%	96 h	1	Published	Rao et al. (1980) A 22299	Data referred to active ingredient
Channa puctata	Continuous flow	Thiodan 35%	96 h	2.5	Published	Devi et al. (1981) A 22297	Data referred to active ingredient
Mystus vittatus	Static	Thiotox 35%	96 h	0.67	Published	Verma et al. (1981) A29130	Data referred to active ingredient
Ophiocephalus punctatus	Static	Thiotox 35%	96 h	22	Published	Verma et al. (1981) A29130	Data referred to active ingredient
Barbus stigma	Static	Endosulfan (not specified)	96 h	4.3	Published	Manoharan & Subbiah (1982) A 27749	
Saccobranchus Fossilis	Static	Thiotox 35%	96 h	6.6	Published	Verma et al. (1982) A 25048	Data referred to active ingredient
Saccobranchus Fossilis	Static	Thiodan 35%	96 h	10.8	Published	Verma et al. (1982) A 25048	Data referred to active ingredient
Rainbow trout	Static	Endosulfan (352 g/l)	96 h	2.1	GLP	Fisher (1984b) A	

Test organisms	Study type	Chemical	Test duration	LC_{50} (µg/l)	Study conditions	Authors, Doc. Nº	Remarks
						30032	
Bluegill sunfish	Static	Endosulfan (352 g/l)	96 h	Between 10 and 5.6	GLP	Fisher (1984c) A 29508	
Lebistes reticulatus	Renewal daily	Endosulfan 35EC	96 h	2.7	Published	Gupta et al. (1984) A 32237	
Channa punctatus	Renewal daily	Thiodan 35%	96 h	3.07	Published	Haider & Inbaraj (1986) A 36292	
Barilius bendelisis	Static	Technical grade Thiodon (35EC)	96 h	13.5 15.6 16.6	Published	Deoray & Wagh (1987) A43067	pH = 6.5 pH = 7.5 pH = 9
Fundulus heteroclitus	Static	Endosulfan (30%)	96 h	1.15	Published	Trim (1987) A 36296	Data referred to active ingredient
Mosquito fish	Static	Thiodan ® (50%)	96 h	1.3	Published	Naqvi & Hawkins (1988) A43065	
Catla catla	Flow trough	Formulation 35% EC	96 h	1.05	Published	Rao (1989) A43108	Data referred to active ingredient
Puntius conchonius	Static	Endosulfan 35% EC	48 h	21.36	Published	Gill et al. (1991) A47588	

In some studies the toxicity of the formulated product has been identified to be higher than that observed for the active substance; when a comparison between studies with similar conditions was done, the results suggest that the toxicity of the formulate is equivalent to that expected according to the proportion of technical endosulfan.

The endosulfan metabolites should be classified as highly toxic or toxic according to the EU regulation and must be included in the risk assessment if relevant. Nevertheless, more information about the toxic effects of these metabolites has to be presented.

The following tables consider the estimated risk of endosulfan for fish assuming worst case conditions.

Table 2.6.2.1-4: Acute TER estimations for fish

Crop	Application rate	Nº	SI Days	Distance m	Drift %	Initial PECsw µg as/L	TER
Citrus	1.05	2	14	3	15.5	54.25	0.002
				10	4.5	15.75	0.008
				50	0.2	0.70	0.18
Vineyards	1.05	2	14	3	7.5	26.25	0.005
				10	1.5	5.25	0.025
				50	0.2	0.70	0.18
Arable crops	0.84	3	14	1	4.0	11.20	0.01
				10	0.4	1.12	0.11
				30	0.1	0.28	0.46
Arable crops	0.53	3	7	1	4.0	7.07	0.018
				10	0.4	0.71	0.18
				30	0.1	0.18	0.72

The results clearly indicate a potential risk for fish even assuming large buffer zones. It is clear that the uncertainty in this assessment is obviously lower than that expected in other cases where the toxicity data are limited to two species with no information on the sensitivity curve distribution. Considering that in this particular case the differences in species sensitivities are already covered by the use of the 95th percentile of a sensitivity distribution curve, the rapporteur considers that in a higher tier assessment, a TER value of 10 on this percentile can be considered as acceptable for the protection of fish species. However, this value is not reached even assuming large buffer zones, and therefore a potential risk for fish is expected. In addition, the estimations for the risk associated to run-off using a generic scenario also provided TER values lower than 1, and therefore suggest a potential risk.

The long-term chronic TER for the initial assessment are included in the following table.

Table 2.6.2.1-5: Chronic toxicity of endosulfan to fish

Test organism	Study type	Test duration		LC_{50} µg/l	NOEC µg/l	Doc. No.:	Author
Cyprinodon variegatus	early life stage test	28	d	n.r.	0.40	A47514	Hansen & Cripe (1991)
Oncorhynchus mykiss	juvenile growth test	21	d	0.28	0.05	A46835	Knacker et al. (1991)
Pimephales promelas	life cycle test	app. 1	y	0.86	0.2	A27951	Maceck et al. (1976)

n.r. not reported

According to chronic toxicity on fish, the rapporteur considers that although the acute toxicity of endosulfan for fish is well document an opposite situation is observed regarding the chronic toxicity because the use of simplified chronic tests for endosulfan seems to be inappropriate and the effects on reproduction must be addressed in life-cycle studies.

Long-term chronic TER estimations for fish are presented in the next table.

Table 2.6.2.1-6: Chronic TER estimations for fish, using the NOEC for rainbow trout

Crop	Application rate	Nº	SI Days	Distance m	Drift %	Initial PECsw µg as/L	TER
Citrus	1.05	2	14	3	15.5	54.25	0.001
				10	4.5	15.75	0.003
				50	0.2	0.70	0.07
Vineyards	1.05	2	14	3	7.5	26.25	0.002
				10	1.5	5.25	0.01
				50	0.2	0.70	0.07
Arable crops	0.84	3	14	1	4.0	11.20	0.004
				10	0.4	1.12	0.04
				30	0.1	0.28	0.18
Arable crops	0.53	3	7	1	4.0	7.07	0.007
				10	0.4	0.71	0.07
				30	0.1	0.18	0.28

All TER values are lower than the trigger value even using large buffer-zones. In addition, these values don't represent the worst case conditions due to the NOEC used correspond to a NOEC for growth . These results suggest a potential long term risk of endosulfan to fish even using an endpoint likely non sensitive. The estimations for the risk associated to run-off using a generic scenario also provide TER values lower than 1, and therefore suggest a potential risk.

From the higher tier studies submitted by the notifier, the rapporteur considers that the study confirms a high risk of endosulfan for fish species if the molecule is able to reach aquatic ecosystems even at concentrations lower than 1µg/l. The development of crop-specific scenarios for the refinement of this assessment is considered the best alternative. Taking into account that the isomer alfa seems to be the most toxic but at the same time the most rapidly degraded in both soil and water, an additional level of refinement could be achieved by an independent assessment of the environmental fate and toxicity of each isomer an the metabolites, particularly endosulfan sulphate, which obviously should include the assessment of synergistic effects among the isomers and the metabolite.

From the available information, a high potential for bioaccumulation in fish tissues but a rapid clearance can be considered. The values suggested by the rapporteur are a BCF in fish of 5000 and a half life of 2 days.

2.6.2.2 Effects on aquatic invertebrates

Data of acute toxicity of endosulfan technical on Daphnia magna are summarised in the next table.

Table 2.6.2.2-1: Acute toxicity to aquatic invertebrates.

Test organisms	Study type	Chemical	Test duration	LC_{50} (µg/l)	Study condition	Authors Doc. Nº	Remarks
Daphnia magna	Static	Technical (96.4%)	48 h	62	Published	Schoettger (1970) A14253	
D.magna	Static	Technical grade	48 h	271	Published	Nebeker et al. 1983	
D.magna	Static	Technical grade	48 h	343	Published	Nebeker et al. 1983	
Daphnia magna	Static	Endosulfan (99%)	48 h	166	Published (parece un informe)	Macek et al (1976)	
Daphnia magna	Static	No specified	48 h	158-740	Published	Nebeker 1982 A 25040	
D.magna	Static	Active ingredient	48h	75	No GLP or published	Knauf 1977b A 16733	
D. carinata	Static	Technical grade	48 h	180	Published	Santharam et al. 1976 A25919	
Cyclops sirenus	Static	Formulado (35% emulsionable)	24 h	1000 LC100	Published	Oeser et al. 1971 A 14255	
Brachionus plicatilis	Static	No especifican	24 h	5600 (5800-5400)	Published	Serrano et al. 1986 A 53745	
Brachionus calyciflorus	Static	endosulfan 96%	24 h	5150	Published	Fdez Caslderrey et al. 1992. A 47492	
Enallagma spec.	Static	Technical grade (90%)	96 h	17.5	Published	Gopal et al. 1981 A23187	
Gammarus lacustris	Static	Not specified	96 h	5.8	Published	Sanders (1969) A 26101	
Gammarus faciatus	Static	Not specified	96 h	6 (4-8)	Published	Sanders (1972) A 28837	
Gammmarus roeselii	Static	Not specified	24 h	5 LC100	Published	Ludemann &Neumann (1960) A 14242	
Caridina weberi	Static	Not specified	96 h	5.1-14.1	Published	Yadav et al. (1991) A47589	
Hydrachna trilobata	Static	Technical grade	48 h	2.8 (2.3-3.4)	Published	Nair (1981) A26111	
Ischnura sp.	Static	Technical grade	96 h	71.8	Published	Schoettger (1970) A	

Test organisms	Study type	Chemical	Test duration	LC_{50} (µg/l)	Study condition	Authors Doc. Nº	Remarks
		(96.4%)				14253	
Moina micrura	Static	Technical grade (90%)	24 h	16.2 (17.1-15.3)	Published	Krishnan& Chockalingam (1989) A 43063	
Oziotelphusa senex	Static	Technical grade (99%)	96 h	570-1490	Published	Naidu et al. (1987) A 43105	
Oziotelphusa senex	Static	Technical grade (95%)	96 h	12200-28600	Published	Reddy et al. (1992)	Data at 38º and 12ª respectively
Pteronarcys californica	Static	Not specified	96 h	2.30 (1.6-3.3)	Published	Sanders &Cope (1968) A 25918	

With these data The rapporteur proposes the use of an LC_{50} of 0.04 µg/l, as the acute toxicity endpoint for the most sensitive aquatic invertebrate; and a 48 h. EC_{50} of 150 µg/l for *Daphnia magna* which corresponds to the 90th percentile for the toxicity data on this species. The use of the pink shrimp data is considered appropriate because of the socio-economic importance of this species in areas near to crops included in the intended uses of endosulfan.

According to the formulated product, the acute toxicity on aquatic invertebrates has been summarised in the following table.

Table 2.6.2.2-2: Acute toxicity of the preparation to aquatic invertebrates

Test organisms	Study type	Chemical	Test duration	LC_{50} (µg/l)	Study conditions	Authors Docs. Nº	Remarks
Chironomus spec.	Static	Thiodan (not specified)	24 hours	53	Published	Ludermann & Neumann (1960) A18837	
Daphnia magna	Static	Endosulfan (35EC)	48 hours	470	Nor GLP or published	Knauf (1976) A16729	
Aedes Aegypti	Static	Endosulfan (35EC)	96 hours	54	Nor GLP or published	Knauf (1977) A16736	
Daphnia magna	Static	Endosulfan (35EC)	48 hours	4	GLP	Fischer (1984) A29798	
Lamellidens marginalis	Semistatic	Endosulfan (35EC)	96 hours	6	Published	Mane & Muley (1984) A31349	
Lamellidens corrianus	Semistatic	Endosulfan (35EC)	96 hours	17	Published	Mane & Muley (1984) A31349	
Procambarus clarkii	Static	Thiodan ®	96 hours	24	Published	Naqvi et al. (1989) A 43061	Data for juveniles

Test organisms	Study type	Chemical	Test duration	LC_{50} (µg/l)	Study conditions	Authors Docs. Nº	Remarks
Procambarus clarkii	Static	Thiodan ®	96 hours	423	Published	Naqvi et al. (1989) A 43061	Data for adults
Penaeus monodon	Renewal daily	Endosulfan (35EC)	48 hours	4.6	Published	Joshi & Mukhopadhyay A 48339	Data for postlarvae
Penaeus monodon	Renewal daily	Endosulfan (35EC)	48 hours	12.2	Published	Joshi & Mukhopadhyay A 48339	Data for juveniles
Diverse microcrustaceans	Static	Thiodan ® (33.7%)	48 hours	0.1-0.9	Published	Naqvi & Hawkins (1989) A43062	

The amount of information reported is lower than for the active substance and it is not easily validable. Therefore, the data presented for the active substance will be used in the assessment.

Due to the large differences of the toxicity data among close species the use of sensitivity distribution curves is not considered appropriate in this case. The rapporteur proposes the use of an LC_{50} of 0.04 µg/l, as the acute toxicity endpoint for the most sensitive aquatic invertebrate; and a 48 h. EC_{50} of 150 µg/l for *Daphnia magna* which corresponds to the 90^{th} percentile for the data on this species.

Both values have been used for the TER calculations. The results are summarised in the next tables.

Table 2.6.2.2-2: Acute TER estimations for Daphnids

Crop	Application rate	Nº	SI Days	Distance m	Drift %	Initial PECsw µg as/L	TER
Citrus	1.05	2	14	3	15.5	54.25	2.7
				10	4.5	15.75	9.5
				50	0.2	0.70	214
Vineyards	1.05	2	14	3	7.5	26.25	5.7
				10	1.5	5.25	28
				50	0.2	0.70	21.4
Arable crops	0.84	3	14	1	4.0	11.20	13
				10	0.4	1.12	134
				30	0.1	0.28	536
Arable crops	0.53	3	7	1	4.0	7.07	21
				10	0.4	0.71	211
				30	0.1	0.18	833

Table 2.6.2.2-3: Acute TER estimations for the most sensitive aquatic invertebrate

Crop	Application rate	Nº	SI Days	Distance m	Drift %	Initial PECsw µg as/L	TER
Citrus	1.05	2	14	3	15.5	54.25	0.0007
				10	4.5	15.75	0.003
				50	0.2	0.70	0.06
Vineyards	1.05	2	14	3	7.5	26.25	0.002
				10	1.5	5.25	0.008
				50	0.2	0.70	0.06
Arable crops	0.84	3	14	1	4.0	11.20	0.004
				10	0.4	1.12	0.04
				30	0.1	0.28	0.14
Arable crops	0.53	3	7	1	4.0	7.07	0.006
				10	0.4	0.71	0.06
				30	0.1	0.18	0.22

The results obtained for the standard species, *Daphnia magna*, must be interpreted in an standard way, and therefore the use of the trigger value of 100 for this assessment is considered appropriate. The data indicate that using large buffer zones the potential risk of endosulfan for aquatic invertebrates can be managed at least in some crops.

The rapporteur considers than from an ecological point of view the risk for this most sensitive aquatic invertebrates should be covered by the risk for fish, and therefore no additional estimations are required. This conclusion is also supported by the information provided by the pond studies, which showed no relevant effects on the invertebrate community at concentrations producing fish kills.

Therefore, appropriate risk management measures should be proposed by the applicant and considered by Member States to avoid toxicity problems of cultured shrimps and related species. The rapporteur considered that due to the localised nature of shrimp culture, indications on the label and buffer zones around these cultures should be efficient enough to provide a proper risk management.

From chronic toxicity to aquatic invertebrates, the reported 21d NOEC for *Daphnia magna* of 63 µg/l as measured concentration will be used in the risk assessment.

The TER long-term estimations are presented in this table.

Table 2.6.2.2-4: Long-term estimations for Dapnids.

Crop	Application rate	Nº	SI Days	Distance m	Drift %	Initial PECsw µg as/L	TER
Citrus	1.05	2	14	3	15.5	54.25	1.1
				10	4.5	15.75	4
				50	0.2	0.70	90
Vineyards	1.05	2	14	3	7.5	26.25	2.4
				10	1.5	5.25	12
				50	0.2	0.70	90
Arable crops	0.84	3	14	1	4.0	11.20	5.7
				10	0.4	1.12	56
				30	0.1	0.28	2.25
Arable crops	0.53	3	7	1	4.0	7.07	8.9
				10	0.4	0.71	90
				30	0.1	0.18	350

The results show a potential long-term risk, with TER values below the trigger, when no buffer zones are applied, while the risk can be reduced to acceptable levels for all crops by requiring appropriate buffer zones.

2.6.2.3 Effects on algae

The information on algae is limited to a reduced number of species and the most relevant information corresponds to the data on an standard species under standard conditions. Therefore, the 72h NOEC obtained for the green alga *Scenedesmus subspicatus* of 560 µg/l and an LC_{50} reported as higher than this value will be used.

Table 2.6.2.3-1: Acute TER estimations for algae

Crop	Application rate	Nº	SI Days	Distance m	Drift %	Initial PECsw µg as/L	TER
Citrus	1.05	2	14	3	15.5	54.25	10.3
				10	4.5	15.75	36
				50	0.2	0.70	800
Vineyards	1.05	2	14	3	7.5	26.25	22
				10	1.5	5.25	108
				50	0.2	0.70	800
Arable crops	0.84	3	14	1	4.0	11.20	50
				10	0.4	1.12	500
				30	0.1	0.28	2000
Arable crops	0.53	3	7	1	4.0	7.07	79
				10	0.4	0.71	800
				30	0.1	0.18	3111

The TER values are higher than the trigger value of 10 and therefore is concluded that endosulfan does not represent a relevant risk for algae and aquatic plants.

2.6.2.4 Effects on dwelling organisms

The available information on the toxicity of endosulfan to sediment dwelling species is summarised in Table 2.6.2.4-1.

Table 2.6.2.4-1: Toxicity effects on sediment species

Test organism	study type	Test duration		LC_{50} µg/kg	NOEC µg/kg	Study	Author
Chironomus plumosus (true midges)	static acute	48	h	25 µg/l	n.r.	Published	Goebel et al. 1982
Chironomus tentans (true midges)	sediment test	96	h	20	<6	GLP	Swigert & Mullen (1988)
Nannopus palustris (benthic copepod)	sediment test	7	d	n.r.	50	Published	Chandler & Scott (1991)
Pseudobradya pulchella (harpacticoid copepod)	sediment test	7	d	n.r.	200	Published	Chandler & Scott (1991)
Streblospio benedicti (polychaete)	sediment test	7	d	n.r.	<50	Published	Chandler & Scott (1991)

n.r.: not reported

The rapporteur concludes that no valid information on the chronic toxicity of endosulfan to sediment dwelling organisms has been submitted.

The acute LC_{50} of 20 µg/kg sediment of endosulfan on the Chironomid midge *Chironomus tentans* has been considered the most valuable information to estimate the acute toxicity of endosulfan for sediment dwelling organisms, while a valid chronic NOEC cannot be estimated from the available laboratory tests.

In addition, no valid chronic toxicity data have been submitted, and no information on the acute and chronic toxicity of the metabolites, and particularly of endosulfan sulphate, has been presented. Therefore a proper risk assessment for sediment dwelling organisms cannot be produced but at least a potential short term risk has been identified.

A pond study confirms the potential of endosulfan to achieve higher concentrations in the sediment. Even for this non-worst case scenario, the concentrations in the sediment are up to 2.5 and 5 times higher than the acute toxicity to chironomids estimated from laboratory species. Therefore, additional information is required for a proper assessment of the potential risk of endosulfan for sediment dwelling organisms.

2.6.3 Effect assessment for bees and other non-target arthropds.

The acute oral toxicity of endosulfan is only available for the formulated product, which showed to be more toxic that the technical substance in contact toxicity tests. Therefore the data for the formulated product, 2 µg a.i./bee for the oral toxicity and 0.82 µg a.i./bee for contact toxicity have been used in the assessment. Results have been summarised in the following table.

Table 2.6.3-1: Hazard quotients for honey bees.

Application rate (kg as/ha)	Crop	Route	Hazard quotient
1.05	Citrus, pome fruit and vineyards	Oral	525
1.05	Citrus, pome fruit and vineyards	Contact	1280
0.53	Tomatoes, Potatoes	Oral	265
0.53	Tomatoes, Potatoes	Contact	646
0.8	Stone fruits	Oral	400
0.8	Stone fruits	Contact	975
0.53	Cucurbits	Oral	265
0.53	Cucurbits	Contact	646

All HQ are higher than the trigger value and therefore a potential risk for bees must be considered. The filed study submitted is not validable and therefore, validable higher tier studies are required.

Regarding other non-target arthropods a set of non standard laboratory data and field studies suggest that endosulfan posses a risk for several species. Additional information for a proper assessment is required.

2. 6.4 Effect assessment for earthworms

The toxicity data for earthworms is summarised in the following table

Table 2. 6.4-1: Summary of the results of the effects of endosulfan on earthworms

Test organism	Study type	Substance	Test duration	LC/EC_{50} ppm	NOEC ppm	Author
Eisenia foetida	Artificial soil test (OECD)	Technical grade (97.7%)	14 days	14	0.1	Fischer 1990 A43674
Pheretima posthuma	Soil pot	Technical grade	24 h	5.01	-	Hans et al. 1990. A 53744
Lumbricus terrestris	Natural soil	Thiodan 35	14 days	23.9	-	Haque and Ebing, 1983. A28776
Eisenia foetida	Artificial soil test	Thiodan	14 days	9.4 (a.i)	-	Heimbach 1985. A 32902
Eisenia foetida andrei	Artificial soil test	Endosulfan 35%	28 days	6.7 (a.i.)	-	Heimbach 1984. A 32903
Eisenia foetida andrei	Artisol test	Endosulfan 35%	14 days	3 (a.i)	-	Heimbach 1984. A 32903
Eisenia foetida	Artificial soil	Endosulfan 35 EC	14 days	30.3	0.32	Fischer 1990. A 43675
Natural population	Semi-arid tropical grassland	Endosulfan 35% EC	80 days	No earthworms at high dose tested. Significantly reduced at normal dose	-	Reddy and Reddy. 1992. A 51812

Several studies on the toxicity of endosulfan to earthworms have submitted. The standard species *Eisenia foetida* showed to be of intermediate sensitivity and the 14 days LC_{50} of endosulfan for earthworms has been estimated using a geometric mean of the validated toxicity data for *Eisenia foetida* obtained under the standard conditions. This value is 11 mg/kg.

The acute risk assessment of endosulfan for earthworms has been estimated for all the crops. The results are summarised in the following table:

Table 2.6.4-2: TER short-term estimations for earthworms

Crop	Application rate	PECs several (ppm)	14 d LC50 (ppm)	TERst
Citrus, pome fruits vine grapes	1.05	1.33	11	8.3
Cotton		1.52	11	7.2
Tomatoes		0.69	11	16
Potatoes		0.67	11	16.4
Stone fruits		1.44	11	7.6
Cucurbits		1	11	11
Sugar beet		0.63	11	17.4
Hazel nuts		1.01	11	10.9

Several values are above the trigger, and therefore the results indicate that endosulfan has a potential acute risk for earthworms in many crops (citrus, cotton and stone fruits).

No information on the reproduction toxicity of endosulfan on earthworms has been presented, and a NOEC cannot be extracted from the field study because the results showed effects even at the lowest application rate. Therefore, the long term risk can not be estimated due to lack of data. At the same time, there are not available information about metabolites.

The rapporteur concludes that a potential acute risk has been identified in certain cases, which must be addressed at a higher tier level, and that information on the long term effects of both the active substance and the metabolites is required.

2.6.5 Effects on soil non target micro-organisms

The submitted data show that no effects of endosulfan on nitrogenase activity, ammonification and nitrification processes and on soil respiration are expected even at application rates of 5 to 10 times higher than the maximum intended rate.

It is concluded that the risk of endosulfan for soil micro-organisms is relatively low.

2.6.6 Effects on other non-target organisms (flora and fauna) believed to be at risk

Endosulfan is also highly toxic for some amphibian species. The risk is covered by the risk assessment for fish.

2.6.7 Effects on biological methods of sewage treatment

No information has been submitted.

APPENDIX 1

STANDARD TERMS AND ABBREVIATIONS

Part 1 Technical Terms

A	Ampere
a	Area
ACCase	Acetyl-CoA-carboxylase
ACh	acetilcholine
AChE	acetilcholinesterase
ADI	Acceptable daily intake
ADP	Adenosine diphosphate
AE	Acid equivalent
AFID	alkali flame-ionization detector or detection
A/G	Albumin/globulin ratio
ai	Active ingredient
ALD_{50}	Approximate median lethal dose, 50%
ALT	Alanine aminotransferase (SGPT)
AMD	Automatic multiple development
ANOVA	Analysis of variance
AOEL	Acceptable operator exposure level
AOLD	Approximate oral lethal dose
AOPP	aryloxyphenoxypropanoates
AP	Alkaline phosphatase
approx.	approximate
appr.	Approximately
AR	Applied radioactivity
AR	Area of cornea involved
ARC	Anticipated residue contribution
ARfD	Acute reference dose
as	Active substance
AST	Aspartate aminotransferase (SGOT)
ASV	Air saturation value
ATP	Adenosine triphosphate
AUC	Area under the curve
AUD	Area under the data
AUD_1	Area under the data at time 1
β	Mean elimination rate constant
BCF	Bioconcentration factor
bfa	Body fluid assay
BOD	Biological oxygen demand
bp	Boiling point
BrdU	Bromocleoxyuridine
BSAF	Biota-sediment accumulation factor
BSE	Bovine spongiform encephalopathie
BSP	bromosulfophthalein
Bt	Bacilus thuringiensis
Bti	Bacilus thuringiensis israelensis
Btt	Bacilus thuringiensis tenebrionis
BUN	Blood urea nitrogen
Bw/bwt	Body weight
c	Centi- (x 10^{-2})
C	Concentrations
C_0	Initial concentration

ºC	Degree celsius (centigrade)
CA	Controlled atmosphere
CAD	Computer aided design
CADDY	Computer aided dossier and data supply (an electronic dossier interchange and archiving format)
CAS name	Chemical abstract name
cd	candela
CDA	Controlled drop(let) application
cDNA	Complemetary DNA
CEC	Cation exchange capacity
cf	Confer, compare to
CFU	Colony forming units
CG	Cytoplasmatic grain
CI	Confidential interval
CL	Confidential limits
cm	Centimetre
CMC	Caarboxymethyl cellulose
C_{max}	Maximum plasma concentrations of total radioactivity
CNS	Central nervous system
COD	Chemical oxygen demand
CPK	Creatinine phosphatase
CPP	Cyclophosphamide
cv	Coefficient of variation
Cv	Ceiling value
CXL	Codex Maximum Resideu Limit (Codex MRL)
d	day
d	Diameter of MN
D	Cell diameter
D	Applied dosage
DAMC	Days after the maximum concentration
DAP	Days after planting
DAT	Day after treatment/application
DCM	dichloromethane
DES	diethylstilboestrol
DFR	Dislogeable foliar residue
DI	deischarge
d.l.	detection limit
DM	Dry matter
DMSO	Dimethylsulfoxide
DNA	Deoxiribonuclei acid
dna	Designated national authority
dns	Unscheduled DNA-synthesis
DO	Dissolved oxygen
DOC	Dissolved organic carbon
dpi	Days pot inoculation
DRES	Dietary risk evaluation system
DT	Disappearance time
DT_{50}	Period required for 50 per cent dissipation (define method of estimation)
$DT_{50, calc}$	Calculated half life
$DT_{50, ref}$	Reference half life
DT_{90}	Period required for 90 per cent dissipation (define method of estimation)
dw	Dry weight
DWQG	Drinking water quality guidelines
ε	Decadic molar extinction coefficient
EC_x	Effective concentration that produces x% of effect
EC_{50}	Median effective concentration
ECD	Electron capture detector

ECU	European currency unit
ED_{50}	Median effective dose
EDI	Estimated daily intake
ELISA	Enzyme linked immunosorbent assay
e-mail	Electronic mail
EMDI	Estimated maximum daily intake
EPMA	Electron probe micro analysis
ETE	Estimated theoretical exposure
Eq	Equivalent
ERC	Environmentally relevant concentration
ERL	Extraneous residue limit
f	female
F	field
^oF	Degree Fahrenheit
F_0	Parental generation
F_1	Filial generation, first
F_2	Filial generation, second
FC	Field capacity
f_{drift}	Drift factor
FIA	Fluorescence immuno assay
FID	Flame ionization detector
FOB	Functional observation battery
fp	Freezing point
FPD	Flame photometric detector
FPLC	Fast protein liquid chromatography
g	Gram
G	Glasshouse
GAP	Good agricultural practice
GC	Gas chromatography
GC-EC	Gas chromatography with electron capture detector
GC-FID	Gas chromatography with flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
GC-MSD	Gas chromatography with mass-selective detection
GEP	Good experimental practice
GFP	Good field practice
GGT	Gamma-glutamyl transferase
G.I.	Gastro intestinal
GIT	Gastro intestinal tract
GLC	Gas liquid chromatography
GLP	Good laboratory practice
GM	Geometric mean
GMM	Genetically modified micro-organism
GMO	Genetically modified organism
GPC	Gel-permeation chromatography
GPPP	Good plant protection practice
GPS	Global positionen system
GR	Growth reduction rate
GS	Growth stage
GSH	glutathion
GST-P	Glutathione-S-Transferase P
GV	granulosevirus
ΔH_{vap}	Molar heat of vaporisation
H	Henry's Law constant (calculated as a unitless value) (see also K)
h/hr	Hour(s)
ha	Hectare
Hb	Haemoglobin

HCG	Human chorionic gonadotropin
Hct	Haematocrit
HDPE	High density polyethylene
HDT	Highest dose tested
HEED	High energy electron diffraction
HID	Helium ionization detector
hl	Hectolitre
HPAEC	High performance anion exchange chromatography
HPLC	High pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	High pressure liquid chromatography – mass spectrometry
HPPLC	High pressure planar liquid chromatography
HPTLC	High performance thin layer chromatography
HRGC	High resolution gas chromatography
H_S	Shannon-Weaver index
Ht	Hematocrit
I	indoor
I_{50}	Inhibitory dose 50%
IC_{50}	Median immobilisation concentration
ICM	Integrated crop management
ID	Ionization detector
i.d.	Internal diameter
IEDI	International estimated daily intake
IGR	Insect growth regulator
im	Intramuscular
inh	Inhalation
ip	intraperitoneal
i.p.	intraperitoneal
IPM	Integrated pest management
IR	infrared
IS	Loamy sand
ISBN	International standard book number
ISSN	International standard serial number
iv	intravenous
IVF	*In vitro* fertilisation
k	Kilo
K	Kelvin or Henry's Law Constant (in atmospheres per cubic meter per mole)
K_{ads}	Adsorption constant
K_d	Distribution coefficient
K_{des}	Apparent desorption coefficient
K_{oc}	Organic carbon adsorption coefficient
K_{om}	Organic matter adsorption coefficient
K_{ow}	n-octanol water partition coefficient
kg	kilogram
l	litre
L	Loam
LAN	Local area network
LASER	Light amplification by stimulated emission of radiation
LBC	Loosely bound capacity
LC	Lethal concentration
LC	Liquid chromatography
LC_{50}	Lethal concentration, median
LC_{Lo}	Lethal concentration low
LCA	Life cycle analysis
LC-MS	Liquid chromatography – mass spectrometry
LC-MS-MS	Liquid chromatography with tandem mass spectrometry
LD_{50}	Lethal dose, median

LD_{Lo}	Lethal dose low
LDH	Lactate dehydrogenase
LOAEC	Lowest observable adverse affect concentration
LOAEL	Lowest observable adverse effect level
LOD	Limit of determination
LOEC	Lowest observable effect concentration
LOEL	Lowest observable effect level
log	logarithm
LOQ	Limit of quantitation
LPLC	Low pressure liquid chromatography
LSC	Liquid scintillation counting or counter
LSD	Least squared denominator multiple range test
LSS	Liquid scintillation spectrometry
LT	Lethal threshold
m	Metre / male
M	Molar
MAT	Month after treatment
MC	Moisturee content
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
µCi	micro curios
MCV	Mean corpuscular volume
MDL	Method detection limit
meq	Miliequivalents
MFO	Mixed function oxidase
µg	microgram
mg	milligram
MHC	Moisture
min	minute
µl	microlitre
ml	millilitre
MLD	Method detection limit
MLT	Median lethal time
mm	Millimetre
µm	Micrometer
MMAD	Mass median aerodynamic diametre
MNPCE	Micronucleated polychromatic erythrocytes
mo	Months
mol	Mole(s)
MOS	Margin of safety
m.p.	melting point
MPC	Maximum plasma concentration
MR	Moderately resistant
MRE	Maximum residue expected
MRL	Maximum residue level
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MS	Moderately susceptible
MSDS	Material safety data sheet
MTD	Maximum tolerated dose
MWC	Maximum water holding capacity
N	Newton
n	Normal (definiting isomeric configuration) or number of observations
n^o	Number
NA	Not applicable
NAEL	No adverse effect level

NCE	Normochromatic erythrocyte
nd	Not determined
n.d.	Not detected
NEDI	National estimated daily intake
NEL	No effect level
NERL	No effect residue level
n.f.	Not found
ng	Nanogram
NNM	N-Nitrosomorpholine
n.m.	Not measurable
nm	Nanometre
NMR	Nuclear magnetic resonance
NG	Nuclear grain
NNG	Net nuclear grains
no/No	Number
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NOED	No observed effect dose
NOEL	No observed effect level
NOIS	Notice of intent to suspend
np	not performed
NPD	Nitrogen-phosphorus detector or detection
NPV	Nuclear polyhedrosis virus
NR	Not reported
ns	Not sampled
NTE	Neurotoxic target esterase
OC	Organic carbon content
OCR	Optical character recognition
ODP	Ozone-depleting potential
ODS	Ozone-depleting substances
O.M.	Organic matter content
OP	Opacity
op	Organophosphorous pesticide
p	para (indicating position in a chemical name)
Pa	Pascal
PAD	Pulsed amperometric detection
2-PAM	2-prlidoxime
PB	Phenobarbitone
pc	Paper chromatography
PC	Personal computer
PCE	Polychromatic erythrocyte
PCV	Haematocrit (packed corpuscular volume)
PEC	Predicted environmental concentration
PEC_A	Predicted environmental concentration in air
PEC_{GW}	Predicted environmental concentration in ground water
PEC_i	Initial PEC
PEC_s	Predicted environmental concentration in soil
$PEC_{s, act}$	Actual PEC_s
$PEC_{s, twa}$	Time-weighed average PEC_s
PEC_{SW}	Predicted environmental concentration in surface water
PED	Plasma-emissions-detector
PEG	Polyethylene glycol
pH	pH - value
PHED	Pesticide handler’s exposure data
PHI	Pre-harvest interval
PIC	Prior informed consent

Pic	Phage inhibitory capacity
PIXE	Proton induced X-ray emission
pKa	Negative logarithm (to the base 10) of the dissociation constant
PNEC	Predicted no effect concentration
po	By mouth
P_{ow}	Partition coefficient between n-octanol and water
POP	Persistent organic pollutants
ppb	Parts per billion (10^{-9})
PPE	Personal protective equipment
ppm	Parts per million (10^{-6})
ppp	Plant protection product
ppq	Parts per quadrillion (10^{-24})
ppt	Parts per trillion (10^{-12})
PRL	Practical residue limit
PrT	Prothrombin residue time
PSP	phenosulfophthalein
PT	Prothrombin time
PTDI	Provisional tolerable daily intake
PTT	Partial thromboplastin time
PVDW	Predicted value drinking water
PVOH	plyvinylalcohol
Q_{10}	Factor for increase of degradation rate with an increase of temperature of 10ºC
QA	Quality assurement
QSAR	Quantitative structure-activity relationship
r	correlation coefficient
r^2	Coefficient of determination
R	Ideal gas constant / resistant
RAC	Raw agriculture commodity
RBC	Red blood cell
RED	Redness
Reg.	Registration
REI	Restrictes entry interval
Rf	Retardation factor
RfD	Reference dose
RH	Relative humidity
RL_{50}	Median residual lifetime
RNA	Ribonucleic acid
RP	Reversed phase
rpm	Rotations per minute
rRNA	Ribosomal ribonucleic acid
RRT	Relative retention time
RSD	Relative standard deviation
S	susceptible
s	second
SAC	Strong adsorption capacity
SAP	Serum alkaline phosphatase
SAR	Structure/activity relationship
SBLC	Shallow bed liquid chromatography
sc	subcutaneous
sce	Sister chromatid exchange
SD	Standard deviation
se	standard Error
SEM	Standard error of the mean
SEP	Standard evaluation procedure
SF	Safety factor
SFC	Supercritical fluid chromatography

SFC	Supercritical fluid extraction
SIMS	Secondary ion mass spectroscopy
SL	Sandy loam
SOP	Standard operating procedures
sp	Species (only after a generic name)
SPE	solid phase extraction
SPF	Specific pathogen free
spp	subspecies
sq	square
SSD	Sulphur specific detector
SSMS	Spark source mass spectrometry
STEL	Short term exposure limit
STMR	Supervised trials median residue
SW	Chemosis
t	Tonne (metric tone)
t_1	Time period
T_3	Tri-iodothyroxine
T_4	thyroxine
T	Absolute temperature
T_{ref}	Reference temperature
T_{calc}	Temperature for which DT_{50} was calculated
$t_{1/2}$	Terminal elimination half-life
T_{max}	Maximum time
TADI	Temporary acceptable daily intake
TBC	Tightly bound capacity
TCD	Thermal conductivity datector
TC_{Lo}	Thermionic concentration, low
TC_{max}	Time to maximum plasma concentration of total radioactivity
$TC_{max/2}$	Time to one-half maximum plasma
TD_{Lo}	Toxic dose low
TDR	Time domain reflectrometry
TID	Thermoionic detector, alkali flame detector
TER	Toxicity exposure ration
TER_I	Toxicity exposure ration for initial exposure
TER_{ST}	Toxicity exposure ration following repeated exposure
TER_{LT}	Toxicity exposure ration following chronic exposure
TEP	Typical end-use product
tert	Tertiary (in a chemical name)
TGAI	Technical grade of the active ingredient
TGGE	Temperature gradient gel electrophoresis
TIFF	Tag image file format
TLC	Thin layer chromatography
Tlm	Median tolerance limit
TLV	Threshold limit value
TMDI	Theoretical maximum daily intake
TMRC	Theoretical maximum residue contribution
TMRL	Temporary maximum residue limit
TOC	Total organic carbon
Tremcard	Transport emergency card
tRNA	Transfer ribonucleic acid
TRR	Total radioactive residue
TSH	Thyroid stimulation hormone
TWA	Time weighted average
UDP-GA	Uridine diphosphate glucoronic acid
UDS	Unscheduled DNA synthesis
UF	Uncertainty factor (safety factor)
ULV	Ultra low volume

UV	Ultraviolet
vl.	volume
V	Volume of the water body
VCR	Vincristine
v/v	Volume ratio (volume per volume)
WBC	White blood cell
wk	week
wt	Weight
wt/vol	Weight per volume
w/v	Weight per volume
w/w	Weight per Weight
XRFA	X-ray fluorescence analysis
yr	year
<	Less than
≤	Less than or equal to
>	Greater than
≥	Greater than or equal to

Part 2 Organisations and Publications

BBA	Federal Biological Research Centre for Agriculture and Forestry
CA	Chemical Abstracts
CAS	Chemical Abstracts Service
CIPAC	Collaborative International Pesticides Analytical Council Ltd.
D/DE	Germany
E	Spain
EC	European Commission
EEC	European Economic Community
ECCO	European Commission Co-ordination
EINECS	European Inventory of Existing Commercial Chemical Substances
EPA	Environmental Protection Agency
EPPO	European and Mediterranean Plant Protection Organisation
ES	Spain
EU	European Union
FAO	Food and Agriculture Organisation of the UN
FR	France
ISO	International Organisation for Standardisation
I	Italy
IUPAC	International Union of Pure and Applied Chemistry
SETAC	Society of Environmental Toxicology and Chemistry
OECD	Organisation for Economic Co-operation and Development
UK	United Kingdom of Great Britain
US	United States
USA	United States of America

APPENDIX 2

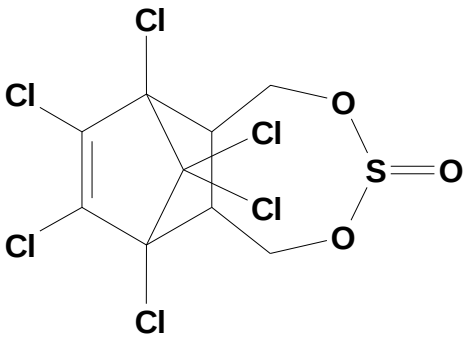
PREPARATION (FORMULATION) TYPES AND CODES

EC	Emulsifiable concentrate	A liquid, homogenous preparation to be applied as an emulsion after dilution in water

Chapter 1: **Identity, Physical and Chemical Properties, Details of Uses, Further Information and Proposed Classification and Labelling**

Active substance (ISO Common Name)	Endosulfan
Function (*e.g.* fungicide)	Insecticide
Rapporteur Member State	Spain

Identity (Annex IIA, point 1)

Chemical name (IUPAC)	6,7,8,9,10,10-hexachloro-1,5,5ª,6,9,9ª-hexahydro-6,9-methano-2,4,3-benzo-dioxathiepin-3-oxide
Chemical name (CA)	6,9-methano-2,4,3-benzodioxathiepin,6,7,8,9,10,10-hexachloro-1,5,5ª,6,9,9ª-hexahydro-3-oxide
CIPAC No	89
CAS No	115-29-7
EEC No (EINECSor ELINCS)	204-079-9
FAO Specification (including year of publication)	CP/228
Minimum purity of the active substance as manufactured (g/kg)	940 +/- 20 g / Kg (FAO)
Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg)	SEE ANNEX C
Molecular formula	$C_9H_6Cl_6O_3S$
Molecular mass	406.96 g/mol
Structural formula	

Physical-chemical properties (Annex IIA, point 2)

Melting point (state purity if not purified)	α - endosulfan: 109.2 °C β - endosulfan: 213.3 °C
Boiling point (state purity if not purified)	**Not required**
Temperature of descomposition	**Not required**
Appearence (state purity if not purified)	Flskes with tendence to aglomeration, cream to tan aminly beige. Odour like sulphur dioxide.
Relative density (state purity if not purified)	1.87 g / cm^3
Surface tension	**Not required.** Solubility < 1 mg / l
Vapour pressure (in Pa. State temperature)	α - endosulfan: 1.05 x 10^{-3} Pa β - endosulfan: 1.38 x 10^{-4} Pa
Henry's law constant (Pa m^3 mol^{-1})	α - endosulfan: 1.1 Pa x m^3 x mol^{-1} at 20 °C. β - endosulfan: 0.2 Pa x m^3 x mol^{-1} at 20 °C.
Solubility in water (g/l or mg/l state temperature)	α - endosulfan: 0.41 mg / l β - endosulfan: 0.23 mg / l Thionex (mixture of isomers): 0.63 mg / l No pH dependency observed
Solubility in organic solvents (in g/l or mg/l state temperature)	dichloromethane > 200 g / l
	ethyl acetate > 200 g / l
	ethanol (aprox) = 65 g / l
	n – hexane = 24 g / l
	acetone = 1164 g / l
	toluene > 200 g / l
Partition co-efficient (log P_{ow}) (state pH and temperature)	log P_{ow} = 4.7 No pH dependence is observed.
Hydrolityc stability (DT_{50}) (state pH and temperature)	**α - endosulfan** T = 25°C pH 5: > 200 days
	pH 7: 19 days
	pH : 0.26 days
	β - Endosulfan T = 25°C pH 5: > 200 days
	pH 7: 10.7 days
	pH : 0.17 days
Dissociation constant	**Not aplicable**
UV/VIS absortion (max.) (if absortion > 290 nm state ε at wavelength)	No significant absorvance above 290 nm.
Photostability (DT_{50}) (aqueous, sunlight, state pH)	Photolitically stable
Quantum yield of direct phototranformation in water at λ > 290 nm	Photolitically stable
Flammability	Not capable of burning
Explosive properties	Non-explosive

Summary of intended uses

CROP	F/G	FORM TYPE	COUNTRY	APPLICATION			APPLICATION RATE			PHI	REMARKS
				Method	Growth stage	N	kg ai/hl	Water l/ha	kg ai/ha		
1. Fruits (i) Citrus fruit	F	EC (350 g/l)	Southern Europe	Medium/High vol spray	During fruiting	1-2	0.035	3000	1.05	21	Spraying interval : 14 – 21
(ii) Hazel nuts	F	EC (350 g/l)	Southern Europe	High volume spray	At any stage	2	0.08	1000	0.8	28	Spraying interval : 14-21
(iii) Pome fruit	F	EC (350 g/l)	Southern Europe	High volume spray	During fruiting	2	0.053 – 0.105	1000 – 1500	max. 1.05	14	Spraying interval : 14 – 21
(iv) Stone fruit (peaches)	F	EC (350 g/l)	Southern Europe	High volume spray	During fruiting	3	0.053	1500	0.8	21	Spraying interval : 14 – 21
(v) Berries and small fruit (a) Table and wine grapes	F	EC (350 g/l)	Southern Europe	Medium/High volume spray	At any syage	2	0.053-0.105	500-1000	max 1.05	28	Spraying interval : 14 – 21 days
2. Vegetables (i) Root and tuber vegetables Sugar beet	F	EC (350 g/l)	Southern Europe	High colume spraying	At any stage	2	0.125	400	0.50	25	Spraying interval: 14 – 21 days
(iii) Fruiting vegetables (a) Solanacea (Tomatoes)	F	EC (350 g/l)	Southern Europe	High volume spray	At any stage	2	0.053 - 0.105	500 - 1000	max. 0.53	3	Spraying interval: 14 – 21 days
	G	EC (350 g/l)	Southern Europe	High volume spray	At any stage	2	0.053	1500	0.8	3	Spraying interval: 7 – 14 days
(c) Cucurbits inedible peel	F	EC (350 g/l)	Southern Europe	High volume spray	At any stage	3	0.053	600 – 1000	0.32 – 0.53	7	Spraying interval: 7 – 14
4. Oil seed											

Summary of intended uses

CROP	F/G	FORM TYPE	COUNTRY	APPLICATION			APPLICATION RATE			PHI	REMARKS
				Method	Growth stage	N	kg ai/hl	Water l/ha	kg ai/ha		
Cotton	F	EC (350 g/l)	Southern Europe	High volume spray	Last application: When balls are partly open	3	0.105	800	0.84	15	Spraying interval: 14-21
5. Potatoes	F	EC (350 g/l)	Southern Europe	High and low volume spray	At any stage	2	0.088	600	0.53	14	Spraying interval: 14 – 21 days

IMPORTED CROPS

Summary of intended uses

CROP	F/G	FORM TYPE	COUNTRY	APPLICATION			APPLICATION RATE			PHI	REMARKS
				Method	Growth stage	N	kg ai/hl	Water l/ha	kg ai/ha		
Citrus fruit	F	EC (350 g/l)	Imported crop	High volume spray	During fruiting	1-2	0.035	3000	max. 1.05	21	Outside Europe, use in citrus is registered in South Africa, Brazil, U.S.A.
Soybeans	F	EC (350 g/l)	Imported crops	High volume spray	At any stage	2	0.13 - 0.26	200 – 400	0.53	30	Outside Europe, use is registered in Brazil, Australia, Argentina a.o. countries
Cotton	F	EC (350 g/l)	Imported crops	High volume spray	Last application: When balls are partly open	1 - 3	0.105	800	0.84	15	Outside Europe registrations exist in Brazil, Columbia, Equador a.o. countries.
Tea	F	EC (350 g/l)	Imported crops	High volume spray	At any stage	3	0.126	350	0.44	7	Amongst other use is registered in India
Coffee	F	EC (350 g/l)	Imported crops	High volume spray	At any stage	3	0.175 – 1.05	100 - 600	1.05	30	Use is registered in Latin american and African countries
Cacao	F	EC (350 g/l)	Imported crops	Medium to low volume spray	At any stage	3	0.21 – 0.875	40 - 120	0.25 – 0.35	28	
Pineaples	F	EC (350 g/l)	Imported crops	Medium to low volume spray	At any stage	2	0.41 – 0.84	200 - 400	1.68	60	Spraying interval 7 –14 days

Remarks:
(a) The EU and Codex classifications (both) should be used
(b) Outdoor or field use, glasshouse application (G) or indoor application (I)
(c) e.g., biting and suckling insects, soil-borne insects, foliar fungi, weeds
(d) e.g., wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GIFAP Codes - GIFAP technical Monograph No. 2, 1989
(f) All abbreviations used must be explained
(m) BBCH scale is used for growth stage identification
(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
(i) g/kg or g/l
(j) Growth stage at last treatment
(k) PHI - Pre-harvest Interval
(l) Remarks may include: Extent of use/economic importance/restrictions (e.g., feeding/grazing/minimal intervals between applications)

Classification and proposed labelling (Annex IIA, point 10)

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

Chapter 2: Methods of Analysis

Analytical methods for the active substance (Annex IIA, point 4.1)

Technical as (principle of method)	CIPAC 89/TC/M2/-(CIPAC hand book 1C, 2110-2113, 1985). GC8-TCD detection.
Impurities in technical as (principle of method)	GC8-TCD detection. See ANNEX C
Plant protection product (principle of method)	CIPAC 89/TC/M2/-(CIPAC hand book 1C, 2110-2113, 1985). GC8-TCD detection.

Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	There are only methods for melons, vines and potatoes. Capillary GC/ECD. LOQ = 0.01 mg / kg. **ILV required.** **Methods to support other uses are required.**
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	Liver, kidney, blood of wistar rat. Capillary GC/ECD. LOQ = 0.02 mg / Kg. **ILV required**
Soil (principle of method and LOQ)	**No acceptable method submitted. Data required.**
Water (principle of method and LOQ)	**No acceptable method submitted or lacking validation data. Data required.**
Air (principle of method and LOQ)	Absortion in Tenax tubes. Eluted with ethyl acetate. GC-ECD. LOQ = 0.5 μg / m^3
Body fluids and tissues (principle of method and LOQ)	To employ the same that for animal products is proposed. **Data required for endosulfan and endosulfan metabolites in fish.**

Chapter 3: Impact on Human and Animal Health

Absortion, distribution, excretion and metabolism in mammals (Annex IIA, point 5.1)

Rate and exent of absortion:	More than 90% of an oral dose of endosulfan was absorbed in rats, with maximum plasma concentrations occurring after 3-8 hours in males an about 18 hours in females. After dermal exposition of endosulfan in male rats the absorption of the doses into the skin was rapid and substantial at all doses (73-89%)at 24 hours. In female rats the dermal absorption was between 20-46% at 168 hours at all doses testes.
Distribution:	After a oral administration of endosulfan the highest tissues concentrations was found mainly in the kidneys, and liver
Potential for accumulation:	The endosulfan residues were below 0.1 ppm in all other examined tissues
Rate and exent of excretion:	The urinary and faecal elimination half-lives for male and female rats were byphasic, with the earlier $t_{1/2}$ of least than 14 h, and the latter $t_{1/2}$ ranging form 33 to 67.5 hours. Excretion was relatively rapid and essentially complete within the first 1-2 days. Urinary elimination was greater in females (2-24%) and males (11-13%). Faecal elimination was 65-82% in males and 60-72% in females
Metabolism in animals	Endosulfan is converted in the animal organism to the following metabolites: endosulfan-sulphate, endosulfan- diol, endosulfan-ether, endosulfan-hydroxyether, and endosulfan-lactone- A number of unidentified polar metabolites are probably the conjugates of the metabolites.
Toxicologically significant compounds (animals, plants and environment)	Parents, no data on plant metabolites.

Acute toxicity (Annex IIA, point 5.2)

Rat LD_{50} oral	10-22.7 mg/kg bw (f)
Rat LD_{50} dermal	500 mg/kg bw (/f)
Rat LC_{50} inhalation	0.0126 mg/l air for 4 hours (/f)
Skin irritation	Not available
Eye irritation	Not available
Skin sensitization (test method used and result)	Buehler Test. No sensitizer

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect	Neurological sings and lethality
Lowest relevant oral NOAEL / NOEL	2.3 mg/kg/day mouse (m/f)./ 2.3 mg/kg/day mouse (m/f)
Lowest relevant dermal NOAEL / NOEL	Not available
Lowest relevant inhalation NOAEL / NOEL	

Genotoxicity (Annex IIA, point 5.4)

	The overall weight of evidence from *in vitro* and *in vivo* studies is that endosulfan does not induce gene mutation. Nevertheless, although it appears to be non-clastogenic, more studies are required in order to give a definitive conclusion.

Long term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target / critical effect	Kidney
Lowest relevant NOAEL / NOEL	0.6 mg/kgbw/day (104-weeks oral study in rats)
Carcinogenicity	Not carcinogenic effects in female mice and rats. No valid conclusion could be drawn about carcinogenicity in male rats and mice.

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction target / critical effect	None identified
Lowest relevant reproductive NOAEL / NOEL	75 ppm (6 mg/kgbw/day) 2.generation reproduction toxicity in rats
Developmental target / critical effect	Fetotoxicity at maternally toxic doses
Lowest relevant developmental NOAEL/NOEL	2 mg/kg bw from developmental toxicity in rats

Neurotoxicity / Delayed neurotoxicity (Annex IIA, point 5.7)

	Endosulfan produce toxic effect due the CNS stimulation and the death may be due to direct depressant effect on some vital organ of the body.

Other toxicological studies (Annex IIA, point 5.8)

90-day, oral, dog. Thiodan Sulphate	NOAEL: 0.75 mg/kg bw/day (m/f)
90-day, oral, dog. Hoe 051329	NOAEL: 9.1 (m) and 8.4 (f) mg/kg bw/day
90-day, oral, rat. Hoe 051329	NOAEL: 7.8 (m) and 8.0 (f) mg/kg bw/day

Genotoxicity testing of metabolites	The available information shows that endosulfan-diol is not genotoxic.

Additional studies (Annex IIA, point 5.8)

Immunotoxicity studies	Immunotoxicity in certain special assays, not confirmed in sensitisation test or histologically.
Endocrine system	Some conflicting evidence of interaction with estrogen receptors in vitro, non in vivo

Medical data (Annex IIA, point 5.9)

	Lowest lethal dose 35 mg/kgbw (oral)

Summary (Annex IIA, point 5.10)

	Value	Study	Safety factor
ADI	0.006 mg/kg bw/day	2-years toxicity study in rats	100
Systemic AOEL	0.006 mg/kgbw/day	104-weeks toxicity in rats	100
Drinking water limit	0.018 mg/litre		

Dermal absorption (Annex IIIA, point 7.3)

At 24 hours, systemic absorption was 21.5%, 21.5% and 8.4% for the LD, MD and HD formulates respectively. Skin penetration increased with time and skin residues declined over time. The % penetrated across all doses was higher for rat than human skin

Acceptable exposure scenarios (including method of calculation)

Operator	It was impossible to obtain an exposition < AOEL (0.0006mg/kg/day)
Workers	
Bystanders	

Chapter 4: Residues

Metaolism in plants (Annex IIA, point 6.1 and 6.7; Annex IIIA, point 8.1 and 8.6)

Plants group covered	Fruits (pome fruit; tomato and cucumber);
Rotation crops	No data available
Plant residue definition for monitoring	Endosulfan (α+β) and endosulfan sulfate (provisional)
Plant residue definition for risk assessment	Endosulfan (α+β) and endosulfan sulfate (provisional)
Conversion factor (monitoring to risk assessment)	

Aditional information should be given on the nature of metabolites found in cucumber. Additional experiments on metabolism in plants are required for oil seeds and root & tuber vegetables.

Metabolism in livestock (Annex IIA, point 6.2 and 6.7; Annex IIIA, point 8.1 and 8.6)

Animals covered	Lactating sheep, goats and cows
Animal residue definition for monitoring	Endosulfan (α+β) and endosulfan sulfate (provisional)
Animal residue definition for risk assessment	Endosulfan (α+β) and endosulfan sulfate (provisional)
Conversion factor (monitoring to risk assessment)	
Metabolism in rat and ruminant similar (yes/no)	
Fat soluble residue: (yes/no)	Yes

Residues in succeeding crops (Annex IIA, point 6.6; Annex IIIA, point 8.5)

The stepwise approach developed by the German BBA was followed for the theoretical estimate of the residues in rotational crops. The uptake factor found for spinach (soil/plant: 2.75/1) make advisable to perform field testing. for selected leafy vegetables in different types of soil.

Stability of residues (Annex IIA, point 6 introduction; Annex IIIA, point 8 introduction)

No data available. Data requirement

Residues from livestock feeding studies (Annex IIA, point 6.4; Annex IIIA, point 8.3)

Intakes by livestock ≥ 0.1 mg/kg diet/day:	Ruminant: yes/no	Poultry: Yes/no	Pig: Yes/no
Muscle	Data requirement		
Liver	Data requirement		
Kidney	Data requirement		
Fat	Data requirement		
Milk	Data requirement		
Eggs	Data requirement		

The available information is clearly insufficient. The worst case diet shouldbe constructed to calculate the 1x dose. Feeding trials should comprise a control group, a group treated with the excess doses (3-5 x dose and 10x dose), according to the Guideline 7031/VI/95 rev. 4.

Summary of critical residues data (Annex IIA, point 6.3; Annex IIIA, point 8.2)

Crop	Northern or Mediterranean Region	Trials results revelant to the critical GAP [a]	Recommendation/comments	MRL	STMR [b]
Citrus	S		Data available are not in accordance to the GAPs. Additional trials required		
Hazelnuts	S		Additional trials required		
Pome fruit	S	3x0.03, 1x0.04, 1x0.05, 1x0.06, 1x0.07, 4x0.08, 1x0.10, 1x0.11, 1x0.14, 2x0.21, 1x0.23, 1x0.26, 1x0.27, 1x0.46		0.5	0.13
Stone fuits (peaches)	N	0.07, 0.09, 0.13, 0.15, 0.19, 0.32, 0.40, 0.49, 0.53	Registered use in S Europe. Residue trials performed only in N Europe. Additional trials required	1.0	0.26
Grapes	S	3x0.15	Insufficient residue trials. Additional trials required	0.2 (d)	0.15 (d)
Fruiting vegetables (tomatoes-Solanacea)	S(F)	4x0.03, 3x0.04, 2x0.06, 3x0.07, 2x0.08, 0.10, 0.12, 2x0.20	Data for field trials	0.5	0.08
	S(G)	0.06, 0.08, 0.10, 0.11, 0.12, 0.20, 0.21, 0.27, 0.29, 0.37, 0.60, 0.72, 1.10, 1.25, 1.78, 1.80	Data for greehouse trials. Use not recommended	(c)	(c)
Cucurbits (inedible peel)	S	6x0.15, 0.19		0.5	0.16

[a] Numbers of trial in which particular residue levels were reported *e.g.* 3 x <0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17

(b) Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the critical

(c) It is recommended the application of endosulfan under green house conditions

(d) Provisional MRL calculated based on an insufficient number of residue trials

Crop	Northern or Mediterranean Region	Trials results revelant to the critical GAP [a]	Recommendation/comments	MRL	STMR [b]
Cotton	S		Data available are not in accordance to the GAPS. Additional trials required	-	-
Potatoes	S	9x0.01, 4x0.015		0.05	0.01
Soybean	Imported crop	0.05, 0.06, 0.08, 2x0.10, 2x0.20, 0.21, 0.25, 2x0.30, 0.40, 0.42, 0.45, 0.60		1.0	0.25
Tea	Imported crop	1.1-5.0, 16.2-24.1	Insufficient and inconsistent data. Aditional trials required	-	-
Coffee	Imported crop	4x0.028	Additional trials required	0.05 (d)	0.03 (d)
Cacao	Imported crop	5x0.015	Additional trials required	0.05 (d)	0.02 (d)
Pineapple	Imported crop		Additional trials required	-	-

[a] Numbers of trial in which particular residue levels were reported *e.g.* 3 x <0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17

(b) Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the critical

(c) It is recommended the application of endosulfan under green house conditions

(d) Provisional MRL calculated based on an insufficient number of residue trials

Consumer risk assessment (Annex IIA, point 6.9; Annex IIIA, point 8.8)

ADI	0.006 mg/kg bw/day
TMDI (% ADI)	75.5% (provisional)
IEDI (European Diet) (% ADI)	
Factors included in IEDI	
ARfD	
Acute exposure (% ArfD)	

The TMDI should be recalculated taking into account the new MRL that have to be proposed by the applicant.

Processing factors (Annex IIA, point 6.5; Annex IIIA, point 8.4)

Crop/proccessed crop	Number of studies	Transfer factor	% Transference
Soybean/steaming	1	0.25-0.5	25-50
Soybean/crude oil	1	1.2-4.3	120-430
Soybean/refined oil	1	about 0.01	about 1
Soybean/cooking of soybean meal	1	0.3-0.5	30-50
Soybean/bread	1	<0.1	<10
Apple/Juice,mash	2	0.05-0.3	5-30
Apple/pomace	1	1.4-1.6	140-160
Plums/puree	1	0.3-0.8	28-80
Tomato/cooked fruit	2	About 1	100
Tomato/pomace (wet and dry)	2	10-20	1000-2000
Tomato/puree, juice	2	0.16-0.43	16-43
Grape/must	2	0.06-0.07	6-7
Grape/wine	2	<0.38	<38
Tea/infusion	1	<0.1	<10

Additional experiments required for oranges (pomace, essential oils and marmelade)

Proposed MRLs (Annex IIA, point 6.7; Annex IIIA, point 8.6)

Crop/Commodity	Proposed MRL
Citrus	(a)
Tree nuts	(a)
Pome fruits	0.5
Stone fruits	1.0 (b)
Grapes	0.2 (c)
Tomatoes (field)	0.5
Cucurbits (inedible peel)	0.5

Crop/Commodity	**Proposed MRL**
Cotton	(a)
Potatoes	0.05
Sugarbeet	(a)
Import tolerance limits	
Soybean	1.0
Tea	(a)
Coffee	0.05 (c)
Cacao	0.05 (c)
Pinapple	(a)

(a) Insufficient data to set MRL
(b) Provisional MRL based on residue trials performed in N Europe
(c) Provisional MRL based on an insufficient number of residue trials.

Chapter 5: Fate and Behaviour in the Environmental

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1.1)

Mineralization after 100 days	< 5 % It was not correctly measured in any study.
Non-extractable residues after 100 days	< 20%
Relevant metabolites – name and/or code, % of applied (range and maximum)	Endosulfan sulphate (34.3-77% at 365 days) The degradation in soil is required

Route of degradation in soil – Supplemental studies (Annex IIA, point 7.1.1.1.2)

Anaerobic degradation	Slower and with no significant differences between the isomers than during the aerobic degradation. Endosulfan sulfate was the main degradation product (15-33 % Applied radioactivity at 53 days)
Soil photolysis	DT_{50} > 200 days

Rate of degradation in soil (Annex IIA, point 7.1.1.2; Annex IIIA, point 9.1.1)

A correct determination of the kinetics of the parent compound and the metabolites are required.

Method of calculation	First order kinetics
Laboratory studies (range or median, with n value, with r^2 value)	Sandy loam $DT_{50\ lab}$ endosulfan (α+β): (20°C aerobic): 98 $DT_{90\ lab}$ endosulfan (α+β): (20°C aerobic): 326 r^2: 0.77; n:12
	Loamy sand $DT_{50\ lab}$ endosulfan (α+β): (20°C aerobic): 128 $DT_{90\ lab}$ endosulfan (α+β): (20°C aerobic): 426 r^2: 0.90; n:13
	Silt loam $DT_{50\ lab}$ endosulfan (α+β): (20°C aerobic): 90 $DT_{90\ lab}$ endosulfan (α+β): (20°C aerobic): 299 r^2: 0.90; n:13
	Sandy loam $DT_{50\ lab}$ endosulfan (α+β): (20°C aerobic): 92 $DT_{90\ lab}$ endosulfan (α+β): (20°C aerobic): 305 r^2: 0.71; n:8
	Sandy loam $DT_{50\ lab}$ endosulfan (α+β): (20°C aerobic): 80 $DT_{90\ lab}$ endosulfan (α+β): (20°C aerobic):265 r^2: 0.84; n:11
	Silty loam $DT_{50\ lab}$ endosulfan (α+β): (20°C aerobic): 25.6 $DT_{90\ lab}$ endosulfan (α+β): (20°C aerobic): 85 r^2: 0.96; n:8
	Loamy sand $DT_{50\ lab}$ endosulfan (α+β): (20°C aerobic): 37.5 $DT_{90\ lab}$ endosulfan (α+β): (20°C aerobic): 124.7 r^2: 0.57; n:8
	$DT_{50\ lab}$ endosulfan (α+β): (28°C aerobic): 37 $DT_{90\ lab}$ endosulfan (α+β): (28°C aerobic):194 r^2:0.99; n:4
	Degradation in the saturated zone: No data
Field studies (state location, range or median with n value)	Germany (silty loam) DT_{50f} (α+β): 91.6 days; DT_{90f} (α+β): 304.2 days (First order kinetics) r^2=0.90; n=10; 29% Endosulfan sulphate 151 DAT

	Germany (sandy silty) DT_{50f} (α+β): 35.9 days; DT_{90f} (α+β): 395.9 days (Root First order kinetics) r^2= 0.64; n=8; 17% Endosulfan sulphate 447 DAT
	Germany (loamy sandy) DT_{50f} (α+β): 38.5 days; DT_{90f} (α+β):424.6 (Root First order kinetics); r2= 0.94; n=10; 50% Endosulfan sulphate 28 DAT
	Germany (Sandy loam) DT_{50f} (α+β): 16.5 days; DT_{90f} (α+β):181.8 (Root First order kinetics); r2= 0.76; n=10; 67% Endosulfan sulphate 336 DAT
	Georgia (Sandy loam) DT_{50f} (α+β): 75.86 days; DT_{90f} (α+β):252 days (First order kinetics); r^2=0.88; n=18
	Georgia (Sandy loam) DT_{50f} (α+β): 89.6 days; DT_{90f} (α+β):297.7 days (First order kinetics); r^2=0.86; n=18
	California (Clay loam) DT_{50f} (α+β): 92.9 days; DT_{90f} (α+β): 308.8 days (First order kinetics); r^2=0.89; n=13
	California (Clay loam) DT_{50f} (α+β): 89.5 days; DT_{90f} (α+β): 297.5 days (First order kinetics); r^2=0.82; n=13
	DT_{50f} of endosulfan sulphate: not determined in any study (Data requirement)
Soil accumulation and plateau concentration	Residues of endosulfan are not expected, residues of endosulfan sulphate could be expected almost 7-9 months after last application. (0.4 mg/kg) Plateu: 20-50% of the initial concentration.

Soil adsorption/desorption (Annex IIA, point 7.1.2)

K_f / K_{oc}	α Endosulfan: 7969-21347; OM= 1.06-4.53%; pH=5.4-5.9
	β Endosulfan: 8612-13906; OM= 1.06-4.53%; pH=5.4-5.9
K_d	α Endosulfan: 81-1022; OM= 1.06-4.53%; pH=5.4-5.9
	β Endosulfan: 89-473; OM= 1.06-4.53%; pH=5.4-5.9
PH dependence (yes / no) (<u>if yes type of dependence</u>)	No data available

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching	No data
Aged residues leaching	<0.2% of the applied radioactivity were found in the leachate
Lysimeter/field leaching studies	No data

PEC (soil) (Annex IIIA, point 9.1.3)

Method of calculation	50% of crop interception. Top 5 cm soil column. Bulk density 1.5 g/cm^3. DT_{50}= 93 days for α+β Endosulfan. Endosulfan sulphate: **60% of the applied concentration (Initial PEC) multiplied by 0.9624. PEC of endosulfan sulphate required.**

Application rate

Crops	Maximum Single Treatment Rate kg a.s./ha	Number of Applications	Spraying interval
Citrus , pome fruit and wine grapes	1.05	2	14
Cotton	0.84	3	14
Tomatoes	0.53	2	7
Potatoes	0.53	2	14
Stone fruits	0.8	3	14
Cucurbits	0.53	3	7
Sugar beet	0.5	2	14
Hazel nuts	0.8	2	14

Calculation of PIEC values for endosulfan

Crops	Maximum Single Treatment Rate kg a.s./ha	Number of Applications	Spraying interval	PIEC mg sa/kg single application	PIEC mg sa/kg several applications
Citrus , pome fruit and wine grapes	1.05	2	14	0.70	1.33
Cotton	0.84	3	14	0.56	1.52
Tomatoes	0.53	2	7	0.35	0.69
Potatoes	0.53	2	14	0.35	0.67
Stone fruits	0.8	3	14	0.53	1.44
Cucurbits	0.53	3	7	0.35	1.00
Sugar beet	0.5	2	14	0.33	0.63
Hazel nuts	0.8	2	14	0.53	1.01

Estimated PEC and TWA PEC after last application in citrus fruit

PEC time after last application		Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial				1.33	1.33
Short term	24h			1.32	1.32
	2d			1.31	1.32
	4d			1.29	1.31
Long term	7h			1.26	1.29
	28d			1.08	1.20
	42d			0.97	1.14
	156d			0.41	0.78

Estimated PEC and TWA PEC after last application in cotton

PEC time after last application		Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial				1.52	1.52
Short term	24h			1.51	1.51
	2d			1.49	1.50
	4d			1.45	1.49
Long term	7h			1.44	1.48
	28d			1.23	1.37
	42d			1.11	1.30
	156d			0.48	0.90

Estimated PEC and TWA PEC after last application in cucurbit

PEC time after last application		Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial				1.0	1.0
Short term	24h			0.99	1.0
	2d			0.99	0.99
	4d			0.97	0.99
Long term	7h			0.95	0.98
	28d			0.81	0.90
	42d			0.73	0.86
	136d			0.36	0.63

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Hydrolysis of active substance and relevant metabolites (DT_{50}) (state pH and temperature)	pH 5: >200 days
	pH 7: α Endosulfan 19 days; β Endosulfan 10.7 days
	pH 9: α Endosulfan 6.2 hours; β Endosulfan 4.1 hours
Photolytic degradation of active substance and relevant metabolites	Stable
Readily biodegradable (yes/no)	No
Degradation in Water/sediment	
-DT_{50} water	15 days ; R^2=0.86; n=8 (River main) (Total endosulfan) 12 days ; R^2=0.85; n=8 (Gravel pit) (Total endosulfan)
-DT_{90} water	
- DT_{50} whole system	21 days ; R^2=0.82; n=8 (River main) Total endosulfan 18 days ; R^2=0.83; n=8 (Gravel pit) Total endosulfan
- DT_{90} whole system	
Mineralization	< 0.1%
Bound residue	20-23 % at the end of the study.
Distribution in water / sediment systems (active substance)	10.8%/37.7% at 4 DAT
Distribution in water / sediment systems (metabolites)	0.8%/10.6% at 51 DAT of endosulfan sulfate 29.6%/43.1% at 4 DAT of total endosulfan Water sediment study required, no information of metabolites in sediment are available

PEC (surface water) (Annex IIIA, point 9.2.3)

Method of calculation	Drift . 10-50 m buffer zone. DT_{50}=15 days
Application rate	See table
Main routes of entry	Drift, runoff.

$PIEC_{sw}$ values for the selected crops after the last application

Crop	Application rate	Nº	SI	Distance	Drift	Initial PECsw (µg as/L)	
			days	m	%	0.3 m depth	1 m depth
Citrus	1.05	2	14	0	100.0	350.00	105
				3	15.5	54.25	16.275
				5	10.0	35.00	10.5
				10	4.5	15.75	4.725
				15	2.5	8.75	2.625
				20	1.5	5.25	1.575
				30	0.6	2.10	0.63
				40	0.4	1.40	0.42
				50	0.2	0.70	0.21
Vineyards	1.05	2	14	0	100.0	350.00	105
				3	7.5	26.25	7.875
				5	5.0	17.50	5.25
				10	1.5	5.25	1.575
				15	0.8	2.80	0.84
				20	0.4	1.40	0.42
				30	0.2	0.70	0.21
				40	0.2	0.70	0.21
				50	0.2	0.70	0.21
Arable crops (cotton)	0.84	3	14	0	100.0	280.00	84.00
				1	4.0	11.20	3.36
				3	1.0	2.80	0.84
				5	0.6	1.68	0.50
				10	0.4	1.12	0.34
				15	0.2	0.56	0.17
				20	0.1	0.28	0.08
				30	0.1	0.28	0.08
Arable crops (Cucumber)	0.53	3	7	0	100.0	176.67	53
				1	4.0	7.07	2.12
				3	1.0	1.77	0.53
				5	0.6	1.06	0.318
				10	0.4	0.71	0.212
				15	0.2	0.35	0.106
				20	0.1	0.18	0.053
				30	0.1	0.18	0.053

TWA-PEC$_{sw}$ values at 48h, 96 h and 21 days for the selected crops after the last application

Crop	Water distance (m)	TWA-PEC$_{sw}$ (µg as/L) Days after last treatment								
		0	**1**	**2**	**4**	**7**	**14**	**21**	**28**	**42**
Citrus fruit	0	533.28	521.14	509.38	486.89	455.62	392.66	341.30	299.14	235.32
	3	82.66	80.78	78.95	75.47	70.62	60.86	52.90	46.37	36.47
	5	53.33	52.11	50.94	48.69	45.56	39.27	34.13	29.91	23.53
	10	24.00	23.45	22.92	21.91	20.50	17.67	15.36	13.46	10.59
	15	13.33	13.03	12.73	12.17	11.39	9.82	8.53	7.48	5.88
	20	8.00	7.82	7.64	7.30	6.83	5.89	5.12	4.49	3.53
	30	3.20	3.13	3.06	2.92	2.73	2.36	2.05	1.79	1.41
	40	2.13	2.08	2.04	1.95	1.82	1.57	1.37	1.20	0.94
	50	1.07	1.04	1.02	0.97	0.91	0.79	0.68	0.60	0.47
Vineyards	0	533.28	521.14	509.38	486.89	455.62	392.66	341.30	299.14	235.32
	3	40.00	39.09	38.20	36.52	34.17	29.45	25.60	22.44	17.65
	5	26.66	26.06	25.47	24.34	22.78	19.63	17.07	14.96	11.77
	10	8.00	7.82	7.64	7.30	6.83	5.89	5.12	4.49	3.53
	15	4.27	4.17	4.08	3.90	3.64	3.14	2.73	2.39	1.88
	20	2.13	2.08	2.04	1.95	1.82	1.57	1.37	1.20	0.94
	30	1.07	1.04	1.02	0.97	0.91	0.79	0.68	0.60	0.47
	40	1.07	1.04	1.02	0.97	0.91	0.79	0.68	0.60	0.47
	50	1.07	1.04	1.02	0.97	0.91	0.79	0.68	0.60	0.47
Cotton	0	503.4	491.9	480.8	459.6	430.1	370.7	322.2	282.4	222.1
	1	20.14	19.68	19.23	18.38	17.2	14.83	12.89	11.3	8.885
	3	5.034	4.919	4.808	4.596	4.301	3.707	3.222	2.824	2.221
	5	3.02	2.952	2.885	2.758	2.581	2.224	1.933	1.694	1.333
	10	2.014	1.968	1.923	1.838	1.72	1.483	1.289	1.13	0.889
	15	1.007	0.984	0.962	0.919	0.86	0.741	0.644	0.565	0.444
	20	0.503	0.492	0.481	0.46	0.43	0.371	0.322	0.282	0.222
	30	0.503	0.492	0.481	0.46	0.43	0.371	0.322	0.282	0.222
Cucumber	0	397	388	379.2	362.5	339.2	292.3	254.1	222.7	175.2
	1	15.88	15.52	15.17	14.5	13.57	11.69	10.16	8.908	7.008
	3	3.97	3.88	3.792	3.625	3.392	2.923	2.541	2.227	1.752
	5	2.382	2.328	2.275	2.175	2.035	1.754	1.525	1.336	1.051
	10	1.588	1.552	1.517	1.45	1.357	1.169	1.016	0.891	0.701
	15	0.794	0.776	0.758	0.725	0.678	0.585	0.508	0.445	0.35
	20	0.397	0.388	0.379	0.362	0.339	0.292	0.254	0.223	0.175
	30	0.397	0.388	0.379	0.362	0.339	0.292	0.254	0.223	0.175

Proper scenarios for the risk assessment of endosulfan in the crops and conditions included in the intended uses are required.

PEC (sediment)

Method of calculation	No data
Application rate	

$PEC_{(8)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial				
Short term				
Long term				

PEC (ground water) (Annex IIIA, point 9.2.1)

Method of calculation and type of study (*e.g.* modelling, monitoring, Lysimeter)	Paren Endosulfan and endosulfan sulfate and endosulfan diol can be regarded as immobile.
Application rate	

$PEC_{(gw)}$

Maximum concentration	
Average annual concentration	

Fate and behaviour in air (Annex IIA, point 7.2.2; Annex IIIA, point 9.3)

Direct photolysis in air	No direct photolysis
Photochemical oxidative degradation in air (DT_{50})	8.5 to 27 days
Volatilization	α isomer > β isomet 25 to 63% (24h) From soil:

PEC (air)

Method of calculation	No data

$PEC_{(a)}$

Maximum concentration	No data

Definition of the Residue (Annex IIA, point 7.3)

Relevant to the environmental	Both isomers of the active substance (α endosulfan; β endosulfan) and endosulfan sulphate. **However this definition must be considered incomplete. A wider investigation of the degradation routes of this compound must be done in order to establish a proper residue definition.**

Monitoring data, if available (Annex IIA, point 7.4)

Soil (indicate location and type of study)	No data available
Surface water (indicate location and type of study)	No data available
Ground water (indicate location and type of study)	No data available
Air (indicate location and type of study)	No data available

Chapter 6: Effects on Non-target Species

Effects on terrestrial vertebrates (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

Acute toxicity to mammals	Rat LD50 10 mg/kg b.w.
Long-term toxicity to mammals	Rat, rabit, mouse chronic NOEL = 1 mg/kg b.w.
Acute toxicity to birds	Mallard duck LD50 = 28 mg/kg b. w.
Dietary toxicity to birds	Bobwhite quail = 805 ppm
Reproductive toxicity to birds	Mallard duck NOEC = 30 ppm

Toxicity/exposure ratios for terrestrial vertebrates (Annex IIIA, points 10.1 and 10.3)

Application rate (kg as/ha)	Crop	Category (*e.g.* insectivorous bird)	Time-scale	TER	Annex VI Trigger
1.05	Citrus, pome fruit, vineyards	Small herbivorous /insectivorous birds	Acute	3.7 4	10
1.05	Citrus, pome fruit, vineyards	Small herbivorous /insectivorous birds	Dietary short-term	25 26	10
1.05	Citrus, pome fruit, vineyards	Small herbivorous /insectivorous birds	Long term	0.92 0.98	5
0.53	Tomatoes, potratoes, cucurbits	Small herbivorous /insectivorous birds	Acute	7 8	10
0.53	Tomatoes, potratoes, cucurbits	Small herbivorous /insectivorous birds	Dietary short-term	49 52	10
0.53	Tomatoes, potratoes, cucurbits	Small herbivorous /insectivorous birds	Long term	1.8 1.9	5
1.05	Citrus, pome fruit, vineyards	Small herbivorous /insectivorous mammals	Acute Long-term	1.2 2.4	10 5
0.53	Tomatoes, potratoes, cucurbits	Small herbivorous /insectivorous mammals	Acute Long-term	0.12 0.24	10 5

The risks for mammals have been calculated assuming a 25% relative feed demand

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point 8.2, Annex IIA, point 10.2)

Group	Test substance	Time-scale	Endpoint	Toxicity (mg/l)
Laboratory tests				
fish	technical	Acute	96h LC50 range	0.0001-0.01
fish	technical	Acute	96h LC50 95th percentile	0.00013
invertebrates	technical	Acute	LC50 most sensitive invertebrate	0.00004
invertebrates	technical	Acute	48h EC50 Daphnia	0.15
algae	Technical	Chronic	72 h NOEC	0.56
fish	technical	Chronic	28 d NOEC	0.00005
invertebrates	technical	Chronic	21 d NOEC	0.063
Microcosm or mesocosm tests				
A pond study is considered the essential work, fish mortalities were observed for water concentrations of 0.4 and 1 µg/l and the percentage of species affected is in agreement with the proportion estimated by the sensitivity distribution curve. No effects on water column invertebrates were observed. No conclusions on the effects on sediment dwelling organisms can be achieved.				

Toxicity/exposure ratios for the most sensitive aquatic organisms (Annex IIIA, point 10.2)

Application rate (kg as/ha)	Crop	Organism	Time-scale	Distance (m)	TER	Annex VI Trigger
1.05	Citrus	Fish	acute	3 10 50	0.002 0.008 0.18	MOS of 10 suggested
0.53	Arable crops	Fish	acute	1 10 30	0.018 0.18 0.72	MOS of 10 suggested
1.05	Citrus	Daphnia	acute	3 10 50	2.7 9.5 214	100
0.53	Arable crops	Daphnia	acute	1 10 30	21 211 833	100
1.05	Citrus	Fish	Chronic	3 10 50	0.001 0.003 0.07	10
0.53	Arable crops	Fish	Chronic	1 10 30	0.007 0.07 0.28	10
1.05	Citrus	Daphnia	NOEC	3 10 50	1.1 4 90	10
0.53	Arable crops	Daphnia	NOEC	1 10 30	8.9 90 350	10
1.05	Citrus	Algae	NOEC	3	10.3	10
0.53	Arable crops	Algae	NOEC	1	50	10

TERs are calculated for the initial PECsw using the BBA spray drift method

Bioconcentration

Bioconcentration factor (BCF)	5000
Annex VI Trigger:for the bioconcentration factor	100
Clearance time (CT_{50}) (CT_{90})	2 days

Effects on honeybees (Annex IIA, point 8.3.1, Annex IIIA, point 10.4)

Acute oral toxicity	LD50 = 2 µg/bee
Acute contact toxicity	LD50 = 0.82 µg/bee

Hazard quotients for honey bees (Annex IIIA, point 10.4)

Application rate (kg as/ha)	Crop	Route	Hazard quotient	Annex VI Trigger
Laboratory tests				
1.05	Citrus, pome fruit, vineyards	Oral	525	50
		Contact	1280	
0.53	Tomatoes, potratoes, cucurbits	Oral	265	50
		Contact	649	

Field or semi-field tests
The submited study cannot be validated

Effects on other arthropod species (Annex IIA, point 8.3.2, Annex IIIA, point 10.5)

Species	Stage	Test Substance	Dose (kg as/ha)	Endpoint	Effect	Annex VI Trigger
Laboratory tests						
						30%
						30%
						30%

Field or semi-field tests
Several non standard laboratory and field data suggest a potential risk for several non-target arthropods

Effects on earthworms (Annex IIA, point 8.4, Annex IIIA, point 10.6)

Acute toxicity	11 mg/kg (geometric mead validable data)
Reproductive toxicity	No data submitted

Toxicity/exposure ratios for earthworms (Annex IIIA, point 10.6)

Application rate (kg as/ha)	Crop	Time-scale	TER	Annex VI Trigger
2x1.05	Citrus, pome fruits vine grapes	acute	8.3	10
3x0.84	Cotton	Acute	7.2	10
2x0.53	Tomatoes	acute	16	10

Effects on soil micro-organisms (Annex IIA, point 8.5, Annex IIIA, point 10.7)

Nitrogen mineralization	No relevant effects for 5x the a.r.
Carbon mineralization	No relevant effects for 10x the a.r.

Classification and proposed labelling (Annex IIA, point 10)

with regard to ecotoxicological data	N R50/53

LEVEL 3

ENDOSULFAN

Proposal for the decision

3 Proposed decision with respect to the application for inclusion of the active substance in Annex I

3.1 Background to the proposed decision

The package of analytical methods for endosulfan residues in animals, plant material, soil, water and wild life is necessary to support the Annex I inclusion.

Based on acute oral toxicity studies in rats, and in accordance with EU criteria for classification, packaging and labelling of dangerous substances, Endosulfan is classified as 'very toxic', assigned the symbol "T+" and the risk phrase 'R28 very Toxic if swallowed'. Based on the dermal LD50 value in rats, it also should be classified as "Harmful" and be associated with the risk phrase "Harmful in contact with skin". Based on results of the acute inhalation study in rat, Endosulfan should be classified as 'very toxic', assigned the symbol "T+" and the risk phrase 'R26 very Toxic by inhalation' in accord with EU Guidelines.

The short term toxicity studies submitted did not allow to establish a correct NOAEL to be used in the AOEL calculation, the dermal and inhalation short term toxicity studies were considered not acceptable.

The overall weight of evidence from the *in vitro* and *in vivo* studies, submitted by AgrEvo, Luxan (Excel) and Calliope, is that endosulfan does not induce gene mutation. Nevertheless, although it appears to be non-clastogenic, more studies are required in order to give a definitive conclusion.

Endosulfan was not carcinogenic at any dose tested on rats, mice or dogs. In addition, endosulfan was not toxic for reproduction; fetotoxicity appear at maternally toxic doses.

It is impossible to obtain solvent acute toxicity data on endosulfan-lactone, endosulfan-hydroxyether, endosulfan-ether, and endosulfan-alcohol because the submitted studies have serious deficiencies, and they have been evaluated as unacceptable. More information is required. The subchronic toxicity study on endosulfan-sulphate was considered unacceptable, since this metabolite was included in the residue definition it should be convenient to clarify its subchronic toxicity. Besides, according to the available information, one endosulfan metabolite, endosulfan-diol , is considered to be a non-genotoxic agent.

The operator exposure should be recalculated taking into account the new GAP, with the available data it is not possible to assure that the risk for operators and workers is negligible.

The residue definition in plant and animal commodities is provisional and it is subject to a confirmation of the validity of the proposed plant metabolic behaviour and the metabolism in animals, which must be carried out in additional experiments that will be required from the applicants.

Many of the residue trials carried out did not follow the GAP conditions. Consequently, only those residue data generated according to the GAPs were considered in MRLs calculation.

In order to assess the residue situation in food of animal origin after feeding of fodder contaminated with endosulfan, a feeding animal studies should be submitted.

Based on the residue data obtained from those residue trials that were performed according to the GAPs, most of MRLs proposed by the applicant were not consistent. Consequently, most of MRLs have to be considered just as provisional until more data is made available from the additional residue trials that have been required to the applicant. The theoretical maximum daily intake (TMDI) of endosulfan residues has to be recalculated taking into account the new MRL resulting from the residue trials required in the Level 4 of this Monograph.

The environmental data provided indicated that endosulfan tends to be degraded in soil and water although pathways should be further investigated. The degradation of endosulfan in soil did not show any alteration of the hexaclor norborene bicycle and showed a very low mineralization (<5%). These two facts suggest a high persistence of a soil residue constituted by a number of chlorinated metabolites, which may not account individually for more than 10% of applied dose but that all together may represent high amount of it. Based on their chemical structure it may be expected that their physico chemical properties of these compound will be similar and generally persistent and bioaccumulable. Therefore, a wider investigation of the degradation routes of this compound must be done in order to define properly the residue in the environment. **As the degradation route in soil is not well defined and complete, it may not be discarded the formation of more polar metabolites able to reach ground water, the available studies demonstrated that parent endosulfan, endosulfan sulphate and endosulfan diol are immobile in soil.**

In water, available data indicated that endosulfan tend to remain in the sediment and it is a source of endosulfan residue for the aquatic system. A correct determination of DT_{50} and DT_{90} values of parent endosulfan and its metabolites in water, sediment and total system should be required, a correct degradation kinetics (route and rates) should be proposed. The field studies submitted clearly showed the importance of the run-off in the endosulfan concentrations in water, therefore proper scenarios for the risk assessment of endosulfan in the crops and conditions included in the intended uses should be required.

The available information, although extensive, does not allow to conduct a proper environmental risk assessment and therefore most risk identifications must be based on low tier assessment.

A potential acute and chronic risk for birds and mammals, particularly small insectivorous vertebrates, has been identified. In addition, potential risk for bees, other arthropods and earthworms should also be assumed.

Endosulfan is highly toxic for aquatic vertebrates and invertebrates, fish and some invertebrate groups are considered the most sensitive populations. A potential risk for fish has been identified using the generic scenario. However the rapporteur considers that these worst-case scenarios are not realistic at least for some of the intended uses. Therefore a refinement of the risk assessment using a crop specific

worst-case scenarios is requested. The assessment should cover both spray drift and run-off exposure routes and also the risk for sediment dwelling organisms.

The risk for algae, aquatic plants and soil micro-organisms is very low.

Endosulfan should be considered as bioaccumulable but due to the rapid clearance no risk for biomagnification through the food chain must be expected.

Due to lack of information the risk associated to the metabolites cannot be assessed.

3.2 Proposed decision concerning inclusion in Annex I

The decision on the inclusion of Endosulfan in Annex I of Council Directive 91/414/ECC is postponed pending receipt and evaluation of the further information data listed in the Level 4 of this monograph.

3.3 Rational for the postponement of the decision to include the active substance in Annex I, or for the conditions and restrictions to be associated with a proposed inclusion in Annex I, as appropiate.

With the available information it is not possible to obtain a correct degradation route and rate of endosulfan in soil and water, a further investigation concerning the environmental fate and behaviour of endosulfan is necessary in order to perform a good risk assessment. Moreover the available information does not allow having a clear profile of the degradation route of endosulfan in soil and water. Proper scenarios based on the intended uses and on the conditions of use should be submitted to do a higher tier risk assessment.

LEVEL 4

ENDOSULFAN

Further information

4. Further information to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in Annex I

4.1 Identity of the active substance

B.V. Luxan (Excel Industries Ltd.) should submit:

The proposed GAPs in the European Union separated in northern and southern zone, because the submitted GAPs are not clear.

Method of manufacture (synthesis pathway) : Not enough details has been submitted on the actual manufacture process employed by EXCEL. Details such as solvent and temperatures should have been submitted

Analytical profile of batches: Information on test material and methods should be submitted to consider these data acceptable.

Composition of the preparation: Emulsifier and stabiliser have not been well specified. No safety data sheet on these components have been provided. This information is required.

4.2 Physical and chemical properties of the active substance

The physico-chemical compatibility must be studied with the formulate Callistar.

Luxan B.V (Excel) has not provided any available documentation (Doc K) on plant protection product Endocel 35EC, this information should be required.

4.3 Data on application and further information

The applicant B. V. Luxan (Excel Industries Ltd.) did not submit any data concerning the packaging and compatibility with packaging materials, this data are essential to calculate the operator exposure.

Moreover the applicant had not take into account the endosulfan toxicity for aquatic organism for the procedures for cleaning application equipment proposed. No data concerning the procedures for destruction or decontamination of the plant protection product and its packaging were submitted.

4.4 Methods of analysis

AgrEvo

For animal products only an acceptable method for liver, kidney and blood of Wistar rats has been submitted. Validation by an independent laboratory is required for this method.

For plant material many old methods, poorly validated, have been submitted. Only the analytical method for melons and vines and the method for potatoes are fully validated. For the rest of the methods no validation data are provided; these data are required to support residue trials that use those methods. Validation by an independent laboratory is also required for plant methods.

For soil method validation data and an English translation of the original report is required.

For drinking water validation data are required.

For surface water no method is provided and it is required.

For wildlife an analytical method to determine endosulfan an its metabolites in fish is required.

Calliope

A method for the determination of technical active ingredient purity and a method for impurities is required for inclusion of Calliope product in Annex 1 of Directive 91/414/EEC because are necessary to establish technical specifications of Calliope product.

As Endosulfan has been classified as very toxic a method for Endosulfan residues in animal and human body fluids and tissues is required.

Methods for analysis of residue sin plants provided by Calliope are not sufficiently validated. Validation and validation by an independent laboratory is required for these methods. It is pointed out that Data Protection is required for the only two fully validated methods submitted by AgrEvo.

Validation data are required to support the method for analysis of soil submitted by Calliope.

A validated method for the determination of endosulfan and its metabolite endosulfan sulphate in surface and drinking water is required to Calliope since the method submitted is not acceptable.

A method for the determination of endosulfan in air is required since the method submitted is not acceptable and Data Protection has been claimed for the method submitted by AgrEvo. A method for the determination of endosulfan in fish tissues is required.

4.5 Toxicology and metabolism

Toxicokinetics

The following studies were presented only as reviews.

Deema *et al* 1996, (AgrEvo: ANRA)
FMC Corporation, 196 (AgrEvo: ANRA)
Maier-Bode , 1996 (Excel, 5/01)
Gupta and Chandra, 1975 (Excel, 5.1.2/03)

Original paper should be provided.

Acute toxicity studies

The following studies were not provided in the original dossier, nevertheless, they were added later by AgrEvo and will be evaluated as addendum to monograph

- Skin irritation in rabbits.
- Eye irritation in rabbits
- Skin sensitisation (maximisation test).

Elsea (1957) , Bracha (1977) and Dikshits (1984) studies were considered as additional information till receiving original paper

Short-term studies

The following studies were not provided in the original dossier, nevertheless, they were added later by AgrEvo and will be evaluated as addendum to monograph

- Short term oral study in rats.
- Short term inhalation study in rats.
- Short term dermal study in rabbits.
- Information about a preliminary study mentioned in the subchronic inhalation toxicity study (B.5.3.3.2-1) which was used to establish a NOAEL value.

- A 90-days feeding study in dogs in required

Genotoxicity

- *In vivo* chromosomal aberration assay in rodent bone narrow cells (chromosomal aberration assay or micronucleus test). Studies should be performed according to specific test guidelines. The highest dose tested should be a dose that produces some indication of toxicity. GLPs should be applied. Depending on the results obtained in this study, more studies could be required.

Toxicity of metabolites

The following studies are required:

- Acute toxicity of endosulfan-lactone, endosulfan-hydroxyether, endosulfan-ether, and endosulfan-alcohol.
- Short term toxicity of endosulfan-sulphate.

4.6 Residue data

Additional information should be provided dealing with the nature of metabolites found in cucumber, in particular about those present in the non-polar and polar fractions. Special attention should also be given to the lactone metabolite due to its high toxicity as it is shown in the toxicity studies.

Additional experiments on metabolism in plants are required for oils seeds and root and tuber vegetables.

Animal metabolism study:

The Table 4.6-1 shows the additional trials required from the applicant in order to establish the adequate MRLs for each crop:

Table 4.6-1: Residue trials required

Crop	Region	No. Trials	No. applications	Rate (kg as/hl)	Rate (kg as/ha)	PHI days
Mandarins	S	4 DC, 4 AH	2	0.035	1.05	21
Oranges	S	4 DC, 4 AH	2	0.035	1.05	21
Hazelnuts	S	2 DC, 2 AH	2	0.08	0.8	28
Peaches	S	4 DC, 4 AH	3	0.053	0.8	21
Grapes	S	5 AH	2	0.105	1.05	28
Cucurbits	S	1 AH	3	0.053	0.53	7
Tea	W	3 DC, 3 AH	3	0.126	0.44	7
Coffee	W	4 AH	3	1.05	1.05	30
Cacao	W	3 AH	3	0.875	0.35	28
Sugar beet	S	8 AH	2	0.125	0.50	25
Cotton	S	4 AH	3	0.105	0.84	15
Pineapple	W	4 AH	2	0.84	1.68	60

Additional experiments in prunes and raisins would be necessary to demonstrate if a residue concentration takes place in these products. The same can be applied for essential oils in citrus.

Residue trials and processing studies in tea.

Animal feeding study on rumiants and poultry considering a worst case animal diet

Field tests which provide information on the actual residue situation in rotational crops are required for selected leafy vegetables in different types of soil and climatic conditions.

4.7 Environmental fate and behaviour

DT values of endosulfan sulfate in soil (laboratory studies and field studies)

A wider investigation of the degradation routes in soil and water must be done.

PEC in soil for endosulfan sulfate.

A correct determination of DT_{50} and DT_{90} values of parent endosulfan and its metabolites in water, sediment and total system.

A correct degradation kinetics (route and rates) should be proposed.

The field studies submitted clearly showed the importance of the run-off in the endosulfan concentrations in water, therefore proper scenarios for the risk assessment of endosulfan in the crops and conditions included in the intended uses should be required.

4.8 Ecotoxicology

Information on the toxicity of all relevant metabolites for all taxonomic groups, including either specific tests or information supporting that the risk is covered by the risk of the active substance.

Semi-field studies on birds and/or relevant information to refine the acute and chronic risk for birds and mammals.

The need of a dietary short-term test on birds must be decided after the ECCO decision on the validity of the existing test.

Specific higher tier scenarios for each crop to assess the realistic risk to aquatic organisms associated to spray drift and run-off exposure of surface water.

A chronic life-cycle study on a sensitive fish species.

Risk management measures for the protection of shrimp cultures.
A chronic toxicity study on sediment dwelling micro-organisms and/or higher tier studies to address the risk for this group.

A field tests on bees.

Enough information to assess the risk for other non-target arthropods

A reproduction toxicity study on earthworms.

A realistic risk assessment of the risk of the active substance and its metabolites to earthworms.

4.9 Classification, packaging and labelling

No data required.