SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride (Chlorendic anhydride)

EC No 204-077-3

CAS RN 115-27-5

Evaluating Member State(s): France

Dated: May 2022

Evaluating Member State Competent Authority

French Agency for Food, Environmental and Occupational Health Safety (ANSES) on behalf of French Ministry of Environment
14 rue Pierre et Marie Curie
94701 Maisons-Alfort cedex
France
Email: reach@anses.fr

Year of evaluation in CoRAP: 2013

Before concluding the substance evaluation two Decisions to request further information were issued on 19 March 2015 and 20 December 2018

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the Registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substance, Chlorendic anhydride (EC number 204-077-3) was originally selected for substance evaluation in order to clarify concerns about:

- CMR
- Suspected sensitiser
- Suspected PBT/vPvB
- Exposure of environment
- Exposure of workers

During the evaluation also other concerns were identified. The additional concerns were:

- Persistence of chlorendic acid in sediment compartment
- Choice of the environmental release categories (ERC) for risk assessment and consequences.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

No other processes at EU level were identified.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	X
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

Skin sensitisation

The evaluating MSCA considers that the registered substance should be classified as **skin sensitiser category 1 (skin sens. 1; H317)**.
In accordance with ECHA Guidance on application of the CLP criteria (2017) where a substance is classified as skin sensitisation category 1 (ie. when the available data does not allow potency categorisation), the risk management measures and operational conditions applicable to the “high hazard” band should be applied. These measures aim to avoid an exposure to the substance.

The evaluating MSCA considers that further review and refinement of the implemented operational conditions and risk management measures are required by the Registrant(s) to ensure that exposure is avoided.

Respiratory sensitisation

The evaluating MSCA considers that the Substance should be classified as a respiratory sensitiser. As stated in a WHO report (2009), allergic respiratory manifestations are well known effects of occupational exposure to cyclic acid anhydrides and specific IgE-antibodies for chlorendic anhydride has been found (see chapter 7.9.3.2). The evaluating MSCA considers that there is insufficient data currently to allow sub-categorisation for respiratory sensitisation. Therefore the substance should be classified as **respiratory sensitiser category 1 (resp. sens. 1; H334)**.

Carcinogenicity

The evaluating MSCA considers that there is sufficient evidence to use carcinogenicity data of the acid form. Indeed, chlorendic anhydride and chlorendic acid are closely related compounds, the acid form being the impurity and hydrolysis product of the anhydride (US EPA, 2009). Chlorendic acid is classified by the IARC (1990)as carcinogenic group 2B (possibly carcinogenic to human) based on evidence of carcinogenicity from two experimental studies conducted by the US NTP (1987). Indeed, 2-year oral exposure to chlorendic acid caused malignant neoplasms in the liver in two rodent species (F344/N rats and B6C3F1 mice), and also benign neoplasms at several different tissue sites.
Consequently, the evaluating MSCA considers that the substance should be classified as **carcinogenic category 1B (carc. cat. 1B)**.

Chronic aquatic toxicity

Based on the data available in the registration dossier (an algal inhibition test with a result of EC50 >97.2 mg/l), the evaluating MSCA considers that chlorendic anhydride must be classified as **Aquatic Chronic 3, H412**: harmful to aquatic life with long lasting effects to aquatic life.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Based on the carcinogenic potential of chlorendic acid (the impurity and degradation product of the anhydride form) that is substantiated by clear evidence in feed studies conducted in two rodent species (US NTP, 1987), an **SVHC identification under Article 57(a) of REACH can be considered**.

In addition, the Substance was identified as respiratory sensitiser by the evaluating MSCA. A proposal to classify the Substance as respiratory sensitiser category 1 under Annex VI of CLP will be made and may also justify consideration of an **SVHC identification due to equivalent level of concern based on the substance respiratory sensitising properties (Article 57(f) of REACH)**.

The relevance of such an identification will be analysed in a further RMOA after harmonisation of the corresponding classifications.

4.1.3. Restriction

Not relevant.

4.1.4. Other EU-wide regulatory risk management measures

Not relevant.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not relevant.

5.2. Other actions

Not relevant.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
CLP Annex VI dossier	2022 at the earliest	France MSCA
RMOA to assess relevance of SVHC identification	After harmonisation of classification	France MSCA

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance, Chlorendic anhydride (EC number 204-077-3) was originally selected for substance evaluation in order to clarify concerns about:

- CMR
- Suspected sensitiser
- Suspected PBT/vPvB
- Exposure of environment
- Exposure of workers

During the evaluation also other concerns were identified. The additional concerns were:

- Persistence of chlorendic acid in sediment compartment
- Choice of the environmental release categories (ERC) for risk assessment and consequences.

All hazard endpoints were evaluated. Since chlorendic anhydride and chlorendic acid are closely related, hazards for both substances were assessed.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome / conclusion**
Skin and respiratory sensitisation	**Concerns confirmed** No sub-categorisation into 1A or 1B is deemed possible based on the current available dataset for the EU harmonized CLP classification. The evaluating MSCA considers that the Substance should be classified as skin sensitiser category 1. In addition, the evaluating MSCA considers that the Substance should be classified as a respiratory sensitise and that there is insufficient data currently to allow sub-categorisation for respiratory sensitisation. Therefore the Substance should be classified as respiratory sensitiser category 1.
Mutagenicity	**Concern refuted** Evaluation, in a weight of evidence approach, of the available *in vitro* and *in vivo* studies related to the genotoxic potentials of chlorendic anhydride and its acid form, leads the evaluating MSCA to consider that the Substance cannot be classified for mutagenicity, even if uncertainty remains with regard to the genotoxic potential of chlorendic acid based on the equivocal results of an *in vivo* comet assay, in the liver especially.
Carcinogenicity	**Concern confirmed** The evaluating MSCA considers that there is sufficient evidence to use carcinogenicity data from the chlorendic acid form for evaluating the carcinogenicity potential of the anhydride form. Chlorendic acid is classified by the IARC as carcinogenic group 2B (possibly carcinogenic to human) based on sufficient evidence of carcinogenicity from oral experimental studies in two rodent species (F344/N rats and B6C3F1 mice), Based on these sufficient evidence for demonstrating animals carcinogenicity of chlorendic acid (i.e. causal relationship established between orally administered chlorendic acid and a significant dose-related increase in malignant neoplasms in two different species), the evaluating MSCA considers that the substance should be classified as carcinogen cat. 1B.

Toxicity to reproduction	**Concern unresolved** The evaluating MSCA considers that uncertainty remains regarding the developmental toxicity of the Substance, and also regarding the potential of the Substance to harm the fertility, considering the limits of the non-GLP studies available. However, investigation of the Substance reproductive toxicity potential was not further evaluated in the context of the SEv, as regulatory management measures are proposed based on carcinogenicity and sensitising properties of chlorendic anhydride.
PBT/vPvB assessment	**Concern refuted** **P based on screening criteria**: from the provided information (ready biodegradation study) on the substance itself and available information (ECHA CHEM database) on its reported impurities, the Substance is considered persistent based on screening assessment. **Not B based on screening criteria**: According to the provided information and available data from literature, the Substance has a low potential for bioaccumulation based on screening assessment. **T based on human health classification:** the Registrant applies a self-classification as STOT RE 2. In addition, the evaluating MSCA warrant a classification as Carc 1B and will propose its harmonisation.
Exposure of environment	**Concern confirmed** A more detailed identification of the lifecycle of chlorendic anhydride and its degradation product, chlorendic acid, including the mixtures and articles into which it is reacted, was provided by the registrant. Based on environmental exposure data available, the eMSCA concludes that no unacceptable risk for environment compartments were identified considering risk mitigation measures implemented. Nevertheless concentrations estimated by models in groundwater are above the parametric drinking water limit of 0.1 µg/L set up for pesticides for all scenarios, therefore there should be no application of the STP sludge on agricultural soil.
Exposure of workers	**Concern confirmed** Hazard properties of chlorendic anhydride, in particular respiratory sensitisation, requires a qualitative risk characterisation to minimise exposure and risks. Such substances should be strictly controlled because they may cause serious health effects for which a dose threshold is not usually identifiable. A regulatory follow-up at EU level is foreseen (CLP classification as skin and respiratory sensitiser and carcinogenic 1B) that should impact the implemented operational conditions and risk management measures at the workplace to avoid exposure. A SVHC identification of the Substance and its potential inclusion on the authorisation list for its carcinogenic and respiratory sensitising properties will be considered also in a next step to protect workers, as exposure to workers have to be minimised in this case.
Persistence in sediment compartment	**Concern refuted** A concern for toxicity to the sediment organisms was identified at the start of the evaluation. The registrant provided clarification of the exposure to demonstrate that there is no risk to the sediment compartment. The update of environmental risk assessment conclude about the absence of environment risk and additional information to clarify the aquatic toxicity of the substance is not needed.
Choice of the environmental release categories (ERC) for risk assessment and consequences	**Concern confirmed** The evaluating MSCA disagrees with Registrants use of ERC 6d. According to the claimed uses, the available data and the description of the ERC, in the guidance R-16, the parameters from the ERC5 must be taken into account for the use of formulated uncured resin to form cured resins in situ.

Additional endpoints	
Acute toxicity	The evaluating MSCA concludes that it is not necessary to classify chlorendic anhydride for acute toxicity as in all studies the values were greater than the classification limit.
Skin irritation	Based on all available data, the evaluating MSCA considers the current harmonized classification Skin Irrit. 2 for chlorendic anhydride as justified.
Eye irritation	The evaluating MSCA considers the current harmonized classification Eye Irrit. 2 for chlorendic anhydride as justified.
Respiratory irritation	Based on all available data, the evaluating MSCA considers chlorendic anhydride as a respiratory irritant and the current harmonized classification as STOT SE 3 H335 as justified.
Repeated dose toxicity	Based on the nature and severity of the observed effects on the available experimental animal studies, the evaluating MSCA considers that the criteria for a classification as STOT RE are not fulfilled. Nevertheless, the registrants apply the following self-classification for chlorendic anhydride: STOT RE2 - H373 (CLP).
Chronic aquatic toxicity	The evaluating MSCA concludes that, based on the data available in the registration dossier (an Algal Inhibition Test with a result of EC50 >97.2 mg/l), chlorendic anhydride must be classified as Aquatic Chronic 3, H412: harmful to aquatic life with long lasting effects to aquatic life.

7.2. Procedure

The Substance chlorendic anhydride was included in the Community rolling Action Plan (CoRAP) in 2013.
All the physico-chemical, human health and environmental hazards that were part of the registration dossier were evaluated.

After discussion at MSC-40, the final decision was issued on 19 March 2015 requesting:

- Vapour pressure determination test (test method EC A.4 / OECD TG 104)
- Adsorption/desorption test (test method EC C.18 / OECD TG 106)
- Short-term invertebrates toxicity test (test method EC C.2 / OECD TG 202)
- Information on short-term fish toxicity test
- Activated sludge respiration test (test method EC. C.11/OECD TG 209)
- Available data on repeated toxicity, carcinogenicity and reprotoxicity of the degradation product chlorendic acid (EC No 204-078-9 and CAS RN 115-28-6)
- Tiered approach strategy for the genotoxic potential assessment on the degradation product chlorendic acid (EC No 204-078-9 and CAS RN 115-28-6)
  - Tier 1: The *in vitro* Mammalian Cell Micronucleus Test should be realised first (test method: OECD TG 487).
  - Tier 2: In case of negative result of the *in vitro* Mammalian Cell Micronucleus Test, an *in vitro* Mammalian Cell Gene Mutation Test in L5178Y mouse lymphoma cells at TK locus (test method: EU: B.17/ OECD TG 476).
    On the basis of the results of the *in vitro* data requested above, the need to perform additional genotoxicity studies *in vivo* will be considered.
- Derive DN(M)EL which covers the concern of chlorendic acid and revise the DN(M)EL of chlorendic anhydride if necessary.
- Detailed description of the life-cycle with identification of the substance(s) of interest along the whole life-cycle and detailed justifications for each contributing scenarios.
- Risk characterization on human health for chlorendic anhydride and chlorendic acid.

After assessment of the data requested, a second decision was issued in December 2018 requesting:

- A combined *in vivo* mammalian erythrocyte micronucleus test in bone marrow with fluorescence in situ hybridisation (FISH) and *in vivo* mammalian comet assay on the following target tissues: liver, glandular stomach, duodenum, gonadal cells and, if

technically feasible, pancreas; test methods OECD TG 474 and OECD TG 489 in male rats, oral route, using the degradation product chlorendic acid;

- An exposure assessment for the whole life-cycle and clarification of environmental release categories (ERC) for risk assessment for the chlorendic anhydride.

The new data were received in October 2020, The updated registration dossier served as thebasis for the substance evaluation conclusion.

Only the hazards were evaluated: the DNELs/DMELs and the occupational exposure scenarios were not evaluated. As a harmonised classification of the Substance as a skin and respiratory sensitiser as well as a carcinogen are deemed necessary, a refinement of the implemented operational conditions and risk management measures by the Registrant will be needed to ensure that risks are adequately managed. The possible concern regarding exposure of workers may be revised if the risk management measures implemented are not considered as sufficient.

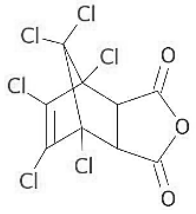
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Chlorendic anhydride
EC number:	204-077-3
CAS number:	115-27-5
Index number in Annex VI of the CLP Regulation:	607-101-00-4
Molecular formula:	$C_9H_2Cl_6O_3$
Molecular weight range:	370.83 g/mol
Synonyms:	1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



Compositions - Chlorendic anhydride SIP

State/form: solid: particulate/powder
Degree of purity: > 92 % (w/w)
Description: Hardener for epoxy resins, paints, and coatings. Other non-specified industry: Flame Retardant in unsaturated polyester resins.

Methods of manufacture of substance (migrated information): Substance manufactured outside the EU.

Table 5: Constituents (Chlorendic anhydride SIP)

Constituent	Typical concentration	Concentration range	Remarks
1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC no.: 204-077-3 CAS RN.: 115-27-5	> 95.0 % (w/w)	>92 - < 98.9 % (w/w)	

Table 6: Impurities (Chlorendic Anhydride SIP)

Constituent	Typical concentration	Concentration range	Remarks
Chlorobenzene EC no.: 203-628-5 CAS RN.: 108-90-7	3.4 % (w/w)	≥1 - ≤3.5 % (w/w)	Considered relevant for the classification and labelling of the substance
1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic acid (Chlorendic acid) EC no.: 204-078-9 CAS RN.: 115-28-6	3.0 % (w/w)	≥0.1 - ≤3.2 % (w/w)	Considered relevant for the classification and labelling of the substance
Furan-2,5-dione (Maleic anhydride) EC no.: 203-571-6 CAS RN.: 108-31-6	≤0.5 % (w/w)	≥0.0 - ≤0.5 % (w/w)	Considered relevant for the classification and labelling of the substance

Conclusion

The registered compositions are in the range that allows - according to REACH guidance for identification and naming of substances - considering this substance as a mono-constituent. Chlorendic anhydride has a content higher than 80% and impurities content is less than 20%. No information is given about the manufacturing process since the substance is manufactured outside the EU. For the Registrant, composition is completed up to 100%. Three different compositions (grades) are provided, based on the impurity profile.

GC-FID and GC-MS are used for characterisation of chlorendic anhydride, completed with UV/Vis, IR and ^{1}H-NMR. UV spectrum shows a main peak 215 nm, effect of acid of alkali conditions are not studied. IR spectra are provided without interpretation. 1H-NMR spectrum is provided with full interpretation. The signals are assigned in detail, NMR spectrum is acceptable. MS spectrum is provided with full interpretation, it matches with the database spectrum of chlorendic anhydride, and the molecular ion peak has an exact mass of 263. Quantification of chlorendic anhydride and impurities is provided, using HPLC-DAD. Chlorendic acid and chlorobenzene contents are determinated first. Then, from the measured concentrations of chlorendic acid and chlorobenzene, the concentration of chlorendic anhydride is calculated by subtraction. All analytical method are considered as acceptable and the information is considered as sufficient to allow the methods to be reproduced. However, evaluating MSCA disagrees with a calculated determination of chlorendic anhydride content because of the following arguments: impurity profile is not clearly specified and all impurities are not quantified, as a consequence chlorendic anhydride content may be overestimated.

7.4. Physico-chemical properties

Table 7

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	White crystalline powder
Vapour pressure	**Value used for CSA:** 2.68 mPa at 25°C (chlorendic anhydride) $1.875.10^{-8}$ Pa at 20°C and $3.685.10^{-8}$ Pa at 25 °C (chlorendic acid)
Water solubility	**Value used for CSA:** < 2.5 mg/L at 20 °C (chlorendic anhydride) 0.499 g/L at 20 °C (chlorendic acid)
Partition coefficient n-octanol/water (Log Kow)	**Value used for CSA:** - 1.59 at 25 °C (chlorendic acid) Not GLP study Chlorendic anhydride is slightly soluble in water, thus, in aqueous solution chlorendic anhydride is rapidly hydrolysed to chlorendic acid. Determination of partition coefficient on chlorendic anhydride is not relevant. According to OECD guideline 107 “measurements should be made on ionizable substances only in their non-ionized form (free acid or free base) produced by the use of an appropriate buffer with a pH of at least one unit below (free acid) or above (free base) the pK”. Two pKa values were observed for chlorendic acid and found to be 3.6 and 5.6 in a new study. Since the aqueous phase is buffered at a pH of 7, the substance is mainly in ionized form (log Pow calculated at pH > pKa). *Method description:* *EU method A.8* *OECD guideline 107 (shake flask method)* *The layers were separated using disposable pipettes into clean vials.* *Analytical method: HPLC (no validation data)* In conclusion, this study is considered as acceptable, partition coefficient was determined with appropriate conditions of pH. At pH 7, relevant pH regarding environmental conditions, Log Pow of chlorendic acid is found to be -1.59 meaning ionized acid form is mainly present in aqueous phase (hydrophilic).
Flammability	**Value used for CSA:** Not flammable
Explosive properties	**Value used for CSA:** Non explosive
Oxidising properties	**Value used for CSA:** Non oxidising
Granulometry	0.1% w/w of chlorendic anhydride was smaller than 10 µm. Chlorendic anhydride does not contain significant content of thoracic or respirable fractions but 31.2% represent an inhalable fraction.

Stability in organic solvents and identity of relevant degradation products	Solvent stability of chlorendic anhydride is not regarded as relevant and in accordance with column 1 of REACH Annex IX, the study was not undertaken as it is only required if stability of the substance is considered to be critical.
Dissociation constant	Chlorendic anhydride rapidly hydrolyses to chlorendic acid in contact with water. **Value used for CSA:** The pKa values for chlorendic acid are 3.6 and 5.6 at 22.8 °C
Melting / freezing point	**Value used for CSA:** 235-239 °C (at 1 atm) The test substance darkened in colour at approximately 237°C and was found to be light brown on cooling.
Boiling point	**Value used for CSA:** 266.5 – 322 °C The boiling point range is wide due to endotherms associated with a change in crystal structure. Although the substance darkened during melting, which may indicate decomposition, in the boiling point study using DSC, no decomposition was observed.
Density	**Value used for CSA:** 1.76 at 20 °C
Surface tension	**Value used for CSA:** 72.0 mN/m (not surface active) Test has been performed at 90% saturated aqueous solution of chlorendic anhydride.

7.5. Manufacture and uses

7.5.1. Quantities

Table 8

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☒ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 9

USES	
	Use(s)
Formulation	This substance is used in the following activities or processes at workplace: transfer of chemicals, closed batch processing in synthesis or formulation, transfer of substance into small containers, mixing in open batch processes, laboratory work and closed processes with no likelihood of exposure. Release to the environment of this substance can occur from industrial use: formulation of mixtures.
Uses at industrial sites	The substance is used in polymers and in the manufacture of plastic products.

	This substance is used in the following activities or processes at workplace: closed batch processing in synthesis or formulation, transfer of chemicals, laboratory work, closed processes with no likelihood of exposure, batch processing in synthesis or formulation with opportunity for exposure, mixing in open batch processes, industrial spraying, roller or brushing applications, treatment of articles by dipping and pouring, production of mixtures or articles by tabletting, compression, extrusion or pelleting and the low energy manipulation of substances bound in materials or articles. Release to the environment of this substance can occur from industrial use: in the production of articles and for thermoplastic manufacture.
Consumer Uses	No uses registered
Article service life	This substance is used in the following activities or processes at workplace: the low energy manipulation of substances bound in materials or articles. Release to the environment of this substance can occur from industrial use: formulation of mixtures. This substance is intended to be released from scented: eraser.

7.6. Classification and Labelling

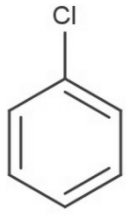
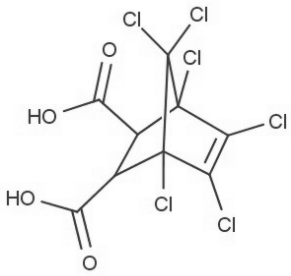
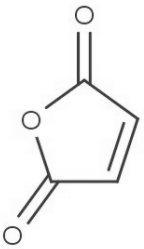
7.6.1. Harmonised Classification (Annex VI of CLP)

Chlorendic anhydride is listed by index number 607-101-00-4 in Annex VI of the Regulation (EC) No 1272/2008 (ATP inserted CLP00/-). The harmonized classification for health hazards of the substance is the following:

Table 10

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS RN**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
607-101-00-4	1,4,5,6,7,7-hexachlorobicyclo [2,2,1]hept-5-ene-2,3-dicarboxylic chlorendic anhydride			Eye Irrit. 2 STOT SE 3 Skin Irrit. 2	H319 H335 H315	C ≥ 1 % C ≥ 1 % C ≥ 1 %	

Table 11: Classification of known impurities in composition (CSR updated in 2020)

Known impurities	Structure	Harmonised classification (CMR and sensitising properties)	Self-classification (CMR and sensitising properties)
Chlorobenzene EC no.:203-628-5		No harmonised classification for CMR or sensitising properties Harmonised classification for environment: Aquatic chronic 2 H411	Self-classification for CMR or sensitising properties : Carc. 1A Muta 1B Resp. Sens. 1
1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic acid EC no.: 204-078-9		No harmonised classification for CMR or sensitising properties No harmonised classification for environment	Self-classification for CMR or sensitising properties : Carc. 1B/Carc 2
Maleic acid EC no.: 203-742-5		Skin Sens 1 - H317 Harmonised classification for environment: not classified	Self-classification for CMR or sensitising properties : Skin Sens 1

7.6.2. Self-classification

- In the registration dossier:
  - Carc Cat. 2 – H351
  - STOT RE 2 - H373: May cause damage to liver and heart by oral route/May cause damage to stomach by dermal route/May cause damage to lungs, stomach and liver by inhalation route.
  - Aquatic Chronic 3 - H412: Harmful to aquatic life with long lasting effects

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*
  - Skin Sens. 1 - H317: May cause an allergic skin reaction.
  - Carc. Cat. 1A - H350 : Causes cancer (no indication of exposure route on ECHA's website).
  - Carc. Cat. 2 - H351: Suspected of causing cancer (by oral route of exposure).
  - STOT RE 2 - H373: May cause damage to organs (lungs, stomach and liver).
    Affected organs: Liver and heart (route of exposure: oral).
    Affected organs: Stomach (Route of exposure: dermal).
    Affected organs: Lungs, stomach and liver (route of exposure: inhalation).
  - Aquatic Chronic 1 - H410: Very toxic to aquatic life with long lasting effects.
  - Aquatic Chronic 3 - H412: Harmful to aquatic life with long lasting effects.
  - Aquatic Chronic 4 - H413: May cause long lasting harmful effects to aquatic life.

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

This information was waived by the Registrant claiming that the test is not feasible.

Anhydrides are a class of compounds, formed by the removal of one molecule of water from two molecules of carboxylic acids. If the carboxylic acid groups are present in the same molecule, a cyclic anhydride, such as chlorendic anhydride is produced.

Anhydrides are well known to react with water (Hendrickson *et al.*, 1970) and revert to the dicarboxylic acid. This means that anhydrides do not usually exist in water but react with it and dissolve as the parent acid. Extensive examples of this behaviour are available in the chemical literature, two of which are given below:

- Phthalic anhydride - soluble in 162 parts water with conversion to phthalic acid (The Merck Index, 1989).
- Acetic - anhydride slowly soluble in water forming acetic acid (The Merck Index, 1989).

Work on the experimental determination of the water solubility of chlorendic anhydride has confirmed that it also dissolves only as the acid with no anhydride being found in aqueous solution (detection limit < 2.5 ppm) (Unpublished study report, 2002b).

In conclusion, on dissolving in water, chlorendic anhydride undergoes immediate hydrolysis to the corresponding di-acid. It is therefore not possible to determine a half-life time for chlorendic anhydride in water and the hydrolysis test described in OECD TG 111 cannot be performed. As chlorendic anhydride undergoes immediate hydrolysis to the corresponding di-acid, the PBT assessment is based only on data obtained for the chlorendic acid.

Moreover, it is stated that chlorendic acid is stable to hydrolysis.

Concerning the phototransformation, chlorendic acid was found to rapidly degrade in UV light, both in water (half-life 16 days) and on solid surfaces (half-life 5 days).

7.7.1.2. Biodegradation

7.7.1.2.1. Biodegradation in water

A ready biodegradation test has been performed according to a method similar to the OECD 301F and OECD 302C guidelines (unpublished study report; 2001a). The biodegradability of chlorendic anhydride (chlorendic acid in solution) is assessed by a manometric respirometry procedure at a concentration of 162.3 mg/L. The inoculum is an activated sludge from a sewage treatment works which treats predominantly domestic wastes. Deviations from the guidelines are reported by the Registrant but not clearly described in the registration dossier where only a temperature variation is stated to be higher (4.1°C) than recommended in the OCDE 301F guideline (maximum 1°C). These deviations are not considered to be significant nor to have significantly affected the integrity of the test.
The evaluating MSCA agrees with the Registrant that no degradation of the tested substance is observed after 31 days.
Therefore, the evaluating MSCA concludes that chlorendic anhydride (chlorendic acid in solution) is not considered to be ready and inherently biodegradable.

7.7.2. Environmental distribution

Considering that the substance is under anionic form at pH environmentally relevant, the substance is considered out of applicability domain of QSAR models of EPISUITE. As a consequence, *in silico* approach by using QSAR models of EPISUITE for estimate Koc is considered not relevant for the environmental risk assessment.

Chlorendic anhydride rapidly hydrolyses to chlorendic acid, therefore the adsorption/desorption values for the acid are used for the anhydride also. As chlorendic acid is an ionisable substance, the batch equilibrium method to calculate Kd was used. According to OECD Guideline 106 and EU Method C.18, five soils were tested and the average value used for the assessment. The log Kp soil was derived from this experimental value: 0.76 at 25°C. In order, to calculate the respective coefficient in the different compartment, a theoritical Koc of 121 has been defined based on the experimental data (mean of koc values of five soils tested).

7.7.3. Bioaccumulation

Under environmental pH conditions, the substance is essentially in the anionic form. According to van Beelen (2000) anions have more affinity for water than for octanol and the log Pow for an anionic form should be lower than the neutral form. Nevertheless, Beelen (2000) reported that for organic acids, the log Pow value of the neutral molecule is a better predictor of the log BCF than the log Pow value of the corresponding anion. A log Pow of -1.59 at 25°C has been determined according to the 107 OECD guideline. This value has been provided by the Registrant.

From HSDB database, BCF values of <2.1 and <0.22 measured in fish, indicate that the bioconcentration in aquatic organisms is low. Such data have not been reviewed.
The evaluating MSCA concludes that, based on screening criteria and the available data, no potential for bioaccumulation in aquatic organisms is expected for chlorendic acid and, hence, secondary poisoning is not considered relevant.

Moreover, the log Kow is <2 for the environmental pH (log D < -1 for the worst case environmental pH = 4), therefore no bioaccumulation in air breathing organisms is expected. Nevertheless, the substance is anionic and a binding to plasma protein cannot be completely excluded. However, the toxicokinetic study support a fast elimination in rats (see 7.9.1). Indeed a half-life of the radiocarbon was less than 2 days except in fat where it was reported to be of 22.5 days According to Goss et al (2018), an elimination half life in rat of 17 days would be sufficient to exclude bioaccumulation concern. It is assumed that this value correspond to the whole organism, higher threshold would be expected based on fat part. It should be reminded that at present, still discussions take place to validate a regulatory threshold value for the elimination in mammals. However, the elimination half-life of chlorendic acid appears low enough to exclude a concern for bioaccumulation in air breathing organism.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

7.8.1.1.1. Short-term toxicity to fish

The results are summarised below in Table 12.

Table 12: Short-term effects on fish

Method	Results	Remarks	Reference
Danio rerio Freshwater According to OECD Guideline 203	LC50 (24h): >100 mg/L test mat. (nominal) based on: mortality LC50 (48h): >100 mg/L test mat. (nominal) based on: mortality LC50 (72h): >100 mg/L test mat. (nominal) based on: mortality LC50 (96h): >100 mg/L test mat. (nominal) based on: mortality NOEC (96h): >=100 mg/L test mat. (nominal) based on: mortality	1 (reliable without restriction) key study experimental study **Test material** (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride	Unpublished report, 2016)

A limit test, with the substance including the relevant impurity as required in the first decision (2013) according to the OECD 203 has been provided by the Registrant and is considered as reliable. No effect on *Danio rerio* is shown in this test.
For fish, the lowest validated endpoint used for CSA is LC_{50}>100 mg/L.

7.8.1.1.2. Long-term toxicity to fish

No data available in the registration dossiers.

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Short-term toxicity to aquatic invertebrates

The results are summarised below in Table 13.

Table 13: Short-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater static according to OECD TG 202	EC50 (24h): >100 mg/L test mat. (nominal) based on: mobility EC50 (48h): >100 mg/L test mat. (nominal) based on: mobility NOEC (48h): >=100 mg/L test mat. (nominal) based on: mobility LOEC (48h): >100 mg/L test mat. (nominal) based on: mobility	1 (reliable without restriction) key study experimental study **Test material** (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride	Unpublished report (2016)

A limit test, with the substance including the relevant impurity as required in the first decision (2013), according to the OECD TG 202 has been provided by the Registrant and is considered as reliable. No effect on *Daphnia magna* is shown in this test.
For aquatic invertebrates, the lowest validated endpoint used for CSA is LC_{50}>100 mg/L.

7.8.1.2.2. Long-term toxicity to aquatic invertebrates

No data available in the Registrant dossier.

7.8.1.3. below in Table 12.Algae and aquatic plants

The results are summarised below in Table 14.

Table 14: Effects on algae and aquatic plants

Method	Results	Remarks	Reference
Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata) (algae) freshwater static EU Method C.3 (Algal Inhibition test) OECD TG 201 (Alga, Growth Inhibition Test)	EC50 (72 h): > 97.2 mg/L test mat. (nominal) based on: growth rate NOEC (72 h): 48.4 mg/L test mat. (nominal) based on: growth rate	1 (reliable without restriction) key study experimental result **Test material** (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride	Unpublished study report, (2002a)

A study on toxicity of chlorendic anhydride to the algae *Selenastrum capricornutum* is considered. The evaluating MSCA agrees with the Registrant conclusions.

7.8.1.4. Sediment organisms

According to the integrated testing strategy for sediment from the guidance R7-b, the evaluating MSCA agrees that no sediment testing is necessary according to a log Kow of -1.59, below the trigger value of 3. Moreover the exposure of sediment compartment is negligible.

7.8.2. Terrestrial compartment

No relevant information available.

7.8.3. Microbiological activity in sewage treatment systems (STP)

The results are summarised in the following table:

Table 15: Effects on micro-organisms

Method	Results	Remarks	Reference
Activated sludge of a predominantly domestic sewage freshwater static according to OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test [before 22 July 2010]	NOEC (3h): 97 mg/L test mat. (nominal) based on: inhibition of total respiration EC10 (3h): 130 mg/L test mat. (nominal) based on: inhibition of total respiration (95% Confidence Interval: 78 – 170 mg/L) EC50 (3h): 670 mg/L test mat. (nominal) based on: inhibition of total respiration (95% Confidence Interval: 490 – 1300 mg/L)	1 (reliable without restriction) key study experimental study **Test material** (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride	Unpublished study report (2016)

The evaluating MSCA agrees with the reliability index of this study with chlorendic anhydride including the relevant impurity as required in the first decision (2013).

7.8.4. PNEC derivation and other hazard conclusions

Table 16: Summary of the PNEC values for the relevant environmental form of the substance: chlorendic acid

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks / Justification**
Freshwater	Hazard assessment conclusion ($PNEC_{freshwater}$): 0.097 mg/L	Assessment factor: 1000 The LC50 from Acute toxicity to Algae, 97.2 mg/l, was used.
Intermittent releases to water	Hazard assessment conclusion ($PNEC_{freshwater\text{-}intermittent\ releases}$): 0.97 mg/L	Assessment factor: 100 The LC50 from Acute toxicity to Algae, 97.2 mg/l, was used.
Sediments (freshwater)	Not relevant	No sediment testing is necessary according to a log Kow of 1.59, below the trigger value of 3 and the exposure of sediment compartment is negligible.
Sewage treatment plant	Hazard assessment conclusion PNEC STP: 9.7 mg/L	Assessment factor: 10 Extrapolation method: assessment factor The NOEC for aquatic micro-organisms: 97 mg/L, was used.
Soil	Hazard assessment conclusion PNEC soil: PNEC = 0.506 mg/kg $_{wwt}$	EPM approach used as screening assessment.

7.8.5. Conclusions for classification and labelling

The substance has no harmonised classification under Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation) for environment.

Nevertheless and based on the data available in the registration dossier (an Algal Inhibition Test with a result of EC_{50} >97.2 mg/l), the **chlorendic anhydride must be classified as Aquatic Chronic 3, H412: harmful to aquatic life with long lasting effects to aquatic life**.

This proposal will be included in the revision of the actual harmonized classification that will be submitted.

7.9. Human Health hazard assessment

Review of all data on chlorendic anhydride and acid (physical-chemical properties, hazard and exposure data) demonstrates that the chlorendic acid and chlorendic anhydride are closely related compounds: chlorendic anhydride is rapidly hydrolysed to chlorendic acid with a half-life of approximately one hour. Conversely when chlorendic acid is heated in an open system, chlorendic anhydride can be formed by deshydratation (IPCS, 1996). The manufacture and the use of chlorendic anhydride lead to the formation of chlorendic acid thus human exposure to both chlorendic anhydride and acid is possible. Furthermore, despite the lack of data on the biotransformation of chlorendric anhydride, it is reasonable to consider that this substance may be transformed to chlorendic acid in mammals.

Based on the above mentioned reasoning, it is clear that the toxicity of chlorendic anhydride and chlorendic acid are closely related. Therefore, an assessment of both, chlorendic anhydride and acid is necessary. Further to the evaluating MSCA request, assessment of human health hazard assessment of chlorendic acid have been introduced

by the Registrant in the registration dossier. Information on both chlorendic anhydride and chlorendic acid, when available, are presented in this section and both are considered relevant for the hazards assessment of the parent compound, i.e. chlorendic anhydride.

7.9.1. Toxicokinetics

7.9.1.1 Non-human information

The results of studies on absorption, metabolism, distribution and elimination with chlorendic anhydride and its acid form are summarised in the following table:

Table 17: Studies on absorption, metabolism, distribution and elimination with chlorendic anhydride and chlorendic acid.

Method	Results	Remarks	Reference
Rat (Holzman's albino), male/female Oral: gavage Exposure regime: single dose Doses/conc.: 3.65 mg/kg (Group 1: 2 females) 4.00 mg/kg (Group 2: 4 females) 5.55 mg/kg (Group 3: 2 females) 3.62 mg/kg (Group 4: 4 males) No OCDE guideline followed Non GLP	One hour after dosing the blood concentration of chlorendic anhydride peaked and was significantly decreased by 96 h After 192 h, the maximum residues present in any tissue were less than 0.1 mg/kg, with the exception of the fat (0.121 mg/kg) and liver (0.296 mg/kg) Regardless of sex, the primary route of excretion was the faeces, 70% of the administered dose being eliminated within the first 72 h. Only 10% of the administered dose was eliminated in the urine. The half-life of the radiocarbon was less than 2 days except in fat (22.5 days) Details on metabolites: No data	4 (not assignable) Key study Secondary literature **Test material: (CAS name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	Unpublished report (1978) cited in IPCS (1996)
Rat (Fischer 344/DuCrj), male Doses/conc.: 3 mg/kg, 7.7 µmol/kg, 11 µCi/kg, 1 ml/kg. Same dose for intravenous and oral route of exposure Vehicle : mixture of emulflor (polyoxyethylated vegetable oil), ethanol and water Details on exposure [^{14}C]Chlorendic acid solution was injected IV into the tail vein of rats (3 mg/kg, 7.7 umol/kg, 11 µCi/kg, 1 ml/kg), which were held for 15 min to 7 d, after which they were	Main ADME results: - <u>absorption</u>: substance absorbed by gastrointestinal tract. - <u>distribution</u>: initially distributed to the blood, liver, muscle, skin, and kidney and did not accumulate in adipose tissue. Most of the dose was located in the liver. Following **intravenous injection**, more than 50% of the administered radioactivity was found in the liver within 15 min. The blood contained 20% of the administered radioactivity at 1 h, and this declined with a half-life of 0.84 h. Muscle contained 14% of the administered radioactivity at 15 min, and this level fell rapidly, with a half-life of 0.57 h. Smaller amounts were detected in other organs. The highest specific activity per gram of tissue (wet weight) was noted in the adrenal gland early after administration. Administration of the same solution of [^{14}C]chlorendic acid by **oral intubation** resulted in a somewhat higher liver concentration and a lower blood concentration at 24 h than those seen after the same time following intravenous administration.	2 (reliable with restrictions) Key study Secondary literature **Test material: (CAS name): 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid (chlorendic acid) EC 204-078-9 /CAS RN 115-28-6** No purity specified	Decad and Fields (1982)

Method	Results	Remarks	Reference
sacrificed by cervical dislocation. For absorption studies, rats received the same dose as in the IV study by oral intubation and were sacrificed by cervical dislocation after 1 d. No. of animals per sex per dose: 3 per time point No control animals No OCDE guideline followed Non GLP	TABLE 2. Distribution of Radioactivity 1 d after Administration of [^{14}C]Chlorendic Acid Percent of total dose ($n \geq 3$ animals) Tissue / Oral / Intravenous Blood 0.033 ± 0.014 0.524 ± 0.026 Liver 1.08 ± 0.035 0.524 ± 0.026 Kidney 0.018 ± 0.008 0.021 ± 0.016 Small intestine 0.188 ± 0.137 0.036 ± 0.062 Contents 0.460 ± 0.194 0.266 ± 0.157 Large intestine 1.16 ± 0.394 0.070 ± 0.035 Contents 12.7 ± 2.76 5.57 ± 2.93 Feces 73.00 ± 5.93 77.80 ± 13.10 Urine 2.98 ± 1.35 5.94 ± 2.14 - distribution: not influenced by exposure route - excretion: rapid excretion, mainly through feces. Excretion of [^{14}C]chlorendic acid-derived radioactivity was analysed by daily collection of urine and feces from individual animals held for 1 d or longer after treatment. The major route of excretion of chlorendic acid was the feces, and approximately 78% of the dose was excreted in the first 24 h. Most of the urinary excretion also occurred within the first 24 h, and less than 0.1% of the dose appeared in the urine on subsequent days. Thus, by the first day, more than 73% of the total dose was recovered in the excreta. Since the feces were the major route of elimination, excretion of radioactivity through the bile was studied. 65% of an IV dose of [^{14}C]chlorendic acid derived radioactivity was excreted in the bile within 5 h. This is in close agreement with the fecal excretion data, suggesting that most of the [^{14}C]chlorendic acid-derived radioactivity in bile was excreted in the feces. Biliary excretion was the primary route of removal of radioactivity from the liver, which occurred with a half-life of 1.19 h. The [^{14}C]chlorendic acid-derived radioactivity in the bile, urine and faeces was attached mainly to parent compound or conjugates resistant to ß-glucuronidase and arylsulfatase Details on metabolites: in the urine, 72% of the radioactivity appeared to represent parent compound. Results suggest that most of the radioactivity excreted in bile, and subsequently in feces, represented metabolites of chlorendic acid. Evaluation of results: no bioaccumulation potential based on study results.		

7.9.1.2. Human information

No data.

7.9.1.3. Summary and discussion of toxicokinetics

Chlorendic anhydride

The pharmacokinetics of chlorendic anhydride was investigated in 4 male and 8 female Holzman's albino rats *via* oral gavage in a single dose of 3.65 mg/kg (Group 1: 2 females), 4.00 mg/kg (Group 2: 4 females), 5.55 mg/kg (Group 3: 2 females) or 3.62 mg/kg (Group

4: 4 males) (Diaz and Atallah, 1978). Blood was drawn from females treated with 4 mg/kg at 1, 4, 8, 17, 24, 48, 72 and 96 h after dosing. Urine and faeces were collected daily in the Group 1, 3 and 4. Animals were sacrificed at respectively 17, 96 and 192 h after treatment and selected tissues were excised.

Regardless of sex, the primary route of excretion was the faeces, 70% of the administered dose being eliminated within the first 72 h. Only 10% of the administered dose was eliminated in the urine. The blood concentration peaked one hour after dosing and was significantly decreased by 96 h.

After 192 h, the maximum residues in any tissue were less than 0.1 mg/kg, with the exception of the fat (0.121 mg/kg) and liver (0.296 mg/kg).

The absorption of chlorendic anhydride followed the two-compartment open model. The first compartment was suggested to consist of the blood and selected tissues which equilibrated rapidly, while the second compartment consisted of the fat which was slow in equilibrating and considered a deep reservoir. The half-life of the radiocarbon was less than 2 days except in fat where it was reported to be of 22.5 days.

Based on the available data, the bioaccumulation potential is low.

No metabolism pathway is reported.

Chlorendic acid

The absorption, distribution, and excretion of chlorendic acid, was studied in the male Fischer 344 rat (Decad and Fields, 1982). [^{14}C]chlorendic acid was absorbed after an oral dose of 7.7 µmol/kg bw. The distribution in various tissues was similar whether the treatment was by oral or intravenous route. The major site of [^{14}C]chlorendic acid deposition was the liver, with smaller amounts found in the blood, muscle, skin, intestines and kidneys.

Chlorendic acid-derived radioactivity was excreted primarily through the bile and into the feces. The urine contained less than 6% of the total dose. Within one day, more than 75% of the total dose was excreted in the feces, primarily as conjugates. Radioactivity in the liver was also primarily metabolites of chlorendic acid. Thus, chlorendic acid was absorbed, metabolized, and excreted primarily in the feces as conjugates.

7.9.2. Acute toxicity and Corrosion / Irritation

7.9.2.1. Acute toxicity

7.9.2.1.1. Non-human information

7.9.2.1.1.1. Acute toxicity: oral

The results of studies after oral administration are summarised below in Table 18.

Table 18: Studies on acute toxicity of chlorendic anhydride after oral administration.

Method	Results	Remarks	Reference
Rat (Charles River CD), male/female Oral: gavage Vehicle : corn oil Doses: 807.1, 1 281, 2 034, 3 229 and 5 126 mg/kg in the diet 5 animals per sex per dose Control animals : not equivalent or similar to OECD TG 420 (Acute Oral Toxicity - Fixed Dose Procedure)	LD50: 2 562 mg/kg bw (male) LD50: 2 130 mg/kg bw (female) LD50: 2 336 mg/kg bw (male/female) **Clinical signs**: At all dose rates, diarrhoea was evident. At 2034 mg/kg and above, hypoactivity,	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-**	Unpublished report (1978a)

Method	Results	Remarks	Reference
Study undertaken and reported scientifically but not GLP-compliant.	ataxia and alopecia were noted. **Body weight (bw):** All animals which survived the dosing increased their bw.	**ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** Form : powder No purity specified	
Mouse (CD-1), male/female Oral: gavage Vehicle : corn oil Doses : 1 000, 1 585, 2 512, 3 980, 6 308 and 10 000 mg/kg in the diet 5 animals per sex per dose No control animals No guideline OCDE Non GLP	LD50: 4 169 mg/kg bw (male) LD50: 2 884 mg/kg bw (female) LD50: 3 349 mg/kg bw (male/female) All surviving mice gained weight.	2 (reliable with restrictions) Supporting study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** Form: powder No purity specified	Unpublished report (1978b)

7.9.2.1.1.2. Acute toxicity: inhalation

The results of the study after inhalation exposure are summarised below in Table19.

Table 19: Study on acute toxicity of chlorendic anhydride after inhalation exposure.

Method	Results	Remarks	Reference
Rat (CD), male/female Inhalation: dust (whole body) **Duration of exposure: 1 h** Concentrations: the chamber dust concentration was calculated to be 203.0 mg/L. The chamber atmosphere was extremely dusty. 5 animals per sex per dose No control animals equivalent or similar to OECD TG 433 (Acute Inhalation Toxicity: Fixed Concentration Procedure) Study undertaken and reported scientifically but not to GLP.	LC50 (1 h): > 203 mg/L air (male/female) **Mortality**: None **Clinical signs**: The immediate response of the rats to the experimental atmosphere was an increase of activity in preening. After several minutes of exposure this activity decreased. After 30 minutes of exposure six rats exhibited salivation. By the end of the exposure all the rats exhibited salivation and one rat exhibited nasal discharge. After the exposure all the rats appeared normal. Body weight: A slight bw loss was observed in 6 rats for 1 day post-exposure. Gross pathology : None	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	Unpublished report (1978c)

7.9.2.1.1.3. Acute toxicity: dermal

The results of the study after dermal administration are summarised below in Table 20.

Table 20: Study on acute toxicity of chlorendic anhydride after dermal administration.

Method	Results	Remarks	Reference
Rabbit (New Zealand White), male/female Coverage: occlusive **Duration of exposure: 24 hours** Doses: 10 000 and 20 000 mg/kg. 2 animals per sex per dose Control animals : yes (vehicle) Equivalent or similar to OECD TG 402 (Acute Dermal Toxicity) Non GLP	LD50: > 10 000 - < 20 000 mg/kg bw (male/female) Mortality: Both male rabbits at the 20 000 mg/kg dosage level died during the 14 day observation period. None of the other rabbits died. Body weight: Apart from the 2 male rabbits at 20 000 mg/kg which died, all other rabbits increased in weight during the study.	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	Unpublished report (1978a)

7.9.2.1.2. Human information

No data.

7.9.2.1.3. Summary and discussion of acute toxicity

Value used for CSA:

Acute oral toxicity: LD50: 2 130 mg/kg bw

Acute dermal toxicity: LD50: 10 000 mg/kg bw

Acute inhalation toxicity: LC50: 203 000 mg/m³

The evaluating MSCA concludes that it is not necessary to classify chlorendic anhydride for acute toxicity as in all studies the values were greater than the classification limit.

7.9.2.2. Irritation

7.9.2.2.1. Skin

7.9.2.2.1.1. Non-human information

The results of the study on skin irritation are summarised below in Table 21.

Table 21: Study on skin irritation

Method	Results	Remarks	Reference
Rabbit (New Zealand White) Coverage: occlusive (abraded) Controls : yes **Duration of treatment / exposure: 4 hours** Observation period	Slightly irritating Overall irritation score: 0.8 (mean) (Time point: 72 h) Erythema score: 0.6 of max. 0.5 (mean) (Time point: 72 h) Edema score: 0.19 of max. 1 (mean) (Time point: 72 h)	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-**	Unpublished report (1978a)

Method	Results	Remarks	Reference
Immediately after bandages were removed and at 24, 48 and 72 hours. 6 animals Equivalent or similar to OECD TG 404 (Acute Toxicity: Dermal Irritation / Corrosion) Non GLP		**trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	

7.9.2.2.1.2. Human information

No data.

7.9.2.2.2. Eye

7.9.2.2.2.1. Non-human information

The results of studies on eye irritation are summarised in the following table:

Table 22: Studies on eye irritation

Method	Results	Remarks	Reference
Rabbit (New Zealand White) Controls: The untreated eye served as a control for each rabbit. **Duration of treatment / exposure: 14 days following instillation** Observation period (*in vivo*) : 14 days following instillation 6 animals equivalent or similar to OECD TG 405 (Acute Toxicity: Eye Irritation / Corrosion) Non GLP	Highly irritating Overall irritation score: 16.4 of max. 17.3 (mean) (Time point: 14 days)	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	Unpublished report (1978d)

7.9.2.2.2.2. Human information

No data.

7.9.2.2.3. Respiratory tract

No data.

7.9.2.2.4. Summary and discussion of irritation

Skin irritation

Chlorendic anhydride has a harmonized classification as Skin Irritant category 2 with the hazard statement H315.

In a skin irritation study on rabbit (IRDC, 1978d); 3 male and 3 female New Zealand White rabbits were treated with 500 mg of the test material as supplied. The test substance was applied dry and not moistened prior to administration to the back of each rabbit. The skin of 3 of the rabbits was abraded with a scalpel blade. The abrasions penetrated the *stratum corneum* but were not deep enough to cause bleeding. The overall irritation score at time point 72 h was 0.8 (mean), the erythema score at time point 72 h was 0.6 (mean) and edema score at time point 72 h was 0.19 (mean).

In the endpoint study record, the irritation/corrosion response scores for each animal at time points 24, 48 and 72 hours were not reported and there was no indication on the reversibility of the skin effects.
Based on the scoring system used in the study, chlorendic anhydride was found to be slightly irritating.

Furthermore, chlorendic anhydride belongs to the category of cyclic anhydrides, which are known to be irritant to the skin. This is due to rapid reactions with water, which form the corresponding acids responsible for the irritation (WHO, 2009).
Therefore, based on all available data, the evaluating MSCA considers the current harmonized classification Skin Irrit. 2 for chlorendic anhydride as justified.

Eye irritation

Chlorendic anhydride has a harmonized classification as Eye Irritant category 2 with the hazard statement H319.

In the dossier under evaluation, the Registrant submitted an eye irritation study on rabbit (IRDC, 1978d). Three male and 3 female New Zealand White rabbits were used for this study. Prior to test material administration the eyes of each rabbit were examined with ultraviolet light following instillation of one drop of a 2.0 percent sodium fluorescein solution. Rabbits exhibiting corneal lesions were discarded. The rabbits received 100 milligrams of the test material. The test material was placed into the cupped conjunctival sac of the right eye of each rabbit following which the eyelids were gently held together for one second. The left eye served as the untreated control for each rabbit. None of the rabbits received a washout. The overall irritation score at time point 14 days was 16.4.

In the endpoint study record, chlorendic anhydride was considered as highly irritating. However the criteria used for interpretation of the results were not specified, the grades of ocular reaction (conjunctivae, cornea and iris) at time point 24, 48 and 72 hours were not reported. No data regarding the reversibility of the effects was given.
In a second eye irritation study (summarised in IPCS, 1996) chlorendic anhydride was found to be severely irritating to the eyes of albino rabbits. The evaluating MSCA notes that only results were reported in the secondary literature.

Furthermore, the chlorendic anhydride belongs to the category of cyclic acid anhydrides which are considered as irritant for mucous membranes of the eyes. This is due to rapid reactions with water, which form the corresponding acids responsible for the irritation. In humans, cyclic acid anhydrides can cause irritation after direct contact with the mucous membranes or after exposure by inhalation (WHO, 2009).
The available data do not allow for a thorough assessment of the potential of chlorendic anhydride to cause eye damage. However, in the absence of newer data that could substantiate a revision of the classification, the evaluating MSCA considers the current harmonized classification Eye Irrit. 2 for chlorendic anhydride as justified.

Respiratory irritation

No human data are available.
Chlorendic anhydride has a harmonized classification as STOT SE 3 with the hazard statement H335.

In an acute inhalation toxicity study (IPCS, 1978c), 5 male and 5 female Charles River CD rats were exposed to a single concentration of 203 mg/L of chlorendic anhydride.
All animals survived to the end of the study. The immediate response of the rats to the experimental atmosphere was an increase of activity in preening. After several minutes of exposure this activity decreased. After 30 minutes of exposure, six rats exhibited salivation. By the end of the exposure all the rats exhibited salivation and one rat exhibited nasal discharge. After the exposure all the rats appeared normal. As all animal survived, no necropsy was performed.
In a sub-acute inhalation toxicity study in rats (IPCS, 1979a), male and female rats were exposed to the dusts of chlorendic anhydride for 6 hours per day, 5 days per week for 20 exposures during a 28 day experimental period. The average nominal exposure concentrations were 0, 0.11, 0.99 and 9.97 mg/L. Immediately after the 6 hour exposure period, the rats exhibited varying degrees of ocular and nasal irritation and salivation in relation to the chlorendic anhydride concentrations. In this study, an increased incidence of dark red foci and dark red area/discolouration was seen at necropsy in the lungs in the treated groups, this was probably compound-related. Furthermore, a compound-related microscopic changes of haemorrhagic inflammatory nature in the lungs and of inflammatory nature in the trachea, nasal turbinates and stomach mucosa occurred in rats from all treated groups.

Furthermore, the chlorendic anhydride belongs to the category of cyclic acid anhydrides which are known to be irritants of mucous membranes of respiratory organs. In humans, cyclic acid anhydrides can cause irritation after direct contact with the mucous membranes or after exposure by inhalation. The irritative symptoms (itching, lacrimation, sneezing, rhinorrhoea, cough and dyspnoea) begin immediately following exposure to high concentrations of dusts or vapours. Irritation is caused by the corresponding dicarboxylic acid that is formed when cyclic acid anhydrides interact with water (WHO, 2009).

Therefore, based on all available data, the evaluating MSCA considers chlorendic anhydride as a respiratory irritant and the current harmonized classification as STOT SE 3 H335 as justified.

7.9.2.2.5. Corrosivity

No data.

7.9.3. Sensitisation

7.9.3.1. Skin

7.9.3.1.1. Non-human information

The results of the study on skin sensitisation is summarised in the following table:

Table 23: Study on skin sensitisation

Method	Results	Remarks	Reference
Guinea pig maximisation test Guinea pig (Albino), male Induction: intradermal Vehicle : Sodium chloride Concentration / amount : 0.9% Challenge: intradermal	**Sensitising** No. with positive reactions: Positive controls (dose: 0.1 ml): 1st reading 24 h after chall.: 4 out of 4 2nd reading 48 h after chall.: 4 out of 4	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-**	Unpublished report (1978e)

Method	Results	Remarks	Reference
Vehicle : Sodium chloride Concentration / amount : 0.9% 8 treated animals Positive control substance: 2,4-Dinitro-1-Chlorobenzene Equivalent or similar to OECD TG 406 (Skin Sensitisation)	Test group (dose: 0.1 ml): 1st reading 24 h after chall.: 5 out of 8 2nd reading 48 h after chall.: 2 out of 8	**hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	

7.9.3.1.2. Human information

A case of contact urticaria due to chlorendic anhydride was described by Keskinen *et al.* (2000). Chlorendic anhydride-human serum albumin (HAS)-conjugate (1%) induced a positive reaction in the skin prick test and also in the open test. Specific IgE antibodies against chlorendic anhydride-HAS-conjugate were found.

7.9.3.2. Respiratory system

7.9.3.2.1. Non-human information

No data.

7.9.3.2.2. Human information

A case of occupational asthma due to the welding fumes of a polyester paint containing phthalic anhydride and chlorendic anhydride was described by Keskinen *et al.* (2000). As previously mentioned, chlorendic anhydride belongs to the category of cyclic acid anhydrides that can induce irritation and sensitization after direct contact with the skin and the mucous membranes or after exposure by inhalation. Cyclic acid anhydrides were widely evaluated and several reported human cases strengthen the evidence on the respiratory sensitization potential.

The evaluating MSCA considers that there is sufficient evidence to consider chlorendic anhydride as a respiratory sensitizer, as stated in the report by WHO on cyclic acid anhydrides (WHO, 2009).

7.9.3.3. Summary and discussion of sensitisation

Skin sensitisation

The dermal sensitisation potential of chlorendic anhydride was evaluated in a guinea-pigs maximisation test (IRDC, 1978e). Twelve male albino guinea pigs were used for this study. The animals were divided into 2 groups consisting of a positive control group of 4 guinea pigs (positive control substance: 2,4- Dinitro-1-Chlorobenzene) and one treated group of 8 guinea pigs. Chlorendic anhydride produced a positive response and should be considered as a possible dermal sensitizing agent in humans. Five out of the 8 tested animals exhibited a positive response at 24 h reading and 2 out of the 8 tested animals exhibited a positive response at 48 h reading. Thus, more than 15% of the treated animals exhibited a positive response at reading time 24 h and 48 h.

Furthermore, the chlorendic anhydride belongs to the category of cyclic acid anhydrides which are considered as irritant and potent sensitizing agents. Indeed, in humans, cyclic acid anhydrides can cause irritation and sensitization after direct contact with the skin and the mucous membranes (WHO, 2009).

Based on the available information, the evaluating MSCA considers that the registered substance meets the criteria for classification as a skin sensitiser but considers that the available data does not allow a decision on sub-categorisation into category 1A or 1B. The available guinea pig maximisation test does not allow a definitive conclusion that category 1A would not be appropriate. Deviations from the current test guideline (OECD TG 406) are noted, such as the number of test animals used (4 animals in the positive control group and 8 treated animals instead of the current requirement of 5 animals in the positive control group and 10 animals in the treated group). No further data was identified which could assist in the sub-categorisation. Therefore the evaluating MSCA concludes that classification of the substance **as skin sensitisation category 1 (skin sens. 1) H317** is appropriate as proposed in the registration dossier.

Respiratory sensitisation

No animal data on respiratory sensitization of chlorendic anhydride are reported in the current dossier since no test guideline exists to investigate this endpoint.

In a sub-acute inhalation toxicity study in rats (IRDC (1979a), described in IPCS (1996)), animals (10 of each sex per group) were exposed for experimental period to chlorendic anhydride (6 h/day, 5 days/week during a 28-day) at a concentrations of 0, 0.11, 0.99 or 9.97 mg/L. An increased incidence of dark red foci and dark red area/discolouration was seen at necropsy in the lungs in the treated groups, which was probably compound-related. All the exposed animals from all treated groups exhibited a compound related microscopic changes of a haemorrhagic inflammatory nature in the lungs and of an inflammatory nature in the trachea.

Furthermore, chlorendic anhydride has a structural alert for respiratory sensitization “cyclic anhydride”. Indeed, chlorendic anhydride belongs to the category of cyclic acid anhydrides, which are considered as potent sensitizing agents. The cyclic acid anhydrides were widely evaluated and several reported human cases strengthen the evidence on the respiratory sensitization potential. In humans, cyclic acid anhydrides can cause irritation and sensitization after direct contact with the skin and the mucous membranes or after exposure by inhalation. Respiratory diseases include occupational allergic rhinoconjunctivitis and occupational asthma, both immediate-type IgE-mediated allergies. Cases of haemorrhagic alveolitis, haemorrhagic anaemia, allergic alveolitis, and allergic laryngitis have also been reported in association with exposure to anhydride (WHO, 2009). The structural similarity within the cyclic acid anhydrides group and the fact that these substances are skin sensitizers support that these substances, including chlorendic anhydride, are also respiratory sensitizers.

Therefore, the evaluating MSCA considers that there is sufficient evidence to consider **chlorendic anhydride as a respiratory sensitiser**.

7.9.4. Repeated dose toxicity

7.9.4.1. Non-human information

7.9.4.1.1. Repeated dose toxicity: oral

The results of studies on repeated dose toxicity after oral administration with chlorendic anhydride are summarised below in Table 24.

Table 24: Studies on repeated dose toxicity after oral administration with chlorendic anhydride

Method	Results	Remarks	Reference
Rat (CD), male/female Oral: feed **Exposure: 28 days (daily)**	No changes in general behaviour and appearance were considered to be related to compound.	2 (reliable with restrictions) Supporting study	Unpublished report (1978f)

Method	Results	Remarks	Reference
Treatment level at : - 500 ppm (compound consumption of 53 mg/kg bw/day for males and 59 mg/kg bw/day for females (actual ingested)) - 1 000 (compound consumption of 108 mg/kg bw/day for males and 115 mg/kg bw/day for females (actual ingested)) - 2 500 ppm (compound consumption of 282 mg/kg bw/day for males and 287 mg/kg bw/day for females (actual ingested)) - 5 000 ppm (compound consumption of 529 mg/kg bw/day for males and 606 mg/kg bw/day for females (actual ingested)) - 10 000 ppm (compound consumption of 1 113 mg/kg bw/day for males and 1 242 mg/kg bw/day for females (actual ingested)). 5 animals per sex per dose Control animals : yes, concurrent no treatment The rats were observed daily for overt signs of toxicity and for mortality. Detailed observations were recorded weekly. Individual bw and food consumption were recorded weekly. Plasma and red blood cell cholinesterase activities were determined at the termination of the study for all rats in the control group and the 2 500, 5 000, and 10 000 ppm dosage levels. Equivalent or similar to OECD TG 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) Non GLP	**Body weight**: Group mean bw slightly to markedly lower for rats at the 500, 2 500, 5 000 and 10 000 ppm dosage levels as compared to control rats. **Food consumption**: The average food consumption during the study was slightly lower for male rats at the 5 000 and 10 000 ppm dosage level as compared to male control rats but was similar for control and treated female rats. **Mortality** : No rats died during the 4 weeks of compound administration, however 1, 2 and 2 rats at the 2 500, 5 000 and 10 000 ppm dosage levels respectively died following the collection of blood for cholinesterase determinations. Plasma and red blood cell cholinesterase levels were similar for control and treated rats. No gross lesions considered to be compound related were seen in rats at necropsy. **NOAEL: > 1 113 mg/kg bw/day (10 000 ppm) (male)** **NOAEL: > 1 242 mg/kg bw/day (10 000 ppm) (female)**	Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	
Rat (Crj:CD) (SD), male/female Oral: feed **Exposure: 90 days (daily)** Treatment level at: - 100 ppm (compound consumption of 8 mg/kg bw/day for males and females (nominal in diet)) - 500 ppm (compound consumption of 39 mg/kg bw/day for males and 45 mg/kg	**Mortality**: three 2 500 ppm-treated females died between the 5th and 13th week of study; no other rats died during the study. **Body weight**: significant decreased group mean bw for the 500 ppm and 2 500 ppm treated males and all three groups of treated females compared with controls at week 13.	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-**	Unpublished report (1980)

Method	Results	Remarks	Reference
bw/day for females (nominal in diet)) - 2 500 ppm (compound consumption of 202 mg/kg bw/day for males and 226 mg/kg bw/day for females (nominal in diet)) Statistics analyses: All statistical analyses compared the treatment groups with the control group by sex Body weights (week 13), food consumption (week 13), haematological biochemical and urinalysis parameters (1, 2 and 3 months) and absolute and relative organ weights (terminal sacrifice) were compared by analysis of variance (one way classification) Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences. 15 animals per sex per dose Control animals : yes, concurrent no treatment The rats were observed twice daily for signs of overt toxicity and mortality. Detailed observations, individual bw and individual food consumption were recorded weekly. Ophthalmic examinations were conducted during the pretest period and at 3 months of study. Hematologic and biochemical tests and urinalyses were performed at 1, 2 and 3 months of study for five rats/sex/ groups. Equivalent or similar to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) Non GLP	**Food consumption**: 500 ppm and 2 500 ppm (males) and 2500 ppm (females): decreased food consumption over the 90-day study when compared with controls (significant only for the 2 500 ppm-treated females). Elevated SAP activities at 1, 2 and 3 months of study (males and females), with statistical significance for the 2 500 ppm-treated females at 2 and 3 months and the 500 ppm-treated females at 2 months. At all doses: statistically significant decreases in the mean absolute weights of hearts of male rats and in the mean absolute and relative weights of livers of male and female rats at all dosage levels, appeared to be treatment related. In the absence of any significant histomorphologic changes in these organs in the test groups, these decreases might be due to the overall reduction in the bw resulting from a reduction of body fat and or extracellular body fluid. No compound-related gross lesions in any of the rats. No compound-related microscopic lesions in any of the tissues from rats from the 2 500 ppm group. **LOAEL:** 2 500 ppm (202 mg/kg bw/day in males and 226 mg /kg bw/day in female)**,** based on: mortality in 3/15 females of the high dose group and decreased group mean bw > 10% in males and females of the high dose) **NOAEL:** 500 ppm (39 mg/kg bw/day in males and 45 mg/kg bw/day in females)	**dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	

The results of studies on repeated dose toxicity after oral administration with chlorendic acid are summarised below in Table 25.

Table 25: Studies on repeated dose toxicity after oral administration with chlorendic acid

Method	Results	Remarks	Reference
Rat (Fischer 344) male/female Oral: feed **Exposure: 14 days** Doses: 3 100 ppm (135 mg/kg bw/d (male); 195 mg/kg bw/d (female)) 6 200 ppm 12 500 ppm 25 000 ppm 50 000 ppm (2177 mg/kg bw/d (male) ; 3 145 mg/kg bw/d (female)) Groups of 5 animals of each sex/dose Non GLP	**Mortality**: 3 male and 2 female rats that received the 50 000 ppm diet died on day 15. **Body weight**: rats of each sex that received 12 500, 25 000, or 50 000 ppm and females that received 6 200 ppm lost weight during the studies. Males that received 6 200 ppm gained no weight. Females that received 3 100 gained notably less weight than the controls. **Gross lesion**: no treatment-related gross lesion observed at necropsy. Histologic examinations not performed	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid EC 204-078-9 /CAS RN 115-28-6 (HET Acid)** Batch: 6287 Purity : approximately 99%	US NTP (1987)
Mice (B6C3F1), male/female Oral: feed **Exposure: 14 days** Doses: 3 100 ppm 6 200 ppm 12 500 ppm 25 000 ppm 50 000 ppm Groups of 4 or 5 animals of each sex/dose Non GLP	**Mortality** : 4 male mice that received the 50 000 ppm diet died on day 7 **Body weight**: male and female mice that received 50 000 ppm lost weight ; mice that received 6 200 ppm or more gained less weight than the controls **Gross lesion**: no treatment-related gross lesion observed at necropsy Histologic examinations not performed	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid EC 204-078-9 /CAS RN 115-28-6 (HET Acid)** Batch: 6287 Purity : approximately 99%	US NTP (1987)
Rat (Fischer 344), male/female Oral: feed **Exposure: 13 weeks** (formulated diets available *ad libitum*) Doses: 0 ppm 620 ppm (eq. 27 mg/kg bw (male) and 39 mg/kg bw (female)) 1 250 ppm (eq. 56 mg/kg bw (male) and 66 mg/kg bw (female)) 2 500 ppm (eq. 112 mg/kg bw (male) and 132 mg/kg bw (female)) 5 000 ppm (eq. 224 mg/kg bw (male) and	All rats survived to the end of the studies, but reduced weight gain was noted at the higher dose. Hepatocytomegaly, mitotic alteration of the liver, and bile duct hyperplasia were observed at increased incidences in rats receiving 5 000 ppm or more of the substance. Severity of bile duct hyperplasia was greater in female rats than in male rats. The liver was the only affected organ identified. The occurrence of liver lesions in rats (centrilobular cytomegaly, mitotic alterations, and bile duct hyperplasia) were dose-related. No clinical signs were observed in the treated animals apart from a thinner appearance in the animals receiving 10 000 ppm of the test substance. A significant reduction of the bw was	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid EC 204-078-9 /CAS RN 115-28-6 (HET Acid)** Purity : approximately 99%	US NTP (1987)

Method	Results	Remarks	Reference
264 mg/kg bw (female)) 10 000 ppm (eq. 448 mg/kg bw (male) and 528 mg/kg bw (female)) Vehicle: unchanged (no vehicle) Statistical analyses: applied for survival analysis (Cox test), calculation of incidence, analysis of tumour incidence and historical control data. Equivalent or similar to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) Non GLP	recorded in female rats receiving 1 250 ppm or more of the test substance and in male rats receiving 2 500 ppm or more of the test substance. **NOAEL: 1 250 ppm (56 mg/kg bw) (male)** based on bw and weight gain **NOAEL: 620 ppm (39 mg/kg bw/d) (female)** based on bw and weight gain		

Method	Results	Remarks	Reference
Mouse (B6C3F1), male/female Oral: feed **Exposure: 13 weeks (formulated diets** available *ad libitum*) Doses: 1 250 ppm (eq. 185 mg/kg bw (male) and 207 mg/kg bw (female)) 2 500 ppm (eq. 370 mg/kg bw (male) and 414 mg/kg bw (female)) 5 000 ppm (eq. 740 mg/kg bw (male) and 828 mg/kg bw (female)) 10 000 ppm (eq. 1480 mg/kg bw (male) and 1656 mg/kg bw (female)) 20 000 ppm (eq. 2960 mg/kg bw (male) and 3312 mg/kg bw (female)) Vehicle: unchanged (no vehicle) Statistical analyses: applied for survival analysis (Cox test), calculation of incidence, analysis of tumour incidence and historical Equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) Non GLP	At the end of the study, surviving animals were subjected to gross necropsy and histopathology. No clinical signs were observed in the treated animals. A reduction of the bw above 7% when compared to the control was observed in all the groups of treated animals. All the mice survived to the end of the studies. Effects were observed in the liver of male and female mice including centrilobular cytomegaly, mitotic alteration and coagulative necrosis with the most significance in animals treated at 10 000 ppm or more. The liver was the only affected organ identified in the two highest concentrations in animals receiving 10 000 ppm or more of the test substance. **LOAEL: 1250 ppm (56/66 mg/kg bw) (male/female) based on bw and weight gain**	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid EC 204-078-9 /CAS RN 115-28-6 (HET Acid)** Purity : approximately 99%	US NTP (1987)

7.9.4.1.2. Repeated dose toxicity: inhalation

The results of the study on repeated dose toxicity after inhalation exposure to chlorendic anhydride are summarised in the following table:

Table 26: Study on repeated dose toxicity after inhalation exposure to chlorendic anhydride

Method	Results	Remarks	Reference
Rat (CD-1), male/female Subacute (inhalation: dust) (whole body) **Exposure: 6 h/day, 5 days/week for 20 exposures**	Dose-related macroscopic and microscopic changes in lungs, trachea and nasal mucosa; changes in haematological	2 (reliable with restrictions) Key study Experimental result	Unpublished report (1979a)

Method	Results	Remarks	Reference
during a 28 day period (daily during weekdays) Doses: 0, 0.11 mg/L, 0.99 mg/L, 9.97 mg/L (nominal conc.) Vehicle: unchanged (no vehicle) 10 animals per sex per dose Control animals: yes, concurrent no treatment Dose selection rationale: not specified Equivalent or similar to OECD TG 412 (Repeated Dose Inhalation Toxicity: 28/14-Day) Non GLP	parameters in both sexes at all dose levels **LOAEC : ca 110 mg/m³ air (male/female)**	**Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3**	

The results of studies on repeated dose toxicity after inhalation exposure to chlorendic acid are summarised below in Table 27.

Table 27: Studies on repeated dose toxicity after inhalation exposure to chlorendic acid

Method	Results	Remarks	Reference
Albino rat, male/female Short-term repeated dose toxicity: inhalation **Exposure: 6 hours per day, 5 days per week for 4 weeks (daily)** Inhalation: dust Doses: 0, 0.134 and 0.273 mg/L air (analytical) Vehicle: clean air 5 animals per sex per dose Control animals : yes, concurrent no treatment Details on study design Statistics not specified Equivalent or similar to OECD TG 412 (Subacute Inhalation Toxicity: 28-Day Study) Non GLP Deviations compared to the OECD Guideline: Age of the rats not recorded. Humidity of the housing conditions not recorded. Only 2 concentrations of test substance used. Chamber concentration only measured once per testing period. Particle size counted only once with one device. Body weights recorded weekly throughout the study. Food consumption and water consumption not measured. Not all clinical or gross pathology assessments carried out. Air flow	No animal died during the treatment due to the test substance. Hepatocytomegaly of centrilobular hepatocytes was observed in male and female rats treated at 0.1234 and 0.273 mg/L (air). Most significant effect was an increase of the weight of the liver and the brain for animals of both sex treated at 0.273 mg/L (air). **NOAEL: 0.134 mg/L (or 134 mg/m³) air (analytical) (male/female)** based on organ weights and organ/bw ratios	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid (HET acid) EC 204-078-9 /CAS RN 115-28-6** Purity not specified	Unpublished report (1983)

rate recorded once during each test period. Parameters analysed / observed: bw, haematological data, clinical chemistry data, urinalysis, pathology and histopathological data were collected.			

7.9.4.1.3. Repeated dose toxicity: dermal

The results of studies on repeated dose toxicity after dermal administration of chlorendic anhydride are summarised below in Table 28.

Table 28: Studies on repeated dose toxicity after dermal administration of chlorendic anhydride

Method	Results	Remarks	Reference
Rabbit (New Zealand White), male/female Subacute **Exposure: 5 days per week for 3 weeks for a total of 15 applications (daily for 5 weekdays, break at week-ends for 3 weeks)** Doses: 100, 500 and 2 500 mg/kg/day (nominal per unit bw) 4 animals per sex per dose Control animals : yes, concurrent vehicle All statistical analyses compared the treatment groups with the control group by sex. At termination of the study, bw, hematologic, biochemical and urinalysis parameters and absolute and relative organ weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences. Equivalent or similar to OECD TG 410 (Repeated Dose Dermal Toxicity: 21/28-Day Study) Non GLP	Evidence of mild skin irritation, characterized by hyperkeratosis, acanthosis and dermal inflammatory cell infiltrate was seen at the application site in most rabbits from the 100, 500 and 2 500 mg /kg/day groups and was considered compound related. Overall skin response based on microscopic examination of the application site was characterized as mild. **NOEL (systemic): > 2 500 mg/kg bw/day (actual dose received) (male/female)**	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3**	Unpublished report (1979b)

7.9.4.2. Human information

No data.

7.9.4.3. Summary and discussion of repeated dose toxicity

7.9.4.3.1. Chlorendic anhydride

Oral Route

In a rat 28-day range finding study (IRDC, 1978f), chlorendic anhydride was administered to 5 male and 5 female Charles River CD rats. In the study, chlorendic anhydride was administered at dosage levels of 0, 500 ppm (equivalent to 53 mg/kg bw/day for males and 59 mg/kg bw/day), 1 000 ppm (equivalent to 108 mg/kg bw/day for males and 115 mg/kg bw/day for females), 2 500 ppm (equivalent to 282 mg/kg bw/day for males and 287 mg/kg bw/day for females), 5 000 ppm (529 mg/kg bw/day for males and 606 mg/kg bw/day for females) and 10 000 ppm (equivalent to 1 113 mg/kg bw/day for males and 1 242 mg/kg bw/day for females). The rats were observed daily for signs of toxicity and for mortality. Detailed observations were recorded weekly. Individual body weights (bw) and food consumption were recorded weekly. Plasma and red blood cell cholinesterase activities were determined at the termination of the study for all rats in the control group and the 2 500, 5 000 and 10 000 ppm dosage levels.
No rats died during the 4 weeks of compound administration. However 1, 2 and 2 rats at the 2 500, 5 000 and 10 000 ppm dosage levels respectively died following the collection of blood for cholinesterase determinations.
No changes considered to be related to compound were seen in general behaviour and appearance.
Group mean body weights were slightly to markedly lower for rats at the 500, 2 500, 5 000 and 10 000 ppm dosage levels as compared to control rats. The average food consumption during the study was slightly lower for male rats at the 5 000 and 10 000 ppm dosage level as compared to male control rats but was similar for control and treated female rats.
Plasma and red blood cell cholinesterase levels were similar for control and treated rats.
No gross lesions considered to be compound related were seen in rats at necropsy.
Based on the study results, the proposed NOAEL is 10 000 ppm (equivalent to 1 113 mg/kg bw/day for males and 1 242 mg/kg bw/day for females mg/kg bw/day).

In a 90 day subchronic toxicity study (IRDC, 1980), chlorendic anhydride was administered in the diet to 15 rats/sex/group. The test substance was administered at dose levels of 0, 100, 500, and 2 500 ppm (approximately 0, 8, 39, or 202 mg/kg-bw/day in males and 0, 8, 45, or 226 mg/kg-bw/day in females). The rats were observed twice daily for signs of over toxicity and mortality.
Detailed observations, individual bw and individual food consumption were recorded weekly. Ophthalmic examinations were conducted during the pre-test period and after 3 months. Hematologic and biochemical tests and urinalyses were performed at 1, 2 and 3 months of study for five rats/sex/groups.
Three high-dosed females died between the 5th and 13th week of the study.
Mid- and high-dose males and all three groups of treated females had decreased group mean bw when compared with controls; the respective mean percentage decreases in bw were 7.6 and 12.4% in the male groups and 3.9, 5.6 and 21.4% in the female groups. Mid- and high-dose males and high-dose females had decreased food consumption over the 90 day study when compared with controls.
No consistent exposure-related effects were found in the ophthalmologic hematologic, clinical chemistry or urine analytic examinations, except that serum alkaline phosphatase activities were elevated in treated males and females at 1, 2 and 3 months of study, compared to the control.
There were statistically significant decreases in the mean absolute weights of hearts of male rats ($p < 0.05$) at and in the mean absolute and relative weights of livers of male and female rats ($p < 0.01$) at all dosage levels. These changes appeared to be treatment related.
No compound-related gross lesions were seen in any of the rats of the treatment groups.
No compound-related microscopic lesions were seen in any of the tissues from rats that were examined of the 2500 ppm group.

Based on the study results, mortalities in 3/15 females of the high dose groups and decreased bw > 10% in male and female of the high dose (2 500 ppm), the LOAEL is considered to be 2 500 ppm equivalent to 202 mg/kg-bw/day in males and 226 mg/kg-bw/day in females.
The NOAEL is considered to be 500 ppm equivalent to 39 mg/kg bw/day in males and 45 mg/kg bw/day in females.

Inhalation route

In a sub-acute inhalation toxicity study in rats (IRDC, 1978c); male and female rats were exposed to the dusts of chlorendic anhydride for 6 hours per day, 5 days per week for 20 exposures during a 28 day experimental period. The average nominal exposure concentrations were 0, 0.11, 0.99 and 9.97 mg/L. The median dust diameter was 6.0 (± 3.16) µm.
All animals survived to the end of the study.
Immediately after the 6 hour exposure period, all rats exhibited dose-related ocular and nasal irritation and salivation. By the following morning these symptoms had generally disappeared. The rats also exhibited alopecia ranging from a slight thinning on the abdomen to large patches lost on the back of some of the rats in the high concentration group.
The low and medium concentration groups and the female rats of the high-concentration group demonstrated weight gains comparable to the rats of the control group. The male rats of the high-concentration exhibited decreased weight gains as compared to the controls.
Statistical differences in hematocrit, erythrocytes and haemoglobin concentrations were observed in male rats while statistical differences in hematocrit, erythrocytes and leucocytes were seen in female rats. Similarly, statistical differences in glucose alkaline phosphatase and SGPT levels were observed in male rats while statistical differences in alkaline phosphatase levels were observed in female rats.
An increased incidence of dark red foci and dark red area/discolouration in the lungs and dark red or brown foci in the glandular part of the stomach seen at necropsy in the treated groups was probably compound-related. Statistically significant decreases ($p < 0.05$), probably compound-related, were noted in the mean relative weights of livers of males from all treated groups and in the mean absolute and relative weights of thyroids in female rats from the 1.0 mg/L and 10.0 mg/L groups.
Compound-related microscopic changes of haemorrhagic inflammatory nature in the lungs and of inflammatory nature in the trachea, nasal turbinates and stomach mucosa occurred in rats from all treated groups.
Based on the study results, dose-related macroscopic and microscopic changes in lungs, trachea and nasal mucosa; significant changes in hematological parameters in both sexes at all dose levels, a LOAEC of 0.1 mg/L is considered.

Dermal route

In a 3-week dermal toxicity study in rabbits (IRDC, 1978a), chlorendic anhydride was administered to the backs of New Zealand White rabbits at dosage levels of 100, 500 and 2 500 mg/kg bw/day, 5 days a week during 3-week. Four male and four female rabbits were used at each dosage level and in the control group. The rabbits were observed daily for signs of over toxicity, general behaviour, dermal irritation, moribundity or mortality. Body weights were recorded weekly. Hematologic, biochemical and urinalysis studies were conducted during the control period and following the 21-day treatment period.
All rabbits survived the treatment period.
One or more of the following signs of dermal irritation were noted for all treated rabbits: erythema, oedema, atonia, desquamation, coriaceousness and fissuring. The number of signs observed severity of the conditions (barely perceptible to moderate) and duration were dose-related.
Incidental findings (primarily at the 2 500 mg/kg bw/day dosage level) included diarrhoea, nasal or ocular discharge, hypoactivity, anorexia and dehydration.
Male and female rabbits at the high-dosage level had decreases in weight when compared with the controls.

No changes considered related to compound were seen in the hematologic and biochemical studies. Urinalyses were considered normal.
Stomach mucosal lesions, described as erosions, ulcerations or light foci and areas at necropsy in rabbits from the 500 and 2 500 mg/kg bw/day were the only gross findings at terminal sacrifice which were considered compound-related.
No compound related organ weight variations were observed.
Microscopically, grossly described stomach changes were confirmed in several rabbits from the 500 and 2 500 mg/kg bw/day groups. These changes were attributed to compound effect. Evidence of mild skin irritation characterized by hyperkeratosis, acanthosis and dermal inflammatory cell infiltrate was seen at the application site in most rabbits from the 100, 500 and 2 500 mg/kg bw/day groups and was considered compound-related. Overall skin response based on microscopic examination of the application site was characterized as mild.
Based on the study results, the systemic NOAEL in this study is 2 500 mg/kg bw per day. The local LOAEL 100 mg/kg bw per day is fixed based on skin irritation founds (macroscopic and microscopic observations) at all dosages and the microscopically stomach changes in several rabbits from the 500 and 2 500 mg/kg bw/day groups.

Overall, based on the nature and severity of the observed effects of the available experimental animal studies, the evaluating MSCA considers that the criteria for a classification as STOT RE are not considered as fulfilled. **Nevertheless, the Registrant apply the following self-classification for chlorendic anhydride: STOT RE2 - H373 (CLP).**

7.9.4.3.2. Chlorendic acid

Oral route

Male and female Fischer 344/N rats and B6C3F1 mice were fed diets containing chlorendic acid for 14 days or 13 weeks. In the 14-day studies, animals received diets containing chlorendic acid at 3 100, 6 200, 12 500, 25 000 or 50 000 ppm. Deaths occurred only in male and female rats and in male mice given the highest dose. No treatment-related gross lesion was observed at necropsy (US NTP, 1987). In the 13-week studies, rats received concentrations of 620, 1 250, 2 500, 5 000 or 10 000 ppm in the diet and mice received 1 250, 2 500, 5 000, 10 000 or 20 000 ppm; all animals survived, but reduced weight gain was noted at the higher doses. In rats, the occurrence of hepatocomegaly and bile-duct hyperplasia was dose-dependent. Liver lesions also occurred in mice and included centrolobular cytomegaly and coagulative necrosis. The liver lesions occurred mainly in rats given 5 000 and 10 000 ppm and in mice given 10 000 and 20 000 ppm (US NTP, 1987).

Dermal route

No data.

Inhalation route

A short-term toxicity study by inhalation was performed on chlorendic acid using a method similar to OECD TG 412 (non GLP) with deviations. Five rats/sex/dose were treated with doses of 0, 0.134, or 0.273 mg/L (air) of chlorendic acid (analytical), 6 hours per day, 5 days per week for 4 weeks. During the treatment, animals were observed for mortality, clinical signs and bw. Before and at the end of the study, blood and urine were collected and parameters analysed. Surviving animals were sacrificed for gross pathology and microscopic examination. One control female rat and one female rat treated at 0.273 mg/L (air) died during the blood sampling on day 28, it was not considered as related to the treatment. Slightly lower weight gains were observed in treated male rats compared to the controls but not in female rats. Liver weights and liver to brain weight ratios for both sexes of rats exposed to 0.273 mg/L (air) of the substance were significantly elevated in comparison to the control rats. Hepatocytomegaly of centrilobular hepatocytes were observed in treated animals. Values of the haematology, clinical biochemistry, and urinalysis in treated animals were comparable to the controls. The subchronic toxicity study by inhalation performed on rats using chlorendic acid allowed to determine a NOAEC of

0.134 mg/L (air) for male and female rats based on the increase of the weight of two organs compared to the control.

Overall, based on the nature and severity of the observed effects of the available experimental animal studies, the evaluating **MSCA considers that the criteria for a classification as STOT RE are not considered as fulfilled.**

7.9.5. Mutagenicity

7.9.5.1. Non-human information

7.9.5.1.1. *In vitro* data

The results of *in vitro* genotoxicity studies with chlorendic anhydride are summarised below in Table 29.

Table 29: *In vitro* genotoxicity studies with chlorendic anhydride.

Method	Results	Remarks	Reference
***In vitro* mammalian cell transformation assay** Mammalian cell line, other: BALB /3T3 cells (met. act.: without) Test concentrations: 0.005, 0.010, 0.020, 0.039 and 0.078 mg/ml Vehicle: DMSO, deionised water Positive control : 3-Methylcholanthrene Equivalent or similar to EU Method B.21 (In Vitro Mammalian Cell Transformation Test) Non GLP	**Evaluation of results:** **ambiguous** (the test compound was evaluated for its ability to produce malignant transformation in BALB / 3T3 cells at several concentrations ranging from 0.005 mg/ml to 0.078 mg/ml. The test compound did not show toxicity over this range. **Test results**: **ambiguous** for mammalian cell line, other: BALB / 3T3 cells; met. act.: without; cytotoxicity: The spontaneous transformation was slightly elevated over what is normally experienced but was acceptable. Vehicle controls valid: yes Negative controls valid: yes Positive controls valid: yes	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3**	Unpublished report (1977a)
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) S. typhimurium TA 1538 (met. act.: with and without) Saccharomyces cerevisiae D4 (met. act.: with and without) Test concentrations: 0.1, 1.0, 10, 100 and 500 µg / plate Vehicle: DMSO, deionised water Positive controls: yes Equivalent or similar to OECD TG 471 (Bacterial Reverse Mutation Assay)	**Evaluation of results**: **negative with metabolic activation** **negative without metabolic activation** **Test results**: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (Overlay plates) ; met. act.: with and without ; cytotoxicity: not determined ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes negative for S. typhimurium TA 1538 (Overlay plates) ; met. act.: with and without ; cytotoxicity: not determined ; vehicle controls valid: yes;	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3**	Unpublished report (1977b)

Method	Results	Remarks	Reference
GLP	negative controls valid: yes; positive controls valid: yes negative for S. typhimurium TA 98 (Overlay plates) ; met. act.: with and without ; cytotoxicity: not determined ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes negative for Saccharomyces cerevisiae (Overlay plates) ; met. act.: with and without ; cytotoxicity: not determined ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes		
Mammalian cell gene mutation assay (gene mutation) **Mouse lymphoma L5178Y cells** (met. act.: with and without) Test concentrations: 0.08, 0.12, 0.16, 0.24 and 0.32 mg/ml Vehicle: DMSO Positive control : Positive control substance(s): methylmethanesulfonate - 15 µg/ml Equivalent or similar to OECD TG 476 (*In Vitro* Mammalian Cell Gene Mutation Test) Non GLP	**Evaluation of results**: **negative with metabolic activation** **negative without metabolic activation** **Test results**: negative for mouse lymphoma L5178Y cells ; met. act.: with and without ; cytotoxicity: no ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3**	Unpublished report (1978a)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells *in vitro* (DNA damage and/or repair) Human WI - 38 cells (met. act.: with and without) Test concentrations: 0.01, 0.05, 0.1 and 0.5 mg/ml Vehicle: DMSO Equivalent or similar to OECD TG 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *In Vitro*) GLP	**Test results**: **positive** for mammalian cell line; met. act.: with and without ; cytotoxicity: yes ; vehicle controls valid: yes; negative controls valid: yes; positive controls valid: yes	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3**	Unpublished report (1978b)

The results of *in vitro* genotoxicity studies with chlorendic acid are summarised below in Table 30.

Table 30: *In vitro* genotoxicity studies with chlorendic acid

Method	Results	Remarks	Reference
Bacterial reverse mutation assay [*in vitro* gene mutation study in bacteria] S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 [bacteria] (Met. act.: with and without) Liver S9 fractions from male SD rats and male Syrian hamster that were injected, IP with Aroclor 1254 at 500 mg/kg Test concentrations: a preliminary toxicity determination was undertaken in order to select the dose range for the mutagenesis assay. Toxicity checked to TA100 strain >10 mg/plate or limit of solubility with and without S9 mix 5 doses tested in addition to a concurrent solvent and positive controls Positive control substance(s): 9-aminoacridine (9-AAD) (Tested on TA1537 without S-9) ; Sodium azide (SA) (Tested on TA100 and TA1535 without S-9) ; 2-aminoanthracene (2-AA)(Tested on all strains in presence of S-9); 4-nitro-o-phenylenediamine (NOPD) (Tested on TA98 without S-9) Triplicate Equivalent or similar to OECD TG 471 (Bacterial Reverse Mutation Assay)	**Negative** for S. **typhimurium TA 1535** [bacteria]; Met. act.: with and without genotoxicity: negative cytotoxicity: not specified vehicle controls valid: yes positive controls valid: yes **Negative** for S. typhimurium **TA 1537** [bacteria]; met. act.: with and without S9mix genotoxicity: negative cytotoxicity: not specified vehicle controls valid: yes positive controls valid: yes **Negative** for S. typhimurium **TA 98** [bacteria]; met. act.: with and without genotoxicity: negative cytotoxicity: not specified vehicle controls valid: yes positive controls valid: yes **Negative** for S. typhimurium **TA 100** [bacteria] met. act.: with and without genotoxicity: negative cytotoxicity: not specified vehicle controls valid: yes positive controls valid: yes	2 (reliable with restrictions) Weight of evidence Experimental study Purity not specified **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid EC No 204-078-9 /CAS RN 115-28-6** Form: solid	Haworth *et al.* (1983)
***In vitro* mammalian cell micronucleus test** Peripheral blood lymphocytes [primary culture from separated lymphocytes] (Met. act.: with and without S9 mix) Source of cells: three human donors Sex, age and number of blood donors if applicable: healthy, non-smoking individuals (36, 34 and 32 years old) with no known recent exposures to genotoxic chemicals or radiation Test concentrations: 62.5, 125, 250, 500, 1 000, 1 250, 1 500, 1 750 and 2 000 µg/ml (2 000 µg/ml was selected as the highest dose according to OECD TG 487 as no precipitate or limiting cytotoxicity was observed).	**Positive** for peripheral blood lymphocytes [primary culture]; met. act.: **with and without S9 mix** genotoxicity: positive cytotoxicity: slight cytotoxicity observed in the group without S9-mix at the highest dose when compared to the concurrent solvent control vehicle controls valid: yes positive controls valid: yes	1 (reliable without restriction) Key study Experimental study **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid (HET acid) EC No 204-078-9 /CAS RN 115-28-6**	Unpublished report (2016c)

Vehicle : DMSO Negative controls: yes, vehicle Solvent controls : yes True negative controls : no Positive controls : yes Positive control substance(s): cyclophosphamide Duplicate; 1 000 cells per dose level for the Cytokinesis-Block Proliferation Index (CBPI), 2 000 binucleated cells per dose Method cytotoxicity: CBPI level for the presence of micronuclei according to OECD TG 487 Deviations: no. The pulse treatment group with S9-mix had to be repeated twice due to a technical error and a notable variation in cytotoxicity between the replicates. GLP : yes		**Form: solid**	
Mouse Lymphoma forward mutation Assay (*in vitro* gene mutation study in mammalian cells) Mouse lymphoma L5178Y cells [mammalian cell line] (Met. act.: with and without) Liver homogenates (S9) Fischer 344 rats that were injected, IP with Aroclor 1254 at 500 mg/kg At least 5 test concentrations: Trial 1 without S9-mix: 0, 62.5, 125, 250, 500, 1000 and 2000 mg/L. Trial 2 and 3 without S9-mix: 0, 700, 900, 1 100, 1 300, 1 500 and 1 700 mg/L. Trial 4 without S9-mix: 0, 1 300, 1 400, 1 500, 1 600 and 1 700 mg/L. cultures per concentrations Vehicle control: culture medium without serum; distilled water; methanol (without S9 mix only); dimethyl sulphoxide (DMSO) Positive control substance(s): 3-methylcholanthrene; ethylmethanesulphonate; methylmethanesulfonate Equivalent or similar to OECD TG 476 (*In Vitro* Mammalian Cell Gene Mutation Test)	**Positive** – in 2/4 experiments for mouse lymphoma L5178Y cells [mammalian cell line] met. act.: **without S9** Genotoxicity: positive in 2/4 experiments cytotoxicity: yes **Significant, mutagenic responses were obtained in 2 of 4 experiments, all of which were conducted in the absence of S9 mix.** No significant response was observed in the first experiment, but the highest acceptable concentration tested was 1 000 µg/ml where the RTG was 81 %. Although a concentration of 2 000 mg/L also was tested, this did not permit adequate growth in suspension during the expression period. Consequently, this result was inconclusive according to the study criteria. In the second experiment, a significant response was obtained at 1 500 µg/ml where the RTG was 21%.The lowest RTG observed in the third experiment was 46%, at a concentration of 1 500 mg/L, and no significant mutagenic response resulted from the treatment. Only when the RTG was reduced to 5%, at a concentration of 1 700 mg/L in the final experiment, was the significant mutagenic response in trial 2 confirmed.	2 (reliable with restrictions) Weight of evidence Experimental study **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid (Het acid), EC No 204-078-9 /CAS RN 115-28-6** Purity not specified	McGregor *et al.* (1988)

	This compound induces a mutagenic response only in a narrow range of dose levels that are highly toxic. Cytotoxicity occurred at the higher doses preventing to determine the genotoxicity at these doses Vehicle controls valid: yes positive controls valid: yes		
***In vivo-in vitro* replicative DNA synthesis (RDS) assay** RDS test is a routine screening procedure for non genotoxic hepatocarcinogens Detection of RDS induction Hepatocytes from male F344 rat (9 week old) [primary culture] Test concentrations: 0, 450, 900 mg/kg chlorendic acid Vehicle(s)/solvent(s) used: corn oil Positive control substance(s): substances with RDS incidence of < 1.0% No guideline followed, non GLP. Chlorendic acid was dissolved or suspended in corn oil, and administered to 9-week-old male F344 rats. RDS was observed at 24, 39 and 48 hr after treatments from primary hepatocyte cultures. RDS incidences were calculated as the % of [3H]thymidine-incorporating cells, relative to 2000 hepatocytes counted per animal under the autoradiograph.	**Negative** for rat ; met. act.: not applicable RDS incidence < 1% : negative response genotoxicity: negative cytotoxicity: not relevant vehicle controls valid: yes negative controls valid: not examined positive controls valid: not examined Remark: non-standard method	3 (not reliable) Weight of evidence Experimental study **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid EC No 204-078-9 /CAS RN 115-28-6** Form: solid Purity not specified	Uno *et al.* (1994)
Sex-Linked Recessive Lethal (SLRL) test Drosophila melanogaster (adult male) Test concentrations: 15 000 and 2 000 ppm Tested both by oral route (feed) and injection Positive control substance(s): no No guideline followed, non GLP	**Negative** for Drosophila melanogaster; met. act.: not applicable genotoxicity: negative cytotoxicity: not applicable vehicle controls valid: yes	3 (not reliable) Weight of evidence Experimental study **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid (Het acid), EC No 204-078-9 /CAS RN 115-28** Purity not specified	Foureman, *et al.* (1994)

Chlorendic acid

The *in vitro* genotoxicity in bacteria of chlorendic acid was determined using a preincubation procedure, which was a modification of the Salmonella/mammalian microsome test of Ames, similar to OECD TG 471 (non GLP) with deviations. At least 5 doses of test chemical, in addition to the concurrent solvent and positive controls, were tested on each bacteria strain in the presence of S9 mix or buffer. Controls were valid. Chlorendic acid was considered to be **non-mutagenic** under the conditions of the test, for both methods, with and without metabolic activation.

The potential of chlorendic acid to induce structural chromosomal aberration *in vitro* was determined in accordance with OECD TG 487 (*In vitro* mammalian micronucleus test on cultured human lymphocytes). A pulse treatment was performed with and without addition of S9-mix at doses up to 2 000 µg/mL. The highest dose was selected as no precipitate or limiting cytotoxicity was observed. Peripheral blood lymphocytes were obtained from three different donors for the purpose of this study. Dimethylsulfoxide (DMSO) was used as a solvent for the test substance. Duplicate cultures were used in all experiments. Cytotoxicity was determined from the Cytokinesis-Block Proliferation Index (CBPI). Cells were incubated for 48 h with phytohaemagglutinin then treated for 4 h with chlorendic acid with and without addition S9-mix. This treatment was followed with a 20 h recovery in presence of Cytochalasin B then cells were harvested 72 h after initiation of the cultures and prepared for microscopic analysis. Two thousand binucleated cells per concentration were examined for the presence of micronuclei. The frequencies of binucleated cells with micronuclei were used for the evaluation of micronuclei induction. It was required to repeat the pulse treatment with S9-mix twice due to a technical error and a notable variation in cytotoxicity between the replicates. At the highest test substance concentration (2 000 µg/ml), chlorendic acid induced a dose-related statistically significant increase in the number of binucleated cells containing micronuclei in the pulse treatment with and without addition of S9-mix. In addition, the number of binucleated cells containing micronuclei in the highest concentration was outside of the historical data range with and without addition of S9-mix. The substance was considered as clastogenic and/or aneugenic under the conditions of the test. As the pulse treatment with and without S9-mix was sufficient to obtain **a clear positive result** in accordance with OECD TG 487, no extended treatment was performed.

An *in vivo- in vitro* replicative DNA synthesis (RDS) assay was conducted according to a non-standard test method. Chlorendic acid, considered by the authors of the study as 'a known nongenotoxic hepatocarcinogen', was investigated along with 46 other chemicals. Acute toxicity testing was first carried out to determine the maximum tolerated dose (MTD), and was followed by a series of time-course experiments to determine RDS incidence. Concurrent control was valid. Chlorendic acid returned **negative results** under the conditions of the test.

The capacity of chlorendic acid to induce gene mutation in mammalian cells was determined according to the method described by Clive *et al*. (1979) and therefore similar to OECD TG 476 (non GLP). Cultures were exposed to the test substance for 4 h, then cultured for 2 days before plating in soft agar with or without trifluorothymidine at 3 µg/ml. Four experiments were performed without metabolic activation and two with metabolic activation at concentrations up to 2 mg/ml. Controls were valid. There was a significant increase in mutation frequency over control counts in L5178Y mouse lymphoma cells during two of the four experiment without metabolic activation. This increase only occurred in a narrow range of concentrations as chlorendic acid was too cytotoxic at higher concentrations. No significant response was obtained with metabolic activation. The substance was considered as **mutagenic** (without metabolic activation) under the conditions of the test.

The sex-linked recessive lethal (SLRL) test detects the occurrence of mutations in the germ line of *Drosophila melangaster*. Chlorendic acid was tested for its ability to induce SLRL mutations in postmeiotic and meiotic germ cells of adult male Drosophila melanogaster. Chlorendic acid was administered by feeding and injection and produce a

negative result for both exposure routes. No further retesting using the reciprocal translocation assay was necessary. Therefore, chlorendic acid is considered to be non-mutagenic under the conditions of the test.

7.9.5.1.2. *In vivo* data

The results of *in vivo* genotoxicity studies on chlorendic anhydride are summarised in the following table:

Table 31: *In vivo* genotoxicity studies on chlorendic anhydride.

Method	Results	Remarks	Reference
Mouse Dominant Lethal assay Mouse (CD-1) male Oral: gavage Doses: 0, 50, 100, 500, 1000 and 5 000 mg/kg Vehicle: DMSO Positive control: triethyleneamine 20 male mice/group Equivalent or similar to OECD TG 478 (Genetic Toxicology: Rodent Dominant Lethal Test) Non GLP	**Evaluation of results**: **Ambiguous** (see section 7.9.7.3)	4 (not assignable) Supporting study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride**	Unpublished report (1978c)

Chlorendic anhydride was considered to be inactive in the mouse Dominant Lethal assay and did not induce dominant lethality under the test conditions (see section 7.9.7.3). Only limited information is available on the experimental conditions and results of this assay (Unpublished report, 1978c).

The results of *in vivo* genotoxicity studies on chlorendic acid are summarised below in Table 32.

Table 32: *In vivo* genotoxicity studies on chlorendic acid.

Method	Results	Remarks	Reference
***In vivo-in vitro* replicative DNA synthesis (RDS) assay** (see above in in vitro section)	**Negative** (see above in in vitro section)	3 (not reliable) Weight of evidence Experimental study **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid (Chlorendic acid)**	Uno *et al.* (1994)
Combined *in vivo* mammalian erythrocyte micronucleus test in bone marrow and *in vivo* mammalian comet assay (*in vivo* alkaline single cell gel electrophoresis assay)	**Test results:** **MN assay: Negative** **Comet assay : Negative in the stomach**	1 (reliable without restriction) Experimental study Weight of evidence Pancreas not tested (no validated method for this tissue)	Unpublished report (2020)

Method	Results	Remarks	Reference
Rat (Sprague Dawley), male Oral (gavage) Doses : 175, 350 and 700 mg/kg/day (nominal) Administrations over 3 consecutive days, at 0 h (Day 1), 24 h (Day 2) and 45 h (Day 3) Necropsy and sampling at 48 h 6 animals/dose of test substance Negative control: yes, concurrent vehicle (corn oil) Positive control: yes, ethylmethanesulfonate (EMS) *via* oral gavage and vinblastine (VIN) *via* intraperitoneal injection; inducing responses compatible with those of the historical positive control database and producing statistically significant increases compared with concurrent negative control Analysis: Examination of at least 4000 polychromatic erythrocytes (PCE)/animal for presence of MN Measurements of tail intensity (% DNA in tail) from 150 cells/animal Statistical tests: Wilcoxon rank sum test to compare numbers of MN PCE in treated *versus* negative control group and Terpstra-Jonckheere test to evaluate dose-response in the MN assay Dunnett's test to compare treated *versus* control group in the comet assay Study complying with OECD TG 489 and 474 (no deviations) GLP-compliant	**Equivocal in the liver, duodenum and gonads** Clinical chemistry: Minimal increases in aspartate transaminase (AST) and alanine transaminase (ALT) activities for some animals dosed at 700 mg/kg/day. Small increases in chloride, urea and creatinine (700 mg/kg/day) Small inconsistent (between animals) increases in potassium and calcium (350 and 700 mg/kg/day), phosphate (700 mg/kg/day), urea and creatinine (175 and 350 mg/kg/day). Histopathology: Abnormal contents in the stomach of animals dosed at 700 mg/kg/day. Decreased hepatocyte glycogen in the liver of animals dosed at 700 mg/kg/day Single cell necrosis in one animal dosed at 700 mg/kg/day Stomach inflammation and/or decreased mucous of animals dosed at 350 or 700 mg/kg/day, with a dose-relationship Vehicle controls valid Positive controls valid	**Test material: chlorendic acid (CAS RN 115-28-6)** Form : solid (powder) Lot number : 8R28J Purity : 99,6%	

An *in vivo* mammalian micronucleus (MN) test (OECD TG 474) in bone marrow combined with an *in vivo* mammalian alkaline comet assay (OECD TG 489) in the liver, glandular stomach, duodenum, pancreas and gonadal cells was requested by ECHA in a decision in December 2018, using the degradation product chlorendic acid, administered by the oral route. As requested and complying with the OECD TG 474 and 489, the study was conducted in rats (Sprague-Dawley (Crl:CD(SD)), considering the large volume of background data for both genetic toxicology end-points for this species. Reproductive

organs were both affected in male and female rats in a 2-year feed study (US NTP, 1987), OECD 453, non GLP), i.e. increased incidence in preputial gland adenoma, carcinomas or squamous cell papilloma in males ; increased incidence of endometrial stromal polyp in females. Gonadal cells were therefore requested to be investigated but the combined *in vivo* assay was performed only in male rats (i.e. testis for the gonadal cells) to reduce animal use. Additionally, the pancreas, one of the target organ for carcinogenicity, was not sampled for the comet assay, the facility not having a validated method for this tissue, which does not compromise the study results. Therefore, the study was considered as valid based on the criteria laid down in the OECD TG 474 and 489.

Prior to the main experiment, a range-finder experiment was conducted in order to estimate the maximum tolerated dose (MTD). Considering the rat acute oral LD50 of 1 770 mg/kg for chlorendic acid (suggested by the IPCS), groups of 3 male Sprague Dawley rats were dosed for three consecutive days (0, 24 and 45 hours) at an initial dose of 500, 700 and 1 000 mg/kg/day. The 1 000 mg/kg/day dose exceeded an appropriate MTD (one dead animal on day 3; mean bw loss of -5.9 % between Day 1 and 3). The 700 mg/kg/day dose was instead considered an appropriate estimate of the MTD (observed piloerection (3/3), mouse rubbing (1/3) and bw loss between Day 1 and 3 (2/3 ; group mean: -2.3%)). Therefore, the following dose levels of chlorendic acid for the main experiment were selected: 175, 350 and 700 mg/kg, equivalent to 25% of, 50% of and the MTD, respectively.

Groups of 6 male rats (7-8 weeks old) were dosed *via* oral gavage for 3 consecutive days (at 0, 24 and 45 hours) by either the concurrent vehicle (i.e. corn oil; negative control group), 175 mg chlorendic acid/kg, 300 mg chlorendic acid/kg or 700 mg chlorendic acid/kg. Similarly, a group of 3 male rats were dosed *via* oral gavage by 150 mg ethylmethanesulfonate (EMS)/kg (15 mg/ml of aqueous solution) as positive control group. Two groups of 6 rats received intraperitoneal injections (at 0 and 24 hours) of 0.25 mg/kg (0.05 mg/ml of aqueous solution) or 0.50 mg/kg (0.10 mg/ml of aqueous solution) vinblastine (VIN) as positive aneugenic control groups. All doses were administered at a dose volume of 10 mL/kg (except VIN positive control: 5 mL/kg).

Bone marrow from the femur (for the MN test), liver, stomach, duodenum and gonad (testis) (for the comet test) were sampled at necropsy on Day 3 (at 48 hours) in all animals. Clinical chemistry tests was performed on a blood sample taken at necropsy from the abdominal aorta and histopathology was performed on samples of liver, stomach, duodenum and gonad.

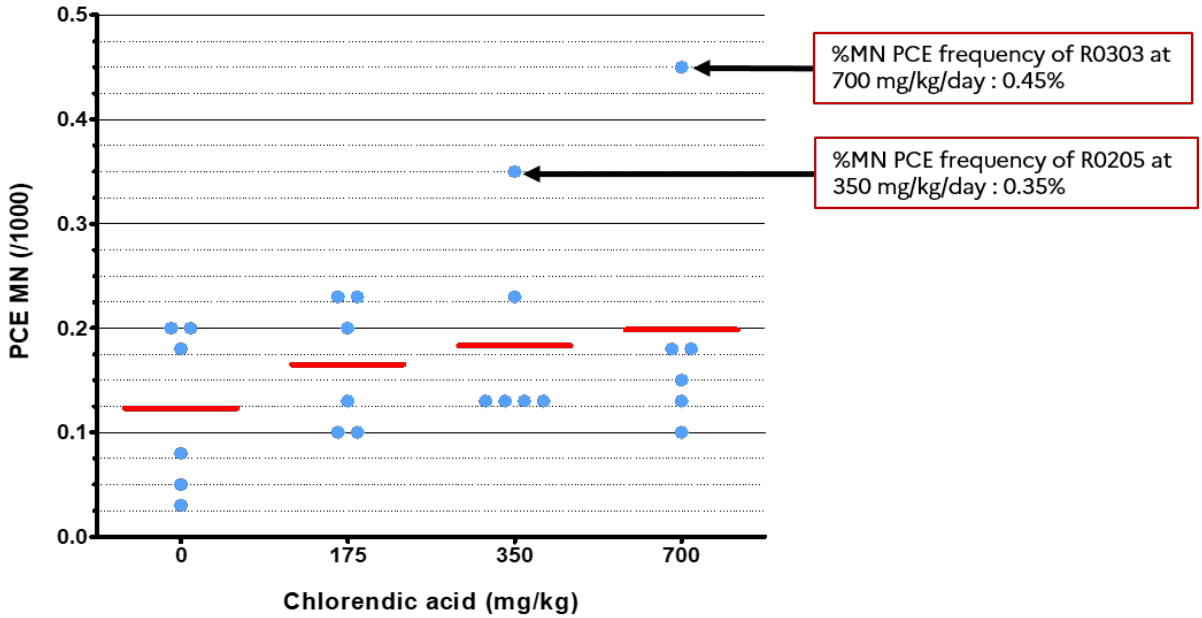
There were no remarkable post-dose observations for any animal, with the exception of mouth rubbing observed immediately after the 3rd dose administration (1/6 animals at 175 mg/kg/day, 3/6 animals at 350 mg/kg/day and 5/6 animals at 700 mg/kg/day). One animal at 700 mg/kg/day was also noted to exhibit paddling behaviour.
A decrease in group mean bw gain was observed at 700 mg/kg/day between Day 1 and 3, (-0.1% compared to +7.3%, +5.3% and +5.6% for the vehicle control group, 175 mg/kg/day group and 350 mg/kg/day group, respectively).

MN assay results

Chlorendic acid showed no evidence of bone marrow toxicity, as animals treated with chlorendic acid at 175, 350 and 700 mg/kg/day exhibited group mean % of polychromatic erythrocytes (PCE) (46.93%, 46.90% and 46.37%, respectively) similar to the vehicle control group mean (47.83%) and falling within the 95% reference range of laboratory's historical vehicle control data (33.30-66.05%).

Group mean % micronucleated PCE frequencies of the animals treated with chlorendic acid at 175, 350 and 700 mg/kg/day (0.16%, 0.18% and 0.20%, respectively) were not statistically different than the vehicle control group mean (0.12%) and fell within the 95% reference range of the laboratory's historical vehicle control data (0-0.33%) (Figure 1). For the positive controls, the group mean % micronucleated PCE frequencies were 1.98 % (EMS controls), 1.45 % (VIN at 0.25 mg/kg day) and 4.48 % (VIN at 0.50 mg/kg day).

Figure 1. Micronucleated polychromatic erythrocytes frequencies (%) in the *in vivo* mammalian micronucleus assay for chlorendic acid.



No statistically significant increases in MN frequency for any of the groups receiving chlorendic acid was observed, compared to the concurrent vehicle control.

However, the MN frequency of one animal at 350 mg/kg/day (R0205, 0.35%) and one animal at 700 mg/kg/day (R0303, 0.45%) exceeded the 95% reference range of the historical vehicle control data (Figure 1), though they fell within the observed range of the historical vehicle control data (0-0.53%). Thus, these data are considered due to normal inter individual variation. The Terpstra-Jonckheere test conducted to evaluate dose-response did not indicate any significance in the results (probability values of $p \leq 0.05$ accepted as significant). **The *in vivo* MN assay for chlorendic acid is thus considered negative.**

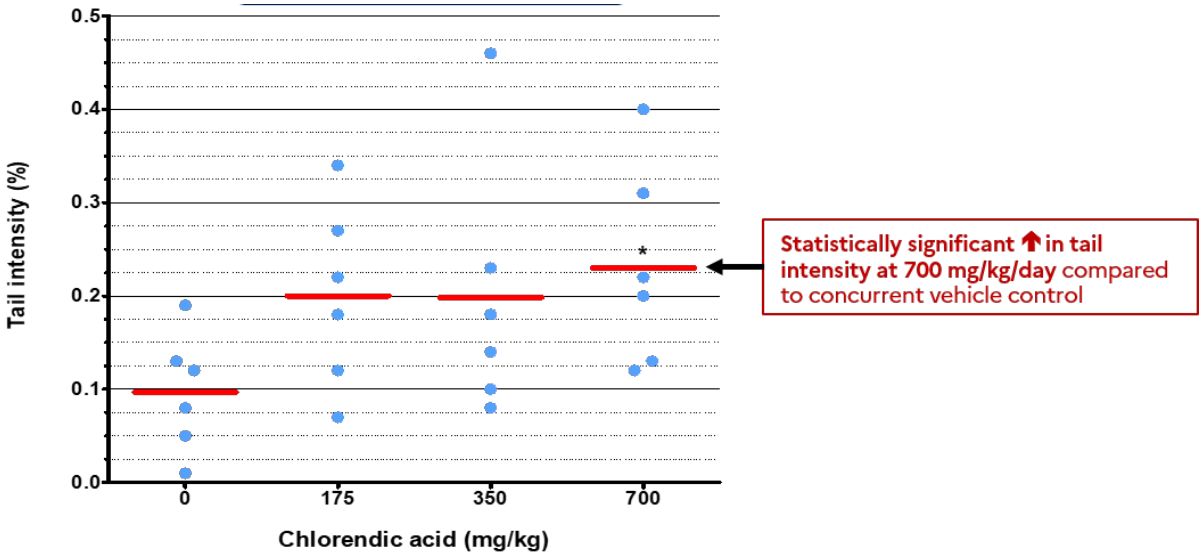
Comet assay results

Liver

The group mean tail intensities in the liver of animals treated with chlorendic acid at 175 and 350 mg/kg/day) were not statistically different (0.20% and 0.20%, respectively) to the concurrent vehicle control group mean (0.10%) and fell within the 95% reference range of the laboratory's historical vehicle control data (0.03-1.57%) (Figure 2).

However, a statistically significant ($p \leq 0.05$) increase in tail intensity at 700 mg/kg/day was recorded (0.23%), compared to the concurrent vehicle control. A significant dose-response relationship (p-value of 0.0159) was also retrieved (Figure 2). The mean tail intensities value of the positive control (EMS) is 35.07%.

Figure 2. Tail intensities (% tail DNA) observed in the liver in the mammalian comet assay for chlorendic acid.



The liver comet data thus do not meet the OECD TG 489 evaluation criteria for a clear positive, nor a clear negative response. But the biological significance of this result is unclear, considering that :

i) the statistical significance at 700 mg/kg/day can be associated with tail intensity values falling towards the lower end of the 95% reference range of the historical vehicle control data and that

ii) slight increases in liver enzymes (AST and ALT) were observed for some animals at this dose, possibly indicating low levels of liver toxicity (5 of 6 animals from the 700 mg/kg/day treated animals were exceeding the mean AST level of the vehicle controls of 84 IU/L (±6.8), with levels between 97 and 151 UI/L and all animals from this dose group were exceeding the mean ALT level of the vehicle controls of 50 IU/L (±10.6), with levels between 51 and 99 UI/L).

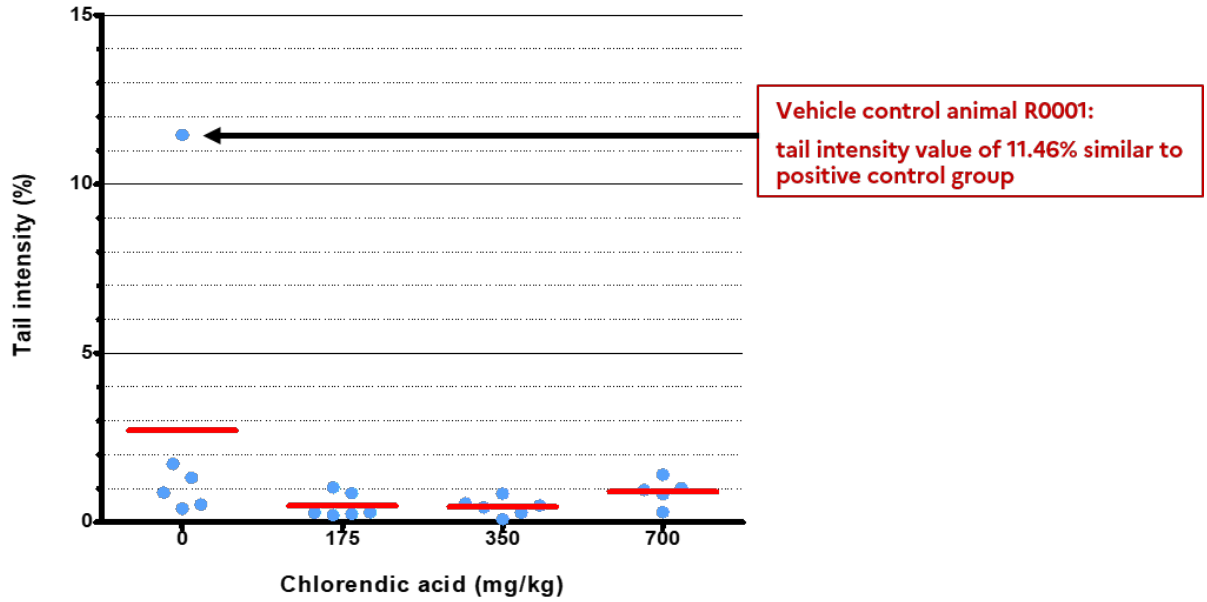
On microscopic examination, decreased hepatocyte glycogen vacuolation was noted in the liver of animals administered chlorendic acid (at 700 mg/kg/day as mentioned in the study report, but also at lower doses according to the available results table). Single cell necrosis was noted in one animal administered 700 mg/kg/day. The ***in vivo*** **comet assay response of chlorendic acid is considered to be equivocal in the liver.**

Stomach

Animals treated with chlorendic acid (175, 350 and 700 mg/kg/day) showed group mean tail intensities in the stomach (0.49%, 0.45% and 0.91%, respectively) falling within the 95% reference range of the laboratory's historical vehicle control data (0.16-7.18%) and not statistically different from control. Mean tail intensities value of the positive control (EMS) is 16.78%.

In an unexplained way, the vehicle control animal R0001 recorded a tail intensity value of 11.46% (± 4.16%) (Figure 3), similar to one of the animal of the positive EMS control group (12.89% ± 4.59%). The hedgehogs' frequency of this vehicle control animal was comparable to other animals in the dataset and no stomach anomalies was observed in the histopathology examination.

Figure 3. Tail intensities (% tail DNA) observed in the stomach in the mammalian comet assay for chlorendic acid.

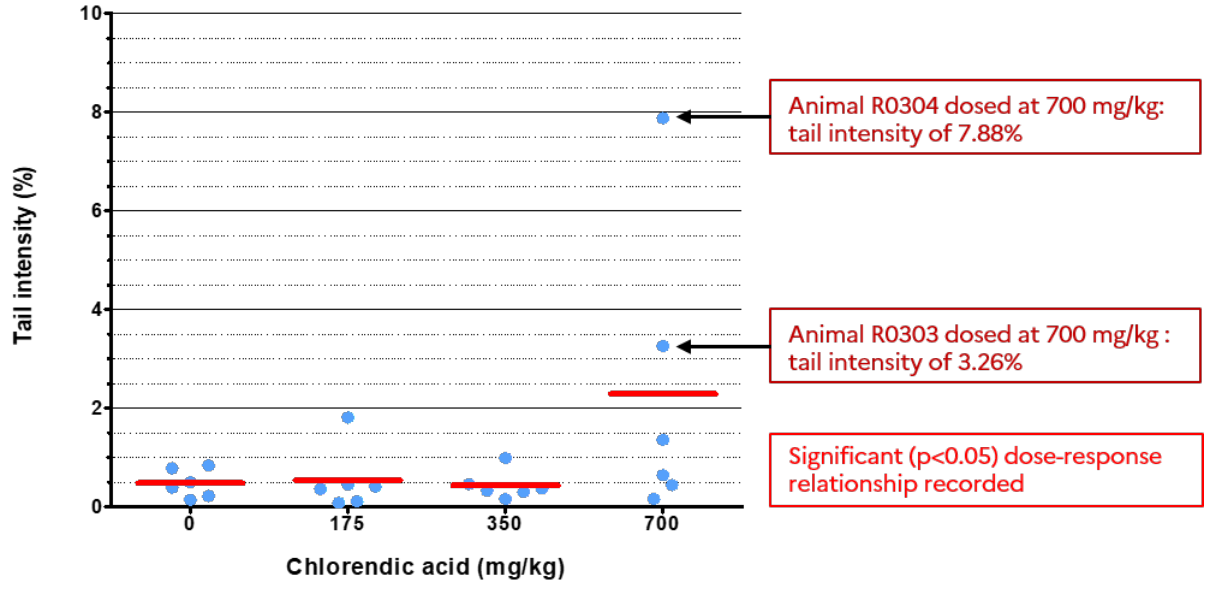


When excluding this animal for the data analysis, the group mean tail intensities of the chlorendic acid treated animals (0.49%, 0.45% and 0.91%, respectively) were similar to the group mean tail intensities of the negative control (0.57%). Excluding this animal from the vehicle control group did not mask any test article statistical significance or dose response. It did not demonstrate an impact on the ***in vivo*** **comet assay clear negative response of chlorendic acid in the stomach**.

Duodenum

Animals treated with chlorendic acid (175, 350 and 700 mg/kg/day) exhibited group mean tail intensities in the duodenum (0.54%, 0.43% and 2.29%, respectively) that fell within the 95% reference range of the laboratory's historical vehicle control data (0.18-7.60%). No statistical significant increases following treatment with chlorendic acid was observed compared to the concurrent vehicle control (Figure 4). Mean tail intensities value of the positive control (EMS) is 10.74%.

Figure 4. Tail intensities (% tail DNA) observed in the duodenum in the mammalian comet assay for chlorendic acid.

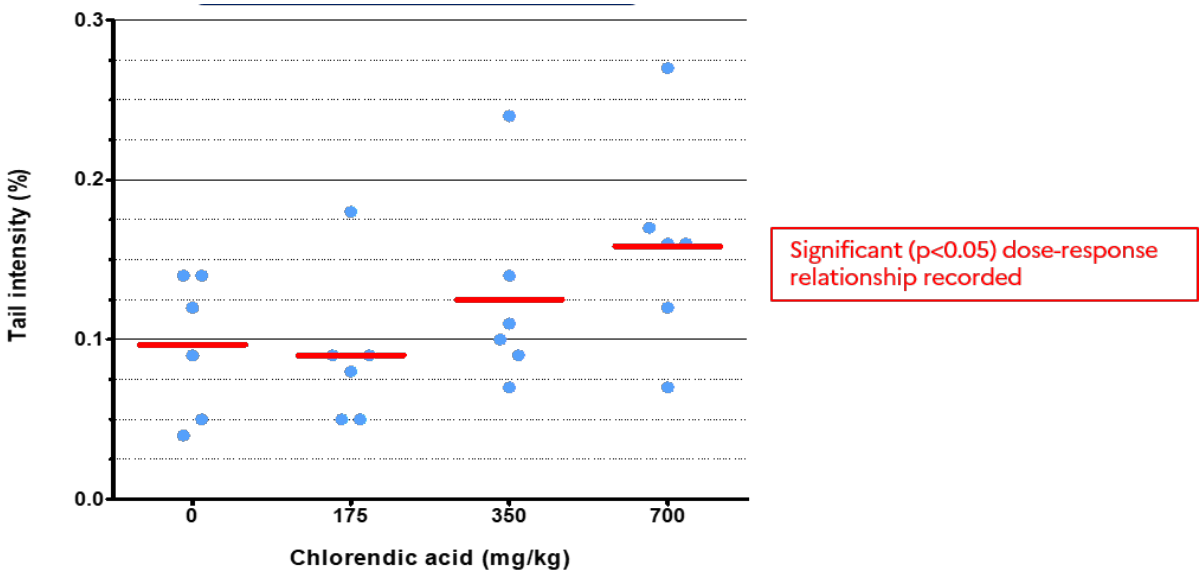


However, a significant ($p<0.05$) dose-response relationship was recorded. One animal dosed at 700 mg/kg/day (R0304) presented a duodenum tail intensity value of 7.88% (± 3.14%), exceeding the 95% reference range of the historical negative control (however falling in the observed range (0.16%-10.74%) of the historical negative control database) This result was almost similar to the tail intensity value of one animal from the positive EMS control group (R0401; 8.41% ± 0.79%). In addition, the tail intensity value of animal R0303 dosed at 700 mg/kg/day (3.26% ± 0.14%) was also noted to be a magnitude higher than all other test article dosed animals or concurrent negative control animals (even if falling within the 95% reference range of the historical negative controls). There were no corresponding pathology findings to suggest target tissue toxicity or inflammation in these two animals. However, % of hedgehogs in these two animals (R0304: 11.29%; R0303: 10.86%) are higher than the group mean % of hedgehogs of the vehicle control animals (7.63%) and of the positive control group animals (10.73%). Overall, the group mean % of hedgehogs of the 700 mg/kg/day-treated animals exceeded the 95% reference range of the historical vehicle control data for hedgehogs, which may be due to a test substance-related cytotoxicity or mechanical/enzyme-induced damage initiated during sample preparation.

Results of these 2 animals from the 700 mg/kg/day-group (out of 6) are raising the group mean, which is resulting in the statistically significant dose-relationship. Nonetheless, 3 out of 6 animals from this group had tail intensities similar to the vehicle control group. Thus, one of three acceptability criteria for a positive response to the test is fulfilled (dose-related increase in tail intensity values). Consequently, the ***in vivo* comet assay response of chlorendic acid is considered equivocal in the duodenum.**

Gonad
Animals treated with chlorendic acid (175, 350 and 700 mg/kg/day) exhibited group mean tail intensities in the testis (0.09%, 0.12% and 0.16%, respectively) not statistically different to the concurrent vehicle control group mean (0.10%) (Figure 5), and falling within the laboratory's historical vehicle control observed data (0.00-0.45%). These, however, were generated from 2 studies only, conducted in 2015, which the evaluating MSCA considers as too little to demonstrate the ability of the testing laboratory to obtain a reproducibility of control responses. Mean tail intensities value of the positive control (EMS) is 17.71%.

Figure 5. Tail intensities (% tail DNA) observed in the gonad (testis) in the mammalian comet assay for chlorendic acid.



No statistical significant increases in tail intensity following treatment with chlorendic acid was noted, compared to the concurrent vehicle control. A significant ($p<0.05$) dose-response relationship was observed. From all chlorendic acid-treated animals, one animal from the 350 mg/kg/day-group and one animal from the 700 mg/kg/day-group are

exceeding the range of the individual tail intensities of the vehicle control animals (but falls within the limited laboratory's historical vehicle control values). The ***in vivo* comet assay response of chlorendic acid in the testis is considered equivocal**.

A summary of the compliance of the chlorendic acid comet assay results along with their evaluation criteria for a positive, equivocal or negative response according to the OECD TG 489 is available in Table 33.

Table 33: *In vivo* comet assay results for chlorendic acid and their evaluation.

Tissue	**Criteria indicative of a positive response to the test**			**Response according to OECD TG 489 criteria**
	Exhibition of a statistically significant increase of at least one of the test doses compared with the concurrent negative control	Dose-related increase when evaluated with an appropriate trend test	Any of the results outside the distribution of the historical negative control data	
Liver	Yes	Yes	No	Equivocal
Stomach	No	No	No	Clearly negative
Duodenum	No	Yes	No (1 animal exceeded the P95 of the historical control data, but the result was included in the observed range of data)	Equivocal
Gonad	No	Yes	No (historical negative control data insufficient)	Equivocal

Overall, these results provide an equivocal response for mutagenicity of chlorendic acid with regards to the OECD TG 489 criteria, as the levels of DNA breaks following treatment are within the distribution of the historical control data and as no statistically significant increase in tail intensity compared with the concurrent negative control are observed, except in the liver at the highest dose tested, i.e. 700 mg/kg. In addition, it is noted that the distribution of responses of treated animals in the liver are differing from that of the negative controls, with a dose-related pattern that would support the biological significance of the result for this tissue.

7.9.5.2. Human information

No data.

7.9.5.3. Summary and discussion of mutagenicity

Based on *in vitro* information, the genetic mutation potential of chlorendic anhydride and acid was questionable and cannot be excluded. On one hand, Ames tests gave negative results for both forms in the presence and absence of metabolic activation. On the other hand, chlorendic acid was mutagenic in an *in vitro* MLA in the absence of metabolic activation, while chlorendic anhydride was not mutagenic in the presence and absence of metabolic activation. Concerning the chlorendic anhydride, the studies were not performed under GLP and not according current OECD guidelines; the study summaries on the IUCLID gave too limited information on the conduct of the test and the results are not sufficiently detailed.

Chlorendic was further investigated for the clastogenic potential in an *in vitro* Mammalian Cell MN Test on chlorendic acid (OECD TG 487). The results were positive and chlorendic acid, was clastogenic and/or aneugenic to cultured human lymphocytes. Since chlorendic anhydride is rapidly hydrolysed into the acid form, the results can be extrapolated to the acid form.

The available existing data regarding the genotoxic potential of chlorendic anhydride and chlorendic acid in bacterial and mammalian cells are presented below:

Genotoxicity tests	Chlorendic anhydride	Chlorendic acid
***In vitro* Ames Bacterial Reverse Mutation Assay** (OECD TG 471) (Haworth *et al.,* 1983)	Not mutagenic in the presence and absence of metabolic activation	Not mutagenic in the presence or absence of metabolic activation
***In vitro* Mouse lymphoma assay L5178Y** (OECD 476) (McGregor *et al.,* 1988)	Not mutagenic in the presence and absence of metabolic activation	**Mutagenic in the absence of metabolic activation**
***In vitro* mammalian micronucleus test on cultured human lymphocytes** (OECD TG 487)	*Not tested*	**Positive in the absence and the presence of metabolic activation without FISH** (clastogenic and/or aneugenic) Whole study Report (TNO Triskelion V20717/04 July 2016) provided by the Registrant
***In vitro* unscheduled DNA Synthesis assay in human WI-38 cells** (Unpublished report, 1978b)	**Significant increases of the unscheduled DNA synthesis**	*Not tested*
***In vitro / in vivo* replicative DNA synthesis (RDS) assay in hepatocytes primary culture cells** (Uno *et al.,* 1994)	*Not tested*	Negative
Mouse Dominant Lethal assay (OECD TG 478) (Unpublished., 1978c)	Ambiguous	*Not tested*
Sex-Linked Recessive Lethal (SLRL) test (genetic toxicity *in vitro*, other) ; Drosophila melanogaster (Foureman *et al*., 1994)	*Not tested*	Negative
***In vitro* transformation test in BALB/3T3 cells**	Ambiguous results in the absence of exogenous activation	**Positive in the absence of exogenous activation**

The available existing data regarding the genotoxic potential of chlorendic anhydride and chlorendic acid in *in vivo* studies are presented below:

Genotoxicity tests	Chlorendic anhydride	Chlorendic acid
Mouse Dominant Lethal assay (CD-1 mice) (OECD TG 478 - *non reliable study*) (Unpublished report, 1978c)	Ambiguous negative results reported, but some statistically non-significant increases in dead implants per pregnant mouse in females mated : - at weeks 2 and 8 at the high dose (5 mg/kg bw) and - at week 5 in the low- (0.1 mg/kg bw) and mid-dose (0.5 mg/kg bw) treated mice	*Not tested*

Combined *in vivo* mammalian erythrocyte micronucleus test in bone marrow and *in vivo* mammalian comet assay (Unpublished report, 2020)	*Not tested*	Micronucleus test: negative Comet assay: negative (but some uncertainties remain especially for the liver)

Available data showed a clastogenic potential *in vitro* of the chlorendic acid, the degradation product of the registered substance chlorendic anhydride, as well as alerts for chlorendic anhydride.

Based on these results, the evaluating MSCA considered that there was a need to perform additional genotoxicity studies *in vivo*. Therefore, an *in vivo* MN test in bone marrow (OECD TG 474) combined with an *in vivo* mammalian alkaline comet assay (OECD TG 489) on the liver, glandular stomach, duodenum, gonadal cells and, if technically feasible, the pancreas, was requested by ECHA in December 2018, using the degradation product chlorendic acid administered by the oral route. These assays are indeed both suitable to follow up positive *in vitro* result for gene mutation and chromosomal aberrations.

The results of this combined *in vivo* study with chlorendic acid are clearly negative for cytogenic damage (MN test) as well as for DNA damage in the stomach (comet test). DNA damage of the other target tissues (i.e. liver, duodenum and gonad) in the comet test are equivocal with regards to the evaluation criteria of the OECD TG 489. The levels of DNA breaks following treatment with chlorendic acid are within the range of the historical control data, however a statistically significant increase compared with the concurrent negative controls is observed in the liver, but only at the highest dose tested (700 mg/kg). A significant trend towards a dose-related increase in the % of tail DNA is also observed in the liver, which would support the relevance of this result. It is not excluded that a low level of liver toxicity could have contributed to this result, considering the slightly increased liver enzymes levels (AST and ALT) observed for some animals at this dose, as also the decreased hepatocyte glycogen vacuolation noted in the liver of animals administered 700 mg/kg/day and the single cell necrosis noted in one animal dosed at this level. However, considering the results of the *in vivo* comet assay in the liver, uncertainty of the mutagenic potential of chlorendic acid remains for this tissue, especially in view of the fact that a statistically significant increase in hepatocellular carcinomas was observed in female F-344/N rats and in male B6C3F1 mice in a two-year feed study (US NTP, 1987).

Considering nonetheless all the results of the available *in vitro* and *in vivo* tests on chlorendic acid, the evaluating MSCA considers that chlorendic acid shows no clear evidence for mutagenicity. By read-across, chlorendic anhydride is not considered as clearly mutagenic either. Therefore, no classification with respect to mutagenicity is proposed for chlorendic anhydride.

7.9.6. Carcinogenicity

7.9.6.1. Non-human information

There is no available data on carcinogenicity performed with chlorendic anhydride.

A series of National Cancer Institute (NCI) carcinogenesis tests on hexachloronorbornene analogue compounds has been completed (NCI 1977a,b; 1978a,b; 1979). These chemicals (aldrin, chlordane, dieldrin, endrin, endosulfan and heptachlor) were mixed individually in feed and supplied to male and female Osborne-Mendel rats and B6C3F1 mice (10 or 20 matched animals per control group, 50 animals per low or high dose group). Pooled controls (at least 50 animals of the same strain, age and sex) from concurrent tests of other chemicals tested under the same experimental conditions were used for statistical purposes. Animals were fed the study compound for at least 80 weeks and then observed for an additional number of weeks (rats, 24-29 weeks; mice, 10 weeks) before necropsy and histologic examination. The results indicate that several of these compounds cause hepatocellular carcinomas in male B6C3F1 mice and some cause hepatocellular carcinomas

in female B6C3F1 mice (Table 34). Follicular cell adenomas of the thyroid gland were associated with chemical administration in male and female Osborne Mendel rats but not in male or female B6C3F1 mice.

Table 34: Results of NCI feed studies on hexachlorinated norbornene structural analogues of chlorendic acid

Chemical	Report No.	Organ Site	Osborne-Mendel Rats (a) Male	Osborne-Mendel Rats (a) Female	B6C3F1 Mice (a) Male	B6C3F1 Mice (a) Female
Aldrin	NCI TR 21 (1978a)	(b) Liver	No effect	No effect	3/20, 16/49, 25/45	No effect
Chlordane	NCI TR 8 (1977a)	(b) Liver (c) Thyroid gland	No effect 0/6, 1/34, 6/31	No effect 0/10, 4/43, 6/32	2/18, 16/48, 43/49 No effect	0/19, 3/47, 34/49 No effect
Dieldrin	NCI TR 21 (1978a)	(b) Liver	No effect	No effect	3/18, 12/50, 16/45	No effect
Endrin	NCI TR 12 (1979)	(b) Liver	No effect	No effect	No effect	No effect
Endosulfan	NCI TR 62 (1978b)		Inadequate study	Inadequate study	Inadequate study	Inadequate study
Heptachlor	NCI TR 9 (1977b)	(b) Liver (c) Thyroid gland	No effect No effect	No effect 1/9, 3/43, 14/38	5/19, 11/46, 34/47 No effect	2/10, 3/47, 30/42 No effect

(a) Incidence--control, low dose, high dose
(b) Hepatocellular carcinomas
(c) Follicular cell adenomas of the thyroid gland

Chlorendic acid was studied by the NTP Carcinogenesis Program after being nominated by the NCI following a review of flame retardants because of the large production, structure-activity considerations, and the potential for human exposure. The dietary route was chosen to obtain systemic exposure to chlorendic acid (US NTP, 1987).

7.9.6.1.1. Carcinogenicity: oral

The results of studies on carcinogenicity after oral administration are summarised below in Table35.

Table 35. US NTP studies (1987) on carcinogenicity after oral administration with chlorendic acid.

Method	Results	Remarks	Reference
Fischer 344/N rat, male/female Oral: feed **Exposure: 103 weeks** (daily, formulated diets available *ad libitum*) 50 animals per sex per dose Control animals : yes, concurrent no treatment No positive control Doses: 0 ppm 620 ppm (estimated mean daily consumption of 27 mg/kg bw for male and 39 mg/kg bw for female rats) 1250 ppm (estimated mean daily consumption of 56 mg/kg bw for male and 66 mg/kg bw for female rats)	**Clinical signs:** no evidence of a compound-related effect on physical appearance or behaviour. **Mortality:** no significant differences in survival were observed between any groups of either sex. **Body weight:** Mean bw of male rats treated at 1 250 ppm: 5% to 10% lower than control throughout the study. Mean bw of female rats treated at 1 250 ppm: 10% lower than control after week 11 and 20 % lower after week 57. Mean bw of female rats treated at 620 ppm:	2 (reliable with restrictions) Key study Experimental study **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid (Chlorendic acid) (Het acid), EC No 204-078-9 /CAS RN 115-28-6** Form: solid Batch: 6745 Purity : approximately 99%	US NTP (1987)

Method	Results	Remarks	Reference
Statistics were applied for survival analysis (Cox test), calculation of incidence, analysis of tumour incidence and historical control data Vehicle: unchanged (no vehicle) Equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	approximately 5% lower than those of the controls by week 10 and 10% lower by week 45. **Food consumption and compound intake:** The average daily feed consumption per animal was 96% and 122% for male and female rats, respectively, treated at 620 ppm compared to the control. The average daily feed consumption per animal was 94% and 96% for male and female rats, respectively, treated at 1 250 ppm compared to the control. **Non-neoplastic lesions**: - increased incidences of liver cystic degeneration and bile-duct hyperplasia in male rats ; - liver granulomatous inflammation and pigmentation and bile-duct hyperplasia in female rats. **LOAEL (carcinogenicity): 620 ppm (27 mg/kg bw/day (male)** based on hepatocellular adenoma and carcinoma and acinar cell adenomas of the pancreas; alveolar/bronchiolar adenomas and preputial gland carcinomas in male **LOAEL (carcinogenicity): 39 mg/kg bw/day (female)** based on: hepatocellular adenoma and carcinoma		
B6C3F1 mouse, male/female Oral: feed **Exposure: 103 weeks** (daily, formulated diets available *ad libitum*) 50 animals per sex per dose Control animals : yes, concurrent no treatment No positive control Doses: 0 ppm	**Clinical signs:** no evidence of a compound-related effect on physical appearance or behaviour. **Mortality:** no significant differences in survival were observed between any groups of either sex. **Body weight :** Mean bw of male mice treated at 620 ppm: 2% above to 9% below those	2 (reliable with restrictions) Supporting study Experimental study **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid (Chlorendic acid) (Het acid)**	US NTP (1987)

Method	Results	Remarks	Reference
620 ppm (estimated daily consumption of 89 mg/kg bw for males and 100 mg/kg bw for females 1250 ppm (estimated daily consumption was 185 mg/kg bw for males and 207 mg/kg bw for females) Vehicle: unchanged (no vehicle) Equivalent or similar to OECD TG 453 (Combined Chronic Toxicity / Carcinogenicity Studies) Statistics were applied for survival analysis (Cox test), calculation of incidence, analysis of tumour incidence and historical control data	of controls throughout the study. Mean bw of male mice treated at 1 250 ppm: 5% to 10% lower than control from week 11 to the end. Mean bw of female rats treated at 620 ppm : 2% above to 7% below those of controls throughout the study Mean bw of female mice treated at 1 250 ppm remained 5%-10% lower than control after week 11 and 20 % lower after week 57. **Food consumption and compound intake:** The average daily feed consumption per animal was 107% and 102% for male and female rats, respectively, treated at 620 ppm compared to the control. The average daily feed consumption per animal was 109% and 102% for male and female rats, respectively, treated at 1 250 ppm compared to the control. **Non-neoplastic lesions** of the liver (coagulative necrosis) observed at increased incidences in dosed male mice; **mitotic alteration** observed in high-dose female mice **LOAEL (carcinogenicity): 89 mg/kg bw/day (male)** based on gross pathology - necrosis of the liver ; histopathology: neoplastic - : hepatocellular adenomas and of hepatocellular carcinomas, hepatocellular adenoma metastasis in lung in male **LOAEL (carcinogenicity): 185 mg/kg bw/day (female)** based on increased hepatocellular	**EC No 204-078-9 /CAS RN 115-28-6** Form : solid Batch 6745 Purity: approximately 99%	

Method	Results	Remarks	Reference
	adenomas or carcinomas (combined) and combined incidence of alveolar/bronchiolar adenomas and carcinomas in female mice		
Rat (Fischer 344 [rat]), male/female **An initiation-promotion assay in rat liver as a potential complement to the 2-year carcinogenesis** Oral: feed Doses: 620 ppm 1 250 ppm Vehicle: unchanged (no vehicle) Positive control : Phenobarbital Exposure: 6 months (Feed was available *ad libitum*) Statistical method of Saltykov No guideline followed, non GLP Principle of test: Four enzyme markers - placental glutathione-S-transferase, 7-glutamyl transpeptidase, canalicular ATPase and glucose-6-phosphatase - are analysed in altered hepatic foci following a 70% partial hepatectomy and a 6-month promotion with the test substance. The purpose of this assay is to identify the carcinogenic agents acting primarily as promoting agents in rat liver.			Dragan *et al.*, 1991
Rat (Sprague-Dawley [rat]), female **Predicting Rodent Carcinogenicity of Halogenated Hydrocarbons by In Vivo Biochemical Parameters** 9 females per doses oral: gavage Doses: 159 and 477 mg/kg/dose Vehicle: corn oil Control : yes, concurrent vehicle Exposure: two doses were administrated (one 21h before sacrifice, the other 4h before sacrifice) No guideline followed, no GLP Four biochemical assays performed:	Negative results in the four biochemical assays.		Kitchin *et al.* 1993

Method	Results	Remarks	Reference
- Hepatic DNA damage by alkaline elution; - Hepatic ornithine decarboxylase activity; - Serum alanine aminotransferase activity; - Hepatic cytochrome P-450 content.			

Chlorendic anhydride

No carcinogenicity study or human carcinogenicity data with chlorendic anhydride are available.

Nevertheless, it is not relevant to consider the sole data on chlorendic anhydride and omit data on chlorendic acid which is considered as possibly carcinogenic to human group 2B by the IARC based on sufficient evidence of carcinogenicity from two experimental studies conducted by the US NTP (1978) (IARC, 1990).

Chlorendic acid

The chlorendic acid was tested for carcinogenicity by oral administration both in rats and mice.

- **Rats carcinogenicity study**

The carcinogenicity potential of the degradation product of chlorendic anhydride, chlorendic acid, was determined using a method similar to OECD TG 453 (non GLP) with deviations.

In the rat carcinogenesis study (US NTP, 1987), chlorendic acid (greater than 98% pure) was administered in feed to groups of 50 male and 50 female F344/N rats at concentrations of 0, 620, or 1 250 ppm for 103 weeks. The estimated mean daily consumption of chlorendic acid was 27 and 56 mg/kg bw for low dose and high dose male rats and 39 and 66 mg/kg for low dose and high dose female rats.

Survival and feed consumption of dosed male and female rats in the 2-year studies were similar to those of controls. Mean bw of high dose male and female rats were lower than those of controls. Mean bw of high dose female rats were 16% to 24% lower than those of controls during the second half of the study (1 250 ppm being the MTD, selected with regards to reductions in mean bw relative to controls at concentrations of 2 500 ppm and greater in the dose range-finding 13-week study).

The incidences of non-neoplastic lesions of the liver in dosed male rats (cystic degeneration) and dosed female rats (granulomatous inflammation, pigmentation, and bile duct hyperplasia) were increased (Table 36).

Table 36: Numbers of rats with liver lesions in the US NTP (1987) two-year feed study of chlorendic acid

	Concentration (ppm)					
	Male			Female		
Lesion	0	620	1,250	0	620	1,250
Number examined	50	50	50	50	49	50
Cystic degeneration	13	32	31	1	1	1
Granulomatous inflammation	1	1	1	10	21	20
Pigmentation	1	1	1	1	3	8
Focal cellular change	15	32	20	30	23	28
Bile duct hyperplasia	31	42	41	3	17	40
Neoplastic nodule	2	21	23	1	3	11
Hepatocellular carcinoma	3	5	1	0	3	5

The neoplastic nodules in male and female rats and hepatocellular carcinomas in female rats occurred with significant positive trends (p<0.001, p=0.001 and p=0.023, respectively) (

Table **37**). The incidences of neoplastic nodules of the liver were significantly increased in dosed males and high dose females when compared to the controls: in males they occurred in 2/50 (4%) controls, 21/50 (42%) low-dose (p<0.001) and 23/50 (46%) high-dose animals (p<0.001); and in females in 1/50 (2%) controls, 3/49 (6%) low-dose and 11/50 (22%) high-dose animals (p=0.004). The incidence of hepatocellular carcinomas was also significantly increased in high dose females when compared to the controls: in 0/50 controls, 3/49 (6%) low-dose and 5/50 (10%) high-dose animals (p=0.044).

Table 37: **Hepatocellular tumors in rats in the US NTP (1987) two-year feed study of chlorendic acid**

MALE RATS	**Vehicle control**	**Dose group (620 ppm)**	**Dose group (1250 ppm)**
		Equivalent to 27 mg/kg bw/d	**Equivalent to 56 mg/kg bw/d**
Neoplastic nodule			
Overall rates	2/50 (4%)	21/50 (42%)	23/50 (46%)
Adjusted rates	8.3%	61.6%	78.6%
Terminal rates	2/24 (8%)	19/32 (59%)	19/25 (76%)
Week of first observation	104	97	83
Life table tests	P<0.001	P<0.001	P<0.001
Incidental Tumor tests	P<0.001	P<0.001	P<0.001
Carcinoma			
Overall rates	3/50 (6%)	5/50 (10%)	1/50 (2%)
Adjusted rates	9.5%	15.6%	4.0%
Terminal rates	1/24 (4%)	5/32 (16%)	1/25 (4%)
Week of first observation	77	104	104
Life table tests	P=0.244N***	P=0.498	P=0.304N***
Incidental Tumor tests	P=0.277N***	P=0.371	P=0.356N***
Neoplastic nodule or hepatocellular Carcinoma*			
Overall rates	5/50 (10%)	22/50 (44%)	23/50 (46%)
Adjusted rates	17.3%	64.6%	78.4%
Terminal rates	3/24 (13%)	20/32 (63%)	19/25 (76%)
Week of first observation	77	97	83
Life table tests	P<0.001	P=0.002	P<0.001
Incidental Tumor tests	P<0.001	P<0.001	P<0.001
FEMALE RATS		**Equivalent to 39 mg/kg bw/d**	**Equivalent to 66 mg/kg bw/d**
Neoplastic nodule			
Overall rates	1/50 (2%)	3/49 (6%)	11/50 (22%)
Adjusted rates	3.2%	8.3%	31.4%
Terminal rates	1/31 (3%)	3/36 (8%)	11/35 (31%)
Week of first observation	104	104	104
Life table tests	P=0.001	P=0.359	P=0.004
Incidental Tumor tests	P=0.001	P=0.359	P=0.004
Carcinoma			
Overall rates	0/50 (0%)	3/49 (6%)	5/50 (10%)
Adjusted rates	0.0%	7.8%	14.3%
Terminal rates	0/31 (0%)	2/36 (6%)	5/35 (14%)
Week of first observation	-	95	104
Life table tests	P=0.028	P=0.146	P=0.044
Incidental Tumor tests	P=0.023	P=0.133	P=0.044
Neoplastic nodule or Carcinoma**			
Overall rates	1/50 (2%)	5/49 (10%)	16/50 (32%)
Adjusted rates	3.2%	13.2%	45.7%
Terminal rates	1/31 (3%)	4/36 (11%)	16/35 (46%)
Week of first observation	104	95	104
Life table tests	P<0.001	P=0.138	P<0.001
Incidental Tumor tests	P<0.001	P=0.130	P<0.001

* Historical incidence in NTP studies: 73/1719 (4.2% ± 3.5%)
** Historical incidence in NTP studies: 48/1766 (2.7% ± 3.0%)
***N indicates a negative trend or lower incidence in a dosed group.

The incidences of acinar cell hyperplasia and acinar cell adenomas of the pancreas were increased in dosed male rats relative to those of controls. The incidence of acinar-cell adenomas of the pancreas occurred with a significant positive trend (p=0.014) and was significantly increased in the high dose group (0/49 controls, 4/50 (8%) low-dose and 6/50 (12%) high-dose animals (p=0.018)) (Table 38). Pancreatic acinar cell adenoma is an uncommon neoplasm in untreated control F344/N rats in NTP studies (3/1667 (0.2%)).

Table 38: Pancreatic tumors in male rats in the US NTP (1987) two-year feed study of chlorendic acid

MALE RATS	Vehicle control	Dose group (620 ppm) Equivalent to 27 mg/kg bw/d	Dose group (1250 ppm) Equivalent to 56 mg/kg bw/d
Acinar cell adenoma*			
Overall rates	0/49 (0%)	4/50 (8%)	6/50 (12%)
Adjusted rates	0.0%	11.3%	24.0%
Terminal rates	0/24 (0%)	3/32 (9%)	6/25 (24%)
Week of first observation		88	104
Life table tests	P=0.011	P=0.104	P=0.018
Incidental Tumor tests	P=0.014	P=0.082	P=0.018

* Historical incidence in NTP studies: 3/1667 (0.2% ± 0.6%)

In dosed male rats, alveolar/bronchiolar adenomas of the lung occurred with a significant positive trend (p=0.014) and the incidence in the high dose group was significantly increased when compared to the controls (0/50 controls, 3/50 (6%) low-dose and 5/50 (10%) high-dose animals (p=0.021)) (Table 39).

Table 39: Lung lesions in male rats in the US NTP (1987) two-year feed study of chlorendic acid

MALE RATS	Vehicle control	Dose group (620 ppm) Equivalent to 27 mg/kg bw/d	Dose group (1250 ppm) Equivalent to 56 mg/kg bw/d
Alveolar Epithelial Hyperplasia			
Overall rates	1/50 (2%)	1/50 (2%)	1/50 (2%)
Alveolar/Bronchiolar Adenoma			
Overall rates	0/50 (0%)	3/50 (6%)	5/50 (10%)
Adjusted rates	0.0%	9.4%	18.5%
Terminal rates	0/24 (0%)	3/32 (9%)	3/25 (12%)
Week of first observation	-	104	100
Life table tests	P=0.019	P=0.175	P=0.036
Incidental Tumor tests	P=0.014	P=0.175	P=0.021
Alveolar/Bronchiolar Carcinoma			
Overall rates	0/50 (0%)	1/50 (2%)	0/50 (0%)
Alveolar/Bronchiolar Adenoma or Carcinoma*			
Overall rates	0/50 (0%)	4/50 (8%)	5/50 (10%)
Adjusted rates	0.0%	12.5%	18.5%
Terminal rates	0/24 (0%)	4/32 (13%)	3/25 (12%)
Week of first observation	-	104	100
Life table tests	P=0.025	P=0.104	P=0.036
Incidental Tumor tests	P=0.019	P=0.104	P=0.021

* Historical incidence in NTP studies: 35/1723 (2% ± 2%)

In the two-year feed study the reproductive organs were also affected in male and female rats in a non-dose-dependent manner. Incidence in preputial gland carcinoma was significantly (p=0.035) increased in low-doses male rats (8/50 (16%)) compared to the control group (1/50 (2%)). Incidence in preputial gland adenoma, carcinoma or squamous

cell papilloma also significantly increased in low-dose male rats compared to control rats (1/50 (2%) controls; 10/50 (20%) low-dose male rats (p=0.012); 4/50 (8%) high-dose male rats) (Table 40). In low-dose males, combined adenoma, carcinoma and squamous cell papilloma rates are the following: overall rates: 10/50 (20%); adjusted rates: 27.8%; terminal rates: 7/32 (22%). These incidences are clearly above the NTP historical control data (6%±5%).

Table 40: Preputial gland tumors in male rats in the US NTP (1987) two-year feed study of chlorendic acid

MALE RATS	Vehicle control	Dose group (620 ppm) Equivalent to 27 mg/kg bw/d	Dose group (1250 ppm) Equivalent to 56 mg/kg bw/d
Carcinoma			
Overall rates	1/50 (2%)	8/50 (16%)	4/50 (8%)
Adjusted rates	4.2%	22.7%	13.2%
Terminal rates	1/24 (4%)	6/32 (19%)	2/25 (8%)
Week of first observation	104	81	82
Life table tests	P=0.194	P=0.047	P=0.189
Incidental Tumor tests	P=0.198	P=0.035	P=0.185
Adenoma, Carcinoma or Squamous cell Papilloma*			
Overall rates	1/50 (2%)	10/50 (20%)	4/50 (8%)
Adjusted rates	4.2%	27.8%	13.2%
Terminal rates	1/24(4%)	7/32 (22%)	2/25 (8%)
Week of first observation	104	81	82
Life table tests	P=0.210	P=0.018	P=0.189
Incidental Tumor tests	P=0.201	P=0.012	P=0.185

* Historical incidence in NTP studies: 105/1727 (6% ± 5%)

Uterus/endometrium was affected with a significant increase in the incidence of endometrial stromal polyps in low dose female rats compared to control rats. The incidence are 6/50 (12%) in control female rats, 15/49 (31%) in low dose female rats (p=0.040) and 10/50 (20%) in high dose female rats. The incidence in the low dose female rats are clearly above the NTP historical control data (22%±8%).

Table 41: Uterine tumors in female rats in the US NTP (1987) two-year feed study of chlorendic acid

FEMALE RATS	Vehicle control	Dose group (620 ppm) Equivalent to 39 mg/kg bw/d	Dose group (1250 ppm) Equivalent to 66 mg/kg bw/d
Endometrial Stromal Polyp			
Overall rates	6/50 (12%)	15/49 (31%)	10/50 (20%)
Adjusted rates	17.8%	39.1%	27.5%
Terminal rates	5/31 (16%)	13/36 (36%)	9/35 (26%)
Week of first observation	58	86	88
Life table tests	P=0.271	P=0.051	P=0.276
Incidental Tumor tests	P=0.274	P=0.040	P=0.315

* Historical incidence in NTP studies: 383/1750 (22% ± 8%)

An increase in incidences of sarcomas, fibrosarcomas, or neuro-fibrosarcomas (combined) of the salivary gland of dosed male rats was observed (1/50 (2%) in control male rats; 2/49 (4%) in low-dose male rats; 4/50 in high-dose male rats (8%)). The incidences in the dosed groups were not significantly different from that in the controls, but these tumors are uncommon in F344/N rats receiving no treatment (3/1689 (0.2%) in NTP studies).

Acinar cell adenomas in male rats occurred with a significant positive trend (p=0.014), and the incidence in the high-dose group was significantly greater than that in the controls (p=0.018) (Table 42). In female rats, adenomas were observed in 1/49 of the low-dosed mice and 1/50 in the high-dosed mice.

Table 42: **Pancreatic tumors in male rats in the US NTP (1987) two-year feed study of chlorendic acid**

MALE RATS	Vehicle control	Dose group (620 ppm) Equivalent to 27 mg/kg bw/d	Dose group (1250 ppm) Equivalent to 56 mg/kg bw/d
Acinar cell adenoma*			
Overall rates	0/49 (0%)	4/50 (8%)	6/50 (12%)
Adjusted rates	0.0%	11.3%	24.0%
Terminal rates	0/24 (0%)	3/32 (9%)	6/25 (24%)
Week of first observation		88	104
Life table tests	P=0.011	P=0.104	P=0.018
Incidental Tumor tests	P=0.014	P=0.082	P=0.018

* Historical incidence in NTP studies: 3/1667 (0.2% ± 0.6%)

The incidence of tumours in other organs (mammary gland, adrenal gland, testis and pituitary gland) occurred with a negative trends, i.e. they were not significantly increased.

A summary of the relevant results regarding carcinogenicity of chlorendic acid in F-344/N rats in the NTP 2-year feed study is provided below in Table 43.

Table 43: Summary of incidence of relevant tumours in F-344/N rats

Type of tumour	Males			Females		
	Control	620 ppm	1250 ppm	Control	620 ppm	1250 ppm
Liver						
Hepatocellular adenoma (neoplastic nodule)*	2/50 (4%)	21/50 (42%)**	23/50 (46%)**	1/50 (2%)	3/49 (6%)	11/50 (22%)**
Hepatocellular carcinoma*				0/50 (0%)	3/49 (6%)	5/50 (10%)**
Pancreas						
Acinar cell adenoma*	0/49 (0%)	4/50 (8%)	6/50 (12%)**			
Lungs						
Alveolar/bronchiolar adenoma*	0/50 (0%)	3/50 (6%)	5/50 (10%)**			
Preputial gland						
Carcinoma	1/50 (2%)	8/50 (16%)**	4/50 (8%)			
Squamous cell papilloma, adenoma or carcinoma	1/50 (2%)	10/50 (20%)**	4/50 (8%)			
Uterus/Endometrium						
Endometrial stromal polyp				6/50 (12%)	15/49 (31%)**	10/50 (20%)
Salivary gland						
Fibrosarcoma/neurofibrosarcoma	1/50 (2%)	2/49 (4%)	4/50 (8%)			

*Significant ($p \leq 0.05$) positive trend in tumour incidences across the groups
**Significant ($p \leq 0.05$) increase in incidence when compared with the control group

Based on the study results, no NOAEL is determined. The LOAEL is set at **620 ppm** (equivalent to 27 mg/kg bw for male rats and 39 mg/kg bw/day in female rats).

In summary, there was clear evidence of carcinogenicity of chlorendic acid for F-344/N male rats, as shown by increased incidences of adenomas in the liver and acinar cell adenomas of the pancreas. Increased incidences of alveolar/bronchiolar adenomas and preputial gland carcinomas in male rats may also have been related to the administration of chlorendic acid. There was also clear evidence of carcinogenicity of chlorendic acid for F-344/N female rats, as shown by increased incidences of adenomas and carcinomas in the liver and by increased incidence in endometrial stromal polyp.

- **Mice carcinogenicity study**

The carcinogenicity potential of chlorendic acid was determined using a method similar to OECD Test Guideline 453 (non GLP) with deviations.

In the mouse carcinogenicity study (US NTP, 1987), diets containing 0, 620 or 1250 ppm chlorendic acid (purity > 98%) were fed to groups of 50 male and 50 female B6C3F1 mice for 103 weeks. The estimated daily intake of chlorendic acid was 89 and 185 mg/kg bw for low and high-dose males and 100 and 207 mg/kg bw for low and high-dose female mice. All survivors were killed at week 112.

Survival and feed consumption of dosed male and female mice were similar to those of controls. Mean bw of high dose male (33.7 gr) and high dose female mice (29.8 g) were lower than those of controls (36.1 g and 31.8 g for control male and female respectively).

The incidences of non-neoplastic lesions of the liver were increased in dosed males (coagulative necrosis). Hepatocellular adenomas, carcinomas and hepatocellular adenomas or carcinomas (combined) occurred with a significant positive trend (incidental tumor test for trend of respectively p=0.041, p=0.023 and p=0.003)) in males (Table 44). The incidences of hepatocellular adenomas in high-dose males were significantly greater than those in the controls (5/50 (10%) controls; 9/49 (18%) low-dose male mice; 10/50 (20%) high-dose male mice (p=0.050)), as also the incidences of hepatocellular carcinomas (9/50 (18%) controls, 17/49 (35%) low-dose male mice ; 20/50 (40%) high-dose male mice (p=0.038). The incidences of hepatocellular adenomas or carcinomas (combined) in dosed males were significantly greater than those in the controls (13/50 (26%) controls; 23/49 (47%) low-dose male mice (p=0.028); 27/50 (54%) high-dose male mice (p=0.005)). Hepatocellular carcinomas metastasized to the lung in 2/50 male controls, 4/49 low-dose males and 7/50 high-dose males.

Table 44: Hepatocellular tumors in male B6C3F1 mice in the US NTP (1987) two-year feed study of chlorendic acid

MALE RATS	Vehicle control	Dose group (620 ppm) Equivalent to 89 mg/kg bw/d	Dose group (1250 ppm) Equivalent to 185 mg/kg bw/d
Hepatocellular Adenoma			
Overall rates	5/50 (10%)	9/49 (18%)	10/50 (20%)
Adjusted rates	13.5%	30.1%	33.3%
Terminal rates	5/37 (14%)	8/28 (29%)	9/29 (31%)
Week of first observation	105	30	102
Life table tests	P=0.038	P=0.077	P=0.047
Incidental Tumor tests	P=0.041	P=0.081	P=0.050
Hepatocellular Carcinoma			
Overall rates	9/50 (18%)	17/49 (35%)	20/50 (40%)
Adjusted rates	22.1%	46.5%	51.8%
Terminal rates	6/37 (16%)	9/28 (32%)	11/29 (38%)
Week of first observation	70	75	60
Life table tests	P=0.004	P=0.018	P=0.005
Incidental Tumor tests	P=0.023	P=0.084	P=0.038
Hepatocellular Adenoma or Carcinoma*			
Overall rates	13/50 (26%)	23/49 (47%)	27/50 (54%)
Adjusted rates	32.2%	61.4%	70.6%
Terminal rates	10/37 (27%)	14/28 (50%)	18/29 (62%)
Week of first observation	70	30	60
Life table tests	P<0.001	P=0.006	P<0.001
Incidental Tumor tests	P=0.003	P=0.028	P=0.005

* Historical incidence in NTP studies: 540/1784 (30% ± 8%)

The incidences of non-neoplastic lesions of the liver were increased in high-dose female mice (mitotic alterations). In dosed female mice, the incidences of hepatocellular adenomas or carcinomas (combined) were somewhat increased but not significantly different from that in the controls (3/50 (6%) controls; 7/49 (14%) low-dosed; 7/50 (14%) high-dosed).

The combined incidences of lung alveolar/bronchiolar adenomas and carcinomas in females occurred with a significant positive trend (p=0.037), but the incidence in the dosed-groups were not significantly increased when compared to the controls (1/50 (2%) controls, 5/50 (10%) low-dose animals and 6/50 (12%) high-dose animals) (Table 45). Moreover, the incidence in the concurrent controls (1/50 (2%)) compared with the historical control mean (7% ± 4%) was low, which makes the biological significance of these results unclear.

Table 45: Lung lesions in female B6C3F1 mice in the US NTP (1987) two-year feed study of chlorendic acid

FEMALE RATS	Vehicle control	Dose group (620 ppm) Equivalent to 100 mg/kg bw/d	Dose group (1250 ppm) Equivalent to 207 mg/kg bw/d
Alveolar Epithelial Hyperplasia			
Overall rates	0/50 (0%)	0/50 (0%)	1/50 (2%)
Alveolar/Bronchiolar Adenoma			
Overall rates	0/50 (0%)	4/50 (8%)	4/50 (8%)
Adjusted rates	0.0%	10.3%	10.5%
Terminal rates	0/39 (0%)	4/39 (10%)	3/35 (9%)
Week of first observation	-	104	74
Life table tests	P=0.047	P=0.063	P=0.054
Incidental Tumor tests	P=0.050	P=0.063	P=0.066
Alveolar/Bronchiolar Carcinoma			
Overall rates	1/50 (2%)	2/50 (4%)	2/50 (4%)
Alveolar/Bronchiolar Adenoma or Carcinoma*			
Overall rates	1/50 (2%)	5/50 (10%)	6/50 (12%)
Adjusted rates	2.6%	12.8%	16.1%
Terminal rates	1/39 (3%)	5/39 (13%)	5/35 (14%)
Week of first observation	104	104	74
Life table tests	p=0.034	P=0.103	P=0.045
Incidental Tumor tests	P=0.037	P=0.103	P=0.053

* Historical incidence in NTP studies: 122/1777 (7% ± 4%)

Follicular-cell adenomas of the thyroid in male mice occurred with a significant positive trend (p=0.039), but the observed increase in incidence in the high-dosed group was not significant when compared to the controls (0/50 controls; 0/47 low-dosed; 3/50 (6%) high-dosed) (Table 46). No follicular lesions were reported in female mice.

Table 46: Thyroid gland lesions in male B6C3F1 mice in the US NTP (1987) two-year feed study of chlorendic acid

MALE RATS	Vehicle control	Dose group (620 ppm) Equivalent to 89 mg/kg bw/d	Dose group (1250 ppm) Equivalent to 185 mg/kg bw/d
Follicular Cell Hyperplasia			
Overall rates	2/50 (4%)	1/47 (2%)	1/50 (2%)
Follicular Cell Adenoma*			
Overall rates	0/50 (0%)	0/47 (0%)	3/50 (6%)
Adjusted rates	0.0%	0.0%	9.1%
Terminal rates	0/37 (0%)	0/28 (0%)	2/29 (7%)
Week of first observation	-	-	67
Life table tests	P=0.030	-	P=0.093
Incidental Tumor tests	P=0.05039	-	P=0.120

*Historical incidence of Follicular Cell Adenoma and Carcinoma (combined) in NTP studies: 28/1680 (2% ± 2%)

Adenomas or carcinomas in the pituitary gland in female mice occurred with a significant negative trend, and the incidences in the dosed groups were significantly lower than that in the controls.

Squamous cell papillomas in female mice occurred with a significant negative trend (control, 3/50; low dose, 0/48; high dose, 0/50). The incidences in the dosed groups were not significantly lower than that in the controls.

A summary of the relevant results regarding carcinogenicity of chlorendic acid in B6C3F1 mice in the NTP 2-year feed study is provided below in Table 47.

Table 47: Summary of incidence of relevant tumours in B6C3F1 mice

Type of tumour	Males			Females		
	Control	620 ppm	1250 ppm	Control	620 ppm	1250 ppm
Liver						
Hepatocellular adenoma*	5/50 (10%)	9/49 (18%)	10/50 (20%)**			
Hepatocellular carcinoma*	9/50 (18%)	17/49 (35%)	20/50 (40%)**			
Hepatocellular adenoma or carcinoma***	13/50 (26%)	23/49 (47%)**	27/50 (54%)**	3/50 (6%)	7/49 (14%)	7/50 (14%)
Lung						
Alveolar/bronchiolar adenoma or carcinoma*				1/50 (2%)	5/50 (10%)	6/50 (12%)
Thyroid						
Follicular cell adenoma*	0/50 (0%)	0/47 (0%)	3/50 (6%)			

*Significant ($p \leq 0.05$) positive trend in tumour incidences across the groups
**Significant ($p \leq 0.05$) increase in incidence when compared with the control group
*** Significant ($p \leq 0.05$) positive trend in tumour incidences across the male groups only

Based on the study results, no NOAEL is determined. The **LOAEL is set at 620 ppm** (equivalent to 89 mg/kg bw for male mice and 100 mg/kg bw for female mice), based on significant increase in incidences of hepatocellular adenoma or carcinoma in male mice.

In summary, there was clear evidence of carcinogenicity of chlorendic acid for B6C3F1 male mice, as shown by a significant increase in incidences of hepatocellular adenomas and of hepatocellular carcinomas. There was no evidence of carcinogenicity of chlorendic acid for female mice given chlorendic acid in the diet at concentrations of 620 or 1 250 ppm for 103 weeks.

- **Other studies**

Additional studies were performed on chlorendic acid to identify its carcinogenicity using alternative methods. Chlorendic acid was identified as a promoting carcinogenic agent in the rat liver according to the initiation-promotion assay (Dragan *et al.,* 1991), while an *in vivo* biochemical assay returned negative results (Kitchin *et al.,* 1993).

An initiation-promotion assay in rat was conducted on chlorendic acid to identify if it was acting as a promoting carcinogenic agent in the liver. No test guideline was followed and the test was conducted according to a non-standard method. Male and female rats were used. A non necrogenic, subcarcinogenic dose of the initiator diethylnitrosamine (10 mg/kg) was administered at 24 hr after the proliferative stimulus provided by a 70% partial hepatectomy. After recovery period of 2 weeks, the positive control or the test agent (chlorendic acid at 620 or 1 250 ppm) was administered to groups of animals. After 6 months of administration (promotion), the rats were killed, the livers removed and the number, size, and phenotypic distribution of altered hepatic foci resulting from the treatment regimens were quantified by stereology. A dose-dependent increase in the number of altered hepatic foci was observed in animals of both sexes when chlorendic acid was administered for 6 months, compared to the control, indicating that chlorendic acid can act as a promoting carcinogenic agent in rat liver (Dragan *et al.,* 1991).

An *in vivo* biochemical assay was performed on chlorendic acid to evaluate its carcinogenic potential (Kitchin *et al*., 1993). No test guideline was followed and the test was conducted according to a non-standard method. The test chemical was administered orally by gavage at two dose levels (either 159 or 477 mg/kg) to female rats, one time at 21h before sacrifice, and the second time 4h before sacrifice. The rats were 90-day-old Sprague-Dawley females (CD strain). Four biochemical assays, indicative of a substance potential for promotion of carcinogenesis, cell toxicity and/or DNA damage were performed (hepatic

DNA damage by alkaline elution, hepatic ornithine decarboxylase activity, serum alanine aminotransferase activity and hepatic cytochrome P-450 content). Chlorendic acid exhibited negative results, i.e. "no change" in these four biochemical assays.

7.9.6.1.2. Carcinogenicity: inhalation

No data.

7.9.6.1.3. Carcinogenicity: dermal

No data.

7.9.6.1.4. Carcinogenicity: other routes

No data.

7.9.6.2. Human information

No data.

7.9.6.3. Summary and discussion of carcinogenicity

Oral exposure to chlorendic acid caused tumors in two rodent species and at several tissue sites. In the 2-year chlorendic acid feed studies (US NTP 1987):

- There was clear evidence of carcinogenicity of chlorendic acid for male F344/N rats as shown by increased incidences of neoplastic nodules of the liver and acinar cell adenomas of the pancreas. Increased incidences of alveolar/bronchiolar adenomas and preputial gland carcinomas in male rats may also have been related to the administration of chlorendic acid.
- There was clear evidence of carcinogenicity of chlorendic acid for female F344/N rats as shown by increased incidences of neoplastic nodules and of carcinomas of the liver.
- There was clear evidence of carcinogenicity of chlorendic acid for male B6C3F1 mice as shown by increased incidences of hepatocellular adenomas and of hepatocellular carcinomas.
- There was no clear evidence of carcinogenicity of chlorendic acid for female B6C3F1 mice given chlorendic acid in the diet at concentrations of 620 or 1 250 ppm for 103 weeks (US NTP, 1987).

No human carcinogenicity data on chlorendic acid are available.

Uncertainty remains regarding the carcinogenic mode of action of chlorendic acid, considering the equivocal results of the *in vivo* comet assay especially in the liver, which is the target organ for increased incidence of malignant tumors in the rodent's carcinogenicity studies (US NTP, 1987).

Chlorendic anhydride is self-classified by the Registrant as a suspected carcinogen (Carc. 2) based on the self-classification of chlorendic acid, present as an impurity at concentration higher than 0.1%.

Nonetheless, in light of the results of the carcinogenicity studies performed with chlorendic acid, the evaluating MSCA considers that a classification of chlorendic anhydride as Carc. Cat 1B H 351 is appropriate.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

7.9.7.1. Effects on fertility

7.9.7.1.1. Non-human information

Table 48: Study on fertility toxicity with chlorendic anhydride

Method	Results	Remarks	References
Rat (CD), male/female Oral: feed **Exposure: 28 days (daily)** Doses: 500 ppm 1 000 ppm 2 500 ppm 5 000 ppm 10 000 ppm 5 animals per sex per dose Equivalent or similar to OECD TG 407 (Repeated Dose 28-Day Oral Toxicity in Rodents) Non GLP	See section 7.9.4.1.1	2 (reliable with restrictions) Supporting study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	Unpublished report (1978f)
Rat (Crj:CD) (SD), male/female Oral: feed **Exposure: 90 days (daily)** Doses: 100 ppm 500 ppm 2 500 ppm 15 animals per sex per dose Control animals : yes, concurrent no treatment Positive control : none Equivalent or similar to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) Non GLP	See section 7.9.4.1.1 Tissues examined included: adrenals; prostate/uterus; mammary gland; testis/ovary; thyroid Gross pathology: the 3 rats from the 2 500 ppm group that died during the course of the study did not show any compound-related lesions. None of the rats that were sacrificed at the termination of the study had any compound-related lesions Histopathology: no compound related microscopic lesions were observed in any of the tissues from rats that were examined from the 2 500 ppm group	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** No purity specified	Unpublished report (1980)
Mouse (CD-1), male/female Oral: feed **Exposure: 5 days (daily)** Doses : 0, 50, 100, 500, 1000 and 5000 mg/kg Vehicle: DMSO	Fetal mortality (PO): >223 mg/kg bw/day (actual dose received) (male/female) **Overall reproductive toxicity:** not specified A statistically significant decrease was observed in	2 (reliable with restrictions) Key study Experimental study **Test material: 1,4,5,6,7,7-**	Unpublished report (1978c)

Positive control: triethyleneamine 20 male mice/group Following a single exposure, each male was rested 2 days and then mated for 5 days/week with two untreated females each week for 7 consecutive weeks. Mated females were sacrificed 14 days after the midweek of the mating period. Pregnant females were scored for dominant lethal indices at mid-pregnancy. Dominant lethality is typically determined from a mutation index derived from the ratio of dead to total implants or the number of dead implants per pregnant female. These ratios are compared with both concurrent and historical control data for significant statistical differences.	the fertility index, relative to controls, for all females mated to treated males during week 5 and females mated to mid-dose level males (not specified) during week 4. There were no differences between females mated to treated and control males with respect to average number of implantations per pregnant female, average resorptions per pregnant female, proportions of dead implants/implants, proportions of females with one or more resorptions, or proportions of females with two or more resorptions. Although the study was reported as giving negative results, there were some statistically non-significant increases in dead implants per pregnant mouse in females mated at weeks 2 and 8 at the high-dose and at week 5 in the low- and mid-dose (not specified) treated mice.	**hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride (chlorendic anhydride); EC 204-077-3 / CAS RN 115-27-3**	

Table 49: Study on fertility toxicity with chlorendic acid

Method	Results	Remarks	References
Rat (Fischer 344 [rat]), male/female Oral: feed **Exposure: 13 weeks (90 days)** 50 animals per sex per dose Control animals : yes, concurrent (no treatment) Doses: 0 ppm 620 ppm (eq. 27 mg/kg bw (male) and 39 mg/kg bw (female)) 1 250 ppm (eq. 56 mg/kg bw (male) and 66 mg/kg bw (female)) 2 500 ppm (eq. 112 mg/kg bw (male) and 132 mg/kg bw (female)) 5 000 ppm (eq. 224 mg/kg bw (male) and 264 mg/kg bw (female))	(see 'subchronic toxicity: oral_chlorendic acid_key study' for details on the results) **Data related to reproductive toxicity:** No effects were identified on reproductive organs during this subchronic toxicity study by oral route on rats.	2 (reliable with restrictions) Weight of evidence Experimental study **Test material : 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid** Form: solid Purity : approximately 99%	US NTP (1987)

10 000 ppm (eq. 448 mg/kg bw (male) and 528 mg/kg bw (female)) Vehicle: unchanged (no vehicle) Statistics were applied for survival analysis (Cox test), calculation of incidence, analysis of tumour incidence and historical control data Equivalent to OECD TG 408			
Mouse (B6C3F1), male/female Oral: feed **Exposure: 13 weeks (formulated diets** available *ad libitum*) Doses: 1 250 ppm (eq. 185 mg/kg bw (male) and 207 mg/kg bw (female)) 2 500 ppm (eq. 370 mg/kg bw (male) and 414 mg/kg bw (female)) 5 000 ppm (eq. 740 mg/kg bw (male) and 828 mg/kg bw (female)) 10 000 ppm (eq. 1480 mg/kg bw (male) and 1656 mg/kg bw (female)) 20 000 ppm (eq. 2960 mg/kg bw (male) and 3312 mg/kg bw (female)) Vehicle: unchanged (no vehicle) Equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) Non GLP	(see 'subchronic toxicity: oral_chlorendic acid_key study' for details on the results) No effects on reproductive organs observed	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid EC 204-078-9 /CAS RN 115-28-6 (HET Acid)** Purity : approximately 99%	US NTP (1987)
Rat (Fischer 344 [rat]), male/female Fertility Oral: feed **Exposure: 103 weeks** Doses: 0 ppm 620 ppm (eq. 27 mg/kg bw (male) and 39 mg/kg bw (female)) 1 250 ppm (eq. 56 mg/kg bw (male) and 66 mg/kg bw (female)) 50 animals per sex per dose Control animals : yes, concurrent no treatment Vehicle: unchanged (no vehicle) Equivalent to OECD TG 453	(see 'carcinogenicity_chlorendic acid_key study' for details on the results) **Gross pathological findings :** The incidence of carcinomas in the **preputial gland** at 620 ppm in male rats was significantly greater than those in the controls. **Uterine cysts** were observed at increased incidence in female rats treated at 1 250 ppm. The incidence of **endometrial stromal polyps** in female rats treated at 620 ppm was significantly greater than that in the controls. The incidence of fibroadenomas in the	2 (reliable with restrictions) Weight of evidence Experimental study **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid** Form: solid Purity : approximately 99%	US NTP (1987)

Non GLP	mammary gland in female rats treated at 1 250 ppm was significantly lower than that in the controls. Interstitial cell tumors in testes of male rats occurred with a significant negative trend, and the incidences in the treated groups were significantly lower than that in the controls. Adenomas and adenomas or carcinomas (combined) in the pituitary gland of female rats occurred with significant negative trends, and the incidences in the group treated at 1 250 ppm were significantly lower than those in the controls. **Overall reproductive toxicity:** yes, lowest effective dose at 620 ppm		
B6C3F1 mouse, male/female Oral: feed **Exposure: 103 weeks** (daily, formulated diets available *ad libitum*) 50 animals per sex per dose Control animals : yes, concurrent no treatment No positive control Doses: 0 ppm 620 ppm (estimated daily consumption of 89 mg/kg bw for males and 100 mg/kg bw for females 1250 ppm (estimated daily consumption was 185 mg/kg bw for males and 207 mg/kg bw for females) Vehicle: unchanged (no vehicle) Equivalent or similar to OECD TG 453 (Combined Chronic Toxicity / Carcinogenicity Studies) Non GLP	(see 'carcinogenicity_chlorendic acid_key study' for details on the results) No effects on reproductive organs observed	2 (reliable with restrictions) Weight of evidence Experimental study **Test material: 1,4,5,6,7,7-Hexachlorendo-5-norbornene-2,3-dicarboxylic acid Chlorendic acid** Form: solid Purity : approximately 99%	US NTP (1987)

7.9.7.1.2. Human information

No data.

7.9.7.2. Developmental toxicity

7.9.7.2.1. Non-human information

The results of the study on developmental toxicity are summarised in the following table:

Table 50: Study on developmental toxicity with chlorendic anhydride

Method	Results	Remarks	Reference
Rat Crj: CD(SD) Oral: gavage **Exposure: from day 6 to day 19 of gestation (daily)** Doses: 25, 100 and 400 mg/kg/day (actual ingested) Vehicle: corn oil (10 ml/kg/day) 25 pregnant females/dose group All statistical analyses compared the treatment groups with the control group with the level of significance at $p < 0.05$. Male to female fetal sex ratio number of litters with anomalies and number of foetuses with anomalies compared using the Chi square test criterion with Yates correction for 2 x 2 contingency tables and / or Fishers exact probability test as described by Siegel to judge significance of differences. The proportion of early and late resorbed foetuses, non-viable foetuses and post implantation losses compared by the Mann Whitney U-test as described by Siegel and Weil to judge significance of differences. Mean number of corpora lutea total implantations and viable foetuses compared by analysis of variance (one-way classification) Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison table to judge significance of differences. Fetal bw compared by analysis of variance (hierarchal classification) and t-test as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences. Equivalent or similar to OECD TG 414 (Prenatal Developmental Toxicity Study) Non GLP	**Maternal toxicity**: no animals died during the study but reduced mean bw gains at highest dose observed when compared to the control group. **NOEL (maternal toxicity): 100 mg/kg bw/day** (based on significant decrease in bw and bw gain) **Embryotoxic / teratogenic effects**: no effects ; - no malformed foetuses in the control group, - 1 malformed foetus in the 25 mg/kg/day group, - 2 malformed foetuses in two litters in the 100 mg/kg/day group, - 1 malformed foetus in the 400 mg/kg/day group. These malformations were not statistically significant and the incidence in malformations in the chlorendic anhydride groups were not biologically meaningful when compared to the control group. The variations observed were similar for all groups. Chlorendic anhydride is not considered teratogenic in rats in dosage levels up to and including 400 mg/kg/day. **Foetotoxicity:** significant increased number of post-implantation losses at 400 and 100 mg/kg bw/day when compared to the concurrent control (however only slightly higher than the mean for historical control **NOEL (foetotoxicity): 25 mg/kg bw/day**)	2 (reliable with restrictions) Key study Experimental result **Test material (EC name): 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-ene-2,3-dicarboxylic anhydride EC 204-077-3 / CAS RN 115-27-3** Purity: 93.81%	Unpublished report (1978g)

7.9.7.2.2. Human information

No data.

7.9.7.3. Summary and discussion of reproductive toxicity

7.9.7.3.1. Effects on fertility

Chlorendic anhydride

Effects on fertility of chlorendic anhydride were not investigated in appropriate studies.

In a 28-day range finding study (IRDC, 1978f), Charles River CD rats (5 animals/sex/dose level) were administered chlorendic anhydride *via* diet at dosage levels of 0, 500 ppm (equivalent to 53 mg/kg bw/day for males and 59 mg/kg bw/day for females), 1 000 ppm (equivalent to 108 mg/kg bw/day for males and 115 mg/kg bw/day for females), 2 500 ppm (equivalent to 282 mg/kg bw/day for males and 287 mg/kg bw/day for females), 5 000 ppm (529 mg/kg bw/day for males and 606 mg/kg bw/day for females) and 10 000 ppm (equivalent to 1 113 mg/kg bw/day for males and 1 242 mg/kg bw/day for females). No compound-related gross lesions were seen in any of the tested animals (see also section 7.9.4.1.1).

In a rat repeated-dose toxicity 90-day study (IRDC, 1980), chlorendic anhydride was administered *via* diet to four groups of rats (see section 7.9.4.1.1). 15 animals of each sex per groups received 0, 100, 500, and 2 500 ppm (approximately 0, 8, 39, or 202 mg/kg bw/day in males and 0, 8, 45, or 226 mg/kg bw/day in females). No compound-related gross lesions were seen in any of the rats from the treatment groups. No compound-related microscopic lesions were seen in any of the tissues from rats that were examined from the 2 500 ppm group.

In a mouse Dominant Lethal assay (Unpublished report, 1978c), 6 groups of 20 male mice were administered chlorendic anhydride *via* oral gavage at 0, 50, 100, 500, 1000 or 5000 mg/kg. Following a single exposure, each male was rested 2 days and then mated for 5 days/week with 2 untreated females each week for 7 consecutive weeks. Pregnant females were scored for dominant lethal indices at mid-pregnancy. There were some statistically non-significant increases in dead implants per pregnant mouse in females mated at weeks 2 and 8 at the high-dose and at week 5 in the low- and mid-dose treated mice. Both pre- and post- implantation losses contribute to dominant lethality. Chlorendic anhydride was considered to be inactive in this Dominant Lethal assay and did not induce dominant lethality under the test conditions employed in this evaluation. However, there was a significant decrease in the fertility index in all females mated to treated male mice. Nevertheless, this study is of poor quality.

No effect on reproductive organs was observed in repeated toxicity studies.

Chlorendic acid

Information on the toxicity to reproductive organs of chlorendic acid were obtained during two repeated-dose toxicity studies by the oral route on Fischer 344 rats and on B6C3F1 mice: a subchronic study (13 weeks) and a 2-year study performed according to OECD TG 453 (non GLP), both conducted by the US NTP (1987).

No effects were identified on reproductive organs during the subchronic toxicity study. During the carcinogenicity study, F-344/N rats were exposed at concentrations of 0, 620, and 1 250 ppm of chlorendic acid in food for 103 weeks. Clinical signs, bw, and food consumption were recorded. At the end of the study, surviving animals were subjected to gross necropsy and histopathology. Effects on preputial gland (significant increase of carcinomas in 620 ppm dosed male rats compared to control rats) along with effects on uterus (significant increase of endometrial stromal polyp in 620 ppm dosed female rats compared to control rats) that could be related to the exposure to chlorendic acid were identified.

No effects on reproductive organs were observed in a subchronic feed study (13 weeks) nor a 2-year feed study conducted on B6C3F1 mice (US NTP, 1987).

7.9.7.3.2. Developmental toxicity

Chlorendic anhydride

The developmental toxicity of chlorendic anhydride was investigated in a prenatal developmental toxicity study on pregnant Charles River CD rats according to a method similar to OECD TG 414 (non GLP) (IRDC, 1978g). The compound was administered by gastric intubation at dosage levels of 25, 100 and 400 mg/kg/day from days 6 through 15 of gestation (25 animals per group). A control group received the vehicle corn oil at 10 ml/kg/day. During gestation, the females were observed for clinical signs of effect for mortality and for changes in bw gains. Caesarean sections were performed on gestation day 20. The number of viable and non-viable foetuses, early and late resorptions corpora lutea and total implantations were recorded. There were no differences in maternal bw or changes in appearance or behaviour for rats in the 25 or 100 mg/kg/day dosage group when compared to the control group. There was a slight increase in matted fur red nasal discharge and anogenital staining in the 400 mg/kg/day dosage group when compared to the control group. Survival was 100% for all groups. Significant differences were observed between treated and control animals in the following parameters:

i) decreased maternal bw (slight mean bw loss during the first 3 days of treatment) and weight gain in the 400 mg/kg/day treated group,

ii) fetal male to female sex ratio in the 25 mg/kg/day dosage group,

iii) increased mean number of post-implantation losses (100 and 400 mg/kg/day dosage groups). The difference in the male to female sex ratio was considered as attributable to random occurrence and not compound-related.

As the significant increase in the mean number of post-implantation losses in the 100 and 400 mg/kg/day dosage groups was only slightly higher than the historical control mean, it is concluded that it is not biologically meaningful. However, the study report including the individual results was not assessed by the evaluating MSCA and the relevance of this conclusion cannot be confirmed. In addition, the evaluating MSCA considers that there is some doubt as to whether this study high dose level, i.e. 400 mg bw/day, is sufficient to demonstrate any effects related to the developmental toxicity (only slight increase in matted fur red nasal discharge and anogenital staining and significant decrease in maternal bw during the first 3 days of treatment and weight gain observed at this dose). No significant differences were noted between the treated and control animals in the mean number of corpora lutea, viable or non-viable foetuses, mean fetal bw and fetal malformation. There were no malformed foetuses in the control group, one malformed foetus in the 25 mg/kg/day group, 2 malformed foetuses in the 100 mg/kg/day and one malformed foetus in the 400 mg/kg/day group. These malformations were not statistically significant and not considered to be treatment related. It is thus concluded that the increase in malformations in the chlorendic anhydride groups are not biologically meaningful when compared to the control group.

NOAEL for maternal toxicity: 100 mg/kg bw/day (rat). The value was set based on the significant decreased maternal bw and weight gain at 400 mg/kg/day.

NOAEL for developmental toxicity: 25 mg/kg-bw/day (rat). The value was set based on the increased number of post-implantation loss at 400 and 100 mg/kg-bw/day.

Chlorendic acid

No data.

Conclusion

Fertility

In a mouse Dominant Lethal assay (Unpublished report, 1978c), chlorendic anhydride was not considered to affect the fertility of male rats in dosage levels up to and including 400 mg/kg/day. However, this study is of poor quality.

A 28-day feed study (Unpublished report, 1978f) and a 90-day feed study (Unpublished report, 1980) on CD rats are also available. No compound-related gross lesions were seen in any of the tested animals in these two studies and no compound-related microscopic lesions were seen in any of the tissues (including adrenals, prostate/uterus, mammary gland, testis/ovary and thyroid) from the 2 500 ppm dosed rats in the 90-day study. No significant weight change of any of the reproductive organs is mentioned in this study. However, this non-GLP 90-day study dating from 1980 lacks examination of the following: epididymis weight and histopathology, sperm parameters, determination of the stage of the oestrous cycle and serum total T4, T3 and TSH levels measurements.

None of the available study provide an adequate assessment of reproductive toxicity.

The evaluating MSCA underlines the fact that the available 28-day and 90-day feed studies on rats do not fully comply with the current requirements to detect adverse effects on endocrine- and reproduction-relevant endpoints. Besides, no study investigating fertility is available. Therefore, uncertainties remain for the reprotoxic potential of chlorendic anhydride, even if the available data do not indicate any significant effect.

Developmental toxicity

A prenatal developmental study for chlorendic anhydride (IRDC, 1978g) is available for the oral route, on one species (rat). This study concludes that chlorendic anhydride is not teratogenic in dosage levels up to and including 400 mg/kg/day. However, a significant increase in the mean number of post-implantation losses was observed in the 100 and 400 mg/kg/day dosage groups. The magnitude of these increases is not reported, as individual data were not available to the evaluating MSCA at the time of the substance evaluation process. Nonetheless, the evaluating MSCA points out that this study may not have been conducted to the limit dose, which potentially prevents the identification of effects in the dams or the offspring. Therefore, uncertainty remains regarding the potential of chlorendic anhydride to cause developmental toxicity.

However, developmental effects were not further evaluated in the context of the SEv as regulatory management measures will be proposed based on carcinogenicity and sensitising properties of chlorendic anhydride.

7.9.8. Hazard assessment of physico-chemical properties

No physical hazard is expected for the substance due to its chemical structure and its use as flame retardant.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

The DNELs proposed by the Registrant(s) were not assessed by the evaluating MSCA.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

Chlorendic anhydride is rapidly hydrolysed into chlorendic acid, therefore data of chlorendic acid can be used to evaluate the human health hazards of chlorendic anhydride. The registered substance is well absorbed and distributed in various tissues, has a low potential of accumulation and is eliminated in urine and feces.

Chlorendic anhydride is already classified under Annex VI of CLP as:

- Skin Irrit. 2; H315: Causes serious skin irritation

- Eye Irrit. 2; H319: Causes serious eye irritation

- STOT SE 3; H335: May cause respiratory irritation

In addition, the Registrant has self-classified the registered substance as:

- Skin sens. 1; H317: May cause an allergic skin reaction

- Carc. 2; H351: Suspected of causing cancer

- STOT RE 2; H373: May cause damage to organs through prolonged or repeated exposure

Upon assessment of the available data, the evaluating MSCA considers that the registered substance should be classified as **skin sensitiser category 1 (skin sens. 1; H317)**.

In addition, the evaluating MSCA considers that the registered substance should be classified as a **respiratory sensitiser category 1 (resp. sens. 1; H334)**.

A proposal for classification as **carcinogenic category 1B (carc. cat. 1B**) is considered adequate, considering the sufficient evidence of the carcinogenic potential of chlorendic acid in two experimental oral studies conducted by the US NTP (1987)

The concern towards the mutagenic potential of chlorendic acid, hence the anhydride form, is not substantiated by the results of a combined *in vivo* mammalian micronucleus assay and mammalian comet assay. The mechanism of action underlying the carcinogenicity of chlorendic acid/anhydride is thus suspected of being non-genotoxic and threshold-based, which is to be considered for the risk assessment to humans.

Uncertainty remains toward the teratogenic potential of the registered substance. Even if a statistically significant increase in malformations in the offspring was not observed in a prenatal developmental rat study with chlorendic anhydride, a significant increase in the mean number of post-implantation losses was observed in the middle and high dosage groups (100 and 400 mg/kg/day). In addition, this study may not have been conducted to the limit dose potentially preventing the identification of effects in the dams or the offspring. No effect on reproductive organs in repeated dose toxicity studies by the oral route were observed for chlorendic anhydride, although these studies are lacking the current requirements to detect sensible endocrine or reproductive-related endpoints. No specific study exploring the reproductive toxicity is available for chlorendic anhydride. The results of a carcinogenicity study on rats with chlorendic acid indicated effects on the following tissues: carcinomas of the preputial gland (male) and endometrial stromal polyp (female). These results do not justify any classification proposal for the reproductive toxicity.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Not evaluated.

7.10.2. Endocrine disruption - Human health

Not evaluated.

7.10.3. Conclusion on endocrine disrupting properties (combined / separate)

No data is available about the chlorendic acid or anhydride potential as endocrine disruptor in the environmental compartments.

7.11. PBT and VPVB assessment

The assessment was conducted on the substance itself and on chlorendic acid since the substance hydrolyses rapidly into the acid.

7.11.1. Persistence assessment

From the provided information (ready biodegradation study) on the substance itself and available information (ECHA CHEM database) on its reported impurities, the substance is considered persistent or very persistent based on screening assessment.

7.11.2. Bioaccumulation assessment

Screening criteria

The Registrant has provided, as required in the decision (2013), a relevant Log Pow value according to an OECD TG 107 conducted with adequate pH buffering conditions. Chlorendic anhydride is slightly soluble in water, thus, in aqueous solution chlorendic anhydride is rapidly hydrolysed to chlorendic acid. Determination of partition coefficient on chlorendic anhydride is not relevant.

According to OECD guideline 107 “measurements should be made on ionizable substances only in their non-ionized form (free acid or free base) produced by the use of an appropriate buffer with a pH of at least one unit below (free acid) or above (free base) the pK”.

Two pKa values were observed for chlorendic acid and found to be 3.6 and 5.6 in a new study. Since the aqueous phase is buffered at a pH of 7, the substance is mainly in ionized form (log Pow calculated at pH > pKa). At pH 7, relevant pH regarding environmental conditions, Log Pow of chlorendic acid is found to be -1.59 meaning ionized acid form is mainly present in aqueous phase (hydrophilic).

According to the provided information and available data from literature, the substance has a low potential for bioaccumulation based on screening assessment.

- Not B / vB based on Log Kow <= 4.5: (The measured LogKow was -1.59 at buffered pH conditions) and **based on the BCF values** from the HSDB database (BCF values < 2.1 and 0.22).

Other evidence of non-B / non-vB properties

- Not B/vB for air breathing organism based on Log Kow <2 and evidence of rapid methabolism and excretion in rats (see section human health): a half-life less than 2 days in rats was determined. According to Goss et al (2018), an elimination half-life in rat of 17 days would be sufficient to exclude bioaccumulation concern.

Conclusion on B / vB properties: not B/vB

7.11.3. Toxicity assessment

7.11.3.1. Fulfilment of the T criterion based on human health classification:

- Carcinogenic Cat 1A or 1B: yes (notification cat 1A on the public ECHA website) and recommendation form the evaluating MSCA to classify as Carc. Cat. 1B
- Mutagenic Cat 1A or 1B: no
- Toxic to reproduction cat 1A, 1B or 2: no
- STOT-RE cat 1, cat 2: yes (self-classification STOT RE2 on the public ECHA website)

7.11.3.2. Fulfilment of the T criterion based on ecotoxicity data:

From the provided data, considering that all relevant NOEC are over 0,01 mg/L from the substance itself and its impurities, the substance is considered not toxic to aquatic organisms according to annex XIII of the REACH Regulation. However, the substance fulfils the human T-criterion.

T according to human health classification.

Overall conclusion:

Based on the assessment described in the subsections above the evaluating MSCA concludes that the substance is not a PBT / vPvB substance.

Justification:

The substance is likely to be persistent as no biodegradation was observed in the screening test in water.

The substance however should not be considered as PBT or vPvB since there is no evidence that bioaccumulation can occur. The log Kow is -1.59 which is well below the screening threshold and there is also evidence of rapid metabolism and excretion in rats.

7.12. Exposure assessment

Chlorendic anhydride is imported from outside the EU. There is no known manufacturer in the EU.

7.12.1. Human health

7.12.1.1. Worker

It should be noted that regarding the human health part the hazards only were evaluated. The DNELs/DMELs and the occupational exposure scenarios were not evaluated. As a harmonised classification of the substance as a skin and respiratory sensitiser as well as a carcinogen are deemed necessary, a refinement of the implemented operational conditions and risk management measures by the Registrant will be needed to ensure that risks are adequately managed. The possible concern regarding exposure of workers may be revised if the risk management measures implemented are not considered as sufficient.

7.12.1.2. Consumer

No consumer uses are identified.

7.12.2. Environment

7.12.2.1. Introduction to the assessment for the environment

Exposure Scenario n°	Registrant	Exposure Scenario name	Environmental Release Categories (ERC)
Life Cycle Stage (LCS) IS: Use at industrial sites			
ES 1	**1**	**Use at industrial sites - Manufacture of uncured polyester resin using chlorendic anhydride**	**ERC 6c**
Relevant information Chlorendic anhydride is expected to react quantitatively during resin formation. Registrant data (Analysis of the resin and waste water) confirms that either all or almost all the chlorendic anhydride is converted into the chlorendic monomer in the resin. Analysis of process waste water shows very low releases of the substance (chlorendic acid) to process waste water. Chlorendic anhydride is reacted with other reactants to form an uncured resin at > 150 °C. This resin is a viscous liquid with a very low vapour pressure. Analysis of the uncured resin shows that the uncured resin contains residual chlorendic acid at up to 750 ppm. Annual tonnage (t/yr) = 750 (registrant data) Annual use amount at site (t/yr) = 250 (max value given by users of the substance)			

Daily tonnage "per site" (t/d) = 5 (max value given by users of the substance)
Release fraction to air = 1E-03%
Registrant's justification: Off-gases are scrubbed through an aqueous sodium hydroxide and then remaining volatile organic compounds (VOCs) are collected and destroyed in a Regenerative Thermal Oxidiser. It should be noted that both chlorendic anhydride starting material and the chlorendic acid hydrolysis product have a very low vapour pressure, so release via air is extremely unlikely even before the treatment of the waste gases.
Release fraction to wastewater = 2.82E-04%
Registrant's justification: Samples of process waste water from two manufacturing sites were analysed. Highest value measured = 14.1 g/day released to wastewater.
Release fraction to non-agricultural soil = 0%
Registrant's justification: ERC 6c default value.
Discharge rate of STP: >= 2E+03 m3/day
Receiving surface water flow rate: 1.8E+04 m3/day
Dilution factor to freshwater: default value of 10
Application of the STP sludge on agricultural soil: yes (municipal wastewater treatment plant)

$Elocal_{wastewater}$ = 0.014 kg/d

Life Cycle Stage (LCS) IS: Manufacture			
ES 2	1	**Synthesis of uncured liquid resin**	

The environmental exposure assessment for this scenario is covered by the emission scenario 1.

Life Cycle Stage (LCS) F: Formulation or re-packing - Formulation of uncured resin			
ES 3	**1**	**Formulation of uncured resin**	**ERC 2**

Relevant information

Product category formulated: PC 9a: Coatings and Paints, Thinners, paint removers; PC 9b: Fillers, putties, plasters, modelling clay

Based on the amount of chlorendic acid present within the uncured resin. Production quantities of the uncured resin is available from manufacturers. The quantity of chlorendic acid present during the use of the uncured resin is calculated from the highest concentration analysed, 750 ppm. The assessment assumes that the chlorendic acid is released proportionally to the release of the uncured resin.

Distribution

Annual tonnage (t/yr) = 1.4 (registrant data)
Annual use amount at site (t/yr) = 0.468 (registrant data)
Daily tonnage "per site" (t/d) = 3.75E-03 (registrant data)

Release fraction to air = 2.5%
ERC 2 default value

Release fraction to wastewater = 2%
ERC 2 default value

Release fraction to non-agricultural soil = 0.01%
ERC 2 default value
Discharge rate of STP: >= 2E+03 m3/day
Receiving surface water flow rate: 1.8E+04 m3/day
Dilution factor to freshwater: default value of 10
Application of the STP sludge on agricultural soil: yes (municipal wastewater treatment plant)

$Elocal_{wastewater}$ = 0.075 kg/d

Life Cycle Stage (LCS) IS: Use at industrial sites			
ES 4	**1**	**Use at industrial sites - Use of formulated uncured resin to form cured resins in situ**	**ERC 5**

Relevant information

The resin product of the reaction of chlorendic anhydride with alcohols and other reactants is a

liquid that contains no chlorendic anhydride and up to 750 ppm of chlorendic acid.

The formulations of the uncured resin (coatings, pastes and putties) are applied to surfaces or mixed with other agents (concretes, marbles). These applications are then cured to form a solid resin. Information provided by registrant from formulators of the uncured resin indicates that the maximum concentration of the uncured resin in these formulations is 65 % prior to curing.

Annual tonnage (t/yr) = 1.4 (registrant data)
Annual use amount at site (t/yr) = 0.0116 (registrant data)
Daily tonnage "per site" (t/d) = 5.8E-04 (registrant data)
Days emitting: 20 days/yr

Release fraction to air = 50 %
ERC 5 default value

Release fraction to wastewater = 50%
ERC 5 default value

Release fraction to non agricultural soil = 1%
ERC 5 default value

Discharge rate of STP: >= 2E3 m3/day
Receiving surface water flow rate: 1.8E+04 m3/day
Dilution factor to freshwater: default value of 10
Application of the STP sludge on agricultural soil: yes (municipal wastewater treatment plant)

$Elocal_{wastewater}$ = 0.29 kg/d

ES 5	1	Use at industrial sites - Use of cured resin	ERC 5

Relevant information

Product category used: PC 32: Polymer Preparations and Compounds
Sector of use: SU 12: Manufacture of plastics products, including compounding and conversion

Annual tonnage (t/yr) = 0.031 (registrant data)
Annual use amount at site (t/yr) = 0.031 (registrant data)
Daily tonnage "per site" (t/d) = 1.56E-03

Release fraction to air = 5%
Refinement by on site RMM: The resin is used in enclosed manufacturing processes and controls are maintained to prevent release from the vessels and associated equipment. Release of 5 % of the resin to either air or water is a very conservative estimate

Release fraction to wastewater = 5%
Refinement by on site RMM: The resin is used in enclosed manufacturing processes and controls are maintained to prevent release from the vessels and associated equipment. Release of 5 % of the resin to either air or water is a very conservative estimate

Release fraction to non agricultural soil = 1%
ERC 5 default value

Discharge rate of STP: >= 2E3 m3/day
Receiving surface water flow rate: 1.8E+04 m3/day
Dilution factor to freshwater: default value of 10
Application of the STP sludge on agricultural soil: yes (municipal wastewater treatment plant)

$Elocal_{wastewater}$ = 0.078 kg/d

7.12.2.2. PNEC derivation

PNECs derivation used in the environmental risk assessment	
Environmental compartment	**Hazard conclusion (see section 7.8.4)**
Fresh water	PNEC aqua (freshwater) = 0.097 mg/L
Sediment (freshwater)	Not relevant
Sewage Treatment Plant	PNEC STP = 9.7 mg/L
Agricultural soil	PNEC soil = 0.506 mg/kg soil ww

7.12.2.3. Fate and distribution parameters

The following substance properties are used in the fate estimation done by the Guidance on information requirements and chemical safety assessment, chapter R.16. The exposure assessment has been carried out with chlorendic acid. The resin product of the reaction of chlorendic anhydride with alcohols and other reactants is a liquid that contains no chlorendic anhydride and residual chlorendic acid.

Physical-chemical, environmental fate data used in the environmental risk assessment		
Input	**Chlorendic acid value**	**Unit**
Molecular weight	388.8	$g.mol^{-1}$ [25°C]
Water solubility	499	$mg.l^{-1}$ at [20°C]
Vapour pressure	1.875E-08	Pa [20°C]
Octanol-water partition coefficient	-1.59	[log10]
Kd (solids-water in soil)	5.78 0.76 (Log) theoretical Koc = 121	$l.kg^{-1}$ [25°C]
Biodegradability	Not biodegradable	[-]
DT_{50} in surface water	1E+40	d [12°C]
DT_{50} in soil	1E+06	d [12°C]

Calculated fate and distribution in the STP – SimpleTreat v4.0	
Compartment	**Percentage [%]**
Air	1.14E-11
Water	98.46
Sludge	1.543

Degraded in STP	0

7.12.3. Combined exposure assessment

The regional predicted environmental concentration (PEC regional). The exposure estimates have been obtained with EUSES 2.1.

Protection target	Regional PEC (evaluating MSCA)
Fresh water	1.24E-2 mg/L
Sediment (freshwater)	9.27E-3 mg/kg dw
Agricultural soil	1.53E-5 mg/kg dw

7.13. Risk characterisation

7.13.1. Human Health

Not evaluated further.

7.13.2. Environment

LOCAL ASSESSMENT

eMSCA's conclusion of the environmental risk assessment for each exposure scenario.

ENV compartment	Exposure concentration	RCR value	Conclusion
ES 1	**Manufacture of uncured polyester resin**		
Sewage Treatment Plant	6.89E-03 $mg.l^{-1}$	RCR < 0.01	Acceptable
Fresh water	6.89E-04 $mg.l^{-1}$	RCR < 0.01	Acceptable
Sediment (fresh water)	NR	NR	NR
Agricultural soil	2.67E-03	RCR < 0.01	Acceptable
Groundwater	> 0.1 µg/l	Above the parametric drinking water limit of 0.1 µg/L[1]	
ES 2	**Synthesis of uncured liquid resin**		
Sewage Treatment Plant	Covered by ES 1		
Fresh water			
Sediment (fresh water)			
Agricultural soil			
Groundwater			
ES 3	**Formulation of uncured resin**		
Sewage Treatment Plant	3.69E-02 $mg.l^{-1}$	RCR < 0.01	Acceptable
Fresh water	3.69E-03 $mg.l^{-1}$	RCR < 0.1	Acceptable
Sediment (fresh water)	NR	NR	NR

Agricultural soil	1.43E-02 mg.kg $_{ww}^{-1}$	RCR < 0.1	Acceptable
Groundwater	> 0.1 µg/l	Above the parametric drinking water limit of 0.1 µg/L[1]	
ES 4	**Use of formulated uncured resin to form cured resins in situ**		
Sewage Treatment Plant	1.43E-01 mg.l^{-1}	RCR < 0.1	Acceptable
Fresh water	1.43E-02 mg.l^{-1}	RCR < 1	Acceptable
Sediment (fresh water)	NR	NR	NR
Agricultural soil	5.53E-02 mg.kg $_{ww}^{-1}$	RCR < 1	Acceptable
Groundwater	> 0.1 µg/l	Above the parametric drinking water limit of 0.1 µg/L[1]	
ES 5	**Use of cured resin**		
Sewage Treatment Plant	3.82E-02 mg.l^{-1}	RCR < 0.01	Acceptable
Fresh water	3.82E-03 mg.l^{-1}	RCR < 0.1	Acceptable
Sediment (fresh water)	NR	NR	NR
Agricultural soil	1.48E-02 mg.kg $_{ww}^{-1}$	RCR < 0.1	Acceptable
Groundwater	0.52 µg.l^{-1}	Above the parametric drinking water limit of 0.1 µg/L[1]	

[1] Maximum allowable concentration for pesticides by the revised Drinking Water Directive 2020/2184.
NR: not relevant

The environmental risk assessment does not demonstrate unacceptable risk for environment compartments. Nevertheless concentrations estimated in groundwater are above the parametric drinking water limit of 0.1 µg/L set up for pesticides for all scenarios.

Environmental risks are acceptable for the manufacture of uncured polyester resin if the daily release to wastewater of mass chlorendic acid does not pass 14 g/day.

The manufacture and use of uncured resin may occur on the same site. The combined exposure to the environment still gives a risk characterisation ratio (RCR) of < 1 for all environmental compartments.

REGIONAL ASSESSMENT

The exposure estimates have been carried out by the registrant and eMSCA agree with the conclusions to consider the impact of the regional scale as negligible on environmental assessment.

CONCLUSION FOR ENVIRONMENT

Environmental risk assessment shows acceptable risk for **Manufacture of uncured polyester resin (ES 1) and Synthesis of uncured liquid resin (ES 2)** considering the **Risk mitigation measures** listed below:

- Daily release to wastewater of mass chlorendic acid does not pass 14 g/day.
- No application of the STP sludge on agricultural soil (incineration), considering that the substance should be classified as carcinogenic category 1B and this non-negligible exposure of groundwater.

Environmental risk assessment shows acceptable risk for **Formulation of uncured resin (ES 3), use of formulated uncured resin to form cured resins in situ (ES 4) and use of cured resin (ES 5)** considering the **risk mitigation measures** listed below:

- No application of the STP sludge on agricultural soil (incineration)

7.13.3. Overall risk characterization

Human health (combined for all exposure routes)

Environment (combined for all exposure routes)

The regional risk for environment is summarized in the following table.

Protection target	RCR
Freshwater (including sediment)	0.13
Agricultural soil	<0.0001

Combined with local assessment, risks are adequately controlled for each emission scenarios.

Local exposure due to all widespread uses

Not relevant as there are not widespread uses covered in this CSR.

Local exposure due to combined uses at a site

The manufacture and use of uncured resin may occur on the same site. The combined exposure to the environment still gives an RCR of < 1, so the exposure to the environment for sites that have all processes occurring is adequately controlled.

7.14. References

Decad G. M., Fields M. T. (1982). Disposition and Excretion of chlorendic acid in Fischer 344 rats. Journal of Toxicology and Environmental health, 9: 5-6, 911-920.

Dragan Y, Rizvi T, Xu Y, Hully J, Bawa N, Campbell H, Maronpot R, Pitot H. (1991). An initiation-promotion assay in rat liver as a potential complement to the 2-year carcinogenesis bioassay. Fundam Appl Toxicol. 16(3):525-47.

Foureman P., Mason J.M., Valencia R., and Zirnrnering S. (1994). Chemical Mutagenesis Testing in *Drosophila.* X - Results of 70 Coded Chemicals Tested for the National Toxicology Program. Environmental and Molecular Mutagenesis 23:208-227.

Goss K.U., Linden L., Ulrich N., Schlechtriem C. (2018) Revisiting elimination half lives as an indicator for bioaccumulation in fish and terrestrial mammals. Chemosphere 210: 341-346.

Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 5.0 July 2017

Haworth S., Lawlor T., Mortelmans K., Speck W and Zeiger E. (1983). Salmonella Mutagenicity Test Results for 250 Chemicals. Environmental Mutagenesis Supplement 1:3 -142.

Hendrickson C. and Hammond, 1970. Organic Chemistry, p135, Third Edition.

IARC (1990). Chlorendric ACID. IARC Monographs Volume 48.

IPCS (1996). Chlorendic acid and Anhydride EnvironmentaL Health Criteria 185. International Programme on Chemical Safety.

Keskinen, H., Pfäffli, P., Pelttari, M., Tupasela, O., Tuomi, T., Tuppurainen, M., Hämeilä, M., Kanerva, L. and Nordman, H. (2000), Chlorendic anhydride allergy. Allergy, 55: 98-99.

Kitchin KT, Brown JL, Kulkarni AP (1993). Predicting rodent carcinogenicity of halogenated hydrocarbons by in vivo biochemical parameters. Teratog Carcinog Mutagen. 1993;13(4):167-84.

McGregor DB., Brown A., Cattanach P., Edwards I., McBride D., and Caspary William J. (1988). Responses of the L5178Y tk+/tk' Mouse Lymphoma Cell Forward Mutation Assay II: 18 Coded Chemicals. Environmental and Molecular Mutagenesis 11:91-118.

NCI (1977a). Bioassay of Chlordane for Possible Carcinogenicity. NCI Technical Report No.8. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health.

NCI (1977b). Bioassay of Heptachlor for Possible Carcinogenicity. NCI Technical Report No. 9. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health.

NCI (1978a). Bioassay of Aldrin and Dieldrin for Possible Carcinogenicity. NCI Technical Report No. 21. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health.

NCI (1978b). Bioassay of Endosulfan for Possible Carcinogenicity. NCI Technical Report No. 62. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health.

NCI (1979). Bioassay of Endrin for Possible Carcinogenicity. NCI Technical Report No. 12. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health.

The Merck Index, p11, Eleventh Edition, 1989.

Uno Y, Takasawa H, Miyagawa M, Inoue Y, Murata T, Yoshikawa K. (1994). An in vivo-in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens. Mutat Res. 1994 Feb;320(3):189-205.

Unpublished report (1977b). Mutagenicity Evaluation of Chlorendic Anhydride.

Unpublished report (1977a). Evaluation of Chlorendic Anhydride; In Vitro Malignant Transformation in BALB / 3T3 Cells.

Unpublished report (1978). Pharmacokinetics of chlorendic anhydride in rats.

Unpublished report (1978a). Acute Toxicity Studies in Rabbits and Rats.

Unpublished report (1978a). Mutagenicity Evaluation of Chlorendic Anhydride in the Mouse Lymphoma Forward Mutation Assay.

Unpublished report (1978b). Acute Oral Toxicity (LD50) Study in Mice.

Unpublished report (1978b). Mutagenicity Evaluation of Chlorendic Anhydride in the Unscheduled DNA Synthesis in Human WI - 38 Cells Assay.

Unpublished report (1978c). Acute Inhalation Study in Rats.

Unpublished report (1978c). Mutagenicity Evaluation of Chlorendic Anhydride in the Mouse Dominant Lethal Assay.

Unpublished report (1978d). Acute toxicity Studies in Rabbits and Rats.

Unpublished report (1978e). Dermal Sensitization Study in the Albino Guinea Pig.

Unpublished report (1978f). Chlorendic Anhydride; Twenty-Eight Day Range-Finding Study in Rats.

Unpublished report (1978g). Chorendic Anhydride; Teratology Study in Rats.

Unpublished report (1979a). Chlorendic Anhydride: Sub-acute Inhalation Toxicity Study in Rats, 20 Exposures in 28 Days.

Unpublished report (1979b). Chlorendic Anhydride; Three Week Dermal Toxicity Study in Rabbits.

Unpublished report (1980). Chlorendric Anhydride; 90-Day Sub-Acute Toxicity Study in Rats.

Unpublished report (1983). 28-Day Subacute Dust Inhalation Toxicity Study with HET acid in rats .

Unpublished report (2010). Koc estimation for chlorendic acid using EPISUITE and Log Pow for chlorendic acid.

Unpublished report (2016a). Determination of the acute toxicity of Chlorendic Anhydride against Danio rerio.

Unpublished report (2016b). Determination of the short term toxicity of Chlorendic Anhydride against Daphnia magna.

Unpublished report (2016c). In vitro mammalian cell micronucleus test with chlorendic acid.

Unpublished report (2020). Chlorendic Acid: Rat Micronucleus and Alkaline Comet Assay.

Unpublished report (2020). Combined in vivo mammalian erythrocyte micronucleus test in bone marrow and in vivo mammalian comet assay with chlorendic acid.

Unpublished study report (2001a). Chlorendic Anhydride; Assessment of Inherent Biodegradability by Manometric Respirometry.

Unpublished study report (2001b). Estimation of Environmental Fate Using the Mackay Level III Fugacity Model.

Unpublished study report (2002a). Chlorendic Anhydride; Algal Growth Inhibition Assay.

US EPA (2009). Screening-level Hazard Characterization on Sponsored Chemical Chlorendic Anhydride (CAS RN 115-27-5) Supporting Chemical Chlorendic acid (CASRN 115-28-6).

US NTP (1987). Toxicology and Carcinogenesis Study of Chlorendic Acid (CAS NO 115-28-6) In F344/N Rats and B6C3F1 Mice (Feed Studies). National Toxicology Program Technical Report Series No. 304.

Van Beelen P. (2000). The risk evaluation of difficult substances in USES 2.0 and EUSES, RIVM Report 679102050.

WHO (2009). World Health Organization Concise International chemical Assessment. Document 75, CYCLIC ACID ANHYDRIDES: HUMAN HEALT ASPECT.

7.15. Abbreviations

ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail [French agency for food, environmental and occupational health & safety]
ALT	Alanine transaminase
AST	Aspartate transaminase
BW	Body weight
CAS RN	Chemical abstracts service registry number
CBPI	Cytokinesis-Block Proliferation Index
CCH	Compliance check
CLP	Classification, labelling, packaging
CORAP	Community rolling action plan
DD	Draft decision
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNEL	Derived no effect level
ECHA	European chemical agency
ED	Endocrine disrupting
ERC	Environmental release categories
EMS	Ethylmethanesulfonate
EU	European union
GD	Gestation day
GLP	Good laboratory practice
HAS	Human serum albumin
IARC	International Agency for Research on Cancer
IgE	Immunoglobulin E
IV	Intravenous
LD50	Median lethal dose
LOAEL	Low observed adverse effect level
MN	Micronucleus
MSCA	Member state competent authority
MTD	Maximum tolerable dose
NCE	Normochromatic erythrocytes
NCI	National Cancer Institute
NOAEL	No observed adverse effect level
OECD	Organisation for economic co-operation and development
NTP	National Toxicology Program
PBT	Persistent, bioaccumulative and toxic
PCE	Polychromatic erythrocytes
QSAR	Quantitative structure-activity relationship
RDS	Replicative DNA synthesis
REACH	Registration, authorisation, restriction of chemicals
RMOA	Risk Management Option Analysis
SAP	Serum Alkaline Phosphatase
SD	Sprague-Dawley
SLRL	Sex-Linked recessive lethal
STOT RE	Specific target organ toxicity, repeated-exposure
STOT SE	Specific target organ toxicity, single-exposure
SVHC	Substance of very high concern
TG	Technical guidance
vPvB	Very persistent very bioaccumulative
VIN	Vinblastine
WHO	World health organisation