Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

4,5-dichloro-2-octyl-2H-isothiazol-3-one; [DCOIT]

EC Number: 264-843-8
CAS Number: 64359-81-5

CLH-O-0000001412-86-258/F

Adopted

30 November 2018

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

30 November 2018

CLH-O-0000001412-86-258/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **4,5-dichloro-2-octyl-2H-isothiazol-3-one; [DCOIT]**

EC Number: **264-843-8**

CAS Number: **64359-81-5**

The proposal was submitted by **Norway** and received by RAC on **15 January 2018.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Norway has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **12 February 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 April 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Ignacio de la Flor Tejero**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **30 November 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	4,5-dichloro-2-octyl-2H-isothiazol-3-one (ISO); [DCOIT]	264-843-8	64359-81-5	Acute Tox. 1 Acute Tox. 4 Skin Corr. 1 Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H302 H314 H318 H317 H400 H410	GHS05 GHS06 GHS09 Dgr	H330 H302 H314 H317 H410	EUH071	Skin Irrit. 2; H315: 0,01 % ≤ C < 5 % Eye Irrit. 2; H319: 0,01 % ≤ C < 3 % Skin Sens. 1A; H317: C ≥ 0,001 % M=100 M=100	
RAC opinion	TBD	4,5-dichloro-2-octyl-2H-isothiazol-3-one (ISO); [DCOIT]	264-843-8	64359-81-5	Acute Tox. 2 Acute Tox. 4 Skin Corr. 1 Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H302 H314 H318 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H330 H302 H314 H317 H410	EUH071	inhalation: ATE = 0,16 mg/L oral: ATE = 567 mg/kg bw Skin Irrit. 2; H315: 0,025 % ≤ C < 5 % Eye Irrit. 2; H319: 0,025 % ≤ C < 3 % Skin Sens. 1A; H317: C ≥ 0,0015 % M=100 M=100	
Resulting Annex VI entry if agreed by COM	TBD	4,5-dichloro-2-octyl-2H-isothiazol-3-one (ISO); [DCOIT]	264-843-8	64359-81-5	Acute Tox. 2 Acute Tox. 4 Skin Corr. 1 Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H302 H314 H318 H317 H400 H410	GHS06 GHS05 GHS09 Dgr	H330 H302 H314 H317 H410	EUH071	inhalation: ATE = 0,16 mg/L oral: ATE = 567 mg/kg bw Skin Irrit. 2; H315: 0,025 % ≤ C < 5 % Eye Irrit. 2; H319: 0,025 % ≤ C < 3 % Skin Sens. 1A; H317: C ≥ 0,0015 % M=100 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

DCOIT (4,5-dichloro-2-octyl-2H-isothiazol-3-one) is an existing biocidal active substance approved as a wood preservative and antifouling agent under Regulation (EU) No 528/2012. DCOIT currently has no harmonised classification in Annex VI of the CLP regulation.

The classification proposal is based on information included in the CLH dossier from studies provided by two applicants (Dow Europe GmBH and Thor GmbH).

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed no classification for physical hazards based on the following study results:

- In a standard study (EEC Method A10) DCOIT was not determined to be flammable
- The self-ignition temperature was found to be 260 °C (EEC Methods A16 and A15)
- Experience in handling and use indicates that it is not a pyrophoric solid and does not emit flammable gas on contact with water.
- DCOIT does not contain any functional groups that can contribute to explosive properties and both the oxygen balance number and the exothermic decomposition energy are below the threshold for explosive substances.
- DCOIT does not possess oxidising properties and so it is not classified as an oxidising solid

Comments received during public consultation

One Member State Competent Authority (MSCA) raised a potential error in the self-ignition temperature but the DS responded that the MSCA used the value from one applicant while the DS used the average of self-ignition from the two applicants.

Assessment and comparison with the classification criteria

RAC agrees with the DS that **classification is not warranted for physical hazards**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify DCOIT for acute oral toxicity in category 4 (H302: Harmful if swallowed) and acute inhalation toxicity in category 1 (H330: Fatal if inhaled) on the basis of lethal doses (LD_{50} values) by the oral route ranging between 500 and 2000 mg/kg bw and lethal concentrations (LC_{50} values) by inhalation ranging between 0.16 and 0.26 mg/L/4h, determined for atmospheres where only aerosol was measured or a mixture of aerosol and vapour was

measured, respectively. The DS did not propose to classify DCOIT for acute dermal toxicity, as the LD_{50} value by the dermal route was higher than 2000 mg/kg bw. Based on evidence for its corrosivity (for details, see the section "Skin corrosion/irritation", below), the DS also proposed labelling the substance with EUH071 (corrosive to the respiratory tract).

Comments received during public consultation

One MSCA highlighted the necessity of setting acute toxicity estimates (ATEs) to correctly classify mixtures containing DCOIT. In their response, the DS agreed and proposed ATEs of 567 mg/kg bw and 0.26 mg/L for the oral and inhalation routes, respectively.

Two manufacturers supported the DS's proposals with respect to acute oral and dermal toxicity. However, they provided further evidence and comments for consideration regarding the applicability of an inhalation classification for solid substances of low volatility and questioned at the same time the DS's proposal for labelling the substance with EUH071 (corrosive to the respiratory tract). The DS responded by clarifying that the classification of substances is based on intrinsic properties and should not take into account exposure considerations. Hence, the results of the acute inhalation toxicity and of the 13-week repeated dose study by inhalation supported the classification for acute inhalation toxicity and the supplemental hazard labelling with EUH071 due to corrosivity, respectively. The DS also disagreed with the manufacturers' position that there is no potential for inhalation exposure to DCOIT during intended, known or reasonably expected use.

One MSCA fully supported the DS's proposal for acute toxicity classification of DCOIT.

Assessment and comparison with the classification criteria

The Table below summarises the acute toxicity studies with DCOIT by the oral route.

Table: *Summary of the acute oral toxicity studies with DCOIT.*

Study	Dose level	Results	Reference
Oral gavage OECD 401 Crl:CD®BR rats Males/Females 6/sex/group 14 day post dose observation period	DCOIT technical product (RH-287 in corn oil) 500, 750, 1000, 1500 or 2000 mg/kg bw Single dose	Mortalities observed from 750 mg/kg bw Treatment-related decrease in body weight gain in the surviving male rats at 750 mg/kg bw and greater, but this effect was not seen in the females. Surviving rats had thickened stomach walls. Clinical signs: irritation around the anal-genital area, passiveness, scant faeces and/or tan-stained muzzle. Pathological findings: high incidence of viscous material in the caecum, intestines, and stomach; black material or black foci adhered to stomach mucosa; reddened stomach and intestinal mucosa; and mottled liver. **Combined male/female LD_{50} = 1636 mg/kg bw**	Dow Anon., 1992 (A6.1.1/01) Valid without restriction
Oral gavage OECD 401	DCOIT technical product	Mortality was observed from 500 mg/kg bw	Dow Anon., 1994 (A6.1.1/02)

Crl:CD-1® (ICR)BR mice Males/Females 6/sex/group 14 day post dose observation period	(RH-287 in corn oil) 100, 250, 500, 1000 or 2000 mg/kg bw Single dose	Body weight gain was not affected in survivors. In the two highest dose groups among males and in the three highest dose groups among females soft and/or scant faeces, passiveness, tremors and ataxia Pathological findings: reddened glandular portion of the stomach and intestines, black material in stomach and mottled liver. **Combined male/female LD_{50} = 567 mg/kg bw**	Valid without restriction
Oral OECD 423 Wistar rats 3/sex/group	200, 500, 2000 mg/kg bw in peanut oil Single dose	200 mg/kg bw: lethargy, abdominal breathing, nostril discharge, no mortalities 500 mg/kg bw; lethargy, abdominal breathing, toe walking, piloerection, no mortalities 2000 mg/kg bw: lethargy, abdominal breathing, gasping, nostril discharge, piloerection, toe walking, salivation, diarrhoea, and unusual locomotion and pathological changes in lung, liver, kidneys and spleen, 100% mortality within 24 hours **500 mg/kg bw < LD_{50} < 2000 mg/kg bw**	Thor Anon. 2000a, (A 6.1.1-01 7.2.1-01) Valid without restriction

The Table below summarises the acute toxicity studies with DCOIT by the dermal route.

***Table**: Summary of the acute dermal toxicity studies with DCOIT.*

Study	Dose level	Results	Reference
OECD 402 New Zealand White rabbits Males/Females 6/sex/group 14 day post dose observation period	>2000 mg C-9211 HQ technical product /kg bw in xylene (equivalent to > 652 mg DCOIT/kg bw)	No mortalities Clinical signs: ataxia, reduced body weights, decreased feed consumption, scant faeces and passiveness. Red fluid filled thoracic cavity and clear fluid filled abdominal cavity was observed at necropsy. Skin irritation (erythema, oedema, pocketing oedema, eschar, blanching). LD_{50} > 2000 mg C-9211 HQ /kg bw equivalent to **LD_{50} > 652 mg DCOIT/kg bw**	Dow Anon., 1989a (A6.1.2/01) Valid without restriction
OECD 402 Wistar rats Males/Females 5/sex/group	2000 mg/kg bw single dermal application	No mortalities. Clinical signs: rough coat and erythema on the skin of the treated animals persisting until the end of the study At terminal sacrifice, varying degree of skin lesions comprised of cutaneous	Thor Anon. 2000b, (A 6.1.2-01 7.2.3-01) Valid without restriction

		thickening, alopecia and erythema in treated animals were recorded. **LD_{50} > 2000 mg/kg bw**	

The Table below summarises the acute toxicity studies with DCOIT by the inhalation route.

***Table**: Summary of the acute inhalation toxicity studies with DCOIT.*

Study	Dose level	Results	Reference
OECD 403 Crl:CD® BR rats Males/Females 6/sex/group 14 day post exposure observation period	0.23, 0.12, 0.46 or 0.20 mg/L (analytical concentration) Single nose-only exposure 4 h Mixture aerosol/vapour Vapour/aerosol ratio unknown although the concentration of DCOIT in the test atmosphere was 2-3 fold higher than the concentration of DCOIT in aerosol MMAD = 1.3-2.1 µm.	Signs of respiratory irritation (gasping and slight to severe rales) were seen in all dose groups. Mortalities in all groups but no clear dose-response Other clinical signs: unkempt appearance, red stained eyes and muzzle, scant faeces, and yellow-stained anogenital area. All groups showed signs of slight to severe redness in lobes of the lung. Scattered incidences of red pinpoint foci on the lungs and gas-filled stomachs were also observed. **Males LC_{50} = 0.21 mg/L** **Females LC_{50} = 0.34 mg/L** **Combined males/females LC_{50} = 0.26 mg/L**	Dow Anon., 1994 (A6.1.3/01) Valid without restriction
OECD 403 Wistar rats Males/females 5/sex/group 14 day post exposure observation period	0.143, 0.221 and 0.289 mg/L Single nose-only exposure 4 h Only aerosols (not vapour) MMAD: not reported	Mortalities 40, 70 and 90% at concentrations of 0.143, 0.221 and 0.289 mg/L air, respectively All the surviving animals showed a gain in body weight over the duration of the experiment. Clinical signs: lethargy, tremors, abdominal breathing, gasping and nasal irritation. Vascular/inflammatory changes in the lungs **Combined LC_{50} = 0.164 mg/L air (95% confidence limits between 0.123 to 0.219 mg/L air)**	Thor Anon. 2001, (A 6.1.3-01 7.2.2-01) Valid with restriction (no MMAD reported in the CLH report)

Comparison with the criteria

Acute oral toxicity

One reliable acute oral toxicity study in rats yielded an LD_{50} of 1636 mg/kg bw. A second more recent reliable study in the same species showed no mortality at 500 mg/kg bw but 100% mortality at 2000 mg/kg bw (no LD_{50} was derived). However, mice seem to be more sensitive to DCOIT than rats by the oral route since a third reliable acute oral toxicity study concluded that

the combined LD_{50} in this species is 567 mg/kg bw. Overall, the available data supports classification of DCOIT for acute oral toxicity within Category 4 (H302: harmful if swallowed) with an ATE of 567 mg/kg bw.

Acute dermal toxicity

One of the available acute dermal toxicity studies demonstrated that the LD_{50} of DCOIT was higher than 2000 mg/kg bw. A second study conducted with a technical product diluted in xylene did not contradict this figure with no mortalities at 652 mg DCOIT/kg bw. Thus, the available information does not support classification of DCOIT for acute dermal toxicity.

Acute inhalation toxicity

The first acute inhalation toxicity study in rats (nose-only) used a mixture of vapours and aerosol (but with 2-3 times more vapour than aerosol) and yielded an LC_{50} of 0.21 mg/L/4h (males), 0.34 mg/L/4h (females) and a combined males/females LC_{50} of 0.26 mg/L. A second study using the same species exposed to an atmosphere where only aerosol was measured, led to a combined LC_{50} of 0.16 mg/L/4h. The first study (majority of vapour in the test atmosphere) would support classification within category 1 ($LC_{50} \leqslant 0.5$ mg/L), while the study conducted with aerosol would support classification in category 2 ($0.05 < LC_{50} \leqslant 0.5$). RAC notes that DCOIT is solid at ambient temperature and that it has a very low volatility. For the first acute inhalation toxicity study, DCOIT was melted in solvent at or above 60 ºC and was vaporised with compressed hot air. Under such test conditions, the vapour/aerosol ratio of DCOIT in the test atmosphere could not be precisely determined but the concentration of DCOIT in the vapour phase was 2-3 fold higher than the concentration of DCOIT in the liquid phase. Partial enrichment of the more volatile fraction cannot be avoided when trapping large aerosol particles to enhance their respirability. RAC also notes that the OECD Test Guideline (TG) does not recommend exposing animals to vapour and aerosol mixtures.

Therefore, RAC considers as key the study performed with aerosol only and supports classification of DCOIT for acute inhalation toxicity within Category 2 (H330: fatal if inhaled) with an ATE of 0.16 mg/L/4h (dust or mist).

Supplemental hazard labelling

According to the Guidance on the Application of the CLP Criteria (version 5.0 July, 2017; henceforth referred to as CLP Guidance) in addition to classification for inhalation toxicity, if data are available that indicates that the mechanism of toxicity is corrosivity, the substance or mixture shall also be labelled as EUH071 (corrosive to the respiratory tract). RAC notes that DCOIT is corrosive to the skin and has produced severe respiratory clinical signs in rats exposed by inhalation. Therefore, RAC agrees with the DS that the supplemental hazard labelling **EUH071** is warranted.

In conclusion, RAC supports the DS's proposal to classify DCOIT for:

- **acute inhalation toxicity in category 2; H330 (fatal if inhaled) with an ATE of 0.16 mg/L/4h**;

- **acute oral toxicity in category 4; H302 (harmful if swallowed) with an ATE of 567 mg/kg bw**.

RAC also agrees that the DCOIT should be labelled with **EUH071 (corrosive to the respiratory tract)**.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS proposed no classification of DCOIT for STOT SE 1 or 2 in the absence of clear (non-lethal) target organ effects from the oral, dermal and inhalation acute toxicity studies. STOT SE 3 was considered superfluous by the DS in view of the proposal to label DCOIT as corrosive to the respiratory tract (EUH071).

Comments received during public consultation

One MSCA supported the DS's proposal for no classification of DCOIT for STOT SE.

One manufacturer provided comments regarding acute inhalation toxicity. The DS responded as stated above regarding acute inhalation toxicity.

Assessment and comparison with the classification criteria

With the exception of the skin and the lungs, no clear evidence of non-lethal effects on a specific target organ or tissues could be derived from the oral, dermal or inhalation acute toxicity studies and therefore classification as STOT SE 1 or 2 is not warranted.

The main adverse effects reported in acute toxicity studies were local effects at the point of contact with the substance due to its corrosivity. The skin effects are already covered by the classification as Skin Corr. 1 (H314). After inhalation, clinical signs indicating severe respiratory irritation (abdominal/laboured breathing, gasping, rales and nasal irritation) occurred. These symptoms were accompanied by severe irreversible histopathological findings in the lungs (including redness in the lobes of the lung, vascular/inflammatory changes) that may lead to mortality. RAC notes that the hazard class STOT SE category 3 (H335) for respiratory tract irritation should cover transient effects and therefore RAC is of the opinion that these effects are too severe to support a classification within this category. The supplementary label EUH071 and the classification as Acute Tox. 2 (H330) by inhalation covers the respiratory system as a target organ after single exposure to DCOIT. RAC also notes that classification as STOT SE category 3 (H336) is not warranted either, since no narcotic effects were reported.

In conclusion, RAC agrees with the DS's proposal for **no classification of DCOIT for STOT-SE**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed classification of the substance as skin corrosive category 1; H314 (causes severe skin burns and eye damage) based on observations in OECD TG 404 compliant tests and supporting evidence from skin sensitisation studies. The DS did not propose sub-categorisation but proposed a Specific Concentration Limit (SCL) of 0.01% for skin irritation since both human patch test studies and animal studies have shown that an irritation response may be induced at concentrations below the General Concentration Limit (GCL).

Comments received during public consultation

One comment from industry highlighted that the potential classification of DCOIT as skin corrosive with the proposed SCL and without considering the real risk might compromise several uses of this substance as a biocide. The DS replied that the classification of substances is set on the basis of the intrinsic properties of the substance and using agreed common criteria, which did not take into consideration either risk or downstream consequences of the classification.

Other comments from industry supported the DS's proposals with respect to skin corrosivity. However, they provided further evidence and comments for consideration regarding the applicability of SCL for irritation at 250 ppm (0.025%). According to Industry, in two primary irritation studies conducted in guinea pigs, the highest reported non-irritating concentrations were 0.03% (300 ppm). Furthermore, human studies showed no responses in volunteers at concentrations up to 250 ppm when formulated in petrolatum. In response, the DS noted that in animal studies the highest non-irritating concentrations reported were in the range 0.01-0.03%.

Two MSCAs supported the DS's proposal for classification of DCOIT as corrosive to the skin. One MSCA also suggested adding an SCL for Skin Irrit. 2 since skin irritation was observed from a concentration of 0.01 %. The DS confirmed in their response that mixtures containing DCOIT should be classified from a concentration of 0.01% as Skin Irrit. 2 (H315). For classification as Skin Corr. 1 (H314), the GCL (5%) should apply.

Assessment and comparison with the classification criteria

The Table below summarises the skin corrosion/irritation studies performed with DCOIT.

Table: *Summary of the skin corrosion/irritation studies with DCOIT.*

Study	Dose level	Results	Reference
New Zealand White rabbits 6 animals OECD 404	0.5 mL of C-9211 HQ formulation in xylene: (32.6% DCOIT) 4 hours	Skin reactions were noted at 1 h and 1, 2, 3, 7 and 14 days post application Average erythema score 24, 48 and 72 hours: 4.0 Average oedema score 24, 48 and 72 hours: 3.9 Five of six animals demonstrated scar formation at 14 days. Moderate to severe erythema and oedema were observed at 1h. At 14 days there was no oedema, but slight to severe erythema, eschar, areas without hair growth and whitened areas. **Conclusion: corrosive**	Dow Anon., 1989b, A6.1.4/01
New Zealand White rabbits OECD 404	LES 1920P formulation in phenoxy-propanol)xylene: (20% DCOIT) 4 hours No further information provided	Average erythema score 24, 48 and 72 hours: 4.0 Average oedema score 24, 48 and 72 hours: 3.3 Severe erythema was evident at all observation periods. Very slight to severe oedema was noted at 1 hour and continued through day 7.	Dow Anon., 1997. (Kathon TM 287 WT B6.2a/04 (PT8)) NK A6.1.4/03 (PT21)

<table>
<tr><td></td><td></td><td>Skin irritation indicative of corrosivity (concave eschar) was evident by 48 hours.

Oedema reversed by day 14.

Erythema did not reverse by day 14.

On day 14, corrosive findings (ulceration/erosion) were confirmed by a veterinary pathologist.

Conclusion: corrosive</td><td></td></tr>
<tr><td>New Zealand White rabbits

OECD 404

3 males</td><td>DCOIT

No further information provided</td><td>Average erythema score 24, 48 and 72 hours: 2.3

Average oedema score 24, 48 and 72 hours: 2.2

No clinical signs other than erythema and oedema were observed in the animals.

Mild to severe erythema and oedema was observed in all three rabbits throughout the experimental period, and the effect increased in severity and was irreversible until termination of the study.

Irritant/corrosive response data for each animal at each observation time up to removal of each animal from the test
<table><tr><td>Score / Reversibility</td><td>Erythema</td><td>Oedema</td></tr><tr><td>60 min</td><td>3/ 3/ 3</td><td>2/ 2/ 2</td></tr><tr><td>24 h</td><td>3/ 2/ 2</td><td>2/ 2/ 2</td></tr><tr><td>48 h</td><td>3/ 2/ 2</td><td>2/ 2/ 2</td></tr><tr><td>72 h</td><td>4/ 2/ 1</td><td>4/ 2/ 2</td></tr><tr><td>Average 24h, 48h, 72h</td><td>3.3/2/1.67</td><td>2.7/2/2</td></tr><tr><td>Reversibility*)</td><td>n</td><td>n</td></tr><tr><td colspan="3">*) Reversibility: c. = completely reversible; n.c. = not completely reversible; n. = not reversible</td></tr></table>
Conclusion: corrosive</td><td>Thor Anon., 2000c (A 6.1.4-01 7.3.1-01)

The applicant gave the study a reliability score of 2.</td></tr>
</table>

In vitro data

The corrosive potential of DCOIT technical was further studied in the EPIDERM™ (EPI-200) *in vitro* skin corrosion test (Anon., A6.1.4/02, non-key study). Twenty-five mg of DCOIT was applied to the reconstructed human epidermis and 25 µL water was applied directly on top. DCOIT only marginally reduced the viability of the tissue (less than 10%) following 3 min or 60 min exposures and was concluded as being non-corrosive according to the prediction models for the EPI-200 provided in the draft revised OECD TG 431. It appears from the study protocol that there was insufficient observation post-exposure and that the maximum exposure time was only 60 min, instead of 240 min. According to the DS, this is probably a false negative result with two possible explanations: i) the low solubility of DCOIT in water (which resulted in a slow and reduced penetration into the skin); or, ii) the EPIDERM™ test is not sensitive to the mechanism of irritation/corrosion of DCOIT.

Human data

The results of a human repeated insult patch test (Anon., A.12.6/01-08) reported a barely perceptible (minimal, faint, uniform, or spotty erythema) to moderate-non-specific or low-grade irritant (pink-red erythema uniform in the entire contact site) with responses of 16/34 individuals during the induction phase with a 0.025% DCOIT solution. In the same study similar effects in 13/34 individuals exposed to 0.035% DCOIT were reported.

Comparison with the criteria

According to CLP criteria, a corrosive substance is one that produces destruction of skin tissue in at least 1 treated animal after a maximum of 4 hours of exposure. The CLH report summarised three independent studies showing severe and irreversible erythema and corrosive findings (ulceration, erosion, eschar, loss of hairs) confirmed by a veterinary pathologist in rabbits (14 days after the exposure) in two studies. These studies provide support for a classification as skin corrosive category 1. RAC notes that the *in vitro* test with human skin produced a negative result. RAC agrees with the view of the DS and the Applicant that this negative *in vitro* EPIDERM™ test is probably a false negative result. Whether the lack of response is related to the low solubility of DCOIT in water resulting in a slow and reduced penetration into the skin or whether the EPIDERM™ test is not sensitive to the mechanism of irritation/corrosion of DCOIT is currently not known.

As the dermal penetration of DCOIT is probably relatively slow, a prolonged tissue incubation time might have been necessary for revealing the corrosive effects of DCOIT in *in vitro* tests. The negative result from the *in vitro* test is therefore not considered to reduce the concern for skin corrosion.

RAC notes that the skin reactivity was not tested for times shorter than 4 hours and therefore classification within category 1A or 1B cannot be ruled out. Thus, RAC concludes that no sub-categorisation within group 1 is possible.

RAC notes that there was evidence in both humans and animals indicating that DCOIT is able to cause skin reactivity at concentrations lower than 5% (the GCL for skin corrosion category 1) and 1% (the GCL for skin irritation category 2). Specifically, a skin sensitisation study with animals (see the Table in the next section) caused skin irritation at 0.02 and 0.03% but not at 0.01%. However, RAC notes that skin sensitisation studies are not designed for testing skin irritation and therefore considers this study non-relevant for setting an SCL for irritation, as proposed by the DS. However, in a human repeated insult patch test moderate to barely perceptible skin irritation was observed at 0.025 and 0.035% without a dose-response relationship; this suggests to RAC that the threshold for irritancy in humans is at or around 0.025%. Based on these results RAC, supports an SCL for skin irritation of 0.025%.

In conclusion, RAC agrees with the DS's proposal for classification of DCOIT as **skin corrosive category 1 H314 (causes severe skin burns and eye damage)** with an **SCL of 0.025% for skin irritation (Skin Irrit. 2; H315)**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed classification of DCOIT as eye corrosive category 1 on the basis of the corrosivity of the substance on the skin.

Comments received during public consultation

One MSCA commented that an SCL should be considered for eye effects because otherwise the GCL of 1% would be applied for setting classification of mixtures containing DCOIT. The DS agreed with the proposal and proposed an SCL of 0.01% for Eye Irrit. 2 (H319) as this is the highest non-irritating concentration identified for skin and there are no data to suggest a lower sensitivity of the eye. The DS confirmed in their response that mixtures containing DCOIT should be classified from a concentration of 0.01% as Eye Irrit. 2 (H319) and from 3% (the GCL) as Eye Dam. 1 (H318).

Two manufacturers supported the DS's proposals with respect to eye corrosivity.

One manufacturer questioned the SCL proposed by the DS. DS replied that the SCL was proposed on the basis of observations in animal studies in which the highest non-irritating concentrations reported were in the range of 0.01-0.03%, rather than on the basis of human data.

Assessment and comparison with the classification criteria

RAC notes that according to the CLP Guidance when a substance or mixture is classified as Skin corrosion Category 1 then serious damage to eyes is implicit as reflected in the hazard statement for skin corrosion (H314: Causes severe skin burns and eye damage). A corrosive substance is consequently also classified for serious eye damage category 1 without labelling with the corresponding hazard statement (H318: Causes serious eye damage) in order to avoid redundancy.

RAC supports a SCL of 0.025% in line with that proposed for skin irritation.

In conclusion, RAC agrees with the DS's proposal for classification of DCOIT for **eye damage in category 1 H318 (Causes serious eye damage)** without the hazard statement in the label and with an **SCL of 0.025% for eye irritation (Eye Irrit. 2; H319)**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed classification of the substance as skin sensitiser category 1A; H317 (may cause allergic skin reactions) on the basis of a local lymph node assay (LLNA) study yielding an EC_3 of 0.03% (extreme potency) which was supported by two Guinea pig maximization tests (GPMT). In addition, studies with humans suggested that DCOIT technical is a skin sensitiser when it is dissolved in ethanol, with an induction threshold at or below 0.025%. Additional data from workers support the sensitisation properties of DCOIT, although there is a low number of cases and insufficient information about the concentration of the substance in the final solution. The DS proposed an SCL of 0.001%, in accordance with the reference value stated in the CLP Guidance for sensitisers with extreme potency.

Comments received during public consultation

Two manufacturers highlighted that the potential classification of DCOIT as a skin sensitiser might compromise the future use of this substance for dry-film preservation. Two Industry or trade associations (European Council of Paint, Printing Ink and Artists' Colours (CEPE) and the German Paint and Printing Ink Association (VdL)) also raised similar concerns regarding the proposed SCL that, according to them, does not take into consideration the risk of the substance. The DS replied

that the classification of substances is set on the basis of the intrinsic properties of the substance and using agreed common criteria, which did not take into consideration either the risk or downstream consequences of the classification.

Regarding the classification, manufacturers supported the DS's proposals with respect to skin sensitisation category 1A. However, they provided further evidence and comments for consideration against the SCL of 0.001% proposed by the DS. They argued that reliable human data with clearly defined exposures should be used to derive SCLs. They submitted the full study report of the 9 repeated insult patch tests (9 RIPT) (main induction study with 34 volunteers; Anon., 1992) and its addendum (re-challenge phase with 8/34 volunteers; Anon., 1993). Based on the 9 RIPT studies with re-challenge, the manufacturers stated that only 1/34 individuals showed a clear and confirmed allergic response upon exposure to DCOIT and all other alterations were in fact due to the vehicle. Furthermore 2 other individuals (giving a total of 3) reacted to DCOIT, questioning whether the dermal reactions in these individuals were due to the presence of DCOIT or were due to the irritant nature of the vehicle used (ethanol). In conclusion, the manufacturers proposed a threshold for induction of dermal sensitisation for DCOIT of 0.035% (350ppm). The DS responded that the SCL was proposed based on reference values for extreme sensitisers rather than on human data, since such data do not normally give sufficient quantitative information to set a SCL. However, in their response, the DS agreed that one of the participants had a positive reaction to the vehicle (ethanol), making the positive re-challenge to DCOIT questionable. The DS however came to a higher figure on the overall sensitisation (induction) rate of the volunteers i.e. 9%,explaineing that this was based on 3 sensitised individuals among 8 of the 34 initial volunteers that accepted a second re-challenge 6 months after the first one. They reacted after testing DCOIT at 350 ppm (induction phase) and 250 ppm (re-challenge phase). Therefore, the DS disagreed with the Industry's arguments to apply an SCL of 0.035%.

Two MSCAs supported the DS's proposal for classification of DCOIT for skin sensitisation.

Assessment and comparison with the classification criteria

The Tables below present skin sensitisation studies with DCOIT in animals and humans, respectively.

***Table**: Summary of the animal studies on skin sensitisation with DCOIT.*

Study	Dose level	Results	Reference
OECD 429 LLNA 4 mice/group	0.005%, 0.01%, 0.1% 0.25%, and 0.5% (w/v) DCOIT Technical in acetone:olive oil, 4:1 (v/v)	Stimulation indexes = 0.8, 1.1, 11.6, 25.7, and 27.0 for 0.005%, 0.01%, 0.1% 0.25%, respectively EC_3 = 0.03% (15 µg/cm^2) No test item-related clinical signs in controls, 0.005%, 0.01% and 0.1% treated animals At 0.25% and 0.5% slight swelling after second application persisting for 4 days **Conclusion: Sensitiser**	Thor Anon., 2003 (Doc III/7.4.1)
OECD 406 GPMT	Induction on day 0: Intradermal injection of 5% DCOIT Technical in	24 hours after challenge: 60% positives (12/20)	Thor Anon., 2001

10 boars (5 exposed + 5 controls) 10 sows (5 exposed + 5 controls)	propylene glycol with and without adjuvant Challenge 1 on day 7: 0.2 mL of 25% DCOIT in 80% ethanol by occlusive dressing during 48 h Challenge 2 on day 21: 0.2 mL of 5% DCOIT in acetone by occlusive dressing during 24 h	48 hours after challenge: 45% positives (9/20) **Conclusion: Sensitiser**	(Doc III/7.4.1)
OECD 406 GPMT Hartley albino guinea pigs 10 animals/sex/group	Induction on day 0 intradermal injection of 0.01%, 0.02% and 0.03% DCOIT in mineral oil Challenge on day 14: topical application for 24 hours of 0.01%, 0.02% and 0.03% DCOIT	Very faint to faint irritation (slight confluent erythema) after challenge with 0.02% and 0.03% DCOIT 24 hours after challenge with 0.01% DCOIT: 75% positives (15/20) 48 hours after challenge with 0.01% DCOIT: 45% positives (9/20) 24 and 48 hours after challenge with 0.02% DCOIT: 95% positives (19/20) 24 and 48 hours after challenge with 0.03% DCOIT: 100% positives (20/20) **Conclusion: Sensitiser**	Dow Anon., 2003 (Doc III-A6.1.5/01)

***Table**: Summary of the human studies on skin sensitisation with DCOIT.*

Study	Dose level	Results	Reference
INDUCTION STUDIES			
Repeated insult patch test	Induction 0.2 mL of 0.025% and 0.035 DCOIT 3 times/week 3 weeks 2 weeks of rest Challenge: 0.01%, 0.025% and 0.035% DCOIT in ethanol during 24 hours	0.025% DCOIT ($12.5 \mu g/cm^2$) 4/34 individuals exhibited sensitisation 0.035% DCOIT 14/34 individuals exhibited sensitisation	Anon., 1992 A6.12.6/06
Open patch test	DCOIT in petrolatum ether or corn oil up to 0.1%	No sensitisation	Anon., A6.12.6/01-05
ELICITATION STUDIES			
Repeated insult patch test (follow-up of the Anon., 1992 A6.12.6/06)	6-month later than the challenge in study Anon., 1992 A6.12.6/06 Re-challenge: 0.025% DCOIT in ethanol 24 hours with an occlusive patch	3 of 8 subjects that positively responded to challenge with 0.035% DCOIT also positively responded after re-challenge	Anon., 1993 A6.12.6/07
Occupational exposure	Induction: Workers were exposed to an unknown	8 out of 19 workers developed itchy reddish	Anon., 1993 (A6.12.6/07)

	volume of a finishing agent of textiles containing un unknown DCOIT concentration in an unknown skin surface	eruptions on exposed areas of skin
Open patch test	Challenge: 0.06% DCOIT onto a 2 cm^2 area	5/6 previously exposed workers showed strong positive reaction The patient without reaction was being orally treated with corticoids

Comparison with the criteria

According to CLP Guidance , the criteria for Skin Sens. in category 1A are met when:

- There are positive responses in humans at ≤ 500 µg/cm^2
- In an LLNA the stimulation index in higher than 3 with a EC_3 lower than 2%
- In a GPMT there are ≥ 30 % responding at ≤ 0.1 % intradermal induction dose

The available data base for skin sensitisation of DCOIT shows:

- One GPMT showing 60% sensitisation after intradermal induction with 5% DCOIT; which would lead to classification for Skin Sens. in category 1B
- One GPMT showing total (100%) sensitisation after intradermal induction with 0.03% DCOIT; which would lead to classification for Skin Sens. in category 1A
- One LLNA with stimulation indexes up to 27 with EC_3 = 0.03%; which would lead to classification for Skin Sens. in category 1A
- Several studies in humans showing positive responses at ≤ 500 µg/cm^2 (RAC also notes that the potency of sensitisation in humans might be vehicle-dependent)

Therefore, the classification of DCOIT as skin sensitiser category 1A is fully warranted.

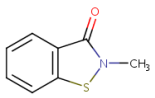
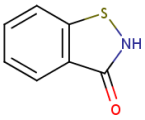
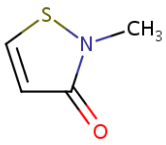
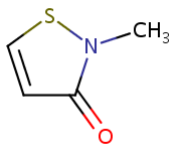
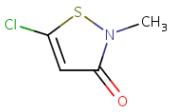
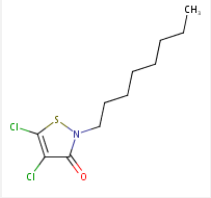
RAC notes that the potency of DCOIT as a skin sensitiser should be considered as extreme based on the results of both LLNA and GPMT tests (Tables 3.6 and 3.7 of the CLP Guidance,). Indeed, the EC_3 was lower than 0.2% and the percentage of sensitisation was higher than 60% at intradermal induction lower than 0.15 % (Table above). According to the CLP Guidance (Table 3.9) an SCL of 0.001% might be considered for DCOIT.

Schwensen *et al*. (2017) demonstrated that cross-reactivity occurs between the non-chlorinated thiazolinones MIT (2-methyl-2H-isothiazol-3-one, ,, BIT (1,2-benzisothiazol-3(2H)-one and OIT (2-octyl-2H-isothiazol-3-one, CAS number: 26530-20-1; see table below). Cross-reactivity between MIT and OIT and as well as between MIT and BIT has been demonstrated in humans according to several publications (Aerts, 2017; Aalto-Korte and Suuronen, 2017; Amsler *et al*., 2017).

RAC notes that no cross-reactivity of skin sensitisation amongst chlorinated thiazolinones (including MBIT) has been reported so far. However, the possibility of cross-reactivity between thiazolinones for skin sensitisation reinforces the view of RAC of similar modes of action, keeping in mind that consumers and professionals may be exposed to different thiazolinones present in different products.

The Table below summarises the chemical structures, LLNA results and human data for different thiazolinones.

Table: *Comparison of skin sensitising properties of several thiazolinones***.** Data taken from RAC opinions on MBIT (2018); MIT (2016); CMIT/MIT (2016) and DCOIT (this opinion). For BIT the LLNA information was taken from the public NICEATM LLNA databank.

	MBIT (CAS: 2527-66-4)	**BIT (CAS: 2634-33-5)**	**MIT (CAS: 2682-20-4)**	**CMIT/MIT (3:1) (CAS: 55965-84-9)**	**DCOIT (CAS: 64359-81-5)**
Chemical structure				 	
LLNA	EC_3 = 1.04 % EC_3 = 0.69 %	EC_3 = 2.3 % EC_3 = 32.4 % EC_3 = 4.8 % EC_3 = 10.4 %	EC_3 = 0.86 %	EC_3 = 0.003 % EC_3 = 0.007 %	EC_3 = 0.03 %
Classification	Skin sens 1A	Skin sens 1B	Skin sens 1A	Skin sens 1A	Skin sens 1A (proposed)
Human data	9/45 (20 %) volunteers showed dermal sensitisation at 500 ppm	5/58 (9 %) at 725 ppm aq., 0/54 (0 %) at 360 ppm aq	1/116 (0.9 %) volunteers at 400 ppm and 1/210 (0.5 %) at 500 ppm	–	5/6 at 0.06% No sensitisation at 0.1% DCOIT (in petrolatum ether) 4/34 at 0.025% 14/34 at 0.035% 3/8 at 0.035% Positive effects at 0.035%
SCL	0.0015 %	0.05 %	0.0015 %	0.0015 %	0.0015 %

Although there are uncertainties regarding human data, RAC considers that at a concentration of 0.025% DCOIT, there is induction of sensitisation in humans. Based on the results of the LLNA study, DCOIT is categorised as an extremely potent skin sensitiser for which the default SCL of 0.001% (10 ppm) should apply, in line with DS's proposal and the CLP Guidance (ECHA, 2017). However, RAC notes that the skin sensitisation potency of DCOIT lies between MIT and the reaction mass CMIT/MIT (3:1). The lowest reported EC3 from a LLNA study was established with CMIT/MIT (3:1) for which an SCL of 0.0015% (15 ppm) was harmonised under the Dangerous Substance Directive (67/548/EC). The SCL of 15 ppm for CMIT/MIT (3:1) was not re-evaluated by RAC in 2016, in the absence of data in the CLH report or in the comments received during public consultation to justify an alternative SCL (ECHA, 2016). RAC acknowledges that both DCOIT and CMIT/MIT (3:1) should deserve the default SCL of 0.001% (10 ppm). However, RAC notes that DCOIT is not more potent than CMIT/MIT for which an SCL of 15 ppm had already been set and that this pointed to an SCL in a similar range, noting that the difference between 15 ppm and 10 ppm is probably of limited relevance for induction of sensitisation.

In conclusion, RAC supports the DS's proposal for **classification of DCOIT as skin sensitiser category 1A; H317 (may cause allergic skin reactions)**. However, RAC proposes **an SCL of 0.0015% (15 ppm)** instead of the default SCL of 0.001% (10 ppm) from the CLP Guidance.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed no classification of DCOIT for STOT-RE because any repeated dose toxicity observed was considered toto be related to local toxicity and/or reduced feed consumption. Moreover, the systemic toxicity observed in most of the cases was considered secondary to reduced body weight and food consumption related to local irritation.

Comments received during public consultation

One MSCA requested discussion of the toxicological significance of the effects reported for spleen, thymus and adrenals in the repeated toxicity studies and in the reproduction studies. The DS argued in their response to the comments that these effects were not of sufficient significance to support classification on the basis of the following:

1. The LOAELs for several of the repeated dose toxicity studies were below the limit qualifying for a STOT RE 2 classification. However, the DS considered local toxicity of the gastrointestinal tract and reduced feed consumption the major drivers of the toxicities observed in the repeated dose toxicity studies. As a consequence, most of the observations can reasonably be regarded as secondary effects. Furthermore, the effects of DCOIT were in general dependent on dose levels and not on the duration of exposure. Consequently, the DS proposed to consider the local toxicity as acute toxicity, and thus classification for STOT RE would not be warranted.
2. Reduced feed consumption is a likely cause of the changes observed in thymus, and this interpretation is supported by rodent and dog restricted feeding studies (Moriyama *et al*., 2008; Takamatsu *et al*., 2015).
3. An increase in granulocytes in the spleen was observed at the high dose (500 mg/kg bw/day) in a rat subacute study (A6.3.1/01) and is considered likely to be related to significant gastrointestinal irritation at this dose level. Similar effects on the spleen were not reported in the other repeated dose toxicity studies, and this effect is thus suggested to be associated with high dose administration.
4. Reduced pup thymus weight and spleen weight were reported in the two available fertility studies. These effects were supported by histopathological findings at the higher dose level in the study by Anon. (2001, A6.8.2-01), and the higher dose levels were also associated with reduced body weights in both studies. Decreases in thymus and/or spleen weights were reported also at the mid doses in F1 and/or F2 pups. These findings suggest that pups might be more sensitive to the cytotoxic effects of DCOIT than adult animals and dams. The findings appear (at least partly) to be related to the reduced body weight compared to control.
5. Lipid accumulation in the adrenal cortex was reported from 100 mg/kg bw/day, and increased absolute and relative adrenal weights were reported at the high dose (500 mg/kg bw/day) in a rat subacute study (A6.3.1/01). The effect was not reversible in the high dose group (500 mg/kg bw/day). An increase in relative adrenal weight in males was reported from 70 mg/kg bw/day in one of the two rat sub-chronic studies (A6.4.1-01; A7.5.1-01), but not in the other at doses up to approximately 250 mg/kg bw/day. The effect on relative adrenal weight in the first study was not associated with histopathological findings and appeared to be related to the reductions in body weight at the same doses. In the two-generation reproductive toxicity study by Anon. (2001; A6.8.2-01), adrenal cortex pathology (hypertrophy/vacuolization) was reported at the high dose (3200 ppm/ 235-259 mg/kg bw/day), a dose for which also reduced body

weight gain and pathology of the stomach (hyperplasia and hyperkeratosis of non-glandular mucosa) were observed. In the two-generation reproductive toxicity study by Anon. (2006; A 6.8.2-01, 7.8.1-01), an increased relative adrenal weight was observed at the high dose (1050 ppm equivalent to 57-71 mg/kg bw/day) in females, which was associated with reduced body weight.

Taken together, the DS did not find sufficient evidence for direct adrenal toxicity at dose levels below ~~300~~ mg/kg bw/day in the subacute studies and at doses below 100 mg/kg bw/day in the sub-chronic studies or in the parental generation in the fertility studies.

One MSCA supported the DS's proposal for no classification of DCOIT for STOT-RE.

Assessment and comparison with the classification criteria

The Table below presents the toxicological findings from the repeated dose toxicity studies by the oral route.

***Table**: Summary table of repeated dose toxicity studies by the oral route with DCOIT.*

Method	Results	Reference
Oral Gavage Japanese guideline 4 weeks 2 week recovery SD (Crj:CD) rats Males and females, 10 animals/sex/ group 0, 20, 100 and 500 mg/kg bw/day; 97.5% DCOIT 97.5 % DCOIT in olive oil Classification limits: Cat 1 ⩽ 30 mg/kg bw/day Cat 2 ⩽ 300 mg/kg bw/day	<u>500 mg/kg bw/day</u> 3 females died on day 4 Reduced spontaneous movement (32/40), anal staining (37/40), salivation (37/40), abdominal distension (37/40). In some animals: hypothermia, cyanosis, reddish lacrimation and gasping. All these clinical effects were reversed during the recovery period. ↓ body weight and food consumption ↓ haemoglobin, MCV, MCH and MCHC, haematocrit, prothrombin time, and lymphocytes ↑ segmented neutrophils. ↑ GOT, GPT, Na, cholesterol; A/G ratio, inorganic phosphorous, chloride ↓ glucose, creatinine and total protein. ↑ absolute and relative adrenal weights, relative brain, adrenal and testes weights ↓ absolute brain, liver, kidney, spleen and testes weights, relative liver weight Thickening of mucosa of stomach (18/18) and small (11/18) and large (6/18) intestine. Hyperplasia of the mucosal epithelium of the stomach (5/18) and small intestine (15/18) and granulation in the stomach (7/18). Liver atrophy of several male animals ↑ granulocytes in the spleen (7/18) considered due to inflammatory changes in the stomach and an increase of lipids in the adrenals <u>100 mg/kg bw/day</u> Salivation (9/20) ↓ haemoglobin, MCV, MCH and MCHC ↑ chloride, GOT, A/G ratio and inorganic phosphorus ↓ glucose and total protein Hyperplasia of the mucosal epithelium of the stomach (14/19) and small intestine (9/19) <u>20 mg/kg bw/day</u> No dose related-effects **Conclusions:** **NOAEL = 20 mg/kg bw/day** **LOAEL = 100 mg/kg bw/day**	Dow Anon., 1991 (A6.3.1/01)
Oral	<u>282-278 mg/kg bw/day</u>	Dow

Diet OECD 408 3 months Crl:CD®BR rats Males and females 10 animals/sex/ group 0, 100, 500, 1000, 4000 ppm Males: 6.2, 32.5, 60.7, 248.2 mg/kg bw/day Females: 7.2, 36.7, 74.7, 278.4 mg/kg bw/day 98.8% DCOIT Classification limits: Cat 1 ⩽ 10 mg/kg bw/day Cat 2 ⩽ 100 mg/kg bw/day	Scant faeces ↓ body weight and food consumption (affecting 100% animals, unknown severity) ↓ mean cell volume (21% males, 6% females), mean cell haemoglobin (21% males, 3% females), haemoglobin (15% males) and haematocrit (15% males) ↑ red blood cell and platelet counts (18%) Altered red blood cell morphology in 8/9 males and 4/10 females ↓ serum triglyceride (62% males, 53% females), total protein (7% males, 6% females) and globulin levels (18% males, 21% females) ↑ inorganic phosphorous (35% males), potassium (16% males, 19% females) and A/G ratio (22% males, 20% females) , GOT (41% males), blood urea nitrogen (28% females) Histopathologic findings affecting 100% of animals: Minimal hyperkeratosis alone to hyperkeratosis in the presence of hyperplasia, oedema, erosion, ulceration, and/or inflammation of the mucosa/submucosa in the forestomach <u>75-61 mg/kg bw/day</u> ↓ reductions in body weight and food consumption in 100% females (unknown severity) ↓ serum triglyceride (41% females) Histopathologic findings: 1/10 males had minimal mucosal erosion and minimal acute inflammation of the mucosa/submucosa, and 1/10 females had minimal mucosal erosion with mild submucosal inflammation and moderate submucosal oedema. <u>37-33 and 6.2-7.2 mg/kg bw/day</u> No treatment-related effects **Conclusions:** **NOAEL: 500 ppm (32.5-36.7 mg/kg bw/day)** **LOAEL: 1000 ppm (60.7-74.7 mg/kg bw/day)**	Anon., 1994 (A6.4.1.a/01)
Oral Diet OECD 409 3 months Beagle dogs Males/females 4 animals/sex/ group 0, 100, 300 and 1500 ppm Males: 3.4, 10.2, 47.5 mg/kg bw/day Females: 3.4, 10.1, 45.9 mg/kg bw/day 98.42% DCOIT Classification limits: Cat 1 ⩽ 10 mg/kg bw/day Cat 2 ⩽ 100 mg/kg bw/day	<u>48-46 mg/kg bw/day</u> ↓ 18% body weight gain by week 12 (females) ↓ 26% Food consumption by week 6 (females) ↓ hemoglobin, hematocrit, red blood cell counts, reticulocytes, total protein and albumin (in all cases in males (not in females) by week 7, but not by week 13) ↓ 12% total protein (females (not in males) by week 13) <u>10-10 and 3.4-3.4 mg/kg bw/day</u> No treatment-related effects **Conclusions:** **NOAEL: 300 ppm (10.2-10.1 mg/kg bw/day)** **LOAEL: 1500 ppm (47.5-45.9 mg/kg bw/day)**	Dow Anon., 2002 (A6.4.1.b/01)
Oral	<u>105 mg/kg bw/day</u>	Thor

Gavage OECD 408 90 days 28 day recovery groups for control and high dose Wistar rats Males/females 10 animals/sex/ group 0, 35, 70, 105 mg/kg bw /day 97.4% DCOIT in peanut oil Classification limits: Cat 1 ⩽ 10 mg/kg bw/day Cat 2 ⩽ 100 mg/kg bw/day	↓ 10% in final body weight (males and females) ↓ 15% in body weight gain (males and females) Local irritation of gastrointestinal tract Significantly increased relative testes and adrenal weights and significantly reduced testicular sperm head count, in males (no morphological abnormalities; alterations absent after 28 day treatment free recovery period) 70 mg/kg bw/day ↓ 10% in final body weight (males) ↓ 15% in body weight gain (males) Local irritation of gastrointestinal tract Significantly increased relative testes and adrenal weights and significantly reduced testicular sperm head count, in males (no morphological abnormalities; alterations absent after 28 day treatment free recovery period) 35 mg/kg bw/day No treatment-related effects **Conclusions:** **NOAEL: 35 mg/kg bw/day** **LOAEL: 70 mg/kg bw/day**	Anon., 2002 (A 6.4.1-01 7.5.1-01)
Oral Diet OECD 409 90 days Beagle dogs Males/females 4 animals sex/dose 100, 300, 1500, 3000 and 4500 ppm 0, 2, 5, 27, 61, 88 mg/kg bw/day (males); 0, 2, 6, 35, 61, 74 mg/kg bw/day (females) 97.1% DCOIT Classification limits: Cat 1 ⩽ 10 mg/kg bw/day Cat 2 ⩽ 100 mg/kg bw/day	88/74 mg/kg bw/day ↓ 26% body weight ↓ 40% food consumption Local irritation in the stomach Reduced cholesterol, phospholipids, total protein, albumin, globulin, and calcium Increased AST, ALT and GLT Reduced absolute thymus (in females also relative), thyroid, epididymides, heart, liver, and prostate weights (primarily at the highest dose). No morphologic abnormalities of the liver (although no histopathological liver assessment) 61/61 mg/kg bw/day ↓ 7% body weight ↓ food consumption Local irritation in the stomach Increased AST, ALT and GLT No morphologic abnormalities of the liver (although no histopathological liver assessment) 35/27 mg/kg bw/day 2 female lean appearance 6/5 mg/kg bw/day 1 male lean appearance 2/2 mg/kg bw/day No treatment-related effects **Conclusions:** **NOAEL: 27-35 mg/kg bw/day** **LOAEL: 61-61 mg/kg bw/day**	Thor Anon., 2007b (A 6.4.1-02 7.5.1-02)

The Table below presents the toxicological findings from the repeated dose toxicity studies by the dermal route.

Table: *Summary table for repeated dose toxicity studies by the dermal route with DCOIT.*

Method	Results	Reference
No guide-line (prior to OECD 410) 21 days New Zealand White rabbits Males/females 6 animals/sex/ group 0, 0.35, 1.75 mg a.i./kg bw/day; Pre-formulation: C-9211M (35% DCOIT) Vehicle: mixed xylene diluted in acetone) Non-occluded dressing 5 days/week 15 doses in 21 days Classification limits: Cat 1 ⩽ 39 mg/kg bw/day Cat 2 ⩽ 390 mg/kg bw/day	<u>1.75 mg/kg bw/day</u> Severe skin irritation: mean erythema score by day 15 (males/females): 2.2/2.2; mean oedema score by day 15 (males/females): 2.8/2.7 Microscopic skin pathology: • marked hyperplasia (10/12), moderate hyperplasia (2/12) • marked hyperkeratosis (9/12), moderate hyperkeratosis (3/12) • parakeratosis (2/12) • diffuse inflammatory cell infiltration (12/12) • focal haemorrhage (1/12) <u>0.35 mg/kg bw/day</u> Slight skin irritation: mean erythema score by day 15 (males/females): 1.2/1.2; mean oedema score by day 15 (males/females): 0.3/0.3 Microscopic skin pathology: • marked hyperplasia (2/12), moderate hyperplasia (4/12), slight hyperplasia (3/12) • moderate hyperkeratosis (1/12), moderate hyperkeratosis (6/12), slight hyperkeratosis (9/12), • diffuse inflammatory cell infiltration (10/12) • focal haemorrhage (2/12) **Conclusions:** **Systemic NOAEL: 1.75 mg/kg bw/day** **Local LOAEL: 0.35 mg/kg bw/day**	Dow Anon., 1983 (A6.3.2/01)
OECD 410 28 days Wistar rats Males/females Occlusive dressing 20 animals/sex for control and high dose 10 animals/sex for low and intermediate dose 0, 3, 15, 60/30 mg /kg bw/day 96.47% DCOIT in corn oil Classification limits: Cat 1 ⩽ 60 mg/kg bw/day Cat 2 ⩽ 600 mg/kg bw/day	Severe local skin reactions were observed (due to corrosivity of the test substance), and the severity of the skin lesions increased with dose. Skin reactions included erythema, oedema, wounds and beginning necrosis. <u>Top dose: 60 mg/kg bw/day from beginning to day 9, rest until day 22 and restart dosing with 30 mg/kg bw/day until day 23</u> Mortality: 6 males and 8 females (one found dead on day 7, 13 killed in extremis on day 9). ↓ body weight in males ↓ blood red blood cell count, haemoglobin and haematocrit ↑ eosinophil granulocyte count and red blood cell distribution volume ↑ spleen- and adrenal-to-body weight-ratio <u>15 mg/kg bw/day</u> Mortality: 1 male and 1 female ↓ body weight in males <u>3 mg/kg bw/day</u> No treatment-related effects **Conclusions:** **NOAEL: 3 mg/kg bw/day** **LOAEL: 15 mg/kg bw/day**	Thor Anon., 2007a (A 6.3.2-01 7.5.2-01)

The Table below presents the toxicological findings from the repeated dose toxicity studies by the inhalation route.

Table: *Summary table for repeated dose toxicity studies in animals by inhalation with DCOIT.*

Method	Results	Reference
Inhalation (nose only) OECD 413 3 months, 6 month and 1 year recovery groups CRL:CD® BR rats Males/females 32 animals/sex/ group 0, 0.02, 0.63 and 6.72 mg ai/m³ (analytical concentrations) Pre-formulation: C-9211M HQ (32.6% DCOIT in o-xylene) Medium mass diameter of DCOIT aerosol = 1.4 µm (respirable fraction 72%) Medium mass diameter of xylene aerosol = 2.5 µm (respirable fraction 58%) 5 days/week 6 hours/day Classification limits: Cat 1 ⩽ 0.2 mg/L/6h/day; Cat 2 ⩽ 0.02 mg/L/6 h/day	Rales, gasping, dyspnoea during the thirteen-weeks of dosing. No clinical signs of respiratory distress were noted during the recovery period. <u>6.72 mg a.i./m³</u> ↓ bodyweight gain Lung: red foci in males (4/16) vs 1/16 in vehicle control, Goblet cell hyperplasia (11/16) and inflammation (11/16) Nose: inflammation (7/32), epithelial hyperplasia (15/32), Goblet cell hyperplasia (20/32) Larynx: inflammation (31/32), hyperplasia (30/32), squamous mataplasia (32/32), hyperkeratosis (16/32) <u>0.63 mg a.i./m³</u> Lung red foci in males (4/16) vs 1/16 in vehicle control Nose: inflammation (6/32), epithelial hyperplasia (13/32), Goblet cell hyperplasia (8/32) Larynx: inflammation (15/32), hyperplasia (22/32), squamous mataplasia (30/32), hyperkeratosis (2/32) <u>0.02 mg a.i./m³</u> Lung red foci in males (3/16) vs 1/16 in vehicle control Nose: inflammation (3/32), epithelial hyperplasia (3/32), Goblet cell hyperplasia (3/32) **Conclusions:** **LOAEC: 0.02 mg a.i./m³**	Dow Anon., 1994 (A6.4.3/01)

RAC notes that the available database shows mainly local toxicity effects at the point of contact (gastrointestinal system, respiratory system and skin depending on the route of exposure). These local effects are related to corrosivity of the substance and are already covered by the classification as skin corrosive category 1 and by the supplementary hazard information label EUH071 (corrosive to respiratory tract). Therefore, RAC is of the opinion that this local toxicity should not be used to support classification for STOT-RE.

Other systemic effects have also been reported (mainly reductions in bodyweight and haematological alterations). However, RAC notes that these effects are probably secondary to the reductions in bodyweight which in turn are the consequence of the reductions in food intake due to gastrointestinal insults caused by DCOIT corrosivity. Thus, RAC does not consider the reported systemic toxicity as sufficient for warranting classification for STOT-RE.

Also alterations in the weight of thymus, spleen and adrenals were consistently reported in the repeated dose toxicity studies and in some reproductive toxicity studies (see section for assessment of reproductive toxicity). However, RAC fully agrees with the arguments stated by the DS (see above) about the lack of robustness of these effects for warranting classification for STOT RE.

In conclusion, RAC agrees with the DS's proposal for **no classification of DCOIT for STOT-RE**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS found no evidence of genotoxicity when DCOIT was tested in *in vivo* and *in vitro* tests. The main DCOIT metabolite was also tested in bacterial gene mutation assays with negative results. Therefore, the DS proposed no classification for DCOIT for germ cell mutagenicity.

Comments received during public consultation

One MSCA supported the DS's proposal for no classification for mutagenicity.

Assessment and comparison with the classification criteria

The Table below summarises the available *in vitro* genotoxicity studies. No positive results were found in any of the reliable studies shown.

Table: *Summary table of relevant in vitro mutagenicity studies with DCOIT.*

Method	Test system	Tested concentrations	Results	Remarks	Reference
Bacterial Gene Mutation Assay OECD 471	S. typhimurium, TA 1535, TA 1537, TA 98, TA 100	0.3 to 300 µg a.i./plate	With S9: Negative Without S9: Negative	Low doses of active ingredient Cytotoxicity was observed from 100 µg/plate (+ metabolic activation) and from 3-10 µg/plate (- metabolic activation)	Dow Anon., 1994 (A6.6.1/01)
Bacterial Gene Mutation Assay OECD 471 and OPPTS 870.5100	S. typhimurium, TA 1535, TA 1537, TA 98, TA 100 and E. Coli WP2 uvrA	1.5 to 5000 µg/plate N-(n-octyl) malonamic acid (NNOMA), a major metabolite of DCOIT identified in metabolism studies	With S9: Negative Without S9: Negative	None	Dow Anon., 2005 (A6.6.1/02)
Chromosome aberration test OECD 473	Chinese hamster ovary (CHO) cells	With S9: 0.1 to 0.7 µg/mL Without S9: 3.0 - 8.0 µg/mL	With S9: Negative Without S9: Negative	In the range finding study cytotoxicity observed at 0.25-0.6 µg/mL (-S9) and 5-10 µg/mL (+S9)	Dow Anon., 1994 (A6.6.2/01)
Gene mutation assay OECD 476	Chinese hamster Ovary (CHO), CHO-K1-BH4	Without S9: 0.005 to 0.75 µg/mL With S9: 0.5 to 25 µg/mL	With S9: Negative Without S9: Negative	Cytotoxicity observed at 0.75 µg/mL (-S9) and 15 µg/mL (+S9)	Dow Anon., 1994 (A6.6.3/01)
Bacterial gene mutation OECD 471	S. typhimurium TA 1535, TA 1537, TA 98, TA 100, TA 102	Without S9: 0.06-1.0 µg/plate With S9: 0.5- 10 µg/plate	With S9: Negative Without S9: Negative	Cytotoxicity: ⩾ 5 µg/ plate (-S9); ⩾ 50 µg/plate (+S9)	Thor Anon., 2000 (A 6.6.1-01 7.6.1-01)
Cytogenetic test OECD 473	Human primary lymphocytes	With and without S9: 0.09-1.5 µg/mL	With S9: Negative Without S9: Negative	Cytotoxicity at ⩾ 1.5 µg/mL	Thor Anon., 2001 (A 6.6.2-01 7.6.1-02)

Gene mutation in mammalian cells OECD 476	Chinese hamster V79 cells	Without S9: 0.03-0.2 µg/mL With S9: 2.5-20 µg/mL	With S9: Negative Without S9: Negative	Cytotoxicity at lower than 0.16µg/L (-S9), ⩾ 20 µg/mL (+S9)	Thor Anon., 2002 (A 6.6.3-01 7.6.1-03)

The Table below summarises the available *in vivo* genotoxicity studies. No positive results were found in any of the reliable studies shown.

***Table*:** *Summary table of relevant in vivo mutagenicity studies with DCOIT.*

Method	Test system	Tested concentrations	Results	Remarks	Reference
Micronucleus test OECD 474 Sampling times: 24 and 48 hours	CD-1 mice Bone marrow cells Male and female 5-9 animals/sex/ group Oral gavage	60, 300, 600 mg a.i./kg bw	Negative	The dose level were selected according to a previous study were it was found that a dose of 600 mg/kg bw induced toxicity and mortality both in females and males	Dow Anon., 2001 (A6.6.4/01)
Mouse bone marrow cytogenetics test OECD 475 Sampling time: 24 hours after last treatment	Swiss mouse Males and females 5 animals/sex/ group	0, 100, 200, 400 mg/kg bw in peanut oil 2 oral applications 24 h between applications	Negative	Pre-test: dose-dependent toxicity, 50% deaths between 750 and 1500 mg/kg bw	Thor Anon., 2001 (A 6.6.4-01 7.6.2-01)
UDS test in rat hepatocytes OECD 486 Sampling times: 2 and 16 hours	Wistar rats 4 males/group	1000, 2000 mg/kg bw in corn oil Single oral application	Negative	Clinical signs but no mortality in all treated animals	Thor Anon., 2002 (A 6.6.5-01 7.6.2-02)

No positive results were found in a wide battery of well-performed and reliable *in vitro* and *in vivo* tests. Thus, RAC agrees with the DS's proposal for **no classification of DCOIT for germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No studies had been performed which addressed the carcinogenicity of DCOIT. Nevertheless, the DS provided a list of arguments in the CLH report that suggest that the substance is of low concern regarding this hazard:

- A common feature of the repeated dose studies is that the toxicity seems related to local toxicity and/or reduced feed consumption. None of the studies showed significant systemic toxicity because the doses or concentrations that may induce systemic toxicity seem to be similar or higher than the concentrations that induce significant local toxicity due to irritation, thus hindering the evaluation of any systemic toxicity.

- The toxicokinetic studies indicate that DCOIT is readily absorbed, metabolised and eliminated and there is no evidence that either DCOIT or its metabolites bioaccumulate.
- Genotoxicity studies on DCOIT were negative, both *in vitro* and *in vivo*, thus arguing against a potential genotoxic mechanism of carcinogenesis.
- Furthermore, no evidence suggestive of an endocrine mechanism of carcinogenesis has been reported in the repeated dose toxicity studies.
- Potential tumour promoting effects caused by chronic tissue irritation will only be relevant if long-term exposure occurs to DCOIT concentrations that give rise to local toxicity.
- Negative results were obtained in three independent studies performed with mixtures of the structurally related thiazolinones MIT and CMIT in rats and mice by oral and dermal routes and with OIT in mice by the oral route.

Therefore, the DS proposed no classification of DCOIT for carcinogenicity.

Comments received during public consultation

One MSCA supported the DS's proposal for no classification regarding carcinogenicity.

Assessment and comparison with the classification criteria

RAC agrees with the DS's proposal for **no classification of DCOIT for carcinogenicity** in the absence of chronic/carcinogenicity data. RAC agrees with the DS that DCOIT is unlikely to be of concern for carcinogenicity based on the arguments provided in the CLH report including its lack of germ cell mutagenicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of DCOIT for fertility and sexual function on the basis of an assessment of two 2-generation studies reported for DCOIT and the following arguments presented in the CLH report:

- No treatment-related alterations of male or female mating or fertility parameters were reported
- There were no treatment-related alterations in the pathology of any of the reproductive organs
- No changes were observed in gestation, lactation or viability indices, oestrus cycle or sperm parameters
- Some indications of delayed puberty in offspring were partly associated with reduced pup body weight and were not accompanied by changes in anogenital distance in the F2-generation
- The DS proposed no classification of DCOIT for development because there was no clear evidence for developmental toxicity at dose levels not causing maternal toxicity. The DS also noted the following:
- The developmental toxicity studies indicated that DCOIT induces a slight foetal developmental delay in rats at high doses as suggested by increases in skeletal variations.
- The incidence of skeletal malformations observed at the two highest doses was low.
- The skeletal variations observed were reversible in nature and may be related to the reduction in maternal body weight gain.
- No increases in skeletal variations or malformations were observed in the rabbit study

- The retinal findings observed were not supported by other available studies.

Comments received during public consultation

One MSCA supported the DS's proposal for no classification for reproductive toxicity.

Assessment and comparison with the classification criteria

Fertility and sexual function

The Table below summarises the available studies assessing the effects on fertility and sexual function.

***Table**: Summary table of relevant reproductive toxicity studies with DCOIT.*

METHOD	RESULTS
Dietary 2-generation reproductive OECD 416 (draft) US EPA OPPTS 870.3800 GLP Crl:CD® BR 26 animals/sex/group Dosing: 10 weeks prior to mating and continuing until sacrifice of parent, F1, and F2 gene-rations 0, 200, 400, 800, 3200* ppm *one generation only due to mortality of F1 off-spring; F1 males: 16, 30, 62 and 235 mg/kg bw/day F2 males: 20, 39 and 88 mg/kg bw/day F1 females: 18, 33, 67 and 259 mg/kg bw/day F2 females: 21, 41 and 93 mg/kg bw/day Addition of 400 ppm group with separate control group DCOIT technical, purity: 100% Dow: Anon. 2001 (A6.8.2/01)	<u>Parental toxicity</u> 3200 ppm F0: ↓ bodyweight gain: 13-45%, 16-31%, 8-18% for premating, gestation and lactation periods, respectively 800 ppm F1: ↓ bodyweight gain (males) **NOAEL: 400 ppm (31-41 mg/kg bw/day)** <u>Toxicity to offspring</u> 400 ppm: F1 pups: ↓ spleen weight; F2 pups: ↓ thymus weight 800 ppm: F1, F2 pups: ↓ body weight; signs of toxicity in various organs (watery blood, enlarged heart, pale lungs, liver, kidney and/or intestine); ↓ thymus weight 3200 ppm: ↓ thymus weight; distended abdomens; ↑ mortality (lactation index 53.8 vs 98.3% in controls concurrent with 200, 800 and 3200 ppm). **NOAEL: F1 400 ppm (31-41 mg/kg bw/day); F2: 200 ppm (16-21 mg/kg bw/day)** <u>Reproductive toxicity</u> No effects on fertility, live litters, live pups/litter, sex ratio, oestrus cycle or sperm parameters. Delay in vaginal opening, F1 (400 ppm: 33.3 vs 31.7 in control run concurrently with 400 ppm; 800 ppm: 35.1 vs 31.9 in controls concurrent with 200, 800 and 3200 ppm) Delays in preputial separation, F1 (400 ppm: 43.7 vs 42.2 in control run concurrently with 400 ppm and vs 43.9 in controls concurrent with 200, 800 and 3200 ppm); 800 ppm: 46.2 vs 43.9 in controls concurrent with 200, 800 and 3200 ppm). Anogenital distance: F2: 400 ppm: slight ↑ in females and males; 800 ppm: no significant effects. **CONCLUSION:** **Parental NOAEL: 30-41 mg/kg bw/day** **NOAEL F1: 30-41 mg/kg bw/day** **NOAEL F2: 16-21 mg/kg bw/day**
Dietary OECD 416 Wistar Crl: (WI) BR rats 24 animals/sex/group	<u>Parental toxicity in F0 generation</u> 350 ppm: ↓ 6.8% by terminal day. 1050 ppm: ↓ body weight: 7.3% by premating day; 16.7% and 19.5% by mating days 8 and 15, respectively; 6.8%, 7.8%, 7.7%

Dosing: 10 weeks pre-mating; up to 2 weeks mating; females: gestation, lactation in addition 97.1% DCOIT 100, 350, 1050 ppm 3-4, 14-16 and 57- 71 mg/kg bw/day Thor: Anon. 2006 A 6.8.2-01; 7.8.1-01	and 8.1% by lactation days 4, 7, 14 and 21, respectively; 8.2% by termination day. 1050 ppm: ↑ relative brain weight (females) 1050 ppm: 9/10 males and 3/11 females hyperplasia of the squamous epithelium of the forestomach 1050 ppm: 6/10 and 2/11 females lymph granulocytic inflammation of the forestomach <u>Parental toxicity in F1 generation</u> 1050 ppm: ↓ decreased absolute prostate, brain and ovaries weights, ↑ relative weights of kidney and adrenals; ↓ terminal body weight 1050 ppm: 2/10 males and 3/10 females hyperplasia of the squamous epithelium of the forestomach 1050 ppm: 2/10 and 1/10 females lymph granulocytic inflammation of the forestomach <u>Toxicity to offspring in F1 generation</u> 1050 ppm: mal-rotated legs, absent/reduced tail and ↓ anus size in 4 pups 1050 ppm: ↓ absolute spleen and thymus weight and ↑ relative brain weight <u>Toxicity to offspring in F2 generation</u> 100 ppm: ↑ relative brain weight 350 ppm: ↓ absolute and relative spleen weight 1500 ppm: ↓ body weight, ↓ absolute and relative spleen weight, ↑ relative brain weight <u>Reproductive toxicity</u> No treatment related effects on sperm count, motility and morphology, and oestrus cycle. Reproduction parameters not affected. F1: Slightly delayed preputial separation at 1050 ppm **CONCLUSION:** **Parental NOAEL: 14-16 mg/kg bw/day** **Reproductive NOAEL: 57-71 mg/kg bw/day** **Developmental NOAEL: 14-16 mg/kg bw/day**

After assessment of the two available 2-generation reproduction studies in rats, RAC notes the following:

- None of the studies reported pathological alterations in reproductive organs and no changes were observed in reproductive performance (mating or fertility) or in gestational, lactation or viability indices.
- Decreased offspring viability was reported concurrently with stomach lesions which reduces the concern for classification purposes.
- Delayed puberty in the offspring (delayed vaginal opening and preputial separation) was reported with low incidence and might be also related to reductions in bodyweight rather than a true reproductive effect. Moreover, these effects were not supported by changes in ano-genital distance. Thus, RAC does not consider these effects sufficiently severe to support classification.

- Alterations in absolute and/or relative weights of several organs such as thymus, spleen and brain. RAC notes that these alterations are not consistently found among different generations and between sexes and also that some of them (specifically the effects in the thymus and spleen) were already reported in the repeated dose toxicity studies. Moreover, the relative variations might also be influenced by reductions in body weight. Therefore, RAC does not consider these effects sufficiently severe and clear to be relevant for classification for sexual function and fertility.

In conclusion, RAC does not consider the effects in offspring viability, delayed puberty and changes in organ weights relevant for classification purposes and therefore agrees with the DS's proposal for **no classification of DCOIT for sexual function and fertility**.

Developmental toxicity

The Table below summarises the available studies assessing developmental toxicity.

***Table**: Summary table for oral developmental toxicity studies in animals with DCOIT.*

METHOD	RESULT
Oral gavage Teratogenicity OECD 414 GLP Crl:CD BR rats 25 females/group Dosing: GD 6-15, 5 days recovery period Doses: 0, 10, 30, 100, (300) mg/kg bw/day 300 mg/kg group terminated due to severe maternal toxicity Extra control group due to addition of 10 mg/kg bw/day dose group Test material: 98.8% DCOIT in corn oil Dow Anon. 1994, A6.8.1b/02	<u>Maternal toxicity</u> 300 mg/kg bw/day: weight loss, soft faeces and/or diarrhoea, altered posture and mortality (group terminated). 100 mg/kg bw/day: One treatment related death, ↓ body weight gain during the treatment period (GD 6-16; 73% of control); ↓ maternal feed consumption throughout the treatment period (88-90% of control); scant/soft faeces and/or diarrhoea in 18/25 dams. 30 mg/kg bw/day: No significant effects on body weight or body weight gain; ↓ maternal feed consumption from GD 10-16 (91% of control); scant/soft faeces and/or diarrhoea in 5/24 dams. <u>Developmental toxicity</u> No treatment-related effects on the numbers of early or late resorptions, live foetuses per litter, foetal body weight or sex ratio were reported No HCD data were available in the CLH report.

	Fetuses with skeletal malformations	**Litters with fetuses with wavy ribs**	**Rudimentary 13th thoracic rib**
Control 1	0/337	1/24	
Control 2	0/383	1/24	
10 mg/kg bw/day	0/380	1/25	
30 mg/kg bw/day	2/347(2/24 litters)	1/24	20/347 foetuses 11/24 litters
100 mg/kg bw/day	1/332 (1/24 litters)	11/24 (21 foetuses: 4 mild, 9 moderate, 8 severe)	6/332 foetuses 5/24 litters

CONCLUSIONS:
Maternal NOAEL: 10 mg/kg bw/day
Developmental NOAEL: 30 mg/kg bw/day

Oral gavage Teratogenicity OECD 414 Crl:CD (SD)BR rats	<u>Maternal toxicity</u> 112.4 mg a.i./kg bw/day: 6/25 mortalities (between days 9-14 of gestation), toxicity (wheezing, salivation, red exudates from nose or eyes, lethargy and difficulty breathing) in 17/25 dams ↓ maternal body weight (30.4 g vs 53.6 g in solvent control group with 2 animals with negative body weight gain between GD 6-16)

25 females/ group

Dosing: GD 6-15

5 days recovery

Doses: 0, 11.2, 33.7, 112.4 mg a.i./kg bw/day

Test material: C-9211M, 48.9% a.i* in xylene
* 40.3% DCOIT and 8.6% mono-chlorinated form

Vehicle: methylcellulose

Solvent control: xylene in methyl-cellulose

Dow

Anon. 1983, A6.8.1b/01

33.7 mg a.i./kg bw/day: ↓ maternal body weight (49.9 g vs 53.6 g in solvent control group); red exudates from nose in 7/25 dams.

Developmental toxicity

No treatment-related effects on the numbers of resorptions or live foetuses per litter

112.4 mg a.i./kg bw/day: ↓ body weight of male foetuses compared to the combined controls (3.3 g vs 3.5 and 3.4 for vehicle and solvent control, respectively);

Teratogenic effects					
	control		**Treated groups (mg/kg bw/day)**		
	vehicle	xylene	11.2	33.7	112.4
Skeletal malformation:					
bent ribs	1.9	3.6	1.9	7.4	10.5
bent limbs	0	0	0	0.5	4.7
Skeletal variations (%)	42.6	60.1	54.4	53.4	61.6
Skeletal variants (%)	4.2	7	6.8	6.8	9.9
Visceral variations (%)	1.8	0.8	1.8	3.7	1.1

CONCLUSIONS:
Maternal NOAEL: 11.2 mg a.i./kg bw/day
Developmental NOAEL: 11.2 mg a.i./kg bw/day

Teratogenicity

US EPA OPP 83-3

New Zealand White rabbits

20 females/group

Dosing: GD 7-19

10 days recovery

Doses: 0, 5, 25, 70 mg a.i./kg bw/day in 10 mL volume

Test material: C-9211, 40% DCOIT in xylene

Vehicle: methylcellulose

Solvent control: xylene in methylcellulose; comparable to high dose

Dow

Anon., 1986 A6.8.1a/01

Maternal toxicity

In all treated group: Decreased defecation and urination; lethargy, ataxia and laboured breathing.

70 mg a.i./kg bw/day: Mortality (5/20 dams); ↓ body weight gain GD 7-19 (-478 g); ↓ body weight GD 15-25

25 mg a.i./kg bw/day: Mortality (3/20 dams). ↓ body weight gain GD 7-19 (-115 g); marked negative body weight gain during the last 4 days of treatment

5 mg a.i./kg bw/day: Mortality: 0/20 dams. Non-significant ↓ body weight gain GD 7-19 (-44 g)

Vehicle control: Mortality: 1/20 dams. Body weight gain GD 7-19 (84 g)

Solvent control: Mortality: 1/20 dams. ↓ body weight gain GD 7-19 (-52 g)

Reproductive and developmental toxicity

No significant differences in resorptions or in foetal body weight

No significant dose-related differences in foetal variations or malformations reported

The total number of viable foetus was 120, 92, 94, 69 and 23 in the vehicle control group, xylene control group, 5, 25 and 70 mg DCOIT/kg bw/day dose groups, respectively.

70 mg a.i./kg bw/day: non-significant ↓ in implantation sites (4.4 vs 6.9 in solvent controls); 6/18 pregnant dams aborted (non-significant) and significant ↓ in live foetuses per litter (3.3 vs 6.1 in solvent control). Number of live foetuses (23) too few for evaluation of teratogenicity, but no malformations reported.

Conclusions:
Maternal NOAEL: 5 mg a.i./kg bw/day
Developmental NOAEL: 25 mg a.i./kg bw/day

Oral (via diet)

OECD 414

New Zealand White rabbits

Maternal toxicity

2 mg/kg bw/day: No treatment-related effects

10 mg/kg bw/day: -12% relative food consumption GD 6-9

24 artificially inseminated females/group Dosing: day 6-28 of gestation 0, 2, 10, 44 mg/kg bw/day (0, 125 ppm, 500 ppm, 2000 ppm, respectively) Test material: 97.1% DCOIT Thor Anon., 2008, A 6.8.1-01 7.8.2-01	44 mg/kg bw/day: ↓ food consumption ($p < 0.05$) ↓ relative food consumption ($p < 0.05$) during GD 6-19 (-58% at GD 6-9) ↓ body weight gain ($p < 0.05$) (GD: 9-28); 1 female died on GD 28 with dark red discoloration of the forestomach and a rupture of the peritoneum; 3 dams with pups with eye findings (in these animals % corrected bw gain: -6.4%, -10.3% and -4.7%) <u>Reproductive and developmental toxicity</u> No statistical significant effects on the numbers of resorptions, live foetuses per litter, foetal body weight.

ppm	**0**	**125**	**500**	**2000**	
mg/kg bw/day	**0**	**2**	**10**	**44**	**HCD**
Fetuses with 12 pairs of ribs	52.8%	47.2%	41.1%	35.9%	62.4% (45.4-73.5%)
Fetuses with 13 pairs of ribs	31%	32%	**47.7%**	**50.4%**	27.2% (19.4-40.3%)
Anomalous cervical vertebral centrum	0.7% (1/20 litters)	0.8% (1/18 litters)	**2.8% (3/17 litters)**	**3.1% (4/17 litters)**	1.2% (0.7-1.8%)
Incisors eruption	39.4% (13/20 litters)	35.5% (10/18 litters)	61.5% (13/16 litters)	55.4% (12/17 litters)	22.2% (15.7-21.1%)

	<u>44 mg/kg bw/day</u> 4 foetuses (3 litters) retinal abnormalities (retina, choroid and sclera detachments). Eye findings outside the HCD range: o dam no. 82/R4 (both eyes); o dam no. 83/R1 (abnormal right eye, and left eye; slightly-moderately folded retina in region of optic nerve) o dam no. 83/L2 (both eyes; slightly-moderately folded retina in region of optic nerve); o dam no. 84/R1 (both eyes). External, visceral or skeletal parameters: no significant difference between these 4 pups and the rest of the pups in the same dose group. **Conclusion:** **Maternal NOAEL: 10 mg/kg bw/day** **Developmental NOAEL: 10 mg/kg bw/day**
Oral Gavage OECD 414 New Zealand White rabbits 27-31 dams/ group Dosing: day 6-29 of gestation 0, 5, 10, 20 mg/kg bw/day Test material: 97.4% DCOIT in peanut oil Thor Anon., 2002, A 6.8.1-02 7.8.2-02	This study considered by DS to be of low reliability because no maternal toxicity was reached and a low maximum dose was employed <u>Maternal toxicity</u> No signs observed <u>Reproductive and developmental toxicity</u> No signs of foetotoxicity or teratogenicity, although high mortality was reported for all groups (including control and low dose groups) **Conclusion:** **Maternal NOAEL: 20 mg/kg bw/day** **Developmental NOAEL: 20 mg/kg bw/day**

After assessment of the 5 available teratogenicity studies (2 in rats and 3 in rabbits), RAC considers the following findings to be the most noteworthy:

- Maternal mortality occurred at and above 30 mg/kg bw in rats and at and above 5 mg/kg bw/day in rabbits.
- In none of the studies was the capability of the pregnant females in either species to conduct the pregnancy to term altered, even in the presence of severe maternal toxicity.
- In none of the studies were treatment-related effects reported on the numbers of early or late resorptions, live foetuses per litter, foetal body weight or sex ratio, even in the presence of severe maternal toxicity. The only exception was a study in rabbits, in which at a maternal dose causing 5 mortalities and a severe reduction in body weight gain during the gestation period, a decrease in live foetuses per litter was reported (3.3 vs 6.1 in controls).
- Statistically significant skeletal malformations and variations were mostly reported in rats only at doses causing marked maternal toxicity (i.e. 24% mortality at 112.4 mg/kg bw/day). Only in one rat study was a statistically significant increased incidence of bent ribs observed (7.4%) at the mid dose, where there was no significant maternal toxicity. RAC does not consider these skeletal malformations sufficiently severe for classification purposes. RAC also notes that some of these variations occurred in solvent controls and were therefore probably incidental.
- Skeletal malformations and variations were reported with incidences higher than the control in rats at non-maternally toxic doses, although the differences were not statistically significant.
- There were no statistically significant increases in the frequency of external or soft tissue malformations at any dose either in rats or in rabbits.
- The incidences of foetuses with 12 pairs of ribs in rabbits were within the HCD range.
- The incidences of foetuses with 13 pairs of ribs and anomalous cervical vertebral centrum in rabbits were just outside the HCD range at a non-maternally toxic dose and slightly above the HCD at a dose causing severe reductions in food consumption (overall during the gestation period but especially between gestation days 6 and 9), reductions in body weight gain (GD: 9-28) and 1 mortality.
- The incidences of incisor eruption in rabbits were higher than those reported in the HCD. However, RAC notes that the incidences in controls were also higher than in the HCD and that no dose-response relationship was observed;, which notably reduces the concern about a possible effect related to substance exposure.
- Four rabbit foetuses (3 litters) showed retinal abnormalities at incidences above the HCD range at a dose causing severe reductions in food consumption (overall during the gestation period but especially between days 6 and 9), reductions in body weight gain (GD: 9-28) and 1 mortality. The corrected maternal body weight in dams with pups with these eye findings ranged between -4.7% and -10.3%. According to the DS the applicant provided a document maintaining that HCD analysis from different research organisations showed an increasing trend in eye findings with New Zealand White rabbits. RAC also notes that these effects were not reproduced in the other two rabbit studies (one of them conducted at even higher doses) and were also not found in rats. All these facts notably reduce the concern regarding these ocular effects.

Overall, RAC does not consider the skeletal malformations and variations in rats at maternally toxic doses and the incidences of variations in rabbits (alterations in number of foetuses with 13 pairs of ribs and anomalous vertebral centrum in rabbits) to be sufficiently consistent to support classification for developmental toxicity. Therefore, RAC agrees with the DS's proposal for **no classification of DCOIT for developmental toxicity**.

Overall, RAC considers that **DCOIT does not warrant classification for toxicity to reproduction**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

Hydrolysis

The DS summarised in the background document two studies for hydrolysis. The first (A7.1.1.1.1/01 and A7.1.1.1.1/02) was performed following OECD TG 111 and U.S. EPA guidelines. The DT_{50} at the environmentally relevant pH of 7 was 71 days at 25°C, and was calculated to be 178-201 days at 12°C. At pH 4, DT_{50} = 736 days and at pH 9 DT_{50} = 10 days at 12ºC. Several degradation products were formed, three of them in quantities above 10%: 2-chloro-2-(noctylcarbamoyl)- 1-ethene sulfonic acid, 1-chloro- 2-(n-octylcarbamoyl)-1-ethene sulfonic acid and N-(n-octyl) propiolic acid amide.

In the second study (A7.1.1.1.1-01) following OECD TG 111, the DT_{50}s were calculated to be 41-47 days at pH 7 and 12°C. At pH 4, DT_{50} = 78 days and at pH 9 DT_{50} = 18 days at 12ºC All degradation products of DCOIT remained < 10%.

Photolysis

In one study (A7.1.1.1.2/01 and A7.1.1.1.2/02), the aqueous photolytic DT_{50} of DCOIT in natural sunlight at 12°C was calculated to be 38 days. In a second study (A7.1.1.1.2-01), the photolytic half-life with simulated natural sunlight at 50°N (25°C) was 7.6-7.9 days. The only degradation product over 10% was (N-(N-octyl) oxamic acid (NNOOA) with 11-31% in aquatic photolysis studies. In the earlier study used as supporting evidence (A7.1.1.1.2-02), non-radiolabelled DCOIT was subjected to photolytic degradation by an artificial light source. The photolysis half-life was 4.5-56 days at 24-hours sunlight at 50°N, depending on the season (9-112 days at 12-hours sunlight).

Ready biodegradability

A ready biodegradability test (A7.1.1.2.1/01) (OECD TG 301B) indicated that DCOIT is not readily biodegradable. However, the toxicity control shows that DCOIT inhibits the bacteria at the test concentration of 32 ppm.

In a second test (A7.1.1.2.1-01) (OECD TG 301B), no CO_2 was evolved during the 28 days test period and therefore the substance is not considered readily biodegradable. However, carbon dioxide evolution in the toxicity control (sodium acetate) was only 28%, *i.e.* just above the trigger value of 25%. Therefore, toxic effects of DCOIT on the microorganisms at the test concentration of 25 mg/L cannot be excluded.

A ready biodegradability test (A7.1.2.3/01) (OECD TG 301B) shows that the DCOIT degradation product NNOMA is readily biodegradable. Over 60% CO_2 evolution was observed within the 10-day window.

Aerobic aquatic biodegradation in estuarine surface water

An aerobic simulation study with estuarine surface water following OECD Draft Guideline 309 was presented (A7.1.2.2.1/01). The recalculated half-lives of DCOIT were 8.7-35 and 6.8-28 hours at 9 and 12°C, respectively. N-(n-octyl) oxamic acid (NNOOA) was found to be the major degradation product (max. 24%), while 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid was found at a maximum concentration of 12%. The other degradation products 1-chloro-2-(n-

octylcarbamoyl)-1-ethene sulfonic acid and 1,2- dichloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid were detected.

Aerobic and anaerobic aquatic biodegradation in a freshwater-sediment system

A simulation study with water and sediment was performed under both aerobic and anaerobic conditions following OECD Draft Guideline 308 (A7.1.2.2.2.a/01). DCOIT half-lives calculated at 12°C were 1.6 and 0.17 days for the water phase for the aerobic and anaerobic studies, respectively. DCOIT was not detected in the sediment, but primary degradation is so rapid that the same rate is considered valid for the whole freshwater-sediment system.

After 101 days, 62% and 50% AR was contained in the bound residues fraction in the aerobic and the anaerobic study, respectively. Metabolism involves cleavage of the isothiazole ring, and $^{14}CO_2$ comprised of about 11% and 5.2% of the applied radioactivity in the aerobic and anaerobic systems, respectively. There were at least 11 non-CO_2 degradation products detected in both studies, all present at < 10%. Identified degradation products were N-(n-octyl) malonamic acid (NNOMA), N-(n-octyl) acetamide (NNOA), 3,3'dithiobis-(n-octyl)-3-chloropropenamide and 2-chloro-3-(formyldithio)-N-octylpropenamide.

Aerobic and anaerobic aquatic biodegradation in a seawater-sediment system

Simulation studies with seawater and sediment were performed under both aerobic and anaerobic conditions following U.S. EPA Guidelines. In the studies, primary half-lives at 25°C were less than one hour. Recalculation to 9°C to reflect marine conditions give half-lives of less than 3.6 hours.

At all sampling intervals, most of the applied radioactivity was detected in the sediment. DCOIT disappeared almost instantly from both water-sediment systems. After 30 days in the aerobic study, 64% of applied radioactivity was contained in the bound residues fraction. About 10-20% and 7-8% was detected as $^{14}CO_2$ in the aerobic and anaerobic studies, respectively.

In both studies, the degradation products were neither identified nor individually quantified.

An additional study (A7.1.2.2.2.c/03) was provided concentrating on identifying the degradation products, using sediment from the same area. Two major degradation products were identified; NNOMA and NNOA in quantities of 16 and 12%, respectively.

In a separate study (A7.1.2.2.2.c/04), the extractability and storage stability of DCOIT in marine sediment was measured. The study demonstrated that in non-sterile water/sediment systems, the ^{14}C-residue remaining in the sediment after solvent extraction (post extract solids) corresponds to degradation products and not to parent compound.

Natural water and water-sediment systems

A simulation study with natural water/sediment was performed under aerobic conditions following OECD Draft Guideline 308 (7.1.2.2.2-01). The primary biodegradation of ^{14}C-DCOIT was very rapid, with half-lives of 1.2-1.5 days (2.5 days at 12°C). DCOIT was mineralised to CO_2 (27% and 30% of applied radioactivity in the river and pond system, respectively). DCOIT was shown to dissipate rapidly from the water phase to the sediment, with < 50% of applied radioactivity remaining in the water after 2 days. A large fraction of radioactivity in the sediment was bound (maximum levels of 57-64% at day 61). Only minor amounts of bound residues (2-3%) were extractable under harsh conditions (acidic reflux), which suggests that the bound residues were substances other than DCOIT. No major degradation products were formed during DCOIT degradation.

Aerobic biodegradation in soil

An aerobic soil simulation (metabolism) study was performed with two soils following U.S. EPA guidelines (A7.2.1/01). The primary half-lives of DCOIT were 2.0 and 0.58-1.1 days at 6 and

25°C, respectively. ^{14}C-label is rapidly incorporated into bound residues, and 41-54% of the applied radioactivity was found in the post extraction solids.

CO_2 was produced at 11-21% of the applied radioactivity. Only one degradation product at only one sampling interval was present at greater than 10% (ca. 11%) of the applied dose, but no definitive degradation product identification analysis was performed.

Conclusion

The hydrolytic and photolytic degradation of DCOIT in aqueous media is moderate and significantly slower than the biotic degradation.

The primary half-life of DCOIT in the environment is short, but mineralisation is limited. Identified degradation products over 10% were N-(n-octyl) oxamic acid (NNOOA, max 24%), N-(n-octyl) malonamic acid (NNOMA, max 16%), N-(n-octyl) acetamide (NNOA, max 12%) and 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid (max 12%). DCOIT has a degradation product (NNOMA) that fulfils the criteria for classification as hazardous to the aquatic environment. DCOIT is therefore regarded as not rapidly biodegradable for classification purposes by the DS, according to the CLP regulation.

Bioaccumulation

The background document indicated that DCOIT has a log K_{ow} > 4 based on OECD TG 107 indicating a potential to bioaccumulate. However, in the Annexes to the CLH report a log K_{ow} = 2.8 determined by the shake flask method (U.S. EPA 40 CFR § 158, Pesticide Assessment Guidelines Subdivision D § 63-11 and OECD 107) was presented.

Bioaccumulation potential in fish

A bioaccumulation study in Bluegill sunfish (*Lepomis macrochirus*) was performed following U.S. EPA guidelines but fish lipid content was unknown. Bioconcentration factors for total ^{14}C-residues (DCOIT + degradates/metabolites) were 56-660 for whole fish. The steady state BCF (BCF_{SS}) based on total ^{14}C-residues in whole fish was 750 ($K_{uptake}/K_{depuration}$). DCOIT in fish was found to be less than 1% of AR by day 28. Taking the highest recorded ^{14}C-BCF of 1300 and multiplying it by 1% gives a parent BCF of less than 13. (A7.4.3.3.1.a/01, A7.4.3.3.1.a/02, A7.4.3.3.1.a/03, A7.4.3.3.1.a/04).

A bioaccumulation study in Carp (*Cyprinus carpio*) was performed following Guidelines of Japanese Ministry of International Trade and Industry. No degradation product identification was performed. The ^{14}C-BCF values (total residue) for whole fish were 198–1126. Calculated BCF_{SS} were 713- 735. Depuration DT_{50} was 11-16 days. For DCOIT, the ^{14}C-residue in fish comprises of several different compounds and the observed BCF values result from the incorporation of degradation products into fish. (A7.4.3.3.1.b/01, A7.4.3.3.1.b/02)

Bioaccumulation potential in aquatic invertebrates – Oyster

A bioaccumulation study in juvenile oysters (*Crassostrea virginica*) was performed following U.S. EPA guideline and OECD TG 305E. The highest estimated BCF for DCOIT in oyster based on total ^{14}C-residues and the use of uptake and depuration rate constants was 44. Depuration DT_{50} was 16-42 days. Depuration at the low dose level did not seem to continue after day 42, indicating that ^{14}C-labelled degradation products might have been incorporated into tissues of the oysters (A7.4.3.3.2/01).

Conclusion

The BCF_{SS} for DCOIT and degradates/metabolites combined was 713-750 in bluegill sunfish and carp. Calculated BCF for DCOIT in bluegill sunfish was <13. The estimated kinetic BCF (BCF_K) for oyster was determined to be 44 based on analysis of total ^{14}C-residues and the comparison of uptake and depuration rates.

Thus, bioconcentration of DCOIT and degradates/metabolites combined was over the CLP trigger value of >500 but DCOIT itself seems to have lower potential for bioconcentration, mainly because of its rapid primary degradation. No firm conclusion on bioaccumulation under CLP was presented by the DS.

Aquatic toxicity

Aquatic toxicity data for DCOIT are summarised in the following table.

Method	Results*	Remarks	Reference	Applicant
Acute toxicity to fish				
US EPA FIFRA 72-1 Rainbow trout (*Oncorhynchus mykiss*)	**96h LC_{50}: 2.7 µg a.s./L (mm)**	**96h, flow-through Reliability (RI) 1**	**A7.4.1.1.a/01**	**Dow**
US EPA FIFRA 72-1 Bluegill sunfish (*Lepomis macrochirus*)	96h LC_{50}: 14 µg a.s./L (mm)	96h flow-through RI: 1	A7.4.1.1.a/02	Dow
US EPA FIFRA 72-3 Sheepshead minnow (*Cyprinodon variegatus*)	96h LC_{50}: 20.5 µg a.s./L (mm)	96h Flow-through RI: 1	A7.4.1.1.b/01	Dow
OECD TG 203 Japanese Blowfish (*Takifugu rubripes*)	96h LC_{50}: 5.66 µg a.s./L (n)	Semi-static RI: 2	A7.4.1.1.b/02	Dow
OECD TG 203 Rainbow trout (*Oncorhynchus mykiss*)	96h LC_{50}: 7.8 µg a.s./L (mm)	Semi-static Reliability: not specified	A 7.4.1.1-01	Thor
OECD TG 203 Sheepshead minnow (*Cyprinodon variegatus*)	96h LC_{50}: 7.3 µg a.s./L (mm)	Semi-static RI: not specified	A 7.4.1.1-02	Thor
Chronic toxicity to fish				
OECD TG 210 Rainbow trout (*Oncorhynchus mykiss*)	97d NOEC: 0.56 µg a.s./L (mm)	ELS flow-through RI: 1	A7.4.3.2.a/01	Dow
US EPA FIFRA 72-4 Sheepshead minnow (*Cyprinodon variegatus*)	35d NOEC: 6.0 µg a.s./L (mm)	ELS flow-through RI: 1	A7.4.3.2.b/01	Dow
OECD TG 210 Zebra fish (*Danio rerio)*	**35d NOEC: 0.47 µg a.s./L (mm)**	**ELS flow-through RI: not specified**	**A 7.4.3.2-01**	**Thor**
Acute toxicity to invertebrates				
US EPA FIFRA 72-2 *Daphnia magna*	48h EC_{50}: 5.2 µg a.s./L (mm)	Flow-through RI: 2	A7.4.1.2.a/01	Dow
US EPA FIFRA 72-3 Mysid (*Mysidopsis bahia*)	96h LC_{50}: 4.7 µg a.s./L (mm)	Flow-through RI: 2	A7.4.1.2.b/01	Dow
US EPA FIFRA 72-3 American oyster embryo (*Crassostrea virginica*)	**48h EC_{50}: 2.1-3.2 µg a.s./L (mm)**	**Static RI: 2**	**A7.4.1.2.b/02**	**Dow**
US EPA OPPTS 850.1055 Bay mussel embryo (*Mytilus edulis*)	48h EC_{50}: 411 µg a.s./L (mm)	Static RI: 2	A.7.4.1.2.b/03	Dow
OECD TG 202 *Daphnia magna*	48h EC_{50}: 9.7 µg a.s./L (mm)	Static RI: not specified	A 7.4.1.2-01	Thor

Chronic toxicity to invertebrates				
US EPA FIFRA 72-4 *Daphnia magna*	21d NOEC: 0.63 µg a.s./L (mm)	Flow-through RI: 2	A7.4.3.4.a/01	Dow
US EPA OPPTS 850.1350 Mysid (*Americamysis bahia*)	28d NOEC: 0.63 µg a.s./L (mm)	Flow-through RI:2	A7.4.3.4.b/01	Dow
OECD TG 211 *Daphnia magna*	**21d NOEC: 0.4 µg a.s./L (mm)**	**Semi-static RI: not specified**	**A7.4.3.4-01**	**Thor**
Toxicity to algae/aquatic plants				
US EPA FIFRA 123-2 *Navicula pelliculosa*	No reliable endpoints could be established.	Static	A7.4.1.3.a/01	Dow
OECD TG 201, US EPA FIFRA 122-2 and 123-2 OPPTS 850.5400 *Selenastrum capricornutum*	No reliable endpoints could be established.	Static	A7.4.1.3.a/02	Dow
US EPA OPPTS 850.5400 and OECD 201 *Navicula pelliculosa*	**24/96h E_rC_{50}: 1.6 µg a.s./L (m) 24/96h NOErC: 0.34 µg a.s./L (m)**	**Static RI: 1**	**A7.4.1.3.a/03**	**Dow**
US EPA FIFRA 123-2 *Skeletonema costatum*	24/120h ErC_{50}: 0.48 µg a.s./L (m) 24/120h NOErC: 0.48 µg a.s./L (m)	Static RI: 2	A7.4.1.3.b/01	Dow
OECD TG 221, US EPA OPPTS 850.4400, US EPA TSCA 797.1160, US EPA FIFRA 122-2 and 123-2, EC 67/548/ EEC Duckweed (*Lemna gibba)*	0-3d EC_{50}: 206 µg a.s./L (m) 0-3d NOEC: 4.54 µg a.s./L (m)	Results based on the first three days, since the effect was declining during the exposure period of 7 days. RI 2	A7.4.3.5.2/01	Dow
OECD TG 201, EPA OPPTS 850.5400 Freshwater green alga (*Scenedesmus subspicatus)*	72h ErC_{50}: 25 µg a.s./L (mm) 72h NOEbC: <15 µg a.s./L (mm)	Static RI: not specified	A 7.4.1.3-01	Thor
EPA OPPTS 850.5400 ISO 10253 Marine diatom, (*Phaeodactylum tricornutum)*	72h ErC_{50}: 25 µg a.s./L (mm) 72h NOEbC: 4.3 µg a.s./L (mm)	Static RI: not specified	A 7.4.1.3-02	Thor
OECD TG 201, US EPA OPPTS 850.5400 *Skeletonema costatum* NNOMA	96h ErC_{50}: 470 µg/L 96h NOEC: 130 µg/L	Static RI: 2	A7.4.1.3.c/02	Dow
OECD TG 201, US EPA OPPTS 850.5400 *Selenastrum capricornutum* NNOMA	96h ErC_{50}: 9700 µg/L (mm) 96h NOEC: 1510 µg/L	Static RI: 1	A7.4.1.3.c/01	Dow

* mm = mean measured. n = nominal concentrations. m = initial measured concentration

Key endpoints used in acute and long-term hazard classification are highlighted in bold.

Acute toxicity fish

Six valid acute toxicity studies with fish are available. DCOIT is highly acutely toxic to freshwater and saltwater fish (96h LC_{50}: 2.7-20.5 µg a.s./L). The lowest valid LC_{50} = 2.7 µg a.s./L is from a test done with rainbow trout (*Oncorhynchus mykiss*) following U.S. EPA Guideline FIFRA 72-1. Mean measured concentrations tested for this test were 0.44-0.93-1.8-3.3-6.3 µg a.s./L. For the lowest tested concentration in the *O. mykiss* test, the validity criteria were not completely fulfilled at 96h, as the measured concentration was only 76% of the concentration measured at test initiation. Nevertheless, the LC_{50} value is above the concentration limit (≥ 80%) and is therefore considered reliable.

Acute toxicity Invertebrates

There are 5 valid studies with aquatic invertebrates. The lowest endpoint is from a US EPA FIFRA 72-3 American oyster embryo (*Crassostrea virginica*) test done in synthetic and estuarine water. The 48h EC_{50} based on mean measured concentrations was 2.1 µg a.s./L in synthetic estuarine water and 3.2 µg a.s./L in natural estuarine water. DCOIT concentrations declined during the test, especially in the test system with natural seawater.

Chronic toxicity fish

Three valid chronic toxicity test are available for fish. The lowest chronic value is from to a 35-days chronic early life stage test performed with juvenile zebra fish (*Danio rerio*), in a flow-through test following OECD TG 210. The NOEC for development of embryos, hatching success, survival and growth was 0.47 µg/L based on mean measured concentrations. (A7.4.3.2-01).

Chronic toxicity invertebrates

There are three chronic studies available for crustacea. In the second *Daphnia magna* study (A7.4.3.4.-01), a NOEC = 0.4µg/L was obtained. This value represents the lowest chronic endpoint for aquatic invertebrates.

Toxicity to algae and aquatic plants

According to the DS, there are four reliable algae studies for classification purposes. The lowest endpoint is from a test done with *Navicula pellicosa* (A7.4.1.3.a/03) according to OECD TG 201 and US EPA OPPTS 850.5400, where a 24h ErC_{50} = 1.6 µg a.s./L and a NOE_rC = 0.34 µg a.s./L were obtained based on initial measured concentrations. Using the 24 hours value is justified by the DS in this case because of the special mode of action of DCOIT. DCOIT, as other isothiazolinones, is rapidly (within hours) taken up by algae and inhibits enzymes by binding to the thiolgroups of the proteins. A consequence of this binding is cleaving of the isothiazole ring. This means that the inhibitory effect on algae is very fast and also will result in the degradation of DCOIT by algae.

The toxicity of the DCOIT degradation product NNOMA towards algae was tested in two algal species, following OECD TG 201 and U.S. EPA guideline. Testing with the marine algae *Skeletonema costatum* gave a 96h ErC_{50} of 470 µg/L (NOEC: 130 µg/L) based on initial measured concentrations.

In addition, there was a test conducted with *Lemna gibba* in a static test system following OECD TG 221 and U.S. EPA guidelines (OPPTS 850.4400, TSCA 797.1160, FIFRA 122-2/123-2). As in the algae tests, most of the observed effects occurred within the initial phase of the test, and the differences in frond numbers or weight observed after 7 days were mainly due to growth inhibition in the initial phase of the test. Since the growth inhibiting effect declined during the exposure period, the calculations of the endpoints were based on the initial phase of the test, in this case days 0-3. This results in an ErC_{50} of 206 µg a.s./L and a NOErC of 4.54 µg a.s./L based on initial measured concentrations.

Based on the above data, the DS concluded that for acute toxicity there were adequate studies with all three trophic levels which show values below 1.0 mg/L, the trigger value for Aquatic Acute 1 (CLP, Annex I: Table 4.1.0 (a)). The lowest value for a standard species was the 24h ErC_{50} of 1.6 µg a.s./L for the freshwater algae *Navicula pelliculosa*. According to the criteria (Annex I: Table 4.1.3), the DS proposed an M-factor of 100.

For chronic toxicity, the DS concluded that adequate studies with all three trophic levels showed values below 0.1 mg/L, the trigger value for Aquatic Chronic 1 (CLP, Annex I: Table 4.1.0 (b) Long-term aquatic hazard (i) Not rapidly degradable substances for which there are adequate chronic toxicity data available). The lowest value for a standard species is the 24h NOErC of 0.34 µg a.s./L for the freshwater algae *Navicula pelliculosa*. According to the criteria (CLP, Annex I: Table 4.1.3), the DS proposed an M-factor of 100, since DCOIT was regarded as not rapidly degradable.

Comments received during public consultation

In total, five MSCAs plus one industry member commented on the classification proposal.

Industry disagreed with the conclusion on degradation. The DS and RAC agree that according to the decision scheme of the CLP Regulation, the substance cannot be considered rapidly degradable.

Two MSCAs agreed on the proposed classification without further comment.

One MSCA supported the proposed classification and commented that the reliability of the *Skeletonema costatum* study should be changed to 3 due to its various deficiencies (lack of analytical monitoring, high variations in cell density at 24h resulting in low statistical power, very steep dose-response curve: +/- no difference between NOEC and EC_{50}). It added that the endpoint for *Navicula pelliculosa* should be based on geomean concentrations using half the detection limit as the lowest concentration of the test.

The DS agreed to the various deficiencies of the *Skeletonema costatum* study and considered it only as additional information. Besides, the DS indicated that using the suggested approach to calculate the 24h ErC_{50} for *Navicula pelliculosa* could be an option and provided the possible results.

Another MSCA also supported the proposed classification but indicated that this should be based on the most reliable studies. They considered that the reliability for the valid *Navicula pelliculosa* test should be changed to 2. Based on this, the lowest acute toxicity with Klimisch score 1 was the LC_{50} value of 2.7 µg/L for rainbow trout and the lowest chronic toxicity values were the NOEC values of 0.4 and 0.43 µg/L for *Daphnia magna* and zebra fish, respectively.

The DS responded regarding the use of RI 1 vs. RI 2 studies, they generally agreed that studies with a RI of 1 should be preferred, however, the CLP guidance allows for the use of RI 2 studies for classification purposes. In their opinion, the *N. pelliculosa* study is well performed. The exponential growth criteria are fulfilled and the results are reliable, even though there are general problems with maintaining the test concentrations of DCOIT and other isothiazolinones over time.

Another MSCA agreed that DCOIT is not rapidly degradable and commented on the results of the two algae studies. For the valid *Navicula* test, the MSCA indicated that the NOEC (24h) = 0.34 µg/L does not match any of the initial measured concentrations. It also questioned the use of 24h endpoint *Navicula pelliculosa* for chronic classification highlighting that chronic endpoints should cover multiple generations. As such, the quoted 24 hour NOEC does not meet this criteria and should not form the basis of the chronic classification. It agreed, however, with the use of initial measured concentrations and required further information to assess the algae tests.

The DS explained that since the NOE_rC at 24 and 48h were below the lowest concentration tested (1.4 µg/L), the calculated EC_{10} values were used instead (0.34 and 0.77 µg/L, respectively). The DS also agreed to the use of chronic endpoints that cover multiple generations. In this case, the 48h endpoint EC_{10} = 0.77 µg/L would be a valid endpoint fulfilling the criteria. This value is higher than the previously calculated 24h value and is also higher than the chronic NOECs for *Daphnia magna* and for zebra fish (NOEC: 0.4 and 0.43 µg a.s./L, respectively), which would then be the lowest chronic values. The final classification would not change.

For the *Skeletonema costatum* test, the MSCA considers that the E_rC_{50} (24h) is not reliable for classification given the problems with the 24 hour cell counts and indicates that a 48h E_rC_{50} should be provided. They also highlighted the need to use an endpoint that covers multiple generations for chronic toxicity. The DS presented additional endpoints at 48, 72 and 96h indicating that the only valid endpoint would be at 72h.

Finally, industry indicated that the substance should be considered rapidly degradable according to the results presented in the test following OECD TG 308.

The DS agreed that according to the decision scheme, DCOIT cannot be considered rapidly degradable.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS that DCOIT inhibits bacteria at the concentrations tested. Hence, a conclusion on ready biodegradability cannot be drawn.

The hydrolytic half-life of DCOIT at 12°C pH 7 was calculated to be 47-201 days. At pH 4, 78-736 days, and at pH 9, 10-18 days. The primary degradation half-lives of DCOIT in the environment are short, being below 16 days. However, the degradation product NNOMA, formed in quantities max 16%, fulfils the criteria for classification as hazardous to the environment (algae 96h ErC_{50} 0.47 mg/L). Therefore, DCOIT cannot be regarded as rapidly degradable according to the CLP regulation.

Bioaccumulation

Due to the uncertainties in the bioaccumulation dataset (see Supplemental information - In depth analyses by RAC in the Background Document), RAC considers that a conclusion on the bioaccumulation potential of DCOIT and it metabolites cannot be reached.

Acute Aquatic toxicity

Acute aquatic toxicity data are available for three trophic levels. The lowest endpoints for fish, invertebrates and algae are all in the same range:

96h LC_{50} = 2.7 µg a.s./L for *Oncorhynchus mykiss*

96h LC_{50} = 2.1 µg a.s./L for *Crassostrea virginica*

24h E_rC_{50} = 1.6 µg a.s./L for *Navicula pelliculosa*

An acute endpoint is not available for the most sensitive chronic endpoint for fish (*Danio rerio).* However, based on the Acute:Chronic ratio (ACR EC50/NOEC = 4.8 / EC50/MATC(0.82) = 3.3) for *O. mykiss* a higher acute toxicity for *D. rerio* impacting on the acute M-factor is not expected.

In addition, the lowest acute endpoint corresponds to algae. DCOIT is an isothiazolinone with a specific mode of action whereby the substance is taken up by algal cells and degraded. It is this process which induces the toxic response. This mode of action justifies the consideration of a 24h endpoint for classification purposes using initial measured concentrations since the highest effect

occurs at this time period. In addition, general validity criteria for the test are met including a growth rate higher than 0.92 per day at 24h.

The lowest study result is an ErC_{50} of 1.6 µg a.s./L for *Navicula pelliculosa*. As this is below 1 mg/L, the substance meets the criteria for classification as Aquatic Acute 1. An M-factor = 100 is justified as the value is in the range $0.001 < L(E)C_{50} \leq 0.01$ mg/L. RAC agrees with the DS that reliability 2 studies can be used also for classification purposes. The CLP Guidance indicates that in general only reliable information (*i.e.* with a Klimisch reliability score of 1 (reliable without restrictions) or 2 (reliable with restrictions)) should be used for classification purposes. The most sensitive organism should therefore be used for classification.

RAC considers that a reliability of 1 for the *Navicula pelliculosa* test is appropriate. The test fulfils the validity criteria including a growth rate higher than 0.92 per day. Losses of test substance are normal for isothiazolinones.

RAC also considers the results from the *Skeletonema costatum* study as additional information for classification purposes. In relation to the use of geomean, RAC considers that the use of initial measured concentrations is a better approach due to the mode of action of DCOIT.

Chronic Aquatic toxicity

Chronic toxicity data are available for all three trophic levels:

35d NOEC = 0.43 µg/L for *Danio rerio*

21d NOEC = 0.4 µg/L for *Daphnia magna*

24h EC_{10} = 0.34 µg/L *Navicula pelliculosa*

48h EC_{10} = 0.77 µg/L for *Navicula pelliculosa*

RAC considers that a 48h endpoint for algae based on initial measured concentrations is more appropriate for chronic toxicity than the lowest 24h EC_{10} of 0.34 µg/L. The reason is that 48h is more relevant to assess the effect over several generations. Whether 24h or 48h is considered does not change the final classification.

Subsequently, the lowest endpoint corresponds to the NOEC for *Daphnia magna* of 0.4 µg/L ($0.0001 < NOEC \leq 0.001$ mg/L). Considering that the substance is not rapidly degradable, a classification as Aquatic Chronic 1 with M = 100 is warranted. The key endpoint considered for chronic toxicity was different to the one originally proposed by the DS (24h algae endpoint) since RAC considered the 48h algae endpoint more appropriate for chronic toxicity and the chronic NOECs for *Daphnia magna* became the lowest chronic value.

In addition, RAC also assesses the chronic toxicity based on the surrogate approach given the absence of chronic toxicity data for the most sensitive invertebrate species, *Crassostrea virginica.* In this case DCOIT would also be classified as Aquatic Chronic 1 with M = 100.

RAC therefore agrees with the classification of the substance as **Aquatic Acute 1 and Aquatic Chronic 1,** both with an **M-factor = 100.**

Additional references

Aalto-Korte K and Suuronen K. 2017. Patterns of concomitant allergic reactions in patients suggest cross-sensitization between octylisothiazolinone and methylisothiazolinone. Contact Dermatitis 77(6): 385-389.

Aerts O. Contact allergy caused by methylisothiazolinone and related thiazolinones. PhD thesis, 2017. https://repository.uantwerpen.be/docman/irua/f43259/142220.pdf

Amsler E, Aerts O, Raison-Peyron N, et al. 2017. Airborne allergic contact dermatitis caused by thiazolinones in water-based paints: a retrospective study of 44 cases. Contact Dermatitis 77(3): 163-170.

ECHA. 2016. Opinion proposing harmonised classification and labelling at EU level of Reaction mass of CMIT/MIT (3:1). https://echa.europa.eu/documents/10162/0b88764c-cd9f-7b3d-b336-caa40b3588ed

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

Moriyama, T. *et al*. 2008. Effects of reduced food intake on toxicity study parameters in rats. The Journal of Toxicological Sciences, 33(5):537-47.

Schwensen JF, Menné Bonefeld C, Zachariae C, Agerbeck C, Petersen TH, Geisler C, Bollmann UE, Bester K, Johansen JD. 2017. Cross-reactivity between methylisothiazolinone, octylisothiazolinone and benzisothiazolinone using a modified local lymph node assay. *Br. J. Dermatol*. 176(1): 176-183.

Takamatsu, K. et al., 2015. Effects of four-week feed restriction on toxicological parameters in beagle dogs. Exp. Anim. 64(3), 269–280.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).