CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name:

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

[DCOIT]

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

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

Index Number:

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Part A.

1 PART A: PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	4,5-dichloro-2-octyl-2H-isothiazol-3-one; [DCOIT]
EC number:	264-843-8
CAS number:	64359-81-5
Annex VI Index number:	
Degree of purity:	95-100% (w/w)
Impurities:	Confidential information

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

Current entry in Annex VI, CLP Regulation	No existing entry in Annex VI
Current proposal for consideration by RAC	**Acute Tox. 1,** H330 **Acute Tox. 4,** H302 **Skin Corr. 1,** H314 **Skin Sens. 1A,** H317 **Aquatic Acute 1,** H400 **Aquatic Chronic 1,** H410 Skin Irrit. 2; H315: C ≥ 0.01 % Skin Sens. 1A; H317: C ≥ 0.001 % EUH071: Corrosive to the respiratory tract. Chronic M-factor: 100 Acute M-factor: 100
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	**Acute Tox. 1,** H330 **Acute Tox. 4,** H302 **Skin Corr. 1,** H314 **Skin Sens. 1A,** H317 **Aquatic Acute 1,** H400 **Aquatic Chronic 1,** H410 Skin Irrit. 2; H315: C ≥ 0.01 % Skin Sens. 1A; H317: C ≥ 0.001 % EUH071: Corrosive to the respiratory tract. Chronic M-factor: 100 Acute M-factor: 100

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.15.	Organic peroxides	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox. 4, H302		Not classified	
	Acute toxicity - dermal	Not classified		Not classified	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	Acute Tox. 1, H330		Not classified	
3.2.	Skin corrosion / irritation	Skin Corr. 1, H314	Skin Irrit. 2; H315: C ≥ 0.01 %	Not classified	
3.3.	Serious eye damage / eye irritation	DCOIT was regarded as corrosive to skin and serious damage to eyes is thus implicit		Not classified	
3.4.	Respiratory sensitisation	Not classified		Not classified	Data lacking
3.4.	Skin sensitisation	Skin Sens. 1A, H317.	Skin Sens. 1A; H317: C ≥ 0.001 %	Not classified	
3.5.	Germ cell mutagenicity	Not classified		Not classified	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified		Not classified	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified		Not classified	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified		Not classified	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified		Not classified	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified		Not classified	Conclusive but not sufficient for classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Acute M-factor: 100 Chronic M-factor: 100	Not classified	
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Hazard pictogram:

GHS05, GHS06, GHS09

Signal word: Danger

Hazard statements:

H302 Harmful if swallowed
H330 Fatal if inhaled
H314 Causes severe skin burns and eye damage
H317 May Cause Allergic Skin Reaction
H410 Very toxic to aquatic life with long lasting effects

Supplemental hazard statement codes:

EUH071 Corrosive to the respiratory tract

2 BACKGROUND TO THE CLH PROPOSAL

DCOIT (4,5-dichloro-2-octyl-2H-isothiazol-3-one) is an existing biocidal active substance reviewed under Regulation (EU) No 528/2012 with Norway as the Rapporteur. DCOIT is not included in Annex VI of the CLP regulation.

The classification proposal is based on information which has been provided by two applicants (Dow/Rohm and Haas and Thor GmbH) for the approval of the substance as a biocidal active substance in product types PT 7, 8, 9, 10, 11 and 21. The study summaries from both applicants are included in the CLH dossier. The study summaries for Dow are taken from the Competent Authority Report (CAR) on DCOIT in PT8/21. As for the study summaries submitted by Thor GmbH, these summaries have not yet been subjected to the peer-review process under Regulation (EU) No 528/2012.

Additional information from the test reports has been included in a few study summaries due to the request in the accordance check for more detailed, quantitative information to be included in the CLH report (additional information included as comments from the Rapporteur Member State).

2.1 Short summary of the scientific justification for the CLH proposal

Concerning physico-chemical properties, DCOIT (4,5-dichloro-2-octyl-2H-isothiazol-3-one) does not fulfil the criteria for a classification according to Regulation (EC) No 1272/2008 (CLP). Therefore, no classification is required regarding physico-chemical hazards.

The active substance is harmful if swallowed and fatal if inhaled. It is corrosive to skin and eyes and may cause an allergic skin reaction. It is also very toxic to aquatic life with long lasting effects.

2.2 Current self-classification and labelling:

At the time of submission there were approximately 250 notifications and 20 aggregated self-classifications for DCOIT in the C&L inventory. Most self-classifications include the current proposal with some variations in the sub-categories in addition to acute toxicity by the dermal route, classification for eye damage, STOT SE 3 for respiratory tract irritation and STOT RE 1 (one aggregated self-classification only).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is an active substance in the meaning of Regulation (EU) No 528/2012 repealing Directive 98/8/EC and a justification is thus not required according to Article 36 of Regulation (EC) No 1272/2008 (CLP).

Part B.

PART B: SCIENTIFIC EVALUATION OF THE DATA

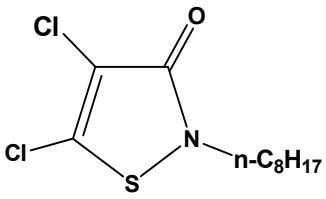
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	264-843-8
EC name:	4,5-dichloro-2-octyl-2H-isothiazol-3-one
CAS number (EC inventory):	-
CAS number:	64359-81-5
CAS name:	3(*2H*)-Isothiazolone, 4,5-dichloro-2-octyl-
IUPAC name:	4,5-Dichloro-2-octylisothiazol-3(2H)-one
CLP Annex VI Index number:	-
Molecular formula:	$C_{11}H_{17}Cl_2NOS$
Molecular weight range:	282.2 g/mol

Structural formula:



1.2 Composition of the substance

DCOIT (4,5-dichloro-2-octyl-2H-isothiazol-3-one) is an active substance with a minimal purity of 95%, as specified from the producers. The 5-batch analyses performed by the two commercial suppliers show a purity between 95-100%. Representative production batches of the active substance were analysed for their content of DCOIT and impurities.

No impurities has been found to be of relevance for the CLP proposal. The identity of the impurities (and/or additives) are confidential to the suppliers.

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
4,5-dichloro-2-octyl-2H-isothiazol-3-one		95-100 % (w/w)	-

1.3 Physico-chemical properties

Table 6: Summary of physico-chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Off-white to beige solid, may have compact agglomerates, specific and pungent odour (>98.6% purity, at 20°C)	A3/01/ A3/02 A3.11-01	Visually determined, average of the two references
Melting/freezing point	41.0 °C	A3/01 A 3.1.1-01	Measured, average of the two references (EC A1 / OECD 102)
Boiling point	Not applicable	A3/01 A 3.10-01	The substance decomposes before the melting point is reached. Decomposes at 300°C
Relative density	1.32 g/cm^3	A3/02 A 3.1.3-01	Measured, average of the two references (OECD 109)
Vapour pressure	0.98*10^{-3} Pa at 25^{0}C 2.2*10^{-3} Pa at 30^{0}C 4.6*10^{-3} Pa at 35^{0}C Henry'law constant at 20^{0}C: 0.033 Pa m^3 mol^{-1} 1.4*10^{-3} Pa, at 20°C 2.7*10^{-3} Pa, at 25°C Henry'law constant at 20^{0}C: 0.21 Pa m^3 mol^{-1}	A3/01 A 3.2-01	The data included are measured directly at the given temperatures. The discrepancies between the results are attributed to differences in the methodology used. (OECD 104, different sub-methods used by the two reports)
Surface tension	62.3 mN/m at 20°C	A 3.13-01	DCOIT is to be considered as a non-surface active substance. (OECD 115)
Water solubility	Solubility at 10°C: pH 5: 2.3 mg/l pH 7: 2.1 mg/l Solubility at 20°C: pH 5: 3.5 mg/l pH 7: 3.1 mg/l Solubility at 30°C:	A3/02 A 3.5-01	Two tests were obtained, one using the shake flask method, the other using the column method (both part of the OECD 105). The presented results are the average values of the two studies.

Property	Value	Reference	Comment (e.g. measured or estimated)
	pH 5: 5.5 mg/l pH 7: 5.1 mg/l		
Partition coefficient n-octanol/water	$logK_{ow}$ at 20°C: pH 5: $LogK_{ow}$ 4.6 pH 6.3: $LogK_{ow}$ 4.4 pH 9.1: $LogK_{ow}$ 4.8	A 3.9-01	OECD 107 - Shake flask method.
Flash point	Not applicable		Product is solid at room temperature and decomposes before it boils.
Flammability	Not flammable	A3/02 A 3.11-01	No flammable properties were found (EEC A10)
Explosive properties	Not explosive	A3/02 A 3.15-01	Does not contain any functional groups that can contribute to explosive properties, in addition both the oxygen balance number and the exothermic decomposition energy are below the threshold for explosive substances.
Self-ignition temperature	Self-ignition temperature: 260^0C Does not self-ignite at room temperature under the presence of oxygen	A3/02 A 3.11-01	The average self-ignition temperature from the two references. (EEC A16/EEC A15)
Oxidising properties	Not applicable	A 3.16-01	The oxygen balance show a negative oxygen count, hence the product does not possess oxidizing properties
Granulometry	Not applicable		-
Stability in organic solvents and identity of relevant degradation products	Solubility: At 30^0C: >704 g/L in n-hexane >587 g/L in ethyl acetate At 10^0C: 133.6 g/L in n-hexane 322.9 g/L in ethyl acetate The substance does not contain any organic solvents, hence stability in organic solvents are not determined.	A3/02	Solubility from only one reference is used, as the other reference used read across. A pre-formulation product showed stability >2 years with xylene as a solvent.
Dissociation constant	Not applicable	A 3.6-01	Does not dissociate in solution
Viscosity	Not applicable		Solid at room temperature

2 MANUFACTURE AND USES

2.1 Manufacture

Not required for biocidal products.

2.2 Identified uses

Intended biocide use areas:

- PT7 – Film preservatives
- PT8 – Wood preservatives
- PT9 – Fibre, leather, rubber and polymerised materials preservatives
- PT10 – Construction materials preservatives
- PT11 – Preservatives for liquid-cooling and processing systems
- PT21 – Antifouling products

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1 Summary and discussion of physical-chemical properties

The data on physical-chemical properties given in Table 6 are conclusive.

3.2 Comparison with criteria

The substance does not meet the criteria for classification for physical-chemical properties (please refer to table 3 and 6). In a standard study (EEC Method A10), DCOIT was not determined to be flammable. Therefore, it does not meet the criteria for classification as a flammable solid. The self-ignition temperature was found to be 260 °C (EEC Methods A16 and A15). Further, 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, in addition both the oxygen balance number and the exothermic decomposition energy are below the threshold for explosive substances. Therefore, it does not meet the criteria for classification as an explosive substance. Finally, DCOIT does not possess oxidising properties and so it is not classified as an oxidising solid

3.3 Conclusions on classification and labelling

No classification required.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Applicant 1, Dow:

Following oral administration of ^{14}C-DCOIT in olive oil to male and female rats (Crl:CD® BR) at dosages of 20 and 250 mg DCOIT/kg bw (Anon., 1994, A6.2.a/01), most of the ^{14}C-label (81-93%) was excreted within a 2 day period primarily in the feces, and by 4 days only negligible amounts of the ^{14}C-label remained in the tissues (≤ 0.5%) and residual carcass (≤ 1.7%). Three out of 26 animals in the high dose group died one day after dosing and were replaced. No overt signs of toxicity were noted in the remaining animals. Peak plasma concentration occurred by 6 - 10 hr (0.9-1.2 ppm) and 24 hrs (4.6-8.6 ppm) following low and high dose respectively. The plasma elimination half-life of ^{14}C-label was 16.1 hr for males and 20.5 hr for females in the low dose group and 19.4 hr for males and 25.0 hr for females in the high dose group. In the low-dose group at 6 hr and the high-dose group at 24 hr, the highest tissue ^{14}C-concentration was found in liver, stomach, intestine and kidney. At 6 hr in the low dose group approximately 2% was found in liver and 0.2% in kidney. In the high dose group approximately 0.7% was found in liver and 0.06% in kidney at 24 hrs. Between 15-18% and 11-12% of the administered dose was eliminated via urine in the low-dose group and high dose group respectively, whereas more than 80% was eliminated via feces. The range finding study indicates that less than 2% of the dose was eliminated through exhalation. The rats were not bile duct cannulated, thus the fraction of fecal radioactivity excreted via bile is not known. In comparison, in the dermal study presented below (Anon. 1994, A6.2.b/01) it is shown that approximately equal amounts of ^{14}C-label is eliminated via urine and feces during the first three days after administration. Pre-dosing animals with non-radiolabelled DCOIT did not alter the pharmacokinetics of ^{14}C-DCOIT. It is judged that ^{14}C-DCOIT would be unlikely to bioaccumulate significantly.

The quantification, identification and characterization of ^{14}C-DCOIT metabolites were conducted on rat excreta collected during the above pharmacokinetic study. Results from this study indicate that DCOIT was rapidly excreted and highly metabolized. The overall metabolic fate of DCOIT in the rat urine and feces was identified, and all major metabolites were identified. Mass spectrometry was used to identify 6 metabolites in the feces and 8 metabolites in the urine. The metabolic profile in the urine proved to be different from the feces profile. In the feces, DCOIT degrades rapidly as there was no parent detected even from Day 1 samples. Degradation primarily involves cleavage of the ring and subsequent oxidation to N-(n-octyl) malonamic acid (NNOMA, major metabolite) 7-18% of the administered dose, N-(n-octyl) acetamide, N-(n-octyl) oxamic acid and N-(n-octyl) b-acetyl propionamide. There was no significant difference in the metabolite profile across dose and sex in the feces. Equally important, there was a general decrease in the percent of metabolites in the feces by days. The major metabolite identified in the urine was N-(2-hydroxyhexyl) malonamic acid, 2.5-5.6% of the administered dose. In the urine, the major degradation pathway appears to involve derivatives of N-(n-octyl) malonamic acid, i.e., N-(2-hydroxyhexyl) malonamic acid and N-(4-hydroxyhexyl) malonamic acid. Subsequent biotransformations involve hydroxylation, dealkylation and acetylation. The metabolic profile for the urine samples was the same irrespective of dose or sex (Anon., 1996, A6.2.a/02).

A proposed metabolic pathway for DCOIT in rats is included in Figure 4.1.

Several dermal toxicokinetic studies have been performed.

In study Anon., 1994, A6.2.b/01, male rats (Crl:CD® BR) were exposed to 3% or 0.045% ^{14}C-DCOIT in acetone for 10 and 24 hrs. Following dermal administration of 3% DCOIT, the dermal delivery (stratum corneum, skin, absorbed dose) was 31-34% (10 hr exposure) and 52% (24 hr exposure). Dermal administration of 0.045% ^{14}C-DCOIT in acetone resulted in a dermal delivery of 44-50% (10 hr exposure) and 70% (24 hr exposure). 22%-23% of the administered dose was absorbed during 3 days following a 10 h exposure and can be considered systemically available in this time period in both low and high dose animals, whereas 22% and 12% of the dose remained in the skin. The excretion of ^{14}C-label via urine preceded the fecal excretion, and slightly more ^{14}C-label was eliminated via urine than via feces over a three day period (11% vs 7 % for the 0.045% dose).

In vivo and *in vitro* dermal absorption studies with ^{14}C-DCOIT in dipropylene glycol monoethyl ether (DPGME) have been performed. In the *in vivo* study (DocIII-B6.4/03, Kathon ^{TM} 287 WT biocide (PT8) and Doc III B6.4/03, theoretical product (PT21)) two groups of male rats (Sprague Dawley) were exposed to 0.25% ^{14}C-DCOIT in DPGME for 24 hours. In the first group of animals skin samples were prepared for autoradiographic analysis and levels of ^{14}C-label were measured in skin and carcass 24 hours, 7 days and 14 days following exposure. In the second group of animals ^{14}C-levels in urine, feces and expired air were measured over a 30 day period and autoradiographic analysis and measurement of ^{14}C-levels in skin and carcass were performed. The blood and plasma measurements indicated that maximum mean concentrations occurred 24 hour post dose. Most of the radioactivity recovered in excreta was found in faeces excreted over the first 11 days (27%). Urinary excretion exceeded the faecal excretion the first three days and accounted for approximately half of the faecal amounts during the 30 day period. The amount of radioactivity recovered from the carcass was 5.5% at 24 h post exposure and was reduced to 4% after 7 and less than 1% after 14 days. In skin 40% radioactivity was found at 24 h and was reduced to 18% and 3% after 7 and 14 days. From this study a dermal delivery value of 51% and an absorption of 49% over a 30 day period are indicated. By combining measurement of ^{14}C-levels in carcass from the first group of animals (5.5% and 4% at 24h and 7 days respectively) with cumulative urine and feces values 3 and 7 days following exposure one can estimate a systemically available fraction of DCOIT of approximately 24% (3 d) and 34% (7 d). Autoradiographic analyses showed that radioactivity penetrated the stratum corneum with time and was distributed into the deeper layers of skin. Hair follicles were shown to be an important route of entry into skin. Radioactivity was still present in the epidermis and underlying musculature at 14 and 30 days postdosing.

In the *in vitro* study (DocIII-B6.4/02, Kathon ^{TM} 287 WT biocide (PT8) and Doc III B6.4/04, theoretical product (PT21)) split-thickness human skin was exposed to 0.25% ^{14}C-DCOIT in DPGME for 24 hours. In this study the dermal delivery (stratum corneum tapestrips 6-20, absorbed dose and skin) was 17%, and the absorbed dose was 1% during 24 hours. This study indicates a significantly lower dermal delivery of DCOIT through human skin than through rat skin. This is supported by dermal absorption studies of ^{14}C-DCOIT following exposure of rat and human skin to an antifouling formulated paints (Doc III-B6.4/01 and Doc III-B6.4/02, theoretical product). However, a study of dermal absorption of DCOIT in a water-based wood treatment solution through human skin established a dermal delivery value of 49% (III-B6.4/01, Kathon ^{TM} 287 WT biocide (PT8) and NK Doc III B6.4/05, theoretical product (PT21).

Summary of toxicokinetics (Dow)

DCOIT is rapidly excreted via feces and urine with plasma half-lives between 16-25 hrs following oral exposure. Close to 20% of DCOIT was excreted in the urine following oral administration. As the contribution of biliary excretion to the elimination of DCOIT is not known the amount of

DCOIT in the feces that has actually been absorbed is uncertain. As the main metabolites in the faeces may have been formed by biodegradation in the GI tract they do not give additional information about the oral absorption of DCOIT. In conclusion, the oral absorption is assumed to be moderate (close to 20%).

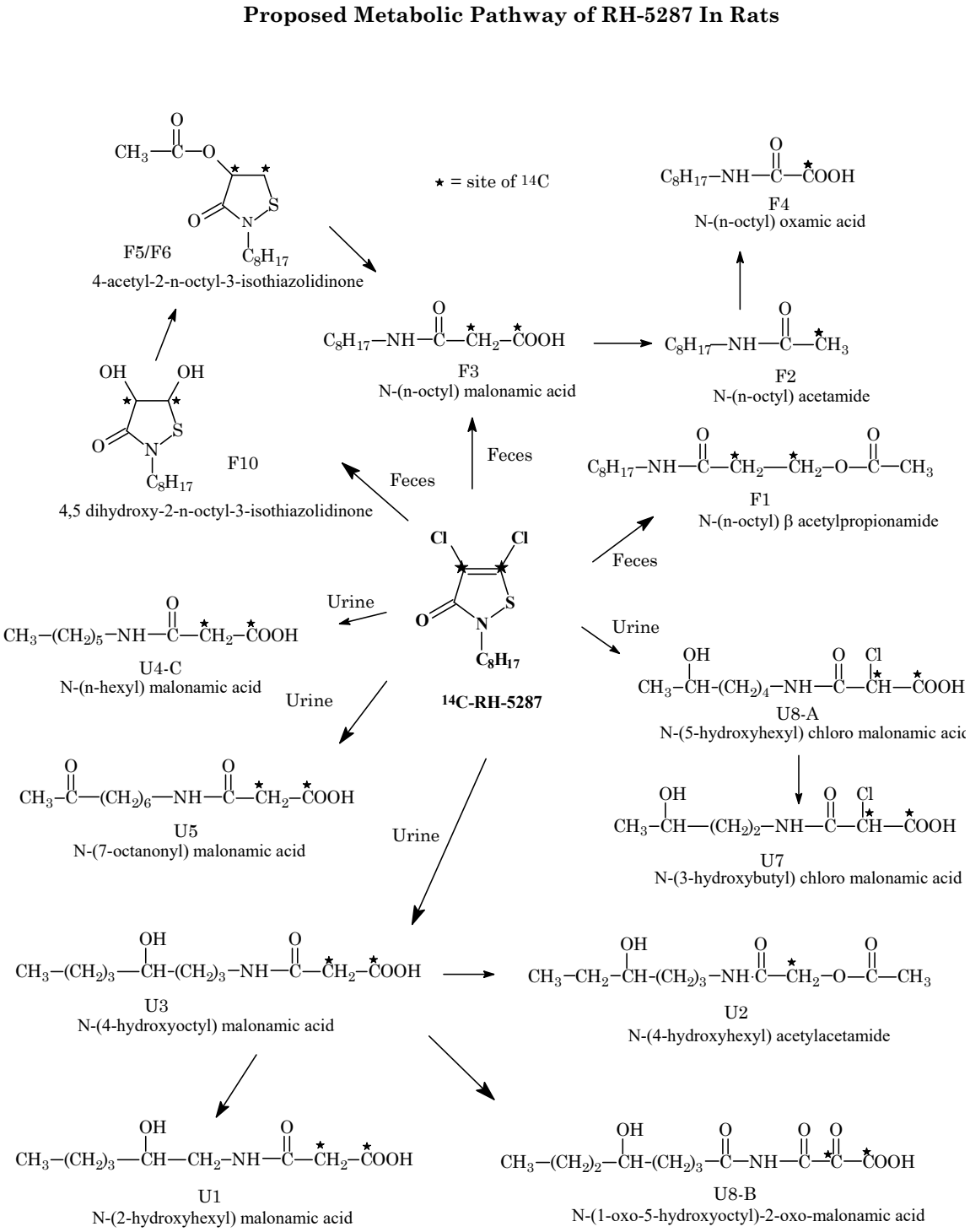
DCOIT is extensively metabolised following oral administration. In the feces, DCOIT degraded rapidly due to ring opening and subsequent metabolism that included oxidation to N-(n-octyl) malonamic acid (NNOMA), the major metabolite. In the urine, the major degradation pathway appears to involve derivatives of NNOMA.

A high penetration of DCOIT dissolved in acetone or DPGME into the skin of rats has been shown and dermal delivery factors between 17% and 70% are estimated, dependent of exposure duration and observation times. The dermal delivery of DCOIT in DPGME through human skin was found to be 17%. However, there are conflicting results of the studies with human skin which makes it uncertain whether it is correct to assume a lower human than rat dermal delivery value. During a 30 day period following a 24h exposure of rats to DCOIT in DPGME 49% was absorbed. This study shows that most of the ^{14}C associated with the DCOIT that penetrates the skin will over time be systemically available, possibly in a metabolised form. However, the absorption rate is relatively low and 24% or less of the administered dose was systemically available in a 3 day period following DCOIT administration. Hair follicles seem to be major portals of entry for DCOIT. A dermal delivery value of 51% for DCOIT in concentrations of 0.25%-1.9% and 31% for DCOIT concentrations of 2% or above is suggested. This is based on rat studies and systemic available doses of 24% for DCOIT in concentrations of 0.25%-1.9% and 22% for DCOIT concentrations above 2%. The systemic available doses are based on data from 10 hour exposure regiments and 3 day absorption periods. (Doc III-A6.2.b/01).

DCOIT is corrosive in higher concentrations and the above mentioned absorption values are not relevant for damaged skin.

Absorption via the respiratory system has not been studied.

Figure 4.1: Proposed metabolic pathway of DCOIT and structure of metabolites



Applicant 2, Thor:

Table 7: Summary of toxicokinetic studies, Thor.

Route	endpoints	Species Strain Sex no./group	dose levels frequency of application	Results	Reference (TNsG IUCLID 5)
Oral gavage[k]	toxicokinetics, mass balance (without bile), metabolite identification	Rat (Wistar) 3-4/sex/ group	35 (single and repeated dose), 105 (single dose) mg/kg bw /day Vehicle (corn oil)	Fecal excretion is the main route of excretion of DCOIT, renal excretion is significantly lower. The small fraction taken up is readily distributed into all organs and extensively metabolized.	Anon., 2008a (A 6.2-01 7.1.1-01)
Oral gavage	excretion in bile, urine and feces, metabolite identification	Rat (Wistar) 3 males/ group	35 and 105 mg/kg bw /day (single dose) Vehicle (corn oil)	Most of the radioactivity quantified in feces can be considered as non-absorbed, because very little of radioactivity was found in bile.	Anon., 2008b (A 6.2-02 7.1.1-02)
Dermal [k]	dermal absorption	Rat (Wistar) 4 males/ group	0.4 and 4 mg/ml (single dose) Vehicle (propylene glycol) Exposure duration 10 h Sample collection at 10, 48 and 72 h	Excretion of absorbed material was via urine and faeces in comparable percentages over a 72 h period. Absorbed dose: 35% and 29% in the low and high dose, respectively Absorbed dose corrected for low recovery: 46% and 39% in the low and the high dose group, respectively	Anon., 2007 (A 6.2-03 7.1.2-01)

k = key study

The absorption, distribution, metabolism and excretion of ^{14}C-DCOIT in male and female Wistar rats were investigated according to OECD guideline 417 following single (high and low dose) and repeated oral administrations to the rat in two studies.

In the key oral toxicokinetic study (Anon. 2008a A 6.2-01, 7.1.1-01), six groups of rats were included: Three groups (4 males and 4 females) for mass-balance analysis and three groups (3 males and 3 females) for toxicokinetic. Rats were treated by oral gavage with a single dose of 35 mg/kg bw or 105 mg/kg bw, or for 10 days with unlabeled DCOIT followed by a single dose of labelled ^{14}C-DCOIT. In mass-balance groups, urine and feces were collected in several intervals during the first 72 h after administration. At the end of the observation period (72 h), animals were euthanized and several tissues and organs were collected. Total radioactivity in urine, feces, tissues and organs was determined. Metabolite profiles in urine- and feces-samples were analyzed by HPLC-RAD and HPLC-MS for structural elucidation and quantitative distribution of the individual metabolites. In toxicokinetic groups, radioactivity in blood was determined at several time points during the first 72 h after administration to determine kinetic behavior of the test material. The oral absorption was fast with T_{max} values of 4 h for all groups (except high-dose females with 24 h). In addition, the dose-normalized C_{max} was 1.6 times lower for the high dose group compared to the single low dose group. Following, the absorption rate of DCOIT is considered to be dose-dependent. The elimination half-life of the radioactivity ranged from 22 to 29 h after a single or repeated low dose. Elimination half-lives were slightly longer in the high dose group (28–33 h). The absorption from orally administered DCOIT was moderately low (14–16 % based on urine, carcass and tissues). Oral absorption after

repeated dosing was comparable to the absorption observed after single dosing. The average total recovery of radioactivity in all groups was between 94 % and 98 % of the applied dose.

The second oral study (Anon., 2008b A.6.2-02, 7.1.1-02) is an additional investigation to a full ADME study with DCOIT. The purpose of this study was to obtain information on excretion via bile of ^{14}C-DCOIT in bile-cannulated Wistar rats. Two groups of male rats (n=3) were dosed with a single dose of 35 mg/kg bw or 105 mg/kg bw. Urine, bile and feces were collected in several intervals during the 48 h after administration. At the end of the observation period (48 h), animals were euthanized, and GI tract and carcass were collected. Total radioactivity in urine, bile, feces, GI tract and carcass was determined. Absorption (based on urine, bile and carcass without GI tract) of DCOIT was moderate low in both groups, 10% (low dose) and 7% (high dose). Only little radioactivity (1–2 %) was observed in bile. These findings indicates that most of the administered dose was excreted without uptake into the body. The average total recovery was 96% (low dose) and 93% (high dose) of the applied dose.

In both studies, excretion via feces was the predominant route of elimination: 78-84% (key study) and 83-86% (second study). In the key study, fecal and urine excretion was similar between the single and repeated low dose groups, and high dose group. However, high-dose administration led to a shift of the peak of fecal excretion to later time points. No relevant excretion via volatiles was observed in a preliminary study. At termination of the studies, the total remaining radioactivity in carcass plus tissues was 1.1–2.0% (key study) and in carcass and GI tract 0.45–0.65% (second study) of the administered dose. The highest residual concentrations of DCOIT equivalents were observed in the liver, kidney and gastrointestinal tract, reflecting extensive metabolism. The concentrations in blood at termination of the study were 1 – 2 times higher than in serum (key study), reflecting distribution of the test substance into red blood cells.

Urinary excretion accounted for 12–15% (key study) and 5.2–8.1% (second study). The bulk (70–92%) of urinary excretion occurred within 24 h (key study). In urine, 22 putative metabolites were observed in both studies, where 7 could be identified (chemical structures are shown in Figure 4.2 accounting for 59–75% of the radioactivity present in urine (key study).

Analysis of feces samples yielded 7 putative metabolites (key study). One could be structurally elucidated in the key study, two in the second study. In the key study, this metabolite accounted for 4 % of the total administered dose of radioactivity, whereas in the second study the two identified metabolites accounted for 7-9% of the total administered dose of radioactivity. Feces metabolites may stem from bacterial metabolism.

Taking all identified metabolites together, 4,5-dichloro-2-octyl-2H-isothiazol-3-one is metabolised via cleavage of the S-N-bond, oxidation (mainly at the octyl chain), dechlorination, decarboxylation, and truncation. N-acetyl conjugates are the only representatives of Phase II metabolism identified.

The dermal absorption of ^{14}C-DCOIT was investigated according to OECD guideline 427 in male Wistar rats (Anon., 2007; A7.1.2-01). Six groups of rats (n=4 per group) were treated with a low (0.4 mg/ml; 0.04%) and a high dose (4.0 mg/ml; 0.4%) of radio-labelled test substance in propylene glycol on a defined area of shaved intact skin over a period of 10 h under occlusive conditions. After exposure, the substance was washed off and the wash was kept to determine radioactivity. Urine and faeces were collected in periods over 72 hours for determination of radioactivity. Animals were euthanized after 10, 48 and 72 h, and blood, skin and carcass were collected. Total radioactivity in urine, faeces, blood, plasma, treated skin, untreated skin and carcass were determined. The radio-activity in treated and non-treated skin was compared. No effects on body weight and no mortality were observed. Administered radioactivity was found in application cover (considerable adsorption to the rubber O-ring (8 -24%) covering the application site was noted),

backwash, treated skin (depot), urine, faeces, blood and carcass. When adsorbed, excretion took place via urine and faeces in comparable amounts. . The dermal delivery (amount retained in exposed skin and absorbed dose (excretion + cage wash + carcass + unexposed skin + blood)) of DCOIT was 52% in the low dose group and 39% in the high dose group during the 72 h period. There seem to be a slow release of DCOIT from the skin to the circulation. The systemically available dose (absorbed dose) was 35% and 29% in the low and high dose groups, respectively, over 72 h. The total recovery of radioactivity in all groups was between 81% and 86% of the applied dose, and was 84% on average, which is lower than the guideline recommendation of > 90%. The cause of the low recovery is unclear. If correcting the mass-balance to 95% (assuming some of the material was adsorbed to the O-ring and not systemically available) and considering the missing 10-11% (from 85 and 84% total recovery after 72 hours) as absorbed, one may derive corrected absorbed doses of 46% and 39% in the low and the high dose group, respectively.

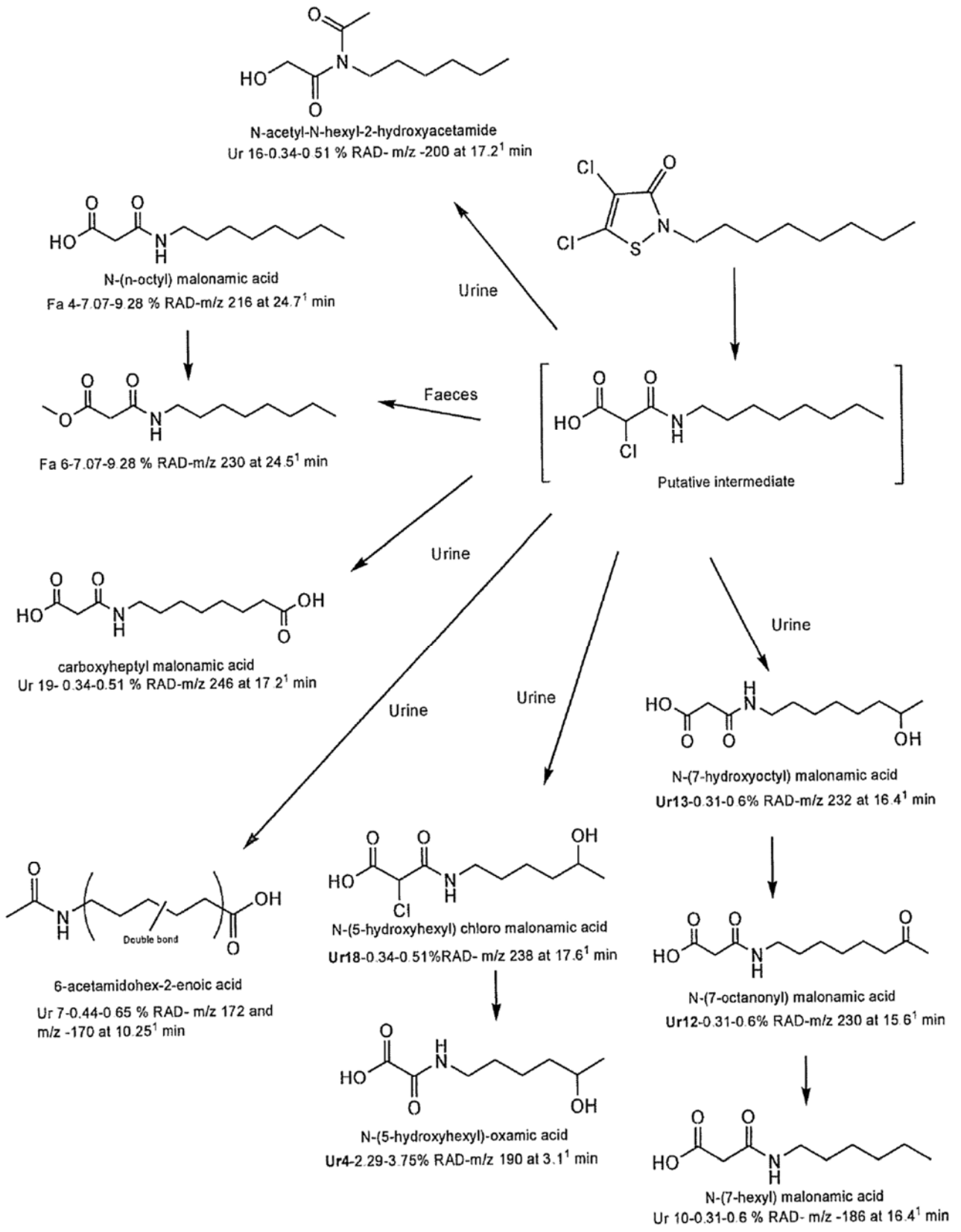
Summary toxicokinetics (Thor)

On average, only moderate amounts of ^{14}C-DCOIT (14–16% (key study), 7–10% (second study)) were absorbed from the oral route, and the majority of the substance (~ 80 %) is excreted via feces, and most of it can be assumed not to be absorbed, as only very little (1–2 %) was observed in bile. A smaller amount (5–15 %) is excreted via urine. The dermal absorption of DCOIT (in propylene glycol) was 46% and 39% in the low and the high dose group, respectively. If taken up, the substance is rapidly metabolized. Metabolism of DCOIT appears to take place via cleavage of the S-N-bond and as oxidation mainly at the octyl chain, dechlorination, decarboxylation and truncation. Phase II metabolism has been observed as N-acetyl conjugation, glucuronic acid and N-acetyl-cysteinyl conjugation.

4.1.1 Combined summary and discussion on toxicokinetics

The oral absorption is assumed to be dose dependent and moderate (below 20 %). DCOIT is extensively metabolised following oral administration. Studies performed by the two applicants' shows that excretion via faeces is the predominant route of elimination (approximately 80 %). The highest residual concentrations of DCOIT equivalents were observed in the liver, kidney and gastrointestinal tract.

Figure 4.2. Proposed metabolic pathway for DCOIT (abbreviations: Ur, urinary metabolite; Fa, faecal metabolite). (From A 6.2-01, 7.1.1-01 and A.6.2-02, 7.1.1-02).



[1]retention time corresponding method 1

4.2 Acute toxicity

Applicant 1, Dow:

Table 8: Summary table of relevant acute toxicity studies, Applicant 1 Dow

Route	**Method Guide-line**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD_{50}/LC_{50}**	**Remarks**	**Reference (Doc III cross-ref)**
Oral gavage	OECD 401	Rat Crl:CD® BR M/F 6/sex/group	500, 750, 1000, 1500 or 2000 mg/kg bw Single dose 14 day post dose observation period	1636 mg/kg RH-287 Combined M/F	(1) valid without restriction DCOIT technical in corn oil	Anon., 1992 (A6.1.1/01)
Oral gavage	OECD 401	Mice Crl:CD-1® (ICR)BR M/F 6/sex/group	100, 250, 500, 1000 or 2000 mg/kg bw Single dose 14 day post dose observation period	567 mg/kg RH-287 Combined M/F	(1) valid without restriction DCOIT technical in corn oil	Anon., 1994 (A6.1.1/02)
Dermal	OECD 402	Rabbits New Zealand White M/F 6/sex/group	2000 mg/kg bw Single dose 24 h occluded dose 14 day post dose observation period	>2000 mg/kg test substance C-9211 HQ equivalent to >652 mg/kg DCOIT	(1) valid without restriction Preformulation, DCOIT in xylene	Anon., 1989a (A6.1.2/01)
Inhalation[k]	OECD 403	Rats Crl:CD® BR M/F 6/sex/group	0.23, 0.12, 0.46 or 0.20 mg /L (analytical concentration) Single nose-only exposure 4 h 14 day post exposure observation period	0.26 mg/L Combined Males 0.21 Females 0.34	(1) valid without restriction DCOIT Technical	Anon., 1994 (A6.1.3/01)

k = key study

Two oral acute toxicity studies with exposure to DCOIT Technical (RH-287) in corn oil have been performed, one in rats (Anon. 1992, A6.1.1/01) and one in mice (Anon. 1994, A6.1.1/02).

The combined male/female acute oral LD_{50} was 1636 mg/kg bw in rats and mortality was observed from 750 mg/kg bw and above. The number of deaths increased dose-dependently. There was a

treatment-related decrease in body weight gain in the surviving male rats at 750 mg/kg and greater, but this effect was not seen in the females. Clinical signs included irritation around the anal-genital area, passiveness, scant feces and/or tan-stained muzzle. Pathological findings included a high incidence of viscous material in the cecum, intestines, and stomach; black material or black foci adhered to stomach mucosa; reddened stomach and intestinal mucosa; and mottled liver. Surviving rats had thickened stomach walls.

The combined male/female acute oral LD_{50} was 567 mg/kg bw in mice and mortality was observed from 500 mg/kg bw and above. The number of deaths increased dose-dependently. 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 feces, passiveness, tremors and ataxia were observed as exposure related effects. Pathological findings included reddened glandular portion of the stomach and intestines, black material in stomach and mottled liver.

No acute dermal toxicity with DCOIT technical was available. However, a study on a preformulation has been performed, No lethality was observed in this acute dermal toxicity study in rabbits following a 24 hrs exposure to 2000 mg/kg bw of the antifoulant preformulation, C-9211 HQ (equivalent to Kathon ^{TM} 930, 32.6% DCOIT in xylene) (Anon. 1989a, A6.1.2/01). The LD_{50} was determined to be greater than 652 mg DCOIT/kg bw, equivalent to 2000 mg/kg bw of the preformulation. Clinical signs included ataxia, reduced body weights, decreased feed consumption, scant feces and passiveness. Red fluid filled thoracic cavity and clear fluid filled abdominal cavity was observed at necropsy. In addition, skin irritation was observed (erythema, edema, pocketing edema, eschar, blanching).

A rat acute inhalation toxicity study has been reported (Anon. 1994, A6.1.3/01). The 4-hr nose-only acute inhalation LC_{50} for DCOIT technical (exposure to mixture of aerosol and vapour) was 0.26 mg /L. Mass median aerodynamic diameter (MMAD) was between 1.3 and 2.1 μm.

The exact ratio of vapour to aerosol during the test is not known. However, the actual concentration of DCOIT in the test atmosphere (collection through isopropanol (impinger method) analysed by HPLC) was 2-3 fold higher than the aerosol concentration of DCOIT (measured gravimetrically and for which the data was not reported). These results indicate that a significant portion of the exposure atmosphere contained vaporised test material.

Mortality was observed in all dose groups, but there was not a clear dose-response relationship. Signs of respiratory irritation (gasping and slight to severe rales) were seen in all dose groups. Other clinical signs were unkempt appearance, red stained eyes and muzzle, scant faeces, and yellow-stained anogenital area. At necropsy at day 14, animals in 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. The effects observed in the inhalation test are consistent with the clinical signs of respiratory irritation, and are considered to be due to the corrosive properties of the active substance. It is likely that the exposure related deaths resulted from excess fluids in the respiratory tract due to the irritant/corrosive nature of DCOIT.

Summary of acute toxicity (Dow)

DCOIT technical is moderately toxic to rats and mice following administration of a single oral dose. The combined male/female acute oral LD_{50} was 1636 mg/kg bw in rats and 567 mg/kg bw in mice, indicating that mice are more sensitive to DCOIT toxicity than rats. In the oral toxicokinetic study (Anon. A6.2.a/01) lethality in rats was observed at 250 mg/kg bw supporting that DCOIT is harmful following oral exposure.

No acute dermal toxicity study with DCOIT technical is reported. However, in an acute dermal study with the preformulation C-9211 HQ no mortality was observed at the highest dose tested (2000 mg/kg bw, equivalent to 652 mg DCOIT/kg bw), but clear toxic effects were reported.

In an acute inhalation toxicity test where rats were exposed for DCOIT (mixture of aerosols and vapour), an LC_{50} value of 0.26 mg/L was determined.

According to 1272/2008/EC, the oral classification for DCOIT should be Acute Tox 4, H302 (harmful if swallowed); based on the oral LD50 > 300mg/kg bodyweight but ≤ 2000 mg/kg bodyweight. An LC_{50} value of 0.26 mg/L/4h (vapour and aerosol) trigger Acute Tox 1 H330 (fatal if inhaled).

The information provided is insufficient for a classification of acute dermal toxicity.

Applicant 2, Thor:

Table 9: Summary table of relevant acute toxicity studies, Applicant 2 Thor

Route	**Method Guideline**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD50/LC50**	**Remarks**	**Reference (TNsG IUCLID 5)**
Oral[k]	OECD 423	Rat Wistar 3/sex/group	200, 500, 2000 mg/kg bw. Vehicle (peanut oil) single oral application (gavage)	500 mg/kg bw < LD_{50}< 2000 mg/kg bw	(1) valid without restriction DCOIT technical mortality at 2000 mg/kg bw 100% within 24 h; clinical signs and pathological changes in lung, liver, kidney and spleen	Anon. 2000a, (A 6.1.1-01 7.2.1-01)
Dermal[k]	OECD 402	Rat Wistar 5/sex/group	2000 mg/kg bw single dermal application	>2000 mg/kg bw	(1) valid without restriction DCOIT technical no mortality; rough coat, persistent erythema of the skin	Anon. 2000b, (A 6.1.2-01 7.2.3-01)
Inha-lation	OECD 403	Rat Wistar 5/sex/group	0.143, 0.221 and 0.289 mg / l air, 4 hours (nose only, only aerosols (not vapour) measured, >85% of exposure was DMSO)	0.164 mg/l air	(1) valid without restriction DCOIT technical mortalities 40, 70 and 90%, respectively; pathological changes of lung and liver	Anon. 2001, (A 6.1.3-01 7.2.2-01)

k = key study

In an acute oral toxicity study (A 6.1.1-01, 7.2.1-01) with DCOIT, dose-dependent onset of clinical signs was observed. At 200 mg/kg bw lethargy, abdominal breathing and nostril discharge was observed. At 500 mg/kg bw lethargy, abdominal breathing, toe walking and piloerection was observed. At 2000 mg/kg lethargy, abdominal breathing, gasping, nostril discharge, piloerection, toe walking, salivation, diarrhea, and unusual locomotion and pathological changes in lung, liver, kidneys and spleen was observed. All animals treated with the highest dose, 2000 mg/kg bw, died within 24 h after treatment. No animal mortality was observed for the other two doses (200 and 500 mg/kg bw). The acute oral lethal dose (LD_{50}) of DCOIT in Wistar rats was set to > 500 mg/kg bw.

The acute inhalation toxicity of DCOIT study was performed according the OECD 403 guideline (A 6.1.3-01, 7.2.2-01). Four groups of Wistar rats (each group consisted of 5 male and 5 female) were treated with aerosols of the DCOIT dissolved in DMSO for 4 h nose-only exposure (concentrations: 0.143, 0.221 and 0.289 mg/l air and DMSO as control). Treated animals were observed for 14 days for mortality, overt signs of toxicity, and body weight changes, and surviving or dead animals were subjected to gross pathological examination. Since exposure was measured only using gravimetric filter analysis (an unspecific method; OECD 403 recommends specific methods) that will not adsorb vapors, it is possible that the animals inhaled higher concentrations than those measured. Also, the substrate solution contained >85% DMSO (solvent) and some effects (e.g. on the lungs) were observed in the control animals, indicating that animals were in a sub-optimal physical state due to DMSO and its presence may also have affected the absorption of DCOIT in the lungs. All the surviving animals showed a gain in body weight over the duration of the experiment.

Clinical signs observed during the exposure period in the various treatment groups were lethargy, tremors, abdominal breathing, gasping and nasal irritation. Gross pathological lesions were observed in all different treatment groups without consistency in nature. The terminally sacrificed animals from different groups revealed vascular/inflammatory changes in the lungs. Overall, the lesions were not consistent in nature and did not increase dose-dependently in severity and incidence. The mortalities observed for the combined male/female rat were 40, 70 and 90 per cent at concentrations of 0.143, 0.221 and 0.289 mg/1 air, respectively. The Finney's Probit method was used to determine LC_{50}-value, and the acute inhalation toxicity (LC_{50}) value of DCOIT in rats was estimated to 0.164 mg/l air with the 95% fiducial limits between 0.123 to 0.219 mg/l air at breathing zone.

DCOIT is of low acute toxicity by the percutaneous route (LD50 > 2000 mg/kg bw). Clinical signs observed in an acute dermal toxicity study (A 6.1.2-01, 7.2.3-01) were 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 thickening, alopecia and erythema in treated animals were recorded. Visceral lesions in both the control and the treated group at comparable level were recorded. No mortality was observed in the control as well as in the group treated with DCOIT 2000 mg/kg bw.

Summary of acute toxicity (Thor)

DCOIT is of moderate toxicity by the oral route (500 mg/kg bw < LD50 < 2000 mg/kg bw). DCOIT is of high toxicity via inhalation based on the high mortality in the acute inhalation toxicity study (LC_{50} = 0.164 mg/L/4h (only aerosol measured)). This may be secondary to corrosivity (as also shown in the skin irritation study below). DCOIT is of low toxicity by the percutaneous route (LD_{50} > 2000 mg/kg bw).

According to 1272/2008/EC, the oral classification for DCOIT should be Acute Tox 4, H302 (harmful if swallowed); based on the oral LD50 > 300mg/kg bodyweight but ≤ 2000 mg/kg bodyweight. An LC_{50} value of 0.16 mg/L/4h (only aerosol measured) trigger Acute Tox 2 H330 (fatal if inhaled) based on an LC50 above 0.05 mg/l, but equal or below 0.5 mg/l.

4.2.1 Combined summary and discussion of acute toxicity

The acute oral studies submitted by the applicant 1 and 2 had LD_{50} values ranging from 500 to 2000 mg/kg bw. The acute inhalation toxicity studies submitted by applicant 1 and 2 had LC_{50} values ranging from 0.16-0.26 mg/L (only aerosol measured, and a mixture of aerosol and vapour measured, respectively). The acute dermal toxicity studies submitted by the applicant 1 and 2 had LD50 values > 2000 mg/kg bw.

4.2.2 Comparison with the CLP criteria

The criteria for classification with Acute Toxic category 4, H302 Harmful if swallowed, is fulfilled. The LC_{50} values of 0.16-0.26 mg/l/4h (only aerosol measured, and a mixture of vapour and aerosol measured, respectively) are below 0.5 mg/l. The seemingly more reliable 0.26 mg/l value from exposure to vapour-aerosol mixture will trigger classification with Acute Tox category 1, H330 Fatal if inhaled) as the value is below the cut off limit for Acute Tox category 1 (vapour).

4.2.3 Conclusions on classification and labelling

According to Regulation (EC) No 1272/2008 (CLP) DCOIT should be classified as:

Acute toxic, Category 4 H302: Harmful if swallowed; Acute toxic 1 H330: Fatal if inhaled

4.3 Specific target organ toxicity – single exposure (STOT SE)

From the acute toxicity studies following oral, inhalation or dermal exposure there was no clear evidence of non-lethal systemic effects on a specific target organ or tissues.

The effects observed in the acute toxicity inhalation tests are consistent with the clinical signs of respiratory irritation (abdominal/laboured breathing, gasping, rales and nasal irritation) and are considered to be due to the corrosive properties of the active substance. Histopathological examinations demonstrated findings in the lungs, (including redness in lobes of the lung, vascular/inflammatory changes). It is likely that the exposure related deaths resulted from the irritant/corrosive nature of DCOIT. Hence, DCOIT should additionally be considered as corrosive to respiratory tract (EUH071 Corrosive to the respiratory tract).

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

4.3.2 Comparison with criteria

From the acute toxicity studies following oral, inhalation or dermal exposure there was no clear evidence of (non-lethal) effects on a specific target organ or tissues. Classifications for acute toxicity and corrosivity cover the toxicological effects of DCOIT after single exposure. An additional classification as STOT SE 1 or 2 is therefore not appropriate.

The hazard class STOT SE 3 should cover 'transient' respiratory tract irritation and narcotic effects. According to the CLP criteria, a classification for corrositivity is generally considered to cover the potential to cause respiratory tract irritation; the additional STOT SE 3 thus is superfluous.

4.3.3 Conclusions on classification and labelling

Although the data suggest that DCOIT is a respiratory irritant, the effects are accounted for by the classification for acute inhalation toxicity (Acute Tox 1) and the application of the EUH071 phrase (see 4.6). An additional classification for STOT SE 3; H335 is not warranted

4.4 Skin irritation/corrosivity

Applicant 1, Dow:

Table 10: Summary table of relevant skin irritation studies, applicant 1 Dow

Species	**Test substance**	**Method**	**Average score** **24, 48, 72 h**		**Reversibility yes/no**	**Result**	**Reference** **(Doc III cross-ref)**
Rabbits New Zealand White	Preformulation, DCOIT in xylene: C-9211 HQ (32.6% DCOIT in xylene)	OECD 404	Erythema 4.0	Edema 3.9	No Not reversible in 5 of 6 animals	Corrosive	Anon., 1989b, A6.1.4/01
Rabbits New Zealand White	LES 1920P mildewcide (20%DCOIT in phenoxy-propanol)	OECD 404	Erythema 4.0	Edema 3.3	No Edema reversed by day 14. Erythema did not reverse by day 14.	Corrosive	Anon., 1997. (Kathon ™ 287 WT B6.2a/04 (PT8)) NK A6.1.4/03 (PT21)
Human epidermal construct (*in vitro* test)	DCOIT technical	OECD 431 EPIDERM ™ (EPI-200)	Na	na	Na	Not corrosive	Anon., 2007, A6.1.4/02

A skin irritation study has been performed with antifoulant preformulation, C-9211 HQ (32.6% DCOIT in xylene (Doc III-A6.1.4/01). 0.5 mL of the preformulation was administered to the skin of 6 rabbits for 4 hours and skin reactions were noted at 1 h and 1, 2, 3, 7 and 14 days post application. The DCOIT/xylene formulation produced severe oedema and erythema in rabbit skin. Moderate to severe erythema and oedema were observed at 1h. After the 72 h severe erythema, moderate to severe oedema, blanching and eschar formation was noted. At 14 days there was no oedema, but slight to severe erythema, eschar, areas without hair growth and whitened areas. Five of six animals

demonstrated scar formation at 14 days. The skin reaction was considered corrosive. Xylene is classified as a skin irritant with H315 (causes skin irritation) and using xylene as a solvent is likely to aggravate the skin reactions to DCOIT.

In another skin irritation study, one male rabbit was exposed for 4 hrs to a formulation containing 20% DCOIT in phenoxypropanol (propylene glycol phenyl ether), (Doc III-B6.2a/04, Kathon ^{TM} 287 WT biocide (PT8) and NK III A6.1.4/03 (PT21)). Severe erythema was evident at all observation periods. 1 hour erythema score was not taken as the test substance had stained the skin application site green. Very slight to severe oedema was noted at 1 hour and continued through day 7. Oedema was reversible by day 14. Skin irritation indicative of corrosivity (concave eschar) was evident by 48 hours. On day 14, corrosive findings (ulceration/erosion) were confirmed by a veterinary pathologist.

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 tissue constructs 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 judged non-corrosive by the criteria for this test. It appears from the study protocol that there was no post-exposure incubation time and maximum exposure time was 60 min. However, due to existing *in vivo* experimental data the Rapporteur supports the view of the Applicant that this is probably a false negative result. Whether the lack of response in the Epi-200-test 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. In the current guidelines for the Reconstructed Human Epidermis Test Methods (OECD TG 431 and 439) up to 240 minutes of exposure is used in some of the corrosion tests and a 42 h post-exposure time is considered optimal for the skin irritation test. As the dermal penetration of DCOIT is relatively slow, a prolonged tissue incubation time might have been necessary for revealing the corrosive effects of DCOIT in *in vitro* tests. The result from the *in vitro* test is therefore not considered to reduce the concern for skin corrosion.

The following information drawn from other animal toxicity studies, human data and comparison with other isothiazolinones support the conclusion that DCOIT technical is a corrosive substance.

Primary irritation was addressed in the two guinea pig sensitization studies described in chapter 4.6 (Sensitisation). In a study (Anon., 2003, A6.1.5/01, very faint to faint irritation (slight confluent erythema) was reported following dermal exposure to 0.02% (8.8 µg/cm^2) and 0.03% (12.12 µg/cm^2) DCOIT technical diluted in mineral oil. The highest non-irritating concentration in the study was 0.01% (4.4 µg/cm^{2}of DCOIT).

Similar results were obtained in a more ancient study (Anon., 1984, A6.1.5/03) where a range-finding primary irritation test was performed. When dissolved in 80% aqueous ethanol, the slightly irritating concentration was 0.1 % DCOIT technical, whereas 0.03% DCOIT technical was the highest non-irritating concentration when dissolved in acetone.

A series of human clinical irritation and sensitization trials patch tests studies have been performed using petrolatum, corn oil and ethanol as the vehicle. In these cumulative irritation/sensitisation studies, essentially no skin irritation was observed and there were no cumulative effects with DCOIT in either petrolatum or corn oil at concentrations up to and including 1000 ppm. In contrast, studies with DCOIT in ethanol demonstrated that 250 ppm (0.025%) to 350 ppm (0.035%) is at or near the threshold concentration for skin irritation (Anon., A6.12.6/01-08).

Taken together, these studies indicate that the concentration of DCOIT leading to local skin irritation is above 0.01% both for acute and subacute exposures. The local effect seems to depend on concentration and solvent formulation rather than on exposure duration.

Summary of skin irritation/corrosivity (Dow)

DCOIT technical has not been tested in the Draize test for skin irritation by Applicant 1. However, a formulation of 32.6% DCOIT in xylene (C-9211 HQ preformulation) was found to be corrosive in a rabbit skin irritation test. Xylene being a skin irritant might aggravate the skin reactions to DCOIT. Furthermore, a product containing 20% DCOIT in phenoxypropanol was found to be corrosive in a single-animal test performed in accordance with the OECD guideline 404. In this study, other product constituents may have contributed to the response.

DCOIT was found to be non-corrosive in an *in vitro* skin corrosion test. However, the result of the *in vitro* test is suspected to be a false negative for reasons discussed above. Based on the corrosive nature of DCOIT in xylene or in phenoxypropanol, and on results on primary irritation in skin sensitisation studies in animals, and clinical irritation and sensitization trials in humans DCOIT is regarded as corrosive to skin and eyes. The irritation/sensitization studies indicate that DCOIT may induce faint irritation at concentrations down to 0.02%.

Applicant 2, Thor:

Table 11: Summary table of relevant skin irritation studies, applicant 2 Thor

Species	**Test substance**	**Method**	**Average score 24, 48, 72 h**		**Reversibility yes/no**	**Result**	**Reference (TNsG IUCLID 5)**
			Erythema	Edema			
Rabbit New Zealand White 3 males[k]	DCOIT Technical	OECD 404	2.3	2.2	No	corrosive upon 4 hour exposure	Anon., 2000c (A 6.1.4-01 7.3.1-01)

k = key study

The acute skin irritation or corrosion potential of Acticide DCOIT was evaluated in vivo on rabbit skin according to OECD guideline 404 (Anon., 2000c, A 6.1.4-01, 7.3.1-01).

No clinical signs other than erythema and oedema were observed in the animals. Mild to severe degree of 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. The control skin sites of rabbits were normal throughout the post-treatment period. The applicant gave the study a reliability score of 2.

Table 12: Irritant/corrosive response data for each animal at each observation time up to removal of each animal from the test

Score at time point / Reversibility	Erythema Max. score: 4	Edema Max. score: 4
60 min	3/ 3/ 3	2/ 2/ 2
24 h	3/ 2/ 2	2/ 2/ 2
48 h	3/ 2/ 2	2/ 2/ 2
72 h	4/ 2/ 1	4/ 2/ 2
Average 24h, 48h, 72h	2.3	2.2
Reversibility*)	n	n

*) Reversibility: c. = completely reversible; n.c. = not completely reversible; n. = not reversible

Summary of skin irritation/corrosion (Thor)

Marked irritation was seen after dermal application of DCOIT to rabbit skin. Mild to severe degree of erythema and oedema were observed from the end of the 4 hour exposure period until the end of the observation period. The study result was re-evaluated by applicant, THOR, from "moderately irritating" to corrosive due to the severity and irreversibility of the observed skin lesions which increased over the time of the study. The study was terminated after 14 days without any signs of reversibility. Full-thickness destruction of the skin after 4 hours exposure can be predicted.

4.4.1 Combined summary and discussion of ski irritation/corrosivity

Skin irritation has been examined in three *in vivo* rabbit studies according to OECD guideline 404; one of the studies used Acticide DCOIT and the two others were performed with antifoulant preformulations. The conclusion from all three studies was that DCOIT causes skin corrosion as the skin lesions were not reversed.

Primary irritation has been addressed in the two guinea pig sensitization studies and a range-finding primary irritation test. In these studies the highest non-irritating concentrations were in the range of 0.01% - 0.03%.

A series of human patch tests studies have shown slight skin irritation from DCOIT in ethanol down to 0.025%-0.035% concentrations. Together the animal and human irritation/sensitisation studies indicate that the concentration of DCOIT leading to local skin toxicity is above 0.01% both for acute and subacute exposures. The local effect seemed to depend on concentration and solvent formulation rather than on exposure duration.

Two isothiazolinones with related structures to DCOIT, namely CMIT/MIT (CAS No. 55965-84-9; 3:1 mixture of 5-chloro-2-methyl-2H- isothiazol-3-one and 2-methyl-2H-isothiazol-3-one) and 2-octyl-2H-isothiazol-3-one (CAS No. 26530-20-1) are corrosives to the skin and are included in Annex VI of CLP with classification as Skin Corr. 1B. A RAC opinion on the former substance of March 2016 proposes a revised classification; Skin Corr. 1C.

4.4.2 Comparison with criteria

According to CLP criteria a corrosive substance is one that produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after

exposure up to a 4 hour duration. The criteria for classification of DCOIT as corrosive to skin are fulfilled based on the observations in OECD 404 tests and supporting information from skin sensitisation studies. This conclusion is supported by the corrosive properties of three structurally related isothiazolinones. The data used for classification does not allow differentiation between the skin corrosion subcategories 1A/1B/1C.Hence, DCOIT should be classified with skin corrosive Category 1.

The CLP guidance 3.2.2.5. states that specific concentration limits (SCLs) shall be set where adequate and reliable scientific information shows that the hazard of a substance is evident when the substance is present at a level below the concentrations set for any hazard class in Part 2 of Annex I or below the generic concentration limits set for any hazard class in Parts 3, 4 and 5 of Annex I. Both human pacht test studies and animal studies have shown that an irritation response may be induced below the generic concentration limit; Skin Corrosion Category 1: ≥ 5%; Skin irritation Category 2: ≥ 1% but < 5%. For this reason the application of a SCL is warranted. Based on the available data, a SCL of 0.01% for skin irritation is proposed as this is the highest non-irritating concentration identified. There is no basis for setting a specific concentration limit for corrosion.

4.4.3 Conclusions on classification and labelling

In accordance with Regulation (EC) No 1272/2008 (CLP) DCOIT should be classified as corrosive to the skin and should be classified with skin corrosive category 1, assigned with the pictogram GHS05 with signal word “Danger” and the hazard statement H314 (Causes severe skin burns and eye damage). SCLs of 0.01% for skin irritation is proposed.

4.5 Eye irritation

Combined Applicant 1 Dow and 2 Thor

DCOIT was regarded as corrosive to skin. As a consequence, and in accordance with the Technical Notes for Guidance on data requirements (chapter 2 section 6.1.4), DCOIT was not tested in the eyes of rabbits.

4.5.1 Summary and discussion of eye irritation

DCOIT was regarded as corrosive to skin and serious damage to eyes is thus implicit. As a consequence, and in accordance with the Technical Notes for Guidance on data requirements (chapter 2 section 6.1.4), DCOIT was not tested in the eyes of rabbits.

4.5.2 Comparison with criteria

DCOIT was regarded as corrosive to skin and serious damage to eyes is thus implicit. As a consequence, and in accordance with the Technical Notes for Guidance on data requirements (chapter 2 section 6.1.4), DCOIT was not tested in the eyes of rabbits.

4.5.3 Conclusions on classification and labelling

DCOIT is proposed classified as corrosive Category 1 and labelling for serious damage to eyes is implicit.

4.6 Respiratory tract irritation

Applicant 1, Dow:
The acute inhalation toxicity study (Anon., 1994-A6.1.3/01) and the 13 week repeated dose study (Anon., 1994-A6.4.3/01) both indicate that DCOIT is a potent respiratory irritant. In addition, a study of upper airway sensory irritation from exposure to the preformulation Kathon^{TM} 930 (equivalent to Antifoulant C-9211 HQ, containing 30% DCOIT in xylene) has been performed (Doc III-B6.1.3/02, Kathon ^{TM} 930 biocide (PT8) and Theoretical product (PT21)). Male mice (Swiss Webster) were exposed for 10 minutes, head only, to analytically determined concentrations of 32, 75, 86, 112, 167, 184, and 198 µg/L of test material formulation. The post-exposure period was 15 minutes. The aerosol particle sizes were 1.6 to 2.1 microns mass median aerodynamic diameter (MMAD). Respiratory rates were moderately decreased at 86 µg/L and higher. Thirty-three percent was the maximum decrease in respiratory rate achieved with the test material at 112 µg/L and greater. The RD_{50} was greater than 198 µg/L, the highest concentration tested. No respiratory rate depression was observed in the xylene control group.

Summary of respiratory tract irritation (Dow)

An acute inhalation toxicity study and a 13 week repeated dose study both indicate that DCOIT is a potent respiratory irritant. In an upper airway irritation test the preformulation Kathon^{TM} 930 (30% DCOIT in xylene) induced a moderate respiratory rate depression. The upper airway irritation test is a measure of sensory irritation and is commonly used for setting up workplace exposure limits, but not for classification purpose. This test indicates that DCOIT is a moderate upper airway respiratory irritant. Together these findings trigger the additional labelling with EUH 071 (corrosive to the respiratory tract).

Applicant 2, Thor:

In the acute inhalation study in rats, clinical signs like gasping and nasal irritation and vascular/inflammatory changes in the lungs was reported. However, the lesions were not consistent in nature and did not increase dose-dependently in severity and incidence. The interpretation of the results with regard to respiratory irritation is probably obscured by effects caused by the high concentration of DMSO solvent in this study. No subacute or subchronic inhalation study is provided by Applicant 2.

4.6.1 Summary and discussion of respiratory tract irritation

The acute inhalation toxicity and the 13 week repeated dose study provided by Applicant 1 indicate that DCOIT is a potent respiratory irritant. The acute inhalation toxicity study provided by Applicant 2 is difficult to interpret in relation to respiratory irritation. Taken together the findings trigger the labelling of DCOIT with EUH071 (corrosive to the respiratory tract).

4.6.2 Comparison with criteria

According to the Guidance on the Application of the CLP Criteria No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, EUH071 (Corrosive to the respiratory tract) applies in addition to classification for inhalation toxicity, if data are available that indicate that the mechanism of toxicity is corrosivity, in accordance with section 3.1.2.3.3 and Note 1 of Table 3.1.3 in Annex I.

The effects observed in the inhalation tests are consistent with the clinical signs of respiratory irritation, and are considered to be due to the corrosive properties of DCOIT. In accordance with these criteria, DCOIT warrants the labelling with EUH071 - corrosive to the respiratory tract).

4.6.3 Conclusions on classification and labelling

The corrosive nature of DCOIT and the indication that corrosivity is important in the inhalation toxicity trigger the labelling with EUH071 (corrosive to the respiratory tract).

4.7 Corrosivity

Please refer to section 4.4.

4.8 Skin sensitisation

Both applicants:

DCOIT is well absorbed in the skin and binds strongly to the various skin layers. DCOIT is presumed to modify nucleophilic sites in skin proteins and thus to have sensitising properties, as have been shown for the related isothiazolinones, 5-chloro-2-methyl-2H-isothiazol-3-one (CMIT) and 2-methyl-2H-isothiazol-3-one (MIT).

Table 13: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD 429 Local lymph node assay (LLNA)	Sensitizer EC3 value 0.03%, 15 µg/cm^2	0.005%, 0.01%, 0.1% 0.25%, and 0.5% (w/v) DCOIT Technical in acetone: olive oil, 4:1 (v/v)	Anon., 2003 (Thor Doc III/7.4.1)
OECD 406 Guinea pig maximization test (GPMT)	Sensitizer at ≥ 5%	Single dose (5% DCOIT Technical in propylene glycol)	Anon., 2001 (Thor Doc III/7.4.1)
OECD 406 Guinea pig maximization test (GPMT)	Sensitizer at ≥ 0.01%, 4.4 µg/cm^2	0.01%, 0.02% and 0.03% of DCOIT Technical in mineral oil.	Anon., 2003 (Dow, Doc III-A6.1.5/01)
Open patch test in patients with itchy reddish eruptions on skin after occupational exposure	Sensitizer	N=6	Anon., 1993 (Dow, Doc III- A6.12.6/09) and Thor, Doc III 7.10.4
Repeat insult patch test (RIPT) - humans	Sensitizer at ≥ 0.025%, 5 µg/cm^2	Dissolved in ethanol Lowest concentration used: 0.025% (12.5 µg/cm^2)	Anon., 1992 (Dow Doc III-A6.12.6/06)
24–hours Occlusive Patch Test – humans	Sensitizer at 0.025%	Dissolved in ethanol Lowest concentration used: 0.025%	Anon., 1993 (Dow Doc III-A6.12.6/07)

24–hours and 48-hours Occlusive Patch Test - humans	Sensitizer at 0.035%	Dissolved in ethanol Lowest concentration used: 0.035% (7 µg/cm^2)	Anon., 1994 (Dow Doc III-A6.12.6/08)
OECD 429 and OPPTS 870.2600 - Mice	Not a sensitizer	NNOMA (metabolite)	Anon., 2006 (Dow Doc III-A6.1.5/02)

4.8.1 Non-human information

DCOIT was tested in the local lymph node assay (LLNA) according to OECD 429 (Anon., 2003 Doc III/7.4.1). Four mice/group were dermally exposed to 0% (two negative control groups), 0.005%, 0.01%, 0.1%, 0.25%, and 0.5% (w/v) DCOIT in acetone:olive oil, 4:1 (v/v) (0, 2.5, 5, 50, 125 and 250 µg/cm^2, respectively) by topical application of 25 µl test solution onto the dorsum (Θ ~ 8 mm) of each ear lobe (left and right) on three consecutive days. The control groups were treated with acetone:olive oil 4:1, (v/v) only. Five days after the first topical application the mice were injected i.v. into a tail vein with ^{3}H-thymidine. Four hours after treatment with ^{3}H-thymidine, the draining lymph nodes were removed and pooled for each experimental group. A single cell suspension were prepared from the pooled nodes and the proliferation capacity was determined by the incorporation of ^{3}H-thymidine in a β-scintillation counter expressing the number of radioactive disintegrations per minute (DPM). A statistical analysis was conducted for assessment of the dose-response relationship, and the EC3 value was calculated. No test item-related clinical signs were observed in any animals of control group or mice exposed to 0.1%, 0.01% or 0.005% test substance. At the second application day, a slight swelling at both dosing sites was observed in all mice of the 0.25% and 0.5% dosing groups, persisting for four days. Stimulation indices of 0.8, 1.1, 11.6, 25.7 and 27.0 were determined with the test item at concentration of 0.005%, 0.01%, 0.1%, 0.25% and 0.5%, respectively. A test item is regarded as a sensitizer in the LLNA if the exposure to at least one concentration results in an incorporation of ^{3}H-thymidine at least 3-fold or greater than that recorded in control mice. Based on these criteria, Acticide DCOIT was found to be a sensitizer at concentrations of 0.1%, 0.25% and 0.5%. An EC3 value (estimated concentration value based on stimulation index of 3) of 0.03% (w/v) (15 µg/cm^2) was derived.

The GPMT procedure comprises of two phases: an induction and a challenge phase (Anon., 2001 Doc III/7.4.1). In the induction phase the animals (10 boars, 10 sows) were exposed to substance by intradermal injection of 5% Acticide DCOIT in propylene glycol with or without adjuvant material (1:1 mixture (v/v) Freund's Complete Adjuvant). In addition, the animals were injected with the adjuvant material alone. The control group (5 boars, 5 sows) was given the same solutions but without Acticide DCOIT. On day 7 patches loaded with 0.2 ml of 25% Acticide DCOIT in 80% alcohol was applied and held in contact by an occlusive dressing for a period of 48 hours. In the control group 80% alcohol was similarly applied. The skin was observed for evaluation of induction of patch reactions at 24 hours from the time of patch removal following the Magnusson and Klingman grading scale. On day 21, an occlusive patch loaded with 0.2 ml of 5% Acticide DCOIT in acetone was applied to all the animals and the patches were held in contact by an occlusive dressing for 24 hours. The skin was observed at 24 and 48 hours after patch removal and skin reactions were graded. Following the challenge, 60% (12/20) of the animals in the treatment group were positive at 24 hours and 45% (9/20) at 48 hours. Due to the use of only one (high) exposure level, the sensitisation potency cannot be derived from this study.

Another study of DCOIT technical was performed in GPMT according to OECD 406 (Anon., 2003 A6.1.5/01). The animals were exposed in the induction phase to 3 different doses (0.01%, 0.02% and 0.03%) of the substance in mineral oil via intradermal injection. The control group was exposed

to vehicle (mineral oil), alone or combined with Freund's adjuvant or adjuvant alone. This was followed by a challenge two weeks later, via topical application for 24 hours at a naïve site (0.01%, 0.02% and 0.03% technical DCOIT). Each group contained 10 animals/sex/group. At the lowest exposure dose 0.01% (4.4 μg/cm^2), 75 % (15/20 animals) were positive at 24 hours and 55 % (11/20) at 48 hours after the challenge. At both time points, 95 % were positive at 0.02% (8.8 μg/cm^2), and 100 % were positive at 0.03% (12.12 μg/cm^2). Due to the lack of lower doses than 4.4 μg/cm^2 (0.01%) without allergic response, the GPMT is not fully according to OECD 406.

4.8.2 Human information

In a textile finishing factory, 8 out of 19 workers developed itchy reddish eruptions on exposed areas of skin (Anon., 1993 A6.12.6/07). Starting about 3 weeks prior to the occurrence of the dermatitis a new type of biocide was added to the finishing agent. The biocide was formulated as a 30% active ingredient solution (DCOIT) in xylene. Open patch tests were performed on 6 patients with the finishing agent without biocide and the finishing agent with 0.2% biocide (0.06%/600 ppm active ingredient). Test substances were applied directly onto a 2 cm^2 area of the skin on the upper arms. Five of the 6 patients showed strong positive reactions to the finishing agents with 0.2% biocide and none showed any reaction to the finishing agents without biocide. The patient who showed no reactions to either solution had taken corticosteroids orally two days prior to the test, and this may have caused false-negative reactions. Although the number of patients is small, this study gives evidence that DCOIT is a skin sensitizer in humans. However, the induction dose during occupational exposure was unknown, and only one concentration of the test substance was tested during the elicitation test. Furthermore, the dose per area applied (μg test substance/cm^2) cannot be determined because the volume of test substance applied is not stated.

Clinical irritation and sensitization trials were performed in humans in the late 1980s and the early 1990s. Several repeated exposure human patch tests demonstrated that DCOIT diluted in petrolatum or corn oil at concentrations up to 1000 ppm did not induce sensitization (Anon., A6.12.6/01-05). In contrast, studies with DCOIT in ethanol demonstrated potency for irritation and sensitization.

- In a repeat insult patch test (Anon., 1992 A6.12.6/06), individuals not previously sensitised were exposed to 0.2 ml of 0.025% and 0.035% DCOIT in ethanol during induction phase (3 times per week for 3 weeks, 34 subjects per dose level). After 2 weeks rest period, naïve sites were challenged for 24 hours with 0, 0.01, 0.025 and 0.035% DCOIT in ethanol. 4/34 (12%) of subjects induced with 0.025% DCOIT and 14/34 (41% subjects) induced with 0.035% DCOIT exhibited sensitization. Converting to a dose per area of exposed skin, the lowest level tested yet inducing sensitisation (0.025%) corresponds to 12.5 μg/cm^2

- As a follow-up of the RIPT study, eight subjects that responded positively to induction and challenge to 0.035% DCOIT in ethanol were re-challenged 6 months later in a 24 hours Occlusive patch test, with 0 (ethanol) and 0.025% DCOIT in ethanol (Anon., 1993 A6.12.6/07). 3 of the 8 subjects each responded positively to re-challenge with 0.025% DCOIT, with a lower, higher, or similar intensity than they did in the initial challenge approximately 6 months earlier. Possible explanations for these results are that a number of the sensitization responses in the previous study may have been irritant responses or that the intensity of the elicitation (sensitization) response in DCOIT sensitized subjects decreases over time.

- 24-hours and 48-hours Occlusive Patch Test were performed in 10 subjects, with 0.035%, 0.05%, 0.075% and 0.1% DCOIT in ethanol (0.01 ml on 8 mm circular chamber discs). The time point for dermal examination was not stated, but it was apparently performed right after removal of the patches. No distinct differences in irritation between the four concentrations were

observed. However, two subjects (20%) reacted adversely to all test concentrations of DCOIT and still exhibited reactions 10-15 days after patch removal, suggesting a sensitising activity to the agent. The study included few individuals and the lowest exposure level induced effects. The lowest dose tested yet giving positive effects (0.035%) corresponds to 7 μg/cm^2.

These human studies suggest that DCOIT technical is a skin sensitizer when it is dissolved in ethanol, with an induction threshold at or below 0.025%. The threshold dose for sensitisation appears to be considerably higher for DCOIT diluted in petrolatum or corn oil (>0.1%). Additional data from already exposed workers are supportive of the sensitisation capacity of DCOIT (Anon., 1993, A6.12.6/09 and 7.10.4) though there is a low number of humans and insufficient information about the concentration of the substance in the finishing solution causing the outbreak of occupational contact dermatitis and potential cross reactions with other substances.

4.8.3 Combined summary and discussion of skin sensitisation

Three reports from appropriate (OECD-guideline) non-human in vivo tests all demonstrate a significant skin sensitising effect (SI>3 and redness in > 30% of the test animals in the LLNA and GPMT, respectively). LLNA is the preferred test for skin sensitisation testing, according to the guidelines (Guidance on the Application of the CLP criteria). The GPMT has limitations compared to the LLNA; the adjuvant used in the GPMT may contribute to false positive results, and the possibility for potency evaluation from GPMT tests are considered limited. Further limitations in the performance of the GPMT-tests were that only one high concentration was tested (Anon., 2001 A7.4.1) and lack of a sufficiently low exposure level (Anon., 2003; A6.1.5/01). In the LLNA test, the derived EC3-level was calculated from the presented LLNA data to be 0.03% (15 μg/cm^2). Thus, according to Table 3.4.2-f in the "Guidance on the Application of the CLP criteria" (version 4.1, June 2015), the skin sensitisation potency category for DCOIT is determined to be "extreme".

This categorisation is supported by the GPMT data where more than 60% of the animals were positive at an induction concentration of 0.01% (4.4 μg/cm^2) (Anon., 2003; A6.1.5/01).

Data from several types of human studies support that DCOIT can lead to sensitisation. These data includes evidence from workers exposed by skin contact, where 5 of 6 patients with previous symptoms showed strong positive reaction to the finishing agent with 0.2% biocide, and none showed any reaction to the finishing agent without biocide. Induction of sensitisation in individuals not previously sensitised was observed after exposure to 0.025% and 0.035% DCOIT in ethanol (but not at concentrations up to 0.1% when suspended in petrolatum or corn oil). Induction of sensitisation in humans appears to be specific for the substance, a significant number of individuals became sensitised and sensitisation occurred already at the lowest concentration tested. The induction threshold for animals and humans seems to be in the same size range.

It is important to notice the strong variation in sensitisation capacity of DCOIT with the different vehicles.

4.8.4 Comparison with criteria

According to the 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, "substances shall be classified as skin sensitizers in accordance with two criteria (Table 3.4.2): i) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of humans, or ii) if there are positive results from an appropriate animal test".

As summarized above, three reports from OECD-guideline *in vivo* tests are in accordance with criteria ii, all demonstrating a significant skin sensitizing effect (according to table 3.4.2.3.4: SI>3 and redness in > 30% of the test animals in the LLNA and GPMT, respectively). According to the criteria proposed by an expert group (nominated by the Technical Committee of Classification and Labelling, Basketter D.A. et al, 2005) for grouping chemicals together in categories of sensitisation potency with the purpose of applying lower concentration limits for labelling for strong and extreme sensitizers (which is implemented in the Guidance on the application of the CLP criteria, ECHA 2015), DCOIT would fall into the category "extreme sensitizer" based on the results from the LLNA study, as well as from the GPMT study. Hence, DCOIT should be classified Skin Sens 1A, H317.

Human data supports that DCOIT can lead to sensitisation. Although the number of individuals appears to be low in each study (but substantial in the RIPT), there are several studies showing similar results i.e. induction of sensitisation appears to be specific for the substance, a significant number of individuals became sensitised and sensitisation occurred already at the lowest concentrations tested.

4.8.5 Conclusions on classification and labelling

DCOIT fall into the category "extreme sensitizer" based on the results from the LLNA-study (EC3-value of 0.03%). This is supported by data from the GPMT studies and the human data. Classification as Skin Sens 1A is thus warranted with H317 (May Cause Allergic Skin Reaction).

According to the guidelines, specific concentration limits for skin sensitisation shall be set when there is adequate and reliable scientific information available showing that the specific hazard is evidently below the generic concentration limit (1%) for classification. This applies for DCOIT. According to table 3.4.2-i (recommendations given by an EU expert group on skin sensitisation (Basketter et al., 2005)), a SCL of 0.001% should be applied for DCOIT.

4.9 Respiratory sensitisation

No studies addressing respiratory sensitisation of DCOIT has been reported and thus it is not possible to evaluate the classification for respiratory sensitisation. However, due to its high skin sensitising potential and considering its mode of action (binding to nucleophilic sites in proteins), there is a possibility that DCOIT may also be sensitising via airway exposure.

4.10 Specific target organ toxicity – Repeated exposure (STOT RE)

Applicant 1 Dow

Table 14: Summary table of relevant repeated dose toxicity studies

Route **Method**	**dura-tion of study**	**Species** **Strain** **Sex** **no/group**	**dose levels** **frequency of application**	**Results**	**LO(A)EL**	**NO(A)EL**	**Reference** **(Doc III-A cross-ref)**
ORAL STUDIES							
Oral Gavage Japa-nese guide-line	4 weeks, 2 week recovery period	Rats, SD (Crj:CD), males and females, 10/sex/ group	0, 20, 100 and 500 mg/kg/day; DCOIT Technical (97.5 % DCOIT) diluted in olive oil	Three females (high dose group) died during the treatment period. Clinical signs reversed (in surviving rats) during the 2 week recovery period. Effects on body weight gain and feed consumption and changes in absolute and relative organ weight and urine analysis (high dose group, males most affected). Most effects reversed during the recovery period. Male animals showed a significant, but slight decrease in relative liver weight from 100 mg/kg bw (body weight unchanged at this dose compared to control). Slight, but significant changes in clinical chemistry and haematology parameters (100 and 500 mg/kg dose group). Gross pathology revealed thickening of the mucosa of the non-glandular stomach and small and large intestine at 500 mg/kg. Atrophy in liver at 500 mg/kg bw of five male animals (considered due to the irritant nature of the test substance). Microscopic histopathological examination revealed slight changes in the stomach and small intestine at 100 and 500 mg/kg and increased fat content in the adrenals. Increase in granulocytes in the spleen at 500 mg/kg.	100 mg/kg	20 mg/kg	Anon., 1991 (A6.3.1/01)

Route Method	dura-tion of study	Species Strain Sex no/group	dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference (Doc III-A cross-ref)
Oral Diet OECD 408	3 months	Rats, Crl:CD® BR, M/F, 10/sex/ group	0, 100, 500, 1000, 4000 ppm equivalent to 6.2-7.2, 32.5-36.7, 60.7-74.7, and 248.2-278.4 mg/kg bw/day (M/F); DCOIT technical (98.8 % DCOIT)	The minimum effect level was 1000 ppm, where significant reductions in body weight gain, feed consumption, and serum triglyceride levels were observed in female animals. Minimal histopathologic changes of the forestomach were observed in one male and one female animal at this dose. At 4000 ppm, forestomach irritation ranging from minimal hyperkeratosis and slight epithelial hyperplasia to erosion/ulcerations with associated inflammation and oedema of the submucosa	1000 ppm (60.7 - 74.7 mg/kg bw/day (M/F)	500 ppm (32.5 - 36.7 mg/kg bw/day (M/F)	Anon., 1994 (A6.4.1.a/01)
Oral Diet OECD 409	3 months	Dogs, Beagle, M/F, 4/sex/ group	100, 300, 1500 ppm DCOIT technical (98.42% DCOIT); Diet offered 2hrs/d	The minimum effect level was 1500 ppm where decreased body weight and food consumption compared to control were observed (significantly in females only). All dogs, but one male exhibited body weight loss during the treatment period. Some changes in haematological and clinical chemistry observed. An increased incidence and severity of thymic atrophy in females at 1500 ppm (considered secondary to decreased body weight and food consumption).	1500 ppm (47.5 - 45.9 mg/kg bw/day (M/F)	300 ppm (10.2 – 10.1 mg/kg bw/day (M/F)	Anon., 2002 (A6.4.1.b/01)
DERMAL STUDIES							
Dermal No guide-line (prior to OECD 410)	21 days	Rabbits, New Zealand White, M/F 6/sex/ group	0, 1, 5 mg/kg bw/day; C-9211M (preformulation, 35 % DCOIT in mixed xylene diluted in acetone); 5 days/week (15 doses in 21 days)	Under these test conditions, skin irritation was the only observed toxic response to the test substance, DCOIT applied in xylene.	Local toxicity: 1 mg/kg bw/day (0.35 mg a.i/kg bw/day)	Systemic toxicity: >5 mg/kg bw/day (1.75 mg ai/kg bw/ day)	Anon., 1983 (A6.3.2/01)
INHALATIONAL STUDIES							

Route Method	dura-tion of study	Species Strain Sex no/group	dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference (Doc III-A cross-ref)
Inhal-ation Nose only OECD 413	3 months, 6 mth and 1 yr recovery groups	Rats, CRL:CD® BR, M/F, 32/sex/ group	0, 0.02, 0.63 and 6.72 mg ai/m³, (analytical concentrations) C-9211M HQ (preformulation, 32.6% DCOIT in o-xylene); 5 days/week, 6 hrs/day	Significant increase in absolute lung weight (6.72 mg/m³) judged to be result of oedema of the lungs. Histopathological evaluations at the 3 month necropsy revealed treatment-related observations in the nose, larynx and lungs (0.63 and 6.72 mg/m³). By the six month necropsy, recovery was seen in all tissues and the lungs no longer showed signs of histopathological lesions. There was no evidence of systemic toxicity at any dose.	0.63 mg a.i./m³, based on histopathological changes in nose and larynx	0.02 mg a.i./m³	Anon., 1994 (A6.4.3/01)

DCOIT technical diluted in olive oil was administered in daily doses of 0, 20, 100 and 500 mg/kg bw/day to rats by oral gavage for 28 days in a study following a Japanese guideline (Anon., 1991, A6.3.1/01). Animals of the control and high-dose groups were also observed during a 14-day recovery period. Three of the 10 females in the highest dose group (500 mg/kg bw/day) died at day 4 of treatment.

DCOIT administration resulted in clear effects on the gastrointestinal tract, spleen and adrenal cortex. Mucosal hyperplasia was reported in the gastrointestinal tract, and these changes were considered due to healing of mucosal damage caused by irritant action of DCOIT. The changes in the stomach were not restored to normal during the recovery period. Animals of the high dose group showed increases in granulocytes in the spleen and hematological tests revealed changes in neutrophil counts. These changes were considered due to inflammatory changes in the stomach. The adrenals were enlarged due to increased fatty content in the cortex during the treatment period and also following the recovery period.

Most male and female rats given 500 mg/kg bw/day had reduced spontaneous movement, diarrhea from the onset of treatment, salivation, and abdominal distension. In some animals hypothermia, cyanosis, reddish lacrimation and gasping were observed during the administration period. These effects were reversed during the recovery period. Whereas male rats in the high dose group exhibited a decrease in weight gain during the treatment period, females had a slightly increased weight gain during treatment, except for the first week. Water intake was measured after three weeks of treatment. Animals exposed to 100 mg/kg bw/day and above had increased water intake and this tendency persisted during the first week of recovery. At a dose of 100 mg/kg, salivation was observed in a few male and female rats. At a dose of 20 mg/kg bw/day, there were no signs of adverse effects due to the test substance.

Male animals of the medium and high dose groups showed a significant, but slight decrease in relative liver weight. Signs of altered liver function were not paralleled by histopathological changes. Male animals of the high dose group had a significant increases in relative weights of the brain and testes. Animals of both sexes showed a significant increase in relative weight of the adrenals in the high dose group.

Among animals administered 100 mg/kg bw/day or above hematological examination revealed significant changes in hematocrit, hemoglobin, reticulocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, prothrombin time, lymphocyte count, and segmented leucocyte count in comparison with controls. Reticulocyte count, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration did not return to normal during the recovery period.

Animals from the high-dose groups showed significant increases in GOT, GPT and A/G ratio (males only), total cholesterol (females only) and significant decrease in serum glucose and total protein compared to controls. Female animals from 20 mg/kg bw/day had significantly increased inorganic phosphorus and from 100 mg/kg bw/day also a decrease in chloride. Male animals at 500 mg/kg had an increase in GPT and sodium and a decrease in creatinine. Several of these changes persisted throughout the recovery period.

These changes suggest that DCOIT may induce liver toxicity, but histology revealed no liver damage.

After 28 days of treatment a significant decrease in urinary pH, increase in urine volume and increase in urine protein were noted in the 100 and 500 mg/kg bw groups. Decrease in specific gravity and in salt (sodium, potassium and/or chlorine) were noted in the high dose groups. The changes in urinalysis were related to the test substance and considered caused by loss of electrolytes due to salivation and diarrhea as well as increased secretion of adrenocortical hormones. These changes reversed during the recovery period.

Microscopic examination revealed that all three animals dying during treatment had severe necrosis of the mucosa of the non-glandular stomach and atrophy of the thymus and spleen. Surviving animals of the 500 mg/kg bw group had slight thickening of the mucosa of the non-glandular stomach and small and large intestine. The livers appeared small in males of the high dose group and this was diagnosed as atrophy in gross pathology. Microscopic examinations revealed no liver changes. Histopathology at 100 and 500 mg/kg bw showed hyperplasia of the mucosal epithelium of the stomach and small intestine and granulation in the stomach. In addition, increased fat content in the adrenals was observed. Hyperplasia of the stomach and intestine were observed in nearly all animals in the high dose group. Animals at 500 mg/kg bw had an increase in granulocytes in the spleen and an increase of lipids in the adrenals. The changes in the stomach and adrenals were still observed at the end of the recovery period.

From this study a NOAEL of 20 mg/kg bw/day and a LOAEL of 100 mg/kg bw/day was derived.

A 21-day dermal toxicity study in rabbits has been conducted (Anon., 1993-A6.3.2/01). Animals were exposed to 1 and 5 mg/kg bw/day, 5 days a week, of a preformulation, C-9211M, containing 35% DCOIT dissolved in mixed xylenes. The test substance was diluted in acetone and administered in 1 mL/kg bw, thus the concentration of DCOIT would be approximately 0.035 and 0.18 % in the low and high dose group respectively. The application site was unoccluded. In a range-finding test, single doses of 0.05 and 0.5 g/kg bw of the preformulation in acetone or propylene glycol induced severe skin irritation.

This study was conducted prior to the adoption of OECD guideline 410 and there are thus several deviations from current recommendations, e.g. fewer animals per group and only use of 2 treatment groups. The preformulation contained high amounts of impurities. No mortalities and no treatment-related systemic effects were reported. Slight skin irritation was seen in the low dose group, and moderate to severe skin irritation was seen in the high dose group. The skin lesions included

hyperplasia, hyperkeratosis or parakeratosis of epidermis, increased amounts of inflammatory cell infiltration in the dermis, and focal dermal hemorrhage.

Under these test conditions, skin irritation was the only toxic response reported following repeated dermal exposure to the preformulation C-9211M. A LOAEL of 1 mg/kg bw/day of test substance for local effects and a NOAEL of 5 mg/kg bw/day for systemic effects is derived for the preformulation. These values corresponds to approximately 0.35 mg DCOIT/kg bw/day and 1.75 mg DCOIT/kg bw/day, respectively, and the concentrations of DCOIT would be approximately 0.035% and 0.18% in the low and high dose group.

As the dermal surface area exposed in this study was not specified, the mg/cm^2 of skin is not known. However, an assumption of 10 % coverage of the animal body could be made based on guideline recommendations. According to the values described in the TGD (ECB, 2003), the total surface body of a rabbit equals 2600 cm² and the mean body weight is 3 kg. Assuming 10% coverage means that the exposure area will be 260 cm^2. Based on these values, a local LOAEC value of 11.5 µg/cm^2 (for the preformulation) and a value of 4.0 µg/cm^2 for DCOIT can be derived. Extrapolating from a LOAEC to a NOAEC using an assessment factor of 3 leads to a NOAEC of 0.01%, equivalent to a dose of 1.3 µg/cm^2, for local skin irritation.

In a subchronic, 90 days study according to OECD guideline 408, rats were administered DCOIT technical in the diet at concentrations of 0, 100, 500, 1000 and 4000 ppm (Anon., 1994-A6.4.1a/01). No mortalities were reported at any dose levels. Significant reductions in body weight gain, primarily during the early weeks of dosing (week 1-3), were observed in females at 1000 ppm and in both sexes in the high dose group (4000 ppm) animals. Feed consumption was significantly, but transiently reduced (88% of control for the three first weeks) in females in the 1000 ppm group. In the high dose group animals, feed and water consumption were reduced throughout the treatment period, but the effects were most prominent during the first weeks of treatment (feed consumption being 53% of control the first week in females versus 41% in males).

No treatment-related effects on organ weights or on gross pathology were reported. Histopathological findings were limited to the forestomach, and were observed in the two highest dose groups of both sexes (in one male and one female at 1000 ppm and in all animals of both sexes at 4000 ppm). The severity of irritation showed a dose-dependency and ranged from minimal hyperkeratosis and slight epithelial hyperplasia to erosion/ulceration with associated inflammation and oedema of the submucosa.

No effects on urine parameters were reported. There were no treatment-related hematologic effects at doses up to and including 1000 ppm. In the high dose animals mean cell volume and mean cell hemoglobin were significantly reduced. In males these changes were accompanied by significantly increased red blood cell and platelet counts, as well as significantly decreased hemoglobin and hematocrit. Altered red blood cell morphology was observed in several animals. The hematological changes were considered related to an assumed persistent, low level blood loss caused by the gastric lesions observed in these animals.

There were no treatment-related changes in any clinical chemistry parameter at doses up to and including 1000 ppm in males and 500 ppm in females. In females of the 1000 ppm dose group, serum triglyceride levels were significantly reduced. In the high dose group animals of both sexes, serum triglyceride, total protein and globulin levels were significantly decreased, potassium and A/G ratio were significantly elevated. Inorganic phosphorous was elevated in both sexes, however not statistically significantly in females. Additional changes included significantly increased serum GOT activity in males, whereas in females, blood urea nitrogen levels were significantly elevated. Although these latter changes were apparently treatment-related, they were of minimal magnitude.

From this study a NOAEL of 500 ppm (32.5 mg/kg body weight/day in males; 36.7 mg/kg body weight/day in females) was derived. The LOAEL was 1000 ppm (60.7-74.7 mg/kg bw, M – F respectively), based on forestomach irritation, reduced body weight gain and reduced serum triglyceride levels.

In a 90-day dietary toxicity study according to OECD guideline 409, dogs were exposed to concentrations of 100, 300 and 1500 ppm DCOIT technical, offered for 2 hours per day in the morning (Anon., 2002-A6.4.1b/01). No mortalities and no clinical treatment related effects were reported. There were no effects on organ weights and no gross histopathological chances were reported. Body weight and food consumption was decreased compared to control in both sexes at 1500 ppm (significantly only in females). Weekly food consumption values (weeks 1-14) for males and females averaged 12 and 23% respectively lower than control values. For the most part, the sex difference was attributed to one of the female animals. All dogs, but one male exhibiting body weight loss during the 13 week period. There was no evidence of significant systemic toxicity at any dose. The haematological (decreased hemoglobin, hematocrit, red blood cell counts and reticulocytes and increased platelet counts in males at 1500 ppm) and clinical chemistry effects (decreased total protein, albumin and globulin at 1500 ppm) are considered secondary to the decreases seen in body weight of both sexes at 1500 ppm. An increased incidence and severity of thymic atrophy in females at 1500 ppm was considered secondary to decreased body weight and food consumption. Atrophic changes in the thymus has been observed in feed restricted beagle dogs (Takamatsu et al., 2015).

From this study a NO(A)EL of 300 ppm (10.2/10.1 mg/kg bw/day in M/F respectively) and a LO(A)EL of 1500 ppm (47.5/45.9 mg/kg bw/day in M/F respectively) was derived based on decreased body weight and food consumption and changes in some hematologic and clinical chemistry parameters seen at 1500 ppm.

A thirteen-week nose-only inhalation toxicity study according to OECD guideline 413, has been performed in rats with exposure to the antifoulant C-9211M HQ preformulation containing 32.6% DCOIT in o-xylene (Anon., A6.4.3/01 and 02).

Animals were exposed 5 days a week for 6 hours per day, and 6 month and 1 year recovery groups were included in the study. Analytical DCOIT concentrations were 0 (air control), 0 (o-xylene control), 0.02, 0.63 and 6.72 mg/m³. Aerosol particle mean mass diameter was 1.4 µm (GSD = 4.6) and the respirable fraction was 72%.

Four males and 15 females died during treatment, however, 12 of these deaths occurred in the control and vehicle groups and there were no dose-response relationship. Mortalities were attributed to over-restraint in the nose-only tubes and not a result of exposure to o-xylene or the test substance. During recovery, 2-6 deaths/dose group occurred, but these deaths were not considered treatment-related. Rales, gasping, and dyspnea were reported during the thirteen-weeks of dosing, the incidence and severity increasing dose-dependently. No clinical signs of respiratory distress were noted during the recovery period. Exposure to o-xylene resulted in statistically significantly decreased body weight gains when compared to the air control. The body weights of the C9211M HQ dosed rats were compared to the body weights of the o-xylene control rats. The high-dose group males and females showed a statistically significant decrease in body weights during most of the exposure period compared to vehicle controls. No body weight changes were seen during the recovery period.

There was an statistical significant increase in absolute lung weights of the high dose group females. The increase in absolute lung weight was judged to be the result of oedema of the lungs and consistent with a respiratory tract irritant. Histopathological evaluations at the 13-week

necropsy revealed treatment-related observations in the nose, larynx and lungs. The occurrence and severity of these observations were dose-dependent. At the two highest doses, inflammation, epithelial hyperplasia and goblet cell hyperplasia in the nose larynx and lungs were reported. Furthermore, chronic inflammation, hyperplasia, squamous metaplasia in epiglottis with dose-dependence in severity and hyperplasia of the low cuboidal epithelium of vocal folds were seen. In addition to the histological findings in the mid-dose, brightly staining eosinophilic material in the olfactory epithelium of the nose, mild to moderate hyperkeratosis in epiglottis was reported in the high dose group. In lungs, goblet cell hyperplasia in the bronchi and acute inflammation in interstitium and in alveoli to a lesser extent, were reported.

By the six month necropsy, recovery was seen in all tissues and the lungs no longer showed histopathological lesions. There was no evidence of systemic toxicity up to and including the highest dose tested (6.72 mg/m^3).

Haematology and clinical chemistry parameters did not reveal significant treatment related effects at 13 weeks or 6 months. Urinalysis was not conducted. Some effects that were considered non-treatment-related were observed in all groups, including lymphoid infiltration, hemorrhage, arterial mineralisation, histiocytic cell infiltration and pigmented macrophages.

In the range-finding study, o-xylene was found to increase lung weight. Being an irritant, o-xylene may contribute to the effect of DCOIT on respiratory irritation. The level of o-xylene in the vehicle control group was lower than the level in the highest exposure group so that the possible contribution of o-xylene to the respiratory irritation observed in high dose animals is not properly addressed.

From this study a LO(A)EC of 0.63 mg DCOIT/m^3 was derived based on the histopathological changes seen in the nose and larynx. The NO(A)EC was 0.02 mg DCOIT/m^3. The NOAEC is considered conservative due to the additional toxicity of xylene and because the spacing of the two lower doses (0.02 and 0.63 mg/m^3) was large. In order to calculate a more realistic NOAEC for inhalation, the LOAEC value (0.63 mg/m^3) was used with an assessment factor of 3 to extrapolate from the LOAEC to a modified NOAEC of 0.21 mg/m^3.

Summary and discussion of repeated dose toxicity (Dow).

DCOIT technical was administered orally by gavage for 1 month in the rat and by diet for 3 months in the rat and dog. In the rat DCOIT technical was administered for 28 days with doses up to 500 mg/kg bw/day and for 90 days with doses up to 4000 ppm (248-278 mg/kg bw/day). Doses as high as the “limit dose” (1000 mg/kg/day) were not administered and severe toxicity was reported in range-finding study with 10 000 and 20 000 ppm. In the dog study, DCOIT technical was administered for 90 days with doses up to ca. 50 mg/kg bw/day.

From the 28 day rat oral gavage study a NOAEL of 20 mg/kg bw/day and a LOAEL of 100 mg/kg bw/day was derived. In the 90-day study with rats, ingestion of DCOIT technical produced dose-dependently increasing irritation of forestomach ranging from minimal irritation to erosion and ulceration. Food and water consumption and body weight gain was significantly decreased in the high-dose group. At this dose, significant changes also in certain parameters of haematology and clinical chemistry were observed. LOAEL is 1000 ppm (60.7 and 74.7 mg/kg/day in males and females, respectively). NOAEL is 500 ppm (32.5 and 36.7 mg/kg/day in males and females). Corresponding effects in the gastrointestinal tract were recorded in the 28-day study with rats.

In dogs reduced body weights and food consumption compared to control (significantly reduction only in females), thymic atrophy (considered secondary to decreased body weight and food consumption) as well as changes in some haematological and clinical chemistry parameters were

observed at 1500 ppm (47.5/45.9 mg/kg bw/day in M/F respectively). A NO(A)EL of 300 ppm (10.2/10.1 mg/kg bw/day in M/F respectively) was derived from the study.

In the dermal exposure study rabbits were exposed for 21 days to the preformulation C-9211M containing 35% DCOIT in xylene at doses up to 1.75 mg a.i./kg bw/day. Mild to moderate local irritation on the application site was reported, and was verified by histological findings. The NOAEL for systemic toxicity in this study was the highest dose tested, i.e. 1.75 mg a.i./kg bw/day. Local effects were observed at 0.35 mg a.i./kg bw/day (0.035%) and 1.75 mg a.i./kg bw/day (0.18%). A local LOAEC value of 4.0 µg/cm^2 for DCOIT can be derived based on this study using the assumptions of 10 % coverage of the animal body (guideline recommendations) and TGD defaults on dermal surface area and body weight. Extrapolating from a LOAEC to a NOAEC using an assessment factor of 3 leads to a NOAEC of 0.01%, equivalent to a dose of 1.3 µg/cm^2 for local skin irritation.

Rats received nose-only inhalation exposure of DCOIT (aerosol, 32.6% DCOIT in o-xylene) for 6 hours per day, 5 days per week for 13 weeks at concentrations up to 6.72 mg a.i/m^3.

Dose-dependent histological changes related to respiratory irritation (chronic inflammation, epithelial hyperplasia, metaplasia, hyperkeratosis), was reported. These lesions were improved to some extent during the one year recovery period. No clear dose-response in the deaths occurred during the exposure period. These may be related to the exposure technique rather than the exposure itself. Based on histological changes in the respiratory organs, a LOAEC of 0.63 mg ai/m^3 and a NOAEC of 0.02 mg ai/m^3 was derived. The NOAEC is considered conservative due to the additional toxicity of xylene and because the spacing of the two lower doses was large. A modified NOAEC of 0.21 mg/m^3 has been calculated extrapolate from the LOAEC using an assessment factor of 3.

The reason for not using higher doses was probably DCOITs irritating effect on the respiratory tract which is likely aggravated by the presence of o-xylene. Higher doses would have been desirable to address potential systemic inhalational toxicity of DCOIT.

An oral NOAEL of 10 mg/kg bw/day from the 90-day dog study was the lowest systemic NOAEL observed in the repeated dose studies. Based on the dermal study with exposure to the preformulation C-9211M a systemic NOAEL 2 mg/kg bw/day (5 mg/kg bw/day of the preformulation) was derived, the highest dose administered. From the rat inhalation, study with exposure to the preformulation C-9211M HQ a NOAEL of 0.02 mg DCOIT/m^3 was derived based on the histopathological changes seen in the nose and larynx at higher exposures.

For the most part, toxicity was observed at the site of dosing (i.e., histopathology of stomach and lower intestinal track). Minimal systemic toxicity was observed.

Chronic toxicity of DCOIT has not been tested and waving of this study was based on the toxic profile of DCOIT seen in the subchronic studies and comparison with structurally related isothiazolinones. The waving of the chronic studies and the argumentation for not performing such a study is further presented in the “Carcinogenesis” chapter.

Applicant 2, Thor:

Table 15: Summary table of relevant repeated dose toxicity studies

Route Method	duration of study	Species Strain Sex no./group	dose levels frequency of application	Results	LO(A)EL	NO(A)EL	Reference (TNsG IUCLID 5)
ORAL STUDIES							
Oral gavage[k] OECD 408	90 days, 28 day recovery groups for control and high dose included	Rat Wistar 10/sex/ group	0, 35, 70, 105 mg/kg bw /day DCOIT Technical (97.4 % DCOIT) Vehicle (peanut oil)	medium and high dose males: a significant reduction (ap. 10%) in final body weight compared to control (ap. 15% reduction in body weight gain during the treatment period). Females were not affected at the mid dose, but was significantly reduced at the high dose. 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	35 mg/kg bw/day	Anon., 2002 (A 6.4.1-01 7.5.1-01)
Oral (*via* diet) [k] OECD 409	90 days	Dog Beagle 4 sex/dose	0, 2, 5, 27, 61, 88 mg/kg bw/day (males); 0, 2, 6, 35, 61, 74 mg/kg bw/day (females) DCOIT Technical (97.1% DCOIT)	at the two highest dose levels palatability problems with food scatter and vomiting, causing reduced body weights (dramatically loss in females), changes in organ weights (thymus, thyroid, epididymides, heart, liver and prostate), effects on blood chemistry parameters and: increase in circulating liver-specific transaminases at two highest doses (not correlated to weight loss). No morphologic abnormalities of the liver.	61 mg/kg bw/day (males and females)	27 / 35 mg/kg bw/day (males/ females)	Anon., 2007b (A 6.4.1-02 7.5.1-02)
DERMAL STUDIES							
Dermal[k] OECD 410	28 days	Rat Wistar 20 sex/dose for control and high dose, 10/sex for low and intermediate dose	0, 3, 15, 60/30 mg /kg bw/day DCOIT Technical (96.47% DCOIT) Vehicle: corn oil	high dose (60 mg/kg/d during 8 days, discontinued until day 21, re-administration of 30 mg/kg/d): severe skin reaction (open wounds, oedema, erythema) leading to mortality, significantly reduced body weights, changes in blood biochemistry; local reaction at 15 mg/kg bw/day	15 mg/kg bw/day	3 mg/kg bw/day	Anon., 2007a (A 6.3.2-01 7.5.2-01)

k = key study

The repeated dose toxicity of DCOIT was evaluated in a 90 day oral toxicity study in rats according to OECD guideline 408 (Anon., 2002, A 6.4.1-01). Wistar rats (10 male and 10 female per group), were daily treated with DCOIT at three dose levels (35 (low dose), 70 (mid dose) and 105 (high dose) mg/kg bw/day) by oral gavage for a period of 90 days. In addition a high dose recovery group and a control recovery group were included to investigate the persistence, recovery or delayed effects of the test substance. Also, a 28-day dose selection pre-study was included. A number of parameters were observed, including mortality, clinical signs, body weight development, food consumptions, clinical biochemistry and neurobehavioral-parameters. In addition, sperm parameters were evaluated in all males.

No mortalities were observed besides one animal in the highest dose group (considered to be incidental). Clinical signs were limited to observations of mild symptoms. No effects on parameters of the functional observation battery for neurobehavorial assessment, ophthalmoscopic parameters, clinical chemistry, haematology or treatment-relatable macroscopic/microscopic abnormalities were identified. Body weight was reduced in males of the mid- and high dose compared to control (significantly in week 6-9 and 11-13 for both doses as well as in week 2 for the high dose) and in females receiving the high dose (significantly reduced compared to control in week 13 only in the normal high dose group and in week 3-17 in the recovery group). Relative organ weights of testes and adrenals were significantly increased and testicular sperm head count was significantly reduced in males in mid- and high dose group at the end of the 90 days treatment period (effects not present after a 28-day recovery period).

The reduced body weight was associated with reductions in feed consumption during the exposure period, although the reduced intake was only significantly different from controls at some time points. Local irritation of the gastrointestinal tract may have contributed to the reduced food intake and body weight gain. The effect on sperm mobility and sperm head count observed at the two highest doses may be related to increased stress due to the local irritation and reduced food consumption. These effects were shown to be reversible. There were no significant effects on absolute organ weights. In males, significant increases in relative adrenal and testes weight were observed at the two highest dose groups. These effect were reversible and are considered related to reductions in terminal body weight and may also be influenced by animal stress, but not associated with changes in gross or histopathological observations.

As a conservative assumption, the NOAEL was set to 35 mg Acticide DCOIT/kg bw.

The repeated dose toxicity of Acticide DCOIT was evaluated in a 90 day oral toxicity study in dogs according to OECD guideline 409 and EU Method B.27 (Anon., 2007b; A 6.4.1-02, 7.5.1-02). Beagle dogs, 24 male and 24 female (4 male and 4 female per group), were daily treated with Acticide DCOIT at five dose levels (100, 300, 1500, 3000 and 4500 ppm corresponding to 0, 2, 5, 27, 61, 88 mg/kg bw (males) and 0, 2, 6, 35, 61, 74 mg/kg bw (females)) incorporated in diet for a period of 90 days. The study was conducted in two phases: i) animals were treated with 100 – 1500 ppm Acticide DCOIT in the diet and ii) animals were treated with 3000 and 4500 ppm Acticide DCOIT in the diet. Also, a 28-day dose selection pre-study was included. A number of parameters were evaluated: clinical signs, body weight development, food consumptions, ophthalmoscope examination, clinical chemistry and pathology, organ weights and histopathology.

In the first phase of the study, no adverse effects were observed. At 1500 ppm treatment there was a slight decrease in body weight compared to controls in females. Lower kidney weights of males at 100 ppm dose group were observed, but the effect was not dose-related.

Therefore, two additional dose groups (3000 and 4500 ppm in diet) were added in the second phase. No substance-related mortalities were observed, but one animal was killed moribund. Clinical signs

(lean appearance) were observed in all animals of the high (4500 ppm) dose group and in two animal of the low (3000 ppm) dose group, two females at 1500 ppm and one male at 100 ppm.. Vomiting occurred in some animals, but was not dose-related. A dramatically decreases in body weight in females at the two highest dose groups (3000 and 4500 ppm; 7% at the end of the study compared to day 1 for 3000 ppm, versus 26% on day 22 compared to day 1 for 4500 ppm) was observed, and body weight gain was statistically significantly reduced during the major part of the study in these groups. Food consumption was initially reduced in all animals of the two highest dose groups and remained reduced in females and one male of the highest dose groups, while food consumption of females at 3000 ppm and the rest of the males normalized to approximately control levels throughout the study. Average food intake of females at 4500 ppm was reduced with approximately 40% compared to control group. Reduced food consumption at the two highest doses may be due to reduced palatability and local irritation in the stomach.

Effects on clinical chemistry parameters were observed in the highest dose level groups (reduced cholesterol, phospholipids, total protein, albumin, globulin, and calcium levels, and increased AST, ALT and GLT-activities) as well as statistical significant reduced absolute thymus (in females also relative), thyroid, epididymides, heart, liver, and prostate weights (primarily at the highest dose). Some of the effects on clinical chemistry parameters were also observed at 1500 ppm. Most of the changes were considered to be secondary to the poor nutritional state resulting from low palatability of the test substance.

The increase in circulating transaminase activities were seen predominantly in males of the two highest dose groups. This effect was not accompanied by morphologic abnormalities of the liver. Although liver damage was not observed in histopathology, this finding is considered treatment-related.

In conclusion, the NOAEL was set to 27/35 mg/kg bw/day (LOAEL 61 mg/kg bw/day).

The repeated dose dermal toxicity of Acticide DCOIT was evaluated in rats according to EU method B.9 and OECD guideline 410 (Anon., 2007a; A 7.5.2-01). Wistar rats, 60 males and 60 females (10 males and 10 females per group for low and intermediate dose groups, 20 males and 20 females per group for control and high dose groups), were daily treated with Acticide DCOIT in corn oil (specific gravity 0.92) at three dose levels (3, 15 and 60/30 mg/kg bw/day) on an exposed area of 10% body surface (intact skin) for 6 hours/day over a period of 28 days under occlusive dressing. After daily exposure, test substance was removed with water. Numbers of parameters were evaluated: clinical signs, body weight, food consumptions, ophthalmoscopy, neurobehavioral abnormalities, clinical chemistry and pathology, organ weights and histopathology.

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. Due to severe skin reactions, treatment of the high dose group was discontinued from day 9 to day 21. At day 22, treatment of this group was re-started with reduced dose and again discontinued after day 23 due to severe skin reactions. Due to the intermittent treatment, animals of the high dose group were necropsied without recovery period. Mortality at 60/30 mg/kg/day was 6 males and 8 females (one found dead on day 7, 13 killed in extremis on day 9). At 15 mg/kg/day, 1 male and 1 female was found dead. At 3 mg/kg/day, no mortality was observed. No dose-related effects on food intake. Body weight was significantly reduced in males in the high and mid-dose group compared to the control. Hematological parameters were affected in the high-dose group (reduced blood red blood cell count, hemoglobin and hematocrit, increased eosinophil granulocyte count and red blood cell distribution volume). Spleen- and adrenal-to-body weight-ratio were slightly

elevated. All observed effects are considered to be secondary to immunological response and stress due to the large corroded and inflamed area of the skin with open wounds[1].

In conclusion, the NOAEL for systemic effects was set to 3 mg/kg bw/day (LOAEL 15 mg/kg bw/day).

Summary and discussion of repeated dose toxicity (applicant 2 Thor).

The repeated dose toxicity of DCOIT was evaluated in a 90 day oral toxicity study in rats according to OECD guideline 408 (Anon., 2002 A6.4.1-01, 7.5.1-01). Body weight was reduced in males of the mid- and high dose and in females receiving the high dose. These effects were associated with reductions in food consumption. Relative, but not absolute, organ weights of testes and adrenals were significantly, but reversibly increased in males in mid and high dose group. The effects were considered related to reductions in terminal body weight and may also be influenced by animal stress. They were not associated with changes in gross or histopathological observations. Testicular sperm head count was significantly reduced in males of the mid- and high dose groups, but these effects were reversible within 28 days. The effect on sperm head count may be related to increased stress due to local irritation and reduced food consumption.

In a 90 day oral toxicity study in dogs reduced body weights, changes in organ weights and blood chemistry parameters and an increase in circulating liver-specific transaminases were observed at the two highest doses. Most of the changes were considered to be secondary to a poor nutritional state resulting from low palatability of the test substance.

In a dermal toxicity study on rats 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. All observed systemic effects were considered to be secondary to immunological responses and stress due to the large corroded and inflamed area of the skin with open wounds[2].

4.10.1 Comparison with criteria

4.10.2 Conclusions on classification and labelling

To get a systematic overview on the information on DCOIT relevant for the STOT RE classification, a table with repeated dose study-specific cut-off levels and the most critical/severe effects at LOAEL is presented.

From the general toxicity data recorded in the two fertility studies described in section 4.13.1 (Effects on fertility) some information on repeated dose toxicity following dietary DCOIT

[1] Increased eosinophil granulocyte count is indicative of inflammation, and reduced blood red blood cell count, haemoglobin and hematocrit are symptoms of blood loss through wounds; Reduced body weight is considered a consequence of reduced food consumption due to stress because of the large corroded area of skin.

administration can be obtained. In the parent generations (F0), reduced food consumption and reduced body weight gain was observed at the high doses of both studies in males and/or females during a 10 week premating exposure. The doses ranged from 200 – 3200 ppm (approximately 16 – 235 mg/kg/day in the Dow Crl:CD® BR (Sprague-Dawley) rat study and from 46 – 651 ppm (corrected values; approximately 3-57 mg/kg bw/day) in the Thor Wistar rat study. Males were sacrificed at the end of the 10 week premating period and females after weaning. Considering both parental generations together (F0 and F1), reductions in body weights compared to controls were observed from doses of approximately 60 mg/kg bw/day in both studies. There were no treatment-related alterations in the pathology of any of the reproductive organs of the parent generations. Some effects were observed on absolute or relative organ weights at the highest doses and these were considered related to reduced body weights and not to organ toxicity. Treatment-related abnormalities (including hyperplasias) of the forestomach were reported for several animals at the higher doses.

Taking all studies together, toxicity seems related to local toxicity and/or to reduced feed consumption. The systemic toxicity observed may in most cases be secondary to reduced body weight/feed consumption and/or local irritation.

As for the local toxicity, the local irritation effect of DCOIT seems to depend on concentration rather than on exposure duration as discussed in section 4.4 (Skin irritation/Corrosivity). Signs of irritation at site of first contact were also observed following acute exposures by all three exposure routes. The doses used in the acute inhalation studies were much higher than in the 90 day RTD-study so a comparison of doses and concentrations is not feasible for this exposure route. The mouse oral acute study provided by Applicant 1 and the rat oral acute study provided by Applicant 2 included doses and concentrations in vehicle close to those used in the oral RTD-studies. A comparison of local toxicity between the acute and repeated dose toxicity studies show that local toxicity is observed at similar concentrations, although at higher total doses in the acute than in repeated studies. Thus it is reasonable to consider the local toxicity as acute toxicity.

In conclusion no classification for Specific Target Organ Toxicity after repeated exposure (STOT RE) is warranted.

Table 16: Summary of repeated dose toxicity studies and comparison with STOT RE criteria

	STOT RE 1	STOT RE 2	NOAEL and LOAEL	Significant/severe effects* at LOAEL
ORAL STUDIES				
STUDIES IN RATS				
Rat 4 wk (gavage) DOW	30	300	NOAEL 20 mg/kg bw/day LOAEL 100 mg/kg bw/day	Statistical significant, but slight decrease in relative liver weight in males at 100 mg/kg bw (body weight unchanged compared to control). Changes in clinical chemistry and haematology parameters (slight, but significant). Microscopic histopathological examination revealed slight changes in the stomach and small intestine and increased fat content in the adrenals. (*Atrophy in liver at 500 mg/kg bw of five male animals. Signs of altered liver function were not paralleled by histopathological changes).*
Rat, 3 mth (diet) DOW	10	100	NOAEL 500 ppm (32.5 (♂) - 36.7 (♀) mg/kg) LOAEL 1000 ppm (60.7 (♂) - 74.7 (♀) mg/kg bw day)	No significant/severe effects at LOAEL. (No treatment-related effects on organ weights or on gross pathology. Statistical significant reduced body weight gain and serum triglyceride levels. Minimal histopathologic changes of the forestomach).
Rat, 90 d (gavage) THOR	10	100	NOAEL 35 mg/kg bw/day LOAEL 70 mg/kg bw	Relative, but not absolute, organ weights of testes and adrenals significantly increased (bw reduced compared to control) and testicular sperm head count significantly reduced in males at the end of the treatment period. No morphological abnormalities, alterations completely absent after 28 days recovery period.
STUDIES IN DOGS				
Dog, 3 mth (diet) DOW	10	100	NOAEL 10.2 – 10.1 mg/kg bw/day LOAEL 47.5 - 45.9 mg/kg bw/day	No evidence of significant systemic toxicity at any dose. (Reduced food consumption and body weight loss, increased incidence and severity of thymus atrophy, changes in some hematologic and clinical chemistry parameters (considered secondary to the decrease in bw)).
Dog, 90 d (diet)	10	100	NOAEL 27/35 mg/kg bw/day (males/ females)	Reduced body weight, reduced absolute thymus (in females also relative) and epididymides weight*, effects on blood

	STOT RE 1	STOT RE 2	NOAEL and LOAEL	Significant/severe effects* at LOAEL
THOR			LOAEL 61 mg/kg bw/day (males and females)	chemistry parameters and increase in circulating liver specific transaminases. No histopathological evidence of organ dysfunction. Most of the changes considered to be secondary to a poor nutritional state. *(* At the topdose, 88/74 mg/kg bw day (M/F)),reduced absolute thyroid, heart, liver and prostate weight were observed in addition to effects on thymus and epidymides.)*
DERMAL STUDIES				
Rabbit, 21 d DOW	86	860	NOAEL > 1.75 mg/kg bw/day (top dose)	No systemic effects *(No guideline study, Test material: DCOIT in xylene)*
Rat, 28 d THOR	60	600	NOAEL 3 mg/kg bw/day LOAEL 15 mg/kg bw/day	All observed effects considered to be secondary to severe dermal irritation. *(Top dose: 30/60 mg/kg)*
INHALATIONAL STUDIES				
Rat, 3 mth (nose only)	0.02 (dust, mist, fume)	0.2 (dust, mist, fume)	NOAEL 0.02 mg a.i./m^3 LOAEL 0.63 mg a.i./m^3,	There was no evidence of systemic toxicity at any dose. Histopathological evaluations at the 3 month necropsy revealed treatment-related observations in the nose, larynx and lungs (0.63 and 6.72 mg/m^3). By the six month necropsy, recovery was seen in all tissues and the lungs no longer showed signs of histopathological lesions. *(Test material: DCOIT in xylene)*

** STOT-RE is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature which significantly impact on health. both factors have to be evaluated by weight of evidence and expert judgement.*

4.11 Germ cell mutagenicity (Mutagenicity)

Applicant 1, Dow:

Table 17: *In vitro* genotoxicity studies (excluding non-key studies)

Test system Method Guideline	**organism/ strain(s)**	**concentrations tested**	**Result**		**Remark**	**Reference (Doc III-A cross-ref)**
			+ S9	**- S9**		
Bacterial Gene Mutation Assay. OECD 471	S. typhimurium, TA 1535, TA 1537, TA 98, TA 100	0.3 to 300 µg a.i./plate DCOIT Technical	not mutagenic	not mutagenic	Low doses of active ingredient. Cytotoxicity was observed from 100 µg/plate (+ metabolic activation) and from 3-10 µg/plate (- metabolic activation)	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 NNOMA	not mutagenic	not mutagenic	-	Anon., 2005 (A6.6.1/02)
Chromosome aberration test in Chinese hamster ovary (CHO) cells. OECD 473	Chinese hamster ovary (CHO) cells	0.1 to 0.7 µg/ml without activation and 3.0 -8.0 µg/ml with metabolic activation DCOIT Technical	not mutagenic	not mutagenic	In the range finding study cytotoxicity observed at 0.25-0.6 µg/ml without activation and 5-10 µg/ml with activation. This leads to the low concentration chosen in the actual study.	Anon., 1994 (A6.6.2/01)
Gene mutation assay. OECD 476	Chinese hamster Ovary (CHO), CHO-K1-BH4	0.005 to 0.75 µg/ml (without activation), 0.5 to 25 µg/ml (with activation) DCOIT Technical	not mutagenic	not mutagenic	Cytotoxicity observed at 0.75 µg/ml without activation and 15 µg/ml with activation	Anon., 1994 (A6.6.3/01)

Table 18: *In vivo* genotoxicity studies (excluding non-key studies)

Type of test Method/ Guideline	**Species Strain Sex no/ group**	**frequency of applica-tion**	**samp-ling times**	**dose levels**	**Results**	**Remarks**	**Reference (Doc III-A cross-ref)**
Micronucleus in mouse bone marrow cells. OECD 474	Mice, CD-1, male and female, 5-9/sex/ group	one oral gavage	24 and 48 hr	60, 300, 600 mg ai/kg bw DCOIT Technical	not mutagenic	The dose level were selected according to a previous study were they found that a dose of 600 mg/kg bw induced toxicity and mortality both in females and males.	Anon., 2001 (A6.6.4/01)

DCOIT Technical was evaluated for its mutagenic potential in four independent studies in *Salmonella typhimurium* TA98, TA100, TA 1535 and TA 1537, with and without (metabolic) activation. The two oldest studies (Anon., A6.6.1/03 and 04) were conducted before guidelines were available and are supportive (non key) studies. A third study (Anon., A6.6.1/05) was performed in accordance with the guideline issued by the Japanese Ministry of International Trade and Industry (1986) and gives additional information in comparison to the key study (Anon., 1994, A6.6.1/01, performed in compliance with OECD guideline 471) as this test was conducted on both *Salmonella typhimurium* and *Escherichia coli*. However the solutions were not submitted for chemical analysis of the test article concentration. Concentrations used in the key study were low compared to what is recommended in current test guidelines.

In the key study, DCOIT did not induce an increase in revertants at doses from 0.3 to 300 µg/plate when compared to solvent controls. Toxicity was observed in all strains with metabolic activation at 100 µg/plate and greater, and without metabolic activation at 10 µg/plate and greater with the exception of TA1537 which was also toxic at 3 µg/plate.

The results of the four gene mutation assays were negative. However it is questionable if the negative findings in the two oldest studies are reliable as there is a substantial lack of information in the reports.

The substance was negative in an *in vitro* cytogenic study in mammalian cells (OECD guideline 473, chromosome aberration test with Chinese Hamster Ovary cells (CHO cells)) with and without exogenous metabolic activation (Anon., 1994, A6.6.2/01). However, the amounts used in this study were low (maximum 8 µg/mL). The result was supported by an older study (Doc III-A6.6.2/02, non-key study) with antifoulant preformulation, C-9211M (DCOIT diluted in xylene at about 40 %). The study complied with the requirement of OECD guidelines 473 except for dose selection and mitotic index estimation.

Three studies were carried out to evaluate DCOIT for its ability to induce gene mutation in mammalian cells. The oldest study (Anon., A6.6.3/02, non-key study) was conducted with antifoulant preformulation, C-9211M (ca 35% DCOIT in xylene) on mouse embryo fibroblast cells.

No guidelines were available at the time the study was conducted. The two other studies evaluate the ability of DCOIT to induce gene mutation at the HGPRT locus in cultured CHO cells with and without exogenous metabolic activation system. Study A6.6.3/03 (non-key study) is compliant with OECD guideline 476 but was conducted with C-9211M (ca. 40 % DCOIT in xylene) as the test material. The study is not considered as a key study as the concentrations at dosing were not corrected for DCOIT content and no analyses for DCOIT were performed on dilutions prepared for the assay. The more recent study referenced (Anon., A6.6.3/01), was conducted with DCOIT Technical as the test material. It is fully compliant with OECD guidelines 476 and has been selected as the key study. In the range finding study concentrations as high as 5000 µg/mL were used and in the main study up to 25 µg/ml. The results of all three tests were negative.

DCOIT was evaluated for its potential to induce chromosomal damage *in vivo*, as assessed by micronucleus assay with mouse bone marrow cells. Two micronucleus assays in CD-1 bone marrow in mice conducted using DCOIT Technical as the test material were negative. The oldest assay (Anon., A6.6.4/02, non-key study) is considered as a supportive study as the maximum tolerable dose was not established and the highest tested dose (325 mg DCOIT/kg bw) did not show signs of cytotoxicity (no statistically significant decrease in the polychromatic/normochromatic ratio). The study was repeated using higher doses of DCOIT Technical i.e., up to 600 mg/kg bw. This second study (Anon., A6.6.4/01) is fully compliant with OECD guidelines 474 and is considered as the key study. The substance was administered orally as a single dose.

In addition an Ames test was conducted on N-(n-Octyl) malonamic acid, one of the major metabolites of DCOIT identified in the metabolism studies (See Toxicokinetic chapter). The result was negative.

The submitted genotoxicity studies on DCOIT were conducted over the period 1981 to 2001. Different vehicles/solvents were used in the studies for the substance: 2 (the in-vivo studies) utilised corn oil, 3 used acetone and the other 6 DMSO. None of the reports provided a rationale for the selection of the vehicle, except the fact that DCOIT has limited water solubility (3.47 mg/l at pH 7 and 20°C to 6.68 mg/l at pH 5 and 30°C). Because of this limited water solubility and in order to conduct a scientifically valid study, an organic solvent compatible with the aqueous environment of the assay had to be chosen. All of the vehicles used in these assays are commonly used in genotoxicity studies and are not expected to have prejudiced their outcome.

Summary of genotoxicity (Dow)

DCOIT Technical produced no evidence of genotoxicity when tested in a battery of *in vitro* and *in vivo* tests. The DCOIT major metabolite, N-(n-octyl) malonamic acid (NNOMA) was not mutagenic when tested in a Bacterial Gene Mutation Assay test.

Applicant 2, Thor:

Table 19: In vitro genotoxicity studies

Test system Method Guideline	**organism/ strain(s)**	**concentra-tions tested**	**Result** + S9	- S9	**Remark**	**Reference (TNsG IUCLID 5)**
Bacterial gene mutation OECD 471[k]	*S. typhimurium* TA 1535, TA 1537, TA 98, TA 100, TA 102	0.06-1.0 µg/ plate (without S-9); 0.5- 10 µg/ plate (with S-9)	negative	negative	cytotoxicity: ≥ 5 µg/ plate (without S-9), ≥ 50 µg/ plate (with S-9)	Anon., 2000 (A 6.6.1-01 7.6.1-01)
Cytogenetic test OECD 473[k]	Human primary lymphocytes	0.09-1.5 µg/mL (with and without S-9); Solvent (acetone (1%))	negative	negative	cytotoxicity at ≥ 1.5 µg/mL	Anon., 2001 (A 6.6.2-01 7.6.1-02)
Gene mutation in mammalian cells OECD 476[k]	Chinese hamster V79 cells	0.03-0.2 µg/ mL (without S-9); 2.5-20 µg/mL (with S-9); Solvent (DMSO (0.5%)	negative	negative	cytotoxicity at lower than 0.16µg/mL (without S-9), ≥20 µg/mL (with S-9)	Anon., 2002 (A 6.6.3-01 7.6.1-03)

k = key study

DCOIT was evaluated for its mutagenic potential in an Ames test according to OECD guideline 471 (Anon., 2000. A 6.6.1-01, 7.6.1-01). Five histidine-dependent, Salmonella typhimurium strains (TA98, TA100, TA 1535 and TA 1537) were used with and without metabolic activation with rat liver S9 mix, and the numbers of surviving colonies (histidine-revertants) were counted. Cytotoxicity in all five Salmonella typhimurium strains with or without metabolic activation was evaluated. Cytotoxicity was observed in all strains with metabolic activation at 50 µg/plate and greater, and without metabolic activation at 5 µg/plate and greater. A statistical significant increase in number of revertants was observed in two stains (one with and one without metabolic activation, 1.44 fold and 1.05 fold increase, respectively). However, the observed increases did not meet the evaluation criteria for a positive outcome – at least at one concentration ≥ 2-fold signal of solvent control. DCOIT was non-mutagenic in all five tester strains of S. typhimurium (TA1537, TA1535, TA98, TA100 and TA102) under these experimental conditions.

The genotoxic potential of DCOIT was investigated in a mammalian cell chromosome aberration test according to OECD guideline 473 (Anon., 2001, A 6.6.2-01, 7.6.1-02). Human primary lymphocytes were treated with DCOIT for 6h or 30h – 36h with/without a metabolic activation system (rat liver S-9 mix) in order to evaluate the genotoxic potential of DCOIT *in vitro*. Five dose levels were used. Colchecine was used as metaphase arresting substance. The study was divided in three phases – in phase 1, cells were exposed for 6 h both without and with 5 % metabolic activation system; in phase 2, cells were exposed for 30 – 36 h without metabolic activation system and in phase 3, cells were exposed for 6 h with 15% metabolic activation system. Positive controls

for mitomycin C (with S-9) and cyclophosphamide (without S-9) were used. Before the chromosomal aberration study, the cytotoxicity was evaluated based on the mitotic index, both with and without metabolic activation with S-9. For the chromosomal aberration study, cells were microscopically examined after fixation and staining. In phase 1, there was a significant increase in the mitotic index at a dose level of 0.09 µg/ml of culture with S-9, and no treatment-related chromosome aberrations were observed. In phase 2, there was a significant decrease in the mitotic index at dose levels of 0.09 and 1.5 µg/ml of culture, and also, there were no treatment-related chromosome aberrations. The observed changes in the mitotic index were not dose-related. In phase 3, there were no significant changes in the mitotic index, and the absence of chromosomal aberrations in presence of 5% metabolic activation system (6 h) was reconfirmed with 15% metabolic activation system (6 h). The positive control group showed an increase in the frequency of aberrant cells and this indicated that the study was valid.

DCOIT was found to be non -mutagenic to human lymphocytes, with and without a metabolic activation system under these experimental conditions.

The genotoxic potential of DCOIT was investigated in a mammalian cell gene mutation study according to the OECD guideline 476 (Anon., 2002, A 6.6.3-01, 7.6.1-03). The study was performed in two independent experiments. Chinese hamster lung fibroblasts (V79) were treated with the test substance for 4 h in the first experiment with (2.5 – 20.0 µg DCOIT/ml) and without (0.05 – 0.15 µg DCOIT/ml) a metabolic activation system. In the second experiment, cells were treated with the test substance (0.0033 – 0.20 µg DCOIT/ml) for 24 h in the absence of a metabolic activation. In both experiments, the cells were cultured for one week after the substance treatment period for (potential) genotoxicity-induced depletion of hypoxanthine-guanine phosphoribosyl transferase (HPRT). Afterwards, cells were incubated for eight days with 6 -thioguanine for elimination of non-mutated cells, while the mutated cells were not affected due to lack of HPRT. Mutated cells were allowed to form colonies, and the numbers of colonies representing mutated cells were counted. Positive controls dimethylbenz[a]anthracene (with S-9) and ethylmethanesulphonate (without S-9) were used. DCOIkT was very toxic to the cells, especially in the absence of a metabolic activation system. Up to the highest investigated concentration with and without metabolic activation, no relevant increase in the number of mutant colonies was observed in both independent experiments. The positive controls showed a distinct increase in induced mutant colonies and this indicated that the study was valid. DCOIT was considered to be non-mutagenic to V79 Chinese hamster lung fibroblasts under these experimental conditions.

No mutagenicity was seen in an Ames test in absence or presence of S9 using different strains of *S. typhimurium*. DCOIT-treatment did not induce chromosome aberrations in human lymphocytes, and no mammalian cell gene mutation in the HPRT-test in V79 Chinese hamster lung fibroblast cells was observed. It was noted that DCOIT had relatively high cytotoxicity, especially in the mammalian cells.

Table 20: In vivo genotoxicity studies

Type of test Method/ Guideline	Species Strain Sex no/group	frequency of application	sampling times	dose levels	Results	Remarks	Reference (TNsG IUCLID 5)
Mouse bone marrow cytogenetics test OECD 475[k]	Mouse Swiss 5 males + 5 females/ group	two peroral applications (24 h between applications)	24 hours after last treatment	0,100,200, 400 mg/kg bw Vehicle (peanut oil)	negative (no increase in chromosome aberrations in bone marrow cells)	Pretest: dose-dependent toxicity, 50% deaths between 750 and 1500 mg/kg bw	Anon., , 2001 (A 6.6.4-01 7.6.2-01)
UDS test in rat hepato-cytes OECD 486	Rat Wistar 4 males/group	Single oral application	2 and 16 hours	0, 1000, 2000 mg/kg bw Vehicle (corn oil)	negative (no increase in net nuclear grain counts)	clinical signs but no mortality in all treated animals	Anon., 2002 (A 6.6.5-01 7.6.2-02)

k = key study

The genotoxic potential of Acticide DCOIT was evaluated in a mouse bone marrow chromosome aberration study according to OECD guideline 475 (Anon., 2001; A 6.6.4-01, 7.6.2-01). Fifty (25 Male and 25 female) healthy, Swiss albino mice (5 males and 5 females per group) were treated with DCOIT by oral gavage on two consecutive days at dose levels of 100, 200 and 400 mg/kg bw, respectively. The DCOIT doses were selected based on dose-range finding study results. The control group received peanut oil and the positive control group received Mitomycin C (4 mg/kg bw). Colchecine injections were given 3h prior to animal scarification to arrest cell cycle in metaphase, and bone marrow cells were isolated. The percentage of aberrant cells was determined and the data on body weight, mitotic index and percent aberrant cells were analysed. No significant change in body weight was observed in mice treated with DCOIT at various dose levels when compared with the control group, except in high dose group. A significant reduction in body weight in the high dose group animals (400 mg/kg bw of DCOIT) was observed. Only mild clinical signs (piloerection, polyurea) were observed. The percentage of chromosomal aberrations from DCOIT treated groups was not significantly different from the control group. Some non significant aberrations including chromatid breaks, fragments and ploidy, was noted. Positive controls showed increased numbers of chromosome aberrations, and this indicated that the study was valid. Two days oral administration of Acticide® DCOIT up to a dose level of 400 mg/kg body weight did not induce chromosomal aberration in mouse bone marrow cells under these experimental conditions.

The genotoxic potential of DCOIT was evaluated in an ex vivo unscheduled DNA synthesis study on rat hepatocytes according to OECD guideline 486 (Anon., 2002; A 6.6.5-01, 7.6.2-02). Male Wistar rats (4 animal per group) were treated with DCOIT (1000 and 2000 mg/kg bw) by oral gavage, and were sacrificed 2 h or 16 h after treatment, respectively. Clinical signs, such as reduction of spontaneous activity, abdominal position, ruffled fur and apathy, were observed in the treated animals. Hepatocytes were isolated by a two-step collagenase technique and were allowed to attach in cell culture. Cells were incubated with [3H]TdR for 4.5 hours, and the amount of incorporated thymidine was determined by visualizing the incorporated radioactivity in the hepatocytes on coverslips. From each group, hepatocytes from 3 animals were assessed for the occurrence of UDS (unscheduled DNA synthesis). Positive control was included (for 2 h: N,N`-dimethylhydrazinedihydrochloride and for 16 h: 2-Acetylaminofluorene). The viability of the hepatocytes from treated animals was not affected by the *in vivo* treatment with DCOIT. None of

the tested dose levels of DCOIT revealed UDS induction in the hepatocytes of the treated animals as compared to control group. Positive controls revealed distinct increases in the number of net grain count, and this indicated that the study was valid. Acticide DCOIT did not induce DNA-damage leading to increased repair synthesis in the hepatocytes of the treated rats under these experimental conditions. No indications for clastogenicity *in vivo* were seen in a chromosome aberration test in mice.

In addition no indication for DNA damage including gene mutations was seen in the *in vivo* unscheduled DNA synthesis (UDS) test in rat (hepatocytes). DCOIT was clearly non-genotoxic *in vivo*.

Summary of genotoxicity (Thor)

No signs of genotoxicity have been observed neither in the *in vitro* studies nor in the *in vivo* study conducted with DCOIT. Therefore DCOIT is considered to be non-genotoxic.

4.11.1 Combined summary and discussion of genotoxicity

There was no evidence of genotoxicity when DCOIT was tested in *in vitro* and *in vivo* tests. The DCOIT major metabolite, N-(n-octyl) malonamic acid (NNOMA) was not mutagenic when tested in a Bacterial Gene Mutation Assay test. Therefore DCOIT is considered to be non-genotoxic.

4.11.2 Combined comparison with criteria

There are no human or animal data indicating genotoxicity, thus classification is not warranted.

4.11.3 Combined conclusions on classification and labelling

There are no human or animal data indicating genotoxicity, thus classification is not warranted.

4.12 Carcinogenicity

Combined applicant 1 and 2

There is no study performed addressing the chronic toxicity and carcinogenicity endpoints for DCOIT. Waving of this study has been justified by both the applicants based on the existing information on genotoxicity of DCOIT, the toxic profile of DCOIT seen in the repeated dose studies and comparison with structurally related isothiazolinones (Anon.,2008, A6.5/01).

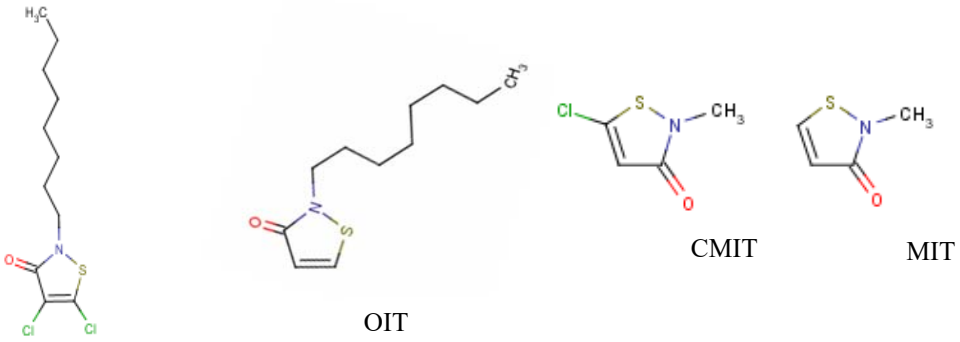
A common feature of the repeat dose studies is that the toxicity seems related to local toxicity and/or reduced feed consumption. None of the studies showed significant systemic toxicity. Doses or concentrations that may induce systemic toxicity seem to be similar or higher than the concentrations that induce significant local toxicity due to irritation, thus hindering the evaluation of systemic toxicity. The toxicokinetic studies indicate that DCOIT is readily absorbed, metabolised and eliminated. 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 studies. Potential

tumour promoting effects caused by chronic tissue irritation will only be relevant if long-term exposure occurs to DCOIT concentrations that gives rise to local toxicity.

Comparison with structurally related isothiazolinones:

Chronic studies have been performed on three structurally related isothiazolinones; 5-chloro-2-methyl-2H-isothiazol-3-one (CMIT), 2-methyl-2H-isothiazol-3-one (MIT), and 2-octyl-2H-isothiazol-3-one (OIT). The four isothiazolinones are structurally quite similar (figure 4.2). Toxicity studies indicate that their main toxic effects are similar. Both the methyl- and the octyl-substituted isothiazolones are irritating/corrosive at the site of contact. Furthermore, all four substances seem to induce limited systemic toxicity at doses that induce local toxicity. Whereas DCOIT is negative in all reported genotoxicity studies, both CMIT/MIT and OIT are positive in some of the *in vitro* genotoxicity studies performed. In a review by an expert in the field of genetic toxicology, Dr. David Brusick (Appendix 3 to Doc IV (Dow)-A6.5/01, "Critical assessment of the genotoxicity of isothiazolone biocides"), it is stated that the positive results reported are the result of hyper toxicity and that the substances should be considered non-genotoxic.

Figure 4.2: Structure of DCOIT and three other isothiazolinones



The most recent carcinogenicity study (1994) was conducted on CMIT/MIT (Kathon™ 886) according to current guidelines in a rat (Sprague-Dawley) 24 months drinking water study. A second study with Kathon™ 886 (Kathon™ CG) was conducted in 1983 in CD-1 mice with dermal administration for 30 months. A third carcinogenicity study has been performed in 1975 on OIT in hybrid mice (C57B/6xC3H/Anf) with dietary exposure for 18 months. These three studies are presented briefly below.

Kathon™ 886 is a mixture of CMIT and MIT, in the ratio of 3:1. CMIT/MIT has been tested in two carcinogenicity studies. CMIT/MIT has demonstrated, like DCOIT, point of contact toxicity (in the form of irritation and/or corrosion) in a range of repeat dose studies. As with DCOIT, metabolism is initiated through a nucleophilic attack on the sulphur-nitrogen bond of the isothiazolone ring, an electrophilic centre, loss of chlorine and sulphur, and subsequent formation of a malonamic acid derivative, in this case, N-methylmalonamic acid. Metabolism then proceeds through malonamic, malonic, acetic, and formic acids to carbon dioxide and methylamine, accompanied by several side products (N-methylglyoxylamide, ethylene glycol and urea). In common with DCOIT, CMIT/MIT

has shown no significant systemic toxicity at the dose levels tested and provides no evidence to suggest a possible endocrine mechanism of carcinogenicity. Like DCOIT, CMIT/MITis not genotoxic *in vivo*, although it does have a genotoxic potential according to some *in vitro* tests as mentioned above. Importantly neither of the carcinogenicity studies conducted on CMIT/MIT, one by the oral (drinking water) route and one by skin "painting" indicate a carcinogenic potential.

The oral drinking water study (1994) was conducted on Crl:CD® BR (Sprague-Dawley) rats 10 rats/sex/group exposed to Kathon™ 886 F in doses of 30, 100, 300 ppm a.i. equivalent to: males: 0, 2.0, 6.6, 17.2 mg a.i./kg bw/day and females: 0, 3.1, 9.8, 25.7 mg a.i./kg bw/day (14.2 % a.i., CMIT: 10.6 %; MIT: 3.5%) for 24 months according to current guidelines (OECD 453). 90 males and 80 females were exposed ad libitum. Tap water and an inorganic stabilizer salt ($MgCl_2/Mg(No_3)_2$) was used as controls. Histopathology was conducted on adrenals, aorta, bone marrow, brain, epididymis, oesophagus, eyes, skin, liver, lung, lymph nodes, female mammary gland, skeletal muscle, ovary, pancreas, prostate, spinal cord, urinary bladder, vagina, zymbal's gland, heart, kidneys, spleen, stomach, intestines, bone and tissues with gross lesions. There were no effects on survival in any treatment group. The study summary states that there were no treatment related effects observed on haematological, clinical chemistry or urinalysis parameters. Furthermore, there were no effects on type or incidence of neoplasms in any group and no signs of systemic toxicity. No adverse effects on the histopathology of any tissues or organs distant from the dosing site were detected. Morphologic changes in the stomach of both sexes in mid and high dose groups were observed. Gastric irritation was the primary effect observed. A slight decrease in food consumption was seen in the males exposed to the 300 ppm dose. In addition, there was detected a treatment-related and concentration-dependent decrease in water consumption in both sexes in all groups treated with Kathon™ 886 F. These decreases appeared to be due to unpalatability of Kathon™ 886 F and not its inorganic stabilizer salts since the water consumption in the salt control group was comparable to the tap water controls. Based on the average daily water consumption, the 300 ppm dose was judged to be a maximum tolerable dose.

A dermal carcinogenicity study was performed in 1983 with Kathon 886 (Kathon™ CG: 1.5% a.i.; 75%:25 % CMIT:MIT), diluted in water. Forty male CD-1 mice were treated with 0 or 400 ppm a.i.(0.04 %, maximum tolerated dose) for 30 months. Twenty-five µl (10 µg/animal) of the test substance was applied 3 times per week. Histopathology was conducted on skin, liver, lung, heart, kidneys, spleen, stomach, intestines, bone and tissues with gross lesions. No other examinations were performed. Ten control and 7 treated mice survived to study termination. The percent survival was lower among the treated animals than in controls, but whether this is a result of treatment is not clear. Skin lesions (very slight to moderate epidermal hyperplasia and hyperkeratosis), was observed with greater frequency in treated mice than in control mice. No treatment-related increases in non-neoplastic lesions in other tissues were reported. No treatment-related increases in neoplastic lesions were observed. The positive control group (3-methylcholanthrene exposed mice) supports the validity of this study and the conclusion that dermally administered Kathon™ 886 is non-carcinogenic.

The third carcinogenicity study was performed in 1975 with dietary exposure to OIT Technical in hybrid mice (C57B/6xC3H/Anf, male and female) for 78 weeks (18 months). One hundred and twenty five mice/sex/group were exposed to 0, 500 or 1000 ppm OIT. The doses were based on observed mortality and reduced body weight gain at 1500 ppm and higher in a 7-week range-finding study. 24-25 mice/sex/group were sacrificed at 30 weeks of treatment. It appears that the following tissues were examined histologically: liver, lung, bladder, spleen, small intestines, kidney, thyroid, stomach, brain, prostate, ovary, mammary gland, gonads, skin and gross lesions. No other examinations were performed. At 30 weeks, body weights and relative liver weights were significantly reduced in male mice of the 1000 ppm group. No histopathologic lesions were

reported in treated animals. At 18 months, body weights were slightly reduced in females of the high dose group, whereas relative liver weights were slightly increased in females of the high dose group and in males of the 500 ppm group. Survival was high and unaffected by treatment. Overall there was no increase in the incidence of any tumour type in the male or female mice that were administered 1000 ppm OIT in their diets for 18 months and OIT was judged to be non-carcinogenic in this study. No description of potential stomach lesions are provided for the main study or for the range-finding study. The positive control groups (2-acetylaminofluorene and diethylnitrosamine exposed mice) support the validity of this study.

When comparing the toxicological data on these materials they seem to demonstrate similar metabolic pathways, toxicity at the site of dosing with no discernible systemic effects and no endocrine or genotoxic mechanism for carcinogenicity.

Based on the results of existing studies it is concluded that the conduct of chronic/carcinogenicity studies is not necessary for the assessment of the human health hazards and risks.

4.12.1 Combined summary and discussion of carcinogenicity

There is no indication of carcinogenic potential of DCOIT based on the existing information on genotoxicity of DCOIT, the toxic profile of DCOIT seen in the repeated dose studies and comparison with structurally related isothiazolinones.

4.12.2 Combined comparison with criteria

Not relevant for DCOIT.

4.12.3 Combined conclusions on classification and labelling

The overall conclusion is that no classification for carcinogenicity is warranted.

4.13 Toxicity for reproduction

4.13.1 Effects on fertility

Table 21: Summary table of fertility study (Dow)

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/ group**	**Exposure Period**	**Doses**	**critical effect**	**NO(A)EL Parental**	**NO(A)EL F1**	**NO(A)EL F2**	**Ref. (Doc III-A cross-ref)**
Dietary	2-generation reproductive, OECD 416 (draft), US EPA OPPTS 870.3800 GLP	Rat, Crl: CD® BR, M/F, 26/ sex/ group	10 weeks prior to mating and continuing until sacrifice of parent, F1, and F2 generations	0, 200, 400, 800, 3200* ppm *one generation only due to mortality of F1 offspring; addition of 400 ppm group with separate control group DCOIT technical, purity: 100%	Parental toxicity: *800 ppm:* none reported in F0. F1 males: ↑paleness; ↓bw gain *3200 ppm F0:* ↓food consumption and ↓bw gain during premating and during gestation and lactation dams Toxicity to offspring: *400 ppm* F1: reduced spleen weight. F2: reduced thymus weight *800 ppm* F1, F2 pups: ↓bw and signs of toxicity in various organs. *3200 ppm* F1 pups: paleness during lactation, distended abdomens; ↑mortality (lactation index 53.8 vs 98.3%[a]). Reproductive toxicity: 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[b]; 800 ppm: 35.1 vs 31.9[a]) and in preputial separation, F1 (400 ppm: 43.7 vs 42.4[b] and 43.9[a]); 800 ppm: 46.2 vs 43.9[a]). AGD, F2: 400 ppm: slight ↑ in F and M; 800 ppm: no significant effects	400 ppm (30-41 mg/kg bw/ day)	400 ppm (30-41 mg/kg bw/day)	200 ppm (16-21 mg/kg bw/day)	Anon. 2001 (A6.8.2 /01)

[a] control run concurrently with 200, 800 and 3200 ppm, [b] control run concurrently with 400 ppm

A 2-generation reproduction study in rats has been performed with exposure to dietary concentrations of 0, 200, 400, 800 and 3200* ppm (*one generation only) DCOIT Technical; equivalent to doses of 0, 16-21, 30-41, 62-93 and 235-259 mg DCOIT/kg bw/day (Anon. 2001, A6.8.2/01).

No treatment-related deaths or clinical signs of systemic toxicity were noted in the first parental animals (F0) during the premating period at doses up to and including 800 ppm. Nor was there any effect on body weights during gestation and lactation at dietary doses up to and including 800 ppm. At 3200 ppm the cumulative body weight gain was reduced (13-45%) in both sexes during the premating period, and in females also during the period of gestation (16-31% on G14-21 and G0-21) and lactation (8-18% on PND0, 4, 7 and 14). The reduction was highest during the first week of treatment and more pronounced in males than in females. During lactation there was an increased incidence of paleness in both F0 females and F1 offspring at 3200 ppm. Offspring at this dose level were observed with distended abdomens. A significant percentage of the F1 offspring died in the 3200 ppm group causing an insufficient number of animals to generate a second generation. The lactation index was 53,8%. (the majority of deaths occurred after PND14). Effects on the pathology of the stomach (hyperplasia and hyperkeratosis of non-glandular mucosa) and adrenal cortex (hypertrophy/vacuolization) were observed at 3200 ppm.

There were no treatment-related deaths or clinical signs of systemic toxicity noted in the second (F1)-generation of parental animals of either sex during the premating period at dose up to 400 ppm, or in females at 800 ppm. Nor was there any effect on body weights during gestation and lactation at dietary doses up to 800 ppm. Body weight gain was reduced in parental F1 males in the 800 ppm dose group and there was an increased incidence of paleness at this dose level.

There were no treatment-related alterations in the pathology of any of the reproductive organs of the parents even in the high dose groups (3200 ppm in F0 animals and 800 ppm in F1 animals). No changes were observed in reproductive performance (mating or fertility); gestational, lactation or viability indices; offspring viability; oestrus cycle or sperm parameters at concentrations up to and including 800 ppm. Offspring viability was decreased at 3200 ppm. From 400 ppm a dose-depended statistically significant delay in vaginal opening was reported in the F1 generation (not measured in the F2 generation) in the absence of decreased body weight. This effect is therefore considered treatment related. The age of vaginal opening was 35.1 days in the 800 ppm group vs 31.9 days in controls. In male pups (F1) in the 400 and 800 ppm dose groups, a statistical significant delay in preputial separation was noted (see table 21). However, the delay at 400 ppm (43.7 versus 42.4) was judged not treatment related since the mean days to preputial separation was comparable to the initial control group (43.7 versus 43.9). Due to these alterations in sexual maturation, anogenital distance (AGD) was measured in all F2 pups. Evaluation of anogenital distance indicated no treatment-related alterations at any dose. An overall evaluation of these data suggests that DCOIT does not have estrogenic or (anti)androgenic effects on the pups.

Treatment-related decreases in pup body weight were observed in both the F1 and F2 offspring at 800 ppm beginning on Postnatal Day (PND) 14. This is approximately the time when pups begin to wean themselves from the dam’s milk and begin to consume the treated feed. The gross findings observed in the F1 and F2 offspring at 800 ppm were similar to those seen in the F1 offspring at 3200 ppm. They included thin and watery blood, enlarged heart, pale lungs, liver, kidney and/or intestines. On PND 21, both sexes had treatment-related decreases in absolute and relative thymus weights in the F1 offspring at 800 and 3200 ppm, as well as the F2 offspring at 400 and 800 ppm, and this effect was accompanied by microscopic changes in the thymus evident as decreased cellularity from 800 ppm in both the F1 and F2 generation. Treatment-related decreases in absolute and relative spleen weights were also noted in both sexes of the F1 offspring at 400 ppm and above.

The NOAEL for parental toxicity was 400 ppm (30-41 mg/kg bw/day) based on clinical signs and body weight changes at 800 ppm and 3200 ppm (62-93 and 235-259 mg/kg bw/day). The NOAEL for systemic toxicity to offspring was 200 ppm (16-21 mg/kg bw/day) based on reduced thymus weight at 400 ppm in the F2 generation. The significance of the latter finding was supported by changes in thymus histopathology at 800 ppm (62-93 mg/kg bw/day). The effects on the thymus are possibly secondary to the reduced weight gain that was observed at the time of weaning when the pups start to depend on the intake of DCOIT treated foods.

Taking the data in its entirety, the weight of the evidence indicates that continuous exposure of rats to DCOIT Technical in the diet up to and including 800 ppm through two generations does not present a reproductive hazard. The delays in vaginal opening and preputial separation observed were not accompanied by changes in other reproductive parameters evaluated in this study. However, the thymus effects indicate that the pups might be more sensitive to the cytotoxic effects of DCOIT than the dams.

Summary of effects on fertility (Dow)

In a 2-generation reproduction study in rats exposed to doses of 0, 200, 400, 800 and 3200 ppm DCOIT technical in the diet, the NOAEL for parental toxicity was 400 ppm (30-41 mg/kg bw/day) based on clinical signs and body weight changes at 800 ppm and 3200 ppm (62-93 and 235-259 mg/kg bw/day). The NOAEL for systemic toxicity to offspring was 200 ppm (16-21 mg/kg bw/day) based on reduced thymus weight in the F2 generation at 400 ppm. The significance of the latter finding was supported by changes in thymus histopathology at 800 ppm (62-93 mg/kg bw/day). The effects on the thymus are possibly secondary to the reduced weight gain that was observed at the time of weaning when the pups start to depend on the intake of DCOIT treated foods.

There were no treatment-related alterations in the pathology of any of the reproductive organs. No changes were observed in reproductive performance (mating or fertility); gestational, lactational or viability indices; offspring viability; oestrus cycle or sperm parameters at concentrations up to and including 800 ppm. Offspring viability was decreased in the high dose group and was associated with stomach lesions. Exposure to DCOIT induced indications of delayed puberty in offspring (delayed vaginal opening and preputial separation). However, these findings were not accompanied by changes in ano-genital distance, and no treatment-related alterations of male or female mating or fertility parameters were reported. Thus, DCOIT is not considered toxic to reproduction based on the Dow 2-generation study.

Applicant 2, Thor:

Effects on reproduction were investigated in a 2-generation feeding study in rats.

Table 22: Summary table of fertility study (Thor)

Route of expo-sure	**Test type Method Guide-line**	**Spec-ies Strain Sex no/ group**	**Expo-sure Period**	**Doses**	**critical effect**	**NO(A)EL Parental**	**NO(A)EL F1**	**NO(A)EL F2**	**Ref.** **(Doc III-A cross-ref)**
Oral (*via* diet)	OECD 416	Rat Wistar Crl: (WI) BR 24 sex/ dose/ genera tion	all parental animals: 10 weeks pre-mating, up to 2 weeks mating. Females : gestatio n, lactation in addition	DCOIT (purity 97.1%) 3-4, 14-16 and 57- 71 mg/kg bw/day (premat-ing dose) (100, 350, 1050 ppm in diet; correcte d after recover y analyses to 46, 179, and 651 ppm) Control: plain diet	Parental toxicity: F0-generation: ↓body weight in females at 1050 ppm compared with controls: - at premating day 64: 7.3% - at mating days 8 and 15; 16.7% and 19.5% - at lactation days 4, 7, 14 and 21: 6.8%, 7.8%, 7.7% and 8.1% ↓body weight gain on females at 1050 ppm: - at lactation days 4-21 (0 vs 5 g weight gain, 1050 ppm vs 0 ppm) decrease in terminal body weights at mid and high dose groups (93.2% and 91.8% of controls, respectively) effects on organ weights, abnormalities in forestomach at 1050 ppm F1- generation: ↓ terminal body weights of females of the 1050 ppm group Effect on organ weights, abnormalities in forestomach at 1050 ppm Toxicity to offspring: reduced bw, effects on organ weights F1- pups: ↓ body weights of males and females of the 1050 ppm group.	14-16 mg/kg bw/day	**repro:** 57-71 mg/ kg bw/day **develop:** 14-16 mg/kg bw/day	Anon. 2006 A 6.8.2-01 7.8.1-01	Oral (*via* diet)

Route of expo-sure	Test type Method Guide-line	Spec-ies Strain Sex no/ group	Expo-sure Period	Doses	critical effect	NO(A)EL Parental	NO(A)EL F1	NO(A)EL F2	Ref. (Doc III-A cross-ref)
					↓absolute spleen and thymus weight and ↑ relative brain weights at 1050 ppm group F2-pups: ↓ body weight at 1050 ppm. ↓absolute spleen weight at 350 ppm and 1050 ppm. ↓Relative spleen weight at 350 ppm and 1050 ppm, but only in female F2-pups. ↑ Relative brain weights for males of the 100 and 1050 ppm group and female of the 1050 ppm. <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 1 050 ppm F2: AGD: No significant effect.				

The reproductive toxicity of ACTICIDE® DCOIT (purity 97.1%) was evaluated in the rat according to EU method B.35 and OECD guideline 416 (Anon.2006, A 6.8.2-01). Two generations of Wistar rats (24 males and 24 females per dose per generation (F0 or F1) were treated with DCOIT in the diet, and the impact on reproductive parameters was investigated. The dose levels for the F0- and F1- parental generation were 100, 350 and 1050 ppm, and the control (0 ppm) animals were treated with plain diet. Dose levels were chosen on the basis of a 28-day range finding study with ACTICIDE® DCOIT and a 90-day toxicity study (Anon. 2002, A 6.4.1-01, 7.5.1-01). Male and female animals (F0 -generation) were treated with the test substance for 10 weeks and were mated. Males were sacrificed after successful mating, and females allowed to litter and raise their offspring (F1-generation). Maternal animals were sacrificed after weaning.

Selected animals of the F1-generation were treated with the test substance for 10 weeks after weaning, and were mated. Males were sacrificed after successful mating, and females allowed to litter and raise their offspring (F2-generation). Maternal animals and their offspring were sacrificed after weaning. All animals were observed for mortality, clinical signs, food or test substance consumption and body weight development through the course of the study. Parental animals were observed for mating performance, and offspring for developmental retardations. After sacrifice, all animals were subjected to macroscopic and microscopic pathological investigation. In general, analytical recovery of test substance was low (35 -73%), but this might not have a negative effect on the study.

In the F0-generation, no treatment related findings were observed in the low (100 ppm) and mid (350 ppm) dose groups. In the high (1050 ppm) dose group, statistical significant ($p<0.05$) decrease in the body weight in females compared to controls were recorded on several occasions; (7.3% at premating day 64; 16.7% and 19.5% at mating days 8 and 15; 6.8%, 7.8%, 7.7% and 8.1% at lactation days 4, 7, 14 and 21, respectively). Further, there was a statistical significant ($p<0.05$) decrease in body weight gain in females on several occasions (particularly at lactation days 4-21) as well as significant decrease in terminal body weights at mid and high dose groups (93.2% and 91.8% of controls, respectively). In the 1050 ppm group, increased relative brain weight was noted for females. Irregular surface and reddish discolouration of the forestomach was observed in one male at necropsy. Increased incidence and severity of hyperplasia of the squamous epithelium of the forestomach was observed in both sexes (9/10 males and 3/11 females) at 1050 ppm, in some cases accompanied by a lymph granulocytic inflammation of the forestomach (6/10 males and 2/11 females). There were no treatment related effects on sperm count, motility and morphology, and oestrus cycle, and the reproduction parameters were not affected by the treatments.

In the 1050 ppm group of the F1 pups, lower body weights were recorded for male and female pups. Clinical signs such as mal-rotated legs, absent/reduced tail and reduced anus size were observed in 4 pups delivered by F0. Decreased absolute spleen and thymus weight and increased relative brain weight were observed in the pups of the 1050 ppm dose group, considered secondary to reduced body weight. Occurrence of preputial separation was slightly delayed in male pups treated at 1050 ppm. This was considered to be caused by a slight delay in development of the pups, which was associated with lower body weights. Treatment related effect on vaginal opening was not reported nor on ano-genital distances (AGD) which was measured in the F2 pups.

In the F1 parental generation, no treatment related findings were observed in the low (100 ppm) and mid (350 ppm) dose groups. In the 1050 ppm group, the following findings were observed: decreased absolute prostate weights in males; decreased terminal body weight at necropsy, decreased absolute weights of the brain and ovaries and increased relative weights of kidneys and adrenals in females. Increased incidence and severity of hyperplasia of the squamous epithelium of the forestomach (2/10 males and 3/10 females) was observed in both sexes, in some cases accompanied by a lymph granulocytic inflammation of the forestomach (2/10 males and 1/10 females). There were no treatment related effects on sperm count, motility and morphology, and oestrus cycle, and the reproduction parameters were not affected by the treatments.

In the F2-generation, lower body weights were recorded for male and female F2-pups at 1050 ppm. Statistical significant absolute spleen weight decrease was noted in males and females as well as statistical significant decreased in relative spleen weights in female F2 pups of the 350 and 1050 ppm group. Relative brain weights were significantly increased for males of the 100 and 1050 ppm group and female pups of the 1050 ppm group. The finding was considered secondary to the lower body weight. Clinical signs such as affected tail apex, reduced size/opaqueness of left eye were observed in four F2-pups.

Summary of effects on fertility (Thor)

Treatment with DCOIT in male and female Wistar rats at dose levels of 100, 350 and 1050 ppm (these dose levels were corrected following recovery analysis, refer Table above) revealed F0- and F1-parental and offspring toxicity at 1050 ppm. The NOAEL for parental toxicity was set to 350 ppm (14-16 mg DCOIT/kg bw/day in males/females), and the LOAEL to 1050 ppm (57-71 mg DCOIT/kg bw/day in males /females). This was based on decreased body weights and body weight gain for F0- dams, effects on organ weights for F1-dams and stomach abnormalities at the high dose level.

The NOAEL for offspring toxicity was set to 350 ppm (14-16 mg DCOIT/kg bw/day) and the LOAEL to 1050 ppm (57-71 mg DCOIT/kg bw/day). This was based primarily on lower body weights for male and female pups of both generations at 1050 ppm. In addition, reduced absolute spleen and thymus weights were observed at the high dose. These effects are likely influenced by the reduction in body weights. A decreased in absolute and relative spleen weights was reported at the mid dose of 350 ppm. However, this finding was only observed in F2 pups and relative spleen weight was only reduced in females. The observed spleen effects in F2pups at the 350 ppm dose were considered of uncertain biological importance and was by itself not considered sufficient for lowering the offspring NOAEL of this study. Exposure to DCOIT induced indications of delayed puberty in male offspring (delayed preputial separation) at the high dose level accompanied by reduced body weight. However, this finding was not accompanied by changes in ano-genital distance in the F2-generation.

Reproduction and breeding parameters were unaffected for both generations at doses up to 1050 ppm. The reproduction and breeding NOAEL was established to be 1050 ppm (57-71 mg/kg bw/day), and no LOAEL for reproductive toxicity was observed.

4.13.2 Developmental toxicity

Applicant 1, Dow:

One developmental study in rats has been performed with exposure to DCOIT technical. In addition, two developmental studies have been performed with exposure to xylene-containing preformulations.

DCOIT technical diluted in corn oil, was administered by oral gavage to pregnant rats from days 6-15 of gestation in a teratogenicity study according to OECD guideline 414 (Anon., 1994, A6.8.1b/02). Animals treated with 300 mg/kg bw/day exhibited signs of severe maternal toxicity, including weight loss, soft faeces and/or diarrhoea, altered posture, and mortality. For humane reasons this group was terminated prior to the scheduled Caesarean section. In the 100 mg/kg bw/day dose group there was one treatment-related death, and maternal body weight gain was significantly reduced. Food consumption was reduced throughout the treatment period in dams treated with 100 mg/kg bw/day, and from days 10-16 in animals treated with 30 mg/kg bw/day.

Treatment-related clinical signs of toxicity were limited to scant faeces, soft faeces, and/or diarrhoea, which were observed in 5 of 24 animals treated with 30 mg/kg bw/day, and 18 of 25 animals in the 100 mg/kg bw/day dose group. No gross pathological changes were reported in dams. There were no treatment-related effects on the numbers of early or late resorptions, live foetuses per litter, foetal body weight or sex ratio.

The number of litters which had foetuses with wavy ribs was 1/24 (corn oil), 1/24 (corn oil), 1/25 (10 mg/kg bw/day), 1/24 (30 mg/kg bw/day), and 11/24 (100 mg/kg bw/day), respectively. Thus a marked and significant increase in the 100 mg/kg bw dose group was observed with a mixture of mild (4), moderate (9) and severe (8) scorings for wavy rib severity (in the 21 foetuses affected). In addition, the number of litters with foetuses with rudimentary ribs (thoracic #13) was significantly increased in the group exposed to 30 mg/kg bw/day of DCOIT. However, there was no apparent dose-response as the incidence of this variation in the 100 mg/kg/day dose group was lower (6/332 foetuses in 5 litters vs. 20/347 foetuses in 11 litters at 30 mg/kg/day) and there was no statistical significant increase in total skeletal variations at the mid dose. Hence, the occurrence of rudimentary ribs was considered to be spontaneous and not treatment related. The slight increase in skeletal malformations reported was not significant. The number of foetuses with skeletal malformations per number of examined foetuses were 0/337 (corn oil), 0/383 (corn oil), 0/380 (10 mg/kg/day), 2/347 (30 mg/kg/day; 2/24 dams), and 1/332 (100 mg/kg/day; 1/24 dams), respectively. Refer to Table 27 for further details.

The NOAELs for maternal and foetal toxicity in rats were determined to be 10 and 30 mg/kg bw/day, respectively. The study suggests a developmental delay in the highest exposure group~~s~~, that was not accompanied by significant increases in structural abnormalities.

The preformulation C-9211M (48.9% a.i. in xylene with 1.0% MgO) was administered to pregnant rats from days 6-15 of gestation in a teratogenicity study according to the OECD guideline 414 adopted in 1981 (Anon. 1983, A6.8.1b/01). The active ingredient (a.i.) consisted of 40.3% DCOIT and 8.6% mono-chlorinated form (4-chloro-2-octyl-2H-isothiazol-3-one). Twenty-five females/group were exposed to vehicle (0.5% methyl cellulose in distilled water), solvent (xylene/MgO in vehicle) control or to the antifoulant preformulation, C-9211M containing 11.2, 33.7 or 112.4 mg a.i./kg bw/day. Six of 25 rats died in the high dose group between days 9 and 14 of gestation. One or more of the physical signs (wheezing, salivation, red exudates from nose and eyes, lethargy and difficulty breathing) occurred in 17 of 25 dams in the high dose group.

There were no compound-related effects on the ability of the exposed dams to maintain a pregnancy to term. Maternal body weight was significantly reduced in the 33.7 and 112.4 mg /kg groups when compared to combined controls. Body weights of male foetuses from the high dose group were significantly reduced and when sexes were combined, the foetal body weights on the high dose group were less than the combined controls.

The frequency of all skeletal variations combined was significantly increased at 112.4 mg/kg, but only when compared to the vehicle control, and there was the suggestion of a solvent effect on the skeletal ossification. For skeletal malformations, there were dose-related increases in foetuses with bent ribs and bent limb bones. The increase was significant for “bent ribs” (associated p-value of 0.024) as well as for “any skeletal malformation” (associated p-value of 0.021) using the modified Jonckheere test with a combined control group (vehicle and solvent group). The percentage of foetuses with skeletal malformations were: vehicle (2.4%), solvent control (3.6%), 11.2 mg/kg bw/day (1.9%), 33.7 mg/kg bw/day (7.4%) and 112.4 mg/kg bw/day (11%). The bent ribs and bent limbs among foetuses from the 112.4 mg/kg groups may be related to maternal toxicity observed at this dose. In particular, one of the dams with a negative body weight gain GD 6-16 had a litter with 8 of 12 foetuses diagnosed with both bent limbs and bent ribs. Only one additional foetus with bent limbs was reported in the mid dose. There were no significant increases in frequency of external or soft tissue malformations at any dose in this study.

Table 23: Summary table of developmental toxicity studies (Dow)

Route of expo-sure	**Test type Metho d Guide-line**	**Species Strain Sex no/ group**	**Expo-sure Period**	**Doses**	**Critical effects dams foetuses**	**NO(A)EL maternal toxicity**	**NO(A)EL Terato-genicity Embryo-toxicity**	**Refer ence (Doc III-A cross-ref)**
Oral gavage **key study**	Teratog enicity, OECD 414, GLP	Rat, Crl:CD BR, female, 25/ group	GD 6-15, 5 days recove ry period	Doses: 0, 10, 30, 100, (300) mg/kg bw/day 300 mg/kg group termi-nated due to severe maternal toxicity Extra control group due to addition of 10 mg/kg bw/day dose group Test material: DCOIT, 98.8% purity. Vehicle: corn oil	<u>Maternal toxicity:</u> *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, diarrhoea observed in 5/24 dams. *100 mg/kg bw/day*: One treatment related death. No significant effects on body weight. ↓ 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, diarrhoea observed in 18/25 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 *30 mg/kg bw/day*: ↑in litters with the skeletal variation rudimentary 13th thoracic ribs (46% vs 8% in controls). No significant increase in total skeletal variations. No significant increase in litters with skeletal malformations (two pups in 2/24 litters affected). *100 mg/kg bw/day*: ↑ in the number of litters which had foetuses with wavy ribs (46% vs 4% in controls) and total skeletal variations (67% vs 25% in controls). Non-significant increase in litters with rudimentary 13th thoracic ribs (21% vs 8% in controls) and skeletal malformations (1 pup in 1/24 litters affected). No HCD data included in study report.	10 mg/kg bw/day	30 mg/kg bw/day	Anon. 1994, A6.8. 1b/02

Route of expo-sure	Test type Metho d Guide-line	Species Strain Sex no/ group	Expo-sure Period	Doses	Critical effects dams foetuses	NO(A)EL maternal toxicity	NO(A)EL Terato-genicity Embryo-toxicity	Refer ence (Doc III-A cross-ref)
Oral gavage	Teratog enicity, OECD 414	Rat, Crl:CD (SD)BR, female, 25/ group	GD 6-15 5 days recove ry	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-chlorinat ed form Vehicle: methylc ellulose Solvent control: xylene in methyl-cellulose	<u>Maternal toxicity</u> *33.7 mg a.i./kg bw/day*: ↓ maternal body weight (adjusted for weight at start of exposure), compared to combined controls. Maternal signs of toxicity (red exudates from nose) were reported in 7/25 dams. *112.4 mg a.i./kg bw/day*: Compound-related deaths (6/25 dams). Maternal signs of toxicity were reported in 17/25 dams and included wheezing, salivation, red exudates from nose or eyes, lethargy and difficulty breathing. ↓ maternal body weight compared to combined controls. <u>Developmental toxicity</u> (See Table 24 for details on malformations. No treatment-related effects on the numbers of resorptions or live foetuses per litter.↓ male foetal body weights in the high dose group. Non-significant reduction of female foetal body weights. ↑ % of pups with skeletal variations, but not malformations in solvent control compared to vehicle control. *33.7 mg a.i./kg bw/day* Non-significant ($0.05 < p < 0.1$), but suggestive ↑ frequency of skeletal variations (53.4%) compared to vehicle control (42.6%), but not to solvent control (60.1%). ↑ frequency of skeletal malformations compared to combined controls ($p = 0.051$) *112.4 mg a.i./kg bw/day* ↓ body weights of male foetuses compared to the combined controls (mean combined male and female bw 3.2 g vs 3.4 g in each of the control groups). ↑frequency of foetuses with skeletal variations (61.6%) compared to vehicle control (42.6%), but not to solvent control (60.1%). ↑ frequency of foetuses	11.2 mg a.i./kg bw/day	11.2 mg a.i./kg bw/day	Anon. 1983, A6.8. 1b/01

Route of expo-sure	Test type Metho d Guide-line	Species Strain Sex no/ group	Expo-sure Period	Doses	Critical effects dams foetuses	NO(A)EL maternal toxicity	NO(A)EL Terato-genicity Embryo-toxicity	Refer ence (Doc III-A cross-ref)
					with skeletal malformations (11%) compared to combined controls. HCD in report.			
	Teratog enicity, US EPA OPP 83-3	Rabbit, NZW, female, 20/ group	GD 7-19 10 days recove ry	Doses: 0, 5, 25, 70 mg a.i./kg bw/day in 10 ml volume Test material: C-9211, 40% DCOIT in xylene Vehicle: methylc ellulose Solvent control: xylene in methylc ellulose; compara ble to high dose	Maternal toxicity: Mortality: 10 dams died during gestation. 5 of these deaths were considered due to intubation error or aspiration of test compound. Dose-response reductions in maternal body weight gain during treatment and also in solvent control. Decreased defecation and urination was considered treatment related. *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) *5 mg a.i./kg bw/day*: Mortality: 0/20 dams. Non-significant ↓ body weight gain GD 7-19 (-44 g) *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 *70 mg a.i./kg bw/day*: Mortality (5/20 dams). ↓ body weight gain GD 7-19 (-478 g) ↓ body weight GD 15-25 Reproductive and developmental toxicity: (Refer Table 28) No significant differences in resorptions or in foetal body weight No significant dose-related differences in foetal variations or malformations reported *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	5 mg a.i./kg bw/day	25 mg a.i./ kg bw/day	Anon. , 1986 A6.8. 1a/01

Route of expo-sure	Test type Metho d Guide-line	Species Strain Sex no/ group	Expo-sure Period	Doses	Critical effects dams foetuses	NO(A)EL maternal toxicity	NO(A)EL Terato-genicity Embryo-toxicity	Refer ence (Doc III-A cross-ref)
					teratogenicity, but no malformations reported.			

↓↑ = significant finding if nothing else is indicated

The preformulation, C-9211M, was maternally toxic and foetotoxic in rats at 33.7 and 112.4 mg DCOIT/kg bw/day. Increased frequencies of total skeletal malformations were reported at the two highest doses, with a positive trend test (Jonckheere). These findings were associated with a marked maternal toxicity at the high dose in the form of mortality and reduced gestational body weight gain. Refer to Table 24 and Table 28 for further details.

In a rabbit study (Anon.A6.8.1a/01), 20 females/group were exposed to vehicle (methyl cellulose vehicle), xylene control, or the antifoulant preformulation C-9211, containing 40% DCOIT in xylene, equivalent to 0, 5, 25 and 70 mg DCOIT/kg bw/day during gestation days 7-19 with a 10-day recovery period. The preformulation produced maternal toxicity at all the dose levels tested and a dose dependent reduction in body weight gain. Mortalities and abortions were observed in most groups including control groups, but the incidence was highest in the two highest dose groups. Some of the deaths were considered related to intubation errors and/or aspiration of the preformulation into the respiratory system during administration. The majority of the animals in all treated groups had decreased defecation and urination, although the incidence was increased in the 70.0 mg DCOIT/kg bw/day group. Clinical signs included lethargy, ataxia, and laboured breathing. Maternal toxicity at the 5.0 mg DCOIT/kg bw/day dose level (reduced body weight gain) was minimal and not statistically significant. 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. The decrease in the total number of foetus is influenced by the number of dams available for examination at the different doses as well as to increased incidence of abortions. The mortality of the dams was 1/20, 1/20, 0/20, 3/20 and 5/20 in controls, xylene controls, 5, 25 and 70 mg DCOIT/kg bw/day groups, respectively. The number of abortions was increased (non-significant) and the number of live foetuses per litter was significantly decreased in the high dose group. A NOAEL for maternal toxicity for the preformulation equivalent to 5 mg DCOIT/kg bw/day is suggested as maternal toxicity was similar in both the vehicle control group and in the group receiving 5 mg DCOIT/kg bw/day. The value 25 mg DCOIT/kg bw/day is suggested as a NOAEL for foetotoxic effects of the preformulation based on a decrease in live foetuses per litter in the 70 mg DCOIT/kg bw/day group. There are indications that xylene contributes to the maternal toxicity of C-9211, as reduced body weight gain was also observed in the xylene control group. No significant increase in foetal skeletal or soft tissue malformations was reported in any of the treatment groups. However, only 23 foetuses were available for evaluation at 70.0 mg DCOIT/kg bw/day which is insufficient for an evaluation of teratogenicity. Some deviations from historical controls and in particular the loss of dams in the high dose group reduce the reliability of this study (refer to Table 28 for further details).

Table 24: Summary table of skeletal malformations from the developmental toxicity study with the DCOIT preformulation C-9211 (Ref: A6.8.1b/01, Dow);

Skeletal malformations data *(skeletal findings extracted from the study report (Table 6 Incidence of malformations, sect IX)*						**Comments**	**Historical control data**
	Vehicle control (0.5% methyl cellulose in water)	Solvent control (xylene/ MgO in 0.5% methyl cellulose solution)	11.2 mg a.i/ kg/day	33.7 mg a.i /kg/day	112.4 mg a.i/ kg/day		HCD data as reported in study report; Appendix 10; Anon. 1983, A6.8.1b/01
Maternal toxicity Body weight gain GD 6-16 (g)	54	53.6	52.5	49.9	30.4	High dose: Two dams with negative bw gain GD 6-16	
Number of foetuses examined skeletally	209	223	206	204	172		736 foetuses examined
No of litters examined	25	25	22	23	19		82 litters
Bent ribs*; moderate or severe no of foetuses (% of foetuses)	4 (1.9%)	8 (3.6%)	4 (1.9%)	15 (7.4%)**	18 (10.5%)	High dose: 6 litters affected; 8 foetuses with bent ribs in one litter from dam with negative bw gain day 6-16	Bent ribs with/without spherical enlargement: 17 (2.3%) of foetuses; 9 (11%) of litters Malformed ribs other than bent: 1 (0.1%) foetus in 1 litter (1.2%)
no of litters (% of litters)	2 (8%)	6 (24%)	4 (18%)	8 (35%)	6 (32%)		
Bent limb bones no of foetuses (% of foetuses)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	8 (4.7%)	High dose: confined to a single litter (all 8 pubs also had bent ribs), dam with neg. bw gain day 6-16	Malformed limb bones (scapulae) 1 (0.1%) foetus in 1 litter (1.2%)
no of litters (% of litters)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.3%)	1 (5.3%)		
All skeletal malformations*; no of pups (% pups)	5 (2.4%) 12% of litters	8 (3.6%) 24% of litters	4 (1.9%) 18% of litters	15 (7.4%)# 35% of litters	19 (11%)** 37% of litters		3% of foetuses / 17% of litters with malformations

*p<0.05 (Jonckheere trend test); **p < 0.05 compared to combined control (pair wise testing); #p = 0.051 compared to combined control. No separate statistical analysis made for bent limb bones.

Summary of developmental toxicity (Dow)

Three developmental toxicity studies are available from Dow.

In the most recent study, pregnant rats were exposed to 0, 10, 30 and 100 mg/kg bw/day of DCOIT technical and the NOAELs for maternal and foetal toxicity were determined to be 10 and 30 mg/kg bw/day, respectively. This is the only study with DCOIT technical and is considered the key developmental study. Maternal signs of toxicity included scant/soft faeces, diarrhoea, reduced feed consumption and at the high dose also reduced weight gain. There was a marked increase in number of litters which had foetuses with wavy ribs in the 100 mg/kg/day dose group and dose-related increase in total skeletal variations that was significant at the high dose (pair-wise testing, no trend test data reported). Only a slight, non-significant, increase in total malformations at the two highest dose groups was observed.

In the two remaining studies, pregnant rats and rabbits were exposed to a preformulation (C-9211M and C-9211) containing 48.9% active ingredient (40.3% DCOIT and 8.6% mono-chlorinated form) and 40% active ingredient in xylene. In the rat study a NOAEL value of 11.2 mg a.i./kg bw/day is suggested for both maternal and foetal toxicity. Compound-related deaths and clinical signs of maternal toxicity was observed at the high dose level. However, there were no compound-related effects on the ability of the remaining dams to maintain a pregnancy to term. Foetal body weights were reduced in the high dose group and the percentage of foetuses with skeletal malformations were increased in the two highest dose groups. The bent ribs and bent limbs among foetuses from the 112.4 mg/kg groups may be related to the marked maternal toxicity observed at this dose. The major contributor to the statistical analysis of the categories “any skeletal malformation” was the incidence of bent ribs. Bent ribs are minor malformation that can be produced by slight alterations in the ossification of these bones. There were no increases in frequency of external or soft tissue malformations at any dose. In the rabbit study a NOAEL value of 5.0 mg DCOIT/kg bw/day is suggested for maternal toxicity and a NOAEL of 25 mg/kg bw/day for foetal toxicity.The number of live foetuses per litter was significantly decreased in the high dose group. Some deviations from historical controls and in particular the mortality of dams in the high dose groups reduce the reliability of this study. These tests indicate that DCOIT is toxic to dams and foetuses. The results indicate that xylene or xylene/MgO in the preformulations could contribute to maternal and foetal toxicity.

Applicant 2, Thor:

Two developmental studies in rabbits have been performed by Thor. In addition, letters of access have been provided to two teratogenicity studies of DCOIT, one in rabbits and one in rats, performed on behalf of R&H Company (See further description of the studies A6.8.1a/01 and A6.8.1b/02 under Applicant 1. Dow).

The prenatal developmental toxicity of Acticide DCOIT was investigated in pregnant New Zealand White rabbits according to OECD guideline 414 (Anon. 2008, A6.8.1-01). Artificially inseminated females were allocated to 4 groups of 24 animals per group. From day 6 to day 28 post-coitum, females of the treatment groups received DCOIT, at dose levels of 0, 125, 500 and 2000 ppm by dietary inclusion (corresponding after correction on average food intake of 0, 2, 10 and 44 mg/kg bw/day). The animals were observed for mortality, clinical signs, body weight development and food consumption during the study. At day 28 of pregnancy, the animals were sacrificed. Maternal

animals were subjected to macroscopic pathological examination. Uterine contents were investigated, and foetuses were investigated for external, visceral and skeletal abnormalities.

Analytical recovery from the diet was low, and thus substance intake was corrected for recovery. Nevertheless, the diet was stable and homogenously prepared. No maternal, reproductive or foetal toxicity was observed at 125 and 500 ppm. Maternal animals treated with the highest dose (2000 ppm) showed a statistical significant reduction in food consumption (GD 6-19), in relative food consumption (GD 6-19; - 58.0 % at GD 6-9) and in body weight gain (GD 9-28) compared to the control group. Further, there was a statistical significant reduction in the relative food consumption in the 500 ppm dose group (GD 6-9; -12% compared to control group). No significant reduction in the food consumption, in the body weight and in the body weight gain was observed in the 125 ppm dose groups.

Compared with the concurrent control and low treatment groups, there was a slight dose related, but not statistical significant decrease in the incidence of foetuses with 12 pairs of ribs in the intermediate and high dose groups (52.8%, 47.2%, 41.1% and 35.9% at 0, 125, 500 and 2000 ppm) and corresponding slight increase in the incidence of foetuses with 13 pairs of ribs. The values were just outside historical control ranges, and there were no associated changes in the number of lumbar vertebrae.

Higher incidence of incisors eruption was observed in all groups of the study (including the control group) compared to historical control foetuses. In the 500 ppm and 2000 ppm groups incisor eruption was slightly in advance of that in the concurrent control (0 ppm) and the 125 ppm groups.

Slight increase in the incidence of unilateral or bilateral slightly folded retina were seen in all groups with values well within the historical control range. This minimal folding was not localised to a particular region of the retina and is considered likely to have been an artefact as a consequence of shrinkage of tissue following fixation.

At 2000 ppm, 4 foetuses (from 3 litters) of 65 examined (~ 6 %) showed retinal abnormalities consisting of detachment of the retina from the underlying choroid and/or detachment of the choroid from the underlying sclera with the retina forming an irregular folded layer adjacent to the lens. The eye findings were outside the historical control range. A document with an expert opinion (Anon., 2008) was prepared by Thor to interpret findings observed in the study. Historical control data analysis from different research organisations showed an increasing trend of eye findings with New Zealand White rabbits, indicative of a specific phenotypic alteration in these animals.

The retinal lesions observed cannot just be regarded as incidental findings, however, such retinal lesions were not observed in the two other rabbit developmental studies (Anon. 1986, A6.8.1a/01 (Dow); Anon. 2002, A 6.8.1-02) nor in the rat developmental study (Anon. 1994, (A6.8.1b/02 (Dow). Thus the concern about the retinal lesions is not supported by these other studies.

In conclusion, based on the reduction in food consumption and in body weight gain, it is reasonable to set the NOAEL for maternal toxicity to 500 ppm (10 mg/kg bw/day) and the LOAEL to 2000 ppm (44 mg/kg bw/day). In view of the eye findings, it is reasonable to consider the LOAEL for developmental toxicity to 2000 ppm (44 mg/kg bw/day).

The second prenatal developmental toxicity of Acticide DCOIT was investigated in pregnant New Zealand White rabbits according to OECD guideline 414 (Anon. 2002; A 6.8.1-02). Pregnant female rabbits were daily treated with the test item by oral gavage over the whole period of organogenesis until the end of pregnancy (days 6 -29). Maternal animals were observed for mortality, clinical signs, and alterations in body weight gain. At day 30 of the pregnancy, animals

were sacrificed and subjected to macroscopic visceral examination. Uterine content and foetuses were in detail examined for abnormalities.

No signs of maternal toxicity were observed. No signs of foetotoxicity or teratogenicity were observed. However, there were high mortalities through all dose groups including the control group and a low maximum dose. No exposure related maternal toxicity was reached. No retinal malformation was observed in this study. The value of this study is limited by its low quality and a low maximum dose.

Table 25: Summary table of developmental toxicity studies (Thor)

Route of exposure	**Test Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses**	**Critical effects dams foetuses**	**NO(A)EL maternal toxicity**	**NO(A)EL Teratogenicity Embryotoxicity**	**Reference (TNsG IUCLID 5)**
Oral (via diet)[k]	OECD 414	Rabbit NZW 24 dams/ group	day 6-28 of gesta-tion	0, 2, 10, 44 mg/kg bw/day (0, 125 ppm, 500 ppm, 2000 ppm, respectively) Purity: 97.1% Control: plain diet	Dams: ↓ food consumption, ↓bw gain in the high dose group Foetuses: eye findings in 4 foetuses (from 3 litters) of 65 examined in the high dose Foetuses: effects on number of ribs in the mid and high dose groups (500 and 2000 ppm; not significant).	NOAEL = 10 mg/kg bw/day (LOAEL = 44 mg/kg bw/day) Based on reduced food consump-tion and reduced bodyweight gain.	NOAEL = 10 mg/kg bw/day (LOAEL = 44 mg/kg bw/day). Based on eye findings	Anon., 2008, A 6.8.1-01 7.8.2-01
Oral (gavage)	OECD 414 (Study of low reliability, RI = 3)	Rabbit NZW 27-31 dams/ group	day 6-29 of gesta-tion	0, 5, 10, 20 mg/kg bw/day Purity; 97.4% Vehicle: peanut oil	Dams: none Foetuses: none High mortalities through all dose groups including the control group.	20 mg/kg bw/day (no LOAEL)	20 mg/kg bw/day (no LOAEL)	Anon., 2002, A 6.8.1-02 7.8.2-02

k = key study ↓↑ = significant finding

Table 26: Maternal toxicity and developmental toxicity in the key rabbit study (Thor)

Ref.	Maternal toxicity	Foetal viability/malformations/variations	Historical control data
Anon. 2008 (A 6.8.1-01/7.8.2-01) Oral (via diet), Rabbit NZW	**Mortality**: one female (1/24) of the 2000 ppm dose group dead on GD 28 with dark red discoloration of the forestomach and a rupture of the peritoneum. **Food consumption from GD 6-19**: 2000 ppm: ↓ GD 6-19 No statistical significant difference for other dose groups **Relative food consumption from GD 6-19**: 2000 ppm: ↓ GD 6-19 (- 58.0 % at GD 6-9) 500 ppm: ↓ GD 6-9 (-12%) No statistical significant difference for other dose groups **Body weight and body weight gain:** *125 ppm & 500 ppm:* no significant reduction in body weight and body weight gain *2000 ppm:* ↓ in body weight gain at GD 9 - 28 **Clinical signs**: No treatment related clinical signs noted **Macroscopic findings**: No statistical significant nor dose related effects ***2000 ppm: three dams with pups with eye findings (Dam no. 82, 83 and 84)***: o No clinical findings noted in these three dams. o No macroscopic findings noted in these dams. o % corrected bw gain of these dams was - 6.4%, - 10.3% and - 4.7%, for dam no. 82, 83 and 84, respectively. o Mean bw and food intake values of these dams were within the dose group mean ± SD	**Foetal viability parameters:** No statistical significant effects on the numbers of resorptions, live foetuses per litter, foetal body weight. No statistical significant differences in the numbers of resorptions, live foetuses per litter, foetal body weight, between the pups with eye findings (Dam no. 82/R4, 83/L2, 83/R1 and 84/R1) and the rest of the pups in the same dose group or the other treatment groups. **External variation/malformation:** No statistical significant increase in numbers of anomalies in the dose groups. **Visceral variations/malformation:** No statistical significant increase in numbers of anomalies in the dose groups. **Skeletal variations/malformation:** Morphological parameters: non-statistical significant effects on number of ribs in the mid and high dose groups (500 and 2000 ppm).The values were just outside historical control ranges. *% of foetuses with 12 pairs of ribs* 0 ppm: 52.8% 125 ppm: 47.2% 500 ppm: 41.1% 2000 ppm: 35.9% *% of foetuses with 13 pairs of ribs* 0 ppm: 31.0% 125 ppm: 32.0% 500 ppm: 47.7% 2000 ppm: 50.4% - Number of foetuses with affected cervical vertebral: (no statistical significant increase, but the values for the mid and high dose groups were just outside historical control ranges). *Anomalous cervical vertebral centrum(a):* *0 ppm*: 1/71 (1/20 litters) (0.7%) *125 ppm*: 1/63 (1/18 litters) (0.8%) *500 ppm*: 3/55 (3/17 litters) (2.8%) *2000 ppm*: 4/66 (4/17 litters) (3.1%)	HCD (2002-2005) from Harlan lab. (Appendix 4, addendum 1 and 2) Both 12 and 13 pairs of ribs are common variants in NLW rabbits % number of ribs: 12/12: 62.4 % (% range: 45.4 – 73.5) 13/13: 27.2% (% range: 19.3 – 40.3) Anomalous cervical vertebral centrum(a): 1.2 %; (% range: 0.7 – 1.8)

Ref.	Maternal toxicity	Foetal viability/malformations/variations	Historical control data
		Soft tissue variations/malformation of heads: (*no statistical significant effects*) - High incidence of incisors eruption in all groups (outside historical control ranges) *0 ppm*: 28/71 cases (13/20 litters) (39.4%) *125 ppm*: 22/62 cases (10/18 litters) (35.5%) *500 ppm*: 32/52 cases (13/16 litters) (61.5%) *2000 ppm*: 36/65 cases (12/17 litters) (55.4%) - Abnormal eye findings in 4 pups (from 3 litters; Dam no. 82, 83 and 84) of 65 examined in the 2000 ppm group. - No pups with abnormal eyes in the other treatment groups **Pups with eye findings**: 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). o external, visceral or skeletal parameters: no significant difference between these 4 pups and the rest of the pups in the same dose group.	Incisors erupted: 22.2% (% range: 15.7 – 21.1) Historical control data for Harlan NZW rabbits (2002-2007): Abnormal eyes in control groups (0.0%), but increased trend with time for retinal findings in the low, mid and high dose group (0.5%, 0.3% and 0.7%, excluding the current study).

↓↑ = significant finding compared to control *($p<0.05$)*

Summary of developmental toxicity (Thor)

Two developmental studies in rabbits have been performed. One study (Anon. 2002, A 6.8.1-02) showed high mortalities through all dose groups including the control group, and can only be considered as supportive. In the key study in rabbits (Anon. 2008, A 6.8.1-01), reduced food consumption and reduced body weight gain was observed in dams of the highest exposure group. This dose (44 mg/kg bw/day) is considered a LOAEL for maternal effects. Regarding foetal development, a slight dose related, but not statistical significant decrease in the incidence of foetuses with 12 pairs of ribs was reported in the intermediate and high dose groups and corresponding slight increase in the incidence of foetuses with 13 pairs of ribs. The values were just outside historical control ranges, and there were no associated changes in the number of lumbar vertebrae. Furthermore, 4 foetuses of 65 examined (6 %) in the highest dose group showed retinal abnormalities. The abnormalities consisted of detachment of the retina from the underlying choroid and/or detachment of the choroid from the underlying sclera with the retina forming an irregular folded layer adjacent to the lens. The applicant have submitted a detailed expert evaluation on the observed eye findings with historical control data analysis from different research organizations showing an increasing trend of eye findings with New Zealand White rabbits (Anon., 2008). The retinal lesions observed in Doc III A6.8.1-01; 7.8.2-01 cannot be regarded as incidental findings, however, such retinal lesions were not

observed in the two other rabbit developmental studies nor in the rat developmental study as mentioned below. Thus the concern about the retinal lesions is not supported by these other studies.

In a teratogenicity study in rabbits performed on behalf of R&H in 1983 (Anon. 1986, A6.8.1a/01), no teratogenicity and no eye malformations were observed up to a dose of 25 mg/kg bw of DCOIT (in a preformulation). No malformations were reported in the highest dose group, but the number of foetuses available for the study was insufficient.

A teratogenicity study in rats performed on behalf of R&H (Anon. 1994, A.6.8.1b/02) on DCOIT technical, is described in the section describing the Dow-registration. The NOAELs for maternal and foetal toxicity were determined to be 10 and 30 mg/kg bw/day, respectively. Maternal signs of toxicity included scant/soft faeces, diarrhoea, reduced feed consumption and reduced body weight gain. Increased number of litters that had foetuses with wavy ribs was observed in the 100 mg/kg/day dose group.

4.13.3 Other relevant information

No data available.

Combined summary and discussion of reproductive toxicity

Effects on fertility

Two rat 2-generation studies with dietary administration of DCOIT are provided, one from Dow and one from Thor.

NOAELs for parental toxicity was between 14-41 mg/kg bw/day, dependent on study and sex of the animals, based on clinical signs and body and organ weight changes and stomach abnormalities at the high dose level. The NOAELs for toxicity to offspring was between 14-21 mg/kg bw/day, dependent on study and sex of the animals, based on reduced pup body weight and reduced spleen and thymus weights.

The weight of the evidence indicates that continuous exposure of rats to DCOIT in the diet up to a dose between 57-93 mg/kg bw/day, depending on the study and on sex of the animals, through two generations does not present a reproductive hazard as no treatment-related alterations of male or female mating or fertility parameters were reported in any of the studies. Exposure to DCOIT induced some indications of delayed puberty in offspring (delayed vaginal opening and/or preputial separation). However, these findings were not accompanied by changes in anogenital distance in the F2-generation and do thus not give rise to concern for oestrogen/androgen disturbance. Reduced thymus and spleen weights were reported in both studies and thymus effects were supported by histopathological findings at the higher dose level in the study by Dow. These findings indicate that pups might be more sensitive to the cytotoxic effects of DCOIT than adult animals and dams and also that the F2 pups may be slightly more sensitive than the F1 generation pups.

Effects on development

Five developmental studies are reported; two in rats of which one was with a pre-formulation containing 48.9% a.i. in xylene and three in rabbits. One of the rabbit studies used a pre-formulation with 40% a.i. in xylene. The main finding from these studies (excluding the low quality rabbit study from Thor) are given in Table 27 and 28. An increase in skeletal variations or malformations in foetuses were observed at higher doses of DCOIT in the two rat studies. In the key rat study with DCOIT, a marked increase in skeletal variations and a slight increase in malformations (not statistically significant) were observed. A more severe increase in skeletal malformations (mostly bent ribs which may be considered minor malformations) was observed in the rat study of a DCOIT-containing preformulation in response to a marked maternally toxic high dose. In the third study, a study in rabbits, there was a slight dose related, but not statistical significant decrease in the incidence of foetuses with 12 pairs of ribs in the intermediate and high dose group and corresponding slight increase in the incidences of foetuses with 13 pairs of ribs. The values were just outside historical control ranges.

An increase in retinae abnormalities (foetal incidence 6%), consisting of detachment of the retina from the underlying choroid and/or detachment of the choroid from the underlying sclera with the retina forming an irregular folded layer adjacent to the lens was observed in one OECD 414 study of DCOIT in rabbits at the highest dose. The findings were not considered incidental, however such retinal lesions were not observed in the two other rabbit developmental studies nor in the rat developmental study. Thus, the concern about the retinal lesions is not supported by the other available studies.

These developmental tests indicate that DCOIT is toxic to dams and foetuses. They also indicate that xylene or xylene/MgO in the preformulations contribute to maternal and foetal toxicity. DCOIT exposure was associated with increases in skeletal variations. Significant increase in malformations (bent ribs and bent limbs) was observed in a rat study with DCOIT in a preformulation with xylene, but not in the rat study with DCOIT technical, or in the rabbit developmental studies. Overall, the developmental studies seems to indicate that DCOIT technical induces a slight foetal developmental delay at high doses as suggested by increases in skeletal variations. This increase was not accompanied by reduced foetal body weights, but with reduced maternal body weight gain. The incidence of skeletal malformations observed at the two highest doses were low.

Table 27: Summary table developmental toxicity studies with DCOIT technical

	RAT DEVELOPMENTAL STUDY (OECD 414)	Historical control data	**RABBIT DEVELOPMENTAL STUDY** (OECD 414)	Historical control data
Ref:	Anon. 1994, A6.8.1b/02 (Dow)	No HCD in study report Control data from CRL (Lang, P.L., 1993)	Anon. 2008, A 6.8.1-01/ 7.8.2-01 (Thor)	Historical control data for NZW rabbit (2002-2005) from Harlan lab HCD for Harlan NZW rabbits (2002-2007)
Species/ strain	Rat, Crl:CD BR	Rat; Crl:CD BR	Rabbit, NZW	Rabbit; NZW

	RAT DEVELOPMENTAL STUDY (OECD 414)	Historical control data	**RABBIT DEVELOPMENTAL STUDY** (OECD 414)	Historical control data
Doses	Doses: 0, 10, 30, 100 mg/kg bw/day		Doses: 0, 2, 10, 44 mg/kg bw/day, equivalent to 0, 125, 500 and 2000 ppm	
Maternal toxicity	*30 mg/kg bw/day*: Body weight: No significant effects, but food intake ↓ GD 10-16 (91% of control) Scant/soft faeces, diarrhoea in 5/24 dams *100 mg/kg bw/d*: Mortality (treatment related): 1/25 dams Body weight: ↓body weight gain GD 6-16 (73% of control) Food consumption: ↓ GD 6-16 (88-90% of control) Scant/soft faeces, diarrhoea in 18/25 dams		*500 ppm* ↓ in food intake from GD 6-9 *2000 ppm* Mortality: 1/24 dams. ↓ in food intake (GD 6-19) ↓ body weight gain at GD 9-28	
Develop-mental toxicity	No significant effects on the numbers of early or late resorptions, live foetuses per litter, foetal body weight or sex ratio Skeletal malformations: no significant effects at any dose Skeletal variations: *30 and 100 mg/kg bw/day*: ↑ number of litters with foetuses with rudimentary 13th thoracic ribs (not significant for the high dose). *100 mg/kg bw/day*: ↑ number of litter with foetuses with wavy ribs and with total skeletal variations		No significant effects on the numbers of resorptions, live foetuses per litter, foetal body weight - High incidence of incisors eruption in all groups (outside historical control ranges) *0 ppm*: 28/71 cases (13/20 litters) (39.4%) *125 ppm*: 22/62 cases (10/18 litters) (35.5%) *500 ppm*: 32/52 cases (13/16 litters) (61.5%) *2000 ppm*: 36/65 cases (12/17 litters) (55.4%) - Abnormal eye findings in 4 pups (from 3 litters) of 65 examined in the 2000 ppm group. - No pups with abnormal eyes in the other treatment groups **Pups with eye findings**: o dam no. 82/R4 (both eyes); o dam no. 83/R1 (abnormal right eye, and left eye; slightly-moderately	HCD (2002-2005) from Harlan lab. Incisors erupted: 22.2% (% range: 15.7 – 21.1) Historical control data for Harlan NZW rabbits (2002-2007): Abnormal eyes in control groups (0.0%), but increased trend with time for retinal findings in the low, mid and high dose group

	RAT DEVELOPMENTAL STUDY (OECD 414)	Historical control data	**RABBIT DEVELOPMENTAL STUDY** (OECD 414)	Historical control data
			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.	(0.5%, 0.3% and 0.7%, excluding the current study).
Skeletal variations (% of foetuses / % of litters)	<u>Rudimentary 13th thoracic ribs:</u> 0 mg/kg bw/d: 0.9% / 8% 0 mg/kg bw/d (control for low dose): 0.5% / 8% 10 mg/kg bw/d: 0% / 0% 30 mg/kg bw/d: 5.8% / 46%* 100 mg/kg bw/d: 1.8% / 21% <u>Wavy ribs</u> 0 mg/kg bw/d: 0.6% / 4% 0 mg/kg bw/d (control for low dose): 0.3% / 4% 10 mg/kg bw/d: 0.3% / 4% 30 mg/kg bw/d: 0.3% / 4% 100 mg/kg bw/d: 6.3% / 46%* <u>Total variations</u> 0 mg/kg bw/d: 3.0% / 25% 0 mg/kg bw/d (control for low dose): 5% / 46% 10 mg/kg bw/d: 3.4% / 32% 30 mg/kg bw/d: 7.8% / 54% 100 mg/kg bw/d: 9.0% / 67%*	<u>Wavy ribs GD 20:</u> average 0.2% (SD 0.4%, max 2.8%) of foetuses; 1.2% (SD 2.6%, max 10%) of litters	Skeletal variations: -Non statistical significant effects on number of ribs in the mid and high dose groups (500 and 2000 ppm), but the values were just outside historical control ranges. *% of foetuses with 12 pairs of ribs* 0 ppm: 52.8% 125 ppm: 47.2% 500 ppm: 41.1% 2000 ppm: 35.9% *% of foetuses with 13 pairs of ribs* 0 ppm: 31.0% 125 ppm: 32.0% 500 ppm: 47.7% 2000 ppm: 50.4% -Number of foetuses with affected cervical vertebral: (no statistical significant effects, but the values for the mid and high dose groups were just outside historical control ranges). *Anomalous cervical vertebral centrum(a):* *0 ppm*: 1/71 (1/20 litters) (0.7%) *125 ppm*: 1/63 (1/18 litters) (0.8%) *500 ppm*: 3/55 (3/17 litters) (2.8%)	12 and 13 pairs of ribs are common variants in NLW rabbits HCD (2002-2005) from Harlan lab. (Appendix 4, addendum 2) % number of ribs: 12/12: 62.4 % (%range: 45.4 – 73.5) 13/13: 27.2% (%range: 19.3 – 40.3) Anomalous cervical vertebral centrum(a): 1.2 %; (%range: 0.7 – 1.8)

	RAT DEVELOPMENTAL STUDY (OECD 414)	Historical control data	**RABBIT DEVELOPMENTAL STUDY** (OECD 414)	Historical control data
			2000 ppm: 4/66 (4/17 litters) (3.1%)	
Skeletal malformations Total malformations: (% of foetuses/ % of litters)	No significant increase 0 mg/kg bw/d: 0% / 0% 10 mg/kg bw/d: 0% / 0% 30 mg/kg bw/d: 0.6% / 8% 100 mg/kg bw/d: 0.3% / 4%	No HCD for total skeletal malformations given in report. HCD data provided for the study with preformulation (more ancient studies, Table 28 below) reports frequencies of 3% / 17%		

↓↑ = significant finding if nothing else is indicated
*Significantly different from control; $p < 0.05$; as stated in study report.

Table 28: Summary table developmental toxicity studies with DCOIT preformulations (DCOIT in xylene)

	RAT DEVELOPMENTAL STUDY (OECD 414)	Historical control data	**RABBIT DEVELOPMENTAL STUDY** (OECD 414)	Historical control data
Ref:	Anon. 1983, A6.8.1b/01 (Dow))	(HCD given in study report)	Anon., 1986, A6.8.1a/01 (Dow)	(HCD given in study report; A:WIL HCD all suppliers B: WIL, Dutchland)
Species/strain	Rat, Crl:CD(SD)BR	Rat; Crl:CD(SD)BR	Rabbit, NZW	Rabbit, NZW
Doses	0, 11.2, 33.7, 112.4 mg a.i./kg bw/day		0, 5, 25, 70 mg a.i./kg bw/day	
Maternal toxicity	*33.7 mg a.i./kg bw/d*: Body weight: ↓ adjusted body weight GD10-18. Mean body weight gain 93% of control.(day 6-16) Red exudates from nose in 7/25 dams. *112.4 mg a.i./kg bw/d*: Mortality (treatment related): 6/25 dams Body weight: ↓ adjusted body weight GD10-20. Mean body		*Vehicle control* Mortality: 1/20 dams. Bw gain GD 7-19 was 84 g. *Solvent control* Mortality: 1/20 dams. ↓ bw gain GD 7-19 (-52 g) *5 mg a.i./kg bw/day*: Mortality:0/20 dams. Non-significant ↓ bw gain GD 7-19 (-44 g) *25 mg a.i./kg bw/day*:	

	RAT DEVELOPMENTAL STUDY (OECD 414)	Historical control data	**RABBIT DEVELOPMENTAL STUDY** (OECD 414)	Historical control data
	weight gain 56% of control (day 6-16). Signs of toxicity were reported in 17/25 dams and included: wheezing, salivation, red exudates from nose or eyes, lethargy and difficulty breathing		Mortality (3/20 dams died). ↓ bw gain GD 7-19 (-115 g). Marked negative body weight gain during the last 4 days of treatment *70 mg a.i./kg bw/day*: Mortality (5/20 dams died). ↓ bw gain GD 7-19 (-478 g). ↓ body weight GD 15-25	
Develop-mental toxicity	No significant effects on the numbers of resorptions or live foetuses per litter *112.4 mg a.i./kg bw/day* Foetal body weight: ↓ in males compared to combined controls. Mean combined male and female bw 3.2 g vs 3.4 g in each of the control groups. Skeletal malformations: ↑ at 33.7 and 112.4 mg a.i./kg bw/d compared to combined controls Skeletal variations: ↑ frequency of foetuses with skeletal variations at 33.7 (ns, 0.5 < p <0.1) and 112.4 mg a.i./kg bw/d compared to vehicle control, but not to solvent control	.	No significant differences in resorptions or in foetal bw ↑ in abortions (non-significant) and ↓ in live foetuses/litter in high dose group (70 mg a.i./kg bw/:day): ↓ in no of live foetus (6/18 pregnant dams aborted) and 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. No statistical significant differences in foetal variations or malformations reported.	
Total skeletal variations % of foetuses	Vehicle control: 42.6% Solvent control: 60.1% 11.2 mg a.i./kg bw/d: 54.4% 33.7 mg a.i./kg bw/d: 53.4% 112.4 mg a.i./kg bw/d: 61.6% Most of the increase related to increases in unossified sternebrae. Also increase in % of foetuses with unossifed metatarsals and reduced ossification of vertebrae Significant increase at high dose compared to vehicle control, but not to the solvent control	Data for total skeletal variations not given Unossified sternabrae: 32% Unossified metatarsals/metacarpals: 0.1% Reduced ossification of vertebrae: 0.8%		
total skeletal malformations* % of foetuses / % of litters	Vehicle control: 2.4% / 12% Solvent control: 3.6% / 24% 11.2 mg a.i./kg bw/d: 1.9%/ 18%	3% / 17%		

	RAT DEVELOPMENTAL STUDY (OECD 414)	Historical control data	**RABBIT DEVELOPMENTAL STUDY** (OECD 414)	Historical control data
	33.7 mg a.i./kg bw/d: 7.4% #/ 35% 112.4 mg a.i./kg bw/d: 11%** / 37%			

↓↑ = significant finding if nothing else is indicated

*p < 0.05Jonckheere trend test; #p=0.051 compared to combined controls; **p<0.05 compared to compared to combined controls;

Comparison with criteria

According to the 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 (v5.0), classification for reproductive toxicity is differentiated into:

– adverse effects on sexual function and fertility, or on development;
– effects on or via lactation

Adverse effects on sexual function and fertility: In the two 2-generation studies reported for DCOIT 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. Exposure to DCOIT induced some indications of delayed puberty in offspring, but these findings were partly associated with reduced pup body weight and were not accompanied by changes in anogenital distance in the F2-generation.

Adverse effects on development of the offspring: The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency. In the 2-generation study from Dow, offspring viability was decreased in the high dose group, an effect that was associated with stomach lesions in the pups and clinical signs of maternal toxicity. Thymus and spleen effects as reported in the fertility studies indicate that pups might be more sensitive to the cytotoxic effects of DCOIT than adult animals and dams.

Skeletal variations or malformations in foetuses were observed at higher doses of DCOIT in the two rat developmental studies. In the key rat study with DCOIT technical, a marked increase in skeletal variations (in particular wavy ribs) and a slight (0.6% of foetuses in mid dose and 0.3% in high dose), non-significant increase in malformations were observed. A more severe increase in skeletal malformations was observed in a rat study with a preformulation of DCOIT in xylene, for which marked maternally toxicity was observed at the highest dose.

An increase in retinal abnormalities (foetal incidence 6%) was observed in one OECD 414 study for rabbits at the highest dose. The findings were not considered incidental, however such retinal lesions were not observed in the two other rabbit developmental studies nor in the rat developmental studies. Thus the concern about the retinal lesions is not supported by the other available studies.

Based on an evaluation of all the available studies, some evidence for developmental toxicity (wavy ribs, bent ribs and bent limbs) is found in some studies. The findings are associated with maternal toxicity. The rat developmental study by Dow, with exposure to a preformulation, suggest that the

solvent in the preformulation tested may aggravate the toxicities of DCOIT technical. It should be noted that not only DCOIT, but also its mono-chlorinated form was present in the preformulation.

In summary, the developmental toxicity studies indicate that DCOIT technical induces a slight foetal developmental delay in rats at high doses as suggested by increases in skeletal variations. This increase was not accompanied by reduced foetal body weights, but with reduced maternal body weight gain. The incidence of skeletal malformations observed at the two highest doses was low. The skeletal variations observed are reversible in nature and may be related to maternal reduced body weight gain. No increases in skeletal variations or malformations were observed in the rabbit study, although there seemed to be a certain change in the % of foetuses with 12 vs 13 pair of ribs. Based on these findings, no classification of DCOIT for developmental toxicity is proposed. This is in line with the CLP criteria that states that "Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity".

The retinal findings were not considered incidental, but is not supported by other available studies. There is no clear evidence for developmental toxicity at dose levels not causing maternal toxicity. Also, no developmental toxicity was apparent in the multi-generation studies performed with DCOIT.

Conclusions on classification and labelling

No classification for fertility is required.

On the basis of the available data no classification of DCOIT for developmental toxicity is proposed.

5 ENVIRONMENTAL HAZARD ASSESSMENT

In the environmental section of this report, studies with the DCOIT degradation product N-(n-octyl) malonamic acid (NNOMA) is included, since this metabolite affect the classification and labelling of DCOIT. Studies with other degradation products, which are not relevant for classification and labelling purposes, are not included in this report.

5.1 Degradation

Table 29: Summary of relevant information on degradation

Method	Results	Remarks	Reference	Applicant
US EPA 161-1 and OECD 111	Hydrolysis DT50 at 25/12°C: pH 4: 260/736 days pH 7: 71/201 days pH 9: 3.5/10 days	-	A7.1.1.1.1/01 A7.1.1.1.1/02	Dow
OECD 111	Hydrolysis DT50 at 20/12°C: pH 4: 18/78 days pH 7: 25/47 days pH 9: 9.4/18 days	-	A7.1.1.1.1-01	Thor
US EPA 161-2	Photolysis in water with natural sunlight at 25/12°C DT50: 13/38 days (dark 80/225 days)	-	A7.1.1.1.2/01 A7.1.1.1.2/02	Dow
OECD draft guideline	Photolysis in acidic buffer and in natural pond water with simulated natural sunlight. DT50: 7.6 and 7.9 days, respectively. Supporting study with artificial light. DT50: 4.5-56 days of 24-hour sunlight at 50°N.	-	A7.1.1.1.2-01 A7.1.1.1.2-02	Thor
OECD 301B Ready biodegradability	Virtually no degradation after 28 days. No results could be obtained due to inhibition of the inoculum at test concentration of 32 ppm.	-	A7.1.1.2.1/01	Dow
OECD 301B Ready biodegradability	No results could be obtained due to inhibition of the inoculum at test concentration of 25 mg/L.	-	A7.1.1.2.1-01	Thor
OECD 309	Aerobic estuarine surface water. Geomean DT50 16.5 hours calculated at 12°C.	-	A7.1.2.2.1/01	Dow

Method	Results	Remarks	Reference	Applicant
OECD 308	Aerobic and anaerobic freshwater-sediment system. DT50 in the water phase calculated as 1.6 and 0.17 days at 12°C in the aerobic and anaerobic systems, respectively. No DCOIT was detected in the sediment, and degradation is so rapid that that the same rate can be used both for water and sediment.	-	A7.1.2.2.2.a/01	Dow
US EPA 162-4	Aerobic and anaerobic seawater-sediment system. DT50 in both studies was less than one hour at 25°C. Recalculated to 9°C gives a DT50 of less than 3.6 hours.	-	A7.1.2.2.2.c/01 A7.1.2.2.2.c/02 A7.1.2.2.2.c/03 A7.1.2.2.2.c/04 A7.1.2.2.2.d/01 A7.1.2.2.2.d/02	Dow
US EPA 162-1	Aerobic soil degradation in two soils. DT50 0.6-1.1 days at 25°C.	-	A7.2.1/01	Dow
OECD 308	Aerobic degradation in natural water/sediment from pond and river. DT50: 1.2-1.5 days. 2.5 if recalculated to 12°C.	-	A7.1.2.2.2-01	Thor

5.1.1 Stability

5.1.1.1 Hydrolysis

Applicant 1, Dow:
An aqueous hydrolysis study with DCOIT was performed following OECD 111 and U.S. EPA guidelines. Kinetics was dependent on temperature and pH. The half-life at the environmentally relevant pH of 7 was 71 days at 25°C, and was calculated to be 178-201 days at 12°C. Several degradation products have been formed; three of them in quantities above 10%: 2-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid (11% at pH 7 at 40°C and 20% at pH 9 at 25°C), 1-chloro-2-(n-octylcarbamoyl)-1-ethene sulfonic acid (16% at pH 7 at 40°C and 40 % at pH 9 at 25°C) and N-(n-octyl) propiolic acid amide (13% at pH 9 at 25°C). **(A7.1.1.1.1/01, A7.1.1.1.1/02)**

Applicant 2, Thor:
The hydrolysis study with DCOIT following OECD guideline 111 showed an increasing hydrolysis rate with increasing pH. At pH 7 (20°C), the half-life was 25 days. The half-lives were calculated to be 41-47 days at the environmentally relevant pH 7 and 12°C. All degradation products of DCOIT remained < 10%. (**A7.1.1.1.1-01**)

5.1.1.2 Photolysis in water

Applicant 1, Dow:
An aqueous photolysis study with DCOIT was performed following U.S. EPA guidelines using sterile buffer and natural sunlight. DCOIT was quantified by HPLC and the half-life in the presence of sunlight was 13 days (38 days at 12°C) while for the dark control the half-life was 80 days (225 days at 12°C). In addition to CO_2 (19%), several degradation products were formed, but only one of them in a quantity above 10% (N-(N-octyl) oxamic acid (NNOOA) at 31%). (**A7.1.1.1.2/01**, **A7.1.1.1.2/02**)

Applicant 2, Thor:
Radiolabelled DCOIT was exposed to simulated natural sunlight (25°C natural summer sunlight at 50°N, diurnal variation accounted for) following OECD draft guideline/U.S. EPA guidelines. The photolytic degradation half-lives were 7.6 and 7.9 days in buffer and natural pond water, respectively. DCOIT was rapidly mineralised in the test, with around 50% of the applied radioactivity (a.r.) present as CO_2 at the termination of the test (after 19 days). Of 19 radioactive fractions detected, only N-(N-octyl)oxamic acid (NNOOA) accounted for over 10% a.r (11 and 20% a.r. in buffer and pond water, respectively). (**A7.1.1.1.2-01**)

In an earlier study following OECD draft guideline, non-radiolabelled DCOIT was subjected to photolytic degradation by an artificial light source. The photolysis half-life was 4.5-56 days at 24-hour sunlight at 50°N, depending on the season (9-112 days at 12-hour sunlight). Degradation products characterisation was very limited and tentative in the study, since non-radiolabelled DCOIT was used. (**A7.1.1.1.2-02**).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not available.

5.1.2.2 Screening tests

5.1.2.3 Ready biodegradability

Applicant 1, Dow:
A ready biodegradability test (OECD 301B) indicates that DCOIT is not readily biodegradable, as there was virtually no degradation after 28 days. However, in the toxicity control containing both DCOIT and the reference item sodium benzoate, the biodegradation of sodium benzoate was totally inhibited after 5 hours compared to sodium benzoate alone. This indicates that DCOIT inhibits the bacteria at the test concentration of 32 ppm. No information on the biodegradability of DCOIT in a STP can therefore be obtained from this test. (**A7.1.1.2.1/01**)

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

Applicant 2, Thor:

A ready biodegradability study (OECD 301B) did not allow determining any degradation rate of DCOIT in activated sludge. No CO_2 was evolved during the 28 days test period, and therefore DCOIT must be regarded as not 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. (**III-A 7.1.1.2.1-01**)

5.1.2.4 Simulation tests

Applicant 1, Dow:

Aerobic aquatic biodegradation in estuarine surface water

A 144 hours aerobic simulation study with estuarine surface water without sediment from Port Penn, Delaware, USA was performed following OECD Draft Guideline 309. (**A7.1.2.2.1/01**) DCOIT was quantified by HPLC and a radioactivity monitor (HPLC-RAM). The half-lives of DCOIT were 20-32 hours and 4.2-12 hours at 10 and 18°C, respectively. Recalculation gives half-lives of 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 at concentrations less than 10% (9.3% and 8.3% respectively). No CO_2 development was detected.

Aerobic and anaerobic aquatic biodegradation in a freshwater-sediment system

A simulation study with water and sediment from a UK pond was performed under both aerobic and anaerobic conditions following OECD Draft Guideline 308. (**A7.1.2.2.2.a/01**)

DCOIT half-lives calculated to 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% of applied radioactivity was contained in the bound residues fraction in the aerobic and the anaerobic study, respectively.

Metabolism involves cleavage of the isothiazolone 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 from York River, Virginia, USA was performed under both aerobic and anaerobic conditions following U.S. EPA Guideline. (**A7.1.2.2.2.c/01, A7.1.2.2.2.d/01, A7.1.2.2.2.c/02 A7.1.2.2.2.d/02**)

In both study types the primary half-lives at 25°C was 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 in both studies, most of the applied radioactivity was detected in the sediment. DCOIT disappeared almost instantly from both water-sediment systems. DCOIT was not detected in the water phase at any sampling time and was only detected in the sediment at day 0. In the water phase, up to 8.2% of applied radioactivity was found, but none of this was DCOIT. 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, but 2 polar compounds were characterised as likely being N-(n-octyl) malonamic acid (NNOMA) and N-(n-octyl) acetamide (NNOA).

An additional study (**A7.1.2.2.2.c/03**) concentrated 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. ^{14}C-DCOIT was applied to aliquots of sterile marine sediment, which were stored frozen or at room temperature. Aliquots were removed periodically over a 224 day period. The extraction procedure was similar to that used for the aerobic and anaerobic seawater-sediment simulation studies. The average recovery of DCOIT as a percent of applied ^{14}C-activity was 97.5 ± 8.1%. This result demonstrates that DCOIT can be quantitatively extracted from marine sediment and that it is stable and does not chemically degrade in sterile sediment. As a consequence, 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.

5.1.2.5 Aerobic biodegradation in soil

Applicant 1, Dow:

An aerobic soil simulation (metabolism) study was performed with two soils following U.S. EPA guidelines. After extraction of the soil samples, DCOIT was quantified by HPLC. (**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. Based on the easy extractability from seawater sediment (see study above), DCOIT is not suspected to be contained in this fraction.

CO_2 was the major degradation product being present 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. Over 87% of the detected peaks were present at less than 5%. The chromatographic behaviour of the degradation products indicated that they were mostly the same compounds as in the sediment-water studies.

5.1.2.6 Natural water and water-sediment systems

Applicant 2, Thor:
A simulation study with natural water/sediment from a river and a pond in Switzerland was performed under aerobic conditions following OECD Draft Guideline 308. (**A7.1.2.2.2-01**)

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 was other substances than DCOIT. No major degradation products were formed during DCOIT degradation in either water or sediment. They were all < 9% of applied radioactivity in at any sampling time. DCOIT is ultimately mineralised to CO_2 or incorporated into natural substances (humic acids and humins).

5.1.2.7 Aerobic biodegradation in soil

No data.

5.1.3 Summary and discussion of degradation

5.1.3.1 Abiotic degradation

The hydrolytic half-life of DCOIT at the environmentally relevant pH 7 and 12°C was calculated to be 47-201 days. The aqueous photolytic half-life of DCOIT in natural sunlight at 12°C was calculated to be 38 days. 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.

The hydrolytic and photolytic degradation of DCOIT in aqueous media is moderate and significantly slower than the biotic degradation. Thus, the primary route of dissipation of DCOIT in the environment is biological.

5.1.3.2 Biodegradation

In ready biodegradation studies, DCOIT could not be classified as readily biodegradable, since the inoculum was inhibited by DCOIT.

Primary half-lives of DCOIT in the environment (water, sediment and soil) are very short, ranging from a couple of hours to a maximum of 4.7 days. The rapid half-life implies that the concentration of parent compound in the environment will be low. Metabolism involves cleavage of the isothiazolone ring and subsequent oxidation. Dissipation of DCOIT in the environment is mainly comprised of biological degradation with subsequent incorporation of the breakdown products into non-extractable residue fraction of soil and sediments. CO_2 development was limited, with a maximum of 30% of applied radioactivity. 41-64% of applied radioactivity was found as bound residues in the different studies. 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%).

Conclusion: The primary half-life of DCOIT in the environment is short, but the mineralisation is limited. In addition, DCOIT has a degradation product that fulfils the criteria for classification as hazardous to the environment. DCOIT is therefore regarded as not rapidly biodegradable for classification purposes.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Applicant 1 Dow:
An adsorption/desorption study was conducted with sewage sludge following U.S. EPA guidelines. The Freundlich sorption constant (Kf) was 2466, which indicates that DCOIT is adsorbed to the activated sludge, and is unlikely to remain in the aqueous phase for the typical concentrations of sludge (3-9 g of sludge/L of solution) expected in a waste treatment plant. (**A7.1.3.a/01**).

The adsorption/desorption of 14C-DCOIT in four soils and one aquatic sediment was determined with five test concentrations following U.S. EPA guidelines. The specific adsorption constants Kaoc in soil were 5659-25237 L/kg. In the sediment, specific adsorption constants were 17232-28320 L/kg at the lowest test concentration of 0.25 mg/L with an outlier of 38237 L/kg at the next lowest test concentration. The results indicate that DCOIT binds very tightly to soil and sediment and will not readily desorb. (**A7.1.3.b/01, A7.2.3.1/01**).

Applicant 2, Thor:
The sorption properties of DCOIT in sewage sludge was investigated in a HPLC study following OECD guideline 121, resulting in a Koc of 2455 mL/g (**A7.1.3-01**).

5.2.2 Volatilisation

Applicant 1, Dow:
No data

Applicant 2, Thor:
The vapour pressure of DCOIT at 20°C is 0.0014 Pa and the Henry's law constant is 0.21 Pa m^3/mol (**A3.2.1-01**). Based on those properties, SimpleTreat in EUSES estimates only negligible volatilisation from sewage treatment plants to air, i.e. 0.043%. Volatilisation of DCOIT from water is therefore considered negligible.

5.2.3 Distribution modelling

No data.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

Table 30: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference	Applicant
EPA guidelines 40 CFR § 158 subdivision N § 165-4	BCF: 56-660 (total residue) BCF: 750 (total residue, steady state) DCOIT BCF: <13 (calc.)	Bluegill sunfish	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	Dow
Guidelines of Japanese Ministry of International Trade and Industry.	BCF: 198-1126 (total residue) BCF: 713-735 (total residue, steady state)	Carp	A7.4.3.3.1.b/01 A7.4.3.3.1.b/02	Dow
U.S. EPA guideline OPPTS 850-1710 and OECD Guideline 305E	BCF: 44 (total residue)	Oyster	A7.4.3.3.2/01	Dow

5.3.1.1 Bioaccumulation estimation

Applicant 1, Dow:
No data

Applicant 2, Thor:
No data

5.3.1.2 Measured bioaccumulation data

Applicant 1, Dow:

Bioaccumulation potential in fish

A bioaccumulation study in Bluegill sunfish (*Lepomis macrochirus*) was performed following U.S. EPA guidelines. Bioconcentration factors for total ^{14}C-residues (DCOIT + degradates/metabolites) were 56-660 for whole fish. The steady state BCF based on total ^{14}C-residues in whole fish was 750 ($K_{uptake}/K_{depuration}$). Depuration DT_{50} in whole fish was 11.6 days (DCOIT + degradates/metabolites). The content of DCOIT in water was 4.5% of total radioactivity at day 21. DCOIT in fish is found to be less than 1% of a.r. 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 content of parent compound in water was 30-70% of total radioactivity. The 14C-BCF values (total residue) for whole fish were 198–1126. Calculated steady state BCF were 713-735. Depuration DT50 was 11-16 days. For DCOIT, the 14C-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 Guideline 305E. Recovery of DCOIT from test solution samples averaged 36% of nominal concentrations, but measured concentrations were stable throughout the 28-day uptake period.

The highest estimated BCF for DCOIT in oyster based on total ^{14}C-residues and the use of uptake and depuration rate constants is 44. Depuration DT50 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**)

Applicant 2, Thor:

Bioconcentration in fish

No data

5.3.2 Summary and discussion of aquatic bioaccumulation

The steady state BCF 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 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 suggested trigger of ≥500, but DCOIT itself seems to have lower potential for bioconcentration, mainly because of its rapid primary degradation.

5.4 Aquatic toxicity

Table 31: Summary of relevant information on aquatic toxicity

Method	Results*	Remarks	Reference	Applicant
US EPA FIFRA 72-1 Rainbow trout (*Oncorhynchus mykiss*)	96h LC_{50}: 2.7 µg a.s./L (mm)	96h, flow-through	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	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	A7.4.1.1.b/01	Dow
OECD Guideline 203 Japanese Blowfish (*Takifugu rubripes*)	96h LC_{50}: 5.66 µg a.s./L (n)	Semi-static	A7.4.1.1.b/02	Dow
OECD Guideline 203 Rainbow trout (*Oncorhynchus mykiss*)	96h LC_{50}: 7.8 µg a.s./L (mm)	Semi-static	A 7.4.1.1-01	Thor
OECD Guideline 203 Sheepshead minnow (*Cyprinodon variegatus*)	96h LC_{50}: 7.3 µg a.s./L (mm)	Semi-static	A 7.4.1.1-02	Thor
OECD Guideline 210 Rainbow trout (*Oncorhynchus mykiss*)	97d NOEC: 0.56 µg a.s./L (mm)	ELS flow-through	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	A7.4.3.2.b/01	Dow
OECD Guideline 210 Zebra fish (*Brachydanio rerio)*	35d NOEC: 0.43 µg a.s./L (mm)	ELS flow-through	A 7.4.3.2-01	Thor
US EPA FIFRA 72-2 *Daphnia magna*	48h EC50: 5.2 µg a.s./L (mm)	Flow-through	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	A7.4.1.2.b/01	Dow
US EPA FIFRA 72-3 American oyster embryo (*Crassostrea virginica*)	48h EC50: 2.1-3.2 µg a.s./L (mm)	Static	A7.4.1.2.b/02	Dow
US EPA OPPTS 850.1055 Bay mussel embryo (*Mytilus edulis*)	48h EC50: 411 µg a.s./L (mm)	Static	A.7.4.1.2.b/03	Dow
OECD Guideline 202 *Daphnia magna*	48h EC50: 9.7 µg a.s./L (mm)	Static	A 7.4.1.2-01	Thor
US EPA FIFRA 72-4 *Daphnia magna*	21d NOEC: 0.63 µg a.s./L (mm)	Flow-through	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	A7.4.3.4.b/01	Dow
OECD Guideline 211 *Daphnia magna*	21d NOEC: 0.4 µg a.s./L (mm)	Semi-static	A 7.4.3.4-01	Thor
US EPA FIFRA 123-2 *Navicula pelliculosa*	No reliable endpoints could be established.	Static	A7.4.1.3.a/01	Dow
OECD 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 ErC50: 1.6 µg a.s./L (m) 24/96h NOErC: 0.34 µg a.s./L (m)	Static	A7.4.1.3.a/03	Dow
US EPA FIFRA 123-2 *Skeletonema costatum*	24/120h ErC50: 0.48 µg a.s./L (m) 24/120h NOErC: 0.48 µg a.s./L (m)	Static	A7.4.1.3.b/01	Dow
OECD 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 EC50: 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.	A7.4.3.5.2/01	Dow
OECD Guideline 201, EPA OPPTS 850.5400 Freshwater green alga (*Scenedesmus subspicatus)*	72h ErC50: 25 µg a.s./L (mm) 72h NOEbC: <15 µg a.s./L (mm)	Static	A 7.4.1.3-01	Thor
EPA OPPTS 850.5400 ISO 10253 Marine diatom, (*Phaeodactylum tricornutum)*	72h ErC50: 25 µg a.s./L (mm) 72h NOEbC: 4.3 µg a.s./L (mm)	Static	A 7.4.1.3-02	Thor
OECD 201, US EPA OPPTS 850.5400 *Skeletonema costatum* **NNOMA**	96h ErC50: 470 µg/L 96h NOEC: 130 µg/L	Static	A7.4.1.3.c/02	Dow
OECD 201, US EPA OPPTS 850.5400 *Selenastrum capricornutum* **NNOMA**	96h ErC50: 9700 µg/L (mm) 96h NOEC: 1510 µg/L	Static	A7.4.1.3.c/01	Dow

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

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Applicant 1, Dow:

DCOIT is highly acute toxic to four species of freshwater and saltwater fish (96h LC50: 2.7-20.5 µg a.s./L). All these studies have LC50 values below 1.0 mg/L, the trigger value for **Category Acute 1.**

Acute flow-through toxicity tests were performed with rainbow trout (*Oncorhynchus mykiss*) and bluegill sunfish (*Lepomis macrochirus*), following U.S. EPA Guideline FIFRA 72-1. The 96 h LC_{50} were 2.7 and 14 µg a.s./L, respectively, based on mean measured concentrations. The 96 h NOECs were 1.8 and 6.5 µg a.s./L, respectively. Test concentrations were measured at 0 and 96 hours. The mean measured concentrations were 0.44-0.93-1.8-3.3-6.3 and 1.6-3.3-6.5-13-26 µg a.s./L, respectively. For the lowest tested concentration in the *O. mykiss* test, the validity criteria were not completely fulfilled at 96 h, 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. (**A7.4.1.1.a/01 A7.4.1.1.a/02**)

An acute flow-through toxicity test was performed with sheepshead minnow (*Cyprinodon variegatus*), following U.S. EPA Guideline FIFRA 72-3. The 96 h LC_{50} was 20.5 µg a.s./L based on mean measured concentrations. The 96 h NOEC was 11.5 µg a.s./L. Test concentrations were measured at 0 and 96 hours, except the two highest concentrations which were terminated after 48 and 24 hours, respectively, since all fish were dead at that time. The mean measured concentrations were 7.6-11.5-21.5-35-70 µg a.s./L. Test substance concentrations were above 80%, but at the highest tested concentration the values measured at end of test are more than 120% of the initial measured concentration. However, the LC50, based on mean measured concentrations, is below this concentration level, and therefore this finding is not considered to have influenced the outcome of the test. (**A7.4.1.1.b/01**)

An acute semi-static toxicity test was performed with Japanese blowfish (*Takifugu rubripes*) following OECD guideline 203. The 96 h LC_{50} was 5.66 µg a.s./L. A NOEC was not calculated, but there were no mortality at test concentrations below 4.0 µg a.s./L. The initial concentrations were 0.25-0.5-1.0-2.0-4.0-8.0-16.0 µg a.s./L. Test concentrations were not measured and the species tested is not one recommended by OECD. However, the test is a semi-static OECD test from 1997, had renewal of medium every 24 hours, gives a clear dose-response-relationship and is fairly well documented. Therefore, the results of this test are nevertheless considered valid. (**A7.4.1.1.b/02**).

Applicant 2, Thor:

DCOIT is highly acute toxic to species of freshwater and saltwater fish (96 h LC50: 7.3-7.8 µg a.s./L). Both these studies have LC50 values below 1.0 mg/L, the trigger value for **Category Acute 1.**

An acute semi-static toxicity test was performed with rainbow trout (*Oncorhynchus mykiss*) following OECD 203 with daily renewal of test medium. Mean measured concentrations of 5.5, 11 and 27 µg a.s./L were determined for the three highest test levels. For the two lowest test levels (dilution levels 1:2000 and 1:909), concentrations were too low to be detected, but were calculated based on the analytically verified concentration of the stock solution to be initially 1.6 and 3.5 µg a.s./L. The 96 h LC_{50} was 7.8 µg a.s./L and the NOEC was 5.5 µg a.s./L., based on mean measured concentrations. (**A7.4.1.1-01)**

An acute semi-static toxicity test was performed with sheepshead minnow (*Cyprinodon variegatus*), following OECD 203 with daily renewal of test medium. Mean measured test concentrations were 1.6-3.8-8.0-16-34 µg a.s./L. The 96 h LC_{50} value was 7.3 µg a.s./L, and the NOEC was 3.8 µg a.s./L based on mean measured concentrations. (**A7.4.1.1-02**)

5.4.1.2 Long-term toxicity to fish

Applicant 1, Dow:

A chronic toxicity test was performed with rainbow trout (*O. mykiss*). This was a 97 days chronic flow-through toxicity test, early life stage (OECD 210). The mean measured concentrations were 0.15-0.30-0.56-1.2-2.6 µg a.s./L. The NOEC values from this test are based on mean measured concentrations of total radioactivity, i.e. they might reflect a mixture of DCOIT and degradation products. However, as this was a flow-through test, it can be assumed that the results refer to parent. This is confirmed by HPLC measurements at the 3.0 µg/L level (nominal) which showed that test concentrations were constant at about 84% of nominal. It can therefore be assumed that the NOEC reflects the toxicity of DCOIT towards fish. The NOEC is 0.56 µg a.s./L for egg hatchability and survival. (**A7.4.3.2.a/01**).

A 35-days chronic early life stage test was performed in a flow-through system with sheepshead minnow (*C. variegatus*), following U.S. EPA guideline (FIFRA 72-4). Test concentrations were measured weekly. The mean measured concentrations were 0.54-1.2-2.9-6.0-14.0 µg a.s./L. The $NOEC_{egg\ hatchability}$ was 6.0 µg a.s./L and the $NOEC_{growth\ and\ survival}$ was 14 µg a.s./L, based on mean measured concentrations. The reliability index was 1. (**A7.4.3.2.b/01**)

Both these studies have NOEC values below 0.1 mg/L, the trigger value for **Category Chronic 1** for non-rapidly degradable substances**.**

Applicant 2, Thor:

A 35-days chronic early life stage test was performed with juvenile zebra fish (*Brachydanio rerio*), in a flow-through test following OECD 210 guideline. The nominal test concentrations were 0.2-0.63-2.0-6.3-20 µg a.s./L. The test media of nominal 0.63 and 2.0 µg a.s./L were analytically determined, and the mean measured test concentrations (calculated as the arithmetic mean over all measurements per test concentration) were 0.47 µg/L and 2.0 µg/L, corresponding to 74 and 97% of nominal, respectively. NOEC for development of embryos, hatching success, survival and growth was 0.47 µg/L, based on mean measured concentrations. Survival of embryos exposed to a mean measured concentration of 2.0 µg/L (LOEC) and above was significantly lower than in the (solvent) control by the end of the test. No embryos hatched at the highest treatment level of nominal 20 µg/L. (**A7.4.3.2-01**)

This study has a NOEC value below 0.1 mg/L, the trigger value for **Category Chronic 1** for non-rapidly degradable substances**.**

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Applicant 1, Dow:

Freshwater Invertebrates

Results from an acute flow-through toxicity study with *Daphnia magna* following U.S. EPA guidelines (FIFRA 72-2) indicate that DCOIT is highly toxic to freshwater invertebrates. The 48 h EC_{50} from this test is 5.2 µg a.s./L based on mean measured concentrations. The 48 h NOEC was 3.9 µg a.s./L. Test concentrations were measured at 0 and 48 hours. The mean measured concentrations were 0.42-0.70-1.5-3.9-7.0 µg a.s./L. Measured concentrations at 48 hours were not in all cases ≥80% and this validity criterion is not fulfilled. At the highest concentration there was no effect after 24 hours and 100% mortality after 48 hours, while in the next lower concentration no mortality was observed at all. The LC50 value was calculated as the geometric mean value of these two concentrations. However, no clear dose-response-relationship can be established with this approach. (**A7.4.1.2.a/01**)

This study has an EC50 value below 1.0 mg/L, the trigger value for **Category Acute 1.**

Saltwater Invertebrates

Results of three acute toxicity tests for marine invertebrates following U.S. EPA guidelines (FIFRA 72-2/72-3 and OPPTS 850.1055, respectively) gave LC/EC_{50} for DCOIT was 4.7-411 µg a.s./L. These studies all have LC/EC50 values below 1.0 mg/L, the trigger value for **Category Acute 1.**

The flow-through test on mysid shrimp (*Mysidopsis bahia*) gave the lowest valid acute value for marine invertebrates exposed to DCOIT. The 96 h LC_{50}, based on mean measured concentrations, is 4.7 µg a.s./L. The 96 h NOEC was 2.8 µg a.s./L. Test concentrations were measured at 0 and 96 hours. The mean measured concentrations were 1.6-2.8-5.2-7.6-13.5 µg a.s./L. Mortality in the solvent control was 10%. Therefore the validity criterion that mortality of control animals should be <10% could not be considered fulfilled. The reliability index was set at 2 (valid with restrictions), but the test result can be accepted, as there are long-term studies available for marine fish, invertebrates and algae. (**A7.4.1.2.b/01**)

The toxicity of DCOIT towards embryos of American oyster (*Crassostrea virginica*) was tested in both natural and synthetic estuarine water in a static test system. Test concentrations were measured at 4 and 48 hours. DCOIT concentrations were declining during the test, especially in the test system with natural seawater. Nominal concentrations were 0.56, 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56 µg a.s./L. The mean measured concentrations with natural estuarine water were 0.13-0.18-0.24-0.32-0.42-0.56-2.12-12.5-40.3 µg a.s./L. The mean measured concentrations with synthetic estuarine water were 0.13-0.18-0.24-0.50-0.75-2.66-6.97-17.8-38.2 µg a.s./L. Biodegradation in the natural test system is faster and hence the EC_{50} higher. The 48 h 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. Corresponding 48h-EC_{10} values are 0.2 and 0.5 µg a.s./L in synthetic and natural estuarine water, respectively. These values are obtained by conducting a probit analysis using the mean measured concentrations. All endpoints are based on mortality. The validity criterion that the concentration of the test substance should be ≥80% of in initial concentrations is not fulfilled, and the reliability is set at 2 (valid with restrictions). (**A7.4.1.2.b/02**)

The 48h EC_{50} from a test with embryos of bay mussel (*Mytilus edulis*) was 411 µg a.s./L based on mean measured concentrations (mortality). The 48 h NOEC was 207 µg a.s./L. Test concentrations were measured at 0 and 48 hours. The mean measured concentrations were 207-426-785-1110-1460 µg a.s./L. At the two highest tested concentrations the values measured at 48 hours are 200-300% of the initial measured concentrations. Considering the fact that DCOIT is rapidly degradable in the aquatic environment and that this test is a static test, there seemed to be problems with the analytical method. However, the EC50 is well below these concentration levels and results are based on mean measured concentrations. Based on the fact that measureable concentrations of DCOIT were found in the control cultures and the deficiencies described above, the reliability is changed from 1 to 2, (valid with restrictions). (**A7.4.1.2.b/03**)

Applicant 2, Thor:

Acute toxicity of DCOIT to *Daphnia magna* was tested in a semi-static system following OECD 202 guidelines. Test concentrations were measured at 0 and 48 hours, and the mean measured concentrations were 0.6-1.2-2.4-4.64-10.04-19.16 µg a.s./L. No significant immobility (>10%) was found in the control and DCOIT treatment groups of up to and including 4.64 µg a.s./L. At 48 hours, 45% immobility was found in the 10.04 µg a.s./L treatment group and 100% immobility occurred in the highest treatment group of 19.16 µg a.s./L. The 48h EC_{50}-value was 9.7 µg a.s./L based on mean measured concentrations. (**A 7.4.1.2-01**)

This study has an EC50 value below 1.0 mg/L, the trigger value for **Category Acute 1.**

5.4.2.2 Long-term toxicity to aquatic invertebrates

Applicant 1, Dow:

Freshwater Invertebrates

The lowest chronic value from a flow-through chronic toxicity test, following U.S. EPA guideline (FIFRA 72-4), with *D. magna* is the 21-day $NOEC_{first\ generation\ survival}$ of 0.63 µg a.s./L, based on mean measured concentrations. Test concentrations were measured at 0, 7, 8, 15 and 21 days, and the mean measured concentrations were 0.63-1.1-1.8-3.1-5.4 µg a.s./L. DCOIT reacts with the algae used as feed for the daphnids and disappears from the test system, resulting in significant differences between nominal and measured test concentrations. This problem was addressed by using a high degree of test solution replacement (103 media exchanges/24 h). However, as a consequence of this high rate of volume exchange in the test system, the density of the feed decreased and thus the (solvent) control daphnids did not reproduce well. Reproductive output of adult daphnids in the control was less than the guideline-required minimum. Despite the very fast turnover, measured test concentrations were > 30% lower than nominal. The results from this study must therefore be interpreted with caution due to the insufficient reproduction of the control daphnids and DCOIT concentrations below 80% of the nominal concentrations. Due to the low reproduction in the control no $NOEC_{reproduction}$ can be derived from this test but only a $NOEC_{first\ generation\ survival}$. The test has been accepted nevertheless because this is a general problem with DCOIT, and the study's reliability is set as 2 (reliable with restrictions). (**A7.4.3.4.a/01**)

It was attempted to repeat the study, but the same problems were encountered. It seems that the chronic *D. magna* study is technically difficult to perform with DCOIT, since DCOIT rapidly reacts with algae and is therefore no longer available in the test system. While increasing the amount of feed might be a way of countering the underfeeding problem, the increased algae amount seems to lead to a decrease of DCOIT from the test solution.

Even though there are methodical problems, the study is accepted, and it has a NOEC value below 0.1 mg/L, the trigger value for **Category Chronic 1** for non-rapidly degradable substances**.**

Saltwater Invertebrates

Chronic toxicity of DCOIT to the mysid shrimp (*Americamysis bahia*) was tested in a 28-day flow-through study following U.S. EPA guideline (OPPTS 850.1350). Test concentrations were measured at 0, 7, 14, 21 and 28 days, and the mean measured concentrations were 0.277-0.627-1.24-2.39-4.97 µg a.s./L The lowest chronic value in this study was $NOEC_{first\ generation\ survival}$ of 0.63 µg a.s./L, based on mean measured concentrations. Reproduction of the control animals in this test was quite low and this indicates that the animals might not have been in a good condition. Due to this low number of offspring produced in this test, an establishment of a NOEC reproduction is not possible. However, the NOEC survival of first generation mysids, which was the most sensitive endpoint in this study, can be used (study reliability 2). (**A7.4.3.4.b/01**)

Even though there are methodical problems, the study is accepted, and it has a NOEC value below 0.1 mg/L, the trigger value for **Category Chronic 1** for non-rapidly degradable substances**.**

Applicant 2, Thor:

Freshwater invertebrates

Long-term effects of DCOIT were investigated in a 21 days semi-static test with *Daphnia magna* following OECD 211. The test media in the test groups were renewed 3 times weekly, and samples were taken from freshly prepared media after 0, 7 and 14 days and from 2 days old media after 2, 9 and 16 days. Calculated/measured concentrations were 0.1-0.4-1.3-4.6-13 µg a.s./L. The stock solution and the medium and highest test concentrations were analytically verified via HPLC. The test substance concentrations of the two lowest concentrations were calculated based on the lowest analytically verified concentration (1.3 µg a.s./L) with the separation factor of 3. The number of stillborn and immobile offspring was significantly increased in daphnia exposed to the 1.3 µg/L treatment level in comparison with the control, whilst survival of parental daphnids and the number of normal juveniles per daphnid were not affected in this treatment group. No difference to the control in any test parameter was found at the nominal 0.4 µg/L treatment level (NOEC). Both the total number of immobile offspring and the number of normal juveniles per daphnid were significantly different from the control at the 4.6 µg/L level and all parental daphnids were dead by day 6 at the treatment level of 13 µg/L. The test concentration representing the NOEC was below the lowest concentration that could be analytically verified, and was calculated based on the next higher concentration and a separation factor. However, the concentration series were prepared in a technically appropriate way and analysis of the middle and two highest concentrations reflect well the separation factor. Therefore, evidence is given that the concentration of the NOEC was estimated correctly. **(A7.4.3.4-01)**

Even though there are methodical problems, the study is accepted, and it has a NOEC value below 0.1 mg/L, the trigger value for **Category Chronic 1** for non-rapidly degradable substances**.**

5.4.3 Algae and aquatic plants

Applicant 1, Dow:

Freshwater Algae

Three DCOIT toxicity tests with freshwater algae are available: Two tests on *Navicula pelliculosa* (**A7.4.1.3.a/01**, **A7.4.1.3.a/03**) and one on *Selenastrum capricornutum* (**A7.4.1.3.a/02**). The results from these tests indicate that DCOIT is highly toxic to freshwater algae, and that *N. pelliculosa* is the most sensitive species.

There are clear methodical problems, and a reliable NOEC cannot be established for one of the *N. pelliculosa* studies and the *S. capricornutum* study. Even though there are methodical problems also with the second *N. pelliculosa* study, the study is accepted, and it shows EC50 below 1.0 µg/L, the trigger value for **Category Acute 1** and NOEC value below 0.1 mg/L, the trigger value for **Category Chronic 1** for non-rapidly degradable substances**.**

In the first test *N. pelliculosa test*, following US EPA FIFRA 123-2 guideline, DCOIT nominal test concentrations were 0.05-0.1-0.2-0.4-0.8 µg a.s./L. Test concentrations were only measured at 0 (0.041-0.145-0.224-0.364-0.798 µg a.s/L) and 120 hours. The applicant calculated 120h ErC50, EbC50 and NOErC based on initial concentrations to be 0.522, 0.371 and 0.224 µg a.s./L, respectively. However, at 120 hours, the test substance concentrations were below detection limit at all concentration levels. The removal of DCOIT from the test system is rapid. This can also be seen from the growth curves. The growth rate was similar to the control at all dose levels below 0.798 µg

a.i./L during the 48-120 hours period. Exposure concentrations in this period were assumed to be too low to affect the growth of the algae. Obviously, the effect on the growth pattern is mainly related to the effects in the early phase of the exposure, which caused a lag phase in cultures above 0.04 µg a.i./L. The NOEC increased from 0.04 µg a.i./L after 48 hours to 0.22 µg a.i./L after 96 hours. Unfortunately, the cell counts were not precise enough to allow an analysis of the growth during the first 48 hours. The 96 hours NOEC based on nominal concentrations cannot be used as an endpoint, as the removal of DCOIT from the test system is rapid. Because of this rapid decline of the test substance concentrations, the use of geometric mean concentrations over 96/120 hours would also not be meaningful. Due to the lack of precise cell density measurements, it is also not possible to calculate effect concentrations for the initial phase of the test. In conclusion, no meaningful NOEC could be established from this study.

The study was later repeated following OECD 201 and US EPA OPPTS 850.5400 guideline. The initial measured test concentrations were 1.3-2.2-3.6-6.0-10 µg a.s./L. Measurements of the test substance concentrations were undertaken every 24 hours, and NOEC values were established for 24, 48, 72 and 96 hours. However, proper monitoring of the test substance concentrations at the three lowest concentration levels after 24 h was impossible because of the rapid degradation resulting in concentrations below the detection limit. The effect of the declining test concentrations is that values for NOEC and EC50 increased during the test. The NOEC increased from 0.34 µg a.i./L at 24 hours to 0.77, 1.4 and 2.2 µg a.i./L at 48, 72 and 96 hours, respectively.

The mode of action of DCOIT implies that the sensitivity of the test is affected by the cell density. DCOIT is rapidly (within hours) taken up by the algae, and inhibits enzymes by binding to the thiol-groups of the proteins. A consequence of this binding is cleaving of the isothiazolone ring and further degradation. This means that the inhibitory effect on algae also will result in a degradation of DCOIT by algae. Therefore, a higher cell density will result in a more rapid degradation of DCOIT, a higher degree of cells that are unaffected by DCOIT and thereby a higher NOEC. The cell density in the first study was lower than in the second study (and lower than recommended in the guideline).

Because of the cell density in the first study being lower than recommended in the guideline, and DCOIT reacting stoichiometrically with the algae, the NOEC from the first study is not used further. Instead, the 24 hours NOEC (0.34 µg a.s./L) from the second test is used for classification. Using the 24 hours value is justified in this case because of the special mode of action of DCOIT. The mode of action is very rapid, leading to primary effects in the order of minutes, with side effects in the order of hours. The validity criterion that the biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72 hours test period was fulfilled. Within the first 24 hours this factor of 16 was not yet reached, and it can impossibly be reached since it would require 4 doublings per day. However, the reasoning behind this criterion is to ensure that the growth rate of the algae culture is exponential over the whole period of the test and that no lag phase occurs at the beginning of the test. The test results from the *Navicula pelliculosa* test show that the growth of the control culture has been exponential from the start of the test without a lag phase and therefore the 24 hours test result can be used. This is especially important because the relationship between exposure dose and toxicity of DCOIT is stoichiometric and given the fact that DCOIT rapidly disappears from the test system.

Using the 24 hours value is not a standard approach, as the general recommendations of the OECD 201 are to use the 72 hours interval with a possibility to reduce the duration to 48 hours. However, for several reasons, the case of DCOIT is specific: DCOIT has a unique mode of action in algae, this is a fast acting biocide and toxicity is stoichiometric and closely associated with degradation. This approach has been agreed at the Technical Meeting for Biocides in October 2007. In

conclusion, the 24h ErC50 (1.6 µg a.s./L) and NOErC (0.34 µg a.s./L) can be used for classification purposes.

In the *S. capricornutum* study, following US EPA FIFRA 123-2 guideline, the applicant calculated 96h ErC50, EbC50 and NOErC based on nomianl concentrations to be 89, 44 and 7.8 µg a.s./L, respectively. The initial measured test concentrations were 3.5-7.8-16-31-63-130 µg a.s./L, and the test substance concentrations were measured at 0, 72 and 96 hours. At 72 and 96 hours, the test substance concentrations were below the quantification limit at concentration levels below 31 µg a.s./L initial level. There was a 48 hours lag phase in the control and the solvent control cultures, where no exponential growth occurred. The removal of DCOIT from the test system was rapid, and due to the lack of analytical monitoring of the test substance concentration, no reliable NOEC could be established. Because of this rapid decline of the test substance concentrations, the use of geometric mean concentrations over 96 hours would also not be meaningful. However, from the results based on initial concentrations it is clear that *S. capricornutum* is not as sensitive as *N. pelliculosa* when exposed to DCOIT. Due to the lack of analytical monitoring of the test substance concentration, no reliable EC50 or NOEC can be established from this test.

Saltwater Algae

The toxicity of DCOIT towards the marine alga *Skeletonema costatum* was tested following U.S. EPA guideline (FIFRA 123-2) (**A7.4.1.3.b/01**) The nominal test concentrations were 0.1-0.2-0.4-0.8-1.6-3.2 µg a.s./L. The test substance concentrations were measured at 0 and 120 hours, and at 120 hours, all concentrations were below the detection limit (0.05 µg a.s./L). The results are therefore based on initial measured concentrations. Because of the rapid removal of DCOIT from the test system, and the lack of DCOIT measurements between 0 and 120 hours, the applicants 96 hour ErC50 of >3.58 µg a.s./L, EbC50 of 1.49 µg a.s./L and NOE_rC of 1.44 µg a.s./L (based on initial measured concentrations) cannot be considered reliable endpoints. Moreover, the growth rate in the controls was not exponential beyond 72 hours exposure. Because of the rapid decline of the test substance concentrations, the use of geometric mean concentrations over 96/120 hours would also not be meaningful. Therefore, results should be based on the initial phase of the test. The 24 hour NOE_rC of 0.479 µg a.s./L is therefore used as an endpoint. However, variations of the cell density measurements at 24 hours are large and the statistical power in the calculation of the NOEC is low. As a result, the apparent growth rate reduction at 0.479 µg a.s./L is statistically not significant. Moreover, the dose-response curve is very steep and therefore there is practically no difference between the NOEC and the EC_{50} at 24 hours. Lack of analytical monitoring of the test substance concentration and large variations of cell density measurements at 24 hours make the establishment of a reliable NOEC difficult. However, the 24 h NOErC of 0.479 µg/l can be used as a first approach. Due to the deficiencies described, the reliability is changed from 1 to 2 (reliable with restrictions).

Even though there are methodical problems, the study is accepted, and it has a NOEC value below 0.1 mg/L, the trigger value for **Category Chronic 1** for non-rapidly degradable substances**.**

Algae toxicity – degradation product NNOMA

The toxicity of the DCOIT degradation product NNOMA towards algae was tested in two algal species, following OECD 201 and U.S. EPA guideline (OPPTS 850.5400) (**A7.4.1.3.c/01, A7.4.1.3.c/02**).

Testing with the freshwater algae *Selenastrum capricornutum* gave a 96 h ErC50 of 9700 µg/L (NOEC: 1510 µg/L), based on mean measured concentrations. The test concentrations were 25-182-384-592-1510-3730-6740-16710 µg/L (mean measured), and the test substance concentrations were measured at 0, 72 and 96 hours. Test concentrations could not be maintained within 80% of nominal, but as results are based on mean measured concentrations, this is acceptable. The 96 hours results based on mean measured concentrations are considered the most reliable endpoints from this study. The study is considered reliable (Reliability index 1).

Testing with the marine algae *Skeletonema costatum* gave 96 h ErC50: 470 µg/L (NOEC: 130 µg/L), based on initial measured concentrations. The mean measured test concentrations were 64-130-250-510-1000 µg/L, and the test substance concentrations were measured at 0, 72 and 96 hours. The control cultures showed little growth during the first 48 hours, indicating that the inoculum culture may not have been in a good condition. The growth was however within the OECD 201 criteria for 96 hours, but below the U.S. EPA guideline criteria. The study is considered reliable, but the reliability index is changed from 1 to 2 (reliable with restrictions).

The ErC50 results from the test with *S. costatum* is below 1.0 mg/L, the trigger value for **Category Acute 1.** All other results are above the triggers for aquatic classification.

Toxicity to aquatic plants

A toxicity test was conducted with duckweed (*Lemna gibba*) in a static test system following OECD 221 and U.S. EPA guidelines (OPPTS 850.4400, TSCA 797.1160, FIFRA 122-2/123-2). The test concentrations were 4.54-11.8-21.8-46.7-104-196-444-632-1370 µg/L (initial measured), and the test substance concentrations were measured at 0 and 7 days. The control cultures grow exponentially during the entire test period, while the cultures exposed to DCOIT are inhibited mainly during the first three days of the test. The cultures with the highest concentration grew at the same rate as the controls between day 3 and 7. This shows clearly the effect of the disappearance of the test material from the solutions. As in the algae tests most of the observed effects occur within the initial phase of the test, and the differences in frond numbers or weight observed after 7 days are mainly due to growth inhibition in the initial phase of the test. Since the growth inhibiting effect is declining during the exposure period, the calculations of the endpoints are based on the initial phase of the test, in this case days 0-3. Although the analysis of the data after 7 days did not show a significant difference between the control and solvent control, the increase in frond numbers after 3 days was significantly different between the control and the solvent control. Therefore, the data for day 3 should be compared to the solvent control. The scientifically preferred endpoint growth rate, calculated for the exposure period 0-3 days, is considered to be the most relevant endpoint from this study. 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. Due to the restrictions described, the reliability index is changed from 1 to 2 (reliable with restrictions). (**A7.4.3.5.2/01**)

Even though there are methodical problems, the study is accepted, and it has a NOEC value below 0.1 mg/L, the trigger value for **Category Chronic 1** for non-rapidly degradable substances**.**

Applicant 2, Thor:

Algae

Effects of DCOIT to algae were investigated in the freshwater green alga *Scenedesmus subspicatus* (**A 7.4.1.3-01**) following OECD 201 guideline. Analytical measurement indicated that the concentration of DCOIT in the test media decreased during the test period potentially due to

sorption to the algae. Toxicity values were calculated based on arithmetic mean measured concentrations. Test substance concentration levels and control were analytically verified at the beginning (0 h) and end (96 h) of the test via HPLC. Mean measured concentrations (0 h and 96 h) were: 15, 26, 65, 175 and 376 µg a.s./L. After 96 h of exposure, algae were transferred from any treatment where growth was inhibited by more than 50% and control into fresh untreated test medium and allowed to grow for further 3-7 days under test conditions. At the start of incubation, no morphological abnormalities were observed in algae cells. At the end of incubation, morphological abnormalities were found in most algae cells at dilution levels 175 and 376 µg a.s./L. At dilution level 65 µg a.s./L, algae cells were clumped together. After 72 h, the algal biomass was statistically significantly inhibited even at the lowest test concentration of 15 µg a.s./L. Growth rate inhibition of algae was statistically significant at test concentrations of 26 µg a.s./L and higher. Therefore, the 0-72 h NOEC for biomass and growth rate were <15 and 15 µg a.s./L, respectively. Growth inhibition of algae at test concentrations of 26, 65, 175 and 376 µg a.s./L were found to be reversible within 3 to 7 days after algae were transferred to fresh untreated water. Based on the measured algae densities, the 0-72 h EbC50 and ErC50 values for the toxicity of DCOIT to *S. subspicatus* were calculated as 19 and 25 µg a.s./L, respectively.

Effects of DCOIT on the marine diatom *Phaeodactylum tricornutum* (**A 7.4.1.3-02**) following U.S. EPA guidelines (OPPTS 850.5400). Analytical measurement indicated that the concentration of DCOIT in the test media decreased during the test period potentially due to sorption to the algae. The toxicity values indicate that there appears to be no significant difference in sensitivity to DCOIT between the algal species from the two different habitats. Mean measured test concentrations were 4.3, 7.8, 16, 30, 127 and 280 µg a.s./L. After 72 h of incubation, the algal biomass and growth rate were statistically significantly inhibited at test concentrations of 7.8 µg a.s./L and higher. Therefore, the 0-72 h NOEC for biomass and growth rate was 4.3 µg a.s./L. Growth inhibition of algae at test concentrations of 30 and 127 µg a.s./L were found to be reversible within 7 and 12 days after algae were transferred to fresh untreated water, respectively. At the highest test concentration of 280 µg a.s./L, growth inhibition was found to be irreversible within 12 days. The 0-72 h NOEC was 4.3 µg a.s./L both for the inhibition of biomass and growth rate. The EC50-values for the inhibition of biomass (EbC50) and growth rate (ErC50) after 72 h were determined to be 12 and 25 µg a.s./L, respectively.

Even though there are methodical problems, the studies are accepted, and they have NOEC values below 0.1 mg/L, the trigger value for **Category Chronic 1** for non-rapidly degradable substances**.**

5.4.4 Other aquatic organisms (including sediment)

No other submitted studies that are relevant for aquatic classification.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

The criteria are taken from the Guidance on the Application of the CLP Criteria (ECHA, 2015).

Rapid degradation (II.4 - Decision scheme):

Point a (readily biodegradable): DCOIT could not be classified as readily biodegradable, due to inhibition of the inoculum by DCOIT in ready biodegradation studies.

Point b (ultimately degraded): The primary half-lives of DCOIT in the environment are very short, ranging from a couple of hours to a maximum of 4.7 days. Metabolism involves cleavage of the isothiazolone ring and subsequent oxidation, but the formation of CO_2 is limited to a maximum of 30%. DCOIT can therefore not be regarded as rapidly degradable according to the criteria.

Point c: DCOIT fulfils the first part of this point, since it shows primary half-lives well below 16 days, but the degradation product NNOMA fulfils the criteria for classification as hazardous to the environment (96h ErC50: 0.47 mg/L). Therefore, according to the decision scheme, DCOIT cannot be regarded as rapidly degradable.

For the purpose of the classification, DCOIT is considered not rapidly degradable.

Aquatic toxicity (Annex I, section 4.1):

Acute toxicity: Adequate studies with all three trophic levels show values below 1.0 mg/L, the trigger value for **Category Acute 1** (Annex I: Table 4.1.0 (a)). The lowest value for a standard species is the 24h ErC50 of 1.6 µg a.s./L for the freshwater algae *Navicula pelliculosa*. According to the criteria (Annex I: Table 4.1.3), this gives an M-factor of 100.

Acute toxicity of the degradation product **NNOMA**: A study with the algae *Skelatonema costatum* shows a 96h ErC50 of 0.47 mg/L, which is below 1.0 mg/L, the trigger value for **Category Acute 1** (Annex I: Table 4.1.0 (a)).

Chronic toxicity: Adequate studies with all three trophic levels show values below 0.1 mg/L, the trigger value for **Category Chronic 1** (Annex I: Table 4.1.0 (b) Long-term aquatic hazard (i) Non-rapidly degradable substances for which there are adequate chronic toxicity data available). The lowest values 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 (Annex I: Table 4.1.3), this gives an M-factor of 100, since DCOIT is regarded as not rapidly degradable.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Symbol: GHS09

Signal word: WARNING

Aquatic Acute 1

H400: Very toxic to aquatic life

M-factor: 100

Aquatic Chronic 1

H410: Very toxic to aquatic life with long lasting effects

M-factor: 100

6 REFERENCES

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A3.5-01	Geffke H	2003a	DCOIT-Standard Water Solubility	THOR GmbH
A3.6-01	Franke J	2008	ACTICIDE® DCOIT 100% - Calculation of the Dissociation Constant	THOR GmbH

A3.9-01	Werle H	2002	Determination of the Partition Coefficient (n-octanol/water) of DCOIT at a Range of Temperatures and pHs	THOR GmbH
A3/01 **A3.1.1** **A3.1.2** **A3.1.3** **A3.2.1** **A3.3.3** **A3.9** **A3.10**	Broughton, H.S.	1994	Product Chemistry Kathon® 287T Biocide Technical grade of Active Ingredient. Rohm and Haas Company, Report N° TR-94-25.	Rohm and Haas
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A6.1.3/02	Anonymous	1992	Kathon™ 930 biocide: acute inhalation toxicity study in rats; Rohm and Haas Company, Report N° 91R-072.	Rohm and Haas
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A6.2-03	Anonymous	2007	Dermal Absorption Study with 4,5-Dichloro-2-octyl-[ring-4,5-14C]isothiazol-3-[2H]-one in the Wistar Rat	THOR GmbH
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A6.4.1-02	Anonymous	2007a	90-Day Oral Dietary Toxicity Study with Acticide DCOIT in Beagle Dogs	THOR GmbH
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A6.8.1-01	Anonymous	2006	Two-Generation Reproduction Toxicity Study with Acticide DCOIT by Dietary Administration	THOR GmbH
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