CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Acrolein

EC Number: 203-453-4

CAS Number: 107-02-8

Index Number: 605-008-00-3

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Version number: 2 **Date: June 2011**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance Acrolein

Table 1: Substance identity

Substance name:	***Acrolein***
EC number:	203-453-4
CAS number:	107-02-8
Annex VI Index number:	605-008-00-3
Degree of purity:	92-96 %.
Impurities:	There are 3 process impurities which are all present individually at a concentration of <1%. These impurities have been taken into account in the proposed classification proposal for acrolein, and are not considered to be of additional concern. The Applicant has requested that impurities remain confidential, further information is provided in the technical dossier.

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Flam. Liq. 2 H225 Acute Tox. 2 *H330 Acute Tox. 3 *H311 Acute Tox. 3 *H301 Skin Corr. 1B H314 Aquatic Acute 1H400	F; R11 T+; R26 T; R24/25 C; R34 N; R50

Current proposal for consideration by RAC	Acute Tox. 1 H330 Acute Tox. 2 H300 Acute Tox. 3 H311 Aquatic Chronic 1 H410 Acute M-factor 100 Chronic M-factor 1	T+; R26/28 T; R24 Cn ≥ 25% N;R50
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Flam. Liq. 2 H225 Acute Tox. 1 H330 Acute Tox. 2 H300 Acute Tox. 3 H311 Skin Corr. 1B H314 Aquatic Acute 1 H400 Aquatic Chronic 1 H410 Acute M-factor 100 Chronic M-factor 1	F; R11 T+; R26/28 T; R24 C; R34 N; R50 Cn ≥ 25% N;R50

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

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	Flam. Liq. 2 H225	Not applicable	Flam. Liq. 2 H225	Not applicable
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
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	Data lacking
3.1.	Acute toxicity - oral	Acute Tox. 2 H300	Not applicable	Acute Tox. 3* H301	Not applicable
	Acute toxicity - dermal	Acute Tox. 3 H311	Not applicable	Acute Tox. 3 H311	Not applicable
	Acute toxicity - inhalation	Acute Tox. 1 H330	Not applicable Not applicable	Acute Tox. 2 H330	Not applicable
3.2.	Skin corrosion / irritation	Skin Corr. 1B H314	Skin Corr. 1B H314 Cn ≥ 1%	Skin Corr. 1B H314	Not applicable
3.3.	Serious eye damage / eye irritation	Not applicable substance is classified as corrosive	Not applicable	Not applicable substance is classified as corrosive	Not applicable substance is classified as corrosive
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	Inconclusive
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 H400 Aquatic Chronic 1 H410	Acute M factor 100 Chronic M-factor 1	Aquatic Acute 1 H400	Not applicable
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	Data lacking

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

Labelling:

Signal word: Danger
Hazard statements:

Flam liq.2 H255

Acute Tox 1 H330

Acute Tox 2	H300
Acute tox 3	H311
Skin Corr 1B	H314
Aquatic acute 1	H400
Aquatic Chronic 1	H410

Precautionary statements:
Annex VI does not include precautionary statements

Proposed notes assigned to an entry**:**

The substance already has agreed classification for the environment in Annex VI. For the environmental classification, this dossier concerns the derivation of concentration limits and M-factors and the Aquatic Chronic classification reflecting the 2nd ATP to CLP in Commission Regulation (EU) No 286/2011. The data presented in this dossier relating to the environment has been reviewed under the EU Existing Substances Regulation and by the UK Competent Authority for biocides registration

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Oxidising properties	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Flammability	F; R11	Not applicable	F; R11	Not applicable
Other physico-chemical properties *[Add rows when relevant]*	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Thermal stability	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Acute toxicity	T+; R26/28 T; R24		T+; R26 T; R24/25	
Acute toxicity – irreversible damage after single exposure	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Repeated dose toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Irritation / Corrosion	C; R34	C; R34 Cn ≥ 1%	C; R34	
Sensitisation	Not classified	Not applicable	Not classified	Data lacking for respiratory sensitization Inconclusive for skin sensitisation
Carcinogenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Mutagenicity – Genetic toxicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – development	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
Environment	N; R50	N; R50 Cn ≥ 0.25%	N; R50	Not applicable

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Indication of danger: F; T+; N
R-phrases: F; R11; T+; R26/28 T; R24 C; R34 - N; R50

S-phrases: S23, S26, S28, S36/37/39, S45, S61

2 BACKGROUND TO THE CLH PROPOSAL

Acrolein is a biocide that has been reviewed under the Biocidal Products Directive (98/8/EC) for use as a slimicide (product type 12).

In accordance with Article 36(2) of EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures, acrolein should now be considered for harmonised classification and labelling. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of acrolein under Directive 98/8/EC. Document IIA (June 2009) of the assessment is attached to the technical dossier.

A risk assessment report (RAR) has been prepared for Acrolein under Commission Regulation (EC) 793/93 (reference 2). The information in this report is consistent with that in the RAR.

The information in this report is consistent with that submitted in the REACH registration dossiers submitted for Acrolein to date.

2.1 History of the previous classification and labelling

The harmonised classification for acrolein was moved into Annex VI of the CLP Regulation when Directive 67/548 was repealed. The original harmonised classification (67/548) position was adopted after discussions by the then TC C&L in 1999.

2.2 Short summary of the scientific justification for the CLH proposal

Acrolein is a biocide, and in accordance with Article 36(2) of the CLP regulation, a full classification proposal is required. This proposal amends the classification for acute toxicity (oral and inhalation).

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Flam liq.2	H255
Acute Tox 2*	H330
Acute Tox 3*	H311
Acute tox 3*	H301
Skin Corr 1B	H314
Aquatic acute 1	H400

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

F; R11, T+; R26, T; R24/25, C; R34, N; R50

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Refer to the current Annex VI entry

2.4.2 Current self-classification and labelling based on DSD criteria

Refer to the current Annex VI entry.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Acrolein is a biocide that has been reviewed under the Biocidal Products Directive (98/8/EC) for use as a slimicide (product type 12).

In accordance with Article 36(2) of EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures, acrolein should now be considered for harmonised classification and labelling. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of acrolein under Directive 98/8/EC. Document IIA (June 2009) of the assessment is attached to the technical dossier.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

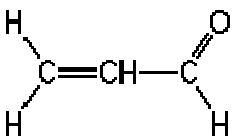
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	203-453-4.
EC name:	acrylaldehyde
CAS number (EC inventory):	107-02-8
CAS number:	107-02-8
CAS name:	2-Propenal
IUPAC name:	acrylaldehyde
CLP Annex VI Index number:	605-008-00-3
Molecular formula:	C_3H_4O
Molecular weight range:	56.0633

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Acrolein	≥ 96 %.	> 92% - <96.3%	

Current Annex VI entry:

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks

There are a number of process impurities which are all present individually at a concentration of <1%. These impurities have been taken into account in the proposed classification proposal for acrolein, and are not considered to be of additional concern. The Applicant has requested that impurities remain confidential, further information is provided in the technical dossier.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

There is 1 additive present at < 1%. This has been taken into account in the proposed classification proposal for acrolein, and is not considered to be of additional concern. Further information is provided in the technical dossier.

1.2.1 Composition of test material

The minimum purity of acrolein is 92% . The toxicity studies detailed in this report were conducted using acrolein with a purity of 92-96%.

1.3 Physico-chemical properties

Table 9: 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	Liquid – clear	Reference 1 Table 1.3	Observation
Melting/freezing point	- 87 °C	Reference 1 Table 1.3	Literature value
Boiling point	52.8 °C	Reference 1 Table 1.3	OECD 103
Relative density	0.8875 at 20 °C	Reference 1 Table 1.3	OECD 109 (Pycnometer)
Vapour pressure	31920 Pa at 25 °C	Reference 1 Table 1.3	OECD 104 (DTA Dynamic Method)
Surface tension	73.2 mN/m	Reference 1 Table 1.3	OECD 115 (Harmonised Ring Method)
Water solubility	237628 mg/L at pH 7 and 25 C	Reference 1 Table 1.3	OECD 105 (Shake Flask)
Partition coefficient n-octanol/water	0.04 at pH 7 and 20 C	Reference 1 Table 1.3	Similar to OECD 107
Flash point	-25 °C	Reference 1 Table 1.3	Dir 92/69/EEC A9 (Pensky Martens)
Flammability	Spontaneous ignition temperature is 234C	Reference 1 Table 1.3	Exothermic polymerisation can occur in contact with light or air. However, acrolein contains a stabiliser to prevent this.
Explosive properties	Not classified	Reference 1 Table 1.3	No test data available. The properties of acrolein are well known, and do not meet the criteria for classification as an explosive.
Oxidising properties	.Not classified	Reference 1 Table 1.3	Examination of the chemical structure of acrolein establishes that it does not contain any chemical groups typical for oxidizing agents. Thus the active substance can be regarded as incapable of reacting exothermically with a combustible material such as powdered cellulose
Dissociation constant	Acrolein has no acidic or basic functional groups	Reference 1 Table 1.3	

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

Acrolein is a slimicide for use in the oil recovery industry (Product type 12 of the EU Biocidal Products Directive).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

See table 9			

3.1 *Flammability]*

3.1.1 Summary and discussion of flammability

Acrolein has a flash point of -25 ^{o}C and a boiling point of 52.8 ^{o}C (Reference 1 table 1.3).

3.1.2 Comparison with criteria

A liquid substance with a flash point of < 23 ^{o}C and a boiling point > 35 ^{o}C is classified as Flam Liq 2 H225 under CLP. A liquid substance with a flash point of < 21 ^{o}C and a boiling point > 35 ^{o}C is classified as F; R11 under DSD.

3.1.3 Conclusions on classification and labelling

Acrolein is classified with Flam Liq 2 H225. Classification with F; R11 under DSD is also applicable.

4 HUMAN HEALTH HAZARD ASSESSMENT

The minimum purity of acrolein is 92% . The toxicity studies detailed in this report were conducted using acrolein with a purity of 92-96%.

A detailed summary of the available studies has been reviewed under the Biocidal Products Directive (98/8 EC), see Document IIA attached to the technical dossier. The key information pertinent to determining a classification position is presented below.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

At low dose levels (of the order of 2.5 mg/kg) acrolein is well absorbed following oral administration and is predicted to be well absorbed following dermal application or inhalation exposure. However, at higher dose levels (15 mg/kg) in the rat, polymerisation of the substance occurs and oral absorption is reduced. Following absorption, radiolabel is widely distributed. Acrolein is extensively metabolised, with the major metabolic pathways likely to involve oxidation/hydrolysis and glutathione conjugation. The majority of radiolabel was eliminated within

48 hours of dosing, with the urine and exhaled $CO_{II\ A}$eing the major routes of excretion. Radiolabel has been found in the milk of lactating goats, therefore it is possible that exposure of infants via human breast milk could occur. As distribution is widespread, *in utero* exposure of the developing foetus is possible. Bioaccumulation is not anticipated based on the low percentage of radiolabel present in tissues 7 days post dose.

4.1.2 Human information

There is no information available on the toxicokinetics of acrolein in humans.

4.1.3 Summary and discussion on toxicokinetics

See section 4.1.1 above

4.2 Acute toxicity

The acute toxicity of acrolein has been well investigated in standard studies, in rats and mice by the oral route of exposure, in rabbits via the dermal route and in rats via the inhalation route.

Table 11: Summary table of relevant acute toxicity studies

Acute Oral Studies			
Method	**Results**	**Remarks**	**Reference**
• Rat, Sprague-Dawley • M&F, 5/sex/group • Vehicle not described • 0, 10, 15, 20, 25, 30 mg/kg • 15d post-exposure period	• M: LD_{50} of 10.3 mg/kg • F: LD_{50} of 11.8 mg/kg	• Mortalities were observed at all doses, 0, 2, 4, 5, 5, 5, and 0, 1, 4, 5, 5, 5, in males and females at doses of 0, 10, 15, 20, 25, and 30 mg/kg respectively. Mortalities occurred within 1-day of dosing. Clinical signs of toxicity were observed in both sexes, comprising lethargy and hypothermia at doses of 15 mg/kg and above, and changes in respiration rate (no further information provided) at 25 mg/kg and above. No treatment-related gross necropsy changes were observed,	Ref 1 - Biocides Document II A David, 1989
• Mouse, CD-1 • Females only • 10/group • Vehicle deionised water • 0, 11.0, 13.2, 15.8, 19.0 mg/kg • 15d post-exposure period	• LD_{50} of 17.7 mg/kg	• Mortalities (0, 0, 3 and 6 at 0, 11, 13.2, 15.8 and 19 mg/kg) were observed at doses of 13.8 mg/kg and above. Lethargy was observed in all treated animals, and respiratory distress at doses of 15.8 mg/kg and above. The most prominent necropsy observation was haemorrhagic stomach and intestine in decedent animals. No adverse necropsy findings were observed in animals sacrificed at study termination .	Ref 1 Biocides Document II A Muni, 1981b
• Mouse, CD-1 • Males only • 10/group • Vehicle deionised water • 0, 11.0, 13.2, 15.84, 19.0 mg/kg • 15d post-exposure period	• LD_{50} of 13.9 mg/kg	• Mortalities (4, 4, 8 and 6 at 0, 11, 13.2, 15.8 and 19 mg/kg) were observed. Lethargy, squinted eyes, rough coat, hunched posture and pilo erection were observed in all treated animals, and respiratory distress at doses of 15.8 mg/kg and above. The most prominent necropsy observation was haemorrhagic stomach and intestine, and reddening of the lungs in decedent animals. No adverse necropsy findings were observed in animals sacrificed at study termination.	Ref 1 Biocides Document II A Mansur, C.A., (1983a)
Acute Inhalation Studies			

Method	Results	Remarks	Reference
• Rat, Sprague-Dawley • M&F, 5/sex/group • 1 hr: 31.2, 49.0, 53.4, 69.0, 180.2, mg/m^3 • 4 hr: 10.7, 15.6, 20.2, 26.9mg/m^3 • Whole body, vapour • 14d post-exposure period	• LC_{50} 57.9 mg/m^3 and 18.5 mg/m^3 for one and four hour exposures, respectively, (equivalent to 0.058 and 0.018 mg/l)	• Mortalities were observed at all concentrations for one hour (0/0 1/0, 1/2, 5/5 and 5/5 - m/f-at 0.031, 0.049, 0.053, 0.069 and 0.18 mg/l respectively) and four hour exposure periods (0/0 0/3, 4/3 and 3/3 - m/f-at 0.011, 0.016, 0.02, and 0.027 mg/l respectively), from day 1 to day 6 post exposure. • Respiratory difficulties (audible respiration, "gasping" and a decrease in respiration rate) were observed in animals of all dose groups. Gross necroscopy revealed fluid in the trachea and thoracic cavity and gas in the stomach and intestines in animals which died during the study. • All clinical signs except perinasal and periocular encrustation, and unkempt fur resolved by the end of week 1 of the 2-week post exposure observation period.	Ref 1 Biocides Document II A Nachreiner & Dodd, 1987

Acute Dermal Studies

Method	Results	Remarks	Reference
• Rabbit, New Zealand white • M&F, 10/sex/group • Vehicle absolute ethanol: water 50/50 vv • 200, 240, 288 mg/kg • 14d post-exposure period • Exposure time not reported	• LD_{50} of 231.4 mg/kg (all animals) • Male 240 mg/kg • Female 233 mg/kg	• Mortality occurred at all dose levels tested (3/4, 7/7 and 5/8 m/f at 200, 240 and 288 mg/kg), occurring from 2 hours post application to 3-9 days post application. • Clinical signs suggesting that the animals were in severe pain and hyperactive behaviour was observed initially in all animals, followed by lethargy, respiratory distress and cyanosis. Ulceration, oedema and haemorrhage of the dermis and skin discoloration occurred in all dose groups. • Necropsy examination found pulmonary petechiae (red spots) and atelectasis (collapsed lung) at all treatment levels. It is possible that the pulmonary effects observed after dermal exposure could be due to vaporisation and inhalation of acrolein, rather than systemic toxicity.	Ref 1 Biocides Document II A Muni, (1981a)

4.2.1 Non-human information

4.2.1.1 Acute toxicity oral:

Acrolein is very toxic following acute oral exposure with LD_{50} values of similar magnitude being reported in both rats and mice (10.3 mg/kg and 11.8 mg/kg for male and female rats, respectively and 13.9 mg/kg and 17.7 mg/kg for male and female mice, respectively).

4.2.1.2 Acute toxicity: inhalation

Overall, acrolein is very toxic to rats following inhalation exposure.

4.2.1.3 Acute toxicity: dermal

Overall, acrolein is toxic following acute dermal administration to rabbits (LD_{50} 231 mg/kg).

4.2.1.4 Acute toxicity: other routes

There is no information available on the acute toxicity of acrolein by other routes of exposure

4.2.2 Human information

There is no information available on the acute toxicity of acrolein in humans, by any route of exposure.

4.2.3 Summary and discussion of acute toxicity

Following single exposure, acrolein is very toxic via the oral (10.3-11.8 mg/kg in rats) and inhalation routes (4-hour LC_{50} of 0.0185 mg/l), and toxic following dermal application (LD_{50} 231 mg/kg).

4.2.4 Comparison with criteria

Comparing the LD_{50} and LC_{50} values with the criteria in CLP (Regulation 1272/2008) and Directive 67/548 indicates that classification is justified for all three routes of exposure.

The relevant CLP criteria are; $\leq$0.5 mg/l for acute inhalation toxicity 1 (vapours), 5 – 50 mg/kg for acute oral toxicity category 2 and 200 – 1000 mg/kg for acute dermal toxicity category 3.

The relevant DSD criteria are; $\leq$0.5 mg/l for R26 (vapours), $\leq$ 25 mg/kg for R28 and > 50 – $\leq$ 400 mg/kg R24.

4.2.5 Conclusions on classification and labelling

CLP Regulation:

Proposal;

Acute inhalation toxicity Category 1 H330

Acute oral toxicity Category 2 H300

Acute dermal toxicity Category 3 H311

Directive 67/548/EEC: Proposal T+; R26/28 T; R24

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

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

Acrolein has been well investigated for acute toxicity, by all three relevant routes of exposure. No evidence was found that acrolein causes Specific Target Organ Toxicity.

As acrolein is a corrosive substance, a separate classification for STOT-SE3 (respiratory tract irritation) is considered unnecessary.

4.3.2 Comparison with criteria

As acrolein did not cause specific target organ toxicity, by any relevant route of exposure, no classification is required for this end point.

4.3.3 Conclusions on classification and labelling

No classification is proposed.

4.4 Irritation

As the substance is corrosive, information on skin irritation is discussed in section 4.5.

4.4.1 Skin irritation

4.4.1.1 Non-human information

4.4.1.2 Human information

4.4.1.3 Summary and discussion of skin irritation

4.4.1.4 Comparison with criteria

4.4.1.5 Conclusions on classification and labelling

4.4.2 Eye irritation

Table 12 Summary table of relevant eye irritation studies

Species/ No./group	Average score 24, 48, 72 h				Reversibility (Y/N)	Result	Reference
	Cornea	Iris	Conjunctiva				
			Redness	Chemosis			
Rabbit, New Zealand White 9 animals	4	2	4	2	Not reversible after 7-days	Irritating	Ref 1 Biocides Document II A Goodband & Dunn, (1980)

Eye irritation was manifested as complete corneal opacity; deepened folds, congestion or swelling of the iris; and crimson red, swollen conjunctiva with the lids more than half closed in all surviving animals. Overall, the findings from this study indicate that acrolein causes severe eye irritation.

4.4.2.1 Non-human information

4.4.2.2 Human information

No data area available

4.4.2.3 Summary and discussion of eye irritation

The available data on acrolein show that the substance causes severe damage to the eyes. As the substance is classified as corrosive this is discussed further in section 4.5.

4.4.2.4 Comparison with criteria

As the substance is classified as corrosive a classification for severe eye damage is considered to be implicit. This is discussed further in section 4.5.

4.4.2.5 Conclusions on classification and labelling

As the substance is classified as corrosive a classification for severe eye damage is considered to be implicit.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

The only useful information comes from single inhalation exposure studies in rats (section 4.2.1.2), and repeated inhalation exposure studies in rats (section 4.1.6.2), rabbits, hamsters, guinea pigs, dogs and monkeys. There was no evidence that acrolein caused histopathological changes to the upper respiratory tract or relevant clinical signs of toxicity after single or repeated inhalation exposure.

4.4.3.2 Human information

There is no information relating to the respiratory tract irritation potential of acrolein in humans

4.4.3.3 Summary and discussion of respiratory tract irritation

There is no evidence from single and repeated exposure studies in a number of experimental animal species that acrolein caused clinical signs of toxicity or damage to the upper respiratory tract consistent with respiratory tract irritation.

4.4.3.4 Comparison with criteria

As there is no information on respiratory tract irritation potential of acrolein in humans, the only useful information comes from single and repeated inhalation exposure studies in experimental animals. As no clinical signs of toxicity or histopathological changes, consistent with respiratory tract irritation, were observed no classification is proposed.

4.4.3.5 Conclusions on classification and labelling

Respiratory tract irritation	
CLP Regulation:	**No classification is proposed**
Directive 67/548/EEC:	**No classification is proposed**

4.5 Corrosivity

Table 13: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
Rabbit, New Zealand White 6 animals	Erythema (average score at 24, 48 and 72 hours) 1, *, 1 (unabraded) 1, *, 1 (abraded) Oedema (average scores at 24, 48 and 72 hours 3, *, 3 (unabraded) 3, *, 3 (abraded)	2 animals died – cause not determined Not reversible in 3/4 survivors after 14 days	Ref 1 Biocides Document II A Goodband (1981)
Human Volunteer patch test 0.01, 0.1, 1 and 10% acrolein in ethanol	1% - 6/48 positive 10% - 20/20 positive	See below	Ref 1 Biocides Document II A Lacroix *et al*., 1976

4.5.1 Non-human information

Erythema and oedema were observed 24 and 72 hours after exposure to acrolein (up to grade 4 oedema). These skin reactions failed to resolve after 14 days, indeed in 3/4 survivors the responses became progressively more severe.

4.5.2 Human information

Human volunteer patch tests were conducted with acrolein in ethanol at concentrations of 0.01, 0.1, 1 and 10% on groups of 8, 10, 48 and 20 volunteers respectively (Lacroix *et al*., 1976). No further information is available, including duration of application. At 1%, positive skin reactions were recorded in 6 out of 48 subjects (12.5%); four of the six with serious oedema and bullae (fluid filled blister - between dermis and epidermis) and the remaining two with erythema. At 10% all subjects (n = 20) showed skin effects with bullae, necrosis, inflammatory cell infiltrate and papillary oedema. No adverse skin reactions were observed at 0.01 (n = 8) or 0.1% (n = 10). Overall, these findings in humans indicate that acrolein is corrosive.

4.5.3 Summary and discussion of corrosivity

Acrolein caused severe adverse skin reactions in a non standard study in human volunteers, indicative of skin corrosivity. Acrolein also caused severe skin reactions in a standard study in rats, which became progressively more severe over the 14-day observation period. Severe skin reactions were also observed in rabbits after single (table 11 and section 4.2.1.3) and repeated dermal application (section 4.7).

4.5.4 Comparison with criteria

There are no criteria regarding the interpretation of severe skin reactions in humans in terms of skin irritation/corrosivity. However, taking a weight of evidence assessment, it can be concluded that acrolein is corrosive and the existing classification (Skin Corrosive 1B) should remain. Classification for corrosivity also includes classification for severe eye irritation.

The study by Lacroix *et al* (1975) suggests that acrolein does not cause adverse skin effects at a concentration of 0.1% and is corrosive at 1%. Therefore a specific concentration limit of 1% is proposed, based on human data.

4.5.5 Conclusions on classification and labelling

CLP Regulation:	**Confirm Skin Corrosivity 1B H314**
Directive 67/548/EEC:	**Confirm Corrosive C; R34**
SCL: Skin Corr 1B (C; R34) Cn ≥ 1%.	

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 14: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
• Guinea pigs • Similar to OECD TG 406 • 0.01% concentration for intradermal induction • 2.5% concentration for topical induction • 0.5% concentration for challenge	• 7/15 test • 1/15 controls		Ref 1 (Biocides Document II A) Susten and Breitenstein, (1990

4.6.1.1 Non-human information

4.6.1.2 Human information

4.6.1.3 Summary and discussion of skin sensitisation

In a brief published report acrolein induced positive skin reactions in 7/15 (46%) test and 1/15 (6.6%) control animals. The protocol employed was similar to the guinea pig maximisation test described in OECD TG 406. Limited information on the skin reactions reported was provided. These changes were described by the authors as 'patches of redness, non-confluent of grade 0.5 severity'. Information, provided by industry on the skin reactions reported in this study suggests they equate to a score of 1 using the scoring system in OECD TG 406. The positive control substance (1-chloro-2,4-dinitrobenzene) gave the expected results. No further information is available on this study.

4.6.1.4 Comparison with criteria

The findings of this study were discussed by the former TC C&L in October 1999 (ECBI/61/99 Rev 2), including the industry interpretation of the skin reactions. The TC C&L concluded that classification was not justified. There is no new information on the skin sensitisation potential of acrolein.

Both the CLP Regulation (1272/2008) and Directive 67/548 indicate that classification is justified with positive skin reactions in at least 30% of the test animals in a maximisation test. It is usual to consider a positive skin reaction to be grade 1 and above. However, there is insufficient information in the brief test report relating to the skin reactions to indicate that a change in classification is justified.

Conclusions on classification and labelling

Skin Sensitisation:

CLP Regulation: **No Classification is proposed**

Directive 67/548/EEC:	**No Classification is proposed**

4.6.2 Respiratory sensitisation

There is no information on the potential of acrolein to induce respiratory sensitisation

4.7 Repeated dose toxicity

The repeat dose toxicity of acrolein has been well investigated by the oral route, with studies available in the rat (90 day and 2 year), the mouse (14 day and 18 month), and the dog (1 year). The dermal toxicity of acrolein has been investigated in a 21 day study in rabbits. The inhalation toxicity of acrolein following repeated exposures has been investigated in studies of up to 90 days duration (Rats, Rabbits, Guinea Pigs, Dogs, Hamsters and Monkeys).

A detailed summary of all the repeated dose toxicity studies is given in the review made by the UK under the Biocidal Products Directive (98/8/EC). This review (Document IIA) is provided as an attachment to the Annex VI report.

Substances are classified for repeated dose toxicity when serious damage (‘clear functional disturbance or morphological change which has toxicological significance’) is seen following repeated or prolonged exposure below guidance values provided in the classification criteria. In this report, there is therefore a focus on whether serious damage is induced by acrolein and, if so, whether the doses at which such effects occur merit classification.

Due to the large number, the relevant studies have, for ease, been separated by route of exposure and species.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Studies in rats

Table 15: Summary table of relevant repeated dose oral toxicity studies

Method	Results	Reference
• Oral Gavage • 5-days per week for 3 months (90-days). • Rats, (Sprague-Dawley) 30/sex/dose • 0.05, 0.50, 5.0 mg/kg day • OECD TG 408, pre GLP	• There were no mortalities or treatment-related overt clinical signs of toxicity, and no changes in haematological, clinical chemistry, urinalysis or gross and histopathological findings in this study at doses of up to 5 mg/kg/day, the highest dose tested. • The NOAEL for this study is 5 mg/kg /day.	Ref 1 (Biocides Document II A) Muni, 1981c
• Oral Gavage, 2-years • Rats, (Sprague-Dawley) 70/sex/dose, and 75 sex/dose at 2.5 mg/kg/day • 0.0, 0.05, 0.5, 2.5 mg/kg/day • OECD TG 453	• 0.05 mg/kg /day: No treatment related effects • 0.5 mg/kg /day: Decrease in survival of females (24% survival) • 2.5 mg/kg /day: decrease in survival of females (33% survival) and males (57% survival). There is no clear reason for the increased mortalities, although it is possible that dosing errors could be a significant factor. • No treatment-related adverse clinical signs of toxicity were noted. • Survival in male rats was 97, 64, and 21% for 12, 18 and 24 months, respectively in controls; 96, 64, 40% at 12, 18 and 24 months, respectively in the 0.05 mg/kg /day dose group; 90, 60, 25% at 12, 18 and 24 months, respectively for the 0.5 mg/kg/day dose group and 76, 57 and 25% at 12, 18 and 24 months, respectively for the 2.5 mg/kg /day dose group. Survival was statistically significantly reduced in the top dose group. • A statistically significant increase in mortality was also observed at the end of the first year, persisting to the end of the study for female rats in the 0.5 mg/kg /day group (84, 61 and 24 % survival at 12, 18 and 24 months, respectively) and 2.5 mg/kg /day group (69, 48 and 33% survival at 12, 18 and 24 months, respectively), compared with survival percentages of 91, 66 and 34% for the 0.05 mg/kg /day dose group and 93, 69 and 40% for controls at 12, 18 and 24 months, respectively. • Histopathological examination did not reveal any toxicologically significant changes. The clinical and pathology data do not indicate why there was a high mortality rate in this study. It is possible that the observed mortalities could be treatment-related or more likely due to poor conditions or dosing technique.	Ref 1 (Biocides Document II A) Long & Johnson, (1989b), Parent., (1992)

	• A NOAEL of 0.05 mg/kg/day can be derived from this study, as mortality was observed at higher dose levels.	

Studies in mice

Table 16: Summary table of relevant repeated dose oral toxicity studies in mice

Method	Results	Reference
• Oral (gavage) • 5 days per week, for 14 days • Mouse, • (CD-1 strain) • 0, 4.6, 5.8, 7.2, 9.0 mg/kg day • 10/sex/dose	• One female mouse in the 5.8 mg/kg/day group died on day 6. Two male mice in the 7.2 mg/kg/day group died within the first week of dosing mg/kg /day group (cause of death was not reported) and one male in the 9 mg/kg/day group died (haemorrhagic lungs were revealed upon necropsy). No effects on bodyweight or food consumption were observed at any dose level. • Gross lesions found upon necropsy were observed mainly in the stomach: effects attributable to local irritation were observed on the gastric mucosa of males (white and thickened gastric mucosa occurred in 0, 0, 1, 2, 9 at 0 4.6, 5.8, 7.2 and 9 mg/kg/day, respectively; and in 6 females in the 9 mg/kg /day dose group). • Stomach ulcers, black flecks in the gastric contents and pin point raised foci/ nodules occurred in isolated animals at the highest dose only. • On the basis of the clinical signs of toxicity, gross pathology and mortality at doses of 5.8 mg/kg /day and above, a NOAEL of 4.6 mg/kg /day can be identified from this study.	Ref 1 (Biocides Document II A) • Mansur, C.A.(1983b)
• Mouse Oral (gavage) • 18 months • OECD TG 453 • Mouse (CD-1 strain) Swiss Albino • 70 per sex per group • 75 per sex per group for high-dose group • 0.0, 0.5, 2.0, 4.5 mg/kg /day	• Survival in male mice in all groups (including controls) and in female mice in the lowest dose group was less than 50% at 18 months of dosing. Mortalities were attributed to mis-dosing, or for reasons unknown. Specifically, a statistically significant increase in mortality was observed in males in the top dose group compared with controls (43, 41, 43 and 36% survival for controls, 0.5, 2, and 4.5 mg/kg /day, respectively). • Reduced mortality was also observed in females, although this was not statistically significant when compared with controls (60, 43, 57 and 57% for controls, 0.5, 2, and 4.5 mg/kg /day, respectively). Gross and histopathological examination did not reveal any toxicologically significant changes. • A NOAEL of 2 mg/kg/day can be derived from this study.	Ref 1 (Biocides Document II A) Long & Johnson, 1989a

Studies in dogs

Table 17: Summary table of relevant repeated dose oral toxicity studies in dogs

Method	Results	Reference
• Dog, (Beagle) • 6 per sex per group • 12 months • GLP but non-guideline test • 0.0, 0.1, 0.5, 2.0 mg/kg /day	• At the top dose, animals were administered 1.5 mg/kg /day for the first 26 days and at 2 mg/kg /day for the remainder of the study. Body weight, food consumption, standard clinical chemistry and haematology parameters were measured and gross and histopathological examinations were performed on all animals. • The only toxicologically significant effect noted was vomiting at doses of 0.5 mg/kg /day and above. This effect increased in frequency and incidence in the top dose. • Overall, a NOAEL of 0.1 mg/kg/day was identified in this study.	Ref 1 (Biocides Document II A) Long,(1987

4.7.1.2 Repeated dose toxicity: inhalation

There are no standard repeated-exposure inhalation studies available. The only information comes from a number of non standard studies, which are limited in terms of design, conduct and reporting, when compared to modern guidelines. In particular, very limited quantitative information is available.

Studies in rats

Table 18: Summary table of relevant repeated exposure inhalation toxicity studies in rats

Method	Results	Reference
• Rat (Fischer 344 strain) • 65 sex/group (24 males/group were examined for effects on respiratory physiology parameters) • 62-day whole body exposure • A non standard study • 0, 0.4, 1.4, 4.0 ppm • (0, 0.9, 3.2, or 9.2 mg/m^3	• A number of changes in lung parameters were observed throughout the study. An elevated expiratory flow rate was seen in the low dose group, (the expiratory flow volume at 50% of vital capacity was 87, 97, 92 and 66 ml for control, 0.4, 1.4 or 4 ppm, respectively; the sex of animal for these data is not known). • Effects similar to controls were observed in the mid-dose group. At the top dose, marked changes in tidal volume (increase of 26% compared with controls), breathing frequency (decrease of 41% compared with controls), pulmonary resistance (increase of 65% compared with controls), residual volume (71% increase compared with controls), functional residual capacity (8% increase compared with controls), total lung capacity (49% increase compared with controls), vital capacity (27% increase compared with controls) and inspiratory capacity (29% increase compared with controls) and an increase in lung compliance occurred when compared with controls. Flow rates were depressed at all lung volumes. • Histopathological changes in the lung occurred in the mid and high dose groups. Exposure to 1.4 ppm resulted in bronchiolar epithelial necrosis in 3 animals. At this concentration, increased numbers of alveolar macrophages and type II cell hyperplasia were observed as well as changes associated with chronic pneumonia or a focal subacute alveolitis. Exposure to 4.0 ppm resulted in bronchiolar necrosis and sloughing, bronchiolar oedema with macrophages present and focal pulmonary oedema in all animals. The severity of the lung lesions was variable and structural effects were not noted. • Effects on lung pathology and physiology occurred at the lowest dose tested (0.4 ppm, equivalent to 8.5 mg/m^3) therefore a NOAEC could not be determined from this study.	Ref 1 (Biocides Document II A) Kutzman, (1981, 1985) Costa., (1986)
• Rat, • 62 days, non standard protocol • 0.4, 1.4, 4.9 ppm (0.9, 3.2, 9.2 mg/m^3) • 6 hrs / day, • 5 days/ week • Whole body exposure	• Three rats/sex died in the high dose group (4.9 ppm). This study was very limited in terms of conduct, design and reporting, compared to modern standards. In particular histopathological changes were reported, but no further information in incidence, severity or type of lesion was included. • Oedema, collapsed areas of lung and haemorrhage were observed in deceased animals. A dose related decrease in body weight was observed in rats in all dose groups (although this was not significant in the low dose group). Decreases were 2/8%, 15/13% and 38/25% for males/females in 0.4, 1.4 and 4.9 ppm dose groups, respectively. An increase in relative organ weights (lung, heart, kidneys and adrenals) was	Ref 1 (Biocides Document II A) Feron 1978

	observed in the top dose group, which is considered to be secondary to the observed decreases in body weight. • Effects on the bronchi were observed in the top dose group (focal bronchopneumonia and bronchitis, bronchiolitis, increased numbers of mucus producing cells and accumulation of alveolar macrophages). Histopathological changes in the respiratory tract (destruction and hyper- and metaplasia of the epithelial lining and inflammatory alterations) were observed with increasing severity, number of sites and numbers affected in all dose groups. All of the animals in the high dose groups had changes in the epithelial lining of the nasal cavity, occasional necrotising rhinitis and tracheal effects. • Overall, based upon histopathological changes in the respiratory tract at 0.4 ppm (0.9 mg/m^3), the lowest dose tested, a NOAEC cannot be derived from this study.	
• 90 days • Non guideline study • 0.7, 3.7 ppm • (1.6, 8.5 mg/m^3) • 8 hrs/day • 5 days/ week • Whole body exposure	• There were no mortalities, body weight changes or clinical signs at the low dose. At the higher exposure, body weight gain was reported to be decreased in both sexes. However, quantitative data cannot be provided because control data were not included in the study report. All animals exposed had mild chronic inflammatory changes in the lungs and occasional emphysema in the low dose group. • Overall, as emphysema was observed at the lowest concentration tested (0.7 ppm or 1.6 mg/m^3), a NOAEC could not be determined from this study.	Ref 1 (Biocides Document II A) Lyon *et al*., 1970
• Rat, • 90 days • Non guideline study • 0.22, 1.0 and 1.8 ppm • (0.5. 2.3 and 4.1 mg/m^3) 24 hrs/day • Whole body exposure	• The nose was not examined microscopically and no organ weights were recorded. No effects were observed in rats at 0.22 ppm. • Weight gain of rats was reported to be significantly lower than controls in the 1 and 1.8 ppm dose groups, however, no quantitative information was provided to support this statement. At 1 ppm, three of nine rats showed occasional pulmonary haemorrhage. Non-specific inflammatory changes were observed in sections of brain, heart, lung and liver. • A NOAEC of 0.22 ppm (0.5 mg/m^3) has been determined for rats for continuous exposure, based on reported decreases in weight gain and pulmonary haemorrhage.	Ref 1 (Biocides Document II A) Lyon *et al*., 1970

Studies in rabbits

Table 19: Summary table of relevant repeated exposure inhalation toxicity studies in rabbits

Method	Results	Reference
• 62 days, non standard protocol • 4, 1.4, 4.9 ppm • (0.9, 3.2, 9.2 mg/m^3) • 6 hrs / day, • 5 days/ week • Whole body exposure	• In the top dose group, clinical signs of toxicity included laboured breathing/ sneezing. A decrease in body weight (12 %) and an increase in relative lung weight were observed. • No toxicologically significant changes were reported in the low dose group. • At the intermediate exposure level, histopathological examination revealed minimal inflammatory changes. At the highest concentration tested, histopathological examination revealed changes in the epithelial lining of the nasal cavity, described as occasional necrotising rhinitis and tracheal change in all animals. A NOAEC of 0.4 ppm (0.9 mg/m^3) was identified from this study, based upon inflammatory changes in the respiratory tract and decreases in body weight at concentrations of 1.4 ppm (3.2mg/m^3) and above.	Ref 1 (Biocides Document II A) Feron 1978

Studies in hamsters

Table 20: Summary table of relevant repeated exposure inhalation toxicity studies in hamsters

Method	Results	Reference
• 62 days, non standard protocol • 0.4, 1.4, 4.9 ppm • (0.9, 3.2, 9.2 mg/m^3) • 6 hrs / day, • 5 days/ week • Whole body exposure	• At the top dose level, salivation and nasal discharge were reported; a decrease in body weight (20/31% for males/females) and an increase in the relative weight of lungs, hearts and kidneys was observed; a statistically significant increases erythrocyte count, packed cell volume, haemoglobin content and number of lymphocytes and decreases in neutrophilic leucocytes were reported; while histopathological investigations revealed changes in the epithelial lining of the nasal cavity, occasional necrotising rhinitis and tracheal effects. • In the mid dose group, histopathological examination revealed minimal inflammatory changes in the respiratory tract. • Overall, a NOAEC of 0.4 ppm (0.9 mg/m^3) was identified, based upon inflammatory changes in the respiratory tract at concentrations of 3.2 mg/m^3 and above	Ref 1 (Biocides Document II A) Feron (1978)

Studies in guinea pigs

Table 21: Summary table of relevant repeated exposure inhalation toxicity studies in guinea pigs

Method	Results	Reference
• Guinea Pig, • 90 days Non guideline study • 0.7, 3.7 ppm • (1.6, 8.5 mg/m^3) • 8 hrs/day • 5 days/ week • Whole body exposure	• In the high dose group, non-specific inflammatory changes were noted in the lungs, liver and kidney. In the low dose group, mild chronic inflammatory changes in the lungs and occasional emphysema were observed. • It was not possible to identify a NOAEC, as inflammatory changes in the lungs and emphysema occurred at 0.7 ppm (1.6 mg/m^3) the lowest concentration tested.	Ref 1 (Biocides Document II A) Lyon *et al*., (1970)
• Guinea Pig, • 90 days • Non guideline study • 0.22, 1.0 and 1.8 ppm • (0.5. 2.3 and 4.1 mg/m^3) 24 hrs/day • Whole body	• Non-specific inflammatory changes were present in sections of liver, lung, kidneys and heart from guinea pigs at 0.22 ppm. Guinea pigs exposed to 1 ppm showed various degrees of pulmonary inflammation and focal liver necrosis occurred without any specific pattern at this level. • Non-specific inflammatory changes were observed at all concentrations. Therefore, a NOAEC could not be determined from this study.	Ref 1 (Biocides Document II A) Lyon *et al*., (1970)

Studies in dogs

Table 22: Summary table of relevant repeated exposure inhalation toxicity studies in dogs

Method	Results	Reference
• , Dog • 90 days • Non guideline study • 0.7, 3.7 ppm • (1.6, 8.5 mg/m^3) • 8 hrs/day • 5 days/ week • Whole body exposure	• In the 3.7 ppm group, dogs salivated excessively, blinked frequently and kept their eyes closed for prolonged periods; signs of eye irritation were present in these animals for the next four weeks. Histopathological examination revealed squamous metaplasia and basal cell hyperplasia of the trachea and bronchopneumonia; while non-specific inflammatory changes were noted in sections of lung, liver and kidney. Animals exposed to 0.7 ppm showed mild chronic inflammatory changes in the lungs and occasional emphysema. • Mild chronic inflammatory changes in the lungs and occasional emphysema occurred at the lowest dose tested (0.7 ppm or 0.5 mg/m^3); therefore a NOAEC could not be identified from this study.	Ref 1 (Biocides Document II A) Lyon *et al*., (1970)
• Dog • 90 days • Non guideline study • 0.22, 1.0 and 1.8 ppm • (0.5. 2.3 and 4.1 mg/m^3) 24 hrs/day • Whole body exposure	• In dogs exposed to 0.22 ppm moderate emphysema, acute congestion, focal vacuolization of the bronchiolar epithelial cells with increased secretory activity and some constriction of the bronchioles was observed in 2 of the dogs, while hyperplasia of the thyroid gland was seen in the other two dogs. Dogs exposed to 1 ppm had ocular and nasal discharge, which decreased in severity as the study progressed; bronchiolitis and early bronchopneumonia was observed in one dog; and inflammatory reactions involving the lung, liver, heart and brain were reported. • Dogs exposed to 1.8 ppm exhibited excessive salivation and ocular discharge. In addition, confluent broncho-pneumonia was observed in all animals; and inflammatory reactions involving the lung, liver, heart and brain were reported. • Moderate emphysema, acute congestion, focal vacuolization of the bronchiolar epithelial cells with increased secretory activity and some constriction of the bronchioles occurred at all concentrations tested therefore a NOAEC could not be identified from this study.	Ref 1 (Biocides Document II A) Lyon *et al*., (1970)

Studies in monkeys

Table 23: Summary table of relevant repeated exposure inhalation toxicity studies in monkeys

Method	Results	Reference
• Monkey, • 90 days • Non guideline study • 0.7, 3.7 ppm • (1.6, 8.5 mg/m^3) • 8 hrs/day • 5 days/ week • Whole body exposure	• In the 3.7 ppm group, 2 animals died: one had several small pulmonary hepatic and splenic lesions, the other had several small liver lesions and haemorrhagic spots in both lungs. Surviving monkeys in this dose group salivated excessively, kept their eyes closed for prolonged periods and when they did open their eyes, they blinked frequently. Squamous metaplasia and basal cell hyperplasia of the trachea was found as well as necrotising bronchitis and bronchiolitis with squamous metaplasia of the lungs. • In the 0.7 ppm (1.6 mg/m^3) group, mild chronic inflammatory changes in the lungs and occasional emphysema were reported, therefore a NOAEC could not be determined from this study.	Ref 1 (Biocides Document II A) Lyon *et al*., (1970)
• Monkey, • 90 days • Non guideline study • 0.22, 1.0 and 1.8 ppm • (0.5. 2.3 and 4.1 mg/m^3) 24 hrs/day • Whole body exposure	• At 1.8 ppm monkeys showed excessive salivation and ocular discharge; all animals showed squamous metaplasia and 6/9 monkeys showed basal cell hyperplasia of the trachea. Non-specific inflammatory changes were observed in sections of brain, heart, lung and liver of the animals. • At the 1 ppm level, animals kept their eyes closed for extended periods. • At 0.22 ppm non-specific inflammatory changes were also present in sections of liver, lung, kidneys and heart. Non-specific inflammatory changes occurred at all concentrations tested (0.7 ppm or 0.5 mg/m^3) therefore a NOAEC could not be determined from this study.	Ref 1 (Biocides Document II A) Lyon *et al*., (1970)

4.7.1.3 Repeated dose toxicity: dermal

Table 24: Summary table of relevant repeated dermal exposure toxicity studies in rabbits

Method	Results	Reference
• Rabbit, New Zealand White, • 10 per sex per dose level • 21-days • OECT TG 410 • 0, 7, 21, 63 mg/kg/day	• One female in the 21 mg/kg /day and one female in the 63 mg/kg/day dose groups died in the first week of dosing; and one female in the 7 mg/kg/day group and one in the 63 mg/kg/day group were sacrificed as a result of treatment-related local toxicity. Dermal application of 7 mg/kg and above resulted in local irritation which became more severe with increased in dose and duration. Slight to moderate erythema and oedema of the skin of almost all rabbits was found in animals receiving 7 or 21 mg/kg/day. Animals given 63 mg/kg/day had a similar severity of erythema to the two lower dose levels, but more pronounced oedema. Increases in the incidences of nasal mucous discharge, interstitial pneumonia and lethargy were observed at all doses, with the incidences occurring in a dose dependant manner. The lung toxicity observed may have been as a result of inhalation of acrolein vapours due to the volatile nature of the substance. • Compared to controls, a marked decrease in body weight gain was observed at all doses (30 – 70%, 30 - 70% and 80-90 % reduction for males/females at 7, 21 and 63 mg/kg/day, respectively). No changes in haematological or clinical chemistry parameters were observed. Histopathological examination revealed dermal necrosis from 7 mg/kg /day and upwards which increased in severity. • No specific evidence of systemic toxicity was observed in this study. A NOAEL could not be determined from this study, the LOAEL is 7 mg/kg/day.	Ref 1 (Biocides Document II A) Muni, 1982

4.7.1.4 Repeated dose toxicity: other routes

None available.

4.7.1.5 Human information

Thee is no human information available on the repeated dose toxicity of acrolein

4.7.1.6 Other relevant information

None available.

4.7.1.7 Summary and discussion of repeated dose toxicity

The effect of repeated exposure to acrolein has been investigated by the oral, dermal and inhalation routes across a number of species.

In the oral studies, no mortalities were observed at dose levels of less than 10 mg/kg /day in the standard 90-day rat study. No clearly treatment-related changes or mortalities were observed in mice and dogs, or in rats dosed orally for longer treatment periods.

The mortalities observed after repeated dermal administration are considered to have occurred secondary to severe local site of contact effects, and not systemic toxicity. Similarly, the lung effects noted after repeated inhalation exposure are also considered to be secondary to repeated exposure to an irritant/corrosive atmosphere. Such local changes are not considered relevant for a discussion of classification for repeated-dose toxicity, and no classification (for any route of exposure) is proposed.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

Refer to section 4.7.1.7.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

A substance is classified for repeated dose toxicity in accordance with DSD when repeated damage (clear functional disturbance or morphological change which has toxicological significance) is likely to be caused by repeated or prolonged exposure and where such effects are observed at or below the specified levels. The effects observed following exposure to acrolein were considered to have occurred secondary to repeated exposure to an irritant/corrosive atmosphere and such local changes are not considered relevant for classification for repeated dose toxicity.,

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

No classification is proposed

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Refer to section 4.7.1.7.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

A substance is classified for STOT-RE in accordance with CLP when specific target organ toxicity arises from repeated exposure to concentrations at or below specified levels. Other specific toxic effects that are addressed elsewhere in the Regulation are not considered here. The effects observed following repeated exposure to acrolein were considered to have occurred secondary to repeated exposure to an irritant/corrosive atmosphere and such local changes are not considered relevant for classification for repeated dose toxicity.,

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

No classification is proposed

4.9 Germ cell mutagenicity (Mutagenicity)

The genotoxic potential of acrolein has been investigated a number of *in vitro* studies: including the potential to induce gene mutations in bacteria and gene mutations, chromosome aberrations and SCE's in mammalian cells *in vitro*. *In vivo* investigations comprise bone marrow chromosome aberrations tests in rats and dominant lethal tests in mice.

4.9.1 Non-human information

4.9.1.1 In vitro data

Table 25: Summary table of relevant in vitro mutagenicity studies

Test system, Method guideline	Organism/strain(s)	Concentrations tested	Result		Remark	Reference
			+S9	-S9		
Ames Test: Similar to OECD471 GLP	*Salmonella typhimurium:* TA98, TA100, TA1535, TA1538, TA1537,	+/-S9: 1, 3, 10, 20, 40 µg/ml	-	-	Toxic at 40 µg/ml For +/ -S9 Some evidence of an increase in TA98 at 20 µg/ml +/-S9. Suspension Method	Ref 1 (Biocides Document II A) Dunn & Seixas (1980)
Ames Test: Similar to OECD 471 GLP unknown	*Salmonella typhimurium:* TA98, TA100, TA1535, TA1538, TA1537, TA102, TA104 *Escherichia coli* WP2 uvra	0.3 to 100 µg/plate	+	+	+ve response in TA100 +/-S9 Equivocal +ve in TA98 +/-S9 -S9: toxic at ≥ 33 µg/plate +S9: toxic at ≥ 67 µg/plate Toxicity was expressed by evaluation of the background lawn. +ve in E. coli + S9 only Mutagenicity observed at non-toxic dose levels. Pre Incubation Method	Ref 1 (Biocides Document II A) Parent (1996)
Ames Test Equivalent To OCED 471 GLP	*Salmonella typhimurium:* TA98, TA100, TA1535,	0.03 to 100 µg/plate	+	+	+ve in TA100 only +S9: toxic at ≥ 25 µg/plate -S9: toxic at ≥ 3.3 µg/plate Toxicity measured by reduced number of	Ref 1 (Biocides Document II A)

Test system, Method guideline	Organism/strain(s)	Concentrations tested	Result		Remark	Reference
			+S9	-S9		
unknown	TA1537,				revertant colonies, decrease in the background lawn Pre-Incubation Method	Haworth (1983)
Modified Ames Test Similar to OECD 471 GLP unknown	*Salmonella typhimurium:* TA98, TA100, TA1535, TA1538, TA1537,	0.001 to 0.1 µl/plate	-	+	+ve in TA98 only –S9 Description of cytotoxicity not provided A statistically significant and dose related increase in revertants in a very narrow dose range (0.04-0.07). due to toxicity a decrease in revertants at ≥0.04 µl/plate. Plate Incorporation Assay	Ref 1 (Biocides Document II A) Lijinsky & Andrews (1980)
Modified Ames Test GLP unknown	*Salmonella typhimurium:* TA98, TA100, TA1535, TA1538, TA1537, TA102, TA104	Not stated	-	+	+ve in TA98 and TA100, and only in the presence of S9 No description of cytotoxicity provided Plate Incorporation Assay	Ref 1 (Biocides Document II A) Khudoley (1987)
Modified Ames Test Similar to OECD 471 GLP unknown	*Salmonella typhimurium:* TA98, TA100, TA1535	0.005 – 1 µmol/plate	-	-	No description of cytotoxicity provided Plate Incorporation Assay	Ref 1 (Biocides Document II A) Loquet (1981)
Modified Ames Test Similar to OECD 471 GLP unknown	*Salmonella. Typhimurium:* TA100	10, 15 µg/2 ml incubation volume	No data	+	No cytotoxicity indicated. An increase in cytotoxicity was investigated (as a decrease in the background lawn) but no cytotoxicity was observed. Pre- Incubation Method	Ref 1 (Biocides Document II A) Waegemaekers & Bensink (1984)
Modified Liquid Suspension Test as described by Rannug GLP unknown	*Salmonella. typhimurium:* TA100	0 – 0.15 µmoles/2 ml incubation volume	-	+	No cytotoxicity indicated at the dose levels used. Cytotoxicity was measured by the degree of survival of treated cultures. Mutagenic at low concentrations Suspension Assay	Ref 1 (Biocides Document II A) Lutz D., (1982)
Modified Ames Test GLP unknown	*Salmonella. typhimurium:* TA102	Up to 5000 µg per plate	-	-	Tested to the limit of cytotoxicity. No information on toxicity Method unknown	Ref 1 (Biocides Document II A) Jung R.,(1992)
Modified	*Salmonella.*	Acrolein was	Not	+	Tested with and without	Ref 1

Test system, Method guideline	Organism/strain(s)	Concentrations tested	Result		Remark	Reference
			+S9	-S9		
Ames Test Similar to OECD 471 GLP unknown	*typhimurium:* TA104	tested to its toxic limit. Maximum non-toxic dose: without GSH 0.9 µmoles with GSH > 1.8 µmoles	tested		glutathione. The addition of 10 mM glutathione decreased toxicity, but not mutagenicity. Pre-Incubation Assay	(Biocides Document II A) Marnett L.J., (1985)
Bacterial Forward and Reverse Mutation Test GLP unknown	*E. coli* K12/343/113	Doses not given	Not tested	No Data	Bacterial Forward and Reverse Mutation Test GLP unknown	Ref 1 (Biocides Document II A) *E. coli* K12/343/113
Liquid Pre-Incubation GLP Unknown	*Salmonella. typhimurium*: TA100, TA104	1 – 13 mM	Not tested	+	There was a drop in the number of revertants seen at 13 mM. >13 mM loss of the background lawn indicated severe toxicity and few if any revertants were present.	Ref 1 (Biocides Document II A) Foiles P.G. *et al.*, (1989)
Bacterial Reverse Mutation Test GLP unknown	*Salmonella Typhimurium:* hisD3052, TA98 and TA100.	*B*-substituted acrolein tested No doses given	Results not provided	+ in hisD3052 - in TA98 and TA100	Insufficient data provided to assess this study	Ref 1 (Biocides Document II A) Basu, A. K. & Marnett, L., (1984)
Bacterial Reverse Mutation Test Not GLP	*Salmonella typhimurium* TA 1535	No information provided	No data	No Data	Insufficient data provided to assess this study	Ref 1 (Biocides Document II A) Pool, B. L. *et al.*, (1988)
Mutagenicity Assay GLP unknown	*Salmonella typhimurium* Strains not specified	No information provided	Loss of mutagenic potential	+	No cytotoxicity information provided. Insufficient data provided to assess this study	Ref 1 (Biocides Document II A) Lutz D. *et al.*, (1980)
Chromosome Aberration Test. Similar To EU Method B10 Not GLP	Chinese Hamster Ovary Cells	+S9: 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 µg/ml -S9: 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 µg/ml	-	-	+S9: non-toxic at < 1.5 µg/ml -S9: non-toxic at < 2 µg/ml Duration of exposure was minimal Number of cells analysed was low	Ref 1 (Biocides Document II A) Gorodecki & Seixas (1982a)
Sister	Chinese	+S9: 0.10, 0.30,	-	-	+S9: toxic at > 0.5 µg/ml	Ref 1

Test system, Method guideline	Organism/strain(s)	Concentrations tested	Result		Remark	Reference
			+S9	-S9		
Chromatid Exchange. Similar To OECD 479. GLP	Hamster Ovary Cells	0.50 µg/ml -S9: 0.30, 0.50, 0.75 µg/ml			-S9: toxic at > 0.75 µg/ml	(Biocides Document II A) Loveday (1982)
Chromosome Aberration and Sister Chromatid Exchange Similar to OECD 473 and 479 GLP unknown	Chinese Hamster Ovary Cells	0.1 – 1 µg/ml	Chromosomal aberration - SCE -	- +(Weak) -	Authors state that acrolein was weakly positive in SCE only at highest concentration level tested (10.4 vs control of 8.1 SCE/cell). No further information is provided. Cytotoxicity information not presented.	Ref 1 (Biocides Document II A) Galloway (1987)
Chromosome Aberration and Sister Chromatid Exchange Similar to OECD 473 and 479 Not GLP	Chinese Hamster Ovary Cells	Chromosome aberration: 0 – 100 µM sce: 0 – 40 µM	Chromab - SCE -	- +(Weak) -	Tangling of chromosomes seen ≥ 40µM at cytotoxic concentrations. Authors state that this is an indication of potential clastogenicity Weak positive in SCE only at highest concentration level tested. -S9: toxic >10 µM +S9: toxic >40 µM	Ref 1 (Biocides Document II A) Au (1980)
Chromosome Aberration and Sister Chromatid Exchange Not GLP	Human Lymphocytes	0.001 – 40 µM	Chromab - SCE -	- +	Metabolic Activation System Used –Sulfhydryl Compound; 2-Mercaptoethanesulfonic Acid (MESNA) Cytotoxicity ≥ 20 µM Positive in SCE only (1.6 Fold increase), without MESNA	Ref 1 (Biocides Document II A) Wilmer (1986)
Mammalian Cell Gene Mutation Assay: Similar to OECD 476 GLP	Chinese Hamster Ovary Cell	+S9:0.04, 0.06, 0.08, 0.1, 0.2 0.3 µg/ml -S9: 0.1, 0.2, 0.3, 0.4, 0.5 µg/ml	-	-	+S9: toxic at > 0.3 µg/ml -S9: toxic at > 0.5 µg/ml Toxicity determined by survival of colonies of treated cultures Authors state that mutation frequencies in duplicate plates too variable for reliable assessment of potential genotoxicity.	Ref 1 (Biocides Document II A) Loveday & Gorodecki. (198II A)
Mammalian Cell Transformation Test. Similar to	Mouse Embryo Fibroblasts C3H/10T½ Clone 8	0.04, 0.06, 0.08, 0.1 µg/ml	-		50 % survival of fibroblasts at 0.1 µg/ml	Ref 1 (Biocides Document II A)

Test system, Method guideline	Organism/strain(s)	Concentrations tested	Result		Remark	Reference
			+S9	-S9		
EU Method B21 GLP						Loveday & Gorodecki (1982a)
Mammalian Cell Gene Mutation Assay: Similar to OECD 476 GLP unknown	Chinese Hamster Ovary Cell Exposure Time 5 hrs Harvest Time 23 – 29 hrs.	0.2 - 2 nl/ml –S9 0.5 – 8 nl/ ml +S9	-	-	HGPRT Gene Studies Cytotoxicity was expressed as relative cloning frequency at doses: -S9 >0.0008 ug/ml + S9 0.003 ug/ ml	Ref 1 (Biocides Document II A) Parent *et al*., (1991)
Mammalian Cell Gene Mutation Assay: Similar to OECD 476 Not GLP	Human Fibroblasts (Normal and Xeroderma pigmentosum)	0.25 - 5µM	- normal cells + Xeroderma pigmentosa (non-standard)		6 TG Gene Studied Normal Cells 37% cytotoxicity <0.8 µM XP cells 37% cytotoxicity <0.3 µM Exposure time of 5 h.	Ref 1 (Biocides Document II A) Curren *et al*., (1988)

Acrolein has been tested in bacterial gene mutation assays which vary in quality, experimental design, strains used and the type of metabolic activation employed. Positive results were obtained, mainly without the addition of S9, and only for certain strains of bacteria.

Acrolein tested negative in 4 standard mammalian gene mutation tests, but a positive finding was reported in a non-standard cell line (DNA repair deficient human fibroblast *xeroderma pigmentosum* cells.

Three chromosome aberration tests (Chinese hamster ovary cells and human fibroblasts) tested negative, with and without metabolic activation. However, chromosome tangling was observed at cytotoxic doses in a further chromosome aberration test.

Overall, acrolein has been found to induce gene mutations in bacterial test systems without S9 and in some instances with the addition of S9. Generally, negative results were obtained in mammalian cell gene mutation assays and chromosome aberration studies.

4.9.1.2 In vivo data

Table 26: Summary table of relevant in vivo mutagenicity studies

Test system, method/ Guideline	Sampling times	Dose levels	Results	Remarks	Reference
Bone Marrow Chromosome Aberration Test OECD 475 GLP Rat, Sprague-Dawley	6, 12, 24 hours after treatment	1.0, 2.1, 4.1, 8.2 mg/kg	1.0 mg/kg 6 h: -ve 12 h: -ve 24 h: -ve 2.1 mg/kg 6 h: -ve 12 h: -ve	Animals dosed at 8.2 mg/kg were not examined due to toxicity (8/18 died). The positive control substance, cyclophosphamide, produced highly significant increases in the incidence of aberrations. Maximum tolerated dose was calculated to be 4.1 mg/kg.	Ref 1 (Biocides Document II A) Gorodecki & Seixas (198II A)

Test system, method/ Guideline	Sampling times	Dose levels	Results	Remarks	Reference
Males 10/ group Single i.p			24 h: -ve 4.1 mg/kg 6 h: -ve 12 h: -ve 24 h: -ve		
Mouse, ICR/Ha Swiss Males/Females 5 males in low dose group 7 males in high dose group females: 3 per dosed male per week	Males: 8 weeks. Females sacrificed 13 days after mating	1.5, 2.2 mg/kg	-ve	Results based on pregnancy rate and total number of implants only Pre GLP	Ref 1 (Biocides Document II A) Epstein SS., (1972)
5 males / group Females: 3 per dosed male per week Single i.p	Males: 8 weeks. Females sacrificed 13 days after mating	1.5 mg/kg	-ve	Results based on pregnancy and total number of implants only. Reporting deficiencies. Pre GLP	Ref 1 (Biocides Document II A) Epstein SS., (1968)

Acrolein tested negative in a well-conducted bone marrow chromosome aberration test in rats at doses of up to 8.2 mg/kg, a dose that resulted in mortalities (Gorodecki, 198II A). It is very likely that there would have been significant exposure of the bone marrow to unchanged acrolein as the ip route will eliminate first pass hepatic metabolism. Acrolein also tested negative in two dominant lethal studies in mice.

4.9.2 Human information

No data are available.

4.9.3 Other relevant information

No data are available.

4.9.4 Summary and discussion of mutagenicity

In vitro, acrolein produced positive results in bacterial gene mutation assays, while in mammalian cells negative results were observed in standard gene mutation and chromosome aberration assays. *In vivo*, acrolein tested negative in a rat bone marrow cytogenetics test and in two mouse dominant lethal assays. It is possible that the positive findings in bacterial test systems are related to the lack of an endogeneous glutathione detoxification pathway. Glutathione has been shown to react readily with reactive electrophiles such as acrolein, protecting sensitive intracellular systems from damage.

4.9.5 Comparison with criteria

The criteria for classification for germ cell mutagenicity (Category 1B and 2) in the CLP Regulation are based on positive findings in appropriate *in vivo* genotoxicity studies. Acrolein tested negative

in a well conducted *in vivo* cytogenetics study and in two dominant lethal tests, it would appear that the *in vitro* genotoxic activity is not expressed *in vivo*. Supporting evidence is provided by the lack of carcinogenicity, particularly at the site of contact, in lifetime gavage studies in rats and mice. Taking a weight of evidence approach, no classification for mutagenicity is proposed.

4.9.6 Conclusions on classification and labelling

CLP Regulation:	**No classification is proposed**
Directive 67/548/EEC:	**No classification is proposed**

4.10 Carcinogenicity

Table 27: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
• Gavage • OECD 453 • Rat, Sprague-Dawley • Male and female • 70/ sex/ group except • 75/ sex/ group for high-dose group 0.0, 0.05, 0.5, 2.5 mg/kg bw/day • 24 months • Interim sacrifice 13 weeks: • 5/ sex high dose group • 12 months: 10/sex, all doses	• No treatment-related increases in the tumour incidence, in this study at doses of up to 2.5 mg/kg/day, the highest dose tested. • Survival was reduced in a dose dependent manner for male and female rats, during weeks 20 through 70, although this is thought to be due to dosing errors		Ref 1 (Biocides Document II A) Long & Johnson 1989b, Parent 1992
• Oral (gavage) • OECD 453 (GLP) • Mice, CD-1 Swiss Albino • Male and female • 70/ sex/ group except • 75/ sex/ group for high dose group • 0.0, 0.5, 2.0, 4.5 mg/kg day • 18 months	• No treatment-related increases in the tumour incidence, in this study at doses of up to 4.5 mg/kg/day, the highest dose tested. • Survival was reduced in all groups which was statistically significant in males in the top dose group. The decreased survival times are though to be due to dosing errors. • A slight decrease of <5% in body weight was observed in both sexes in the top dose groups and in females at the mid dose group.		Ref 1 (Biocides Document II A) Long & Johnson 1989a

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

The carcinogenic potential of acrolein has been investigated in standard lifetime studies in rats and mice. Acrolein did not cause an increase in the tumour incidence in rats and mice following lifetime gavage dosing. Non-neoplastic findings are summarised in the repeated dose section (4.6.1.1).

4.10.1.2 Carcinogenicity: inhalation

There is no information on the carcinogenic potential of acrolein via the inhalation route of exposure.

4.10.1.3 Carcinogenicity: dermal

There is no information on the carcinogenic potential of acrolein via the dermal route of exposure.

4.10.2 Human information

No data available.

4.10.3 Other relevant information

No data available.

4.10.4 Summary and discussion of carcinogenicity

The carcinogenic potential of acrolein has been investigated in lifetime gavage studies in rats and mice, and no treatment-related increases in tumour incidence were observed.

4.10.5 Comparison with criteria

Classification for carcinogenicity in category 1A is based on information in humans. As there is no information in humans, consideration of the relevant classification is between category 1B, 2 and no classification based on available animal data. As there was no treatment-related increase in tumour incidence, no classification for carcinogenicity is proposed.

4.10.6 Conclusions on classification and labelling

CLP Regulation:	**No classification is proposed**
Directive 67/548/EEC:	**No classification is proposed**

4.11 Toxicity for reproduction

The potential of acrolein to adversely effect fertility has been investigated in two well-conducted standard 2-generation studies, in rats. The potential of acrolein to adversely effect development has been investigated in two well-conducted standard studies, in rabbits, rats and mice.

Table 28: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
• **Reproductive Toxicity** • Similar to OECD 416 • GLP • Dose levels 0.0, 4.0, 5.4, 7.2 mg/kg/day • F0: 115 days • F1: 135 days • F2: Not stated. • Rat, Sprague-Dawley • Male and female • 40 females, 20 males per group	• Dam: respiratory irritation at low, mid and high dose levels; wheezing, dyspnoea, rales, stomach lesions at high dose. • Compared to controls, body weight was statistically significantly reduced in the F0 males at the highest dose, of 7-9%, and in all treatment groups of F1 males (7-13%). • There were no treatment-related effects on any of the fertility parameters investigated, at doses of up to 7.2 mg/kg/day, the highest dose tested.		Ref 1 (Biocides Document II A) King. & Mione 1982
• **Reproductive toxicity** • OECD 416 • GLP • Doses 0.0, 1.0, 3.0, 6.0 mg/kg /day • F0: 10 weeks • F1: 10 weeks (72 d) • F2: Not stated. • Rat, Crl:CD® (SD) BR • Male and female	• Mortality rate was statistically significantly increased in parental animals at the highest dose (3/30 males and 9/30 females in F0 and 8/40 and 7/40 in F1compared to none in controls) and gastric lesions at high dose. • Statistically significantly decreased body weight gain (10-15% compared to controls) was observed at the highest doses. • Compared to controls, statistically significant decreases in pup weight of around 10% were observed at high dose in F1 pups only • There were no treatment-related effects on any of the fertility parameters investigated, at doses of up to 6.0 mg/kg/day, the highest dose tested		Ref 1 (Biocides Document II A) Hoberman 1991
• **Developmental toxicity** • New Zealand White Rabbits (14-17 per dose) • 0, 0.1, 0.75 or 2 mg/kg /day acrolein via gavage from day 7 to day 19 of gestation	• Although deaths occurred in the study, these were attributed to either misdosing or aspiration of acrolein. • There was transient decrease in body weight observed in dams of the top dose group after three days of dosing (day 10 of gestation), associated with a decrease in food consumption. • No effect was seen on pregnancy indices, implantation sites, or the number of live foetuses. A slight increase in the number of early resorptions was seen in the 2 mg/kg/d group, but this was not statistically significant (0.4, 0.2, 0.3 and 0.7 mean resorptions per litter, for control, 0.1, 0.75 and 2 mg/kg/day, respectively) and did not remarkably reduce live litter size compared with controls. No external, soft tissue or skeletal malformations or variations were observed at any dose level. • Overall, no developmental toxicity was		Ref 1 (Biocides Document II A) Reference: Hoberman 1987

	observed in this study at doses of up to 2 mg/kg /day, the highest dose tested.		
• **Developmental Toxicity** • Sprague Dawley rats (28-37//dose) • 0, 3.6, 6 or 10 mg/kg /day on days 7 to 19 of gestation via gavage	• An increase in mortality was observed in the 10 mg/kg /day group (14/40 females died, compared with no deaths in controls). Body weight (and body weight gain) was statistically significantly reduced in the dams administered 6 and 10 mg/kg /day by 7% and 18%, respectively, when compared with controls. Clinical signs (notably wheezing and dyspnoea) were also observed with greater frequency in these two dose groups. No treatment related effects were observed in the low dose group. • No treatment related, toxicologically significant changes were reported for the number of resorptions, or the ratio of live/dead foetuses per litter. A decrease in total litter size (24% decrease compared with controls) and mean foetus weight (21% decrease compared with controls) was observed at the top dose. At this dose there was also an increase in incidence of delayed ossification (occurring in 36% of foetuses, compared with 27%, 8% and 14%, for control, 3.6, and 6 mg/kg /day groups, respectively). • It is noted that the changes in litter size and foetus weight occurred at a dose that caused significant maternal mortality and are not considered to be a specific treatment-related effect. • Overall, the changes observed in this study are considered to be secondary to maternal toxicity.		Ref 1 (Biocides Document II A) King & Mione 1982
• **Developmental toxicity** • Mice (CD-I 13-20/dose) • gavage from day 7 until 17 of gestation at doses of 0, 4, 6.3 or 10 mg/kg /day	• Evidence of maternal toxicity was observed in the 10 mg/kg/day dose group, manifested as a smaller increase in weight gain compared with the other dose groups (increases of 18, 13, 13 and 9 % for control, 4, 6.3, and 10 mg/kg /day groups, respectively from body weights at the start of the study of 27, 27, 27, and 28g, for control, 4, 6.3, and 10 mg/kg /day respectively). Clinical signs of toxicity occurred in the top dose group and consisted of lethargy, squinted eyes, dyspnoea and hunched posture. Slight lethargy and rough coats were also observed in the control, 4 and 6.3 mg/kg /day dose groups. • No effect was seen on the number of live foetuses. The number of resorptions was increased at 10 mg/kg /day (3.1%, compared with 0.6% in controls).		Ref 1 (Biocides Document II A) King 1982

	• Increase in the incidence of subcutaneous oedema was observed in foetuses (0, 0, 2 and 32% for control, 4, 6.3 and 10 mg/kg/day, respectively). The trend for the effect above is suggestive of a dose-related response, which is unlikely to be a secondary non-specific consequence of maternal toxicity. • An increase in incidence of cleft palate was observed, however, this abnormality occurs in mice with a high background incidence. Although haemorrhage occurred, it was with low frequency and is unlikely to be related to treatment. • Delayed ossification was also observed, but only at a dose that caused some maternal toxicity.		

4.11.1 Effects on fertility

4.11.1.1 Non-human information

There were no adverse effects on fertility observed in 2 well conducted, standard studies, in rats at doses of 6 and 7.2 mg/kg/day, the highest doses tested.

4.11.1.2 Human information

There is no information available on the potential of acrolein to adversely effect reproduction in humans.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

In rabbits, no toxicologically significant, treatment-related developmental toxicity was reported. In rats and mice, evidence of developmental toxicity was observed but in most cases occurring at doses causing marked maternal toxicity. The only evidence of possible specific developmental toxicity was observed in mice at a dose level of 6.3 mg/kg/day and above, reported as the presence of subcutaneous oedema. Unfortunately the test report is poorly written and provides no information on the severity of the subcutaneous oedema. As a consequence, it is not known whether this recorded change is a slight localised oedema, which is considered to be a very minor change and unlikely to have adverse health consequences; or anasarca (a generalised accumulation of fluid in the subcutaneous tissues and body cavities). Anasarca is considered to be a severe change.

4.11.2.2 Human information

There is no information available on the potential of acrolein to adversely effect development in humans.

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

The only adverse reproductive effect was anasarca in mice, therefore, this section concentrates specifically on this finding.

To try to gain a better understanding of the toxicological significance of the actual change that occurred in the mouse study, industry has provided additional background information on the condition of anasarca which is considered below and summarised in Annex I to this report.

Industry has identified two possible aetiologies for anasarca (also described as hydrops fetalis), one immune-related and the other non-immune-related. In the immune-related condition, anasarca is associated with alloimmune general foetal haemolysis (as a result of maternal antibodies passing through the placenta into the foetus). However, no evidence of haemolysis in the foetuses was reported in the mouse study, indicating that it is unlikely the change recorded as subcutaneous oedema was an immune-related anasarca. It should be noted that although an increased incidence of haemorrhage was present in the acrolein treated groups, this is likely to be the result of extravasation of whole blood rather than lysis of erythrocytes, possibly as a result of the procedures used to handle the foetuses. Supporting evidence for the absence of haematotoxicity following exposure to acrolein comes from the repeated doses studies, in which no evidence for haematotoxicity was observed.

Non-immune anasarca can have a more diverse aetiology, but tends to be associated with cardiovascular disease, including arrhythmias, myocardial infarction, angiomas, premature closure of the foramen ovale, right or left heart hypoplasia and single ventricle. The cardiovascular disease is thought to lead to fluid balance problems, which manifest as widespread and marked oedema. Major morphological changes in the cardiovascular system would probably be detectable in the mouse developmental toxicity study, but effects such as arrhythmias and myocardial infarction would not. In the mouse study, the increased incidence of subcutaneous oedema was not associated with any cardiac malformations. This suggests that the change recorded as subcutaneous oedema was less likely to be non-immune anasarca.

Additional evidence that the reported subcutaneous oedema was unlikely to be anasarca is provided by an analysis of the foetal bodyweight data. If anasarca was present it would be expected that foetal bodyweight would be increased. However, in the mouse study, group mean foetal bodyweight in the highest dose group, in which over 30 % of the foetuses examined were reported to show subcutaneous oedema, is slightly lower than controls. Unfortunately, individual foetal data are not available to conduct a more detailed analysis of the relationship between foetal bodyweight and the presence of subcutaneous oedema.

There is no clear evidence from the repeated dose studies in a number of species, including a 21-day and lifetime studies in mice, that acrolein induces any systemic toxicity. Consideration of the toxicokinetics of acrolein suggests that this could be because acrolein is rapidly and extensively cleared by hepatic metabolism.

Overall, the weight of evidence suggests that the change in mice recorded as subcutaneous oedema is a minor variation. As a result, classification for developmental toxicity is not appropriate. Support for this position is provided by the standard studies in rats and rabbits in which no evidence of developmental toxicity was observed.

4.11.5 Comparison with criteria

There are no data in humans, for either fertility or developmental toxicity, therefore, consideration of classification is between category 1B, 2 or no classification based on available animal data.

There is no evidence, from standard animal studies, to show that acrolein caused an adverse effect on fertility therefore no classification is proposed.

Taking a weight of evidence assessment of the relevant studies in experimental animals, no classification is proposed for developmental toxicity.

4.11.6 Conclusions on classification and labelling

CLP Regulation:	**No classification is proposed**
Directive 67/548/EEC:	**No classification is proposed**

4.12 Other effects

There is no information available on non standard end points

4.12.1 Non-human information

.

4.12.1.1 Neurotoxicity

4.12.1.2 Immunotoxicity

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

5 ENVIRONMENTAL HAZARD ASSESSMENT

A detailed summary of the available studies has been reviewed under the Biocidal Products Directive (98/8/EC), see Document IIA attached to the technical dossier (Ref 1). The key information pertinent to determining a classification position is presented below. In addition, an EU

Existing Substances Regulation (ESR) EEC No 793/93 Risk Assessment Report (Ref 2) is available for acrolein also presenting fate and ecotoxicity data.

5.1 Degradation

5.1.1 Stability

Acrolein reacts reversibly with water to give the hydration product 3-hydroxypropanal (HPA). HPA can react with water to form reversibly 3,3-dihydroxy-1-propanal. HPA can also condense with acrolein to give 3,3'-oxydipropoionaldehye, which can react further to give more complex products.

An hydrolysis study is available according to US EPA FIFRA Guideline 161-1 (Haag et al, 1988a – see ref 1). Three pHs were tested, 5, 7 and 9, at 25 ºC with two concentrations of 5 and 10 mg/l. The 10 mg/l concentration returned mean half lives of 14 hours, 37 hours and 92 hours for pH 9, 7 and 5, respectively (HPLC-UV analysis). The main product of the hydrolysis was HPA. Analysis showed that, after more than seven half lives, about 9% acrolein remains at all pHs, indicating that the equilibrium of the reaction lies far to the right.

In their assessment under the biocides directive (UK 2009 – see ref 1), the UK competent authority adjusted the study's results to 9 ºC in accordance with the EU Technical Guidance Document (TGD) on Risk Assessment (2003 – see ref 1); DT50 values were 13.7, 5.4 and 2.2 days at pH 5, 7 and 9, respectively.

A study carried out according to US EPA FIFRA Guideline 161-2 (Haag et al 1988b – see ref 1) showed that aqueous photolysis is negligible with a half life of 70d at 40 ^{o}N.

In air, acrolein is expected to react with hydroxyl radicals. Both the alkene and aldehyde groups may react, with the aldehyde being the more susceptible of the two. A calculated half-life of acrolein in the atmosphere for indirect photo-oxidation (using a hydroxyl radical concentration of 5 x 10^5 molecules/cm^3 and a daylight duration of 12 hours) is less than one day.

A phototransformation study in air according to US EPA FIFRA Guideline 161-4 (Haag et al, 1988b – see ref 1) gave a photolysis half life of 10.9 days (which compares well with a calculated half life of 7.7 days).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not relevant for this dossier**.**

5.1.2.2 Screening tests

No valid ready tests are available for the substance. A number of BOD5 studies, with acclimated and non-acclimated inoculum, showed no or only low levels of degradation, but this was probably due to toxicity of the substance to microorganisms. In a study by Tabak et al (1981 – see ref 1), most similar to a ready test using non-acclimated inoculum from a domestic STP, acrolein was totally degraded within seven days (DOC and TOC, GC detection). However, yeast was used as a carbon source in the experiment and only primary degradation may have been measured, so the validity of this test is questionable.

In an inherent biodegradation study 100% of the substance was degraded in 2-6 days (WHO, 1992 – see ref 1), and in another study measuring primary degradation 100% of the substance was removed after seven days (Tabak et al, 1981 – see ref 1).

In anaerobic studies 42% of the substance was degraded after 20 days using acclimated inoculum (WHO, 1992 – see ref 1) at a concentration 0f 10 mg/l, however in a study using unacclimated inoculum and a concentration of 500 mg/l no degradation was observed (WHO, 1992 – see ref 1). Again it is likely that the substance was toxic to microorganisms at the higher concentration.

A study assessing the biodegradability of the substance in a commercial formulation (at a concentration of ca. 95% w/w) in seawater is available, according to OECD TG 306 (Manley, 2003 – see ref 1). The study used two concentrations of 2 and 3.5 mg/l in a closed bottle test. Inoculum consisted of coarse filtered seawater. Analysis was conducted using DOC ThOD. Limited potential for biodegradation was observed, and the inhibition control in the study indicated that the substance was toxic to microorganisms.

Two tests in soil are available, conducted according to US EPA FIFRA Guidelines 162-1 and 162-2 (Chou and Spanggord, 1990 and 1991, respectively – see ref 1). In the aerobic study acrolein was added at 10 mg/l to a sandy loam soil (61% sand, 25% silt, 14% clay, 0.4% organic matter) and allowed to stand for seven days at 20 – 22 ºC. After 48 hours acrolein was not detected; acrylic acid and 3-hydroxypropionic acid were detected from 2 hours onwards. Both degradation products were entirely mineralised by the end of study. The half-life for acrolein was 4.2 hours (converted to 12 ºC this equates to 9.4 hours) for unbound acrolein, and an extrapolated half-life for bound acrolein was 410 days. Half-lives of the degradation products of acrolein were estimated to be in the region of 29 days. It should be noted, however, that the soil microorganisms in the study were acclimated to acrolein. In the anaerobic study, 4.2 mg/l of acrolein was added to two soil types at 20 – 23 ºC. Both soil types, a sandy loam and a loam, gave similar results. Acrolein was found to degrade completely after 25 days. As for other studies, acrolein was first converted to HPA, and then was converted to 1,3-propanediol and subsequently to 23-hydroxypropionic acid. The half-life of acrolein was 11 days (adjusted to 12 ºC this is about 21 days). The half life for complete mineralisation of acrolein's degradation products was predicted to be 80 – 110 days.

5.1.2.3 Simulation tests

Two degradation studies have been conducted using a ^{14}C radiolabelled acrolein test substance in aerobic and anaerobic freshwater, according to US EPA FIFRA Guidelines 162-4 and 162-3 (Smith, 1993a and 1993b – see ref 1). Both studies are summarised in the UK Competent Authority assessment conducted under the Biocidal Products Directive 98/8/EC(UK, 2009 – see ref 1). Canal sediment (sandy loam; 75% sand, 19% silt, 6% clay, 0.5% organic matter) and water (hardness 56 mg/l $CaCO_3$; suspended solids <0.002 mg/l; total solids 0.122 mg/l) were obtained for use in both studies, and acrolein was added at a concentration of 15 mg/l in both. In the 32 day aerobic study conducted at 25 ºC, biodegradation was observed with the production of carbon dioxide (expressed as bicarbonate ion, representing greater than 90% on days 5 and 32). Hydrolysis was observed to be a major degradation pathway (production of HPA) with various oxidative reactions further transforming the hydrolysis product. Subsequent and competing microbial transformation of acrolein and HPA to acrylic acid and allyl alcohol also occurred. No acrolein was detected after 48 hours. The half life of acrolein in the test was 33.7 hours. The UK CA adjusted this to 9 ºC (121.2 hours). In the anaerobic study, conducted at 22 ºC for 182 days, no acrolein was detected beyond the first day of the study. Carbon dioxide was the major degradation product, representing greater than 60% of the initial test dose on days 30, 93 and 178. As for the aerobic study, biodegradation of the hydrolysis products was likely to be the major pathway. Both studies show rapid degradation in water

5.1.3 Summary and discussion of degradation

Acrolein reacts reversibly with water to give 3-hydroxypropanal as the major product. The equilibrium has been shown to lie far to the right at all pHs (ie mainly the hydrolysis product present in water). Reaction is faster at alkaline pHs, as the reaction is driven by nucleophilic attack. Further reversible reaction with water may occur to give 3,3-dihydroxy-1-propanal. Hydrolysis products may condense with acrolein to give more complex secondary products. Degradation due to hydrolysis far outweighs degradation due to photolysis in water.

Based on the available data, the substance was considered readily biodegradable although no single valid ready biodegradable study was available in the EU ESR risk assessment of the substance. The assessment of acrolein conducted under the Biocidal Products Directive 98/8/EC concluded that the substance was rapidly degraded through both biodegradation and hydrolysis and subsequent oxidation under freshwater aerobic conditions, and that degradation and subsequent mineralisation were faster under anaerobic conditions.

For the purpose of classification and labeling, acrolein is considered rapidly degradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

A K_{oc} of 2.8 l/kg can be estimated for acrolein according to the EU TGD (using a log K_{ow} of -1.1). Experimental Koc values ranged between 51 and 270 for two soil types in studies following US EPA-FIFRA guideline 163-1 (Irwin, 1988 - see ref 1. The estimated and measured data indicate that acrolein is likely to be mobile in soils; however some of the data recorded in the freshwater and soil biodegradation studies show an appreciable level of binding.

5.2.2 Volatilisation

Given the substance's high vapour pressure, estimated Henry's Law constant (HLC) of 6.1 $Pa.m^3/mol$ (according to the EU TGD) and measured HLC of 3.1 $Pa.m^3/mol$ (NTIS, 1990 – see ref 1), volatilisation of acrolein from surface waters is expected to be high.

5.2.3 Distribution modelling

Not relevant for this dossier.

5.3 Aquatic Bioaccumulation

5.3.1.1 Bioaccumulation estimation

A low potential for bioaccumulation can be estimated from the substance's log Kow of -1.1 (and taking into account its high water solubility and degradation).

5.3.1.2 Measured bioaccumulation data

Bluegill sunfish were exposed to ^{14}C-radiolabelled acrolein at a concentration of 0.013 mg/l for 28 days (Barrows et al, 1980; WHO, 1994 – see ref 1). The half time for removal of the radiolabel in

fish was greater than seven days. The study describes assimilation of radiolabelled substance/metabolites in fish tissue rather than bioconcentration – a BCF of 344 can be derived based on total radioactivity – and therefore overestimates bioconcentration of the parent substance.

In studies conducted according to US EPA FIFRA Guideline 171-4 (Biever, 1994 – see ref 1), bluegill sunfish, channel catfish, northern crayfish and freshwater mussels were exposed to two single applications of ^{14}C radiolabelled acrolein. Concentrations of 20 µg/l and 101 µg/l were used for fish and invertebrates, respectively, with a period of seven days between each application. 26 hours after application of the second dose the study was terminated, and fillet tissues were analysed for the parent compound. Results showed that the parent was rapidly degraded/metabolised such that it was not detected in any tissue.

5.3.2 Summary and discussion of aquatic bioaccumulation

Based on the substance's physico-chemical properties, its estimated bioconcentration factor, and supporting information from assimilation studies, the substance is considered to have has a low potential for bioaccumulation, below the GHS BCF criterion of 500 and 100 for Regulation (EC) No 1272/2008 and Directive 67/548/EEC.

5.4 Aquatic toxicity

Studies with flow through conditions with analytical verification of test concentrations are to be preferred for Acrolein, given its degradation in water and reasonably high potential for volatilisation.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The most sensitive species identified in valid short term fish toxicity tests were bluegill sunfish (*Lepomis macrochirus*), in a test following US EPA-FIFRA guideline 72-3 (96h LC50 22.4 µg/l, based on mean measured concentrations, under flow through conditions; Bowman, 1990– see ref 1) and fathead minnow (*Pimephales promelas*) in a study following the ASTM guideline (96h LC50 14 µg/l, based on measured concentrations; Holcombe, 1987 – see ref 1).

5.4.1.2 Long-term toxicity to fish

A long term NOEC is available from a 60 day reproduction study conducted in fathead minnow, according to a (non-stated) ASTM guideline (Macek et al, 1976 – see ref 1). The study gave a NOEC of 11.4 µg/l (measured) for effects on mortality of adults, number of spawning, number of eggs per female, number of eggs per spawn, length of offspring and hatchability.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

A number of studies for invertebrates are available. The lowest result with *Daphnia magna* is a 48h EC50 of 23 µg/l (with 95% confidence limits 21-26 µg/l) in a flow through test according to US EPA-FIFRA guideline 72-2 with analytical measurement (Blakemore and Burgess, 1990 – see ref 1).

5.4.2.2 Long-term toxicity to aquatic invertebrates

A long term study was conducted with *Daphnia magna* over three generations and a duration of 64 days under flow through conditions (Macek et al, 1976 – see ref 1). Five concentrations were tested, with mean measured concentrations recorded as 3.2, 7.1, 16.9, 33.6 and 42.7 µg/l. The 64 day NOEC was 16.9 µg/l, based on survival of female and offspring per female. Over the first 22 days, significant effects were observed for parental survival in the 33.6 and 42.7 µg/l test concentrations

5.4.3 Algae and aquatic plants

A 72 hour study has been conducted in the marine species *Skeletonema costatum*, according to ISO Standard 10253 (Sullivan, 2007 – see ref 1). The study used nominal concentrations of acrolein of 0, 8.6, 13.0, 19.4, 29.2, 43.2 and 64.8 µg/l. However, the test material would have significantly degraded over the course of the study. In the absence of any chemical analysis of the test preparations, the data were reanalysed and presented in the assessment conducted under the Biocidal Product Directive 98/8/EC (see ref 1) using the hydrolysis data to estimate concentrations of acrolein after 72 hours. Using a worst-case hydrolysis half-life of 14 h (pH 9.3), it was estimated that approximately 17 % of the initial test material concentration would remain after 72 h (determined using the Xlfit 4 software package). The geometric mean test concentrations were therefore predicted using the nominal test concentrations at 0 hours and according to an 83 % decline in concentration after 72 h. A logistic regression model provided the best fit to the data using non-linear regression, and was therefore used to calculate the toxicity endpoints.

Based on growth rate and geometric mean estimated concentrations of acrolein, an acute 72 h ErC50 of 11 µg/l was obtained. This results compares well with the acute data for fish and invertebrates, and algae are expected to be sensitive to a substance used as a slimicide active ingredient.

The 72 h NOEC, using the same estimated mean concentrations and based on the growth rate endpoint, was 5.1 µg/l.

5.4.4 Other aquatic organisms (including sediment)

A flow through test according to ASTM acute toxicity to fish, macroinvertebrate and amphibian guidelines has been conducted with the tadpole of the African claw frog, *Xenopus laevis* (Holcombe, 1987 – see ref 2). This is reported in the same publication as the acute fathead minnow

study detailed above, and was conducted in the context of water quality criteria setting in the USA. The study used lake water with simultaneous exposure for different species. A 96h test an LC50 of 7 µg/l (measured) was obtained. This result was considered valid and used in the EU ESR risk assessment for PNEC derivation. It was not considered suitable for effects assessment under the Biocidal Products Directive 98/8/EC.[1] The result is considered valid and is relevant for classification and labelling purposes under Directive 67/548/EEC and EC 1272/2008.

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

Acrolein is considered rapidly degradable for the purpose of classification and labelling. The acute classification follows from acute aquatic toxicity < 1 mg/l in species from three trophic levels. Three valid NOECs are available for three trophic levels - the lowest is 0.005 mg/l for algae.

Acrolein is classified as N; R50 according to Directive 67/548/EEC

According to Regulation (EC) No 1272/2008 acrolein is classified Aquatic Acute 1 (H400) and Aquatic Chronic 1 following the 2nd ATP in Commission Regulation (EU) No 286/2011.

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

Acrolein is classified as N; R50 according to Directive 67/548/EEC with classification of the preparation/mixture for the environment:

N; R50 (H400): Cn ≥ 0.25%

Not classified: Cn ≤ 0.25%

Were Cn is the concentration of acrolein in the preparation/mixture.

Acrolein is classified as Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410) according to Regulation (EC) No 1272/2008.

M-factor

The acute M-factor is 100 based on the tadpole of the African clawed frog (*Xenopus laevis*) in a 96-h LC_{50} value of 0.007 mg/l i.e. $0.001 < LC_{50} \leq 0.01$ mg/l. The chronic M-factor is 1 based on the algal NOEC of 0.005 mg/l for a rapidly degradable substance i.e. $0.001 < NOEC \leq 0.01$ mg/l.

Justification for selection of concentration limit and M-factor:

The selected acute result is lower than, but comparable to (and within a factor of ca. 3), $L(E)C_{50}$ values for those of the other species tested in reliable studies (*Pimelas Promelas*, 96h LC50 14 µg/l;

[1] The four reasons given were: 1) it is from a non-guideline study conducted as part of a series of multispecies experiments; 2) the authors of the study were able to compare favourably results from their study with literature results for standard species, but not for this non-standard test species (no literature data available); 3) the adult of this species is adapted for stagnant water conditions and so flow-through conditions may cause stress to the organism (although it is recognised that this may have had a limited effect on the tadpole phase); 4) and, in the context of assessment for the marine environment, there is no equivalent marine amphibian species.

Daphnia magna 48 h EC50 23 ug/l; *Skeletonema costatum* 72h ErC50 11 µg/l[2]). All of these results lead to the same classification. However the *Xenopus laevis* result leads to concentration limits and an acute M-factor a factor of 10 lower. The result was derived in a valid study previously accepted for the EU ESR Risk Assessment and is relevant for classification and labelling purposes.

Three valid chronic NOECs covering three trophic levels are available to determine the chronic classification. Using the lowest NOEC of 0.005 mg/l this results in Aquatic Chronic 1 and a chronic M-factor of 1 given the substance is rapidly degradable. The NOEC is considered valid and has been applied in the Biocidal Products Directive assessment.

[2] this result is based on a prediction of the mean concentrations the organism would have been exposed to over the duration of the study, as predicted by the hydrolysis data (i.e. an 83% decrease in concentration during the 72h study).

6 OTHER INFORMATION

6.1 Microbiological activity in sewage treatment systems

Two “contact” studies are available for acrolein with microorganisms with uncertain validity. The bacterium *Proteus vulgaris* gave a 2 hour EC_{50} of 20 ug/l (Brown, 1967), and the 30 min EC_{50} for activated sludge bacteria (municipal STP) was 400 mg/l (Degussa, 1992).

Four longer duration studies are available, with NOECs ranging from 210 – 1700 ug/l for 16 – 72 hour exposure periods in specific bacterial species and protozoa (Bringmann 1977, 1978, 1980a, 1980b).

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ANNEXES

Annex I: The Association of Foetal Anasarca with other Foetal Abnormalities

The Association of Foetal Anasarca with other Foetal Abnormalities

The purpose of this review is to demonstrate the view that foetal anasarca is not observed in isolation but is commonly found with other foetal abnormalities.

Foetal anasarca is described as a generalised accumulation of fluid in the subcutaneous connective tissue and in body cavities [1]. It is one of the clinical conditions associated with Hydrops Fetalis (HF), a birth defect observed as a spontaneous malformation in both humans and other species. In certain cases, both terms have been used to describe the same abnormality. Hydrops Fetalis (oedema of the foetus) has been characterised and it's pathology investigated. A full review of this condition has been described [2, 7]. The condition has been classified into two distinct aetiologies; Immune-related Hydrops Fetalis (IHF) and Non-Immune Hydrops Fetalis (NIHF). In both cases the condition is not observed in isolation and therefore it is the particular aspect that separates this condition (including foetal anasarca) from the condition of localised subcutaneous oedema.

Human and Veterinary Reports of Hydrops Fetalis (foetal anasarca)

Case reports of HF show the two aetiologies of this condition. Ishmail *et al* [3] studied 63 cases of which 12.7 were classified as IHF and the remainder were classified as NIHF. The review of HF has shown the type of findings often seen as the potential causes [2]. For IHF, it is the result of alloimmune haemolytic disease and hence a general haemolysis of the foetus. The final report for the mouse teratology study where subcutaneous oedema was seen [4] showed no evidence of foetal anaemia, and only minor delays in foetal sketetal development gave any indication of foetal toxicity. The aetiology of NIHF is more diverse. Other conditions cited include:

i) Cranial - cerebral tumours, intracranial haemorrhage

ii) Neck and thoracic tumours

iii) Gastrointestinal tract abnormalities

iv) Chromosomal disorders including Trisomy 21 and 18 [8]

v) Genetic disorders such as Gauchers and Hurlers disease

vi) Skeletal dysplasias such as achondroplasia

vii) Fetal hypokinesis

viii) Maternal disorders including Graves disease

ix) Placental disorders such as cholangioma

x) Infection - including cytomegatovirus, coxsackie virus. In a separate report polycystic kidney was linked to HF [5]

The most commonly cited abnormalities associated with NIHF is cardiovascular disease. These include arrhythmias, myocardial infarction, angiomas, premature closure of the foramen ovale, right or left heart hypoplasia and single ventricle. One publication discusses the association between HF and cardiovascular abnormalities in foetal mice which are lacking a functional gene for adrenomedullin, an endogenous potent vasodilator [6]. Foetal congestive heart failure has been linked to HF [9]. Some questions have been raised as to whether congenital heart disease is the cause of HF [10] but for the purposes of this review, it is the presence of other foetal abnormalities that is the key issue, as it is more than likely that the true condition may be multifactoral in its aetiology. Because there is some question as to whether foetal anasarca and HF are separate conditions, evidence from one publication provides evidence to support the view that severe subcutaneous oedema, in the absence of ascites and hydrothorax, may well be more likely a consequence of cardiac defects [11]. Ultrasound readings of 132 patients with NIHF showed that a decreased incidence of pleural effusion and ascites was seen amongst HF patients with an underlying cardiac abnormality. Foetal anasarca has also been reported in domestic animals. A syndrome has been used to describe foetal anasarca in domestic breeds of dog [12] and is known as "water puppy" syndrome. The likely cause cited include malformation of the lymphatic drainage system or congenital cardiopathy. Anasarca has also been reported in a flock of sheep [13] which has been associated with agenesis of lymphoid tissue.

Experimental Observations

There is limited evidence to suggest HF or foetal anasarca are common abnormalities seen with experimental induction of teratogenesis. The injection of betamethasome into pregnant rats between Days 12 to 17 of gestation resulted in cardiovascular and pulmonary effects, plus pale oedematous skin in foetuses [14]. The potential link between HF and constriction of the ductus arteriosus was described in this publication. A study on the effects of perfluoroctane sulfonate on pregnant rats and mice exposed from Day 2 to 20 and 1 to 17 of gestation respectively showed a large number of birth defects including cardiovascular and foetal anasarca with the mouse less sensitive than the rat [15]. It is also of note that anasarca is not limited to the foetus. Spontaneous anasarca has been linked with hypertension, renal and cardiovascular lesions in certain strains of mice [16]. Foetal anasarca was also seen at a low incidence in a developmental toxicity study of N,N Dimethylacetamide [18] at the highest dose level of 600 ppm to maternal rats by inhalation exposure from Day 6 to 19 of gestation. At this dose level, which showed signs of toxicity to the adult female, there was a high incidence of cardiovascular malformations amongst foetuses. There was a low incidence of foetal anasarca at this level. The type of cardiovascular malformations included septal defects, persistent truncus arteriosus and malpositioning of vessels. The fact that anasarca was not present in every case of cardiovascular malformation may well be dependent upon whether the foetus was able to compensate for the structural rearrangements, thereby limiting elevations in hypertension.

Experimental induction of Hydrops Fetalis has been conducted using foetal sheep. Infusions of low doses of angiotension into nephractomised fetal sheep produced Hydrops Fetalis. The conclusions of the study were also analysed using computer simulated models [17]. These models showed that fetal cardiac failure constituted the strongest stimulus for the formation of foetal oedema.

Conclusions

The observation of foetal oedema, whether classified as foetal anasarca or as part of the condition Hydrops Fetalis has generally been associated with other conditions, when considering the spontaneous appearance of this condition amongst humans or domestic animals. Experimental induction or observation of this condition has generally been associated with cardiovascular defects.

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