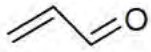
Section A1 **Annex Point IIA1**	**Application for the Annex I inclusion of a new active substance**
1.1 Applicant	Baker Petrolite Kirkby Bank Road Knowsley Industrial Park Liverpool L33 7SY Tel: ~~+44 (0)151 545 3742~~ *Fax: ~~+44 (0)151 547 3590~~* *E-mail: ~~peter.jacques@bakerpetrolite.com~~*
1.2 Manufacturer of Active Substance (if different)	~~Micheal Harless~~ Baker Petrolite/Manufacturing/ Plant Management 19815 South Lake Road Taft, California 93268 USA
1.3 Manufacturer of Product(s) (if different)	As above

Section A2	Identity of Active Substance	
Subsection (Annex Point)		**Official use only**
2.1 Common name (IIA2.1)	Acrolein	
2.2 Chemical name (IIA2.2)	2-propenal	x
2.3 Manufacturer´s development code number(s) (IIA2.3)		
2.4 CAS No and EC numbers (IIA2.4)		
2.4.1 CAS-No	107-02-8	
2.4.2 EC-No	203-453-4	
2.4.3 Other		
2.5 Molecular and structural formula, molecular mass (IIA2.5)		
2.5.1 Molecular formula	C_3H_4O	
2.5.2 Molecular structure		
2.5.3 Molecular mass	56.06	
2.6 Method of manufacture of the active substance (IIA2.1)	[redacted]	
2.7 Specification of the purity of the active substance, as appropriate (IIA2.7)	[redacted]	
2.8 Identity of impurities and additives, as appropriate (IIA2.8)	[redacted]	
2.9 The origin of the natural active substance or the precursor(s) of the active substance (IIA2.9)	[redacted]	

Section A2	Identity of Active Substance	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11/05/06	
Materials and methods	The applicant's version is acceptable with the following amendments: 2.2 Chemical name 2-propenal (CAS name), acrylaldehyde (IUPAC and EINECS name).	
Conclusion	Adopt applicant´s version with the amendments as detailed in materials and methods.	
Reliability	0 (studies not applicable to these end-points – stated information only)	
Acceptability	Acceptable	
Remarks	UK CA agrees with the applicant's summary and conclusion with UK CA amendments (see materials and methods). Further information can be found in the "Confidential Appendix IX for Doc IIIA".	
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A2.8 **Annex Point IIA2.8**	**Identity of impurities and additives (active substance)**				
Subsection					**Official use only**
2.8.1.1 Common name	[redacted]				
2.8.1.2 Function					
2.8.2 IUPAC name					
2.8.3 CAS-No					
2.8.4 EC-No					
2.8.5 Other					
CIPAC					
2.8.6 Molecular formula					
2.8.7 Structural formula					
2.8.9 Concentration of the impurity or additive *typical and range of concentrations*	g/kg	g/l	% w/w	% v/v	

Section A2.8 Annex Point IIA2.8	Identity of impurities and additives (active substance)	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	1/5/2008	
Materials and methods	n.a.	
Conclusion	Applicants version is acceptable with comments form the UK CA in the remarks section.	
Reliability	0 (studies not applicable to these end-points – stated information only)	
Acceptability	Acceptable.	
Remarks	[redacted]	
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A2.10 Annex Point IIA2.10	Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC	
Subsection		**Official use only**
2.10.1 Human exposure towards active substance		
2.10.1.1 Production		
i) Description of process	[redacted]	
ii) Workplace description		
iii) Inhalation exposure		
iv) Dermal exposure		
2.10.1.2 Intended use(s)		
1. Professional Users		
i) Description of application process	[redacted]	
ii) Workplace description	[redacted]	
iii) Inhalation exposure	[redacted]	

Section A2.10 Annex Point IIA2.10	Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC	
iv) Dermal exposure	[redacted]	
2. Non-professional Users including the general public		
(i) via inhalational contact	Slimicide products based on acrolein are used exclusively by professional workers on offshore oil rigs. There are no non-professional uses for this product.	
(ii) via skin contact		
(iii) via drinking water		
(iv) via food		
(v) indirect via environment		
2.10.2 Environmental exposure towards active substance	[redacted]	
2.10.2.1 Production		
(i) Releases into water	[redacted]	
(ii) Releases into air		
(iii) Waste disposal		
2.10.2.2 Intended use(s)		
Affected compartment(s):	[redacted]	
water		
sediment		
air		
soil		
Predicted	[redacted]	

Section A2.10 **Annex Point IIA2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**	
concentration in the affected compartment(s)	[redacted]	
water		
sediment		
air		
soil		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	1/5/2008	
Materials and methods	n.a	
Conclusion	Applicants version is acceptable.	
Reliability	1	
Acceptability	Acceptable.	
Remarks	[redacted]	
	COMMENTS FROM ...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA3.1)								
3.1.1 Melting point								
Melting pt. 1	Not specified	Not specified	***result:*** -87.0°C ***pressure:*** not measured	Literature data and experience in use indicates that the substance will not freeze above -20°C. Therefore, in accordance with the TNsG on Data Requirements a study to determine the freezing point for a liquid to satisfy the requirements for this end-point is not scientifically justified.	n.a	2	Caravello H, (1988), Physical Properties of Acrolein: A summary, Baker Performance Chemicals Inc. Study no. RD 0070.188	X
3.1.2 Boiling point								
Boiling pt. 1	OECD 103	95.62%	**result:** 52.8°C **pressure:** not measured		Y	1	Sarff P, (2005b), Determination of the Boiling Point, Density, Viscosity and Flash Point of Acrolein, ABC	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
							Laboratories, Inc. Study No. 49925	
3.1.3 Bulk density/ relative density								
Bulk/rel. density 1	OECD 109	95.62%	Specific gravity = 0.8875 at 20°C Density = 0.8859 g/ml at 20°C		Y	1	Sarff P, (2005b), Determination of the Boiling Point, Density, Viscosity and Flash Point of Acrolein, ABC Laboratories, Inc. Study No. 49925	
3.2 Vapour pressure (IIA3.2)								
Vapour pressure 1	OECD 104 FIFRA 63-9	96.43%	**temperature:** 25°C **result:** 31920 Pa		Y	1	Robillard KA, (1988) Vapour Pressure of Acrolein. Health and Environment Laboratories, Eastman Kodak Company. Study no:	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
							EN-030-UKA001-1	
3.2.1 Henry´s Law Constant (Pt. I-A3.2)	Mackay, D., Shiu, W.I. and Sutherland, R.P. Determination of Air-Water Henry's Law Constants or Hydrophobic Pollutants. Environ. Sci. Technol. 13:333-337 (1979).	96.17%	**Calculated:** Log H = 1.289 **result:** 19.465 kPa/mol		Y	1	Irwin, K (1987) Henry's Constant for Acrolein (Magnicide H Herbicide and Magnacide B Microbiocide). SRI International, SRI Project PYU 3562	X
3.3 Appearance (IIA3.3)								
3.3.1 Physical state	FIFRA 63-3	92-96%	Liquid		n.a	2	Caravello H, (1988), Physical Properties of Acrolein: A summary, Baker Performance Chemicals Inc. Study no. RD 0070.188	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.3.2 Colour	FIFRA 63-2	92-96%	Clear		n.a	2	Caravello H, (1988), Physical Properties of Acrolein: A summary, Baker Performance Chemicals Inc. Study no. RD 0070.188	X
3.3.3 Odour	FIFRA 63-4	92-96%	Pungent		n.a	2	Caravello H, (1988), Physical Properties of Acrolein: A summary, Baker Performance Chemicals Inc. Study no. RD 0070.188	X
3.4 Absorption spectra (IIA3.4) **UV/VIS**		96% - 96.5%	T: ambient, pH: Not stated solvent: methanal. Absorbance (0.64322) at 207 nm				Baham, G.J. Hackerott, J.A & Patek G.J., (2004) UV/Vis, IR, NMR, and GC/MS Spectra of Acrolein.	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
							Project number 0408A031	
IR	FTIR	96% - 96.5%	T: Not stated, pH: Not stated, Absorbance at 2847w, 2812w, 1761w, 2702w, 1692s, 1618w, 1422m, 1363m, 1278vw, 1158s, 977s, 917m.					X
NMR	Proton	96% - 96.5%	T: 305K, pH: Not stated, peak groups at 1 and 3.29					X
	^{13}C		T: 305K, pH: Not stated, peaks at 137, 138 and 194.					
MS	Quadruple GC/MS	96% - 96.5%	T: Not stated, pH: Not stated, GC peak at 1.75 min, major fragments at 26, 27, 29, 55 and 56.					X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.5 Solubility in water (IIA3.5)	*including effects of pH (5-9)*							
Water solubility 1	OECD 105, FIFRA 63-8	96.43%	**result:** 237,628 mg/l ± 2856 mg/l. **temperature:** 25°C **pH:** not measured	The active substance is highly soluble in water at room temperature. Acrolein has no functional groups that would undergo dissociation, hence it would not ionise in solution. Measuring at different pHs would therefore have no effect on the water solubility of acrolein. Since acrolein is so highly water soluble, measuring at different temperatures would have little effect on the outcome of the solubility study, relative to its water solubility at 25 °C i.e. acrolein would still be highly soluble in water regardless of the temperature at which it was measured.	Y	1	Robillard, KA, (1988), Water Solubility of Acrolein, Health and Environment Laboratories, Eastman Kodak Company, EN-040-UKA001-1	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.6 Dissociation constant (-)	Not applicable			The active substance does not contain any functional groups that would undergo dissociation				
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA3.1)	CIPAC Method MT 181	95.28 %	**Result:** Acrolein solubility > 214 g/L in acetone, dichloromethane, ethyl acetate, methanol, n-heptane and toluene. **Temperature:** 24 °C	For dichloromethane, heptane and toluene, small masses of solids were observed to adhere to the test tube walls after mixing. Otherwise the solutions were clear. The examination of the effect of temperature on the solubility of acrolein in organic solvents is unjustified. At 24 °C, acrolein is highly soluble in organic solvents; hence the use of different temperatures would have little effect on the solubilities observed. The use of higher temperatures would increase the volatility and polymerisation of acrolein and at lower	Y	1	Reimer, G.J. (2007), CANTEST Project No. 4-06-0135	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
				temperatures it is expected that the results would be very similar to those at 24 °C.				
	FIFRA 63-8	92 – 96%	**result:** Alcohol - soluble Ether - soluble **temperature:** no data	Peer reviewed literature data presented on physical and chemical properties.	n.a.	2	Doane, B (1991) Solubility of Acrolein: Representative Polar and Non-Polar Solvents. Baker Performance Chemicals Inc.	
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)				The active substance will not be used in products containing organic solvents, therefore a GLP study is considered to be scientifically unjustified.				

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.9 Partition coefficient n-octanol/water (IIA3.6)	*including effects of pH (5-9)*							
log Pow 1	FIFRA 63-11	99.9%	**result:** log Pow = 0.04	The active substance is highly soluble in water at room temperature. Acrolein has no functional groups that would undergo dissociation, hence it would not ionise in solution. Measuring at different pHs would therefore have no effect on the Pow. Since the log Pow is so low, measuring at different temperatures would not change the outcome of the study since any differences obtained would be very small compared to the result of 0.04. The samples which indicate a purity of 67.58 % (NNBT-481-94D – NNBT-481-94F) show an average deviation of	Y	2	Matherly R, et al (1987) Octanol/Water Partition Coefficient of Acrolein, Baker Performance Chemicals. Study no. RD0008.287	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
				15 % between them. This represents the lower acrolein concentration used in the test (0.00129 M). This increase in relative standard deviation is due to the samples being very close to the detection limits of the instrument. Also, at this low level, the base lines are not nearly as stable as in the higher concentration. Any interferences from contaminants in either the water or 1-octanol would have a 10 fold greater effect on the integration of the acrolein peak.				
3.10 Thermal stability, identity of relevant breakdown products (IIA3.7)				The EU risk assessment on Acrolein performed for the existing substances review programme (European Chemicals Bureau			European Risk Assessment Report on acrylaldehyde (acrolein) (European Chemicals	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
				[ECB], 2001) states that the active substance is thermally unstable (dimerisation, polymerisation) and should be stabilised by a radical annihilator such as hydroquinone. Therefore, further stability studies are considered to be unnecessary.			Bureau [ECB], 2001)	
3.11 Flammability, including auto-flammability and identity of combustion products (IIA3.8)	FIFRA 63-15	92-96%	The substance is a liquid therefore flammability is covered by the flash-point test. Auto-ignition: 234°C		n.a	2	Doane B, (1989) Flammability and Viscosity of Acrolein: A summary, Baker Performance Inc, Study no. RD 0115.189	
3.12 Flash-point (IIA3.9) Flash-point 1	Official Journal of the European Communities Part A.9	95.62 %	-25 °C		Y	1	Sarff P, (2005b), Determination of the Boiling Point, Density, Viscosity and Flash Point of	X

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
							Acrolein, ABC Laboratories, Inc. Study No. 49925	
3.13 Surface tension (IIA3.10) Surface tension	OECD 115	96.63%	**result:** 73.2mN/m **temperature:** 19.7 ± 0.2°C		Y	1	Sarff P (2005a) Determination of the Surface Tension Of Acrolein, ABC Laboratories Inc, Study No. 49281	
3.14 Viscosity (-)	OECD 114	95.62 %	**Kinematic viscosity:** **result:** 1.45 mm^2/s **temperature:** 20°C **result:** 1.16 mm^2/s **temperature:** 40°C **Dynamic viscosity:** **result**: 1.28 cP **temperature:** 20°C		Y	1	Sarff P, (2005b), Determination of the Boiling Point, Density, Viscosity and Flash Point of Acrolein, ABC Laboratories, Inc. Study	

Section A3 Physical and Chemical Properties of Active Substance

Subsection (Annex Point)	Method	Purity/ Specification	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
							No. 49925	
3.15 Explosive properties (IIA3.11)	Not applicable	-	-	Please refer to the justification for non-submission of data.	-	-	-	
3.16 Oxidizing properties (IIA3.12)	Not applicable	-	Not oxidising	The substance does not contain any oxidising reagents.	-	-	-	
3.17 Reactivity towards container material (IIA3.13)	EPA 63-17	103.29%	>95% stable – decline of 1.98% after 1 year	Acrolein in Magnicide H Herbicide with two other potential degradants, methacrolein and acrolein dimer. No change in storage stability for methacrolein, whereas acrolein dimer degraded 9.5±0.15% after one year	Y	1	Kuo, A.Y. et al (1991) Determination of the Storage Stability of Acrolein in Magnacide® H Herbicide. PTRL-West. PTRL Report No. 264W-1.	X

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	18/04/2008
Materials and Methods	The applicants version is acceptable

	Evaluation by Competent Authorities
Results and discussion	The applicants version is acceptable with the following comments from the U.K. CA: 3.1.1 Melting point Purity/Specification 92-96 % acrolein Results Literature value stated (Encyclopaedia of Chemical Technology) 3.2.1 Henry's Law constant Results Calculated result (from experimental data) = 19.465 kPa $m^3.mol^{-1}$ 3.3.1 Physical state Reliability 0 (studies not applicable to this end-point – stated information only) 3.3.2 Colour Reliability 0 (studies not applicable to this end-point – stated information only) 3.3.3 Odour Results Extremely sharp, piercing odour Remarks The odour is irritating and may also be described as acrid or pungent Reliability 0 (studies not applicable to this end-point – stated information only) 3.4 Absorption spectra GLP N Reliability 1 3.9 Partition coefficient Method Similar to 92/69/EEC method A.8 (shake flask) GLP N (GLP not required as study was conducted before 30/6/88) Reference N/A 3.12 Flash point Method 92/69/EEC method A.9 (Pensky-Martens) 3.17 Reactivity towards container material Remarks *370 lb capacity steel container is compatible with the active substance.*

Evaluation by Competent Authorities	
Conclusion	Adopt the applicants version with the addition of the U.K. CA comments
Reliability	Adopt the applicants reliability indicators with the addition of the U.K. CA comments
Acceptability	Acceptable
Remarks	The U.K. CA agrees with the applicants summary but with the above mentioned comments
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A3.6 Annex Point IIIA III3.6	Dissociation constant	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The active substance does not contain any functional groups that would undergo dissociation	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11/03/2008	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A3.8 Annex Point IIIA III.2	Stability in organic solvents used in biocidal products and identity of relevant breakdown products	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	As the active substance is known to be unstable and highly soluble in organic solvents (Section A3.7, Annex Point IIIA, II.1.), further stability studies are considered to be unnecessary. In addition, hydroquinone will act as a stabiliser in the biocidal product.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11/03/2008	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A3.10 Annex Point IIA III3.7	Thermal stability, identity of relevant breakdown products	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The EU risk assessment on Acrolein performed for the existing substances review programme (European Chemicals Bureau [ECB], 2001) states that the active substance is thermally unstable (dimerisation, polymerisation) and should be stabilised by a radical annihilator such as hydroquinone. Therefore, further stability studies are considered to be unnecessary.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11/03/2008	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A3.15 Annex Point IIA III.3.11	Explosive properties	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [X]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Explosive properties of Acrolein in accordance with EU Method A14 will not be submitted on the grounds that the test can not be performed on the active substance and that such a test is not scientifically justified as the explosive properties of the active substance are already known. Risk mitigation measures are already in use to prevent explosive atmospheres of acrolein vapour and air being formed. Acrolein is a colourless liquid with a very low vapour pressure (VP = 31920 Pa at 25°C See Document IIIA Section 3.2). The A14 test for explosive properties investigates the potential for the test substance to explode under the effect of flame or sensitivity to friction or shock. The test for friction is not applicable for liquids therefore only the tests for shock and thermal sensitivity would apply to acrolein. *Safety-in-handling tests* Before performing the main tests, a small sample (10 mg) would be subjected to heating in a gas flame and to shock in any apparatus to ascertain if the substance is so sensitive that the prescribed tests should be performed with special precautions to the operator. *Thermal sensitivity*: The method involves heating the substance in a steel tube closed by orifice plates with differing diameters of hole to determine if the substance is liable to explode under conditions of intense heat and defined confinement. *Mechanical Shock.* The method involves subjecting the substance to shock from a specified mass dropped from a specified height. Acrolein at room temperature and pressure is very volatile and will go to the gaseous state very quickly. It is not possible to perform either the thermal sensitivity test or the mechanical shock test on a gas. In addition, it is well known that acrolein in the gaseous state forms explosive atmospheres with air, thereby preventing any safety tests with a naked flame being considered safe to the operator. (Further consideration must be given to the toxic nature of acrolein vapours and whether it would be possible to prevent exposure to the operator during any form of explosivity testing.) The reported flammable limits for acrolein in air were originally reported by Coward, H F and Jones, G W (1952) (Limits of Flammability of Gases and Vapours. Bull. Bureau of Mines 4th Ed.) as 2.12% and 15.5% (upward propagation of a flame in closed tube, 10.2 cm diameter and 96 cm length). Further investigation of the explosion limit has been performed by Ohta Y and Furutani M (Evaluation of hydrocarbon explosion limit in a chemical plant. Published on the website: www mech nitech.ac.jp). Vapourised acrolein and air mixtures	

Section A3.15 Annex Point IIA III.3.11	Explosive properties	
	were placed under temperatures and pressures similar to those found in acrolein processing plants. The mixtures were then compressed rapidly to determine the temperature and pressure at which the mixture would be most likely to ignite. The paper specifies that at pressures lower than 7 atm, it was not possible to ignite the acrolein/air mixture. The published EU Existing Substances Risk Assessment for acrolein indicates that although there are no published data for explosive properties of the active substance in liquid form, no further testing for this property would be required as the explosive potential of the vapour in air is well known and that the risk mitigation measures already in place to prevent explosive atmospheres being formed were sufficient to prevent the perceived hazard. As discussed in Document IIIA Section 2.10.1.2, Confidential Appendix XI, Acrolein is supplied in specialised containers (cylinder containing 168 kg active substance or skid tank 1113.6 kg active substance). Acrolein contains hydroquinone as a stabiliser to prevent polymerisation occurring (acrolein can undergo rapid polymerisation in a highly exothermic reaction in the presence of air, acid or alkali). The containers are pressurised and the active substance is kept under nitrogen to keep the substance as a liquid and out of contact with air. Containers are not opened to air but are attached to the feed system which is also maintained under nitrogen. Acrolein will only come into contact with dissolved oxygen in the water in the pipes to be treated, therefore explosive atmospheres will not be formed during application. There are only two reported incidents of explosions involving acrolein. 1) Taft, USA 11 Dec 1982 Listed on UNEP APELL Disasters Database (www.uneptie.org/pc/apell/dosasters/lists/disasterloc) A report of the incident was made by the University of Delaware Disaster Research Centre (Quarantelli, EL et al (1983) Evacuation Behaviour: Case study of the Taft, Louisiana chemical tank explosion incident. www.udel.edu/DRC/preliminary/miscreport34.pdf). The report records that monitoring of the internal temperature of a storage tank containing 45.000 gallons acrolein showed an increase over several hours and this resulted in the tank exploding causing a fire. A full report on the cause of the tank heating has not been published, but the internal heating in the tank would suggest that the substance was undergoing rapid polymerisation and therefore the explosion was due to increases in pressure and temperature in the tank as a result of an oxidation reaction not due to an intrinsic explosion potential of the active substance. 2) Niihama, Ehime, Japan 23 Dec 1998 Listed on the JST Failure Knowledge Database (http://shippai.jst.go.jp/en/Detail?fn=0&id=SC1200108&) In this case hot liquid nitrate coolant leaked through a hole in the heat exchanger (caused by corrosion) into the reactor at an acrolein manufacturing plant. The resulting high temperature reaction material blow out of the rupture disk and caused a fire in surrounding forests.	

Section A3.15 Annex Point IIA III.3.11	Explosive properties
	Again in this case it was the high temperature oxidation reaction which caused increases in pressure and temperature and thereby causing an explosion. In conclusion, explosive properties of the active substance to EU test method A14 will not be performed as it is technically impossible to perform the study and in addition the test is not scientifically justified as the explosive potential of the active substance is already well established. Risk mitigation measures for prevention of formation of explosive atmospheres of acrolein vapour and air are already in place.
Undertaking of intended data submission [ ]	
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10/03/2008
Evaluation of applicant's justification	
Conclusion	Acceptable
Remarks	None
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A3.16 Annex Point IIA III.3.12	Oxidizing properties	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	In accordance with the TNsG on Data Requirements for the Biocidal Products Directive, a study for oxidising properties is considered to be scientifically unjustified as there are no structural indications of oxidising potential.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11/03/2008	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A4.2 (a) **Annex Point IIA IV.4.2**	**Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, and where relevant in/on the following:** **Soil**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The use pattern of acrolein (off-shore oil-rigs) would lead to negligible exposure to soil, therefore it is considered that studies into analytical methods in soil are not necessary.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11/03/2008	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A4.2 (b) **Annex Point IIA IV.4.2**	**Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, and where relevant in/on the following:** **Air**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	Acrolein is a highly volatile active substance and therefore would be released to air under general use conditions. However, the active substance is applied via a closed system from sealed containers. Releases to the environment are via the aqueous environment where the substance undergoes rapid degradation. The application system and containers are neutralised by purging with nitrogen gas followed by flushing of the system with methanol before opening to prevent vapour release. The use pattern would lead to negligible exposure to air, therefore it is considered that studies in addition to the estimation of photolysis rate in air and the identification of the degradation products (Section A7.3.1, Annex Point IIIA, VII.5), are not necessary.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11/03/2008	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	

Section A4.2 (d) **Annex Point IIA IV.4.2**	**Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, and where relevant in/on the following:** **Animal and human body fluids and tissues**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	As there will be no exposure to humans, this study is not necessary. A review into the disposition and metabolism of acrolein, hydroquinone and 3-hydroxypropanal has been performed (Section A6.2, Annex Point IIA, VI. 6.2.). The data suggests rapid excretion of acrolein when administered orally to rats, mainly in the urine but with a significant amount being exhaled. Only very limited amounts of radioactivity were found in tissues at 7 days post dose. There is very limited information on human metabolism; it is likely that acrolein metabolism is similar in rats and humans. It is therefore considered that studies into analytical methods in animal and human body fluids and tissues are not necessary. The review into the disposition and metabolism of hydroquinone showed that significant amounts of radioactivity were still present in the carcass 7 days after the dermal dose was administered. However as this level is below the no adverse effect level, it is considered that studies into analytical methods in animal and human body fluids and tissues are not necessary.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11/03/2008	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	

Section A4.2 (d) **Annex Point IIA IV.4.2**	**Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, and where relevant in/on the following:** **Animal and human body fluids and tissues**
Remarks	

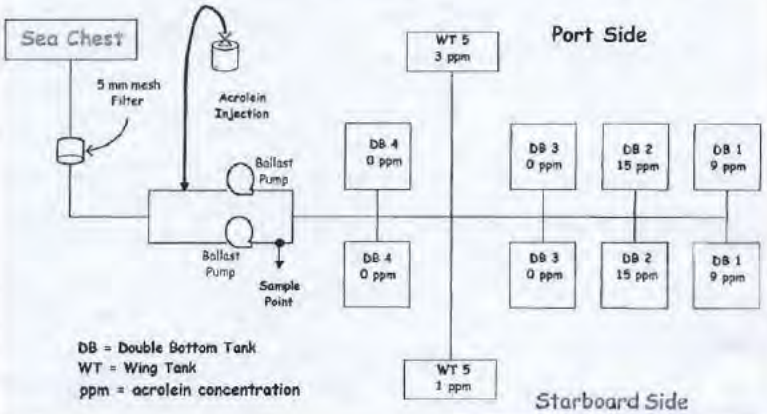
Section A4.3 **Annex Point IIIA IV.1**	**Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, in/on food or feedingstuffs and other products where relevant**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [X]	**Other justification [ ]**	
Detailed justification:	The use pattern of Acrolein (off-shore oil rig) would lead to negligible contamination of food or feeding stuffs. In accordance with the TNsG on Data Requirements for the Biocidal Products Directive, it is therefore considered that these studies are not necessary.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11/03/2008	
Evaluation of applicant's justification		
Conclusion	Acceptable	
Remarks	None	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A5	Effectiveness against target organisms and intended uses	
Subsection (Annex Point)		**Official use only**
5.1 Function (IIA5.1)	PT 12: Slimicide	
5.2 Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)	Targeted on metal surfaces to prevent corrosion. Acrolein is injected into the well-bore to prevent plugging.	
5.2.1 Organism(s) to be controlled (IIA5.2)	Sulphate-reducing bacteria (SRB) *Desulfovibrio*. General aerobic and facultative anaerobic bacteria (GAB), Iron bacteria (Crenothrix sp.), fungi, moulds and encapsulated bacteria.	
5.2.2 Products, organisms or objects to be protected (IIA5.2)	Metal surfaces are protected against corrosion. Injection into the well-bore to prevent plugging.	
5.3 Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)		
5.3.1 Effects on target organisms (IIA5.3)	Acrolein is absorbed by the bacterial cell where it reacts with protein and ultimately destroys the microorganism. The effects of acrolein on its target organisms are discussed in section B5.10.2. Penkala J.E. et al (2004 and addendum to the report) summarised the results of 28 laboratory tests. In 25 of the 28 tests conducted, acrolein exhibited complete control of SRB and GAB in tests which compare anywhere from 2 to 8 different biocide chemistries for contact times ranging from 2 to 24 hours (simulating batch or continuous applications). In 26 out of 28 tests, the minimum inhibitory concentration ($Conc_{MI}$) for acrolein ranged from 50 to 200 ppm. In 19 of the 24 batch treatment simulations (contact time = 2 to 8 hours), the $Conc_{MI}$ for acrolein ranged from 25 – 135 ppm. In the two tests simulating continuous applications (contact time = 24 hrs) the $Conc_{MI}$ for acrolein was 25 and 121 ppm. The efficacy data presented supports the use of acrolein as a slimicide to control bacteria in injection waters in oil field water systems at a concentration of 50 – 250 mg/L.	**X**
5.3.2 Likely concentrations at which the A.S. will be used (IIA5.3)	Acrolein is a slimicide used in the control of bacteria in injection waters in oil field water systems at a concentration of 50 – 250 mg/L.	
5.4 Mode of action (including time		

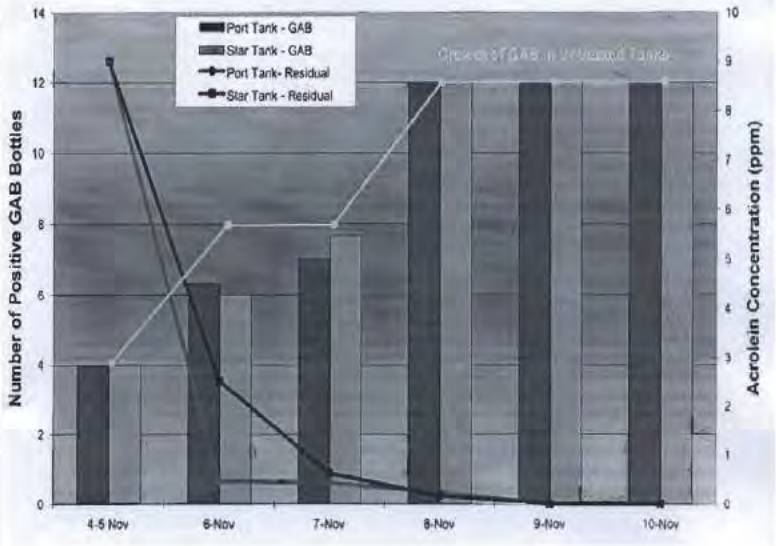
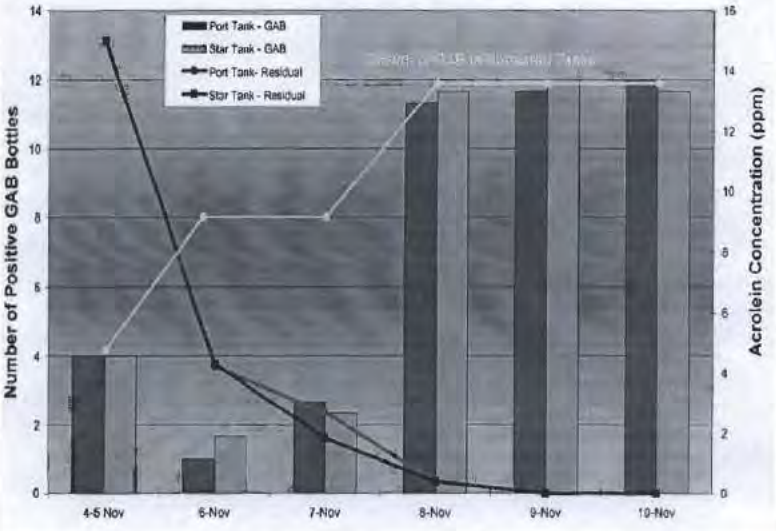
Section A5		Effectiveness against target organisms and intended uses	
	delay) (IIA5.4)		
5.4.1	**Mode of action**	The biocidal efficacy of acrolein stems from its ability to denature proteins and inhibit several enzyme systems within the living cell. Acrolein reacts with the free SH- groups of cysteine residues and the e-amino groups of lysine and cysteine residues in proteins. The electrophillic attack on sulfhydryl groups results in the rapid formation of very stable irreversible adducts. The attack on proteins affects enzyme systems and structural proteins, including those in the bacterial cell wall.	**X**
5.4.2	**Time delay**	Speed of kill is one hour.	**X**
5.5	**Field of use envisaged (IIA5.5)**		
	MG01: Disinfectants, general biocidal products		
	MG02: Preservatives	Product type 12: Slimicide	
	MG03: Pest control		
	MG04: Other biocidal products		
	Further specification		
5.6	**User (IIA5.6)**		
	Professional	Industrial professional only	
	General public	No	
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**	No information is available	
5.7.1	**Development of resistance**	No resistance of target organisms to Magnacide® B Microbiocide is known	**X**
5.7.2	**Management strategies**	Not applicable	
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	[redacted]	

Section A5	Effectiveness against target organisms and intended uses	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	8/2/2008	
Materials and methods	N/A	
Conclusion	N/A	
Reliability	N/A	
Acceptability	Applicant's version is considered incomplete.	
Remarks	**5.3.1** The Applicant has not included a tabulated summary of the key data. The Applicant's statement regarding section B5.10.2. refers to the robust study summaries (RSS) for the active substance. The data that has been cited is data summarised in Penkala, *et al*, 2004b – see section B5.10.2(8). Although Penkala, *et al*, 2004b has been cited as a key study, the Applicant has been unable to obtain the original study reports presenting the data in more detail. For this reason, no RSS, relating specifically to this data, has been produced by the Applicant. Therefore, although cited in this section, this data is not key data. The UK CA agrees with the Applicant's statement that the efficacy data presented supports the use of acrolein as a slimicide to control bacteria in injection waters in oil field water systems at a concentration of 50 – 250 mg l^{-1}. **5.4.1** The word 'affects' should be 'effects'. **5.4.2** The UK CA is unclear of the origin of this statement, and unclear as to why the Applicant has stated a specific number. The UK CA does not consider this to be a significant point. **5.7.1** The Applicant has provided further information on this aspect. This information has been included in the UK CA's evaluation in Doc. IIA, Section 2.5.	

Section A5/01 (Annex point IIA5)		Efficacy data on the active substance	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Penkala J.E., Law M.D. and Cowan J.K. 2003. Acrolein as a potential treatment alternative for control of microorganisms in ballast tanks: Five day sea trial. Institute of Marine Engineering, Science and Technology. Technical paper presented at the 2nd International Ballast Water Treatment R&D Symposium, International Maritime Organisation, London, England, July 21-23, 2003.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite Corporation	
1.2.2	Criteria for data protection	Data on new a.s. / b.p. for first entry to Annex I	
1.3	**Guideline study**	No	
1.4	**Deviations**	Not applicable	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**	Acrolein	**X**
2.1.1	Trade name/ proposed trade name	MAGNACIDE® B Microbiocide and MAGNACIDE® H Herbicide	**X**
2.1.2	Composition of Product tested	Not specified	**X**
2.1.3	Physical state and nature		**X**
2.1.4	Monitoring of active substance concentration	Acrolein residuals were measured.	
2.1.5	Method of analysis	The most sensitive and accurate field method to date for determining acrolein residuals is differential pulse polarography (DPP). This method employs an EG&G PARC Model 394 electrochemical trace analyzer connected to an EG&G PARC Model 303A static mercury drop electrode (SMDE). DPP analysis allows for the determination of a trace chemical, in this case acrolein, which can be electrochemically oxidized or reduced (in this case, reduced) in a sample. A potential is applied to a sample via a conductive electrode. The potential, which serves as the driving force in the experiment, is scanned over a region of interest. When measuring acrolein residuals, all samples are scanned from an initial potential of -0.9 V to a final potential of -1.5 V. At a potential of approximately -1.2 V the acrolein in solution is reduced, producing a current at the working mercury electrode. The magnitude of current produced is proportional to the concentration of acrolein in the solution	
2.2	**Reference substance**	No	
2.2.1	Method of analysis for reference substance		**X**

2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	General aerobic and facultative anaerobic bacteria (GAB) and sulfur reducing bacteria (SRB)	
2.3.2	Toxicity System	**Intermarine Industrial Century: Ballast Line Schematic**  	
2.3.3	Application of TS	Acrolein was injected into the line on the suction side of one pump. Both the acrolein cylinder and the sample drum were placed on the weather deck, and chemical/sample lines were run down to the ballast room via an escape hatch. The acrolein was delivered from a 26 kg (net weight) cylinder via a standardized BPC manifold using nitrogen pressure. Chemical volumes were metered using a Sponsler digital flowmeter at a rate to achieve maximum chemical injection time during ballast tank filling. Injection rates varied between 114ml/min and 280ml/min.	**X**
2.3.4	Test conditions	**Hydrographic characteristics of water column in Port Guanta, Venezuela prior to ballast uptake** Depth in Water Column Parameter / 1.0 m / 5.5 m / 10.9 m Temperature (°C) / 27.73 / 25.59 / 25.21 Conductivity / 48.7 / 49.2 / 49.3 Salinity (ppt) / 31.9 / 32.2 / 32.3 Dissolved Oxygen (ppm) / 5.15 / 3.56 / 3.81	
2.3.5	Duration of the test / Exposure time	The trial took place over 6 days (November 4-10)	
2.3.6	Number of replicates performed	There were 2 tanks with an applied concentration of acrolein of 9 ppm and 2 with 15 ppm. One wing tank received a concentration of 1 ppm and the other 3 ppm.	
2.3.7	Controls	There were 4 controls used in the trial.	
2.4	**Examination**		
2.4.1	Effect investigated	Growth inhibition of general aerobic and facultative anaerobic bacteria (GAB) and sulfur reducing bacteria (SRB).	
2.4.2	Method for recording / scoring of the effect	Immediately following collection, the water samples were prepared for semi-quantitative enumeration of viable SRB and viable GAB using the serial dilution technique. Samples were diluted into 3.5% TDS modified Postgate's SRB media and 3.5% TDS modified aerobic phenol red dextrose media for GAB growth (C&S Laboratories, Inc. Broken Arrow, OK). Serial dilutions were performed according to the NACE	**X**

		Standard Test Method 0194-94 "Field Monitoring of Bacterial Growth in Oilfield Systems". The serially diluted culture vials were then incubated at 28°C for 28 days at which time the log_{10} number of bacterial growth for each sample was recorded.	
2.4.3	Intervals of examination	Three 100 ml water samples were obtained in triplicate during the filling operation of the ballast tanks approximately 15 minutes apart. During the voyage each of the test ballast tanks were sampled daily. Each tank was sampled in triplicate and tested for acrolein residuals and bacterial cultures.	
2.4.4	Statistics	Not specified	
2.4.5	Post monitoring of the test organism	No	
		3 RESULTS	
3.1	**Efficacy**	**General aerobic and facultative anaerobic bacteria (GAB)** The GAB concentrations in the ballast uptake samples ranged from 4-5 log_{10} GAB/ml for each of the 10 tanks. The average concentration for all samples collected was 4.3 x 10^4 GAB/ml. In the four control tanks, the GAB numbers increased dramatically from 10^4 to 10^8 GAB/ml within 24 hours to 36 hours after filling the tanks. 48 hours after filling the tanks, the same concentration was encountered in all control samples. For each of the subsequent time points, samples collected from the control tanks showed positive cultures in all 12 bottles, indicating that the GAB concentrations tanks were greater than or equal to 10^{12}/mL. In Wing Tanks #5 Port and Starboard, treatments of 3 ppm and 1 ppm of acrolein were used, respectively. The GAB concentrations were the same as reported for the untreated tanks described above. It can be concluded that the acrolein treatment applied had no impact on the concentration of GAB in these tanks. This finding is not surprising, since chemical monitoring revealed no residual acrolein in the tanks immediately after treatment. The Double Bottom Tanks #1 Port and Starboard were both treated with 9 ppm acrolein. On November 6th, a reduction in GAB concentration to approximately 10^6 GAB/ml was achieved as compared to the control tanks. However, this still represented a 2 log_{10} increase over the intake water. Since approximately 10% of the tank volume remained following discharge and prior to uptake and chemical treatment, it is presumed that bacteria in this residual ballast water contributed to the rapid increase in GAB seen the day after filling. On November 7th, the GAB concentration had increased to approximately 10^7 GAB/ml. This was still less than the controls but steadily increasing as the acrolein residuals decreased to less than 1 ppm. The Double Bottom Tanks #2 Port and Starboard were each treated with 15 ppm acrolein. On November 6th, the GAB had decreased to well below the concentration in the uptake water: less than 10^2 GAB/ml. This represents an approximate 99.9% reduction of the bacteria in the uptake water. Furthermore, this represents greater than 99.9999% decrease in GAB compared to the untreated tanks. On the November 7th, the GAB concentration was slightly greater than 10^4 GAB/ml, approximately the same concentration as seen in the uptake water. However, this represents a greater than 99.99% GAB reduction as compared to the untreated tanks. **Sulfate reducing bacteria (SRB)** On November 6th (24 – 36 hours) only Double Bottom Tank #3 Starboard (control) showed positive cultures of SRB. Until November	

		8th (72 hours), no SRB were detected in any of the treated tanks. At this time point, three tanks, one control, one at 1 ppm, and one at 15 ppm were positive for SRB. The maximum number of SRB detected (10^2/mL) was observed in Double Bottom Tank #3 Starboard. At the time of ballast discharge (November 10th), seven of the nine tanks discharged for the test contained SRB. Concentrations ranged up to 10^3 SRB/ml in Double Bottom Tank #2 Starboard (15 ppm). The contamination by SRB may be due to residual populations that have become established in the tanks over time.	
3.1.1	Dose/Efficacy curve	**Figure 4** **Acrolein Residual Versus GAB Concentration in Tanks Treated at 9 ppm**  **Figure 5** **Acrolein Residual Versus GAB Concentration in Tanks Treated at 15 ppm**  	**X**
3.1.2	Begin and duration of effects	24 h after treatment with 15 ppm acrolein the bacterial levels were reduced by at least 6 $\log_{10}$ units or greater than 99.9999%.	**X**
3.1.3	Observed effects in the post monitoring phase	n/a	

3.2	**Effects against organisms or objects to be protected**	No adverse effects were noted in the report.	
3.3	**Other effects**	None	
3.4	**Efficay of the reference substance**	n/a	
3.5	**Tabular and/or graphical presentation of the summarised results**	**Average Log_{10} Number of GAB/ml in Ballast Water**	
3.6	**Efficacy limiting factors**		
3.6.1	Occurrences of resistances	No occurrences of resistance were noted in the report.	
3.6.2	Other limiting factors	Although the GAB levels in the port water during ballast filling were 10^4 to 10^5 GAB/ml, within 24-36 hrs the concentrations in the controls, 1 ppm and 3 ppm-treated tanks were greater than or equal to 10^8/mL using a 8 bottle dilution series. This may have been due to rapid growth within the tank environment or it might have been due to a high initial concentration of GAB in the residual water residing in the tanks prior to filling. Given that the estimated residual water in each tank was 1/10 of the total volume, had it contained 10^9 bacteria/ml, then a 10-fold dilution with incoming ballast water would only reduce this population to 10^8/mL. The concentrations of bacteria in the residual water of the tanks are fairly important and should be accounted for prior to treatment. Not only that, but any sediment or sludge in the tanks which most likely contain sessile bacterial populations could also significantly impact the initial concentration in the tanks once they are filled. In the future, it is important to obtain data on the final concentration of bacteria in the tanks immediately after filling.	

Ballast Tank	Applied Concentration of Acrolein (ppm)	4-5 Nov uptake	6-Nov	7-Nov	8-Nov	9-Nov	10-Nov discharge
DB #3 Port	Control	5.00	8.00	8.00	12.00	12.00	12.00
DB #3 Star	Control	5.00	8.00	8.00	12.00	12.00	12.00
DB #4 Port	Control	4.00	8.00	8.00	12.00	12.00	12.00
DB #4 Star	Control	4.00	8.00	8.00	12.00	12.00	12.00
WT #5 Star	1 ppm	5.00	8.00	8.00	12.00	12.00	12.00
WT #5 Port	3 ppm	4.00	8.00	8.00	12.00	12.00	12.00
DB #1 Port	9 ppm	4.00	6.33	7.00	12.00	12.00	12.00
DB #1 Star	9 ppm	4.00	6.00	7.67	12.00	12.00	12.00
DB #2 Port	15 ppm	4.00	1.00	2.67	11.33	11.67	12.00
DB #2 Star	15 ppm	4.00	1.67	2.33	11.67	12.00	11.67

Average Log_{10} Number of SRB/ml in Ballast Water

Ballast Tank	Applied Concentration of Acrolein (ppm)	4-5 Nov uptake	6-Nov	7-Nov	8-Nov	9-Nov	10-Nov discharge
DB #3 Port	Control	NT	0.00	0.00	0.00	0.00	1.00
DB #3 Star	Control	NT	1.67	0.00	2.00	0.00	x
DB #4 Port	Control	NT	0.00	0.00	0.00	0.00	0.67
DB #4 Star	Control	NT	0.00	0.00	0.00	0.00	1.67
WT #5 Star	1	NT	0.00	0.00	0.33	2.00	2.00
WT #5 Port	3	NT	0.00	0.00	0.00	0.00	0.00
DB #1 Port	9	NT	0.00	0.00	0.00	0.00	2.00
DB #1 Star	9	NT	0.00	0.00	0.00	0.00	0.00
DB #2 Port	15	NT	0.00	0.00	0.00	0.33	0.67
DB #2 Star	15	NT	0.00	0.00	1.33	1.67	3.00

		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	Laboratory experiments were carried out examining the biocidal efficacy of acrolein against marine microorganisms under conditions mimicking exposure time in ballast tanks during a voyage.	**X**
4.2	**Intended actual scale of biocide application**	Not specified	
4.3	**Relevance compared to field conditions**		
4.3.1	Application method	A series of experiments were conducted to evaluate the efficacy of acrolein against common marine microorganisms.	**X**
4.3.2	Test organism	The test organisms used were: 1). Common marine bacterial strains: *Pseudomonas fluorescens*, a Gram negative, non-sporulating bacterium *Bacillus cereus* a Gram positive, spore-forming bacteria *Bacillus subtilis*, a Gram positive, spore-forming bacteria *Staphylococcus epidermidis*, a Gram positive, non-sporulating bacterium. 2). General aerobic and faculative anaerobic bacteria (GAB) and sulfate reducing bacteria (SRB) were tested to model populations that might be encountered in port water used for ballast. *3).* The marine dinoflagellate, *Gymnodinium sanguineum.*	**X**
4.3.3	Observed effect	The results from the common marine bacterial strains indicate that significant reductions in bacterial number occurred at all acrolein concentrations tested. The control organisms (0 ppm) exhibited at least 10^6 bacteria per ml for all strains tested. At 10 ppm acrolein no greater than 10^1 bacterial per ml were observed for any strain at either 24 or 72 hours contact (>99.999% reduction). At 3 ppm acrolein, no greater than 10^2 bacteria per ml were detected (>99.99% reduction). There was no detectable growth of GAB or SRB at 10 ppm of acrolein, an 11 order of magnitude reduction. At 3 ppm acrolein limited SRB growth to 10^2/ ml at 24 hour contact and to below detection with 72 hour contact compared to 10^{11} /ml in the controls. At 3 ppm, GAB growth was limited to 10^3 /ml after 24 hours contact and 10^1/ml after 72 hours contact compared to 10^{11}/ml in the controls. These results show that acrolein is effective at 3 and 10 ppm for control of microorganisms in Galveston Port water. The results indicated that all concentrations of acrolein were able to reduce the concentration of viable dinoflagellates to below the detectable limit of the assay. No viable or motile dinoflagellates were observed in any of the acrolein-treated samples. The integrity of the dinoflagellate cell was completely destroyed by the treatment with acrolein.	**X**
4.4	**Relevance for read-across**	n/a as field studies were conducted. The demand for acrolein in the tanks is much higher than what was predicted from laboratory testing. Applied concentrations of 1 and 3 ppm were immediately undetectable by the time the tanks had been filled.	

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was conducted to investigate the efficacy of acrolein as a potential ballast water treatment alternative. Ten ballast tanks were selected (5 pairs) so that discharge and some of the tank filling could be done on parallel tanks. The ballast water enters the ship via a single line and then passes through a 5mm mesh filter. It then separates into two parallel lines each feeding a charge pump. The normal operating rate of each pump is 250 m^3/hour. The line pressure was approximately 15 psi. The two lines then converge downstream of the pumps to a common line that transports ballast water to the parallel ballast tanks. The application and sampling points were set up on each of the parallel lines feeding the charge pumps. Acrolein was injected into the line on the suction side of one pump and water samples were obtained on the parallel line on the discharge side of the second pump. In that way, acrolein treatment and sampling could be carried out simultaneously. Both the acrolein cylinder and the sample drum were placed on the weather deck, and chemical/sample lines were run down to the ballast room via an escape hatch. Untreated control tanks were filled first in order to ensure that the ballasting operation, flow rates and sampling were proceeding properly. The first treated tank (Wing Tank #1 - Port) received 3 ppm (v/v) of acrolein. The parallel starboard tank (Wing Tank #1 – Starboard) received 1 ppm of acrolein. After measuring residuals in these tanks using differential pulse polarography (DPP) it was determined that the acrolein in these two tanks had been immediately consumed as no residual was detectable. Therefore adjustments were made and subsequent tanks were treated with 15 ppm acrolein (Double Bottom #2 Port and Double Bottom #2 Starboard) and 9 ppm of acrolein (Double Bottom #1 Port and Double Bottom #1 Starboard). Discharge and then reballasting were conducted. At the time of discharge, the chemical residuals in the treated tanks were below the 10 ppb detection limit of the DPP.	
5.2	**Reliability**	n/a	
5.3	**Assessment of efficacy, data analysis and interpretation**	Acrolein at 15 ppm had a significant impact on the bacterial load in the tanks as compared to the controls. This comparison is the more critical one when determining efficacy of biocide, not the comparison with intake water. If one uses this comparison, then 24 hours after treatment with 15 ppm acrolein the bacterial levels were reduced by at least 6 $\log_{10}$ units or greater than 99.9999 %. The residual at that time was approximately 4.0 ppm. On the following day (48 hours), when the residual had decreased to approximately 2 ppm, there was still at least a 6 $\log_{10}$ reduction in the number of bacteria in the tank. Acrolein applied at 9 ppm had a lesser impact on the bacterial load in the tanks as compared to the controls. However, it is important to review what the actual biocide residual is at the time the readings are being made. After 24 hours, the residual was 0.5 ppm in the Port tank and 3 ppm in the Starboard tank. The bacterial load at this time point had been reduced by at least 2 $\log_{10}$ units as compared to the control, or 99 % reduction. However, since the dilution limit had been exceeded in the control tanks, the maximum reduction at this time point could not be obtained. Not having data on the initial load in the tanks limits our conclusions. Bacterial control in the 9 ppm-treated tanks becomes further reduced as the residual decreases. In all cases, the tanks no longer exhibited substantial bacterial control after the third day, assuming the untreated tanks had concentrations no more than 10^{12}	

	GAB/ml (the maximum detection limit for this test). The other class of bacteria examined was sulfate reducing bacteria (SRB). Although none of these organisms were detected in the port water used to fill the tanks, their growth was detected in the tanks, presumably due to SRB resident in the residual water and sediments in the tanks. By the end of the voyage, 7 out of 9 tanks had established planktonic populations of SRB, up to 1000 SRB/ml. These planktonic populations only became established as the acrolein residuals had become negligible and bacterial control was lost.	
5.4 Conclusion	Acrolein at a concentration of 15 ppm was required to have a significant impact on bacteria present in the ballast tanks after filling the tanks. A concentration of 9 ppm, exhibited a lesser degree of effectiveness. Whereas, 1 and 3 ppm acrolein was ineffective. At 15 ppm, acrolein controlled the bacteria for at least 48 hours, but regrowth occurred by 72 hours as the acrolein residual had diminished below 0.5 ppm. It is estimated that a minimum residual of $\geq$ 2 ppm would be required to maintain control. SRB were present in 7 of the 9 ballast tanks tested at the end of the voyage (1-3 $\log_{10}$/mL), although none were present in the seawater that was used to fill the tanks. Overall, the results were encouraging. A very high level of control was maintained by 15 ppm of acrolein, especially given the high concentrations of bacteria observed in the untreated tanks. This study supports the feasibility of acrolein as an alternative ballast water treatment method by showing its potential for high efficacy, safe and simple installation and application, economic viability, and potential for safe discharge.	**X**
5.5 Proposed efficacy specification	Acrolein at a concentration of 15 ppm was required to have a significant impact on bacteria present in the ballast tanks after filling the tanks. A concentration of 9 ppm, exhibited a lesser degree of effectiveness. Whereas, 1 and 3 ppm acrolein was ineffective. The study therefore supports the proposed use concentration of acrolein at 50-250 mg/l and demonstrates its efficacy at lower concentrations.	**X**
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	8/2/2008	
Materials and methods	The UK CA accepts the Applicant's version, with the following comments. **2.1, 2.1.1 & 2.1.2** In their dossier, the Applicant has specified (Doc. IIIB, Section 2.2) that the biocidal product Magnacide B contains 99.7 – 99.8 % w/w/ acrolein. Given that 99.7 – 99.8 % of Magnacide B is acrolein, the UK CA considers that, from the efficacy point of view, the active substance and the biocidal product are the same. For this reason, the UK CA is satisfied that the efficacy data presented in this robust study summary (RSS), if acceptable, can be used in support of the active substance. Magnacide B is also marketed under the name Magnacide H. Magnacide H is, however, an aquatic herbicide widely used in irrigation canals to control submerged plants and algae that can impede water flow. Magnacide H is thus marketed for use in an area that is outside of the scope of the BPD.	

	2.1.3 & 2.2.1 The Applicant has not completed these Sections. The UK CA does not consider these to be significant omissions. **2.3.3** The applicant stated the cylinder weight in pounds. The UK CA has re-expressed this weight as kilogrammes. **2.4.2** The bacterial numbers were determined using the serial dilution methodology set out in the National Association of Corrosion Engineers (NACE) standard test method 0194-94. NACE International is a long established, worldwide organisation dedicated to protecting people and the environment from the effects of corrosion. The NACE standard test method 0194-94 serial dilution methodology is therefore a well established and commonly used methodology for determining the effect of a biocide on the size of bacterial populations. The UK CA considers the methodology used to be acceptable. The efficacy template does not require the Applicant to state a number for the reliability indicator. However, the UK CA considers the reliability indicator to be 2 (see below).
Results and discussion	The UK CA accepts the Applicant's version, with the following comments. **3.1.1** Using the serial dilution method, the number of positive bottles is a direct measure of the number of bacteria in the samples. For example, where the data shows that 4 positive bottles were obtained, this indicates that the samples contained 4 $\log_{10}$ bacteria ml^{-1}, that is to say 10,000 bacteria ml^{-1}. This is illustrated in the tabulated data presented in Section 3.5. **3.1.2** The Applicant has stated that '24 h after treatment with 15 ppm acrolein the bacterial levels were reduced by at least 6 $\log_{10}$ units or greater than 99.9999 %'. This was the reduction in bacterial numbers measured in relation to the untreated control tanks, and not to the bacterial levels in the up-take water in the treated tanks at the time of treatment. As the bacterial levels in the up-take water in all of the treated and untreated tanks were very similar, the UK CA considers that the Applicant's comparison of the treated tanks with the untreated control tanks is the correct comparison to make, and is thus acceptable. **4.1, 4.3.1, 4.3.2.& 4.3.3** The Applicant's statements in these Sections relate to a series of initial laboratory tests conducted before field testing was begun. These tests were designed to establish the minimum effective concentrations of acrolein, under laboratory conditions, against common marine bacterial strains, general aerobic and facultative anaerobic bacteria (GAB) and sulphate reducing bacteria (SRB). These tests were 'range finding' tests, with the results used as a basis for the concentrations of acrolein used in the field test. Based on the results of these tests, the field test was conducted using 1, 3, 9 and 15 ppm acrolein. The Applicant has not presented the results of these laboratory tests in the RSS. However, the UK CA considers that the field results, which have been presented in the RSS, demonstrated the efficacy of 9 ppm and 15 ppm acrolein against GAB for < 24 hours and up to 48 hours, respectively. As the UK CA considers field data to be a superior way of demonstrating efficacy than laboratory data, the UK CA does not consider the absence of the laboratory data to be a significant omission.

Conclusion	**5.4** The UK CA agrees with the Applicant's conclusions regarding the effectiveness of acrolein against GAB. The UK CA also agrees with the Applicant's conclusion regarding the presence of SRB in the ballast tanks. However, no SRB were present in the seawater used to fill the tanks, and most of the ballast water samples from the treated and control tanks contained no SRB (see Section 3.5). For this reason, although agreeing with the Applicant's statement regarding SRB, the UK CA does not consider the field test as providing any evidence for the effectiveness of acrolein, under field conditions, against SRB. **5.5** The UK CA agrees with the Applicant's statements, but only in relation to the effectiveness of acrolein against GAB. The UK CA considers the field test results as demonstrating the efficacy of 15 ppm acrolein against GAB, but not against SRB.
Reliability	2
Acceptability	The UK CA considers the data to be acceptable in support of Annex I inclusion.
Remarks	All data and endpoints presented in the study summary have been checked against the original study and are correct.
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

Criteria	Details
Nature	General aerobic and facultative anaerobic bacteria and sulfate reducing bacteria.
Origin	
Initial biomass	The GAB concentrations in the ballast uptake samples ranged from 4 – 5 $\log_{10}$ GAB/ml for each of the 10 tanks
Reference of methods	Not specified
Collection / storage of samples	Water samples were collected by delivery topside via a ½ inch industrial hose. Three 100 ml samples were obtained in triplicate during the filling operation of the ballast tanks approximately 15 minutes apart. These samples were immediately diluted into culture bottles and parallel samples were fixed for microscopy.
Preparation of inoculum for exposure	Not specified
Pretreatment	Not specified
Initial density of test population in the test system	Not specified

1.5 Test conditions

Criteria	Details	
Substrate	Port Guanta water used for ballast uptake	
Incubation temperature	Depth in water column (m):	Temperature(°C):
	1.0	27.73
	5.5	25.59
	10.9	25.21
Moisture	Not specified	
Aeration	Not specified	
Method of exposure	Not specified	
Aging of samples	Not specified	
Other conditions	Not specified	

Section A5/02 (Annex point IIA5)		Efficacy data on the active substance	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Harless M.L. 1996. An acrolein pilot plant treatment program for Exxon Pipeline Company's Grand Isle, Louisiana Water Treatment Facility. American Filtration Society Produced Water Seminar, League City, Texas, January 1996.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Performance Chemicals Inc.	
1.2.2	Criteria for data protection	Data on new a.s. / b.p. for first entry to Annex I	
1.3	**Guideline study**	No	
1.4	**Deviations**	Not applicable	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**	Acrolein	**X**
2.1.1	Trade name/ proposed trade name	MAGNACIDE®B	**X**
2.1.2	Composition of Product tested		**X**
2.1.3	Physical state and nature		**X**
2.1.4	Monitoring of active substance concentration	Acrolein residuals were measured.	
2.1.5	Method of analysis	Acrolein residuals were monitored using a Princeton Applied Reasearch model 364 A polarographic analyser with a 303 static mercury drop electrode and an XY plotter. Samples were collected and analysed periodically from the Tank No. 2100 outlet, the commingled IGF-101&102 outlets, the commingled DF-104 A&B outlets, the commingled outlet of the clean water tanks and the injection pump outlet.	
2.2	**Reference substance**	No	
2.2.1	Method of analysis for reference substance		**X**
2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	General aerobic and facultative anaerobic (GAB) bacteria and sulfate reducing bacteria (SRB) were enumerated.	**X**
2.3.2	Test system	Water treatment at Exxon Pipeline Company (EPC), Grand Isle began at a primary separation vessel (Tank No.2238). The water leg of Tank No. 2238 flowed to a skim tank (Tank No. 2100) and then through two parallel induced gas flotation vessels (IGFs 101 and 102). Water from the IGFs passed through two parallel primary filtration vessels (DFs 104	

		A&B) and flowed to a set of clean water tanks. From the clean water tanks, the water flowed through two of the three guard filter units in service at the time. From the guard filter units, the water was delivered to a set of high pressure pumps for disposal into the three SWD wells. Water through-put varied with respect to production parameters, but averaged approximately 40000-45000 barrels of water per day (BWPD).	
2.3.3	Application of TS	Acrolein was injected into the water leg of Tank No. 2238 using a skid mounted pump equipped with mounted valves and pressure gauges, a site-glass and an explosive vapour monitoring device. The treatment rate was varied throughout the test to evaluate the effectiveness of different acrolein concentrations.	
2.3.4	Test conditions	Not specified	
2.3.5	Duration of the test / Exposure time	The microbiocide treatment regime consisted of a seven day continuous application of acrolein.	
2.3.6	Number of replicates performed	Not specified	
2.3.7	Controls	Tank outlet 2238 was untreated .	
2.4	**Examination**		
2.4.1	Effect investigated	Bacterial growth	
2.4.2	Method for recording / scoring of the effect	Bacteria were enumerated using the serial dilution method with six bottle strings of media. Using this convention, 0 positive bottles represents a range of 0 to 1 bacteria/ml and is reported as 1 bacteria/ml, 1 positive bottle represents a range of 1 to 10 bacteria/ml and is reported as 10 bacteria/ml and so on for the remaining number of bottles in the string.	**X**
2.4.3	Intervals of examination	Bacteria were enumerated once two days prior to the test, once each day during the test and once on each of the three days following chemical injection. Enumeration of SRB was performed daily on samples taken from Tank No. 2238 outlet, Tank No. 2100 outlet and the injection pump outlet. Two samples of sessile bacteria were collected: one prior to the start of the test and one during the guard filter change out which occurred on the 5th day of the test.	
2.4.4	Statistics	Not specified	
2.4.5	Post monitoring of the test organism	Not specified	
		3 RESULTS	
3.1	**Efficacy**	Extremely high numbers of sessile bacteria were present on the interior surface of the guard filter units prior to initiation of treatment with acrolein. Continuous treatment with acrolein at low levels provided the control of planktonic bacteria throughout the system specified by EPC. The results of the residual monitoring, the bacterial monitoring and the time-kill test suggest that a continuous acrolein residual of 10 ppm in the system should be sufficient to provide bacterial control. The continuous low level treatment significantly reduced the number of sessile bacteria present in the iron sulfide sludge build-up on the interior surface of the guard filter units. Continuous treatment for a longer period of time may reduce the numbers even further. Both the bacterial control and sulfide scavenging provided by acrolein	**X**

		substantially prolonged guard filter life. The cost savings due to less frequent filter change-outs would be considerable with continuous acrolein treatment due to its biocidal properties as well as its sulfide scavenging capabilities. Prolonged treatment with acrolein may also result in less frequent well work-overs as well as reduced corrosion and failure of equipment due to bacteria.	
3.1.1	Dose/Efficacy curve		X
3.1.2	Begin and duration of effects	Not specified	
3.1.3	Observed effects in the post monitoring phase	Not specified	
3.2	**Effects against organisms or objects to be protected**	Not specified	
3.3	**Other effects**		X
3.4	**Efficacy of the reference substance**	n/a	
3.5	**Tabular and/or graphical presentation of the summarised results**		X
3.6	**Efficacy limiting factors**		
3.6.1	Occurrences of resistances	There were no occurrences of resistance noted in the report.	
3.6.2	Other limiting factors		X
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	A laboratory test was not carried out	
4.2	**Intended actual scale of biocide application**	n/a	
4.3	**Relevance compared to field conditions**		
4.3.1	Application method	n/a	
4.3.2	Test organism	n/a	
4.3.3	Observed effect	n/a	
4.4	**Relevance for read-across**	n/a	
		5 APPLICANT'S SUMMARY AND CONCLUSION	

5.1	**Materials and methods**	The purpose of the pilot plant work was to determine the most efficient and economical microbiocide for controlling bacteria at this facility. The microbiocide treatment regime consisted of a 7 day continuous application of acrolein into the water leg of Tank No. 2238.	
5.2	**Reliability**	n/a	
5.3	**Assessment of efficacy, data analysis and interpretation**	Continuous treatment with acrolein at low levels provided the control of planktonic bacteria throughout the system specified by EPC. The results of the residual monitoring, the bacterial monitoring and the time-kill test suggest that a continuous acrolein residual of 10 ppm in the system should be sufficient to provide bacterial control. The continuous low level treatment significantly reduced the number of sessile bacteria present in the iron sulfide sludge build-up on the interior surface of the guard filter units. Continuous treatment for a longer period of time may reduce the numbers even further.	**X**
5.4	**Conclusion**	Continuous treatment with acrolein at low levels provided the control of planktonic bacteria throughout the system specified by EPC. The results of the residual monitoring, the bacterial monitoring and the time-kill test suggest that a continuous acrolein residual of 10 ppm in the system should be sufficient to provide bacterial control.	**X**
5.5	**Proposed efficacy specification**	The results of the residual monitoring, the bacterial monitoring and the time-kill test suggest that a continuous acrolein residual of 10 ppm in the system should be sufficient to provide bacterial control	**X**
		Evaluation by Competent Authorities	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		8/2/2008	
Materials and methods		The UK CA accepts the Applicant's version, with the following comments. **2.1 &2.1.1** In their dossier, the Applicant has specified (Doc. IIIB, Section 2.2) that the biocidal product Magnacide B contains 99.7 – 99.8 % w/w/ acrolein. Given that 99.7 – 99.8 % of Magnacide B is acrolein, the UK CA considers that, from the efficacy point of view, the active substance and the biocidal product are the same. For this reason. the UK CA is satisfied that the efficacy data presented in this RSS, if acceptable, can be used in support of the active substance. **2.1.2, 2.1.3. &2.2.1**. The Applicant has not completed these Sections. The UK CA does not consider these to be significant omissions. **2.3.1** The statement in this Section should read 'General aerobic bacteria (GAB) and general and facultative anaerobic bacteria and sulfate reducing bacteria (SRB) were enumerated'. **2.4.2** The information provided does not properly describe the methodology followed. However, the study report provides a full description. The planktonic and sessile bacterial counts were obtained using a serial dilution methodology according to NACE Standard TM0194-94. The time-kill test on planktonic bacteria was also conducted according to this standard.	

	The efficacy template does not require the Applicant to state a number for the reliability indicator. However, the UK CA considers the reliability indicator to be 2 (see below).
Results and discussion	The UK CA accepts the Applicant's version, with the following comments. **3.3. & 3.6.2** The Applicant has not completed these Sections. The UK CA does not consider these to be significant omissions. **3.1, 3.1.1, 3.5 & 5.3**. Although the Applicant has provided a written summary of the results, the data have not been presented in Sections 3.1.1 & 3.5 . As Section 3.1. refers to the results for planktonic and sessile bacterial monitoring, acrolein residual monitoring, and a time-kill test, the UK CA has presented these data in Appendix 1 to this RSS. The bacterial count results presented are for planktonic and sessile GAB, SRB and general anaerobic and facultative anaerobic bacteria. Although not stated in the RSS, the study report states that thioglycolate media was used for the enumeration of the latter. This is why the results for general anaerobic and facultative anaerobic bacteria have been presented under the heading of 'Thio'. The SRB were enumerated in two ways, namely, by the use of a modified Postgate's media and by the use of 'RapidChek II' test kits. The results for the former have been presented under the heading 'SRB', and those for the latter under the heading 'RC'. The method used to record/score the effect is described in Section 2.4.2. For example, in the serial dilution method used in the study, if 6 positive bottles are obtained, this indicates a bacterial count in the range $10^5 – 10^6$ microorganisms ml ^{1}. Following the convention in the serial dilution method, the upper value is reported. So, in this example, the count is reported as 10^6 microorganisms ml^{-1}. In this particular study, the counts have been expressed as log values. So, a count of 10^6 microorganisms ml^{-1} would be expressed as '6'. In Section 3.1. the Applicant has stated that 'continuous treatment with acrolein at low levels provided the control of planktonic bacteria throughout the system specified by EPC'. Although the RSS provides no further information on this, the study report states that 'the purpose of the pilot work was to determine the most efficient and economical microbiocide for controlling bacteria at this facility. Control of bacteria was defined by the Exxon Pipeline Company (EPC) as maintaining a bacterial level not in excess of 1 - 9 to 10 - 99 bacteria/ml throughout the system'. The objective of the acrolein treatment was, therefore, to produce and maintain a viable bacterial count of < 100 microorganisms ml^{-1} i.e. a $\log_{10}$ value of 1 – 2. For those sampling points that were used to monitor acrolein residues (Table 2), the monitoring results can be compared with the planktonic counts (Tables 1a & 1b). This showed that an acceptable level of planktonic bacteria (< 100 microorganisms ml^{-1}) was maintained during the 7 day treatment period. From the residue data, the UK CA has calculated that the average concentration of acrolein at these sampling points during this period was 10.36 ppm After the treatment period, the residue levels, as would be expected, decreased sharply and the bacterial counts increased to unacceptable levels. The results from the time kill tests (Table 4), which were also based on samples taken from the same sampling points, also demonstrated the effectiveness of 10 ppm acrolein against planktonic bacteria. The results for sessile bacteria (Table 3) demonstrated the ability of 10 ppm acrolein to markedly reduce the size of the sessile population on

	the guard filter during the first 5 days of treatment. On the basis of these results, the UK CA agrees with the Applicant's statements in Sections 3.1 and 5.
Conclusion	**5.4 & 5.5** The UK CA agrees with the Applicant's statements.
Reliability	2
Acceptability	The UK CA considers the data to be acceptable in support of Annex I inclusion.
Remarks	All data and endpoints presented in the study summary have been checked against the original study and are correct.
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

APPENDIX 1

Table 1a Planktonic bacterial sampling results pre- and post-treatment with variable concentrations of Magnacide B

Time after initiation of treatment (days)	Area of system													
	Tank 2238 outlet				Tank 2100 outlet				IGF 101 outlet			IGF 102 outlet		
	Number of Bacteria				Number of Bacteria				Number of Bacteria			Number of Bacteria		
	SRB	GAB	THIO	RC	SRB	GAB	THIO	RC	SRB	GAB	THIO	SRB	GAB	THIO
-1	3	5	≥6	ND	4	4	5	ND	4	4	4	4	≥6	5
+1	5	4	≥6	ND	≤1	≤1	2	ND	≤1	1	4	≤1	3	3
+2	5	5	5	1E+05	≤1	≤1	2	1E+04	≤1	≤1	2	≤1	2	2
+3	5	3	4	1E+05	≤1	1	2	1E+04	≤1	≤1	2	≤1	1	2
+4	4	3	4	ND	≤1	≤1	1	ND	≤1	≤1	≤1	≤1	≤1	≤1
+5	3	2	3	1E+04	≤1	≤1	1	1E+04	≤1	1	1	≤1	≤1	≤1
+6	4	2	4	1E+04	≤1	≤1	2	1E+04	≤1	1	3	≤1	1	2
+7	5	2	4	1E+04	≤1	≤1	2	1E+04	≤1	≤1	2	≤1	1	2
+8	≥6	3	4	1E+05	2	3	3	1E+04	1	3	4	2	2	3
+9	4	4	4	1E+05	4	3	5	1E+05	4	3	4	4	2	5
+10	3	2	4	ND	5	3	5	ND	5	2	5	3	2	3

Table 1b Planktonic bacterial sampling results pre- and post-treatment with variable concentrations of Magnacide B

Time after initiation of treatment (days)	Area of system									
	DF-104 A&B outlet			Clean water tank outlet			Injection Pump outlet			
	Number of Bacteria			Number of Bacteria			Number of Bacteria			
	SRB	GAB	THIO	SRB	GAB	THIO	SRB	GAB	THIO	RC
-1	4	4	5	4	4	5	4	5	5	ND
+1	≤1	2	3	2	5	4	≤1	≤1	4	ND
+2	≤1	1	4	≤1	≤1	2	≤1	≤1	2	1E+03
+3	≤1	≤1	2	≤1	≤1	2	≤1	≤1	2	1E+04
+4	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	≤1	ND
+5	≤1	≤1	2	≤1	≤1	≤1	≤1	≤1	1	1E+04
+6	≤1	≤1	2	≤1	≤1	2	≤1	≤1	3	1E+03
+7	≤1	1	1	≤1	≤1	2	≤1	≤1	1	1E+04
+8	2	3	3	1	2	3	2	3	3	1E+03
+9	3	4	4	4	3	4	4	4	4	1E+04
+10	3	3	4	4	5	4	4	4	4	ND

Table 1c Planktonic bacterial sampling results pre- and post-treatment with variable concentrations of Magnacide B

Time after initiation of treatment (days)	**Area of system**					
	SWD well No. 2			**Recycling unit**		
	Number of Bacteria			**Number of Bacteria**		
	SRB	**GAB**	**THIO**	**SRB**	**GAB**	**THIO**
-1	4	3	4	4	3	≥6
+1	≤1	1	5	4	3	≥6
+2	≤1	≤1	2	3	4	≥6
+3	-	-	-	1	3	4
+4	≤1	≤1	1	≤1	2	3
+5	≤1	1	≤1	≤1	≤1	≤1
+6	≤1	≤1	2	≤1	1	5
+7	≤1	≤1	2	1	1	4
+8	1	1	3	2	3	2
+9	3	2	4	4	2	4
+10	3	4	4	4	4	5

Table 2 Acrolein concentrations following treatment with Magnacide B

Time after initiation of treatment (days)	Area of system				
	Tank 2100 outlet	**IGF 101 outlet**	**DF-104 A&B outlet**	**Clean water tank outlet**	**Injection Pump outlet**
	Acrolein concentration (ppm)				
0	**0**	**0**	**0**	**0**	**0**
+1	**5.6**	**2.1**	**1.4**	**0.3**	**0**
+1	**9.3**	**8.3**	**5.6**	**4.1**	**2**
+1	**12.4**	**11**	**9**	**6.5**	**4.1**
+1	**9.2**	**8.3**	**5.7**	**5**	**3.4**
+1	**7.2**	**6.6**	**4.7**	**3.8**	**2.9**
+2	**7.5**	**7**	**6.3**	**6.6**	**6.1**
+2	**11.5**	**10.4**	**9.2**	**8.8**	**8.3**
+2	**15.3**	**13.3**	**12**	**11**	**9.5**
+2	**20**	**17.8**	**14.9**	**13.3**	**12.2**
+3	**20.7**	**21.4**	**20.1**	**18.7**	**17.4**
+3	**18.9**	**18.2**	**16.4**	**15.3**	**15.8**

+3	**16**	**15.1**	**14.4**	**14.4**	**13.1**
+3	**18.9**	**18.3**	**15.8**	**15.1**	**13.3**
+3	**24.5**	**22.8**	**23.6**	**21.4**	**16.9**
+4	**19.2**	**19.1**	**17.8**	**17.8**	**17.3**
+4	**18.7**	**18.3**	**16.2**	**15.5**	**15.6**
+4	**20**	**19.2**	**13.5**	**13.7**	**13.8**
+5	**25.5**	**20.5**	**23.6**	**18.7**	**19.6**
+5	**14.7**	**13.3**	**12.9**	**13.8**	**13.8**
+5	**12.2**	**12.2**	**11.5**	**11.5**	**12**
+5	**9.9**	**8.8**	**8.6**	**8.4**	**8.6**
+5	**9.9**	**9.7**	**8.6**	**9**	**8.8**
+6	**8.8**	**8.3**	**8.3**	**7.2**	**7.7**
+6	**10.2**	**9.2**	**8.4**	**8.6**	**8.1**
+6	**8.3**	**7.7**	**8.3**	**7.7**	**7.5**
+6	**8.3**	**7.5**	**6.8**	**7**	**6.5**
+6	**7.2**	**7.5**	**6.6**	**7.2**	**7.5**
+6	**5.2**	**5.6**	**5**	**5.2**	**5**
+7	**2.5**	**2.3**	**1.6**	**2.3**	**2.3**
+7	**3**	**2.5**	**2.1**	**2.5**	**2**
+7	**4.5**	**4.1**	**4.1**	**3**	**3.2**
+7	**7.2**	**5.6**	**4.7**	**3.6**	**3.8**
+7	**9.9**	**8.6**	**8.4**	**8.1**	**7.5**
+8	**1.4**	**1.4**	**1.4**	**1.4**	**1.4**
+8	**0**	**0**	**0**	**0**	**0**

Table 3 Sessile bacterial sampling results pre- and post-treatment with variable concentrations of Magnacide B

Time after initiation of treatment (days)	**Area of system**			
	Guard filter			
	Number of Bacteria			
	SRB	**GAB**	**THIO**	**RC**
-1	≥10	≥10	≥10	5E+073
+5	3	3	5	1E+04

Table 4 Time-kill results following treatment with variable concentrations of Magnacide B

Acrolein Concentration (ppm)	**Contact time (h)**					
	6			**12**		
	Number of Bacteria			**Number of Bacteria**		
	SRB	**GAB**	**THIO**	**SRB**	**GAB**	**THIO**
5	3	1	4	≤1	1	3
10	1	1	4	≤1	1	1
20	≤1	≤1	1	≤1	≤1	≤1
Control	≥6	2	≥6	5	2	≥6

Section A5/03 (Annex point IIA5)		Efficacy data on the active substance	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Penkala JE, Soto H, Carranza D and Gimenez C (Date Unknown) Acrolein (2-Propenal) Mitigates Sessile Biofilm and Biogenic Sulfides in a Large Waterflood in Neuquen Province, Argentina. Baker Petrolite Corporation Internal Report. Case History I referenced in: Penkala, J.E et al, 2004, Acrolein, 2 Propenal: A Versatile Microbiocide for control of Bacteria in Oilfield Systems. NACE Paper No. 04749, NACE International Corrosion/2004, New Orleans, LA, 30/3/04-02/4/04	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new b.p. for first entry to Annex I	
1.3	**Guideline study**	No	
1.4	**Deviations**	Not applicable.	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**	Acrolein	**X**
2.1.1	Trade name/ proposed trade name	Not specified	**X**
2.1.2	Composition of Product tested	Not specified	**X**
2.1.3	Physical state and nature	The acrolein skid container contains a nitrogen blanket over the liquid acrolein.	
2.1.4	Monitoring of active substance concentration	Yes	
2.1.5	Method of analysis	Acrolein residuals were monitored during the course of a one skid application to ensure that an adequate residual was being delivered throughout the injection system. Samples were collected at intervals from various monitoring points and residuals measured by differential pulse polarography (DPP). This method employs an EG&G PARC Model 394 electrochemical trace analyzer connected to an EG&G PARC Model 303A static mercury drop electrode (SMDE).	**X**
2.2	**Reference substance**	No	
2.2.1	Method of analysis for reference substance	Not applicable	

2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	Sessile and planktonic sulphate reducing bacteria (SRB) General aerobic and facultative anaerobic bacteria (GAB) Location: Flowlines and injection wells at the Chihuido waterflood in Neuquen Province, Argentina.	
2.3.2	Test system	The Repsol-YPF facility is located in the northeastern corner of Neuquen Province near the town of Rincon de los Sauces in Central Argentina. The Repsol YPF facility processes 9000 cubic meters per day of oil and 86,000 cubic meters per day of water. The water is separated in a free water knockout (FWKO) and wash tank and is then transported through two parallel water treatment systems (Figure 1). The open system consists of an API pit, decanter pit and sand filters. The closed system, which treats approximately 75 % of the produced water, consists of three flotation units and sand filters. Both systems send the treated water to a 5000 m^3 tank from which the water is pumped to the injection system. The injection system includes 13 branch points, 63 satellites, and 506 injection wells. Fresh water used for make-up is added directly to the 5000 m^3 tank, resulting in a total volume of 92,000 cubic meters per day in the secondary recovery system.	
2.3.3	Application of TS	On December 13, 2000, the first skid application of acrolein was made into the injection point upstream of the system filters in the water plant. A volume of 1111 kg of acrolein was applied over a one hour period The acrolein skid container contains a nitrogen blanket over the liquid acrolein and is delivered from the container through an internal dip tube into a closed chemical manifold by applying positive pressure from an external nitrogen bottle connected to the manifold which in this case was set at 80 psig. The treatment consisted of 8 skids per month (2 treatments per week) initially. The study concluded in June of 2004.	**X**
2.3.4	Test conditions	Not specified	
2.3.5	Duration of the test / Exposure time	The trial was performed from December 13 200 until June 2004.	
2.3.6	Number of replicates performed	Not applicable, field application	
2.3.7	Controls	None The efficacy of acrolein was evaluated by comparing the results with those obtained prior to treatment commenced.	**X**
2.4	**Examination**		
2.4.1	Effect investigated	Levels of GAB and SRB	
2.4.2	Method for recording / scoring of the effect	Concentrations of planktonic sulfate-reducing bacteria (SRB) were determined by serial dilution of the water samples into a modified Postgate B medium (C&S Laboratories Inc., Broken Arrow, OK) according to the NACE Standard TMO 194-94 ("Field Monitoring of Bacterial Growth in Oilfield Systems"). Similarly, general aerobic and facultative anaerobic bacteria (GAB) were enumerated by serial dilution into a phenol red dextrose medium (C&S Laboratories Inc., Broken Arrow, OK). Incubation of cultures was carried out for a maximum of 28 days to score for positive growth to determine the log concentration of bacteria in the original samples. For sessile bacterial monitoring,	**X**

		biocoupons were installed at selected sampling points throughout the water plant and at various injection wells located at near, intermediate, and outlying distances from the water plant. To obtain sessile samples, a 1 cm^2 area of the biocoupons was swabbed with a sterile cotton swab and the adhering material was dispersed into appropriate media followed by serial dilution as described above.	
2.4.3	Intervals of examination	Bacterial levels were measured approximately once per month.	
2.4.4	Statistics	None specified	
2.4.5	Post monitoring of the test organism	No	
		3 RESULTS	
3.1	**Efficacy**	**Bacteria at the Water Plant** Results of bacterial sampling during the course of the 3 ½ year program at the water plant are presented in Figure 4. The data represent an average of the bacterial concentrations per cm^2 for sessile samples and per ml for planktonic samples for the entire treatment program. Prior to the start of the program, concentrations of sessile SRB at the filters were 10^3 per cm^2 (planktonic SRB samples were not obtained), whereas GAB were 10^6 for both sessile and planktonic samples. For the 5000 m^3 tank the sessile SRB and GAB were determined to be 10^6 per cm^2 or ml (planktonic samples were not measured for the 5000 m^3 tank prior to treatment). During the course of the acrolein program the bacterial concentrations at the filters were maintained at less than 10^4 for sessile GAB and less than 10^3 for all other samples. At the 5000 m^3 tank the levels of sessile SRB were reduced to approximately 10^2 per cm^2 or a 4 log_{10} reduction and sessile GAB were reduced by 5 log_{10} units to 10^1 per cm^2. Control of bacterial levels in this tank which feeds the injection system is critical to downstream control of bacteria and water quality throughout the injection system. **Bacteria at Injection Wells** Profiles of bacterial concentrations for representative injection wells during the course of the program are given in Figures 5-8. A total of 8 injection wells were sampled for both sessile and planktonic bacteria. Each data point represents the average of all injection wells sampled. These results are summarized in Table 2 which gives the average for pre-treatment samples vs the average for course of the acrolein program. As was observed at the water plant, the initial concentrations of sessile SRB and GAB in the injection system prior to initiation of the acrolein treatment was at least 10^6 per cm^2, indicating that sessile biofilm was well established throughout the system (Figures 5 and 6). Following the initial treatment, these concentrations were reduced by 5 orders of magnitude to ~ 10^1 per cm^2. Although these levels fluctuate during the course of the program, the SRB concentrations generally remain at 10^3 per cm^2 or less and the GAB concentrations are generally less than 10^2 per cm^2. The average values during the course of the program are 10^2 SRB per cm^2 and 10^1 GAB per cm^2, representing 4 and 5 log_{10} reductions, respectively (Table 2).	**X**

		These findings show significant bacterial control for sessile populations well established in the flow lines, providing protection against bacteria problems throughout the injection system including the outlying injection wells and wellbore. The acrolein treatment also had a significant impact on the concentrations of planktonic bacteria throughout the injection system (Figures 7 and 8). The initial planktonic concentrations were 10^5 SRB per ml and 10^6 GAB per ml. Following treatment, there was a gradual decline in planktonic SRB and GAB to 10^1 per ml. Planktonic SRB were maintained on the average at $<10^3$ per ml and planktonic GAB at $< 10^2$ per ml during the course of the program (see Table 2). The overall pattern is a reduction of > 3 $\log_{10}$ of planktonic SRB and 4 $\log_{10}$ planktonic GAB for the duration of the study.
3.1.1	Dose/Efficacy curve	None specified
3.1.2	Begin and duration of effects	Effects were seen within the first month of treatment.
3.1.3	Observed effects in the post monitoring phase	Not applicable
3.2	**Effects against organisms or objects to be protected**	Not specified
3.3	**Other effects**	**Residual monitoring of acrolein:** In order to ensure that adequate residual was being delivered throughout the system and to aid in treatment optimization, a profile of acrolein residuals was monitored during various periods of the program at the water plant and at the injection wells. At the 5000 m^3 tank acrolein was detected at the tank outlet in less than 5 minutes after the start of the application. A peak of 266 ppm was detected at 55 minutes into the application. By the 3 hours, the residual leaving the tank was less than 10 ppm. The duration of the residual at > 50 ppm was approximately 1.5 hours and the residual exceeded 100 ppm for > 1 hour. At an intermediate distance injection well T2 (3 hour transit time from the water plant) the acrolein residual peaked at 241 ppm, indicating that the majority of the chemical exiting the 5K tank was not spent before reaching this injection well. The residual was sustained at > 88 ppm for at least 1 hour and 15 minutes. Table 1 summarizes the overall pattern of acrolein residuals in the injection system, giving profiles for near, intermediate, and outlying injection wells. Based on the transit of acrolein through the system, the half life was estimated to be approximately 7.89 hours. It can be seen that from Table 1 that an acrolein residual of 50 ppm is being sustained at the near injection wells for 70-90 minutes, at the intermediate injection wells for 60-75 minutes, and at the outlying injection wells for >30 minutes. This was considered to be sufficient for providing

		bacterial control of the entire system as is described in the following Sections.	
3.4	**Efficacy of the reference substance**	Not applicable	
3.5	**Tabular and/or graphical presentation of the summarised results**	See Table 2 and Figures 4 – 8.	
3.6	**Efficacy limiting factors**		
3.6.1	Occurrences of resistances	Not specified	
3.6.2	Other limiting factors	Not specified	
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	Not applicable – a field study was performed	
4.2	**Intended actual scale of biocide application**	Not applicable	
4.3	**Relevance compared to field conditions**	Not applicable	
4.3.1	Application method		**X**
4.3.2	Test organism		**X**
4.3.3	Observed effect		**X**
4.4	**Relevance for read-across**	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In the current study, a 92,000 m^3 per day waterflood in the Rincon de los Sauces region of Argentina (Neuquen Province) operated by Repsol-YPF was impacted by sulfate reducing bacteria in the water injection system enumerated at > 10^6 planktonic SRB/mL and > 10^6 sessile SRB /cm^2. Subsequently, those water injection lines with low flow rates and injection wells located furthest from the water treatment facility had elevated concentrations of iron sulfide resulting in deteriorated water quality. An acrolein treatment was designed using 1111 kg (1314 liters) of acrolein applied over one hour, twice per week at the water treatment facility.	
5.2	**Reliability**	The method shows that the product is efficacious in a similar use pattern to that proposed for off-shore oil-rigs.	**X**

5.3	**Assessment of efficacy, data analysis and interpretation**	As a biocide acrolein was effective in reducing: • sessile SRB by 4 orders of magnitude (from 10^6 to 10^2 SRB per ml) and sessile GAB by 5 orders of magnitude (from 10^6 to 10^1 GAB per ml) throughout the injection system at representative monitoring points. Paramount to the success of this program was implementing bioprobes at strategic points though out the injection system to accurately detect bacterial regrowth and to keep the program optimized. • planktonic SRB by approximately 3 orders of magnitude from 10^5 to 10^2 SRB per ml and planktonic GAB by approximately 5 orders of magnitude from 10^6 to 10^1 GAB per ml throughout the injection system. The study demonstrates bacterial control in a large volume waterflood by treating with acrolein at a single injection point upstream in the system. Although the transit time to outlying injection wells, ranged from 10-12 hours, chemical throughput resulted in acrolein residuals at these outlying wells at a concentration of 50 ppm for a minimum of 30 minutes. Inlying wells received residuals of up to 50 ppm for a minimum of 1.5 hours.	**X**
5.4	**Conclusion**	The study demonstrates that acrolein is effective in reducing levels of sessile and planktonic SRB and GRB in water injection lines and injection wells.	**X**
5.5	**Proposed efficacy specification**	The data shows that the product is efficacious towards the bacteria to be controlled.	**X**
		Evaluation by Competent Authorities	
Date		8/2/2008	

Materials and methods	The UK CA accepts the Applicant's version, with the following comments. **2.1, 2.1.1 & 2.1.2** In their dossier, the Applicant has specified (Doc. IIIB, Section 2.2) that the biocidal product Magnacide B contains 99.7 – 99.8 % w/w/ acrolein. Given that 99.7 – 99.8 % of Magnacide B is acrolein, the UK CA considers that, from the efficacy point of view, the active substance and the biocidal product are the same. For this reason. the UK CA is satisfied that the efficacy data presented in this RSS, if acceptable, can be used in support of the active substance. **2.1.5** The term 'one skid application' refers to the way in which acrolein was dosed. The biocide was contained in a skid tank. These are enclosed metallic containers which, following dosing, are cleaned and then re-used. The skid tanks used for acrolein are specifically built for use with the product. Following use, they are returned to the Applicant for cleaning. The term 'injection system' is the system responsible for pumping water into rock formations for the purpose of forcing the oil and gas out of the formations and into the production wells. **2.3.3** Although not mentioned in this Section, the original study report states that, in the treatment reported in this study, the acrolein was applied as a batch treatment. The Applicant has stated that a volume of 1111 kg of acrolein was applied. This is, in fact, a weight of product and not a volume. **2.3.7**. The Applicant has stated that no controls were conducted. However, as the results obtained prior to treatment allow a comparison to be made with the results following treatment, the UK CA considers the pre-treatment results to be acceptable as control data.
	2.4.2 The serial dilution methodology used for measuring the levels of planktonic bacteria is taken from the NACE Standard TMO 194-94. This standard, produced by NACE International, formerly the National Association of Corrosion Engineers, is a well-established and commonly used standard within the oil industry. The use of biocoupons, in which specimens of material (the coupons) are exposed to the environment to be monitored, is a commonly used method for obtaining samples of sessile bacteria. As described above, the serial dilutuion method for measuring the sessile populations, is a well established and common technique. **5.2** This Section refers to the methodologies followed in the study. The UK CA considers the methodologies used to be acceptable. The efficacy template does not require the Applicant to state a number for the reliability indicator. However, the UK CA considers the reliability indicator to be 2 (see below).

Results and discussion	The UK CA accepts the Applicant's version, with the following comments. **4.3.1, 4.3.2 & 4.3.3** The Applicant has not completed these Sections. The UK CA does not consider these omissions to be significant. **3.1** The Applicant has stated that, in the 5000 m^3 tank, no samples of planktonic SRB and GAB were measured before treatment began. Neither the RSS nor the original study report indicate the reason for this. Although the absence of such pre-treatment data means that there is no control data for planktonic SRB and GAB in the 5000 m^3 tank, the UK CA does not consider this to be a significant omission. The reason for this is that sessile bacteria are attached to surfaces and are usually contained within biofilm which shields them from biocide attack. For this reason, if a biocide is shown to be efficacious against sessile bacteria, this means that it will also be efficacious against planktonic bacteria. Therefore, as control data have been reported for sessile SRB and GAB in the 5000 m^3 tank, and in the filter and injection wells, the UK CA considers that the absence of control data for planktonic bacteria in the 5000 m^3 tank is not a significant deficiency in the reporting of the study. **5.3** In the RSS the Applicant has expressed the sessile bacterial numbers as SRB and GAB per ml. However, the numbers should be expressed as SRB and GAB per cm^2 (the area of biocoupon swab sampled) and this is how they were reported in the original study report. The UK CA considers this to be a minor error. The UK CA agrees with the Applicant's statements regarding the efficacy of the acrolein batch applications on the levels of sessile and planktonic SRB and GAB. These statements are in accordance with the data presented in Tables 1 and 2 and in Figures 4 – 8. The UK CA considers the results as demonstrating that, when acrolein was used under field conditions, a concentration of 50 ppm acrolein, when dosed as a batch treatment for 1 hour twice weekly, was efficacious against both sessile and planktonic SRB and GAB.
Conclusion	**5.4 & 5.5**.The UK CA agrees with the Applicant's statements.
Reliability	2
Acceptability	The UK CA considers the data to be acceptable in support of Annex I inclusion.
Remarks	All data and endpoints presented in the study summary have been checked against the original study and are correct.
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	

Acceptability
Remarks

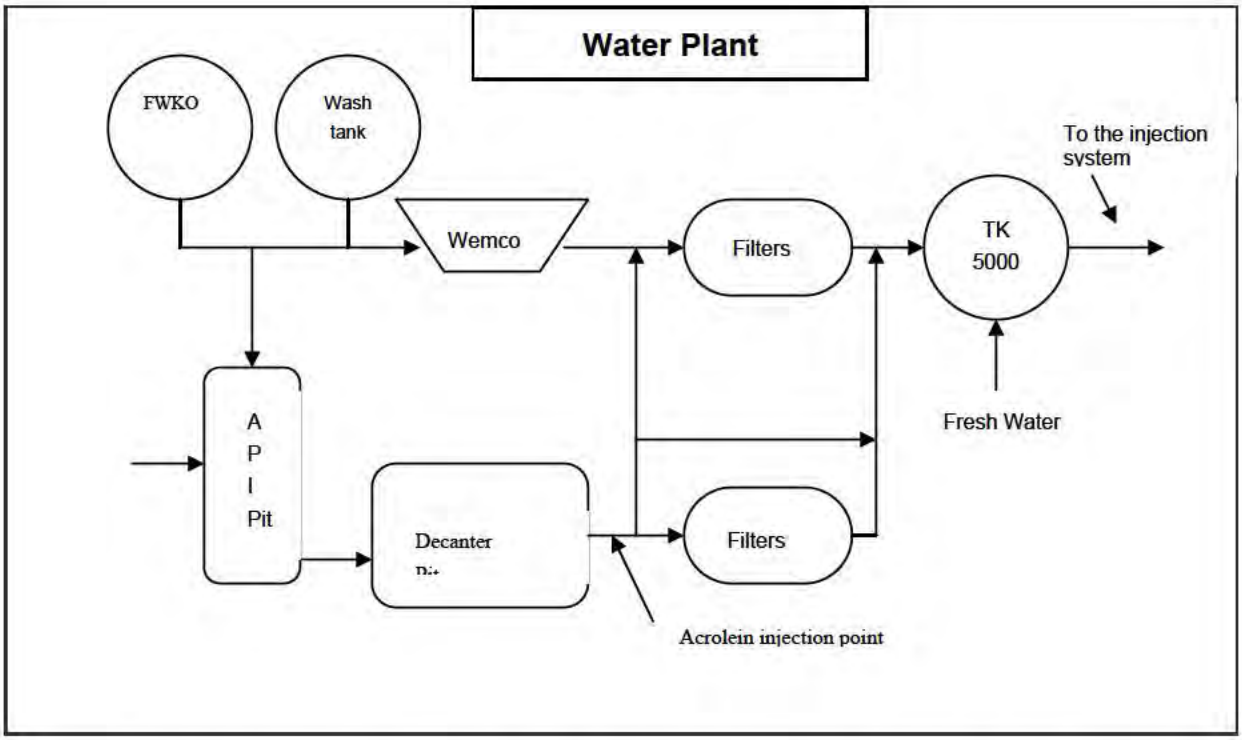


Figure 1.- Diagram of Chihuido Water Plant showing acrolein injection point.

Table 1.- Summary of acrolein residual throughput in Chihuido water injection system during one skid acrolein application

Injection Well	Distance from Injection Plant	Time Until Residual Detected (h)	Maximum Residual Detected (ppm)	Duration for Biocidal Concentration (≥ 50 ppm) (min)
5K Tank	-----	< 5 min	266	> 120
D6	near	1.6	217	> 60
D44	near	2.0	135	70
D30	near	2.4	113	90
T2	intermediate	3.0	241	> 75
78	intermediate	4.0	146	> 60
A30	intermediate	4.0	101	> 60
240	outlying	10.5	99	> 30
247	outlying	10.5	108	> 30

Table 2.- Summary of impact of acrolein program on bacterial concentrations at injection wells

Type of Bacteria Monitored	Before Acrolein Program	During Acrolein Program
Sessile SRB	**6.0**	**2.2**
Planktonic SRB	**4.8**	**2.3**
Sessile GAB	**6.0**	**1.4**
Planktonic GAB	**6.0**	**1.7**

Data expressed as $\log_{10}$ bacteria per ml for planktonic bacteria and $\log_{10}$ bacteria per cm^2 for sessile bacteria. Each number represents an average of all wells sampled before treatment and during the course of the treatment program.

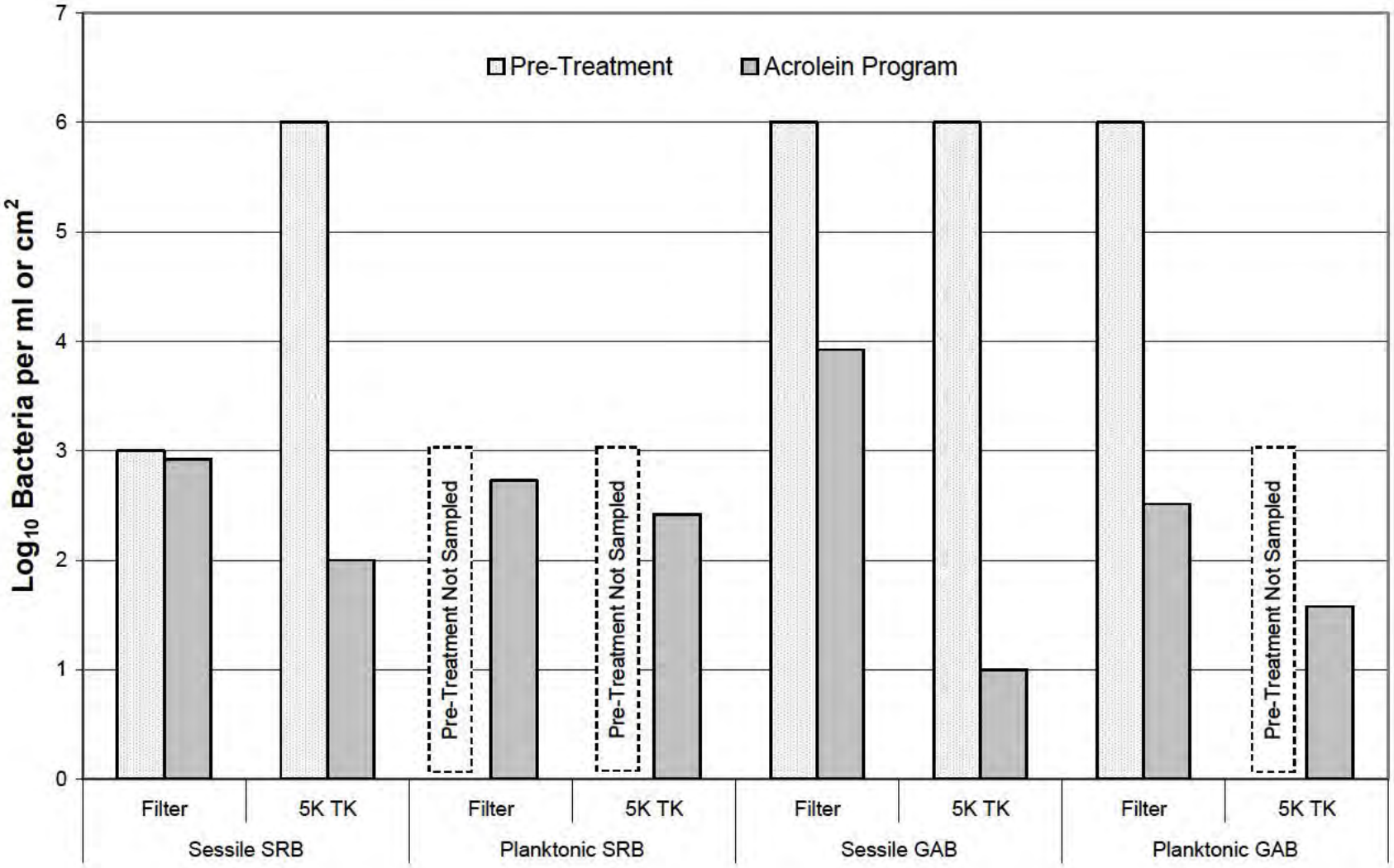


Figure 4.- Bacterial concentrations in water processing plant during acrolein treatment program

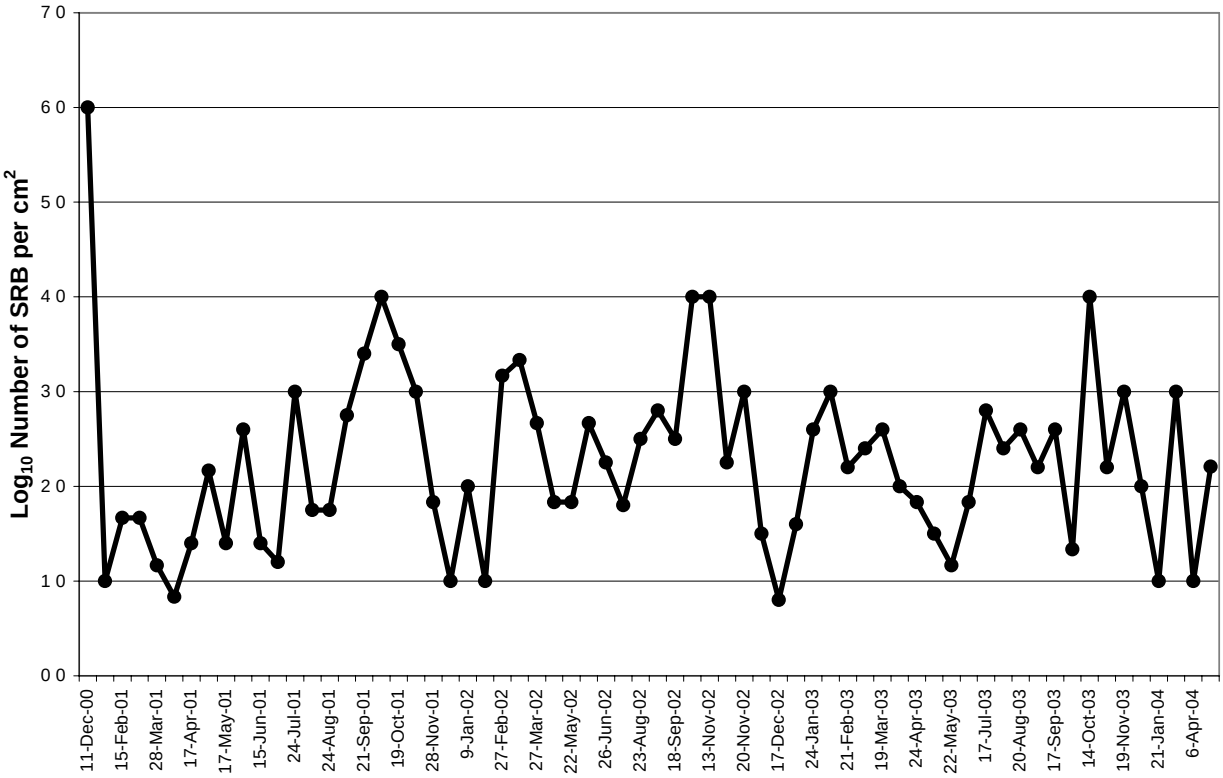


Figure 5.- Sessile SRB at injection wells during acrolein treatment program

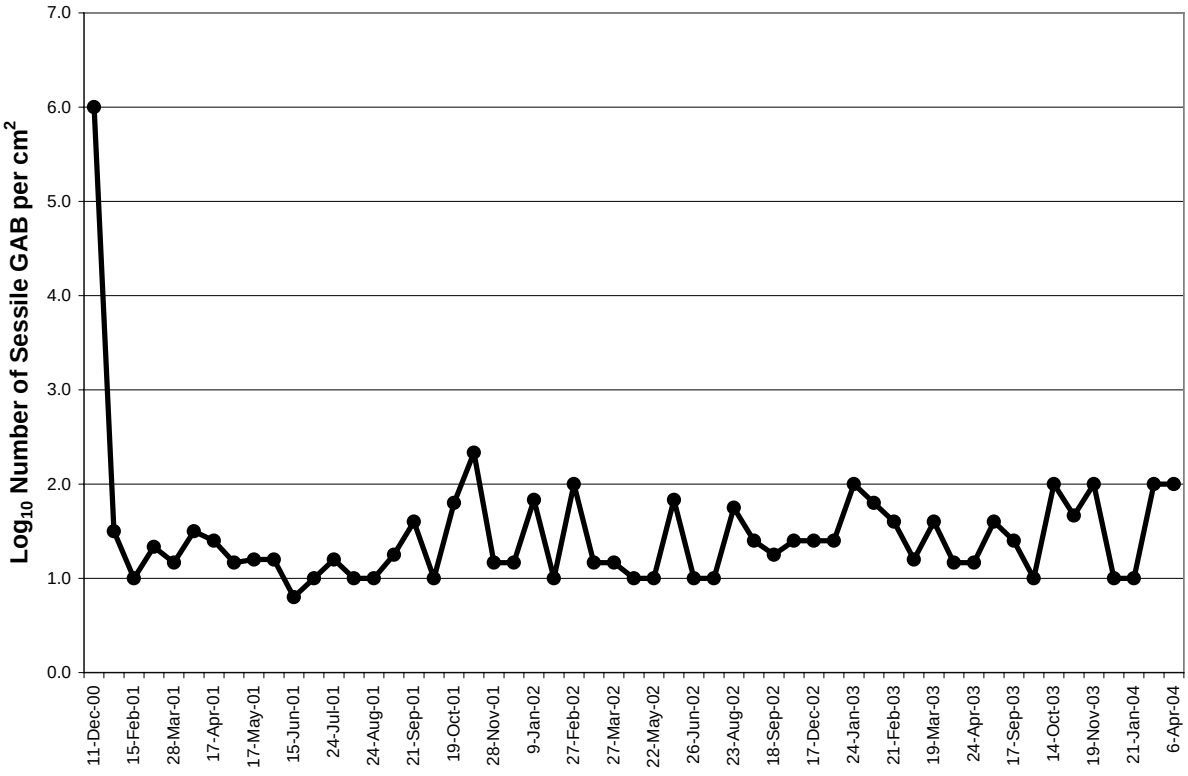


Figure 6.- Sessile GAB at injection wells during acrolein treatment program

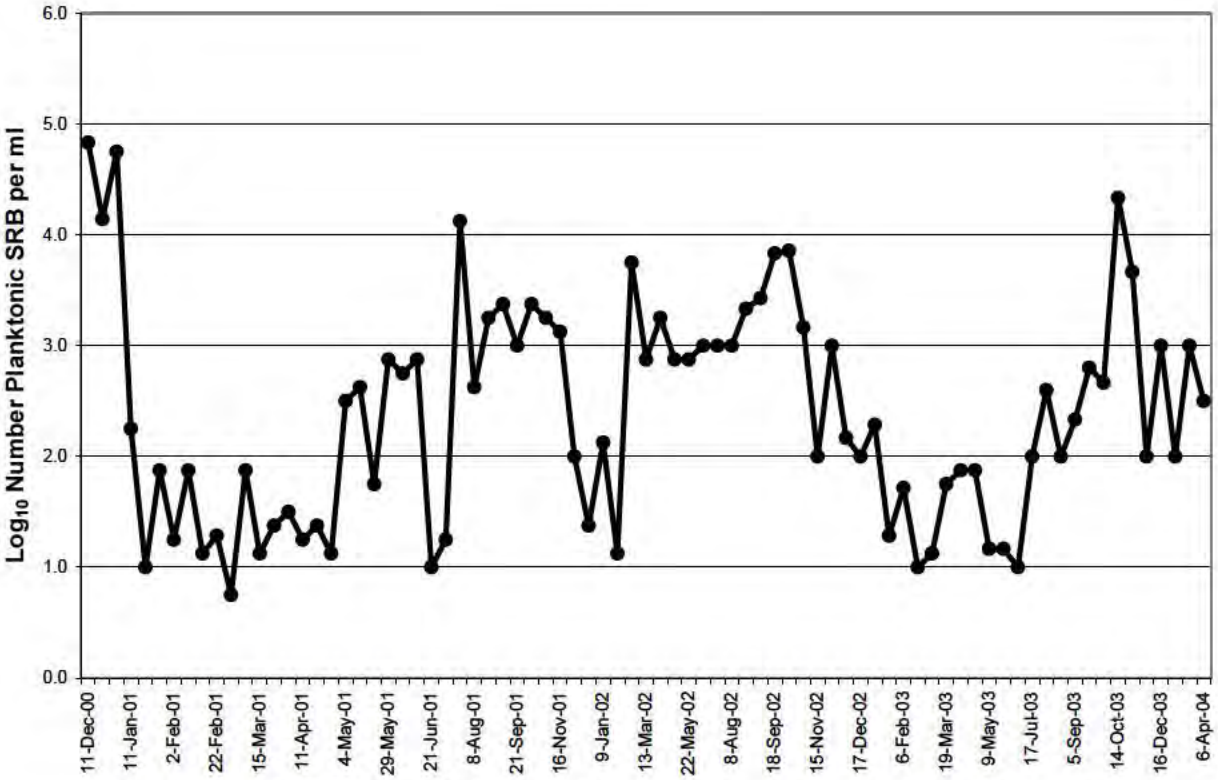


Figure 7.- Planktonic SRB at injection wells during acrolein treatment program

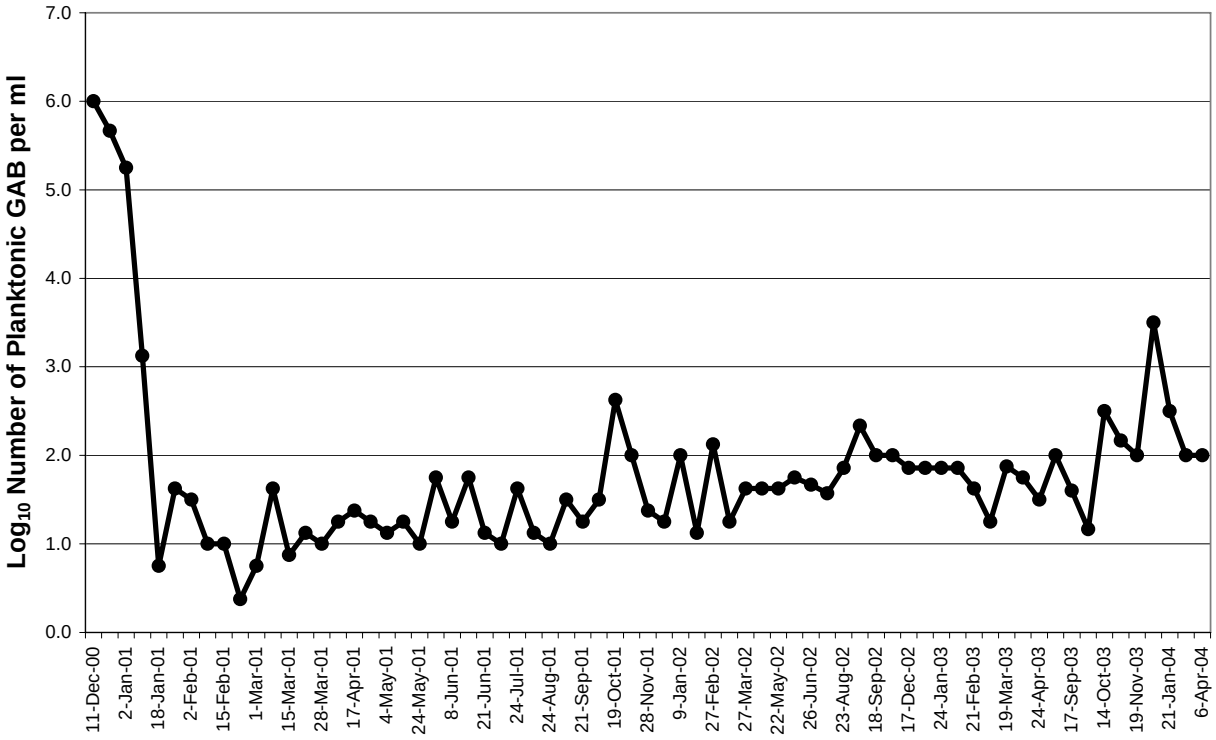


Figure 8.- Planktonic GAB at injection wells during acrolein treatment

Section A5/04 (Annex point IIA5)		Efficacy data on the active substance	
		1 REFERENCE	Official use only
1.1	**Reference**	[redacted]	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Criteria for data protection	[redacted]	
1.3	**Guideline study**	[redacted]	
1.4	**Deviations**	[redacted]	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**		X
2.1.1	Trade name/ proposed trade name	[redacted]	X
2.1.2	Composition of Product tested	[redacted]	
2.1.3	Physical state and nature	[redacted]	
2.1.4	Monitoring of active substance concentration	[redacted]	
2.1.5	Method of analysis	[redacted]	
2.2	**Reference substance**		
2.2.1	Method of analysis for reference substance	[redacted]	
2.3	**Testing procedure**	[redacted]	X
2.3.1	Test population / inoculum / test organism	[redacted]	

2.3.2	Test system	[redacted] X
2.3.3	Application of TS	[redacted]
2.3.4	Test conditions	[redacted]
2.3.5	Duration of the test / Exposure time	[redacted]
2.3.6	Number of replicates performed	[redacted]
2.3.7	Controls	[redacted] X
2.4	**Examination**	
2.4.1	Effect investigated	[redacted]

2.4.2	Method for recording / scoring of the effect	[redacted]	X
2.4.3	Intervals of examination	[redacted]	
2.4.4	Statistics	[redacted]	

2.4.5	Post monitoring of the test organism	[redacted]	
		3 RESULTS	
3.1	**Efficacy**	[redacted]	**X**
3.1.1	Dose/Efficacy curve	[redacted]	
3.1.2	Begin and duration of effects	[redacted]	
3.1.3	Observed effects in the post monitoring phase	[redacted]	
3.2	**Effects against organisms or objects to be protected**	[redacted]	
3.3	**Other effects**	[redacted]	
3.4	**Efficacy of the reference substance**	[redacted]	

3.5	**Tabular and/or graphical presentation of the summarised results**	[redacted]	X
3.6	**Efficacy limiting factors**		
3.6.1	Occurrences of resistances	[redacted]	
3.6.2	Other limiting factors	[redacted]	
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	[redacted]	X
4.2	**Intended actual scale of biocide application**	[redacted]	
4.3	**Relevance compared to field conditions**	[redacted]	X
4.3.1	Application method	[redacted]	X
4.3.2	Test organism	[redacted]	X
4.3.3	Observed effect	[redacted]	
4.4	**Relevance for read-across**	[redacted]	X
		6 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	[redacted]	
5.2	**Reliability**	[redacted]	
5.3	**Assessment of efficacy, data analysis and interpretation**	[redacted]	
5.4	**Conclusion**	[redacted]	X

5.5 Proposed efficacy specification	[redacted]	X
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Materials and methods	[redacted]	
Results and discussion	[redacted]	

	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table 1 [redacted]

[redacted]

Table 2 [redacted]

[redacted]

Table 3 O[redacted]rchand facilities microbiological analyses

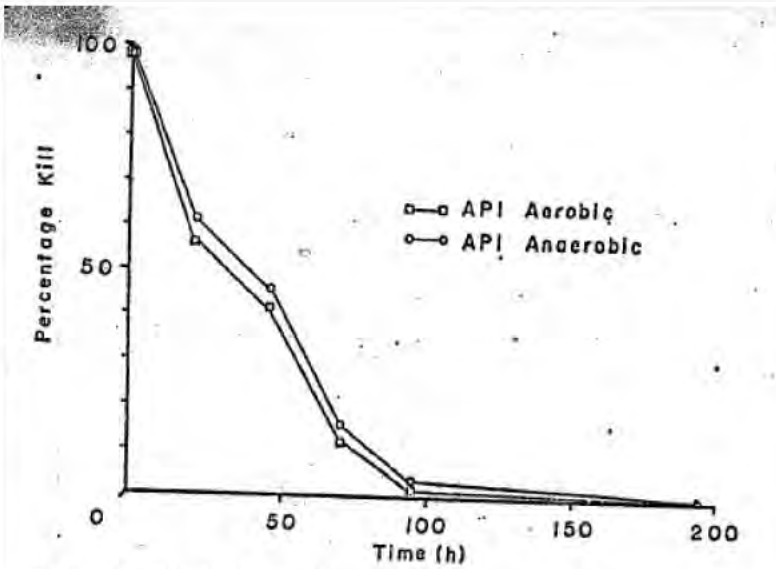
[redacted]

Table 4 [redacted]

[redacted]

Section A5/05 (Annex point IIA5)		Efficacy data on the active substance	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Kissel C.L., Brady J.L., Guerra A.M., Meshishneck M.J., Rockle B.A. and Caserio F.F. 1980. Monitoring acrolein in naturally occurring systems. In "Water for Subsurface Injection", J.L. Johnson et al. (eds), ASTM STP 735, American Society for Testing and Materials, Philadelphia, 102 pp.	
1.2	**Data protection**	No	
1.2.1	Data owner	Not applicable	
1.2.2	Criteria for data protection	Not applicable	
1.3	**Guideline study**	No	
1.4	**Deviations**	Not applicable	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**	Acrolein	**X**
2.1.1	Trade name/ proposed trade name	Magnacide B Microbiocide	**X**
2.1.2	Composition of Product tested	Not specified	**X**
2.1.3	Physical state and nature	Not specified	
2.1.4	Monitoring of active substance concentration	Yes	
2.1.5	Method of analysis	Acrolein concentrations in natural waters were measured using 2,4-dinitrophenylhydrazine colorimetry (DNPH), gas liquid chromatography (GLC) and ultraviolet spectroscopy (UV). Differential pulse polarography was also used, with a certified oxygen free nitrogen atmosphere and the hanging drop mode of the Model 303 Electrode unit using a 2 *M* drop size.	
2.2	**Reference substance**	No	
2.2.1	Method of analysis for reference substance	n/a	
2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	See Table 1.2	
2.3.2	Test system	Natural waters: Each of the naturally occurring field waters was placed in 0.5 L amber volumetric flasks and charged with various amounts of commercial acrolein. The 0.5 L solutions were stored at 22 °C. As these solutions aged, aliquots were removed from each solution and the acrolein	

		concentrations were determined using several analytical and bioassay procedures.	
2.3.3	Application of TS	Natural waters: Acrolein was introduced to the naturally occurring waters without further purification in the following initial concentrations: 49 ppm and 151 ppm acrolein in Water C (C-49 and C-151 respectively); 17 ppm, 49 ppm and 151 ppm acrolein in the Water Q (Q-17, Q-49 and Q-151 respectively); and 10 ppm acrolein in Water P (P-10).	
2.3.4	Test conditions	Characteristics of some natural waters: TABLE 1—*Characteristics of some natural waters.* Value of Parameter[a] Chemical Parameter / Water C / Water Q / Water P pH 8.0 7.3 7.9 Specific gravity, 15.6°C (60°F) 0.999 1.040 0.995 Total solids, g/litre 0.48 63.6 1.1 Dissolved oxygen, ppm 1 1 1 Nitrogen (Kjeldahl), ppm <0.2 <0.2 <0.2 Bicarbonate, ppm 138 124 207 Carbonate, ppm 15 <1 <1 Chloride, ppm 36 33 280 178 Sulfate, ppm 98 4 700 46 Sulfide, ppm <0.1 <0.1 <0.1 Arsenic, ppb <5 <5 <5 Antimony, ppm <1 <1 <1 Barium, ppm <1.0 <1.0 <1.0 Calcium, ppm 26 500 56 Iron, ppm <0.5 <0.5 <0.5 Magnesium, ppm 13 2 778 17 Tin, ppm <1 4.2 <1 Zinc, ppm <0.1 <0.1 <0.1 Sources of naturally occurring field waters: Waters C and Q – oil field floodwater system Water P – commercial cooling tower The 0.5 L solutions were stored at 22 °C.	
2.3.5	Duration of the test / Exposure time	The biocidal potency of acrolein was assessed using solutions that were aged for up to 192 h.	
2.3.6	Number of replicates performed	Not specified	
2.3.7	Controls	Bacterial levels were assessed in blank solutions.	
2.4	**Examination**		
2.4.1	Effect investigated	The study assesses the biological activity of acrolein solutions.	
2.4.2	Method for recording / scoring of the effect	The biocidal potency of acrolein in each of the aging stock solutions of naturally occurring Waters C, Q and P was determined by withdrawing aliquots at various times. These aliquots were then measured using standard American Petroleum Institute (API) and adenosinetriphosphate (ATP) bioassay techniques.	X
2.4.3	Intervals of examination	Aliquots of the test solutions were removed after 0, 24, 48, 72, 96 and 192 hours.	
2.4.4	Statistics	Not specified	
2.4.5	Post monitoring of the test organism	No	

		3 RESULTS	
3.1	**Efficacy**	The purpose of the study was to demonstrate the efficacy of acrolein/ Magnacide B as a biocide against bacteria.	
3.1.1	Dose/Efficacy curve	 FIG. 4—*Loss of biocidal potency of acrolein (initial concentration 50 ppm) versus time in natural Water C at 22°C in the dark.*	X
3.1.2	Begin and duration of effects	Biocidal effects were seen in solutions that had not been aged i.e. after 0 hours of exposure.	
3.1.3	Observed effects in the post monitoring phase	None specified	
3.2	**Effects against organisms or objects to be protected**	No adverse effects were noted in the report.	
3.3	**Other effects**	None specified	
3.4	**Efficacy of the reference substance**	n/a	
3.5	**Tabular and/or graphical presentation of the summarised results**	TABLE 3—*Bacteria bioassay data.* Water System[a] / API Bioassay Method / Percent Kill at Various Solution Aging Times, h (0, 24, 48, 72, 96, 192) / Blank (col/ml) C-49 Aerobic 99 56 41 8 2 <1 37.8 × 10⁵ C-49 Anaerobic 99 61 46 11 3 <1 5.4 C-151 Aerobic 99 63 46 28 8 <1 37.8 C-151 Anaerobic 99 69 50 33 9 <1 5.4 Q-17 Anaerobic 95 51 10 <1 ... ... 2.8 Q-49 Anaerobic 99 78 43 8 <1 ... 2.8 Q-151 Anaerobic 99 85 55 23 3 ... 2.8 P-10 Aerobic 91 70 44 26 9 <1 21.0 [a]The letter denotes the water system, the numeral denotes the initial acrolein concentration (ppm).	X
3.6	**Efficacy limiting**		

	factors		
3.6.1	Occurrences of resistances	There were no occurrences of resistance noted in the report.	
3.6.2	Other limiting factors	None specified	
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	The purpose of the study was to demonstrate the efficacy of acrolein/ Magnacide B as a biocide against bacteria.	
4.2	**Intended actual scale of biocide application**	Not specified	
4.3	**Relevance compared to field conditions**		
4.3.1	Application method	The application technique chosen in this study is considered to be representative of that used under field conditions.	
4.3.2	Test organism	The study used naturally occurring water from oil field flood water systems and commercial cooling towers. The organisms present in these waters are therefore representative of those found under field conditions.	
4.3.3	Observed effect	The observed effect is identical to that seen under field conditions.	
4.4	**Relevance for read-across**	This study is considered to demonstrate the efficacy of acrolein/ Magnacide B as a biocide and is relevant to the use of acrolein under field conditions.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The purpose of the study was to demonstrate the efficacy of acrolein/ Magnacide B as a biocide against bacteria. Three naturally occurring field waters were selected from the following sources: oil field flood water systems (Waters C and Q) and a commercial cooling tower (Water P). Different concentrations of acrolein were added to these naturally occurring waters and allowed to age at 22 °C in the dark. Aliquots were removed from each system and the acrolein concentrations were measured and bioassays obtained.	
5.2	**Reliability**	n/a	
5.3	**Assessment of efficacy, data analysis and interpretation**	The study demonstrates that the use of acrolein in water obtained from oil field flood water systems and cooling towers at concentrations above 10 ppm resulted in a > 90 % kill in un-aged solutions. Freshly prepared solutions with acrolein concentrations of 30 ppm resulted in a percent kill of 99 % in both Waters C and Q, when analysed using the API-Aerobic and API-Anaerobic methods.	
5.4	**Conclusion**	The report demonstrates that acrolein is efficacious.	**X**
5.5	**Proposed efficacy specification**	Excellent (90-99 %)	

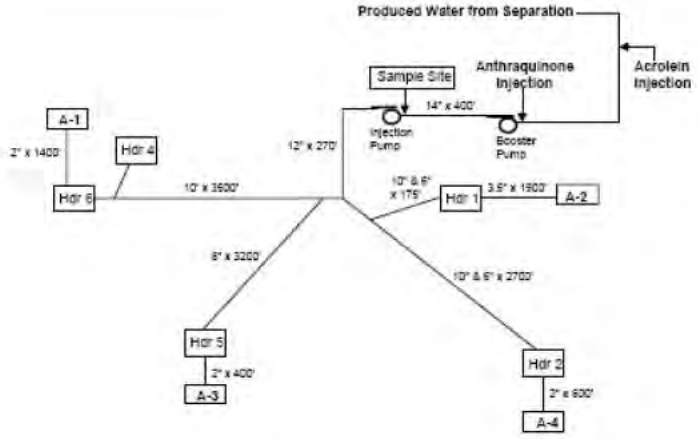
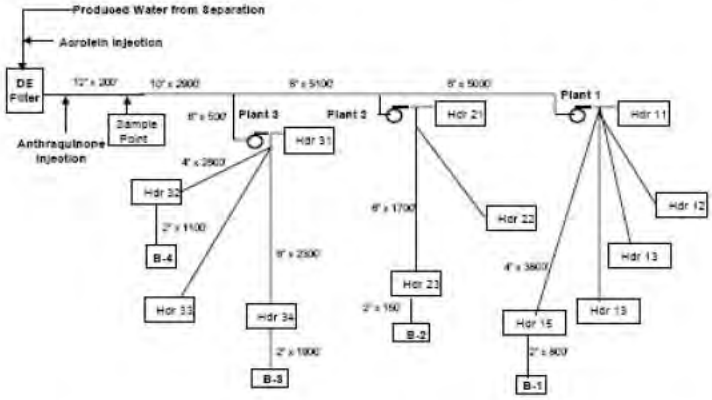
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	8/2/2008	
Materials and methods	The UK CA accepts the Applicant's version, with the following comments. **2.1, 2.1.1 & 2.1.2** In their dossier, the Applicant has specified (Doc. IIIB, Section 2.2) that the biocidal product Magnacide B contains 99.7 – 99.8 % w/w/ acrolein. Given that 99.7 – 99.8 % of Magnacide B is acrolein, the UK CA considers that, from the efficacy point of view, the active substance and the biocidal product are the same. For this reason. the UK CA is satisfied that the efficacy data presented in this RSS, if acceptable, can be used in support of the active substance. **2.4.2** Although not mentioned in the RSS, the study report indicated that the API bacterial bioassay technique was commonly used as an industry standard, with the ATP technique representing a newer approach. The report also indicated that the API technique was the more accurate one. The API methodology was used to determine the effectiveness of different concentrations of acrolein against aerobic and anaerobic bacteria, at various time intervals. For each concentration of acrolein, the concentration was added to a sample of naturally occurring field water in a 0.5 litre flask. Aliquots were then immedietely removed and the bio-assay used to determine the efficacy of each initial concentration against the bacteria. Each flask was then stored at 22°C and aliquots removed after 24, 48, 72, 96 and 192 hours. The efficacy of each aged acrolein containing solution was then determined using the bio-assay. The efficacy template does not require the Applicant to state a number for the reliability indicator. However, the UK CA considers the reliability indicator to be 2 (see below)..	
Results and discussion	The UK CA accepts the Applicant's version, with the following comments. **3.1.1 & 3.5** The bacterial bio-assay data presented are those generated using the standard API technique. The bacterial data in Figure 4 are the same data as that presented for water C-49 in Table 3, but presented in graphical form. The data in Figure 4 and Table 3 showed that an initial concentration of 10 ppm acrolein produced a 91 % kill of aerobic bacteria, and 17 ppm produced a 95 % kill of anaerobic bacteria. The data also showed that initial concentrations of 49 & 151 ppm produced a 99 % kill of both aerobic and anaerobic bacteria. The results for aged acrolein solutions showed a decrease in efficacy. The UK CA considers that this would be expected due to the loss of acrolein residues with time. The UK CA considers the results for the initial concentrations as demonstrating the ability of 49 & 151 ppm acrolein to produce a high level of efficacy against both aerobic and anaerobic bacteria.	

Conclusion	**5.4** The UK CA agrees with the Applicant's conclusion.
Reliability	2
Acceptability	The UK CA considers the data to be acceptable in support of Annex I inclusion.
Remarks	All data and endpoints presented in the study summary have been checked against the original study and are correct.
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

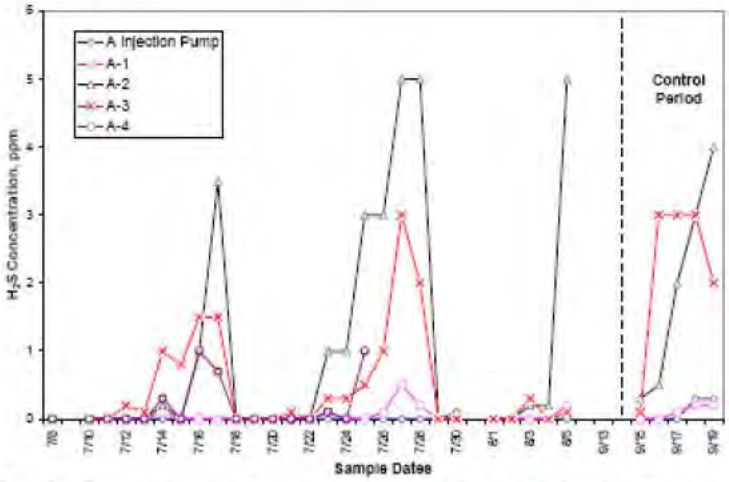
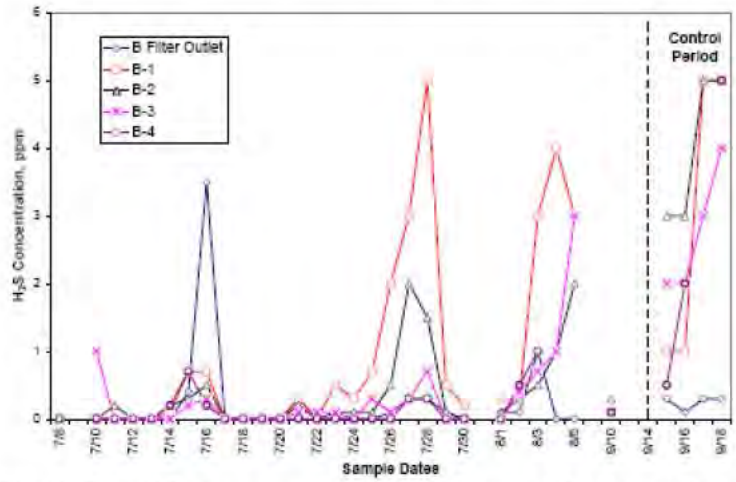
1.2 Test organism (*if applicable*)

Criteria	**Details**
Species	Aerobic and anaerobic bacteria
Strain	Not specified
Source	Naturally occurring water (Waters C, Q and P). Waters C and Q: from oil-field floodwater systems Water P: from a commercial cooling tower
Laboratory culture	No – the bacteria tested were those present in naturally occurring waters.
Stage of life cycle and stage of stadia	Not specified
Mixed age population	Not specified
Other specification	Not specified
Number of organisms tested	Not specified
Method of cultivation	Not specified
Pretreatment of test organisms before exposure	No
Initial density/number of test organisms in the test system	Not specified

Section A5/06 (Annex point IIA5)		Efficacy data on the active substance	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Johnson M.D., Harless M.L., Dickinson A.L. and Burger E.D. 1999. A new chemical approach to mitigate sulfide production in oilfield water injection systems. SPE Paper No. 50744, Presented at the International Symposium on Oilfield Chemistry, Houston, Texas, February 16-19, 1999.	
1.2	**Data protection**	No	
1.2.1	Data owner		**X**
1.2.2	Criteria for data protection		**X**
1.3	**Guideline study**	No	
1.4	**Deviations**	Not applicable	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**	Acrolein (used on its own to collect baseline (control) data) and anthraquinone	**X**
2.1.1	Trade name/ proposed trade name	Not specified	**X**
2.1.2	Composition of Product tested	Anthraquinone was injected as a 10 % by weight solution of the soluble anthrahydroquinone disodium salt in caustic.	**X**
2.1.3	Physical state and nature	Not specified	
2.1.4	Monitoring of active substance concentration	Chemical residuals were monitored at most of the designated wells.	
2.1.5	Method of analysis	The anthraquinone solution is easily detectable in field water by an increase in pH and a characteristic green colour when in the presence of iron (II). After these changes were noted samples were collected in clean bottles at 5 minute increments as the slug of anthraquinone passed the sample site. Each of these samples were analysed for anthraquinone residuals with a proprietary colorimetric analytical technique.	**X**
2.2	**Reference substance**	No	
2.2.1	Method of analysis for reference substance	n/a	
2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	Sulfate reducing bacteria	

2.3.2	Test system	 **Fig. 1—Schematic diagram of flow path in the "A" water injection system. Only monitored wells are shown.**  **Fig. 2—Schematic diagram of flow path in the "B" water injection system. Only monitored wells are shown.**	
2.3.3	Application of TS	Acrolein and anthraquinone were injected in three cycles over the course of the trial. Each cycle was started with the injection of acrolein followed by the injection of anthraquinone. The first treatment cycle lasted 9 days, the second 11 days and the third 7 days. Each cycle began the day of acrolein injection and ended when the next acrolein slug was injected. Slug treatments of acrolein were injected via a pump truck into both the A and B systems. The total amount of acrolein injected each day during nominal 50 minute injection periods was 81 kg (A system, 36 kg acrolein/treatment and B system 45 kg). Required treatment volumes of the anthrahydroquinone solution were estimated to be 57 and 95 l for the A and B systems respectively. Following each acrolein treatment, anthraquinone was injected with a diaphragm pump upstream of the A system injection pump and downstream of the B system DE filters.	**X**
2.3.4	Test conditions	Not specified	
2.3.5	Duration of the test / Exposure time	Test period of approximately 3 months	
2.3.6	Number of replicates	Not specified	

	performed		
2.3.7	Controls	Acrolein was used during the baseline (control) data determination. The purpose of the control period was to gather baseline data using the same sample points and parameters used during the trial. Normal acrolein treatment was applied to both systems. Total suspended solids (TSS) and H_2S concentrations were monitored at all of the sample points used during the field trial. Semi-quantitative enumeration of viable SRB using the serial dilution technique was done on the first and last day of the control period. The field control was stopped when all of the B system injection wells and one of the three A system injection wells had H_2S concentrations equal to or greater than those H_2S concentrations observed during the entire trial.	X
2.4	**Examination**		
2.4.1	Effect investigated	H_2S concentrations and growth inhibition of SRB.	
2.4.2	Method for recording / scoring of the effect	All sample sites were monitored daily for total suspended solids (TSS). H_2S concentrations were monitored daily with HACH HS-C test kit at all sample points. Twice weekly planktonic samples were collected at each sample point and processed immediately for semi-quantitative enumeration of viable SRB using the serial dilution technique. The culture vials were incubated for 2 weeks at 37 °C and visually inspected after the incubation period to determine the log number of viable bacteria in the original produced water samples.	
2.4.3	Intervals of examination	H_2S concentrations were monitored daily and twice weekly planktonic samples were collected at each sample point.	
2.4.4	Statistics	None specified	
2.4.5	Post monitoring of the test organism	Not specified	
		3 RESULTS	
3.1	**Efficacy**	**Free H_2S measurements:** In the A-system, the produced water flowing to injection well A-2 became sour most rapidly during each of the treatment cycles. The only other A system well to experience significantly increased H_2S in the injection water during each of the cycles was A-3. In the B system, no injection waters soured during the first treatment cycle. The water transported to only the most remote B-system well, B-1, soured significantly during the second and third cycle. In the B-system H_2S concentrations increased most rapidly overall during the third cycle possibly because the ambient temperature increased about 10 °C throughout that cycle. Since most of the pipelines were not buried, flowing water temperature also increased, thereby probably contributing to higher SRB activity. This may have accounted for the shorter period before the H_2S began to increase. During the generation of baseline (control) data, produced water collected from A system injection well A-3 had a significant increase in H_2S after one day. Produced water collected from A system injection well A-1 and A-2 had increased concentrations of H_2S after 2 days. Produced water collected from all B system injection wells had increased concentrations of H_2S after one to two days following initiation of the control. These results confirmed that daily acrolein treatments were required to maintain stable H_2S levels in both injection systems. **SRB measurements:**	X

	Results from the SRB serial dilutions for the A system indicate that the population remained relatively stable throughout the trial and control periods for each sample site monitored. Except for the sampling period on July 11th and the A-4 water sample taken on July 22nd, the wellhead SRB populations were equal to or less than those in the system influent water taken at the injection pump. The SRB levels in the B system influent water (downstream of the DE filter) varied more than those in the A system, although overall they were slightly lower than those entering the A system. Variability was most likely due to growth of SRB in the filter cake coupled with backwashing frequency. Except for 2 wellhead water samples, the SRB levels were between 10^1 and 10^3 cells/ml throughout the treatment and control periods. As with the A system, no definite trends with treatment cycle were evident.	
3.1.1 Dose/Efficacy curve	 Fig. 8—Free H_2S measurements in the "A" water injection system. Acrolein treatments were made on July 6th, 8th, 17th, 28th, and 29th. Anthraquinone treatments were made on July 9th, 10th, 18th, and 30th. The control period began with the cessation on September 15th of acrolein treatments  Fig. 9—Free H_2S measurements in the "B" water injection system. Acrolein treatments were made on July 6th, 8th, 17th, 28th, and 29th. Anthraquinone treatments were made on July 9th, 18th, and 30th. The control period began with the cessation on September 15th of acrolein treatments.	X

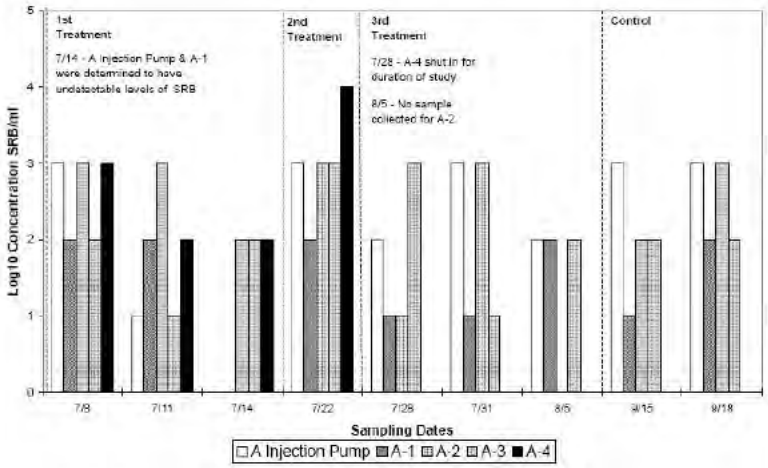


Fig. 12—Results from SRB serial dilutions for the "A" system wellhead and inlet water samples.

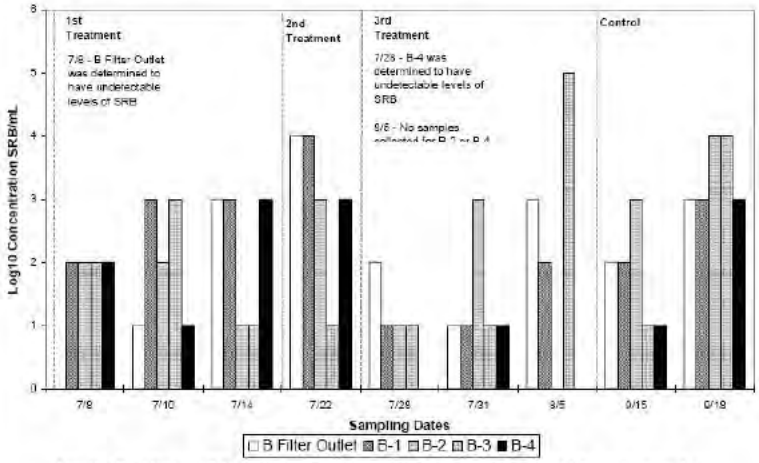
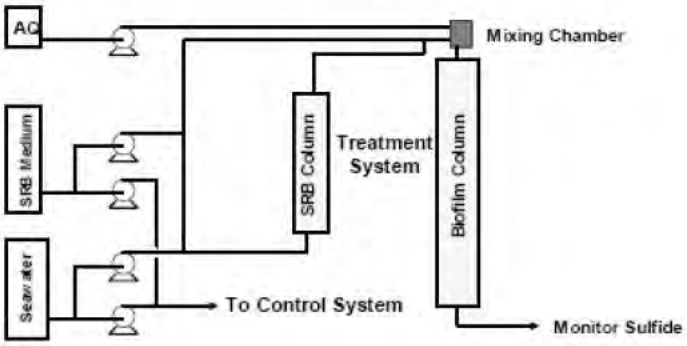


Fig. 13—Results from SRB serial dilutions for the "B" system wellhead and inlet water samples.

3.1.2	Begin and duration of effects	Not specified	
3.1.3	Observed effects in the post monitoring phase	Not specified	
3.2	**Effects against organisms or objects to be protected**	No adverse effects were noted in the report.	
3.3	**Other effects**		**X**
3.4	**Efficacy of the reference substance**	n/a	
3.5	**Tabular and/or graphical presentation of the summarised results**	Not specified	
3.6	**Efficacy limiting factors**		
3.6.1	Occurrences of resistances	There were no occurrences of resistance noted in the report.	

3.6.2	Other limiting factors		X
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	n/a	
4.2	**Intended actual scale of biocide application**		X
4.3	**Relevance compared to field conditions**		
4.3.1	Application method	 **Fig. 3—Schematic diagram of the laboratory dynamic biofilm inhibition system.** Two systems were run simultaneously: one as an untreated control and the second as the treated system. In the latter, the influent was treated with anthrahydroquinone solution for 20 h. At the conclusion of this treatment period, the flow paths for each system were switched so that SRB- free medium was allowed to flow directly into the 2 columns, bypassing the SRB columns. Effluent flow from the two columns was monitored for sulfide several times per day. Inhibition of biofilm development was determined by comparing the two effluent sulfide levels.	
4.3.2	Test organism	SRB	

4.3.3 Observed effect

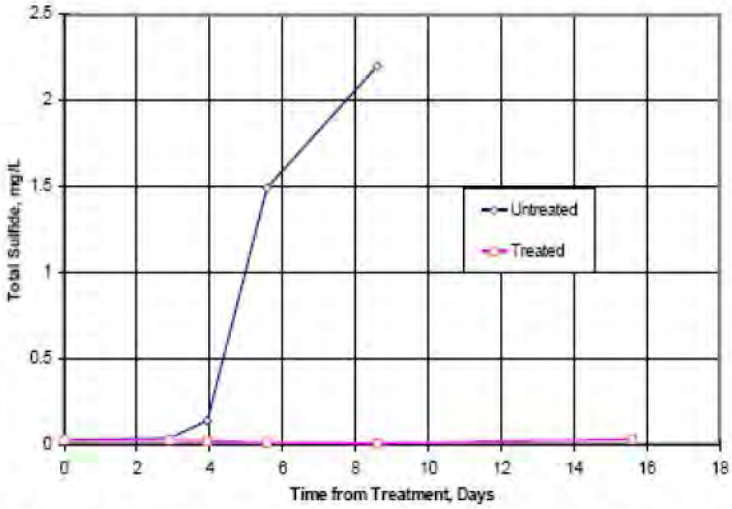


Fig. 4—Sulfide production from bottle study initiated on-site with water sampled at the B-2 wellhead. Treatments made with 1100 ppm of the anthrahydroquinone solution.

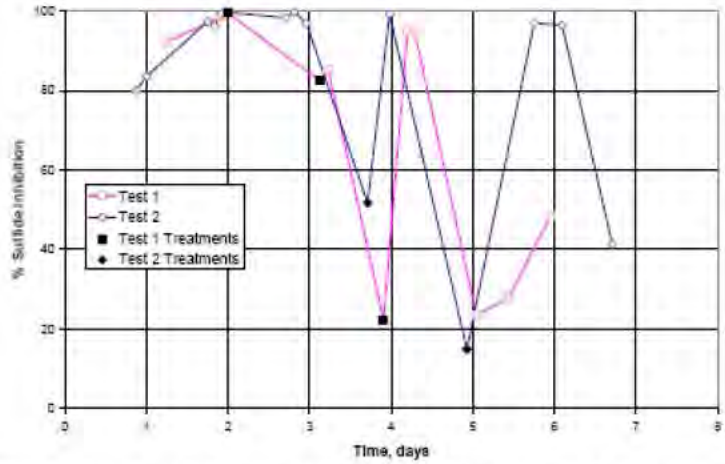


Fig. 6—Results of dynamic biofilm inhibition study. Solid points represent times for direct anthrahydroquinone solution treatments into the biofilm column. The initial treatments were made beginning at Time = 0.

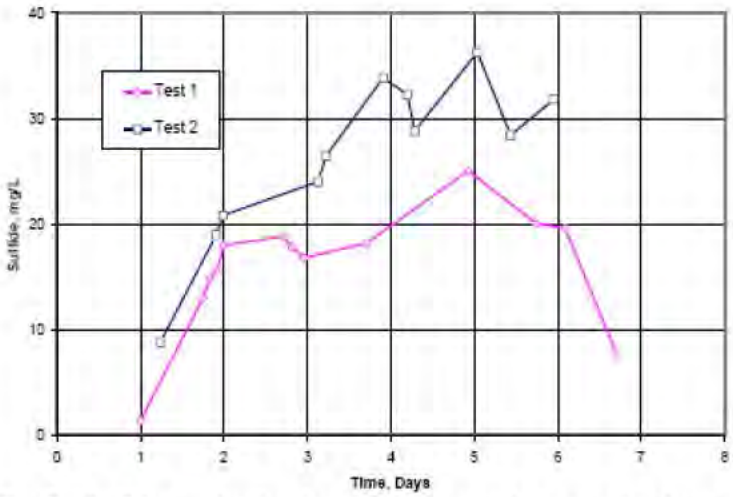


Fig. 7—Sulfide production in effluents from the control columns during the dynamic biofilm inhibition study.

		Fig. 6 shows significant inhibition of sulphide production for about 3 days. Effluent sulphide levels for the control columns are shown in Fig.7. Subsequent repeat treatments (500 ppm of the anthraquinone solution for 2-4 h) directly into the biofilm column once sulphide production had increased restored inhibition for at least one day. During the second test, 2 treatments were applied before inhibition ceased, but these treatments did not appear to extend the inhibition period beyond that observed for the first test. The initial treatment of the influent SRB flow allows the anthrahydroquinone solution to intimately contact the SRB for about 1 minute before entering the biofilm column. This allows molecules of anthraquinone to partition into the SRB cell membrane and effect inhibition of sulphate respiration after an initial lag period. The inhibition duration for this laboratory system using synthetic medium apparently is limited to about 3 days.	
4.4	**Relevance for read-across**	Yes Laboratory studies have confirmed field results that biogenic sulfide production within this California oilfield's water injection system can be inhibited with anthraquinone treatments. Extended duration inhibition was obtained in the laboratory when using the original SRB population and unamended natural water as the test medium and using synthetic medium and the SRB consortium cultured from this medium.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	This report is included as supporting data regarding the efficacy and use of acrolein.	**X**
5.2	**Reliability**	n/a	**X**
5.3	**Assessment of efficacy, data analysis and interpretation**	During the field trial, H_2S concentrations remained stable for up to 9 days in both the A and B systems following each acrolein/anthraquinone treatment cycle. The SRB population in the wellhead water samples generally remained stable throughout both water injection systems.	**X**
5.4	**Conclusion**	During the field trial, H_2S concentrations remained stable for up to 9 days in both the A and B systems following each acrolein/anthraquinone treatment cycle. Following these stable periods, sharp increases in H_2S concentrations indicated that the available anthraquinone concentrations within the biofilm had dropped below inhibitory levels. H_2S level appears to be the most responsive parameter for monitoring treatment efficacy. The SRB population in the wellhead water samples generally remained stable throughout both water injection systems. As with TSS levels during the first and second treatment cycles, variability was most likely due to changes in the influent water quality. The increased H_2S concentrations towards the end of each acrolein/anthraquinone treatment coupled with a relatively stable SRB population throughout the field trial indicates that anthraquinone was acting as an inhibitor of sulphate reduction rather than as a biocide. Furthermore, the anthraquinone treatment is acting to control any further growth and reproduction of the SRB population resulting in a stable population over the period of each acrolein/ anthraquinone treatment.	**X**
5.5	**Proposed efficacy specification**	The total amount of acrolein injected each day during nominal 50 minute injection periods was 81 kg (A system, 36 kg acrolein/treatment and B system 45 kg). Required treatment volumes of the anthrahydroquinone solution were estimated to be 57 and 95 l for the A and B systems respectively. At these concentrations acrolein/ anthrahydroquinone were efficacious.	**X**

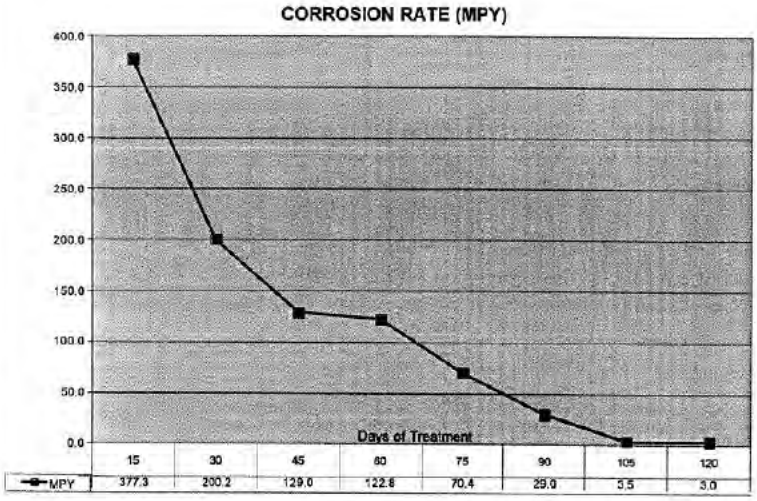
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	8/2/2008	
Materials and methods	**1.2.1, 1.2.2, 3.3, 3.6.2 & 4.2** The Applicant has not completed these Sections. The UK CA does not consider these omissions to be significant. **2.1, 2.1.1, 2.1.2, 2.1.5, 2.3.3, 2.3.7 & 5.1** In their dossier, the Applicant has specified (Doc. IIIB, Section 2.2) that the biocidal product Magnacide B contains 99.7 – 99.8 % w/w/ acrolein. Given that 99.7 – 99.8 % of Magnacide B is acrolein, the UK CA considers that, from the efficacy point of view, the active substance and the biocidal product are the same. For this reason, the UK CA is satisfied that the efficacy data presented in this RSS, if acceptable, can be used in support of the active substance. In Section 2.3.3 the applicant stated the weights in pounds and the volumes in gallons. The UK CA has re-expressed these weights and volumes as kilogrammes and litres. Although anthraquinone is stated as being present in Magnacide B as a stabiliser, it is, within the oil industry, also considered to be a biostat. As a biostat, it is thought to act by interfering with those metabolic pathways in sulphate-reducing bacteria (SRB) involved in the reduction, by the SRB, of sulphate to sulphide. Its role is therefore to reduce the level of H_2S via this mechanism, rather than by killing the SRB. This study did not investigate the efficacy of acrolein, but investigated the efficacy of anthraquinone as a biostat. The study report indicates that the reason for the study was to investigate the use of anthraquinone as a biostatic supplement to biocide treatment. More specifically, the study was designed to determine whether the use of anthraquinone, as a supplement to biocide treatment, could reduce H_2S concentrations to levels low enough to enable the frequency of biocide treatment to be reduced. To investigate the effectiveness of anthraquinone as a biostat, the study Authors' selected an oilfield water injection system that had previously experienced 'a very active SRB population and resulting production of iron sulfide solids'. Given the very active SRB population, it had previously been decided to use acrolein as a biocidal treatment. So, • At the beginning of the field test on anthraquinone, acrolein was already being applied as a daily slug treatment of 50 minutes injection per day. • During the field test, anthraquinone was applied in conjunction with acrolein. • Following the 3 cycles of anthraquinone/acrolein treatment, the anthraquinone treatment was stopped and acrolein was again applied on its own. The role of acrolein was therefore to act as a baseline control by which the efficacy of anthraquinone as a biostat could be evaluated.	

	As the focus of the study was on anthraquinone rather than acrolein, and as acrolein was used as the control, the study provided no baseline control data on the original levels of SRB at the site. In other words, no control data were presented which would allow the SRB levels during acrolein treatment to be compared with pre-acrolein treatment levels. **5.2** The efficacy template does not require the Applicant to state a number for the reliability indicator. However, the UK CA considers the study design to be an unsuitable test system for the investigation of the efficacy of acrolein. For this reason, the UK CA considers the reliability indicator to be 4 (see below).
Results and discussion	**3.1, 3.1.1 & 5.3** On the basis of the SRB results presented in Section 3.1.1, the UK CA agrees with the Applicant's statements. The UK CA notes, in particular, the Applicant's statements (Section 3.1) that 'the results from the SRB serial dilutions for the A system indicate that the population remained relatively stable throughout the trial and control periods', that 'except for 2 wellhead water samples, the SRB levels were between 10^1 and 10^3 cells/ml throughout the treatment and control periods', and that 'as with the A system, no definite trends with treatment cycle were evident'. The UK CA can accept that the fact that acrolein was already being used before the anthraquinone test began, indicates that the operators of the system were sufficiently satisfied with its ability to reduce the SRB levels at the tests site. However, • The SRB levels tended to remain stable during the test and control periods. • In both parts of the test system, no definite trends were evident. • No baseline control data was available to enable a comparison to be made between the SRB levels found during acrolein treatment, and those found before acrolein treatment began. For the above reasons, the UK CA considers that the data presented are not usable in support of the efficacy of acrolein against SRB.
Conclusion	**5.4** The UK CA agrees with the Applicant'statement regarding the stability of the SRB population throughout the water injection system. However, the UK CA does not consider the Applicant's other statements to be relevant to the efficacy of acrolein as a biocide. **5.5** The Applicant stated the weights in pounds and the volumes in gallons. The UK CA has re-expressed these weights and volumes as kilogrammes and litres.
Reliability	4
Acceptability	In the UK CA's opinion, the study is not acceptable in support of Annex I inclusion.
Remarks	All data and endpoints presented in the study summary have been checked against the original study and are correct.
	COMMENTS FROM...
Date	
Materials and methods	
Results and discussion	
Conclusion	

Reliability	
Acceptability	
Remarks	

Section A5/07 (Annex point IIA5)	Efficacy data on the active substance	
	1 REFERENCE	**Official use only**
1.1 Reference	Penkala, J.E et al, 2004, Acrolein, 2 Propenal: A Versatile Microbiocide for control of Bacteria in Oilfield Systems. Case History IV. NACE Paper No. 04749, NACE International Corrosion/2004, New Orleans, LA, 30/3/04-02/4/04	
1.2 Data protection	Yes	
1.2.1 Data owner	Baker Petrolite	
1.2.2 Criteria for data protection	Data on new b.p. for first entry to Annex I	
1.3 Guideline study	No	
1.4 Deviations	Not applicable.	
	2 METHOD	
2.1 Test Substance (Biocidal Product)	Acrolein	**X**
2.1.1 Trade name/ proposed trade name	Not specified.	**X**
2.1.2 Composition of Product tested	Not specified.	**X**
2.1.3 Physical state and nature	Not specified.	
2.1.4 Monitoring of active substance concentration	Not specified.	
2.1.5 Method of analysis	Not specified.	
2.2 Reference substance	The original treatment program consisted of alternating batch treatments of glutaraldehyde and a quaternary amine biocide into the injection system.	**X**
2.2.1 Method of analysis for reference substance	n/a	
2.3 Testing procedure		
2.3.1 Test population / inoculum / test organism	Sulfate reducing bacteria Location: Ecuador waterflood	
2.3.2 Test system	Not specified	
2.3.3 Application of TS	An acrolein biocide program was established utilising weekly batch treatments applied over a 4 hour period at a concentration of 200 ppm.	
2.3.4 Test conditions	A biogenic sulphide inhibitor supplement, anthraquinone was batched into the system every 10 days at an applied concentration of 25 ppm to extend the life of the acrolein biocide treatment level.	**X**
2.3.5 Duration of the test / Exposure time	4 hour exposure once per week for 120 days	

2.3.6	Number of replicates performed	Not specified	
2.3.7	Controls	Not specified	X
2.4	**Examination**		
2.4.1	Effect investigated	Killing SRB Corrosion rates were also measured as an indirect way of assessing the SRB concentration.	X
2.4.2	Method for recording / scoring of the effect	Serial dilution into culture media.	X
2.4.3	Intervals of examination	Not specified	
2.4.4	Statistics	Not specified	
2.4.5	Post monitoring of the test organism	Not specified	
		3 RESULTS	
3.1	**Efficacy**		
3.1.1	Dose/Efficacy curve	The efficacious acrolein dose was 200 ppm.	
3.1.2	Begin and duration of effects	Not specified	
3.1.3	Observed effects in the post monitoring phase	Not specified	
3.2	**Effects against organisms or objects to be protected**	Not specified	
3.3	**Other effects**	Not specified	
3.4	**Efficay of the reference substance**	The concentration of SRB in the injection system with the original treatment program using glutaraldehyde and a quaternary amine biocide, was greater than 10^6 bacteria/ml.	
3.5	**Tabular and/or graphical presentation of the summarised results**	The graph shows the effect of decreasing SRB concentration on corrosion rates.	X

		 **Figure 9. Case History IV: Corrosion rates in Ecuador waterflood after initiation of acrolein treatment.**	
3.6	**Efficacy limiting factors**		
3.6.1	Occurrences of resistances	No resistance observed.	
3.6.2	Other limiting factors	Not specified	
		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	The Case History presents results collected from a field trial.	
4.2	**Intended actual scale of biocide application**	n/a	
4.3	**Relevance compared to field conditions**		
4.3.1	Application method	n/a	
4.3.2	Test organism	n/a	
4.3.3	Observed effect	n/a	
4.4	**Relevance for read-across**	n/a	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Case History IV: An operating company in Ecuador reinjecting 12,000 BWPD (1908 m^3 water/day) had severe corrosion problems in the injection flow lines due to the presence of SRB. The corrosion rate in the injection lines established by weight loss coupons was consistently greater than 300 mpy. The treatment program originally consisted of alternating batch treatments of glutaraldehyde and a quaternary amine biocide into the injection system. As verified by serial dilution into culture media, the concentration of SRB in the injection system was greater than 10^6 bacteria/ml. Consequently, an acrolein biocide program	**X**

		was established utilising weekly batch treatments applied over a 4 hour period at a concentration of 200 ppm. In addition, a biogenic sulphide inhibitor supplement, anthraquinone was batched into the system every 10 days at an applied concentration of 25 ppm to extend the life of the acrolein biocide treatment level.	
5.2	**Reliability**	Yes: The method used and the test results are reliable and relevant for efficacy assessment.	**X**
5.3	**Assessment of efficacy, data analysis and interpretation**	After 120 days of treatment with acrolein and the anthraquinone sulphide inhibitor the results obtained are as follows: • The bacterial biofilm and associated solids have been removed and the system remains clean. • The SRB contamination levels have decreased by 99 %. • The corrosion rates have decreased to 3 mpy. • Periodic purging of the injection lines is no longer necessary. • The treatment cost per barrel of water has been lowered 50 %. • Costs associated with injection line maintenance have been reduced by 95 %.	**X**
5.4	**Conclusion**	Weekly batch treatments of acrolein applied over a 4 h period at a concentration of 200 ppm, reduced SRB contamination levels by 99 %, after 120 days of treatment.	**X**
5.5	**Proposed efficacy specification**	The findings from this study support the use of acrolein in batch treatments at the proposed label concentration of 50-250 ppm for 4 to 6 hours on a weekly basis.	**X**
		Evaluation by Competent Authorities	
		EVALUATION BY RAPPORTEUR MEMBER STATE	
Date		8/2/2008	
Materials and methods		**2.1, 2.1.1 & 2.1.2** In their dossier, the Applicant has specified (Doc. IIIB, Section 2.2) that the biocidal product Magnacide B contains 99.7 – 99.8 % w/w/ acrolein. Given that 99.7 – 99.8 % of Magnacide B is acrolein, the UK CA considers that, from the efficacy point of view, the active substance and the biocidal product are the same. For this reason, the UK CA is satisfied that the efficacy data presented in this RSS, if acceptable, can be used in support of the active substance. **2.2** The Applicant has indicated, in Sections 3.4. and 5.1, that following the original treatment programme, consisting of glutaraldehyde and a quaternary amine, the levels of SRB remained high, with counts of 10^6 SRB ml^{-1} observed. As the SRB levels remained high, it was decided to use acrolein instead. **2.3.4 & 5.1** The Applicant has indicated (Section 5.1) that the corrosion problem at the location was so severe that the use of acrolein needed to be supplemented by the use of anthraquinone. W ithin the oil industry, anthraquinone is considered to be a biostat which is thought to act by interfering with those metabolic pathways SRB involved in the reduction, by the SRB, of sulphate to sulphide. Its role is therefore to reduce the level of H_2S via this mechanism, rather than by killing the SRB. **2.3.7** The Applicant has stated that no controls were conducted. Although no control test has been conducted, the Applicant has indicated (Section 5.1) that, following the original treatment with glutaraldehyde and a quaternary ammonium	

	biocide, the level of SRB in the location remained high (10^6 SRB ml^{-1}). This high SRB level therefore acted as a baseline control against which the efficacy of the acrolein treatment could be assessed. **2.4.1 &2.4.2** The method stated is that for obtaining bacterial counts. However, in the study, corrosion rates were also measured. The methodology employed in measuring corrosion rates was the corrosion coupon (weight loss) method. This is a well established and commonly used methodology in this area. Essentially, the method involves exposing specimens of material (the coupons) to the environment to be monitored, and then removing them for analysis. The basic measurement determined from corrosion coupons is weight loss. The coupons are pre-weighed, and, following the selected exposure period, are cleaned and re-weighed. The difference between the two weights represents the amount of metal lost. This is the corrosion rate and is measured as millilitres penetration per year (MPY). **5.2** The original study report has not been submitted, and the Applicant has been unable to locate it. For this reason, although the UK CA has commented on the RSS, the UK CA cannot verify the accuracy of the information presented in the RSS. In addition to this, no results have been presented on SRB numbers during the test period. For these reasons, the UK CA considers the reporting of the methods and results to be insufficient, and therefore considers the reliability indicator to be 4 (see below).
Results and discussion	**3.5** The data presented are the results of the monitoring of microbiologically induced corrosion rates at the location. The graph presents the corrosion rates in MPY both before and during acrolein and anthraquinone treatment. The results showed that a corrosion rate of approximately 380 MPY was measured 15 days after the initiation of acrolein/anthraquinone treatment. The corrosion rate then decreased steadily to < 10 MPY at 120 days after initiation of treatment. Although the UK CA accepts that there is a well-established link between levels of SRB in aquatic environments such as those found in off-shore oil facilities, and the levels of corrosion found in such environments, no data on SRB have been presented. For this reason, the RSS presents no direct evidence for the efficacy of acrolein as a biocide against SRB. **5.3** The UK CA accepts the Applicant's statement that the corrosion rates decreased to 3 MPY. However, as no data on SRB levels have been presented, the UK CA cannot determine the accuracy of the Applicant's statement that the SRB contamination levels decreased by 99 %, nor the other statements made in this Section.
Conclusion	**5.4 & 5.5** As no data have been presented to demonstrate that the SRB contamination levels were reduced by 99 %, the UK CA does not agree with the Applicant's'statements.
Reliability	4
Acceptability	In the UK CA's opinion, the study is not acceptable in support of Annex I inclusion.
Remarks	All data and endpoints presented in the study summary have been checked against the original study and are correct.
	COMMENTS FROM ...

Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A5/08 (Annex point IIA5)		Efficacy data on the active substance	
		1 REFERENCE	**Official use only**
1.1	**Reference**	Penkala, J.E et al, 2004, Acrolein, 2 Propenal: A Versatile Microbiocide for control of Bacteria in Oilfield Systems. NACE Paper No. 04749, NACE International Corrosion/2004, New Orleans, LA, 30/3/04-02/4/04 (The report also includes the results tables submitted as an addendum)	**X**
1.2	**Data protection**	Yes	
1.2.1	Data owner	Baker Petrolite	
1.2.2	Criteria for data protection	Data on new a.s. / b.p. for first entry to Annex I	
1.3	**Guideline study**	No	
1.4	**Deviations**	Not applicable.	
		2 METHOD	
2.1	**Test Substance (Biocidal Product)**	Acrolein	**X**
2.1.1	Trade name/ proposed trade name	Not specified.	**X**
2.1.2	Composition of Product tested	Not stated.	**X**
2.1.3	Physical state and nature	Not stated.	
2.1.4	Monitoring of active substance concentration	Not stated.	
2.1.5	Method of analysis	Not stated.	
2.2	**Reference substance**	The performance of acrolein was compared to tetrakishydroxymethyl phosphonium sulfate (THPS), glutaldehyde, DBNPA, MBT, isothiazalone, methylene bis(thiocyanate), Diamine Quat/Quat blend, Glut/Quat blend, formaldehyde, bronopol, hypochlorite, Diamine Quat, Glutaraldehyde, and quat.	**X**
2.2.1	Method of analysis for reference substance	Not stated.	
2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	General aerobic and facultative anaerobic bacteria (GAB) and SRB: Locations: Gulf of Mexico, Kansas, California, Texas, Utah, Canada, New Mexico, Argentina, Trinidad and Alaska.	
2.3.2	Test system	Not Stated.	
2.3.3	Application of TS	Not stated.	
2.3.4	Test conditions	Not reported.	
2.3.5	Duration of the test / Exposure time	Various, between 1 and 24 hours.	

2.3.6	Number of replicates performed	28 different studies, comparing differing chemistries.	
2.3.7	Controls	Not stated.	X
2.4	**Examination**		
2.4.1	Effect investigated	Killing GAB, SRB.	
2.4.2	Method for recording / scoring of the effect	Not stated.	
2.4.3	Intervals of examination	Not stated.	
2.4.4	Statistics	Not stated.	
2.4.5	Post monitoring of the test organism	Not stated.	
		3 RESULTS	
3.1	**Efficacy**		
3.1.1	Dose/Efficacy curve	The effective dose required varied: majority: 25 – 150 ppm, 3 exceptions where, 200, 500, and 620 ppm were required.	X
3.1.2	Begin and duration of effects	None described.	
3.1.3	Observed effects in the post monitoring phase	None described.	
3.2	**Effects against organisms or objects to be protected**	Not described.	
3.3	**Other effects**	Not described.	
3.4	**Efficacy of the reference substance**	Acrolein comparison reported.	
3.5	**Tabular and/or graphical presentation of the summarised results**	See table 1 at the end of this robust study summary	X
3.6	**Efficacy limiting factors**		
3.6.1	Occurrences of resistances	No resistance observed.	
3.6.2	Other limiting factors	Acrolein reacts slowly with water to form a water soluble, nontoxic hydration product, ßhydroxypropanal. This product ultimately breaks down to form CO_2 and water. The halflife of acrolein in most produced water systems without H_2S or iron sulfide is 8-24 hours (average = 10 hours), which provides adequate time for biocidal actions, but may limit its life when treating long transit water injection lines. Acrolein reacts with H_2S in a 2:1 molar ratio; normally 1 ppm of H_2S consumes approximately 4 ppm acrolein.	

		4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	Use cases also reported.	
4.2	**Intended actual scale of biocide application**	Not reported. Supported by actual use cases.	
4.3	**Relevance compared to field conditions**		
4.3.1	Application method	Not stated.	
4.3.2	Test organism	Acrolein efficacy was tested against four common bacterial strains: 1) *Pseudomonas fluorescens*, a Gram negative, non-sporulating bacterium, 2) *Bacillus cereus* a Gram positive, spore-forming bacteria, 3) *Bacillus subtilis*, a Gram positive, spore-forming bacteria, and 4) *Staphylococcus epidermidis*, a Gram positive, non-sporulating bacterium. General aerobic and facultative anaerobic bacteria (GAB) and SRB. (See Document IIIB, Section 5.10.2(5), Annex Pt. IIB V 5.10)	**X**
4.3.3	Observed effect	Yes. Laboratory tests show effective 6 log kill at 1-3 ppm against SRB and GAB cultured from seawater from the Texas Gulf Coast. In the same study, acrolein efficacy was tested against four common bacterial strains. At 3 ppm acrolein, a >99.99 % reduction was observed in all strains after 24 h exposure and at 10 ppm, a >99.99 % reduction was observed. During routine laboratory testing, acroelin exhibits effective control over field bacteria cultured on agar plates (aerobes), phenol red dextrose (GAB), thioglycollate (anaerobes) and various SRB media. In general, the minimum inhibitory concentration ($Conc_{MI}$) ranges from 25 ppm to 100 ppm for contact times of 2-8 h. The speed of kill for acrolein, 1 h compares with other fast acting organic biocides.	**X**
4.4	**Relevance for read-across**	Yes. The laboratory tests show function of acrolein as desired, the mobility required to kill sessile bacteria is then demonstrated in the 5 case histories.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The review of the published and unpublished efficacy data is supported by 5 case histories in the use of acrolein industrially: I. Effective treatment of sessile bacteria in water injection lines II. Mitigation of biogenic H_2S in gas production wells III. Control of Microbial Induced Corrosion (MIC) and biogenic FeS in a water injection plant IV. Control of sessile biofilm and MIC in an oil separation facility V. Control of SRB mediated underdeposit corrosion in an offshore production facility.	**X**
5.2	**Reliability**	Yes. The laboratory tests show function of acrolein as desired at dose levels comparable to those advised (50-250 mg/l), the mobility required to kill sessile bacteria is then demonstrated in a number of the case histories.	**X**
5.3	**Assessment of efficacy, data**	As a microbiocide, acrolein is broad spectrum and has been demonstrated to be highly effective against SRB and GAB, which	**X**

	analysis and interpretation	include acid producing bacteria (APB), two common classes of bacteria encountered in the oilfield. Laboratory tests show effective 6 log kill at 1-3 ppm against SRB and GAB cultured from seawater from the Texas Gulf Coast. Acrolein efficacy was tested against four common bacterial strains: 1) Pseudomonas fluorescens, a Gram negative, non-sporulating bacterium, 2) Bacillus cereus a Gram positive, spore-forming bacteria, 3) Bacillus subtilis, a Gram positive, spore-forming bacteria, and 4) Staphylococcus epidermidis, a Gram positive, non-sporulating bacterium. At 3 ppm acrolein, a >99.99% reduction was observed in all strains after 24 hours exposure and, at 10 ppm, a >99.999% reduction was observed. During routine laboratory testing, acrolein exhibits effective control over field bacteria cultured on agar plates (aerobes), phenol red dextrose (GAB), thioglycollate (anaerobes), and various SRB media. In general, the minimum inhibitory concentration (ConcMI) ranges from 25 ppm to 100 ppm for contact times of 2-8 hours. The speed of kill for acrolein, 1 hour, compares with other fast-acting organic biocides, such as glutaraldehyde and tetrakishydroxymethyl phosphonium sulfate (THPS).	
5.4	**Conclusion**	In 26 out of 28 tests, the minimum inhibitatory concentration (ConcMI) for acrolein ranges from 50 to 200 ppm. However, there were two exceptions. In one case, Utah-1999, there were incompatibilities in the water due to the presence of high concentrations of ammonia. In the other case, Texas-1999, acrolein was still the most cost effective biocide out of 7 tested but a high ConcMI was required. In 19 of the 24 batch treatment simulations (contact time = 2 to 8 hours), the ConcMI for acrolein ranged from 25 – 135 ppm (excluding the exceptions noted above). In the two tests simulating continuous applications (contact time = 24 hrs) the ConcMI for acrolein was 25 and 121 ppm. As is well understood, the nature of the planktonic kill test does not faithfully reproduce the challenge to the biocide in the actual system being treated. Various parameters contribute to this difference between laboratory tests and applications in the field, one of the most significant being the need to control sessile bacteria in the system. The performance of a biocide against sessile populations in a system cannot be easily predicted by laboratory tests. In many cases, however, acrolein performs well in the field system at or below the ConcMI identified in the planktonic selection tests. Acrolein is a non-surface active, water-soluble biocide that will partition into the oil phase. This important feature allows acrolein to penetrate oil wet surfaces and solids to target elusive bacterial populations protected by this film. In most oilfield systems, even with refined water for secondary injection, there is generally oil coating of tanks, vessels, and flow lines. The ability of a biocide to penetrate these coatings is essential to its performance. This property also renders acrolein as an effective biocide in mixed production and in oil separation facilities. It allows acrolein access to the oil/water interface where microbial activity is frequently present.	**X**
5.5	**Proposed efficacy specification**	The findings from this study support the efficacy of acrolein at the proposed concentration of 50-250 ppm.	**X**
		Evaluation by Competent Authorities	

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	8/2/2008
Materials and methods	**2.1, 2.1.1&2.1.2** In their dossier, the Applicant has specified (Doc. IIIB, Section 2.2) that the biocidal product Magnacide B contains 99.7 – 99.8 % w/w/ acrolein. Given that 99.7 – 99.8 % of Magnacide B is acrolein, the UK CA considers that, from the efficacy point of view, the active substance and the biocidal product are the same. For this reason. the UK CA is satisfied that the efficacy data presented in this RSS, if acceptable, can be used in support of the active substance. **2.2** The entry in this Section is a list of all of the reference substances used in the original studies summarised in this report, including those for which the results have been presented in Table 1. **2.3.7**. The controls are 'not stated' because the report is a summary of a number of original studies on acrolein. **5.2** The efficacy template does not require the Applicant to state a number for the reliability indicator. However, the UK CA considers that this RSS has major reporting deficiences. For this reason, the UK CA considers the reliability indicator to be 3 (see below).
Results and discussion	**3.1.1 & 3.5** The results in Table 1 are a summary of the results obtained from 28 different studies conducted at various times and in various geographical locations. The entry in Section 3.1 is a summary of these summary results. **4.3.2** The entry in this Section refers to the test organisms in the key study by Penkala, *et al*, 2003. Please refer to RSS B5.10.2(1). **4.3.3 & 5.3** The first pragraph relates to results obtained by Penkala, *et al*, 2003. Please refer to RSS B5.10.2(1). The second paragraph relates to data that are not in any of the studies considered by the Applicant to be key in support of acrolein. **5.1**. Three of the case studies have been cited as key studies by the Applicant, and are presented elsewhere in this evaluation. Please refer to: 1. RSS B5.10.2(4) – Case history I 2. RSS B5.10.2(7) – Case history IV 3. RSS B5.10.2(3) – Case history V Case histories II & III have not been cited as key by the Applicant.
Conclusion	**5.4** As the results presented in Table 1 are only summary results, and the original studies have not been cited as key studies, the UK CA cannot conduct an evaluation of the data. For this reason, the UK CA cannot verify the accuracy of the results presented and therefore cannot agree with the Applicant's conclusion. The UK CA does, however, agree with the Applicant that if laboratory based data demonstrate efficacy against planktonic bacteria, this does not demonstrate efficacy against sessile bacteria under field conditions. **5.5** For the reasons stated above for 5.4 the UK CA does not agree with the Applicant's statement.
Reliability	3

Acceptability	In the UK CA's opinion, the study is not acceptable in support of Annex I inclusion
Remarks	**1.1** This report does not report on an original study, but summarises the results from a number of original studies that have been conducted on acrolein.
	COMMENTS FROM ...
Date	
Materials amd methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table 1

Table 2. Biocide Selection Tests Comparing Acrolein Performance to Other Biocides

Date	Region	Acrolein Results				Chemistries Tested	Product Most Competive to Acrolein	Best Performing Product	Cost Ratio for Same Degree of Kill: Competing Product / Acrolein
		Contact Time (hrs)	MIC (ppm)	Log No. GAB Killed / Log No. in Control	Log No. SRB Killed / Log No. in Control				
1997	Gulf of Mexico	24	121	8/8	7/7	A,G,T,Q2,M,I	Glutaridehyde	Acrolein	2.09
1998	Kansas	2	150	4/4	1/1	A,G,T,I	Isothiazalone	Acrolein	2.00
1998	California	2	50	6/6	6/6	A,G,T,D,DQ1	Glutaraldehyde	Acrolein	4.04
1998	Texas	1	150	—	12/12	A,G,T,Q1,F	THPS	Acrolein	3.06
1998	Utah	2	500	12/12	6/6	A,G,T,Q1D	Diamine Quat	Diamine Quat	0.19
1999	Gulf of Mexico	2	100	12/12	6/6	A,G,T,D,GQ1,GQ2	Glutaraldehyde	Acrolein	2.02
1999	Texas	6	620	12/12	12/12	A,Q1,Q2,D,DQ,GQ,B	Glut/Quat	Acrolein	1.48
1999	Texas	4	57	6/6	7/7	A,G,T,Q1,Q2,D,DQ,GQ1	Glut/Quat	Glut/Quat	0.89
1999	Canada	3	104	12/12	6/6	A,G,Q1,D,GQ1,DB	Glut/Quat	Acrolein	1.86
1999	Canada	3	104	12/12	6/6	A,G,Q1,D,GQ1,DB	Glut/Quat	Acrolein	1.86
1999	Canada	3	108	12/12	12/12	A,G,Q1,D,GQ1	Glut/Quat	Acrolein	9.90
1999	Canada	3	134	12/12	6/6	A,G,Q1,D,GQ1	Glut/Quat-1	Acrolein	3.64
1999	Texas	3	135	12/12	12/12	A,G,Q1,D,GQ1,DQ,H,F	Glutaridehyde	Acrolein	3.66
1999	Canada	3	134	12/12	11/11	A,G,Q1,D,GQ1,	Glut/Quat-1	Acrolein	1.82
1999	New Mexico	2	200	7/7	6/6	A,G,T,D	Glutaraldehyde	Acrolein	1.52
1999	Argentina	3	61	—	7/7	A,G,T,Q1,Q2,Q3,D,DQ	Glut/Quat-2	Acrolein	1.80
1999	California	3	100	6/6	6/6	A,G,T	Glutaridehyde	Acrolein	2.75
1999	Trinidad	4	100	9/9	9/9	A,T	THPS	Acrolein	1.99
1999	Gulf of Mexico	2	88	12/12	12/12	A,G,Q1,D,DB,H	Glutaraldehyde	Acrolein	6.16
2000	Texas	4	50	7/7	12/12	A,G,T	Glutaraldehyde	Acrolein	1.84
2000	Kansas	4	100	7/7	8/8	A,T	THPS	Acrolein	1.99
2000	Canada	2	200	12/12	7/7	A,G,T,D,GQ1,GQ2	Diamine Quat	Diamine Quat	0.74
2000	Gulf of Mexico	6	50	8/8	6/6	A,G,T,D	Glutaraldehyde	Acrolein	2.50
2000	Alaska	6	100	12/12	12/12	A,G,T,Q1,GQ2,D	Diamine Quat	Acrolein	1.25
2000	Texas	3	36	8/8	11/11	A,G,Q1,Q2,D,DQ,GQ2	Glut/Quat	Acrolein	4.25
2000	Kansas	2	36	7/7	6/6	A,G,T,Q1,Q2,D,DQ,GQ2	Glut/Quat	Acrolein	2.52
2001	Gulf of Mexico	24	25	6/6	6/6	A,T	THPS	Acrolein	9.91
2001	Gulf of Mexico	4	100	12/12	12/12	A,G,T,H	Glutaraldehyde	Acrolein	2.50

A = Acrolein
G =Glutaraldehyde
T = THPS
Q(1,2,3) = Quat
D = Diamine Quat
I = Isothiazalone
H = Hypochlorite
B = bronopol
F = formaldehyde
DB = DBNPA
GQ (1,2) = Glut/Quat blend
DQ = Diamine Quat/Quat blend
M = methylene bis(thiocyanate)

Number in parentheses indicates different products in this chemistry class