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 Melting pt. 2	Capillary method: EEC Method A1	99.7% difenacoum	***result:*** 216.3-226°C ***pressure:*** Atmospheric	Material did not appear to melt but was observed to darken and decompose.	Y	1	Drake RM (2003) Determination of the Melting Point and Boiling Point of DIFENACOUM TECHNICAL. Chemex Environmental International Ltd report ENV5799/120139 GLP, Unpublished	X
3.1.2 Boiling point Boiling pt. 1	EEC Method A2	99.7% difenacoum	**result:** The test substance did not boil before decomposing at 226.3º C. **pressure:** Atmospheric	Not determined as material was a powder and decomposed	Y	1	Drake RM (2003) Determination of the Melting Point and Boiling Point of DIFENACOUM TECHNICAL. Chemex Environmental International Ltd	X

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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
Boiling pt. 2	Differential Scanning Calorimeter (DCS)	99.5%	No boiling under 150ºC, thermally stable up to its melting point		Y	1	report ENV5799/120139 GLP, Unpublished Drake RM (2005) Chemex Environmental International Ltd., Report No. ENV7063/120139	
3.1.3 Bulk density/ relative density								
Bulk/rel. density 1	Pycnometer method: CIPAC MT3.2. OECD 109	>99% difenacoum	Relative Density = 1.1363		Y	1	Garofani S (2001) DIFENACOUM Determination of the Relative Density. ChemService report CH-152/2000 GLP, Unpublished	
3.2 Vapour pressure (IIA3.2)								
Vapour pressure 1	Gas saturation method: EEC Method A.4 OECD 104	99% difenacoum	**temperature:** 45ºC **result:** <0.05 mPa		Y	1	Fabbrini Dr. R (1997) DIFENACOUM Determination of the Vapour Pressure. ChemService	

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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
Vapour pressure 2							report CH-14/96-C-DIF GLP, Unpublished	
3.2.1 Henry´s Law Constant (IIA3.2)	Calculation		**calculated**:<0.046 Pa m^3 $mole^{-1}$ vapour pressure = < 5 x 10^{-5} Pa (45ºC) water solubility = 4.83 x 10^{-4} g/l (20ºC)	Scarcely volatile	N	2	Worthington, M (2006), SafePharm report, calculation of Henry's Law Constant., non-GLP, unpublished.	
3.3 Appearance (IIA3.3)								X
3.3.1 Physical state	Solid; fine powder			It is accepted that a study is needed. The applicant has been quoted for this study.			Tomlin, 13th ed. Pesticide manual, page 303	
3.3.2 Colour	buff/beige			See above			Tomlin, 13th ed. Pesticide manual, page 303	
3.3.3 Odour	No odour			See above			Tomlin, 13th ed. Pesticide manual, page 303	
3.4 Absorption spectra (IIA3.4)								
3.4.1 UV/VIS	OECD 101	98.8% Difenacoum	259 nm (28515 and 29085 l/mol.cm) & 308 nm (12926 and 13279 l/mol.cm) in both methanol solution and in 10% 1 N HCL solution		Y	1	Garofani S. (2001) DIFENACOUM UV/Vis, MS, IR and NMR Spectra. ChemService report CH-	

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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
			in methanol 252 nm (28004 l/mol.cm) and 312 nm (14901 l/mol.cm) in 10% 1 N NaOH in methanol All at 12.6 mg/ml conc.				132/2001 GLP, Unpublished	
3.4.2 IR	OECD 101 Performed at room temperature, of the compressed solid mix.	98.8% Difenacoum	O – H Stretching 3269 cm^{-1} C – H stretching 2925 cm^{-1} C = C Ring stretching 1665 – 1430 cm^{-1} C(aryl) – O Stretching 1300 – 1100 cm^{-1}	Although there is no signal corresponding to the wavenumber of 3269 on the spectrum, there seems to be signal for the hydrogen bonded O - H group around 3400 cm^{-1}	Y	1	Garofani S. (2001) DIFENACOUM UV/Vis, MS, IR and NMR Spectra. ChemService report CH-132/2001 GLP, Unpublished	
3.4.3 NMR	OECD 101 ^{1}H	98.8% Difenacoum	ppm (no. of H) / ppm (multiplicity) 2.4 (2), 2.8 (4), 2.9-3.2 (2), 3.4 (2), 3.6 (2), 4.7 (2), 5 (1, broad), 7.0-7.8 (16), 8.1 (1, singlet)	deuterated acetone and deuterated pyridine were used as solvent.	Y	1	Garofani S. (2001) DIFENACOUM UV/Vis, MS, IR and NMR Spectra. ChemService report CH-132/2001 GLP, Unpublished	
	^{13}C		36.4 (triplet), 39.0 (droplet), 39.5 (triplet), 42.7 (droplet), 117-133, 137 (droplet), 151 (droplet)					
3.4.4 MS	OECD 101	98.8% Difenacoum	MS/MS^{-} fragments: 93, 143, 293	Fragments identified in the report.	Y	1	Garofani S. (2001) DIFENACOUM UV/Vis, MS, IR	

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
			MS/MS^{+} fragments: 174, 179, 189, 257, 291				and NMR Spectra. ChemService report CH-132/2001 GLP, Unpublished	
3.5 Solubility in water (IIA3.5)	*including effects of pH (5-9)*							
Water solubility	Flask method: EEC Method A.6	99.70% purity/ 99.0% min. specification	**Result**: ≤ 4.82 x 10^{-5}g/l (pH 5.08) 4.83x10^{-4} g/l (pH 6.50) 3.72 x 10^{-3}g/l (pH8.86) **temperature** : 20±0.5°C	Very low water solubility over pH range 5-9. The flask method was chosen because the results in the pesticide manual suggested that the solubility would actually be greater than 10mg/l. Also data exists suggesting that difenacoum is sensitive to light and hence the flask method is easier to protect from light compared to the column elution method. In the opinion of the test laboratory, the flask method tends to obtain the highest possible solubility when compared to the column elution method since elevated . temperatures and significant energy is applied to the test system during the initial saturation process. The reason why the results from	Y	2	Woolley.S (2005) Difenacoum: Determination of water solubility report,number 1558/011. July 2005	

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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
				sample 1 during the neutral pH study were not included is that it was deemed they were inconsistent with the other 2 samples. As the 3 samples are analysed in duplicate, 2 results out of the 6 were inconsistent. When the Grubbs outlier test was applied for a 95% confidence, the results were on the criteria for discarding. Therefore it was decided that as samples 2 and 3 were within the required accuracy of the method (+/- 15%) and that the saturated solutions of sample 1 had probably not been completely cleaned of excess material during the centrifugation stage, they were not included in the mean. In addition to this argument, the guideline states that if the last two successive solubilities are within 15% of eachother the test is acceptable.				
3.6 Dissociation constant (-)	ACDI Labs QSAR, Uses a unique structure-fragment approach and an internal database of		pKa = 4.5 ± 1.00			2	ACD/I- Lab Web Service (ACD/pKa 8.03), SafePharm Laboratories	

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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
	nearly 16000 structures with over 31000 experimental pKa values, ensuring a fast and highly-reliable prediction						(2004)	
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA3.1)	Based on OECD 105	99.7%w/w	**Results: (mean values, g/l)** Toluene : 1.49g/l Ethyl acetate**:** 3.60g/l **Methanol:** 1.00g/l **Acetone:** 8.12g/l **Dichloromethane:** 17.39 g/l All solubilities at 20°C	With regards to the low solubility of difenacoum in n-hexane, this is attributed to two factors; firstly there is a notable difference in polarity between n-hexane and the other solvents. Due to the localised nature of the difenacoum molecule, dissolution will be favoured in a solvent with a dipole moment where energetically favourable interactions between solvent and solute are possible. The very low polarity of hexane implies that, although organic, it would not easily interact electrostatically with difenacoum. It is worthy of note that Brodifacoum has an additional bromine atom which gives an extra dipole moment. This extra dipole moment and its relationship with solubity is supported by the relative	Y	1	Staniland, J.(2005) Solubility of Difenacoum in organic solvents. Chemex Environmental International ltd, Report ENV7059/120139. (May 2005)	

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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
				increase in solubility with the more polar solvents (by a factor of 2-3). This information implies that the polarity (induced dipole moment) has a direct influcen on the solubility of the molecule.				
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)	-			Not required. See data waiver below.				

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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	EEC Method A8, Shake-Flask method. The concentration was determined in both phases by HPLC.	99.0%	**result**: >3.3 **temperature:** 20ºC **pH:** This result has been queried by the Finnish CA as being derived from an inappropriate method. Please see calculated value below.	Buffer was not used in the study to obtain the non-ionised form because , as reported in the pesticide manual for brodifacoum, a molecule with a very similar structure, " the brodifacoum is a very weak acid which is too liphophilic to form water soluble salts". Although not stated in the A8 guideline, it states in the data requirements that the A8 is partly based on OECD 117 and 107. In OECD 117 it states that the shake-flask method can be used for log Pow of 5 or even higher, if care is taken in sampling. It also states in the data requirements (chapter 2, part A, point 3.9) that " for those substances which are extremely soluble in one of the phases a limit value should be provided. If necessary it can be based on the individual soulbilities in n-octanol and water" The A.8 EC method	Y	3	Fabbrini Dr R (1997) DIFENACOUM Determination of the Partition Coefficient. ChemService report CH-14/96-B-DIF GLP, Unpublished	

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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
log Pow 2	EPIWIN model, agreed by Finnish CA	n/a	7.62	recommends a maximum concentration in both phases of 0.01M. The Mwt of difenacoum is 444.5, therefore this solution in n0octanol is 4.445mg/ml; the water solubility is less than 0.1mg/l. So, the Pow is calculated as >44450 and a log Pow >4.65. The application of the HPLC method for this molecule would not be easy due to the small number of molecules to use as standards for the comparison. In any event as stated earlier, OECD 117 itself states that the shake-flask method can be used for higher Pow than 4.	N	2	Worthington, M (2006), SafePharm report, calculation of Partition-coefficient., non-GLP, unpublished.	
3.10 Thermal stability, identity of relevant breakdown products (IIA3.7)	OECD guideline 113 - Screening test for Thermal Stability (TGA and DSC) CIPAC Accelerated Storage Test	99.70%	Result: No significant change was noted on storage for 14 days @54°C.Thermally stable below 150°C.	It was also found to be stable up to its melting point which was noted to be 5°C higher than the manufacturer's data. Note: the tin correction of 7.2°C	Y	1	Drake RM Determination of the thermal stability and breakdown products of	X

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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
				was subtracted incorrectly from the Difenacoum melting point. The result for the peak melting point should be 226.3ºC (supports melting point result in 3.1.1 – Drake 2003)			difenacoum. Chemex Environmental International Ltd, Report no. ENV7063/120139	
3.11 Flammability, including auto-flammability and identity of combustion products (IIA3.8)	EEC Method A.10 EC Method A.16	> 99% Difenacoum > 99% Difenacoum	Not highly flammable No self-ignition detected from room temperature to the mpt 215°C	Sample did not ignite, but melted and decomposed when flame approached.	Y Y	1 1	Garofani S (2001) DIFENACOUM Determination of the Flammability. ChemService report CH-153/2000 GLP, Unpublished Garofini S (2001), Difenacoum determination of the self-ignition temperature for solids, Chemservice report CH155/2000	
3.12 Flash-point (IIA3.9) Flash-point 1 Flash point 2				Difenacoum is a solid, therefore a flash-point study is not required.				

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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
3.13 Surface tension (IIA3.10) Surface tension 1	EC A5	99.7%	**result:** 72.9mN/m Not surface active **temperature:** 20° C	Study is unreliable due to low solubility of difenacoum. However, not a data-gap since solubility is < 1mg/l.	Y	3	Sacker, D.J (2005) Determination of the surface tension of Difenacoum, Chemex Environmental International Ltd. Ref: ENV7163/120139	
3.14 Viscosity (-)	-		**result: temperature:**	Dienacoum is a solid at atmospheric pressure and at room temperature, therefore does not require a viscosity study..				
3.15 Explosive properties (IIA3.11)	EEC Method A.14	> 99% difenacoum	Does not exhibit explosive properties	Tested to thermal sensitivity (flame), Mechanical sensitivity (shock and friction). It was decided to submit a justification for non-submission of data on the grounds that this study was deemed inadequate by the Competant Authority. Please see separate data waiver for A.3.15.	Y	4	Garofani S (2001) DIFENACOUM Determination of the Explosive Properties. ChemService report CH-154/2000 GLP, Unpublished	

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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
3.16 Oxidizing properties (IIA3.12)	EEC Method A.17	> 99% difenacoum	. Does show oxidizing properties. No test performed to demonstrate not a false positive result. Please see data waiver for more information.		Y	2	Garofani S (2001) DIFENACOUM Determination of the Oxidising Properties. ChemService report CH-156/2000 GLP, Unpublished	
	EEC Method A.17	>99%	This additional study shows that the 'wick effect' was observed and therefore confirms the above study (CH-156/2000) result that difenacoum is not an oxidising substance.		Y	1	Garofani S, (2006) Difenacoum Techmical: Determination of the Oxidising Properties. ChemService report CH-267/2006	
3.17 Reactivity towards container material (IIA3.13)	Experience in use and chemical structure (data requirements, pg 33)		-Not considered reactive. See Data waiver below.	Difenacoum does not come out of the reaction vessel as the technical material.It is never stored in bulk as technical. However, should the pure material need to be stored for sending a sample for phys-chem studies, this could be done in a glass jar, since the reaction vessel used in the process to make difenacoum is glass-lined.	Not applicable	Not applicable		

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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
				Alternatively, looking at the structure, it is highly likely that samples could be stored in HDPE (high density polyethylene) jars. Many organic solids can be stored in HDPE jars.				

	EVALUATION BY THE RAPPORTEUR MEMBER STATE
Date	21st January 2008
Materials and Methods	The applicant's version is acceptable with the following amendments (below).
Results and discussion	The applicant's version is acceptable with the following amendments (below).
Conclusion	The applicant's version is acceptable with the following amendments (below).
Reliability	The applicant's version is acceptable with the following amendments (below).
Acceptability	The applicant's version is acceptable with the following amendments (below).
Remarks	The applicant's version is acceptable with the following amendments (below).

3.1.1 Melting point
Result: Observations at 216.3-226 ºC included signs of melting (formation of droplets at 216.3 ºC and above) and some signs of degradation (appearance of brown colour). The findings and the reporting were not exact in the report, but suggest at least partial melting. The DSC study (3.10) further supports the interpretation that in the proximity of 220 ºC melting takes place. The sum of evidence suggests that the melting point is 226.3 ºC.
Reliability: 3

3.1.2 Boiling point
Result: No boiling point detected, in tests (3.1.1 and 3.10) up to the temperature of 250 °C in the 3.10

3.3 Appearance
Result: For Appearance (3.3.1-3.3.3) ownership of data, for the technical substance, should be demonstrated or a study should be submitted..In 3.10 "off-white powder" is reported for the purified substance.

3.4.2 IR
Result: no 3269 cm^{-1} signal detected.

3.10 Thermal stability
Result: a single endotherm at 226.3 ºC (a tin-corretd value) was found in a sealed crucible. The tests ranged up to the temperature of 250 ºC. The 226.3 ºC is most likely a temperature of melting.

3.11 Flammability and autoflammability
Flammability. Method: A train test.

Autoflammability
Result: no self ignition detected from room temperature to 215 °C (high end temperature of the test).

Other indications of Flammability: According to the TNsG on Data requirements "tests A12/A13 can be omitted if experience in use indicates that negative results would be obtained". Also, from the structural formula and composition of the substance it can be concluded that the substance does not evolve any flammable gases in contact with water or humid air and that the substane is stable at room tempratureand is not pyroforic.

Section A3.15 **Annex Point IIA III.3.11**	**Explosive properties**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	Consideration of structure and physico-chemical properties does not suggest any explosive potential. Widespread experimental and commercial use over many years has not shown any exothermic or explosive activity. There is no reason to believe that difenacoum's structure gives any cause for concern as it does not contain certain reactive groups e.g. nitro, peroxide etc. The data requirements also state that the oxygen balance can be used to establish a low risk of explosive properties. Using the well know equation for O2 balance : = -(1600 [2a + b/2 – c])/mwt Where a = number of carbon atoms, b = number of hydrogens and c= number of oxygens, we get – (1600 [2x31 +23/2 -3])/444.5 = -253.7. This oxygen balance is considered as a low risk. When both the structure together with the oxygen balance, as well as experience in use, are all taken into account, it is considered that a study on the explosive properties of difenacoum is not scientifically justified.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	21st January 2008	
Evaluation of applicant's justification	The applicant's version is acceptable	

Section A3.15 **Annex Point IIA III.3.11**	**Explosive properties**
Conclusion	The applicant's version is acceptable
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:	Consideration of structure and physico-chemical properties does not suggest any oxidising potential. Widespread experimental and commercial use over many years has not shown any signs of oxidising activity. There are no examples of structural alerts in the molecule, for example peroxide, to indicate that difenacoum would act as an oxidiser towards combustible material. On this basis it is believed that it is scientifically unjustified to perform a study to determine oxidising potential.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed 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	21st January 2008	
Evaluation of applicant's justification	The applicant's version is of supportive value. The Rapporteur is of the opinion that in the experimental tests (3.16) only false positive results were demonstrated	
Conclusion	The Rapporteur is of the opinion that there is neither experimental data nor structural data supporting the oxidizing property of difenacoum. Therefore the substance should be considered as non—oxidising.	
Remarks		

Section A3.17 **Annex Point IIA III.3.13**	**Reactivity towards container material**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Consideration of chemical structure and physico-chemical properties show the compound is stable and largely unreactive. Widespread experimental and commercial use over many years, since 1975, has not shown any signs of reaction with container materials. The pure active substance is immediately diluted within manufacturing plant to form the mastermix concentrate. Difenacoum does not come out of the reaction vessel as the technical material.It is never stored in bulk as technical. However, should the pure material need to be stored for sending a sample for phys-chem studies, this could be done in a glass jar, since the reaction vessel used in the process to make difenacoum is glass-lined. Alternatively, looking at the structure, it is highly likely that samples could be stored in HDPE (high density polyethylene) jars. Many organic solids can be stored in HDPE jars.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	21st January 2008	
Evaluation of applicant's justification	The applicant’s version is acceptable	
Conclusion	The applicant’s version is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	

Section A3.17 **Annex Point IIA III.3.13**	**Reactivity towards container material**
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	