Table 4-2: Standard form for justification of the non-submission of data

Section 6.1.4 **Annex Point IIA, VI, 6.1.3**	**Acute Eye Irritation** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [4]	
Detailed justification:	It is not technically possible to determine the eye irritation potential of carbon dioxide. Notwithstanding this, it should be noted that the some of the acute inhalation studies summarised in Section 6.1.3, and some of the repeated dose toxicity studies summarised in Section 6.3 and 6.4 were not 'nose-only' exposure. This means that some continuous exposure to eyes would have occurred during these studies and contributed to the overall effect and end-point. In addition, the potential for exposure to carbon dioxide when it is manufactured for use as an insecticide is minimal [redacted] This means that there is no exposure to workers, bystanders or the environment during manufacture.	
Undertaking of intended data submission [ ]	Not applicable.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(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	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.1.5 **Annex Point IIA, VI, 6.1.5**	**Skin Sensitisation** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	It is not technically possible to determine the skin sensitisation potential of carbon dioxide. Notwithstanding this, it should be noted that the some of the acute inhalation studies summarised in Section 6.1.3, and some of the repeated dose toxicity studies summarised in Section 6.3 and 6.4 were not 'nose-only' exposure. This means that some continuous dermal exposure would have occurred during these studies and contributed to the overall effect and end-point.	
Undertaking of intended data submission [ ]	Not applicable.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(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	

Table 4-2: Standard form for justification of the non-submission of data

Section A6.2 **Annex Point IIA, VI, 6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [4]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [4]	
Detailed justification:	A study to determine how carbon dioxide is metabolised by the body is not considered scientifically necessary for five reasons: 1. Carbon dioxide is constantly produced in the body as a result of the numerous metabolic reactions involving carbon-containing compounds. An adult man, at rest, can be expected to contribute approximately 12 litres of carbon dioxide per hour to his blood stream. If undergoing sustained work, carbon dioxide production can increase to around 100 litres of carbon dioxide per hour. The body has an ability to excrete carbon dioxide in amounts that correspond to over 12,000 mEq of acid per day without causing any toxic effects. 2. The production, transport and excretion of carbon dioxide by the human body has been established for decades, and are well understood. It is reported in many different sources from students' textbooks to scientific papers, and all of these sources are in agreement. *Refer to attached study summary for details about how carbon dioxide is produced and metabolised by the human body.* Continued....	

[redacted]

Section A6.2 Annex Point IIA, VI, 6.2	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study** Section 6: Toxicological and Metabolic Studies	
Detailed justification:	3. The normal working practices of carbon dioxide as an insecticide fumigant are within a sealed enclosure (fumigation bubble) and therefore additional exposure to the gas is not expected. 4. The potential for exposure to carbon dioxide when it is manufactured for use as an insecticide is minimal [redacted] This means that there is no exposure to workers, bystanders or the environment during manufacture. 5. EC method B36 for toxicokinetics studies states that the route of administration should be by the oral, dermal or inhalation route. As carbon dioxide is a gaseous substance, dermal or oral exposure will not be significant routes of exposure so does not need to be considered in the metabolism study. Given the reasons above, it seems unnecessary to conduct a metabolism study on carbon dioxide, given the need to minimise unnecessary vertebrate animal testing whenever possible.	Official use only
Undertaking of intended data submission [ ]	Not applicable.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(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 A6.2
Annex Point IIA, VI, 6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study.

Section 6: Toxicological and Metabolic Studies

Official use only

1. REFERENCE

1.1 Reference [redacted]

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2

1.2.3 Criteria for data protection [redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No.

Not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC.

2.2 GLP No.

GLP was not compulsory at the time study was performed.

2.3 Deviations Yes.

No set guideline followed.

3. MATERIALS AND METHODS

3.1 Test material As given in section 2.

3.1.1. Lot/Batch number Not reported.

3.1.2 Specification [redacted]

[redacted]

Section A6.2
Annex Point IIA, VI, 6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study.
Section 6: Toxicological and Metabolic Studies

3.2	**Test Animals**	
3.2.1	Species	Man.
3.2.2	Strain	Not applicable.
3.2.3	Source	Not applicable.
3.2.4	Sex	Not reported.
3.2.5	Age/weight at study Initiation	Not reported.
3.2.6	Number of animals per group	Not reported.
3.2.7	Control animals	Not reported.
3.3	**Administration/ Exposure**	Inhalation. As carbon dioxide is a gas, dermal exposure is not considered a significant route of exposure.
3.3.1	Preparation of test site	Not reported.
3.3.2	Concentration of test substance	Not reported.
3.3.3	Specific activity of test substance	Not reported.
3.3.4	Volume applied	Not reported.
3.3.5	Size of test site	Not reported.
3.3.6	Exposure period	Not reported.
3.3.7	Sampling time	Not reported.
3.3.8	Samples	Not reported.
		4. RESULTS AND DISCUSSION
4.1	**Toxic effects, clinical signs**	As carbon dioxide is constantly produced in the body as a result of the numerous metabolic reactions involving carbon-containing compounds. An adult male, at rest, can be expected to contribute approximately 12 litres of carbon dioxide per hour to his blood stream. If undergoing sustained work, the normal human body can produce, transport and excrete 90 or 100 litres of carbon dioxide per hour. The body has an ability to excrete carbon dioxide in amounts which correspond to over 12,000 mEq of acid per day, while the pH of the blood remains constant within a few hundredths of a pH unit while the tension of the blood carbon dioxide is stable within a few millimetres of mercury without major dislocations of water or electrolytes. This means that exposure to carbon dioxide from it's use as a biocide is extremely unlikely to lead to toxic effects, given that it's use will not cause any detectable elevation in the level of carbon dioxide in air. A full description of how the body metabolises carbon dioxide is given in section 5.2 (below).
4.2	**Dermal irritation**	As carbon dioxide is a gas, dermal exposure is not considered a significant route of exposure.
4.3	**Recovery of labelled compound**	Not reported.
4.4	**Percutaneous absorption**	Not reported.
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	Not reported.
5.2	**Results and discussion**	Carbon Dioxide is constantly produced in the body as a result of the numerous metabolic reactions involving carbon-containing compounds. An adult male, at rest, can be expected to contribute approximately 12 litres of carbon dioxide per hour to his blood stream. If undergoing sustained work, the normal human body can produce, transport and excrete 90 or 100 litres of carbon dioxide per hour. The body has an ability to excrete carbon dioxide in amounts

5.2 Results and discussion

(Continued)

which correspond to over 12,000 mEq of acid per day, while the pH of the blood remains constant within a few hundredths of a pH unit while the tension of the blood carbon dioxide is stable within a few millimetres of mercury without major dislocations of water or electrolytes. This demonstrates an impressive homeostatic mechanism, and is described in detail below.

Movement of carbon dioxide

The movement carbon dioxide through body fluids is dependent upon a pressure gradient. Gases move from an area of high partial pressure to one of lower partial pressure.

The partial pressures of carbon dioxide in the body tissues, the blood and the alveolar air determine the rapidity with which

- carbon dioxide is transferred across cell and capillary membranes
- the quantity of carbon dioxide held in physical solution, and
- the extent to which the reversible reactions in the transport of carbon dioxide approach completion.

The movement of gases through body fluids is also dependent of the diffusability of the substance, and this is determined by it's solubility, molecular weight and the permeability of the medium. Carbon dioxide is a larger molecule than oxygen, but given it's much higher solubility in body fluids, it can diffuse through the tissues twenty to thirty times more rapidly than oxygen. The higher diffusability of carbon dioxide accounts for the successful removal of carbon dioxide from the tissues with a lower pressure gradient than is necessary to move oxygen from the atmosphere to the tissues.

Forms of carbon dioxide in the blood

Carbon dioxide originates in the tissues by the decarboxylation of organic acid intermediates through the process of respiration. The carbon dioxide produced diffuses freely from it's intracellular site of formation into interstitial fluid and blood. It dissolves into these body fluids, and can be carried in the blood in three principle forms:

Carbon dioxide dissolved in solution.
Bicarbonate ions in red blood cells and blood plasma
Combined with haemoglobin in the red blood cell, in the form of carboaminohaemoglobin.

The blood carries the carbon dioxide in the above forms to the alveoli in the lungs where it is converted back into carbon dioxide before being exhaled to the atmosphere.

The biochemical reactions leading to these different forms of carbon dioxide that are carried in the blood are described below.

a) Carriage of Carbon Dioxide Dissolved in Solution
Carbon dioxide produced by respiring cells diffuses freely from it's site of production into interstitial fluid and blood. A proportion of this carbon dioxide (around 0.5 ml of every 5ml carbon dioxide produced by cells) is carried in the blood as dissolved carbon dioxide.

b) Carriage of Carbon Dioxide as Bicarbonate Ions
Carbon dioxide dissolves in bodily fluids, and a proportion (around 3.5 ml of every 5 ml carbon dioxide produced by cells) then chemically reacts with the water present in the fluids to form carbonic acid thus:

$CO_2 + H_2O \surd H_2CO_3$ $\Delta F = 2.01$ kg.-cal./mol

5.2 Results and discussion

(Continued)

The low ΔF value of this reaction means that, without a catalyst, the reaction is so slow that only an insignificant of carbonic acid is formed from carbon dioxide in extracellular fluid. Within certain cells, particularly the red blood cells, the above reaction is catalysed by a specific zinc-containing enzyme called carbonic anhydrase so that the majority of carbon dioxide is hydrated to form carbonic acid.

Carbonic acid behaves as a weak acid, disassociating to form the bicarbonate ion:

$H_2CO_3 \surd H^+ + HCO_3^-$ $\Delta F = 8.81$ kg.-cal./mol

When a base is present, which is generally the case in body fluids, the following reaction takes place:

$BOH + H_2CO_3 \surd H_2O + B^+ + HCO_3^-$

B represents whatever basic radical or cation is present, usually potassium (K+).

The theoretical *p*K for the isolated dissociation of carbonic acid is 3.6, but under physiological conditions, carbonic acid is always in equilibrium with carbon dioxide. Since the equilibrium of the hydration of carbon dioxide to form carbonic acid strongly favours carbon dioxide and water, the amount of carbonic acid undergoing dissociation is decreased by this competing reaction. Hence, the observed *p*K for the dissociation of carbonic acid is 6.11.

At the pH of blood, pH 7.4, the bicarbonate ion has a concentration twenty times of that of undissociated carbonic acid.

It should be noted that the bicarbonate ion, in addition to acting as the base of carbonic acid, is an acid in it's own right since it can ionise to form the carbonate ion CO_3^{2-}. Since the *p*K of this ionisation is 9.76, it has been assumed to be of no significance.

c) Carriage of Carbon Dioxide combined with Haemoglobin
It has been estimated that around 83% of the total carbon dioxide carried by the blood is transported either directly or indirectly through haemoglobin molecules. Haemoglobin is a protein in red blood cells that helps transport gases such as oxygen and carbon dioxide to and from respiring cells.

Maintenance of pH in the red blood cell
At any given pH, oxyhaemoglobin (haemoglobin when combined with oxygen) will disassociate to a greater extent than haemoglobin, in the reaction:

$HHb + O_2 \surd HbO_2^- + H^+$

This reaction is vitally important in the maintenance of a stable pH in the red blood cell. When the arterial blood permeates over the peripheral tissues, two events occur (termed the I sohydric shift) in order to maintain pH in the red blood cell.

The disassociation of the newly formed carbonic acid from the carbon dioxide which has just entered the red blood cell from the tissues tends to lower pH. But the transformation of oxyhaemoglobin to reduced haemoglobin converts a strong acid into a relatively weak one, tending to raise pH. A consequence of these events means that hydrogen ions formed in the

(Continued.....)

5.2 Results and discussion

(Continued)

disassociation of carbonic acid are accepted by the imidazole groups of the reduced haemoglobin thereby leaving the overall pH of the red blood cell essentially unchanged.

Transport of carbon dioxide through direct combination with haemoglobin
In addition to the dissolved carbon dioxide and bicarbonate which circulate in the blood, carbon dioxide can combine directly with haemoglobin to form carbaminohaemoglobin. About one fifth of the total carbon dioxide in the blood is carried as carbaminohaemoglobin.

$R\text{-}NH_2 + CO_2 \surd R\text{-}NHCOO^- + H^+$

The binding of carbon dioxide to the amino groups of the globin molecule does not require an enzyme catalyst. It is mainly a function of the level oxygen saturation of the haemoglobin, and is largely independent of carbon dioxide tension. In addition, a small amount of carbon dioxide can be carried in the blood in the carboamino form with the plasma proteins, but this form of transport is small when compared to the carbon dioxide being carried as carbaminohaemoglobin in the red blood cells. This is simply because haemoglobin exists in far larger quantities than any other blood protein and it also has a higher content of the amino acid lysine, which provides a free amino group for such binding.

Haemoglobin when combined with oxygen (oxyhaemoglobin) does not form carboaminohaemoglobin with the same facility as haemoglobin does. It has been calculated that a given quantity of oxyhaemoglobin can carry approximately one third of carbon dioxide in the form of carboaminohaemoglobin that haemoglobin can.

Summary of Carbon Dioxide Transport
Arterial blood arrives at the tissue capillaries containing approximately 50 ml carbon dioxide at a carbon dioxide partial pressure of 40 mmHg. This carbon dioxide is distributed in the blood as 3ml in physical solution (exerting a partial pressure of 40 mmHg), 3 ml in carboamino form (given that arterial blood has 95% oxygen saturation) and 44ml bicarbonate.

In the tissues, carbon dioxide is constantly produced as an end-product of oxidative metabolism. The intracellular carbon dioxide partial pressure is estimated to be 46-50 mmHg. Carbon dioxide passes by simple diffusion from the tissues to the blood, adding approximately 5 ml of carbon dioxide to each 100 ml of blood. Venous blood thus leaves the capillary bed with a content of 55 ml carbon dioxide and a carbon dioxide partial pressure of about 46 mmHg. The additional 5 ml of carbon dioxide entering the blood from the tissues will be distributed approximately as follows: 0.5ml dissolved carbon dioxide, 1 ml carboamino form (since the oxygen saturation has dropped from 95% to 70%), and 3.5 ml as bicarbonate.

The largest segment of the carbon dioxide carried in the blood is present as bicarbonate in plasma. There is very little carbonic acid formed in blood plasma, because of the absence of the enzyme carbonic anhydrase which catalyses the reaction to transform carbon dioxide to carbonic acid. This is in contrast to the large amounts of carbonic acid produced in red blood cells, where carbonic anhydrase

5.2 Results and discussion

(Continued)

is present. Carbonic acid in the red blood cell yields bicarbonate with potassium as the principle intracellular cation. Given the increased bicarbonate concentration present in the red blood cell, relative to the blood plasma, some bicarbonate ions move out of the red blood cells into the plasma (with the corresponding movement of chloride ions to preserve electrical neutrality, and maintain osmotic pressure. Some water is redistributed between cells and plasma in order to maintain osmotic pressure).

This entire sequence is reversed in the pulmonary capillaries in order to unload the three forms of carbon dioxide from the blood into the alveoli in the lungs, where it is exhaled to the atmosphere.

5.3 Conclusion

5.3.1 Reliability: 3

5.3.2 Deficiencies: Yes

This study indicates that carbon dioxide is carried in the blood in three principle forms which are dissolved in solution, bicarbonate ions in red blood cells and blood plasma, and combined with haemoglobin in the red blood cell, in the form of carboaminohaemoglobin. The body produces large volumes of carbon dioxide as a result of normal metabolic processes and is able to excrete it while keeping the pH of the blood constant within a few hundredths of a pH unit and the tension of the blood is kept within a few millimetres of mercury without major dislocations of water or electrolytes.

It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols.

Despite the major reporting deficiencies in this study, it explains how carbon dioxide is produced, transported and excreted by the body.

This study, notwithstanding it's deficiencies, can be used to support the metabolism of carbon dioxide because:

1. Carbon dioxide is constantly produced in the body as a result of the numerous metabolic reactions involving carbon-containing compounds. An adult male, at rest, can be expected to contribute approximately 12 litres of carbon dioxide per hour to his blood stream. If undergoing sustained work, carbon dioxide production can increase to around 100 litres of carbon dioxide per hour. The body has an ability to excrete carbon dioxide in amounts which correspond to over 12,000 mEq of acid per day without causing any toxic effects.

2. The production, transport and excretion of carbon dioxide by the human body has been established for decades, and therefore is very well understood. It is reported in many different sources from students textbooks to scientific papers, and all of these sources are in agreement.

3. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges. We can therefore expect it to be metabolised in the same way as the carbon dioxide naturally inhaled into the body as part of ventilation, and that produced by respiring cells.

5.3.2 Deficiencies (continued...)	4. EC method B36 for toxicokinetics studies states that the route of administration should be by the oral, dermal or inhalation route. As carbon dioxide is a gaseous substance, dermal or oral exposure will not be significant route of exposure so does not need to be considered in the metabolism study. Given the reasons above, it seems unnecessary to conduct a metabolism study on carbon dioxide, given the need to minimise unnecessary vertebrate animal testing whenever possible.	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if applicants version is acceptable, or indicate relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers.*
Conclusion	Other conclusions: *(adopt applicant's version or include revised version)*
Reliability	*Based on assessment of materials and methods include appropriate reliability indicator.*
Acceptability	acceptable / not acceptable *(give reasons if necessary e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat if necessary).*
Remarks	
	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	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.3.1 **Annex Point IIA, VI, 6.3**	**Repeated Dose Toxicity (Oral)** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [**4**] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	A 28-day repeated dose toxicity test by the oral route cannot be submitted for carbon dioxide because it is not technically possible to determine the toxicity of a gas by the oral route. It should be noted that the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market : Guidance on Data Requirements for Active Substances and Biocidal Products" states that short term repeated dose toxicity (28 days) is intended as a range-finding test and is not required when an adequate subchronic (90 day) toxicity study is available in a rodent. As the subchronic toxicity of carbon dioxide has been considered in Annex Point IIA6.4 Repeated Dose Toxicity, it is therefore not necessary to meet the requirements of the 28-day repeated dose toxicity study.	
Undertaking of intended data submission [ ]	Not applicable.	

Section 6.3.1 Annex Point IIA, VI, 6.3	**Repeated Dose Toxicity (Oral)** Section 6: Toxicological and Metabolic Studies
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(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	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.3.2 **Annex Point IIA , VI, 6.3**	**Repeated Dose Toxicity (Dermal)** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	A 28-day repeated dose toxicity test by the dermal route cannot be submitted for carbon dioxide because it is not technically possible to determine the toxicity of a gas by the dermal route. It should be noted that the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market : Guidance on Data Requirements for Active Substances and Biocidal Products" states that short term repeated dose toxicity (28 days) is intended as a range-finding test and is not required when an adequate subchronic (90 day) toxicity study is available in a rodent. As the subchronic toxicity of carbon dioxide has been considered in Annex Point IIA6.4 Repeated Dose Toxicity, it is therefore not necessary to meet the requirements of the 28-day repeated dose toxicity study.	
Undertaking of intended data submission [ ]	Not applicable.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(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	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.3.3 **Annex Point IIA ,VI, 6.3**	**Repeated Dose Toxicity (Inhalation)** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data **[4]** **Limited exposure** **[4]**	**Technically not feasible** [ ] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	It is not scientifically necessary to submit a 28-day repeated dose toxicity study for carbon dioxide, by the inhalation route for three reasons. 1. The normal working practices of carbon dioxide as an insecticide fumigant are within a sealed enclosure (fumigation bubble) and therefore additional exposure to the gas is not expected. 2. In addition to the above, the potential for exposure to carbon dioxide when it is manufactured as an insecticide is minimal [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture. 3. The "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market : Guidance on Data Requirements for Active Substances and Biocidal Products" states that short term repeated dose toxicity (28 days) is intended as a ange-finding test and is not required when an adequate subchronic (90 day) toxicity study is available in a rodent. As the subchronic toxicity of carbon dioxide has been considered in Annex Point IIA6.4 Repeated Dose Toxicity, it is therefore not necessary to meet the requirements of the 28-day repeated dose toxicity study.	
Undertaking of intended data submission [ ]	Not applicable.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(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	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.4.1 **Annex Point IIA, VI, 6.4**	**Subchronic Oral Toxicity Test** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	A 90-day subchronic toxicity test by the oral route cannot be submitted because it is not technically possible to determine the toxicity of a gas by the oral route. Notwithstanding the above, the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that the route of administration chosen for the 90-day subchronic toxicity study should be the most significant route of exposure. As carbon dioxide is a gas the most significant route of exposure is by inhalation, and therefore it is not scientifically necessary to determine the repeated dose toxicity of carbon dioxide by the oral route.	
Undertaking of intended data submission [ ]	Not applicable.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(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	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.4.2 **Annex Point IIA, VI, 6.4**	**Subchronic Dermal Toxicity Test** Section 6: Toxicological and Metabolic Studies	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	A 90-day subchronic toxicity test by the dermal route cannot be submitted because it is not technically possible to determine the toxicity of a gas by the dermal route. It should be noted that the "Technical Guidance Document in Support of Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market: Guidance on Data Requirements for Active Substances and Biocidal Products" states that the route of administration chosen for the 90-day subchronic toxicity study should be the most significant route of exposure. As carbon dioxide is a gas the most significant route of exposure is by inhalation, and therefore it is not scientifically necessary to determine the repeated dose toxicity of carbon dioxide by the dermal route.	
Undertaking of intended data submission [ ]	Not applicable.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(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	

Table 4-2: Standard form for justification of the non-submission of data

Section 6.4.3 **Annex IIA, VI, 6.4**	**Subchronic Inhalation Toxicity Test**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [4]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [4]	**Other justification** [ ]	
Detailed justification:	A 90-day subchronic inhalation toxicity test for carbon dioxide is not considered necessary for a number of reasons including: • Carbon dioxide is not a foreign material, but is a fundamental part of the metabolism of organisms. Carbon dioxide is naturally produced by the body, and is effectively regulated by a series of homeostatic mechanisms designed to maximise the carbon dioxide-carrying capacity of the blood. Cells produce carbon dioxide as part of the normal catabolic process. This carbon dioxide diffuses in solution from the cell to the blood plasma and thence to the red cells. Under normal circumstances, in the resting human, the dissolved concentration of carbon dioxide in the blood is between 48 (arterial) and 52 (venous) ml/100 ml blood. Very low levels of carbon dioxide may lead to failure to stimulate inspiration. Vigorous exercise increases the amount of carbon dioxide carried and exhaled (mainly by increased heart rate and respiratory rate), but as the excretion of the gas depends on a diffusion gradient across the alveolar wall, the amount of carbon dioxide already present in the air will govern the efficiency of excretion. Normal alveolar partial pressure of carbon dioxide is approximately 5-6% carbon dioxide. Typically, normal air contains 0.03% carbon dioxide. If extra carbon dioxide is added such that alveolar concentration increases by just 0.2%, the resting pulmonary ventilation is doubled [6]. Continued on next page……..	

[redacted]

Section 6.4.3 Annex IIA, VI, 6.4	Subchronic Inhalation Toxicity Test

Detailed justification:

(continued)

The normal working practices of carbon dioxide as an insecticide fumigant are within a sealed enclosure (fumigation bubble) and therefore additional exposure to the gas is not expected.

[redacted]In addition to the above, the potential for exposure to carbon dioxide when it is manufactured and used as an insecticide is minimal [redacted] This means there is no exposure to workers, bystanders or the environment, during manufacture.

[redacted]

- Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers [2]. Such data have been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement, for example the US OSHA permissible exposure level (PEL) is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm.[3] The long-term workplace exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term workplace exposure limit is 15,000 ppm (15 minutes reference period)[4].

  As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value.

- There is a substantial volume of information available for carbon dioxide, including a number of studies that consider the subchronic toxicity of carbon dioxide. While this data were not generated in accordance with modern scientifically acceptable protocols, it is considered sufficient to permit the necessary calculations regarding safety in use.

  Refer to study summaries for details about the data available on the subchronic inhalation toxicity of carbon dioxide.

Conclusion

On the basis of exposure alone, it is not scientifically necessary to conduct a 90-day subchronic inhalation toxicity test for carbon dioxide.

Existing data on the subchronic toxicity of carbon dioxide are available, including data on man. However, because the occupational exposure standard for safe working conditions is well established, this value can be used for the risk assessment. Given the above, it seems unnecessary to conduct a 90-day subchronic inhalation toxicity test for carbon dioxide in rats given the need to minimise unnecessary vertebrate animal testing whenever possible.

Section 6.4.3 **Annex IIA, VI, 6.4**	**Subchronic Inhalation Toxicity Test**
Undertaking of intended data submission [ ]	Not applicable.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Evaluation of applicant's justification	*Discuss applicant's justification and, if applicable, deviating view*
Conclusion	*Indicate whether applicant's justification is acceptable or not. If unacceptable because of the reasons discussed above, indicate which action will be required, e.g. submission of specific test/study data*
Remarks	
	COMMENTS FROM OTHER MEMBER STATES *(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 A6.4.3 **Subchronic Inhalation Toxicity Test**
Annex IIA, VI, 6.4

1.1 Details

Official use only

WORKPLACE EXPOSURES TO CARBON DIOXIDE

and the choice of the UK short term exposure limit 1.5% carbon dioxide (15 minutes time weighted average) in the risk assessment for use of carbon dioxide in insecticide products (PT 18)

As demonstrated in the enclosed dossier, there is a substantial volume of information available for carbon dioxide. This data has been used by a number of regulatory authorities to set maximum exposure limits for safe working conditions when working with carbon dioxide. All of these exposure limits, set by different regulatory authorities in different countries worldwide are in general agreement. For example:

a) The US OSHA permissible exposure level (PEL) for carbon dioxide is 10,000ppm (1.0%). The short-term exposure limit is 30,000ppm (3.0%). [1]

b) The European occupational exposure limit for carbon dioxide (as set in Exposure Standard - Directive EC/98/24 (1St IOELV Directive) is 5,000ppm (0.5%) (8h time weighted average) while the short term occupational exposure limit for carbon dioxide is 15,000ppm (1.5%) (15 minutes time weighted average). The UK workplace exposure limit is the same as the European standard. [2]

The first three studies cited in Document IIIA, Section 6.4.3 Subchronic Inhalation Toxicity are related to occupational exposure work with humans exposed to an environment with high carbon dioxide concentrations in the workplace. One of these studies cites the occupational exposure limits for carbon dioxide set in the US, and the other two studies use the UK/European occupational exposure limit.

It has been decided to use the established maximum occupational exposure limits for safe working conditions with carbon dioxide, for the risk assessment for its use as an insecticide (PT18). This is because this limit is well established, accepted and used across the many different uses of carbon dioxide in industry. Also, it is acknowledged that none of the numerous studies submitted for the data end point, Section 6.4.3 Subchronic Inhalation Toxicity have a reliability indicator of 1 or 2.

The UK/European occupational exposure limit for carbon dioxide has been used in the risk assessment for carbon dioxide use as an insecticide, over the US limit (see point a and b, above). This is because the European occupational exposure limit for carbon dioxide is lower, therefore giving a more conservative margin of safety to the end user.

The short-term workplace exposure limit of 15,000ppm (1.5%) has been chosen for use in the risk assessment for the following reasons:

In the work that was carried out to monitor operator exposure to carbon dioxide whilst using a fumigation bubble (Ref A6.4.3/30) it was seen that in all activities, except immediately after venting, the CO_2 levels did not exceed 15,000ppm (1.5%).

This is backed up in a second venting of a fumigation bubble (Ref A6.4.3/31) in which it was also seem that CO_2 levels were well below the Workplace Exposure Limits.

(Continued...)

Section A6.4.3
Annex IIA, VI, 6.4

Subchronic Inhalation Toxicity Test

1.1 Details
Continued

Official use only

[redacted]

The CO_2 levels observed during various stages of a fumigation alongside the 0.5% monitoring system in place confirm the suitability of using the short-term workplace exposure limit of 15,000ppm in the risk assessment.

[redacted]

Section A6.4.3
Annex Point IIA, VI, 6.4

Subchronic Inhalation Toxicity Test (1 of 11)

Official use only

1. REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2		
1.2.3	Criteria for data protection	[redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Not carried out to Guideline B.29. in Annex V of Directive 67/548/EEC.
2.2	**GLP**	No. GLP was not compulsory at the time study was performed.
2.3	**Deviations**	Yes. No set guideline followed.

3. MATERIALS AND METHODS

3.1	**Test material**	As given in section 2.
3.1.1.	Lot/Batch number	Not reported.
3.1.2	Specification	[redacted]

[redacted]

Section A6.4.3 Subchronic Inhalation Toxicity Test (1 of 11)

Annex Point IIA, VI, 6.4

3.2	**Test Animals**	
3.2.1	Species	Human.
3.2.2	Strain	Not applicable.
3.2.3	Source	Not applicable.
3.2.4	Sex	Exposed workers were all male, two of the controls were female.
3.2.5	Age/weight at study Initiation	Average age of exposed workers: 38 yr (range 20 to 55 yr) Average age of controls: 50 yr (range 23 to 62 yr)
3.2.6	Number of animals per group	19 exposed workers / 20 controls.
3.2.7	Control animals	Yes.
3.3	**Administration/ Exposure**	Inhalation
3.3.1	Duration of treatment	5 days.
3.3.2	Frequency of exposure	Daily.
3.3.3	Post exposure period	Not reported.
3.3.5	**Inhalation**	
3.3.5.1	Concentrations	Carbon dioxide levels in the workplace varied (see table A6.3-2 at the end of this study summary for raw data), but yielded a time weighted average of 1.08% carbon dioxide. No analytical concentration reported.
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.5.4	Type of exposure	Whole body.
3.3.5.5	Vehicle	Air.
3.3.5.6	Concentration in vehicle	Not applicable – background levels of carbon dioxide in the workplace was monitored.
3.3.5.7	Duration of exposure	8h/day.
3.3.5.8	Controls	20 control subjects were exposed to ambient carbon dioxide levels.* *"Exposed workers" are those who normally work in the cellar area of the brewery, and are expected to be exposed to high levels of carbon dioxide. Control workers are those who normally work in other areas of the brewery away from the cellar.
3.4.	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Not reported.
3.4.1.2	Mortality	No mortalities reported. Timescales for observation of mortality is not reported.
3.4.2	Body weight	Not reported.
3.4.3	Food consumption	Not reported.
3.4.4	Water consumption	Not reported.
3.4.5	Ophthalmoscopic examination	Not reported.

3.4.6	Haematology	Yes. Number of subjects: All. Time points: Day 1 (Monday before work, Monday after work) and Day 5 (Friday after work). Parameters: Blood samples were analysed for oxygen partial pressure, carbon dioxide partial pressure and pH. Standard bicarbonate*, haemoglobin concentration and oxygen saturation were also measured. For raw data, see table A6.3-1 at end of this study summary. *Standard bicarbonate is, by definition, the plasma bicarbonate concentration when the blood is fully saturated with oxygen and carbon dioxide partial pressure is 40 mm Hg, and the analysis is carried out at 37°C. When venous blood is taken (as in this study), the partial pressure of carbon dioxide is higher than 40 mm Hg by a variable amount, and the blood is unsaturated with oxygen to a variable extent (table 1). Correction factors are applied to calculate the plasma bicarbonate under the standard conditions defined above. Note that the "normal" value for standard bicarbonate is given as 24.5 mEw/L at which time base excess is zero.
3.4.7	Clinical Chemistry	Not reported.
3.4.8	Urinalysis	Not reported.
3.5	**Sacrifice and Pathology**	
3.5.1	Organ weights	No. No mortalities in test. No sacrifices made.
3.5.2	Gross and histopathology	No. No mortalities in test. No sacrifices made.
3.5.3	Other examinations	Parameter: Carbon dioxide content in air breathed by cellar workers. Number of subjects: Three (one cellar worker per shift 11pm to 7 am, 7 am to 3 pm, 3 pm to 11 pm). Time points: Continuous.
3.5.4	Statistics	Not reported.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Not reported.
4.1.2	Mortality	No mortalities reported.
4.2	**Body weight gain**	Not reported.
4.3	**Food consumption and compound intake**	Not reported.
4.4	**Ophthalmoscopic examination**	Not reported.

Section A6.4.3 **Subchronic Inhalation Toxicity Test (1 of 11)**
Annex Point IIA, VI, 6.4

4.5	**Blood analysis**	
4.5.1	Haematology	For data, see table A6.3.1 at the end of this study summary. No obvious trends appear on inspection of the mean values of the venous blood analyses except that haemoglobin values for both groups declined progressively. The calculated standard bicarbonate values were consistently higher in the cellar workers than in the controls when bloods drawn at the same time were compared. These differences are not statistically significant. Neither the test group nor the controls showed any change in standard bicarbonate during the work shift on Monday (day 1). Both groups showed significantly lower values on Friday after work (day 5).
4.5.2	Clinical chemistry	Not reported,
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No mortalities in test. No sacrifices made.
4.6.2	Gross and histopathology	No mortalities in test. No sacrifices made.
4.7	**Other**	Air breathed by cellar workers at the brewery were monitored. Eight hour time-weighted averages (TWA) were calculated for the three cellar workers who were monitored continuously during their working hours from the continuous strip chart recordings of carbon dioxide concentration and the log kept by students, who were helping take measurements in the study. The highest acute exposure (carbon dioxide concentration x time) for each work shift was also identified (see table A6.3-2 at the end of this study summary for details).
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC. Levels of carbon dioxide encountered by cellar workers in a medium sized brewery was studied. The authors of this study undertook continuous monitoring of the air breathed by one cellar worker on each shift during an entire 5-day work week (three different workers were monitored each day on the shifts 11pm to 7am, 7am to 3pm and 3pm to 11pm). Venous blood samples were drawn on three occasions from 19 exposed cellar workers, and on 20 non exposed controls working in other parts of the brewery (away from the cellar). The blood samples were drawn before work on Monday, after work on Monday (day one) and after work on Friday (day 5). This was in order to compare carbon dioxide levels after a weekend away from carbon dioxide exposure, with levels after a single day's work and levels after five consecutive days of intermittent carbon dioxide exposure. <u>Blood samples</u> Blood was collected in heparinized Vacutainer tubes and iced immediately. The blood was taken to The John Hopkins School of Hygiene and Public Health for analysis. There was a delay of 1 to 2.5h between sampling and analysis of the blood. Carbon dioxide concentration, oxygen concentration and pH of the blood were

determined in a micro blood gas analyser (BMS 3 Mk2 blood micro system, Radiometer, Copenhagen). Haemoglobin concentration and

(Continued....)

5.1 Materials and Methods

(Continued)

oxygen saturation were determined with an Instrumentation Laboratories CO-oximeter, Model 182. Standard bicarbonate, as determined with the Blood Gas Calculator (Radiometer, Copenhagen) corrects for haemoglobin concentration but not for oxygen unsaturation. Since determinations were made on venous blood, oxygen unsaturation was corrected using the Siggaard-Andersen correction method. The results were also expressed in terms of base excess or deficit.

Monitoring Air breathed by Cellar Workers

Air breathed by cellar workers at the brewery were monitored by continuous monitoring the air breathed by one cellar worker on each shift during an entire 5-day work week (three different workers were monitored each day on the shifts 11pm to 7am, 7am to 3pm and 3pm to 11pm). Carbon dioxide content of the air was measured with an infra-red analyser (a Wilks-Miran High-Level Carbon Dioxide Monitor), which measures and records carbon dioxide concentration continuously. A record was also kept of the worker's activities by two students who conducting the measurements. The infra-red carbon dioxide analyser was calibrated twice during each work shift and readings were referred to a carefully drawn calibration curve. When possible, the sampling tube of the infra-red carbon dioxide analyser was clipped to the worker at shoulder height. When the worker was in and out of the sampling area so quickly that this was not possible, the student held the sampling tube at shoulder height, but not in the path of the worker's expired air. When the worker went to other floors of the brew house, one of the students went along and took "grab samples" with a large syringe which were brought back for analysis in the carbon dioxide monitor. Note that the infra-red carbon dioxide analyser failed to operate during the first shift of the study (11 pm Sunday to 7 am Monday), and the analyser that was used instead was too unstable to give usable data. Repairs were made, and good data obtained for all other shifts during the five day study duration.

5.2 Results and discussion

Comments about experimental design

It should be noted that as this study was conducted in a workplace, rather than a laboratory, certain allowances have to be made regarding choice of subjects monitored and measurements taken (particularly as the workers participation was entirely voluntary). The authors of this study make the following points:

1. Test and control groups were not perfectly matched to age and sex Since standard bicarbonate is not significantly affected by age in a healthy working population, this failure of matching is not considered important. (Note that spot samples from the places where control subjects worked demonstrates that they satisfy the essential condition for the purposes of this study i.e. non-exposure to carbon dioxide).

2. The delay of 1 to 2.5h between sampling and analysis of the blood samples was greater than the authors expected, and may have caused slight changes in some of the blood samples.

Section A6.4.3 Subchronic Inhalation Toxicity Test (1 of 11)

Annex Point IIA, VI, 6.4

5.2 Results and discussion

(continued)

Choice of HCO_3^- as an index of carbon dioxide exposure.

During continuous exposure to carbon dioxide, there is an immediate increase in the partial pressure of carbon dioxide of the blood, followed by a slow adaptation leading to an increase in plasma bicarbonate. The pH, which depends upon the HCO_3^-/P_{CO2} ratio decreases at first and then slowly returns towards normal during continued exposure to carbon dioxide. The slowness of the HCO_3^- adjustment to changes in carbon dioxide partial pressure, coupled in the steady-state with the approximate proportionality between changes in HCO_3^- and carbon dioxide partial pressure makes HCO_3^- attractive as an index of carbon dioxide exposure. In order to deal with a situation in which carbon dioxide exposure is intermittent and variable, as in the workplace, standard bicarbonate was used as an indicator which averages out the physiological effects of the elevated carbon dioxide of the workplace and the absence of carbon dioxide in the non-work environment. Standard HCO_3^- is a personal monitor operating on a 24-hr-a-day basis, yielding a time-weighted average of the body's adjustment to carbon dioxide.

Difficulty of monitoring exposures in workplace

The authors of this study take time to report the difficulties in monitoring the air breathed by workers in this study. The cellar workers were few in number and seldom stayed in one place more than a few minutes at a time. The cellar worker may be in an area of relatively high carbon dioxide for a few minutes, but between bouts of activity will relax in a nearby lounge, where the carbon dioxide level is at normal background levels. The time weighted averages and maximum acute exposures provide a reasonable picture of carbon dioxide exposures of the three cellar workers monitored, but this time weighted average was inordinately difficult to establish under the working conditions of the brewery and was irrelevant with respect to acutely hazardous exposures.

Discussion of results

Results show a 0.6 mEq/L difference in standard bicarbonate between test and control groups. (See table A6.3.1 at the end of this study summary for details). This reflects occupational exposure to carbon dioxide, although it is statistically insignificant. The authors of this study therefore conclude that intermittent industrial exposure to carbon dioxide at a time weighted average concentration of about 1.0% causes no significant alteration in blood acid base balance.

The lack of change in standard bicarbonate between before work on day 1 (Monday) and after work on day 1, was expected given the long half-time for HCO_3^- adjustments. However, the standard bicarbonate level was lower on day 5 (Friday) for both the test and control groups, along with a progressive lowering of blood haemoglobin. . (See table A6.3.1 at the end of this study summary for details). It is believed that blood haemoglobin levels were actually unchanged, but was diluted in a larger total volume of blood. HCO_3^- was also diluted but in a larger volume of distribution since HCO_3^- is not confined to the vascular bed. The source of the diluent, which increased blood volume and total body water, is believed to have been beer or other fluids which the workers ingested in large amounts during working hours. The more

	rapid drop in haemoglobin than HCO_3^- during working hours on day 1 (Monday) may have been related to the different volumes of (Continued……)

5.2	**Results and discussion** (continued)	distribution or to factors effecting HCO_3^- selectively. Note that the decline in haemoglobin and bicarbonate between Monday before work and Friday after work was roughly proportional – in keeping with the dilution hypothesis. When the values for oxygen saturation and oxygen partial pressure are plotted on a standard dissociation curve, the points almost invariably fall to the left on the expected position at the measured value of pH. Despite a number of hypothesis' being investigated (including the effect of smoking), this finding remains unexplained.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not reported.
5.3.2	NO(A)EL	Intermittent industrial exposure to carbon dioxide at a time weighted average concentration of about 1.0% causes no significant alteration in blood acid base balance.
5.3.3	Other	Not reported.
5.3.4	Reliability	3
5.3.5	Deficiencies	Yes It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of intermittent exposures to carbon dioxide under normal working conditions in a medium sized brewery. The time weighted average exposure to carbon dioxide in the brewery, for one working week (5 days) was approximately 1.0%. While this study was not generated to modern, scientifically accepted protocols, nor was it a full 90 day investigation, it does provide useful data on some of the parameters measured in a subchronic study. Despite the deficiencies in this study, it does gives an indication about carbon dioxide normally encountered in a typical workplace (where high levels of carbon dioxide are expected). The authors of this study concluded that intermittent industrial exposure to carbon dioxide at a time weighted average concentration of about 1.0% causes no significant alteration in blood acid base balance. This study, notwithstanding it's deficiencies, can be used to support the inhalation toxicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges. 2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal, and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum

exposure limits for safe working conditions.

(Continued....)

Section A6.4.3
Annex Point IIA, VI, 6.4

Subchronic Inhalation Toxicity Test (1 of 11)

5.3.5 Deficiencies

(Continued)

3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary.

4. There is sufficient data available concerning the subchronic toxicity of carbon dioxide in various species (including rats, man and mammals). However, because the occupational exposure standard for safe working conditions with carbon dioxide is well established, this value can be used for the risk assessment*.

*The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period).

Section A6.4.3 **Subchronic Inhalation Toxicity Test (1 of 11)**

Annex Point IIA, VI, 6.4

Table A6 3-1 Blood Studies

		Raw data					Calculated values	
No. of subjects		PH	P_{CO2} * mm Hg	P_{O2} ** mm Hg	S_{O2} $ %	Hb # gm %	Standard HCO_3^- [] MEq/L	B.E. ~ MEq/L
Monday before work	19 test	7.34 (0.026)	54 (6.1)	26 (6.6)	53 (13.6)	16.6 (0.92)	24.0 (0.88)	-0.7 (1.14)
	20 control	7.34 (0.040)	53 (8.2)	26 (5.3)	53 (12.0)	16.3 (1.14)	23.4 (1.29)	-1.4 (1.64)
Monday after work	19 test	7.35 (0.036)	51 (6.7)	26 (8.3)	52 (12.9)	15.7 (0.84)	23.8 (1.10)	-0.9 (1.37)
	17 control	7.36 (0.023)	48 (4.7)	25 (6.5)	50 (13.2)	15.8 (1.04)	23.3 (1.14)	-1.6 (1.43)
Friday after work	17 test	7.33 (0.089)	47 (4.5)	27 (9.5)	56 (14.1)	15.4 (0.93)	22.7 (1.04)	-2.3 (1.27)
	18 control	7.34 (0.028)	48 (4.7)	24 (5.3)	51 (11.5)	15.6 (0.88)	22.0 (1.10)	-3.2 (1.37)

Key:
NOTE: Mean values and standard deviations (in brackets) of venous blood
*P_{CO2} : Partial pressure of carbon dioxide.
**P_{O2} : Partial pressure of oxygen
$ S_{O2} : Oxygen saturation
# Hb : Haemoglobin concentration
[] Standard HCO_3^- : Standard bicarbonate of plasma corrected for the effect of oxygen unsaturation
~ B.E. : Base excess corrected for oxygen unsaturation

Table A6 3-2
Carbon dioxide Exposures (% CO2, 8 h Time Weighted Average TWA) and Maximum Acute Exposures (% CO2 x Time in Minutes)*

*Measurements taken on one cellar worker during each work shift

Shift	11 pm to 7 am		7 am to 3 pm		3 pm to 11 pm	
	TWA	Max. acute	TWA	Max. acute	TWA	Max. acute
Monday	---*	---*	0.95	(1.3% x 240 min)	0.50	(1.7% x 5 min)
Tuesday	0.94	(8% x 2 min)	0.95	(2.7% x 8 min)	1.11	(3.4% x 15 min)
Wednesday	0.78	(8% x 3 min)	0.80	(1.8% x 20 min)	1.36	(6.5% x 6 min)
Thursday	1.29	(4% x 17 min)	1.95	(4.5% x 18 min)	1.41	(3% x 48 min)
Friday	1.11	(1.8% x 25 min)	1.38	(2.6% x 9 min)	0.57	(3% x 7 min)
Mean TWA	1.08 % carbon dioxide					

*As explained in section 5.1 Materials and Methods, the infra-red carbon dioxide analyser failed to operate during the first shift of the study (11 pm Sunday to 7 am Monday), and the analyser that was used instead was too unstable to give usable data.

Section A6.4.3 **Subchronic Inhalation Toxicity Test (1 of 11)**
Annex Point IIA, VI, 6.4

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if applicants version is acceptable, or indicate relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers.*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(adopt applicant's version or include revised version)*
Reliability	*Based on assessment of materials and methods include appropriate reliability indicator.*
Acceptability	Acceptable / not acceptable *(give reasons if necessary e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat if necessary).*
Remarks	
	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 A6.4.3
Annex Point IIA, VI, 6.4

Subchronic Inhalation Toxicity Test (2 of 11)

Official use only

1. REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2		
1.2.3	Criteria for data protection	[redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Not carried out to Guideline B.29. in Annex V of Directive 67/548/EEC.
2.2	**GLP**	No. GLP was not compulsory at the time study was performed.
2.3	**Deviations**	Yes. No set guideline followed.

3. MATERIALS AND METHODS

3.1	**Test material**	As given in section 2.
3.1.1.	Lot/Batch number	Not reported.
3.1.2	Specification	[redacted]

[redacted]

Section A6.4.3 Subchronic Inhalation Toxicity Test (2 of 11)

Annex Point IIA, VI, 6.4

3.2	**Test Animals**	
3.2.1	Species	Human.
3.2.2	Strain	Not applicable.
3.2.3	Source	Not applicable.
3.2.4	Sex	Not reported.
3.2.5	Age/weight at study Initiation	Not reported.
3.2.6	Number of animals per group	4.
3.2.7	Control animals	Yes.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of treatment	7 x 15 minutes exposures (see 3.3.2 and 3.3.5.7 for details).
3.3.2	Frequency of exposure	Seven times. One repetition with the following: No surgical helmet Each of the four surgical helmets available to the medical practice Two NIOSH-approved powered air-purifying respirators with high efficiency particulate air (HEPA) filters. (NIOSH is the US National Institute for Occupational Safety and Health).
3.3.3	Post exposure period	Not reported.
3.3.5	**Inhalation**	
3.3.5.1	Concentrations	Less than the recommended US National Institute for Occupational Safety and Health (NIOSH) short term exposure limit recommended for carbon dioxide: 30,000 ppm.
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.5.4	Type of exposure	Head only.
3.3.5.5	Vehicle	Air.
3.3.5.6	Concentration in vehicle	Not applicable – background levels of carbon dioxide in air , inside the surgical helmet, was monitored.
3.3.5.7	Duration of exposure	The test was halted after 15 minutes unless: a. Carbon dioxide concentration in the surgical helmet reached the US National Institute for Occupational Safety and Health (NIOSH) short term exposure limit recommended for carbon dioxide: 30,000 ppm. b. Carbon dioxide concentration reached a steady state for 5 minutes.
3.3.5.8	Controls	Test protocol was repeated on same test subjects but they did not wear the surgical helmet. The “no helmet” test was the first test performed by the test subject.
3.4.	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Not reported.
3.4.1.2	Mortality	No mortalities reported. Timescales for observation of mortality is not reported.
3.4.2	Body weight	Not reported.
3.4.3	Food consumption	Not reported.
3.4.4	Water consumption	Not reported.

Section A6.4.3 **Subchronic Inhalation Toxicity Test (2 of 11)**
Annex Point IIA, VI, 6.4

3.4.5	Ophthalmoscopic examination	Not reported.
3.4.6	Haematology	No. (See note below). The authors of this study planned to measure the heart rate and blood oxygen saturation with a pulse oximeter. However, the difficulties encountered in the use of the pulse oximeter on a hand in motion in the beginning of this study precludes the report of the measurements with it.
3.4.7	Clinical Chemistry	Not reported.
3.4.8	Urinalysis	Not reported.
3.5	**Sacrifice and Pathology**	
3.5.1	Organ weights	No. No mortalities in test. No sacrifices made.
3.5.2	Gross and histopathology	No. No mortalities in test. No sacrifices made.
3.5.3	Other examinations	Yes. Parameter: Body temperature (determined by taking oral temperature). Time period: Beginning and end of test.
3.5.4	Statistics	Not reported.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Not reported.
4.1.2	Mortality	No mortalities reported.
4.2	**Body weight gain**	Not reported.
4.3	**Food consumption and compound intake**	Not reported.
4.4	**Ophthalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	Not reported (see 3.4.6 for details).
4.5.2	Clinical chemistry	Not reported,
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No mortalities in test. No sacrifices made.
4.6.2	Gross and histopathology	No mortalities in test. No sacrifices made.

4.7	Other	Raw data was not given in the study report that was available to the applicant. However results are discussed as follows: The mean difference between pre- and post-test oral temperatures was 0.2°C. In 12 of the 56 instances, the oral temperatures dropped during the trial. The maximum decrease was 0.9°C. In three cases, oral temperature rose more than 1°C. In one case, this increase was 2°C (from 35°C to 37°C). However, the oral temperature did not exceed 38°C in any of the trials. Therefore no heat strain was noted as a result of using the surgical helmets or powered air-purifying respirators (PAPRs).
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	Materials and Methods	This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC. Potential carbon dioxide exposure among surgeons, nurses and other operating room personnel were evaluated, when they perform or assist with surgical procedures while wearing surgical isolation suits. Surgical isolation suits are worn particularly for orthopedic surgery to prevent the infection of patients by operating personnel, and also to prevent the potential transmission of blood borne pathogens from infected patients to healthcare providers. The authors of this study measured carbon dioxide concentrations inside the helmet of the surgical isolation suit (the surgical helmet), during an experimental exercise routine. The exercise routine was designed to simulate the effort required during orthopedic surgery. Note that a simulation was performed because of surgeons concerns that testing the helmet atmosphere during surgical procedures might prolong the operation, thus increasing the patient's risk of infection. The surgical helmet typically consists of a helmet frame with disposable cover, a window that may or may not be an integral part of the cover, air filters for inhaled and exhaled air, and one or more fans. Surgical helmets resemble powered air-purifying respirators (PAPRs). To provide a comparison for the surgical helmet, two NIOSH-approved PAPRs were used. (NIOSH is the US National Institute for Occupational Safety and Health). <u>Exercise protocol</u> A brief questionnaire was administered to the all study participants to assess the potential for risk to their health from participating in the exercise protocol. If there was a risk, the person was not allowed to participate in the study. Test subjects were asked to perform light exercise (< 4 kcal/min) approximating the effort of a total joint replacement while standing at an upper extremity ergometer. The ergometer was set at a work load of 20 W, and the subjects were asked to maintain an exercise rate of 60 revolutions per minute (60 rpm) on the ergometer's hand cranks. This exercise level was intended to be no more demanding than the work of orthopedic surgery which may involve the use of hand or power tools (e.g. hammers and chisels) during operations such as hip or knee joint replacement. (Continued....)

Section A6.4.3 **Subchronic Inhalation Toxicity Test (2 of 11)**
Annex Point IIA, VI, 6.4

5.1 Materials and Methods

(Continued)

Test subjects wore typical surgical clothing (i.e. a surgical gown) with:
1. No helmet
2. Each of the four surgical helmets available to the medical practice
3. Two NIOSH-approved powered air-purifying respirators (PAPR) with high efficiency particulate air (HEPA) filters.

The test was halted after 15 minutes unless:
1. Carbon dioxide concentration in the surgical helmet reached the US National Institute for Occupational Safety and Health (NIOSH) short term exposure limit recommended for carbon dioxide: 30,000 ppm.
2. Carbon dioxide concentration reached a steady state for 5 minutes.

Subjects rested between exercise periods until their heart rate, blood oxygen saturation and oral temperature returned to base-line values or for as long as the duration of the exercise period which preceded the rest period (whichever was the longer).

Biological monitoring
Heart rate and blood oxygen saturation were measured with a pulse oximeter. This gauged the effect of the different surgical helmets on the cardiovascular system at equal levels of physical exertion. The probe was attached to the test subject's left index finger. Because a subject's arm motion affected the instrument's performance, subjects held their left hand up every two minutes while continuing to exercise with their right arm, and kept it up until a stable reading was attained. Note that the difficulties encountered in the use of the pulse oximeter on a hand in motion in the beginning of this study precludes the report of the measurements with it.

Body temperature was measured before and after each trial, with an oral thermometer. Measuring oral temperature indicated whether wearing a surgical helmet or powered air-purifying respirators (PAPR) placed an individual at increased risk of heat stress compared with the exercise protocol alone.

Environmental monitoring
Carbon dioxide measurements were made using a Gastech model R1-411A portable infrared (IR) indicator. This instrument is battery-powered, weighs approximately 2.6 kg and is 23 cm wide, 19 cm high and 11cm deep. It is a chopped, single-beam non-dispersive IR analyser which monitors absorbance of carbon dioxide in a selective (unspecified) narrow frequency range. An internal pump continuously draws sample air through the detection chamber where absorbance is measured, compared with a background signal, and converted to an output signal used to show carbon dioxide concentration on an LCD display. The output signal can also be sent to an analog data collection device for storage. Normal instrument range is 0 to 4975 ppm carbon dioxide in air, with the instrument reading in 25-ppm increments.

For the purposes of this study, the sample air stream was diluted by drawing the total sample from two air streams (one stream was the sample from the surgical helmet, and the other was ambient air). Two Hasting mode CPR-4SA mass flow controllers were used to adjust the flow of the two air streams. Note that the ambient air sample was drawn through a scrubber containing Ascarite II to remove the carbon

(Continued.....)

Section A6.4.3 Subchronic Inhalation Toxicity Test (2 of 11)

Annex Point IIA, VI, 6.4

5.1	**Materials and Methods** (Continued)	dioxide. Scrubbed ambient air was then mixed with the air coming from the surgical helmet system, before being drawn into the carbon dioxide indicator. Dilution of the air in the surgical helmet in a 1:9 ratio with scrubbed ambient air enabled the analytical range of the assembed instrumentation package to be extended from 4950 to 49500 ppm, measured in 250 ppm increments. No measurements or calibrations were made above 20,000 ppm. A multi-point calibration of the assembled analytical instrumentation was conducted in the laboratory and validated on site using standards prepared by injecting known volumes of pure carbon dioxide into known volumes of room air using 50 ml and 1 litre syringes. A zero setting was accomplished by adding a second carbon dioxide scrubber to the air stream from the surgical helmet, so that carbon dioxide was eliminated from both ambient air, and the air from the surgical helmet. Combined data from on site, pre-calibration and post-calibration produced a correlation coefficient of 0.971 for 110 data points. The carbon dioxide concentration in room air was monitored continuously during all testing using a second, non-diluted carbon dioxide analyser. This second monitor was calibrated using a commercial scan gas. Continuous voltage output (corresponding to carbon dioxide concentration) from both analysers was downloaded onto a computer. The carbon dioxide concentration in the surgical helmet was recorded every two minutes during the exercise trials, and the room carbon dioxide concentration was recorded before and after each trial.
5.2	**Results and discussion**	Raw data was not given in the study report that was available to the applicant. However results are discussed as follows: The surgical helmets tested were selected from the operating room inventory or provided by sales representatives. Mean carbon dioxide concentrations measured ranged from 5,500 to 11,700 ppm. These results indicate that if the surgical helmet or powered air-purifying respirators (PAPRs) are used during operations lasting 8h or more, the users will be exposed to carbon dioxide levels exceeding the 8h Time weighted exposure limits set in the US (US OSHA's permissible exposure level is 10,000 ppm). For the highest mean carbon dioxide levels measured (11,700 ppm), a user would be overexposed if an operating procedure lasted for 3.5h or longer. Three of the four test subjects reported headaches following several hours of trials. The concentration of carbon dioxide in room air ranged from 275 ppm to 575 ppm with a mean of 450 ppm. The mean difference between pre- and post-test oral temperatures was 0.2°C. In 12 of the 56 instances, the oral temperatures dropped during the trial. The maximum decrease was 0.9°C. In three cases, oral temperature rose more than 1°C. In one case, this increase was 2°C (from 35°C to 37°C). However, the oral temperature did not exceed 38°C in any of the trials. Therefore no heat strain was noted as a result of using the surgical helmets or powered air-purifying respirators (PAPRs).

(Continued...)

Section A6.4.3
Annex Point IIA, VI, 6.4

Subchronic Inhalation Toxicity Test (2 of 11)

5.3	**Results and discussion** (continued)	The difficulties encountered in the use of the pulse oximeter on a hand in motion in the beginning of this study precludes the report of the measurements with it. In conclusion, none of the carbon dioxide concentrations measured during any of the 15 minute tests exceeded the short term exposure limit recommended in the US (30,000 ppm). The results of this study indicate that wearing a surgical helmet or powered air-purifying respirator during orthopedic surgical procedures may result in a user being over-exposed to carbon dioxide, depending on the duration of the surgery. While the carbon dioxide concentrations noted in this study have not been associated previously with adverse health effects, they may explain the symptoms reported by employees at the medical centre.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not reported.
5.3.2	NO(A)EL	None of the carbon dioxide concentrations measured during any of the 15 minute tests exceeded the short term exposure limit recommended in the US (30,000 ppm).
5.3.3	Other	Not reported.
5.3.4	Reliability	3
5.3.5	Deficiencies	Yes It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of short term exposures to carbon dioxide under simulated normal working conditions in an operating theatre. None of the carbon dioxide concentrations measured during any of the 15 minute tests exceeded the short term exposure limit recommended in the US (30,000 ppm). Despite the deficiencies in this study, particularly the lack of raw data, it does gives an indication about carbon dioxide normally encountered in a typical workplace (where high levels of carbon dioxide are expected). The authors of this study concluded that intermittent industrial exposure to carbon dioxide at a time weighted average concentration of less than 30,000 ppm has not been associated with adverse health effects. This study, notwithstanding it's deficiencies, can be used to support the inhalation toxicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges. 2. The potential for exposure to carbon dioxide when it is

manufactured and used as a rodenticide is minimal, and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions.

(Continued....)

Section A6.4.3 Subchronic Inhalation Toxicity Test (2 of 11)

Annex Point IIA, VI, 6.4

5.3.5 Deficiencies

(Continued)

3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary.

4. There is sufficient data available concerning the subchronic toxicity of carbon dioxide in various species (including rats, man and mammals). However, because the occupational exposure standard for safe working conditions with carbon dioxide is well established, this value can be used for the risk assessment*.

   *The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period).

Section A6.4.3 **Subchronic Inhalation Toxicity Test (2 of 11)**
Annex Point IIA, VI, 6.4

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if applicants version is acceptable, or indicate relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers.*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(adopt applicant's version or include revised version)*
Reliability	*Based on assessment of materials and methods include appropriate reliability indicator.*
Acceptability	Acceptable / not acceptable *(give reasons if necessary e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat if necessary).*
Remarks	
	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 A6.4.3
Annex Point IIA, VI, 6.4

Subchronic Inhalation Toxicity Test (3 of 11)

Official use only

1. REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2		
1.2.3	Criteria for data protection	[redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Not carried out to Guideline B.29. in Annex V of Directive 67/548/EEC.
2.2	**GLP**	No. GLP was not compulsory at the time study was performed.
2.3	**Deviations**	Yes. No set guideline followed.

3. MATERIALS AND METHODS

3.1	**Test material**	As given in section 2.
3.1.1.	Lot/Batch number	Not reported.
3.1.2	Specification	[redacted]

[redacted]

Section A6.4.3 Subchronic Inhalation Toxicity Test (3 of 11)

Annex Point IIA, VI, 6.4

3.2	**Test Animals**	
3.2.1	Species	Human.
3.2.2	Strain	Not applicable.
3.2.3	Source	Not applicable.
3.2.4	Sex	Not reported.
3.2.5	Age/weight at study Initiation	Not reported.
3.2.6	Number of animals per group	Not reported.
3.2.7	Control animals	Not reported.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of treatment	Carbon dioxide levels were monitored in a room for one day. (10.15 am to 12.30pm and 2.15 pm to 4.30pm).
3.3.2	Frequency of exposure	Continuous.
3.3.3	Post exposure period	Not reported.
3.3.5	**Inhalation**	
3.3.5.1	Concentrations	Background carbon dioxide levels in the treatment room was found to be 1,000 ppm. Background carbon dioxide levels in the patient waiting area were found to be between 980 and 1250 ppm. Carbon dioxide levels in the fresh air would be expected to be about 300 ppm. With the window open, and the exhaust tube vented out, the typical reading in the treatment room was 1500 ppm carbon dioxide. With the window closed and the carbon dioxide released into the treatment room, the highest reading recorded was 4,000 ppm.
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.5.4	Type of exposure	Whole body.
3.3.5.5	Vehicle	Air.
3.3.5.6	Concentration in vehicle	Not applicable – background levels of carbon dioxide in air were monitored.
3.3.5.7	Duration of exposure	Carbon dioxide levels were monitored in a room for one day. Measurements were taken every 15 minutes. (Morning session: 10.15 am to 12.30pm, afternoon session: 2.15 pm to 4.30pm).
3.3.5.8	Controls	Not reported.
3.4.	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Not reported.
3.4.1.2	Mortality	No mortalities reported. Timescales for observation of mortality is not reported.
3.4.2	Body weight	Not reported.
3.4.3	Food consumption	Not reported.
3.4.4	Water consumption	Not reported.
3.4.5	Ophthalmoscopic examination	Not reported.
3.4.6	Haematology	Not reported.

Section A6.4.3 **Subchronic Inhalation Toxicity Test (3 of 11)**
Annex Point IIA, VI, 6.4

3.4.7	Clinical Chemistry	Not reported.
3.4.8	Urinalysis	Not reported.
3.5	**Sacrifice and Pathology**	
3.5.1	Organ weights	No. No mortalities in test. No sacrifices made.
3.5.2	Gross and histopathology	No. No mortalities in test. No sacrifices made.
3.5.3	Other examinations	Not reported.
3.5.4	Statistics	Not reported.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Not reported.
4.1.2	Mortality	No mortalities reported.
4.2	**Body weight gain**	Not reported.
4.3	**Food consumption and compound intake**	Not reported.
4.4	**Ophthalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	Not reported.
4.5.2	Clinical chemistry	Not reported.
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No mortalities in test. No sacrifices made.
4.6.2	Gross and histopathology	No mortalities in test. No sacrifices made.
4.7	**Other**	Not reported.
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC. Cryotherapy is regularly used in clinics to treat genital warts. Nitrous oxide was used as the cryogenic gas. Following a health and safety review, it was found that high levels of nitrous oxide were being detected in the working areas of the clinic, and venting of this gas to adjacent areas could present a hazard. Carbon dioxide was proposed as an alternative cryogenic gas to nitrous oxide. The study cited here investigated the acceptability of carbon dioxide as an alternative to (continued....)

5.1	**Materials and Methods**	nitrous oxide, the cost impact of any changes and monitored levels of carbon dioxide under different conditions of room ventilation.
	(Continued)	When monitoring background levels of carbon dioxide, a Gastec precision gas detector system comprising of a syringe pump and Gastec carbon dioxide tube 2LL (range 100-11,500 ppm and 300-5000 ppm were used to take grab samples every 15 minutes (Morning session: 10.15 am to 12.30pm, afternoon session: 2.15 pm to 4.30pm).
5.2	**Results and discussion**	Background carbon dioxide levels in the treatment room was found to be 1,000 ppm. Background carbon dioxide levels in the patient waiting area were found to be between 980 and 1250 ppm. Carbon dioxide levels in the fresh air would be expected to be about 300 ppm. With the window open, and the exhaust tube vented out, the typical reading in the treatment room was 1500 ppm carbon dioxide. With the window closed and the carbon dioxide released into the treatment room, the highest reading recorded was 4,000 ppm. The occupational exposure standard for carbon dioxide, in the UK is 5,000 ppm (8h time weighted average) with a short term exposure limit at 15,000 ppm (15 minute reference period). All the readings obtained, with the exhaust vented out as well as the gas released into the room were well below 5,000 ppm carbon dioxide. Carbon dioxide could therefore be used safely as a cryogenic gas with the windows closed, if necessary.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not reported.
5.3.2	NO(A)EL	Carbon dioxide concentrations measured were well below the UK occupational exposure standard for carbon dioxide (5,000 ppm, 8h time weighted average) and therefore carbon dioxide can be used safely as a cryogenic gas under the conditions described.
5.3.3	Other	Not reported.
5.3.4	Reliability	3
5.3.5	Deficiencies	Yes It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of long term exposures to carbon dioxide under normal working conditions in a medical clinic. None of the carbon dioxide concentrations measured exceeded the UK occupational exposure standard for carbon dioxide (5,000 ppm, 8h time weighted average). Despite the deficiencies in this study, it does gives an indication about carbon dioxide normally encountered in a typical workplace (where high levels of carbon dioxide are expected). The authors of this study concluded that long term, 8h, exposure to carbon dioxide at a time weighted average concentration of less than 5,000 ppm is acceptable.

(Continued....)

Section A6.4.3 Subchronic Inhalation Toxicity Test (3 of 11)

Annex Point IIA, VI, 6.4

5.3.5	Deficiencies (Continued)	This study, notwithstanding it's deficiencies, can be used to support the inhalation toxicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges. 2. The potential for exposure to carbon dioxide when it is 2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal, and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions. 3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary. 4. There is sufficient data available concerning the subchronic toxicity of carbon dioxide in various species (including rats, man and mammals). However, because the occupational exposure standard for safe working conditions with carbon dioxide is well established, this value can be used for the risk assessment*. *The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period).

Section A6.4.3 Subchronic Inhalation Toxicity Test (3 of 11)

Annex Point IIA, VI, 6.4

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if applicants version is acceptable, or indicate relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers.*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(adopt applicant's version or include revised version)*
Reliability	*Based on assessment of materials and methods include appropriate reliability indicator.*
Acceptability	Acceptable / not acceptable *(give reasons if necessary e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat if necessary).*
Remarks	
	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 A6.4.3 Subchronic Inhalation Toxicity Test (4 of 11)

Annex Point IIA, VI, 6.4

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1. REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2		
1.2.3	Criteria for data protection	[redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC.
2.2	**GLP**	No. GLP was not compulsory at the time study was performed.
2.3	**Deviations**	Yes. No set guideline followed.

3. MATERIALS AND METHODS

3.1	**Test material**	As given in section 2.
3.1.1.	Lot/Batch number	Not reported.
3.1.2	Specification	[redacted]

[redacted]

Section A6.4.3 Subchronic Inhalation Toxicity Test (4 of 11)

Annex Point IIA, VI, 6.4

3.2	**Test Animals**	
3.2.1	Species	Human
3.2.2	Strain	Not applicable.
3.2.3	Source	Not applicable.
3.2.4	Sex	Male.
3.2.5	Age/weight at study Initiation	Not reported.
3.2.6	Number of animals per group	23.
3.2.7	Control animals	23.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of treatment	Other: 42 days.
3.3.2	Frequency of exposure	Other: Continuous.
3.3.3	Post exposure period	Other: 9 day control period on atmospheric air under identical test conditions immediately after the carbon dioxide exposure period, in addition to a 35 day post test period, where further laboratory investigations were carried out.
3.3.5	**Inhalation**	
3.3.5.1	Concentrations	Nominal concentration 1.5% carbon dioxide. (Maintained between 1.4-1.6% CO_2 in air). No analytical concentration reported.
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.5.4	Type of exposure	Whole body.
3.3.5.5	Vehicle	Gas.
3.3.5.6	Concentration in vehicle	Gas mixture contains 1.5% carbon dioxide and 20.5% oxygen.
3.3.5.7	Duration of exposure	42 days, continual exposure.
3.3.5.8	Controls	Test subjects breathed atmospheric carbon dioxide under identical test conditions for nine days immediately before and after exposure to 1.5% carbon dioxide for 42 days.
3.4.	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	None reported.
3.4.1.2	Mortality	No mortalities reported. Subjects were observed during all 42 days of exposure to 1.5% carbon dioxide, and for 9 days after the carbon dioxide levels were returned to normal levels. In addition, there was a 35-day post-test period where mortality was monitored.
3.4.2	Body weight	Yes. The study report includes a graph charting the measurement of weight (Refer to graph 1 at the end of this study summary for further details). This chart shows that weight measurement was recorded regularly throughout the test period (42 days exposure to 1.5% carbon dioxide and 9 days post-exposure), however the exact times for determination of weight changes has not been specifically recorded.
3.4.3	Food consumption	Not reported, but note body weight observations.
3.4.4	Water consumption	Not reported, but note body weight observations.
3.4.5	Ophthalmoscopic examination	Not reported.

Section A6.4.3 **Subchronic Inhalation Toxicity Test (4 of 11)**

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3.4.6	Haematology	Yes. Number of subjects: All. Time points: Prior to CO_2 exposure, After 28 days (4 weeks) exposure to CO_2 After 42 days (6 weeks) exposure to CO_2 7 days (1 week) after the end of CO_2 exposure. 28 days (4 weeks) after the end of CO_2 exposure. Parameters: Other: Haematocrit, haemoglobin concentration, erythrocyte count, white blood cell count, reticulocyte count and differential.
3.4.7	Clinical Chemistry	Yes Number of animals: All. Time points: Not reported. Parameters: Other. Electrolyte (bicarbonate, sodium, chloride, calcium, phosphate) and water composition of the plasma and urine. Nitrogen balance and serum pH.
3.4.8	Urinalysis	Yes Number of animals: All. Time points: Not reported. Parameters: Other: urine pH.
3.5	**Sacrifice and Pathology**	
3.5.1	Organ weights	No. No mortalities in test. No sacrifices made.
3.5.2	Gross and histopathology	No. No mortalities in test. No sacrifices made.
3.5.3	Other examinations	None.
3.5.4	Statistics	Statistical analysis not reported.
3.6	**Further remarks**	Pulse rate, weight, oral temperature and systolic and diastolic blood pressure measurements were taken. In order to measure any variations during the day (diurnal variations), blood pressure, pulse rate, oral temperature, total leukocytes, total eosinophils and total lymphocytes were made on a group of six subjects for two consecutive days prior to carbon dioxide exposure, then during the first two weeks of carbon dioxide exposure and again three to four weeks post exposure to carbon dioxide. Alveolar carbon dioxide tensions, respiratory minute volume, oxygen consumption, and carbon dioxide excretion were measured throughout the experiment on two groups of ten subjects each. Carbon dioxide sensitivity tests were carried out prior to the carbon dioxide exposure, at the end of carbon dioxide exposure and three weeks post carbon dioxide exposure. They consisted of breathing 1.5% carbon dioxide and 5% carbon dioxide over a period of 15 minutes. Pre- and post-exposure to carbon dioxide were periods on air of equal length. Electroencephalographic studies were conducted. Repeated EEGs were taken from the subjects prior to and during carbon dioxide exposure, and post exposure to obtain information as to the response to light stimulus, and during sleep in an attempt to evaluate changes Which might occur in cortical functions as a result of carbon dioxide Exposure. Note that a study was also carried out to determine the Change in frequency and amplitudes of the EEG, but the results to this

3.6	**Further remarks** (Continued)	study has not been fully reported. Flicker frequencies were carried out on 20 subjects once or twice a week, in order to determine response to light stimulus. Sleep electroencephalograms were carried out using an EEG, and counting the number of movements occurring in comparable periods: during the first hour (which was recorded continuously), during the first 10 minutes of the second hour, and during the first 10 minutes of the 3rd, 4th and 5th hours. As every movement during sleep is connected with a state near awakening, the number of alpha-wave movements on an EEG can be used as an indication of the depth of sleep (where an increased number of movements would indicate a decreased depth of sleep). 15 subjects were investigated prior to exposure to carbon dioxide, during the first two weeks of exposure to carbon dioxide, during the fifth and sixth weeks of exposure to carbon dioxide, eight days post exposure and four weeks post exposure to carbon dioxide.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No clinical signs reported.
4.1.2	Mortality	No mortalities reported.
4.2	**Body weight gain**	No changes in weight were observed.
4.3	**Food consumption and compound intake**	Not reported, but note body weight observations.
4.4	**Ophthalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	Red blood count, white blood count and haemoglobin concentration did not change. The eosinophil count dropped consistently from control levels of 143 per cubic millimetre to reach the lowest value of 49 per cubic millimetre eight days after the end of exposure to carbon dioxide. After four weeks post exposure, eosinophils still had not reached the initial level. The total lymphocytes did not change significantly throughout the carbon dioxide exposure. Eight days after the end of carbon dioxide exposure, a remarkably high value was found. Four weeks post exposure the lymphocyte counts returned to the initial level. The total neutrophils dropped slightly throughout carbon dioxide exposure, reaching a very low value eight days post exposure, but came back to initial level four weeks post exposure. The reticulocyte counts did not show any significant changes throughout the experiment. These results suggest that prolonged exposure to 1.5% carbon dioxide does not influence the erythropoietic system. However, the consistent downward trend of the eosinophils throughout the carbon dioxide exposure and eight days following the end of carbon dioxide exposure indicates an increasing stress on the adrenal system. The leucopoietic system seems more affected, as shown in the consistent decrease of total neutrophils throughout exposure to carbon dioxide and eight days post exposure.
4.5.2	Clinical chemistry	Measurements of the electrolyte and water composition of the plasma and urine were made to determine what degree of respiratory acidosis might occur under exposure to 1.5% carbon dioxide for a long period. The average changes which occurred in the electrolyte composition of the serum was small and in keeping with the appearance of a very mild respiratory acidosis, which was well compensated at all times.

4.5.2	Clinical chemistry (Continued).	Serum bicarbonate showed a slight increase above control values during the period of increased carbon dioxide, which was in keeping with the small rise in alveolar carbon dioxide tension (refer to question "4.7 Other" for details). The values returned to the normal range in about one week post exposure (when the test subjects were exposed to normal air). The control levels of serum sodium showed considerable variation, but it appears that there may have been a slight increase in the middle period of carbon dioxide exposure. Serum chlorides showed a slight rise at the same time as the serum sodium, however this change may be due to an analytical error or to some change in the water content in the serum, which was not investigated further. Serum calcium was approximately constant throughout the experiment. Serum phosphate showed a rise during the entire period of exposure to increased carbon dioxide, with a peak at the end of the tenth day. These values returned to control levels within a week of returning to normal atmospheric levels of carbon dioxide. Serum water showed no apparent change. Changes in electrolyte composition of the urine are probably only significant in terms of total balance. Not all findings have been reported, however urine bicarbonate remained within the control range until the 13th day of exposure to increased carbon dioxide. At this time, it began to rise reaching a peak on the 26th day of exposure. By this time it was some seven times the control value (0.5 millimoles per day). During the last week of the experiment, in a five-day period the urine bicarbonate fell to control levels, only to return again for two days before the finish of the experiment to 15 mM per day. The reasons for this drop, which parallels a similar fall in carbon dioxide excretion through the lungs, are unknown. Following exposure there was a sharp increase in the bicarbonate excretion, which reached levels of 35 mM. This occurred on the second day after returning to normal atmospheric levels of carbon dioxide. The excretion then returned to normal ranges over the ensuing four days. Urine calcium, chloride and phosphate excretions roughly parallel each other but show no apparent consistent trend. The daily average of potassium excretion fell to about one half the control value during exposure to carbon dioxide and later returned somewhat towards normal. Sodium excretions were not completely evaluated, but appeared to show no spectacular change. Nitrogen balance was not completely evaluated, but there appears to be no significant variation in the nitrogen content of either the urine or faeces, resulting in little or no gain or loss in total body nitrogen. The average serum pH was 7.34 in the control period. This fell to 7.30 and remained at about this level for the first 20 days of exposure to increased carbon dioxide. It then rose to the control level, where it remained for the rest of the experimental period. There was a slight and probably not significant fall at the end of the period on air that followed the carbon dioxide exposure.
4.5.3	Urinalysis	Urine pH mirrored the bicarbonate curve (described under question "4.5.2 clinical chemistry"), which was to be expected.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No mortalities in test. No sacrifices made.

4.6.2	Gross and histopathology	No mortalities in test. No sacrifices made.

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Annex Point IIA, VI, 6.4

4.7	**Other**	Pulse rate, weight, oral temperature and systolic and diastolic blood pressure measurements were taken under resting conditions in the morning, after awakening in order to indicate whether there was any change in circulation or metabolism. The results showed no significant change in these parameters during prolonged exposure to 1.5% carbon dioxide. (Refer to graph 1 at the end of this study summary for further details). Changes in the daily cycles (diurnal cycles) of functions such as blood pressure, pulse rate, oral temperature, total leukocytes, total eosinophils and total lymphocytes suggest a deep change in physiological adaptation to the environment. The daily pattern of blood pressure, temperature and pulse rate showed no systemic change during exposure to carbon dioxide, although the pulse rate tended to be slightly higher during the control period. Similarly, no change occurred in the pattern of lymphocytes, leukocytes and eosinophils. Eosinophils were, however at a lower level at the end of the carbon dioxide exposure and for two weeks thereafter than during the control period and the first two weeks of exposure to carbon dioxide. In general, no systemic consistent change in the diurnal cycles could be detected, especially no reversion: cycles were not reversed. Alveolar carbon dioxide tensions, respiratory minute volume, oxygen consumption, and carbon dioxide excretion were measured throughout the experiment on two groups of ten subjects each. For further details refer to graph 2 at the end of this study summary. The two test groups varied from each other in their responses to carbon dioxide. This is due to the fact that these groups had different subjects who were more sensitive to carbon dioxide. Observations indicate that, as expected, under increased carbon dioxide of 1.5%, alveolar carbon dioxide tension rises. It is worth noting that it takes two days before a significant increase in alveolar carbon dioxide tension is reached. Following transition to air, the alveolar carbon dioxide tension remains elevated above the initial level throughout the control period. This is in contrast to the usual finding of an undershooting of alveolar carbon dioxide tension below the initial alveolar carbon dioxide level in acute exposure to 1.5% carbon dioxide. The prolonged exposure apparently has the effect of eliminating the usual transitory response to carbon dioxide. Ventilation was increased throughout the 1.5% carbon dioxide exposure, as expected, and then dropped and returned to initial levels within the first three days post exposure. The final increase, at the end of the control period may be due to external reasons such as alcohol consumption. Oxygen consumption appeared to be somewhat increased throughout the first part of the carbon dioxide exposure up to 14 days, and apparently returns to the initial level in the latter part of carbon dioxide exposure. Following transition to air, oxygen consumption remains at the same level for the first three or four days. Carbon dioxide excretion drops continuously up to the fifth to the eighth day, respectively of carbon dioxide exposure and then shows an increase for the following two weeks. During the last two weeks of carbon dioxide exposure, carbon dioxide excretion drops again but on transition to air returns promptly to initial levels. These three phrases of carbon dioxide excretion during the carbon dioxide exposure parallel the carbon dioxide excretion in the urine. The initial drop is to be

	expected, because of the well-known fact that breathing gas with increased carbon dioxide will result in a degree of uptake of excess carbon dioxide by body alkali in an endeavour to maintain a (Continued....)	

4.7	**Other** (Continued)	constant pH. Until a new equilibrium is reached between the increased carbon dioxide tension and the carbon dioxide content of the body buffers, the amount of carbon dioxide in the expired air must be less than the sum of carbon dioxide normally expired by breathing air and the carbon dioxide added in the experiment. The low point of carbon dioxide excretion at the 5th to 8th day represents the point, apparently, at which the buffers are saturated. A new equilibrium begins to develop during the following two weeks between the increased carbon dioxide tension and the increased carbon dioxide content of the blood buffers. No explanation has been given for a second drop during the end of carbon dioxide exposure. The respiratory gas ratio follows essentially the curve of carbon dioxide excretion. Results to the carbon dioxide sensitivity tests to 5% carbon dioxide show that there was a significant drop in the rate of ventilation at the end of carbon dioxide exposure, and the variations were much less than in the tests prior to exposure to carbon dioxide. Post exposure to carbon dioxide, ventilatory response increased but still did not reach the initial level – it was still significantly less than the initial response. These carbon dioxide sensitivity tests demonstrate that the sensitivity of the respiratory centre to carbon dioxide is significantly decreased at the end of 42 days exposure to 1.5% carbon dioxide. Electroencephalographic studies. Flicker fusion frequency is a good way of measuring the excitability of the visual pathways. The results indicated that the flicker fusion frequency did not show any significant drop throughout the exposure to carbon dioxide. Judged from these measurements no depressive effects of carbon dioxide on the excitability of visual pathways could be detected. Results to the sleep electroencephalogram show that the number of alpha-wave movements during the first hour and during the following test periods was increased under carbon dioxide exposure when compared with the pre- and post-control periods. This means that during exposure to increased carbon dioxide the depth of sleep is reduced. **5. APPLICANTS SUMMARY AND CONCLUSION**	
5.1	**Materials and Methods**	This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC. Twenty three human male volunteers went aboard a naval submarine called the USS Haddock. For the purposes of the test, the forward engine room and all spaces forward to it were activated. The forward escape trunk was used as the primary airlock and the forward torpedo room as the secondary airlock. The actual test compartments were the forward battery, control room and after battery. For the first nine days within the submarine, the volunteers breathed atmospheric air. After nine days, the submarine hull ventilation openings were shut thus sealing the atmosphere within the submarine from contact with atmospheric air. At this time, carbon dioxide concentration was built up to 1.5%. The atmosphere within the submarine was controlled by electronic devices and regulated so that the carbon dioxide concentration was maintained at 1.5% and the oxygen concentration at 20.5%.	

		The carbon dioxide concentration of 1.5% was maintained within the submarine atmosphere for a period of 42 days. Oxygen was supplied from a modified Helium-Oxygen diving manifold that was located (Continued.....)

5.1	**Materials and Methods** (Continued)	nearby. Excess amounts of carbon dioxide that accumulated within the test compartment were removed by a carbon dioxide removal device (called the North-western caustic scrubber) located in the forward torpedo room of the submarine. After 42 days, the hull ventilation openings on the submarine were opened, establishing communication between the interior of the submarine and atmospheric air. The volunteers breathed atmospheric air for nine days, before emerging from the test compartments of the submarine. After leaving the submarine the volunteer group underwent extensive physiological tests. The five phases of testing is as follows: 1. Initial laboratory testing (35 days). 2. Control period on atmospheric air within the submarine (9 days). 3. Under 1.5% carbon dioxide within the submarine (42 days). 4. Control period on atmospheric air within the submarine (9 days). 5. Final laboratory testing (35 days).
5.2	**Results and discussion**	No changes in the basal (resting) pulse rate, weight, oral temperature, or blood pressure were observed during the exposure period. (Refer to graph 1 at the end of this study summary for details). Measurement of the diurnal (daily) variations of blood pressure, pulse rate, oral temperature and total leukocytes revealed no consistent change in the diurnal cycles. The alveolar carbon dioxide tension was elevated to approximately 8% above control values during the exposure period. (Refer to graph 2 at the end of this study summary for details). Pulmonary ventilation was also increased by approximately 8% during the exposure period, and returned to the initial level within the first three days following the exposure period. (Refer to graph 2 at the end of this study summary for details). The oxygen consumption appeared to be somewhat increased during the first part of the exposure period, and then later returned to the initial level. (Refer to graph 2 at the end of this study summary for details). The pulmonary carbon dioxide excretion showed a cyclic variation during the exposure period - being decreased during the first week, increased during the second, third and fourth weeks, and decreased again during the fifth and sixth weeks. (Refer to graph 2 at the end of this study summary for details). The ventilatory response to inhalations of higher concentrations of carbon dioxide (5%) was found to be decreased as compared to the control level during the sixth week of exposure, indicating a decrease in sensitivity to carbon dioxide. It was further found that this ventilatory response to higher concentrations of carbon dioxide did not completely return to the initial level as long as four weeks following the exposure period.

Haematological studies revealed that prolonged exposure to 1.5% carbon dioxide does not seem to influence the erythropoietic system.

The leucopoietic system, however, seems to be effected more, as shown in a consistent decrease of the total neutrophils throughout exposure to 1.5% carbon dioxide, and eight days following exposure.

(Continued.....)

5.2	**Results and discussion** (Continued)	Throughout the exposure period the eosinophil count showed a consistent downward trend. This trend persisted for eight days following the exposure period, indicating an increasing stress on the adrenal system. Electroencephalograms made during sleep indicated that the depth of sleep was reduced during the exposure period. The excitability of the visual pathways, as measured by the flicker fusion frequency, showed no change during the exposure period. The changes that appeared in the electrolyte composition of the serum were small and consistent with a very mild respiratory acidosis. The electrolyte composition of the urine showed no appreciable change during the experimental period. Urine bicarbonate remained within the control range for the first two weeks of exposure. Following this, it began to rise, reaching a peak near the end of the fourth week. There was a brief fall to control levels at the end of the fifth week. Immediately following the return to atmospheric concentrations of carbon dioxide, there was a sharp rise in excretion, which returned to normal at the end of five days.
5.3	**Conclusion**	
5.3.1	LO(A)EL	LOEL: 1.5% carbon dioxide* * Despite there not being a range of carbon dioxide levels tested, the results to this study show a low observable effect level to humans when exposed to 1.5% carbon dioxide.
5.3.2	NO(A)EL	Not reported.
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of pronged exposure to 1.5% carbon dioxide to humans. While this study was not generated to modern, scientifically accepted protocols, nor was it a full 90 day investigation, it does provide useful data on the majority of parameters measured in a subchronic study. Despite the deficiencies in this study, and the fact that test subjects were only exposed to one level of carbon dioxide, it does gives an indication about the level of carbon dioxide that can be tolerated by humans over a pronged period. This study, notwithstanding it's deficiencies, can be used to support the inhalation toxicity of carbon dioxide because:

1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges.

(Continued……)

5.3.2 Deficiencies

(Continued).

2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal, and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions.

3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary.

4. There is sufficient data available concerning the subchronic toxicity of carbon dioxide in various species (including rats, man and mammals). However, because the occupational exposure standard for safe working conditions with carbon dioxide is well established, this value can be used for the risk assessment*.

*The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period).

Section A6.4.3 Subchronic Inhalation Toxicity Test (4 of 11)

Annex Point IIA, VI, 6.4

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted.
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	*State if applicants version is acceptable, or indicate relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.*
Results and discussion	*Adopt applicant's version or include revised version. If necessary, discuss relevant deviations from applicant's view referring to the (sub)heading numbers.*
Conclusion	LO(A)EL: NO(A)EL: Other conclusions: *(adopt applicant's version or include revised version)*
Reliability	*Based on assessment of materials and methods include appropriate reliability indicator.*
Acceptability	Acceptable / not acceptable *(give reasons if necessary e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat if necessary).*
Remarks	
	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 A6.4.3 Subchronic Inhalation Toxicity Test (5 of 11)

Annex Point IIA, VI, 6.4

Official use only

1. REFERENCE

1.1 Reference [redacted]

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2

1.2.3 Criteria for data protection [redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No.

Not carried out to Guideline B.29.in Annex V of Directive 67/548/EEC.

2.2 GLP No.

GLP was not compulsory at the time study was performed.

2.3 Deviations Yes.

No set guideline followed.

3. MATERIALS AND METHODS

3.1 Test material As given in section 2.

3.1.1. Lot/Batch number Not reported.

3.1.2 Specification [redacted]

[redacted]

Section A6.4.3 Subchronic Inhalation Toxicity Test (5 of 11)

Annex Point IIA, VI, 6.4

3.2	**Test Animals**	
3.2.1	Species	Human
3.2.2	Strain	Not applicable.
3.2.3	Source	Not applicable.
3.2.4	Sex	Male.
3.2.5	Age/weight at study Initiation	Ages range of test subjects: 18 to 45 years. Weight of test subjects not reported.
3.2.6	Number of animals per group	4 to 77.
3.2.7	Control animals	No.
3.3	**Administration/ Exposure**	È
3.3.1	Duration of treatment	Other: five experiments, each lasting between 35 and 72 hours.
3.3.2	Frequency of exposure	Other: Continuous.
3.3.3	Post exposure period	Not reported.
3.3.5	**Inhalation**	
3.3.5.1	Concentrations	Test subjects were exposed to carbon dioxide in increasing amounts over the test period. Details of the test conditions are given in Table A6_3-1 at the end of this study summary. Graphs 1-4 at the end of this study summary also summarise the carbon dioxide levels and temperatures for experiments 3, 4 and 5. The carbon dioxide concentrations reported are the nominal concentrations. No analytical concentrations have been given.
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.5.4	Type of exposure	Whole body.
3.3.5.5	Vehicle	Gas.
3.3.5.6	Concentration in vehicle	Not reported.
3.3.5.7	Duration of exposure	Exposure was between 35 and 72 hours. Full details of the exposure duration are given in Table A6_3-1 at the end of this study summary.
3.3.15	Controls	Details of control subjects not reported.
3.4.	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes. Timescales for observation of clinical signs is given in Table A6_3-4 at the end of this study summary.
3.4.1.2	Mortality	No mortalities reported. Timescales for observation of mortality is not reported.
3.4.2	Body weight	Not reported.
3.4.3	Food consumption	Not reported.
3.4.4	Water consumption	Not reported.
3.4.5	Ophthalmoscopic examination	Not reported.

Section A6.4.3 **Subchronic Inhalation Toxicity Test (5 of 11)**
Annex Point IIA, VI, 6.4

3.4.6	Haematology	Yes. Number of subjects: All. Time points: Not reported. Parameters: Other: Effect of carbon dioxide on oxygen saturation of haemoglobin at various ambient oxygen concentrations was measured. Refer to graph 5 at the end of this study summary for details about oxygen saturation of haemoglobin in test subjects.
3.4.7	Clinical Chemistry	Yes Number of animals: All subjects Time points: Not reported Parameters: Other. Blood plasma pH. Refer to Table A6_3-2 at the end of this study summary for details of blood plasma pH data taken for test subjects.
3.4.8	Urinalysis	Not reported.
3.5	**Sacrifice and Pathology**	
3.5.1	Organ weights	No No mortalities in test. No sacrifices made.
3.5.2	Gross and histopathology	No. No mortalities in test. No sacrifices made.
3.5.3	Other examinations	None
3.5.4	Statistics	Statistical analysis not reported.
3.6	**Further remarks**	Pulse rate, respiration rate blood pressure and body temperature were measured. Refer to graphs 6, 7, 8 and 9 at the end of this study summary for details about pulse rate, respiration rate blood pressure and body temperature measurements taken for test subjects.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	When subjects were exposed to 5% carbon dioxide and ambient oxygen is as low as 12%, there is an approximate 2.5% increase in minute breathing volume, a rise in pulse rate of approximately 10 beats per minute, some impairment in specific sensorimotor performance, headache affecting 20% of the test subjects, and occasionally nausea. Throughout all experiments involving 130 man exposures, only three men were removed from the closed spaces. One of these men showed apprehension, another exhaustion, and a third, a steadily increasing blood pressure. At no time were these men in a critical condition. The period immediately following inhalation of outside air was occasionally accompanied by transient dizziness and headache. Observers exposed periodically for one to two hours to the re-circulated compartment air repeatedly developed headaches and experienced a transient taste and smell of ammonia upon leaving the compartment. However, the apparently complete recovery of subjects and observers was rapid. The data given in Table A6_3-1 (refer to end of this study summary for details), shows that the highest carbon dioxide concentration was 6.75% and the lowest oxygen concentration 10.45%. Although these

4.1.1	Clinical signs (Continued)	concentrations were tolerated, the symptoms of headache and respiratory difficulty especially during physical effort sharply increased whenever the carbon dioxide rose appreciably above 5%. It is also noted that the alveolar carbon dioxide pressure, respiratory minute volume and pulse rate show very little change until the atmospheric carbon dioxide approaches the 3% level. Above the 3% level, these functions begin to increase rather sharply. Subjective reactions were recorded in experiments 5 and 6 (refer to table A6_3-1 at the end of this study summary for details of the conditions that test subjects were exposed to in experiments 5 and 6. Graphs 1-4 also summarise the carbon dioxide levels, oxygen levels and temperature conditions in experiments 5 and 6. The subjective reactions recorded in experiments 5 and 6 are given in Table A6_3-4 at the end of this study summary. The usual symptoms of sore throat, nasal congestion and headache were experienced about 40 hours after the start of the experiments. In experiment 5, about 40% of the subjects complained of all of these symptoms, in experiment 6 dry throats and headaches occurred in 18% and nasal congestion in about 10%. However all personnel felt well in the morning after the conclusion of each experiment. In the first four experiments, transient headaches occurred frequently after leaving the sealed spaces. A reaction of interest was the fleeting smell and taste of ammonia when outside air was breathed following exposure in high carbon dioxide atmospheres.
4.1.2	Mortality	No mortalities reported.
4.2	**Body weight gain**	Not reported.
4.3	**Food consumption and compound intake**	Not reported.
4.4	**Ophthalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	The percentages of oxyhaemoglobin were measured in experiment 4 and 5. Refer to table A6_3-1 at the end of this study summary for details of the conditions that test subjects were exposed to in experiments 4 and 5. Graphs 1-4 at the end of this study summary also summarise the carbon dioxide levels, oxygen levels and temperature conditions in experiments 4 and 5. When carbon dioxide in ambient air is 5%, oxyhaemoglobin levels show considerable elevation over percentages expected in the absence of carbon dioxide when the oxygen pressure in inhaled air is reduced (refer to graph 5 and Table A6_3-2 at the end of this study summary for details). These relatively high saturation values are due to the maintenance of a high alveolar oxygen pressure resulting from hyperventilation. (Refer to Table A6_3-3 at the end of this study summary for details).
4.5.2	Clinical chemistry	The blood plasma pH values given in table A6_3-2 at the end of this study summary indicate a slight increase in acidity, 7.44 to 7.38 in one experiment and 7.40 to 7.38 in another, when the carbon dioxide of the ambient air increased from 0.03% to 5%. Plasma carbon dioxide increased from 58.6 to 59.5 % vol. in one experiment and from 58.2 to

64.6 % vol. in another. These results show only slight differences in

(Continued....)

4.5.2	Clinical chemistry (Continued)	plasma carbon dioxide levels when exposed to increased carbon dioxide concentrations and demonstrates the role of hyperventilation in protecting the body against the accumulation of carbon dioxide in the presence of high ambient concentrations of this gas.
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No mortalities in test. No sacrifices made.
4.6.2	Gross and histopathology	No mortalities in test. No sacrifices made.
4.7	**Other**	The most prominent physiologic response to the altered oxygen and carbon dioxide concentrations was the change in respiration. In the course of each experiment, the respiratory rate and tidal volume approximately doubled and the minute volume was two to three times its normal value (refer to graph 6 at the end of this study summary for further details). It was found that an increase in normal ventilation minute volume of over 300% could be maintained for many hours without serious or persistent effects. Some subjects complained of soreness of the respiratory musculature at the end of the experiments but this symptom disappeared within one or two days. The increase in respiratory minute volume produced by 3% carbon dioxide was in the order of one and a half times normal, compared with a two-to-three fold increase brought about by 5% carbon dioxide. Measurements of pulse rate showed a characteristic mean increase of approximately 10 beats per minute over the normal resting rate occurred when the carbon dioxide concentration reached 5% (refer to graph 7 at the end of this study summary for further details). This increase was in response to the increased carbon dioxide (rather than lowered ambient oxygen), as proved in experiment 3 where the rising carbon dioxide concentration was accompanied by a similar rise in pulse rate, even though the oxygen concentration was maintained between 19% and 21%. Refer to table A6_3-1 at the end of this study summary for details of the conditions to which test subjects were exposed. Graphs 1-4 (at the end of this study summary) also summarise the carbon dioxide levels, oxygen levels and temperature conditions in experiment 3. Graph 7, given at the end of this study summary, also shows an approximate difference of 10 beats per minute at equivalent carbon dioxide concentrations between experiments 3 and 4 and 5 and 6. This difference is attributed to the effect of temperature on pulse rate. Experiments 3 and 4 were carried out at Effective Temperatures of 85 and 88°, and experiments 5 and 6 at 75° and 60° respectively (refer to graphs 1-4 at the end of this study summary for further details). Regardless of the effect of temperature on pulse rate, a rise always accompanied an increase in carbon dioxide concentration. There was no characteristic changes in blood pressure following exposure to increased carbon dioxide (refer to graph 8 at the end of this study summary for details). In two of the experiments, both the systolic and diastolic pressures showed a tendency to increase but pulse pressure did not change. (Continued...)

Section A6.4.3 **Subchronic Inhalation Toxicity Test (5 of 11)**
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4.7	**Other** (Continued)	Graph 9, at the end of this study summary, shows the changes in rectal and/or oral temperatures of the test subjects in experiment 3, 4,5 and 6. Refer to table A6_3-1 at the end of this study summary for details of the conditions to which test subjects were exposed. Also, graphs 1-4 at the end of this study summary summarise the carbon dioxide levels, oxygen levels and temperature conditions for experiments 3, 4, 5 and 6. In experiments 3 and 4 rectal temperatures were taken, and in experiments 5 and 6 oral temperatures were taken. Control measurements showed that the oral temperature is approximately one-half degree lower than rectal temperatures. In experiments 3 and 4, the rectal temperature rose within 5 to 10 hours after the start of the experiment to higher levels and dropped almost as rapidly at the close of the experiment. This rise is attributed to the high Effective Temperature of the experimental chamber (88° and 85°). However, experiment 5 (Effective Temperature 75°) and experiment 6 (Effective Temperature 60°) imposed a heat conservation problem as indicated by the considerable decrease in oral temperature. The subjects were inadequately clothed, particularly in experiment 6 and felt cold. The fall in oral temperature may in part be due to mouth breathing which many subjects felt necessary at high ambient carbon dioxide concentrations as well as to the increased heat loss due to hyperventilation.
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC. In six experiments of 35 to 72 hours' duration, groups of 4 to 77 male subjects (age range 18 to 45 years) occupied sealed steel chambers, which allowed a free air space of approximately 500 cu. ft. per man. The first experiment was run to learn the test system. In the second experiment of 52 hours duration (4 subjects) the exhaled carbon dioxide was not absorbed and oxygen was not replenished. In the third experiment (4 subjects) carbon dioxide was not absorbed (like in the second experiment), but the ambient oxygen was not permitted to fall below 19%. In the fourth experiment of 72 hours duration (4 subjects), carbon dioxide in excess of 5% was absorbed and oxygen was not replenished. In the fifth experiment carbon dioxide in excess of 5% was absorbed and oxygen was not replenished. The 37 test subjects in this experiment breathed re-circulated air for 60 hours. In the sixth experiment, carbon dioxide in excess of 5% was absorbed and oxygen was not replenished. The 77 test subjects in this experiment breathed re-circulated air for 50 hours. In the first four experiments an Effective Temperature of approximately 85° was maintained to simulate hot tropical conditions with a dry bulb of 90°F and a relative humidity of 75%. In experiment 5, the Effective Temperature averaged 75° with a dry bulb of 80°F, and a relative humidity of 65%. In experiment 6, the Effective Temperature averaged 59°, with a dry bulb of 60°F, and a relative

humidity of 90%.

(Continued...)

Section A6.4.3 Subchronic Inhalation Toxicity Test (5 of 11)

Annex Point IIA, VI, 6.4

5.1 Materials and Methods

(Continued)

Biochemical and physiological measurements and observations were made. The following testing schedule was adhered to daily for the first four experiments (slight modifications were made in experiments 5 and 6):

0800-1030	Psychological tests
1030-1130	Physiological tests
1130-1200	Biochemical tests
1200-1400	Lunch- rest period
1400-1800	Test programme repeated
1800-2000	Dinner – rest period
2000-2400	Test programme repeated
2400-0800	Sleep period- breakfast

Note that the results of the psychological tests have not been reported in this study summary.

For biochemical tests, blood was drawn from the brachial artery. Due to the frequency of needle insertion as well as technical difficulties, samples of "arterialized" venous blood were drawn in the later experiments. These were obtained by immersing the hand in hot water (45°C) for 20 minutes and with the hand still immersed, drawing blood from one of the dorsal veins of the hand. Blood obtained in this manner was used for gas analysis in lieu of arterial blood.

Alveolar air samples were taken according to the technique described by Dill[1]. All subjects were trained for several days before the start of the experiments to ensure a proper sampling technique.

The plasma pH was calculated by means of the Henderson-Hasselbalch equation from data obtained from analysis of alveolar air and arterial blood, or "arterialized" venous blood.

The following physiological measurements were made in the course of the experiments: pulse rate, blood pressure, body temperature and respiratory rate and minute volume. Those taking the measurements followed a strict routine in making all measurements in order to reduce to a minimum the variability in data. During a typical test procedure, the subject reclined quietly for 15 minutes after which the pulse rate, blood pressure and body temperature was obtained. The test subject then assumed a sitting position while the respiratory measurements were made.

Pulse beats were counted for 30 seconds. Blood pressure was measured by auscultation, the diastolic pressure being taken at the point of sound disappearance. Pulse pressure was calculated as the difference between systolic and diastolic pressures. Body temperature was obtained with standard clinical thermometers, rectal temperatures being employed in the first four experiments and oral temperatures in the last two. To obtain the respiratory data in the first four experiments, expired air was collected by means of a face mask connected to a Tissot spirometer, dry gas meters were employed in experiments 5 and 6 in place of spirometers. The respiratory rate was counted for a full minute, and minute volume was measured for a period of 5 minutes.

[redacted]

5.2	**Results and discussion**	In six experiments men breathed re-circulated air for periods of 35 to 72 hours in sealed spaces of such size as to provide 500 cu. ft. of air (Continued....)

5.2	**Results and discussion** (Continued...)	volume per man. Exposure in atmospheres of carbon dioxide concentrations up to 5% and reduced oxygen concentrations as low as 12% did not seriously impair the physical condition and efficiency of the subjects as evaluated by biochemical and physiological tests. Minor symptoms of headache, nasal congestion and dryness of the throat quickly disappeared when outside air was breathed. In an atmosphere of 5% carbon dioxide and 12% oxygen, healthy men are able to maintain an adequate oxygen pressure in the lungs, blood and tissues because an increase in respiratory minute volume (hyperventilation) and an increase in pulse rate (circulation) prevent a corresponding reduction in oxygen concentration in lungs and blood, despite the increase in ambient oxygen from 21% to 12%. Consequently, in long exposures to atmospheres of high carbon dioxide content (5%) it is not necessary to maintain the oxygen concentration of the recirculated air at the normal value. Concentrations of carbon dioxide much above 5% are not well tolerated. This value appears to be a limiting level for healthy young men if exposures are prolonged. Under these test conditions the carbon dioxide output was found to be 0.326 l/min STP (0.69 cu. ft per man hour) and the oxygen consumption was 0.387 l/min STP (0.82 cu. ft. per man hour).
5.3	**Conclusion**	
5.3.1	LO(A)EL	LOEL: 5% carbon dioxide.
5.3.2	NO(A)EL	Not reported.
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of pronged exposure to levels up to 5% carbon dioxide to humans. While this study was not generated to modern, scientifically accepted protocols, nor was it a full 90 day investigation, it does provide useful data on some of the parameters measured in a subchronic study. Despite the deficiencies in this study, it does gives an indication about the level of carbon dioxide that can be tolerated by humans over a pronged period. This study, notwithstanding it's deficiencies, can be used to support the inhalation toxicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges.

(Continued.....)

Section A6.4.3 Subchronic Inhalation Toxicity Test (5 of 11)

Annex Point IIA, VI, 6.4

5.3.2	Deficiencies (Continued)	2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal, and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions. 3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary. 4. There is sufficient data available concerning the subchronic toxicity of carbon dioxide in various species (including rats, man and mammals). However, because the occupational exposure standard for safe working conditions with carbon dioxide is well established, this value can be used for the risk assessment*. *The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period).

Section A6.4.3 **Subchronic Inhalation Toxicity Test (5 of 11)**

Annex Point IIA, VI, 6.4

Table A6 3-1 Summary of Conditions to which Test Subjects were Exposed.

Experiment number	Number of test subjects	Duration (h)	Highest CO_2 (%)	Lowest O_2 (%)	Hour when ambient CO_2 approached 5%
1	4	34	5.95	14.18	29
2	4	52	6.54	13.45	34
3	4	51	6.75	19.22	37
4	4	72	5.42	10.45	32
5	37	60	5.27	12.21	34
6	77	50	5.18	13.21	34

NOTE: Graphs 1-4 also summarise carbon dioxide levels, oxygen levels and temperature in experiments 3,4,5,6

Table A6 3-2 Effects of Increased Ambient Carbon Dioxide on Gas Equilibria in Blood

Exp. No.	Date	Time	Ambient air		Alveolar air		Blood				Plasma	
			PCO_2 mmHg	PO_2 mmHg	PCO_2 mmHg	PO_2 mmHg	O_2 Cont. % vol.	O_2 Cap'y % vol.	HbO_2 % sat	CO_2 Cont. vol. %	CO_2 Cont. Vol. %	pH
4 [1]	6/6	0830	0.2	149.0	39.2	101.3	18.68	19.63	95.3	47.8	58.6	7.44
	6/1	0800	17.4	127.5	38.0	102.2	19.79	20.85	95.0	48.4	58.9	7.45
	6/2	0800	32.3	106.8	46.0	91.2	18.71	20.22	92.6	51.2	59.9	7.38
	6/3	0800	32.4	92.6	44.3	70.5	19.24	21.27	90.5	50.2	60.4	7.40
	6/3	1400	36.0	73.5	44.6	61.2	18.16	20.33	89.3	48.9	59.5	7.38
5 [2]	7/13	0830	0.2	149.0	42.4	97.3	19.94	20.34	95.5	47.9	58.2	7.40
	7/16	0700	35.2	90.8	48.5	72.1	18.44	19.93	92.5	53.3	64.6	7.38

Key: 1 Average of 4 subjects. For details of test conditions in experiment 4, refer to Table A6_3-1 and graphs 1-4.

2 Average of 5 subjects For details of test conditions in experiment 5, refer to Table A6_3-1 and graphs 1-4.

Cont. Content
Cap'y Capacity
Vol. Volume
Sat Saturation

Section A6.4.3 **Subchronic Inhalation Toxicity Test (5 of 11)**

Annex Point IIA, VI, 6.4

Table A6 3-3 Effects of Increased Ambient Carbon Dioxide on Alveolar Air

Experiment No.	No. of Subjects	Date	Hours of exposure	Ambient Air				Alveolar Air				ΔpCO_2[1]	ΔpO_2[1]
				CO_2	O_2	PCO_2	PO_2	CO_2	O_2	PCO_2	PO_2		
				%		mm. Hg		%		Mm Hg		mmHg	mmHg
1	4	11 May	Rest	0.03	20.94	0.2	150.1	5.76	14.18	40.8	100.4	40.6	49.7
	4		4	1.28	19.62	9.2	140.5	5.49	14.60	39.3	103.7	30.1	36.8
	4		10	2.41	18.32	17.3	131.2	5.94	13.75	42.3	98.5	25.0	32.7
	4	12 May	23	3.84	16.63	27.5	119.1	5.81	13.28	43.5	95.0	16.0	22.1
	4		28	4.79	15.50	34.3	111.0	6.50	13.01	46.4	92.5	12.1	18.5
	4		34	5.95	14.18	42.6	101.5	7.08	12.55	50.4	89.3	7.8	12.2
2	4	17 May	Rest	0.03	20.94	0.2	150.1	5.92	14.55	41.9	103.0	41.7	47.0
	4		4	0.75	20.23	5.3	143.4	4.90	16.05	33.7	112.8	26.6	30.6
	4		10	1.79	18.97	12.6	134.4	5.74	14.20	40.4	100.0	27.8	34.4
	4	18 May	22	3.15	17.48	22.3	123.7	5.95	13.49	42.2	95.2	19.9	28.5
	4		28	4.07	16.42	28.8	116.3	6.22	13.09	44.1	92.9	15.3	23.4
	4		34	4.83	15.50	34.1	109.6	6.61	12.95	46.3	89.0	12.2	20.0
	4	19 May	46	5.66	14.52	40.0	102.8	6.93	12.39	49.3	88.2	12.3	18.4
	4		51	6.54	13.45	46.2	95.2	7.87	11.45	55.8	81.4	9.6	13.8
3	4	25 May	Rest	0.03	20.94	0.2	150.1	5.81	13.85	41.1	98.1	39.9	52.0
	4		18	2.21	19.34	15.9	138.5	5.85	14.84	41.4	105.6	25.5	32.9
	4		34	4.32	20.57	31.0	147.5	6.57	17.79	46.8	127.0	15.8	20.5
	4	26 May	42	5.41	19.54	38.8	140.0	7.10	16.86	50.7	123.7	11.9	16.3
	4		51	6.72	20.52	48.2	147.2	7.92	18.98	56.7	135.8	8.5	11.4
4	4	31 May	Rest	0.030	20.94	0.2	148.5	4.95	15.08	35.5	108.2	35.3	40.3
	4	1 June	17.5	2.47	18.13	17.4	127.5	5.39	14.50	38.0	102.2	20.6	25.3
	4		28	4.19	16.25	29.4	114.4	6.14	13.53	42.9	95.0	13.4	19.4
	4	2 June	42	4.60	15.22	32.3	106.8	6.54	13.01	46.0	91.2	13.5	15.6
	4		52	4.98	13.27	35.0	93.2	6.64	10.85	46.5	76.1	11.5	17.1
	4		58	4.78	12.45	33.6	87.3	6.54	10.73	45.9	73.5	12.3	13.8
	4	3 June	66	4.36	13.21	30.6	92.6	6.33	10.04	44.4	70.5	13.8	22.1
	4		72	5.13	10.45	36.2	73.5	6.35	8.72	44.5	60.7	8.3	12.8
5	10	13 July	Rest	0.03	20.94	0.2	148.8	5.96	13.77	42.3	97.6	42.0	51.2
	8	14 July	19	3.07	17.53	22.2	122.7	6.38	12.73	45.5	90.6	22.3	32.1
	10	15 July	31	4.32	15.50	30.7	110.0	6.98	11.91	49.6	84.4	18.9	25.6
	7	16 July	54	4.98	12.83	35.2	90.8	6.88	10.23	48.5	72.1	13.3	18.7

Key: 1 ΔpCO_2 and ΔpO_2 are defined as the difference between ambient and alveolar pO_2 or pCO_2
Experiment No.: For details of test conditions in experiment 1,2,3,4 and 5 refer to Table A6_3-1 and graphs 1-4 for additional details about test conditions in experiment 3,4,5 and 6..

Table A6_3-4 Subjective Symptoms with Respect to the Subjects, Recorded as a Fraction (Personnel Effected / Personnel Interviewed)

	Hour of the Test	Ambient air		Cerebral fullness	Headache [1]	Nasal congestion	Nausea	Sore throat	Dry throat	Dry or sore throat	Felt good	Felt fair
		CO_2	O_2									
		%										
Exp. 5 Chamber closed	46.5	4.8	13.5		15/35	14/34	5/35			16/34		
	58.5	5.0	12.5		16/34		2/34					
Chamber Open	1.5 h after test	0.03	20.9		7/34		1/34					
Exp. 6 Chamber Closed	3	0.5	20.5	-	2/20	3/20		1/20	0/20		19/20	1/20
	7	1.3	19.5	1/18	0/18	0/18		0/18	0/18		17/18	1/18
	11	1.9	18.6	1/15	0/13	0/15		0/15	0/15		15/15	0/15
	15	2.4	18.3	1/16	0/16	3/16		1/16	0/16		16/16	0/16
	19	2.8	17.7	1/35	0/35	0/35		3/35	0/35		31/35	4/35
	23	3.3	17.3	1/17	0/17	1/17		0/17	0/17		17/17	0/17
	27	3.9	16.5	0/17	3/17	4/17		1/17	0/17		15/17	2/17
	29	4.2	15.9	1/10	2/10	2/10		0/10	1/10		6/10	4/10
	31	4.4	15.6	1/18	1/18	1/18		0/18	2/18		15/18	3/18
	35	5.0	15.1	0/16	0/16	1/16		0/16	0/16		16/16	0/16
	39	4.8	14.6	0/14	4/14	4/14		2/14	1/14		11/14	3/14
	43	4.8	14.1	4/16	3/16	0/16		0/16	0/16		8/16	8/16
	47	5.1	13.7	1/10	3/10	0/10		0/10	3/10		8/10	2/10
	50	5.2	13.2	6/76	18/76	8/76		6/76	18/76		50/76	23/76 [2]
Exp. 6 Chamber open	1.5h after test	0.03	20.9	4/76	3/76	-		-	-		-	-

Key: 1 Headaches for the most part were transient and not severe. One man was removed from the chamber because of headache, nausea, vomiting and a blood pressure rise to 146 mm.

2. All personnel were in good condition the following morning. Only 3 of 76 individuals complained of malaise at the 50th hour.

Experiment No. For details of test conditions in experiment 5 and 6, refer to Table A6_3-1 and graphs 1-4.