Detailed justification:

[redacted]

Undertaking of intended data submission [ ] Not applicable.

Section 6.1.2	Acute Toxicity : Dermal
Annex Point IIA, VI, 6.1.2	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 6.1.3 **Annex Point IIA, VI, 6.1.3**	**Acute 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:	An acute inhalation toxicity study for carbon dioxide is not considered necessary for three reasons: 1. It is not scientifically necessary on the basis of low exposure to carbon dioxide during its normal use as a biocide. 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. *Refer to next page for full details of the scientific calculation, which supports this statement.* 2. In addition to the above, the potential for exposure to carbon dioxide when it is manufactured for use as a rodenticide is minimal. [redacted] 3. There are sufficient data already available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals). The data cited are in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide. (Rats tolerated exposure to 100,000 ppm carbon dioxide (10%), LC_{Lo} values for humans and mammals have been reported to be 90,000 ppm). *Refer to study summaries for details about the data available on the inhalation toxicity of carbon dioxide.* Continued on next page....	

[redacted]

Section 6.1.3 Annex Point IIA, VI, 6.1.3	Acute Toxicity : Inhalation Section 6: Toxicological and Metabolic Studies
Detailed justification:	Given that substantial rises in atmospheric carbon dioxide are never going to be reached under the normal conditions of use of Rentokil Initial's biocidal products and the data cited in this application gives a definitive inhalation toxicity value of 90,000 ppm (note that all of the data used are in broad agreement), it seems unnecessary to conduct an inhalation toxicity for carbon dioxide in rats, given the need to minimise unnecessary vertebrate animal testing whenever possible. [redacted]

Official use only

[redacted]

Undertaking of intended data submission [ ]	Not applicable.

Section 6.1.3 **Annex Point IIA, VI, 6.1.3**	**Acute Toxicity : Inhalation** 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	

Section A6.1.3 **Acute Toxicity: Inhalation (1 of 14)**
Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

Official use only

1. REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	No.
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 A6.1.3 Acute Toxicity: Inhalation (1 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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	Volunteers were aged 18-37 years. Weight of volunteers is between 122 lb and 280 lb. (Exact weight and age of each subject is given in the report.)
3.2.6	Number of animals per group	Twelve.
3.2.7	Control animals	Not reported.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other: No post-exposure period reported. **Inhalation**
3.3.8	Concentrations	Test atmospheres contained 7-14% carbon dioxide in oxygen. No analytical concentrations reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Mouth only.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	Gas mixture contains desired concentration of carbon dioxide being tested with oxygen.
3.3.14	Duration of exposure	Periods between 10 and 20 minutes.
3.3.15	Controls	No information about controls reported.
3.5	**Method of determination of LD_{50}**	Other. LD_{50} not reported, but expressed as maximum tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	Twelve male volunteers inspired concentrations of carbon dioxide in oxygen ranging from 7% to 14% carbon dioxide for periods of 10-20 minutes. Systolic, diastolic and pulse pressures, heart rate and respiratory minute volume all increased significantly during exposure to all of the concentrations administered ($P = 0.01$). The diastolic pressure was the least affected. With rare exceptions, the systolic, diastolic and pulse pressures, heart rate and respiratory minute volume increased as Pco_2 increased. At high concentrations of CO_2 the dicrotic notch of the arterial pressure pulse became less pronounced and sometimes disappeared completely. Profuse sweating, severe headache and auditory and visual hallucinations were not infrequent. Vomiting into the mouthpiece interrupted some studies. At Pco_2 levels above 80 mm Hg (10.6%) most subjects lost consciousness. In these subjects, involuntary movements occurred, ranging from tremors and twitching of fingers to gross movements of the body requiring restraint. Continued....

Section A6.1.3 **Acute Toxicity: Inhalation (1 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

4.3	**Other** (Continued)	Soon after substitution of oxygen for the carbon dioxide-oxygen mixture, the arterial pressure returned towards normal levels and a minimum pressure was observed in 22 of 27 cases within 3 minutes after discontinuing carbon dioxide. In only three observations was the minimum pressure below the control level of 10 mmHg or more. The heart rate at this time was still above control values. In some instances, the heart rate increased further after termination of the period of elevated alveolar Pco_2. The arterial pressure usually increased briefly after the initial minimum and then declined to reach another low 2-48 minutes later. The second minimum was, as a rule, not as low as the first and in no case was the mean pressure as much as 10 mm Hg below the control level. The heart rate was slower than during the first minimum, but exceeded the initial rate in all observations but one. Cardiac arrhythmias were not observed during Pco_2 reduction. The subjects' symptoms usually diminished during the posthypercarbia period but headache persisted in some cases for as long as 3 hours after the end of carbon dioxide administration.
4.4	**LD_{50}**	Refer to "4.3 Other"
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC. The effect of increased carbon dioxide levels on twelve healthy male volunteers (3 Negro, one oriental and 8 Caucasian), aged between 18 – 37 years were studied. All had fasted for 6 hours prior to the study. Nine subjects were exposed to elevated concentrations in the supine position (lying on back, face upwards), and three were studied in both the supine and sitting positions. Placed on an operating table, electrocardiograph needle electrodes were placed subcutaneously into the right arm and left leg, and a thin walled, 21-gauge needle was placed into the brachial artery. The intra-arterial needle was connected by polyethylene tubing to a manifold through which blood samples could be drawn. Arterial pressure pulses were transduced with a strain gauge, and lead 2 of the electrocardiogram was recorded. All measurements were recorded on a four-channel Polygraph, ordinarily at a paper speed of 10 or 25 mm/sec. The subject's nose was occluded with a clip and the subject breathed through a rubber mouthpiece and a non-rebreathing valve. Exhaled gases passed through a dry gas volume meter to the atmosphere. A portion of respired gas mixture was drawn continuously from the lumen of the mouthpiece through a needle to a carbon dioxide analyser. The carbon dioxide analyser was calibrated with five known gas mixtures, which covered the observed range of expired carbon dioxide tension (3.1% - 18.8%). Respiratory minute volume was measured with the gas mixture, the pointer of which touched a capacitance microphone producing an electrical signal with each revolution. On some occasions, the volume of gas passing through the meter was monitored visually and timed with a stop watch. Continued.....

Section A6.1.3 **Acute Toxicity: Inhalation (1 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

5.1 Materials and Methods

(Continued)

The inspired gas mixture was delivered from a McKesson anaesthesia machine with a 'Nargraf proportioning demand valve' to a 5 litre reservoir bag and thence via the non-breathing valve to the subject. The anaesthesia machine was supplied with gases from a tank of pure oxygen and a second tank of about 15% carbon dioxide in oxygen. The two gases were mixed in the desired proportions in the Nargraf mixer, which supplied the gases to the subject as demanded.

Blood plasma was analysed for epinephrine and norepinephrine using the Price and Price method [1], and plasma steroid analysis were performed by the Nelson-Samuels method [2].

With the subject in position and the measuring equipment operating, the mouth piece and nose clip were applied and the subject were permitted to breathe oxygen for an average of 23 minutes (range 4 – 30 minutes). Inspired carbon dioxide tension was then elevated by adding the desired proportion of the CO2-O2 mixture. When the end-expired Pco_2 had been maintained with +/- 5 mm of the desired level for 10-20 minutes or longer, CO_2 was removed from the inspired gas mixture and the subject breathed pure oxygen for an average of 7 minutes (range 0 – 17 minutes). The total period of elevated inspired carbon dioxide concentration averaged 22 minutes (range 8 – 35 minutes). The total period of elevated inspired carbon dioxide concentration averaged 22 minutes (range 8 – 35 minutes). The mouthpiece and nose clip were then removed and the subject permitted to rest in the supine position (lying on back, face upward) while breathing air. After a rest period of about 30 minutes, the mouthpiece and nose clip was replaced and the subject breathed pure oxygen for an average of 7 minutes before the next CO_2 elevation (range 4-10 minutes).

In six of the twelve subjects, carbon dioxide was elevated three times, in three subjects twice, and in three subjects once.

[redacted]

5.2 Results and discussion

Systolic, diastolic and pulse pressures, heart rate and respiratory minute volume increased significantly ($P < 0.01$) during exposure to all of the concentrations of carbon dioxide administered. The diastolic pressure was the least effected. When measurements in the same subject at different Pco_2 levels were compared, systolic diastolic and pulse pressures, heart rate and respiratory minute volume (with rare exceptions) increased progressively as Pco_2 increased.

At high levels of carbon dioxide, the dicrotic notch of the arterial pressure pulse became less pronounced and sometimes disappeared completely.

Profuse sweating, severe headache and auditory and visual hallucinations were not infrequent. Vomiting into the mouthpiece interrupted some studies. At Pco_2 levels above 80 mm Hg most subjects lost consciousness. In these subjects, involuntary movements occurred, ranging from tremors and twitching of fingers to gross movements of the body requiring restraint.

Continued...

Section A6.1.3 Acute Toxicity: Inhalation (1 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

5.2 Results and discussion

(Continued)

Soon after substitution of oxygen for the carbon dioxide-oxygen mixture, the arterial pressure commenced its return toward normal levels and a minimum pressure was observed in 22 of 27 cases within 3 minutes after discontinuing carbon dioxide. In only three observations was the minimum pressure below the control level of 10 mmHg or more. The heart rate at this time was still above control values. In some instances, the heart rate increased further after termination of the period of elevated alveolar Pco_2. The arterial pressure usually increased briefly after the initial minimum and then declined to reach another low 2-48 minutes later. The second minimum was, as a rule, not as low as the first and in no case was the mean pressure as much as 10 mm Hg below the control level. The heart rate was slower than during the first minimum, but exceeded the initial rate in all observations but one.

Cardiac arrhythmias were not observed during Pco_2 reduction. The subjects' symptoms usually diminished during the posthypercarbia period but headache persisted in some cases for as long as 3 hours after the end of carbon dioxide administration.

These results show that 7% to 14% carbon dioxide in oxygen can be tolerated for periods of at least 10-20 minutes, although at concentrations above 10% most subjects lost consciousness. All observed effects were reversible when the subject was removed from the atmosphere containing the increased carbon dioxide level.

5.3 Conclusion

5.3.1 Reliability: 3

5.3.2 Deficiencies: Yes

This study gives an indication about the maximal tolerated dose for carbon dioxide in humans, rather than determining the LC_{50}.

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 gives an indication about the level of carbon dioxide that can be tolerated by humans (approximately 10% carbon dioxide, after which most of the subjects in this experiment lost consciousness).

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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{LO} values for humans and mammals have been reported to be 90,000 ppm.)

Section A6.1.3 **Acute Toxicity: Inhalation (1 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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	

Section A6.1.3 **Acute Toxicity: Inhalation (2 of 14)**
Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in humans.

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]

Section A6.1.3 **Acute Toxicity: Inhalation (2 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in humans.

3.2	**Test Animals**	
3.2.1	Species	This study uses mathematical extrapolation to determine the toxicity of carbon dioxide to humans, from data available on warm blooded mammals. No details have been reported about the raw data on warm blooded mammals.
3.2.2	Strain	Not reported (see 3.2.1).
3.2.3	Source	Not reported (see 3.2.1).
3.2.4	Sex	Not reported (see 3.2.1).
3.2.5	Age/weight at study Initiation	Not reported (see 3.2.1).
3.2.6	Number of animals per group	Not reported (see 3.2.1).
3.2.7	Control animals	Not reported (see 3.2.1).
3.3	**Administration/ Exposure**	This study uses mathematical extrapolation to determine the toxicity of carbon dioxide to humans, from data available on warm blooded mammals. No details have been reported about the raw data on warm blooded mammals.
3.3.1	Post exposure period	Not reported (see 3.3)
		Inhalation
3.3.8	Concentrations	Not reported (see 3.3)
3.3.9	Particle size	Not reported (see 3.3)
3.3.10	Type or preparation of particles	Not reported (see 3.3)
3.3.11	Type of exposure	Not reported (see 3.3)
3.3.12	Vehicle	Not reported (see 3.3)
3.3.13	Concentration in vehicle	Not reported (see 3.3)
3.3.14	Duration of exposure	Not reported (see 3.3)
3.3.15	Controls	Not reported (see 3.3)
3.5	**Method of determination of LD_{50}**	Lethal dose, toxic level and tolerated level of carbon dioxide in humans was determined mathematically.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	This section is not relevant because the report discusses mathematical extrapolation to determine the toxicity of gases to humans. (See "4.3 Other" for details).
4.2	**Pathology**	This section is not relevant because the report discusses mathematical extrapolation to determine the toxicity of gases to humans. (See "4.3 Other" for details).

Section A6.1.3 Acute Toxicity: Inhalation (2 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in humans.

4.3	**Other**	Lethal dose at 5-10 minutes continuously inhaled: 90,000 cm^3 carbon dioxide per m^3 air. Toxic levels have been cited as 50,000 cm^3 carbon dioxide per m^3 air (continuously inhaled for 0.5-1h). The tolerated level in humans is 30,000 carbon dioxide per m^3 air (continuously inhaled for 0.5-1h).
4.4	**LD_{50}**	Refer to "4.3 Other".

5. APPLICANTS SUMMARY AND CONCLUSION

5.1	**Materials and Methods**	This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC. The activity of a gas with regard to quantitative effects can be demonstrated with a geometric co-ordination system, which contains the two important values concentration (c) and the inhalation time (t). The resulting products can simply be displayed as rectangular areas. This formula has in many instances proved successful in establishing simple gas effects, however the author acknowledges that the mathematical method can only be applied to certain situations (e.g. exposure to only a single gaseous poison). Using the formulae described in the reference, the quantitative relationships of both purely locally acting gases and re-sorptive gases and their transitions can be simply and clearly demonstrated. Each gas has its own curve of activity. These curves can only be assembled from simple experimental conditions, but they are indispensable in determining the type of effect.
5.2	**Results and discussion**	The author quotes the following toxicity values for carbon dioxide for humans: Lethal dose at 5-10 minutes continuously inhaled: 90,000 cm^3 carbon dioxide per m^3 air. Toxic levels have been cited as 50,000 cm^3 carbon dioxide per m^3 air (continuously inhaled for 0.5-1h). The tolerated level in humans is 30,000 carbon dioxide per m^3 air (continuously inhaled for 0.5-1h).

Section A6.1.3 **Acute Toxicity: Inhalation (2 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in humans.

5.3	**Conclusion**	
5.3.1	Reliability	4
5.3.2	Deficiencies	Yes

As described below, it is duly acknowledged that there is insufficient reporting of methods and results data, and this data has not been generated in accordance with scientifically acceptable protocols.

This study gives an indication about the lethal dose for carbon dioxide in humans, and also the toxic level and tolerated level. These values have been extrapolated from work carried out on warm blooded mammals using mathematical formulae. The raw data on the warm blooded mammals has not been reported, nor has details about this data was generated been given.

Despite the fact that the raw study has not been made available, this report gives an indication about the levels of carbon dioxide that can be tolerated by humans, the levels which are toxic to humans and the lethal concentration.

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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{L0} values for humans and mammals have been reported to be 90,000 ppm.).

Section A6.1.3 **Acute Toxicity: Inhalation (2 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in humans.

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (3 of 14)

Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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 A6.1.3 **Acute Toxicity: Inhalation (3 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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	Age and weight of test subjects not reported.
3.2.6	Number of animals per group	One and six.
3.2.7	Control animals	Not reported.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other: No post-exposure period reported. **Inhalation**
3.3.8	Concentrations	Various levels between 6-7 % carbon dioxide. No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Whole body.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	Nitrogen and carbon dioxide gas mixture. Pressure was adjusted in exposure chamber so that carbon dioxide content was 2.5% and oxygen was around 20%. The carbon dioxide content was then allowed to rise (and oxygen levels fall) owing to normal respiration of the test subject.
3.3.14	Duration of exposure	14.5 h
3.3.15	Controls	Details of control animals not reported.
3.5	**Method of determination of LD_{50}**	Other. LD_{50} not reported, but expressed as a tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	The first test period began at 10.10 p.m. when the carbon dioxide content in the exposure chamber was 2.5% and oxygen was around 20%. The carbon dioxide content was then allowed to rise (and oxygen levels fall) owing to normal respiration of the test subject. The first test subject slept intermittently from 12.30 to 8.30 am, when the carbon dioxide level was 5.1%. Panting was marked but there was no distress. At 10.50 am, carbon dioxide levels had reached 5.8%. Panting was severe with slight headache, photophobia and transient nausea. At noon, the respiration rate was 44 per minute but the pulse rate was only 60 (compared to a normal figure of about 75). At 12.40 p.m., the carbon dioxide level had reached 6.6% and there was slight confusion though the headache was not severe and there was no vomiting. At 12.40 p.m. the test subject left the exposure chamber and breathed pure oxygen through submarine escape apparatus. 2-3 minutes later, he vomited, at intervals bringing up a pint of clear fluid. Note that the test subject had not eaten or drunk

4.3	**Other** (Continued)	for the previous sixteen hours. A violent headache developed, mainly frontal but somewhat diffuse. This severe headache persisted for about an hour, and passed at about 5pm. Later the test subject remarked that he felt unusually well. Five subjects entered the exposure chamber for one hour. At the beginning of the test period the carbon dioxide level was 6.1%, but it rose rapidly to 6.7% by the end of the test period of 60 minutes. Oxygen content in the exposure chamber was approximately 18.7%. At the end of one hour exposure all test subjects were panting severely, and one was in serious distress. Upon leaving the exposure chamber, all test subjects except one breathed pure oxygen through submarine escape apparatus. Of those that breathed pure oxygen immediately after leaving the carbon dioxide exposure chamber, one test subject vomited repeatedly, even though he had not eaten for the previous seven hours. This subject had a moderate headache. Two subjects had very severe headaches which developed after a few minutes of leaving the exposure chamber and both were temporarily incapacitated. The remaining subject who was exposed to pure oxygen immediately after leaving the carbon dioxide exposure chamber had a slight headache, which developed 10-15 minutes after leaving the exposure chamber. The one subject who was not exposed to pure oxygen after leaving the carbon dioxide exposure chamber also developed a slight headache 10-15 minutes after leaving the exposure chamber. All five test subjects had recovered three hours after exposure to 6.7% carbon dioxide.
4.4	**LD_{50}**	Refer to "4.3 Other"
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC. A steel chamber of 5800 litres was used as the exposure chamber. The pressure in this chamber was kept at approximately 1.1 atmospheres and the carbon dioxide was allowed to rise to between 6 and 7%. The pressure in the exposure chamber fell from time to time due to a slight leak, but the pressure was maintained with compressed air when necessary. The dry bulb-temperature for the first investigation, where one test subject was exposed to increasing levels of carbon dioxide for 14.5 h, varied from 70-74°F and the wet bulb was 2-4° lower.
5.2	**Results and discussion**	In each of the six subjects who have breathed air containing carbon dioxide at a partial pressure of over 6% with normal or subnormal oxygen for an hour there was an aggravation of symptoms other than panting when air or pure oxygen was breathed, and three of the six subjects vomited. It should be noted, however, that there were marked differences in individual symptoms of the test subjects.

Section A6.1.3 **Acute Toxicity: Inhalation (3 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

5.3	**Conclusion**	
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes

This study gives an indication about a tolerated dose for carbon dioxide in man, rather than determining the LC_{50}.

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 gives an indication about the level of carbon dioxide that can be tolerated by man.

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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{L0} values for humans and mammals have been reported to be 90,000 ppm.).

Section A6.1.3 **Acute Toxicity: Inhalation (3 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (4 of 14)
Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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 A6.1.3 Acute Toxicity: Inhalation (4 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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	Ages of subjects are: 23 years, 50 years, 60 years, 64 years and 69 years. Weights of subjects not reported.
3.2.6	Number of animals per group	5.
3.2.7	Control animals	Yes. No details about numbers of controls given.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other: No post-exposure period reported. **Inhalation**
3.3.8	Concentrations	Step-wise increase in carbon dioxide up to a maximum of 9.3% (range of carbon dioxide tested 2.6%-9.3%). No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Via an endotracheal tube into the nose and/or mouth.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	Subjects were kept anaesthetised with nitrous oxide and oxygen via an endotracheal tube Ventilation was adjusted to produce end-tidal carbon dioxide concentrations of approximately 3%. Then carbon dioxide was added to the inspired gas mixture in increments of 1-2% until an inspired concentration of approximately 10% was attained. Range of carbon dioxide tested: 2.6%-9.3%
3.3.14	Duration of exposure	12-15 minutes at each concentration of CO_2 tested.
3.3.15	Controls	Control readings were taken after ventilation was adjusted to produce end-tidal carbon dioxide concentrations of approximately 3%. (refer to 3.3.7, above).
3.5	**Method of determination of LD_{50}**	Other. LD_{50} not reported, but expressed as a tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	Carbon dioxide exerts dual effects on the cardiovascular system. Its direct effect on the heart is as a depressant, but this effect is masked by an indirect stimulant action through the sympathetic nervous system. Previous studies show that $1/PEP^2$ measurements correlate well with the myocardial inotropic state of the heart and therefore can act as an indicator of aortic blood flow acceleration. As carbon dioxide levels increased, $1/PEP^2$ measurements increased which indicates an acceleration of blood flow through the heart. When there was excessive amounts of carbon dioxide in the blood (hypercapnia), the response varied with age. The youngest subject had the greatest response, whereas the least noticeable response was in the two oldest patients. The oldest patient's $1/PEP^2$ measurements actually decreased when $PaCO_2$ increased over 7.5% carbon dioxide.

Continued.... .

Section A6.1.3 **Acute Toxicity: Inhalation (4 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

4.3	**Other** (Continued)	In the subjects studied, the $PaCO_2$ level ranged from 2.6-9.4% Alterations in $1/PEP^2$ measurements were slight when the $PaCO_2$ levels were below 5.3% carbon dioxide, but the response curve became steeper in the higher ranges. No cardiac arrhythmias occurred. In general there were slight increases in pulse rate with increases in $PaCO_2$ but there were no consistent changes in mean arterial pressure.
4.4	**LD_{50}**	Refer to "4.3 Other"
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC. Studies were carried out to evaluate the response of the heart (via measurements of myocardial inotropy) to step-function alterations of inspired carbon dioxide in anaesthetised ventilated man. Studies of five human subjects were performed during constant-volume ventilation at constant levels of surgical anaesthesia and at $Paco_2$ values as high as 70 torr. (70 torr is equivalent to 9.3% CO_2). Changes in myocardial inotropy were expressed as changes in $1/PEP^2$. PEP was measured as the difference between total electromechanical time (EMT) and left ventricular ejection time (LVET), from measurements taken on the electrocardiogram (ECG) and phonocardiogram. The ECG was recorded as standard lead I or II. The microphone used for the phonocardiogram was either a Philips crystal microphone placed over the precordium, or a catheter tip microphone (U.S. Catheter Corporation) passed into the oesophagus. Four of the human subjects had either a radial or a brachial arterial catheter (Becton-Dickinson 18T Teflon, internal diameter 0.042 inch, and length 2.5 inches) inserted transcutaneously for essential monitoring. The pressure waveforms were transduced with Statham P23 Gb strain gauges. In patient 1, the arterial pulse waveform was obtained transcutaneously by placing a Statham UC3 (gold cell) force transducer over the external carotid artery. All data were recorded on a Hewlett-Packard (Sanborn) multichannel recorder. Paper speeds of 100 mm/s were used to facilitate measurements of the relevant time intervals necessary for the calculation of PEP. Times were measured to the nearest 5 ms and each measurement of PEP was derived as the mean of 10 or 20 consecutive complexes recorded during a corresponding period of apnea. No correction for blood pressure or pulse rate was considered necessary. Carbon dioxide concentrations in the inspired, end-tidal and mixed expired gases were monitored with infrared CO_2 analysers (Beckman LB-1 and Uras). $Paco_2$, pH and Pao_2 of the blood were measured directly using radiometer microelectrodes. Inspired and mixed expired halothane and oxygen concentrations were monitored with an ultraviolet halothane analyser (Hook and Tucker) and a paramagnetic oxygen analyser (Servomex), respectively. Intraesophageal temperatures were measured with a thermocouple probe (Ellab). Blood-gas results were corrected for temperature differences between the subject and the measuring electrodes, using the correction factors of Kelman and Nunn [1].

Continued.....

Section A6.1.3 **Acute Toxicity: Inhalation (4 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies

Special investigation in man.

5.1	**Materials and Methods** (Continued)	The patients studied were undergoing prolonged elective surgical operations. Anaesthesia was maintained with nitrous oxide and oxygen via endotracheal tube, and full muscle relaxation was produced with tubocurarine. Constant-volume controlled ventilation was used. The level of ventilation was initially adjusted to produce an end-tidal carbon dioxide concentration in the order of 3%. After the control readings, carbon dioxide was added to the inspired mixture in increments of 1-2% until an inspired concentration of approximately 10% was attained. Measurements were made at each inspired carbon dioxide level when an acute steady state had been obtained. This was evidenced by a constant inspired-to-end tidal carbon dioxide gradient, usually attained in 12-15 minutes. In all but patient1, recordings were also made during similar decrements of inspired carbon dioxide. [redacted]
5.2	**Results and discussion**	Carbon dioxide exerts dual effects on the cardiovascular system. Its direct effect on the heart is as a depressant, but this effect is masked by an indirect stimulant action through the sympathetic nervous system. Previous studies show that $1/PEP^2$ measurements correlate well with the myocardial inotropic state of the heart and therefore can act as an indicator of aortic blood flow acceleration. As carbon dioxide levels increased, $1/PEP^2$ measurements increased which indicates an acceleration of blood flow through the heart. When there was excessive amounts of carbon dioxide in the blood (hypercapnia), the response varied with age. The youngest subject had the greatest response, whereas the least noticeable response was in the two oldest patients. The oldest patient's $1/PEP^2$ measurements actually decreased when $PaCO_2$ increased over 7.5% carbon dioxide. In the subjects studied, the $PaCO_2$ level ranged from 2.6-9.4% Alterations in $1/PEP^2$ measurements were slight when the $PaCO_2$ levels were below 5.3% carbon dioxide, but the response curve became steeper in the higher ranges. No cardiac arrhythmias occurred. In general there were slight increases in pulse rate with increases in $PaCO_2$ but there were no consistent changes in mean arterial pressure. The results to this study give an indication about how the heart adjusts to increased carbon dioxide concentrations. For the purposes of the application of carbon dioxide as a biocide, it shows that the human body can tolerate atmospheres containing approximately 9% Carbon dioxide for at least 12 minutes without it being lethal.
5.3	**Conclusion**	
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes This study gives an indication about a tolerated dose for carbon dioxide in man, rather than determining the LC_{50}. 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 gives an indication about the level of carbon dioxide that can be tolerated by man.

Continued.....

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (4 of 14)
Section 6: Toxicological and Metabolic Studies
Special investigation in man.

5.3.2 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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{L0} values for humans and mammals have been reported to be 90,000 ppm.).

Section A6.1.3 Acute Toxicity: Inhalation (4 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (5 of 14)

Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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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.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 A6.1.3 Acute Toxicity: Inhalation (5 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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	Male.
3.2.5	Age/weight at study Initiation	Controlled respiration group (using volume limited ventilator): Age 24.2 years (+/-0.8), Height 175 cm (+/- 2), Weight 70 kg (+/-2.2) Spontaneous respiration group (same level of carbon dioxide as controlled respiration group but without a volume limited ventilator): Age 24 years (+/-0.6), Height 174 cm (+/- 1.4), Weight 70.4 kg (+/-1.4)
3.2.6	Number of animals per group	15 (controlled respiration group – using volume limited ventilator) and 26 (spontaneous respiration group – same level of carbon dioxide as controlled respiration group but without a volume limited ventilator).
3.2.7	Control animals	Each test subject acted as his own control.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other: No post-exposure period reported. **Inhalation**
3.3.8	Concentrations	Step-wise increase in carbon dioxide up to a maximum of 6.7%. No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Exposure via mouth piece.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	Controls were exposed to oxygen in an anaesthetic circle system. For test subjects, carbon dioxide was added to the oxygen to produce step-wise increases of carbon dioxide. There was an increase of 0.3-0.5% carbon dioxide each time. Maximum amount of carbon dioxide administered was 6.7%.
3.3.14	Duration of exposure	Eighteen minutes (Note there were three separate levels of carbon dioxide administered per subject, and there was six minutes exposure at each concentration of carbon dioxide)
3.3.15	Controls	Each subject was his own control with measurements being taken when breathing oxygen in an anaesthetic circle system. Once accustomed to the breathing system, carbon dioxide was administered.
3.5	**Method of determination of LD_{50}**	Other. LD_{50} not reported, but expressed as a tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	The cardiovascular response to increased carbon dioxide was analysed by linear regression. The mean carbon dioxide pressure at which regression lines began was 5.16% carbon dioxide. The mean Carbon dioxide pressure at which the last measurement was obtained was 6.7%.

Continued.....

Section A6.1.3 **Acute Toxicity: Inhalation (5 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

4.3	**Other** (Continued)	Several circulatory variables (cardiac output, cardiac index, stroke volume, stroke index, mean arterial pressure, left ventricular stroke work, left ventricular work, forearm blood flow and heart rate) were significantly increased, while one (peripheral resistance) decreased. The slopes of the various carbon dioxide response curves were similar during controlled and spontaneous respiration (a volume-limited respirator was used to control respiration in the so called controlled respiration group, where it was not used in the spontaneous respiration group). For example, although the control cardiac output was lower during controlled respiration, the responsiveness of cardiac output to carbon dioxide administration was not different.
4.4	**LD_{50}**	Refer to "4.3 Other"
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC. Forty-one healthy, young adult male volunteers fasted overnight, and the studies began the following morning. Using local anaesthesia, an arterial catheter was inserted percutaneously into the brachial or radial artery and a right atrial catheter was inserted through a 14-gauge needle into a basilic vein. In most cases, the catheter was advanced until a right ventricular pressure trace appeared and was then withdrawn until an atrial pressure wave was observed. A peripheral venous catheter was inserted into the forearm. Whitney strain gauges (mercury-filled plastic tubing) were placed about the fleshy portion of the forearm. Venous occlusion cuffs were placed at the base of the wrist and on the arm. A lead II electrocardiogram was used for recording heart rate. In 34 subjects, a precordial phonocardiogram and carotid pulse monitor were attached for determining intervals of electrical and mechanical systole. Each subject lay on an inflated air mattress, which rested on an ultra-low frequency air-bearing ballistocardiogram bed. The air mattress was deflated during ballistocardiographic and other cardiovascular measurements. Mean arterial pressure, mean right atrial pressure and peripheral venous pressure were transduced with Statham strain gauges. Duplicate cardiac outputs were obtained by dye dilution with indocyanine green using a Beckman Cardiodensitometer. Arterial Po_2, Pco_2 and pH were measured with blood-gas electrodes. Forearm blood flow was obtained by venous occlusion plethysmography with the Whitney strain gauge. The amplitude of the IJ wave of the ballistocardiogram was recorded. Oral and skin temperatures were measured with thermistors. Calculated variables included stroke volume, stroke index, cardiac index, total peripheral resistance, ejection time, mean rate of left ventricular ejection, left ventricular stroke work, left ventricular work, forearm venous compliance, forearm vascular resistance and base excess. Continued....

Section A6.1.3 Acute Toxicity: Inhalation (5 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

5.1	**Materials and Methods** (Continued)	Control measurements were obtained with the subject breathing oxygen from an anaesthetic circle system via a mouth piece. The nose was occluded by nose clips. After the subjects had become accustomed to the breathing system, ventilation of 15 of the 41 subjects were changed from spontaneous to controlled with a volume-limited ventilator. There was no sign that the subjects resisted ventilation over the range of carbon dioxide studied. End-tidal Pco_2 was monitored with an infra-red carbon dioxide analyser, and maintained at normal levels. After control measurements had been obtained, carbon dioxide was added in incremental amounts to the breathing system to produce 2-4 torr stepwise increases in $Paco_2$. (2-4 torr is equivalent to 0.3-0.5%). All measurements were repeated after equilibrating for 6 minutes at each level of $Paco_2$. At the end of 6 minutes, an arterial sample was obtained and the $Paco_2$ value was used in the regression analysis. Measurements at three separate levels of $Paco_2$ were obtained for each subject. The cardiovascular response to carbon dioxide was analysed by linear regression, comparing the 15 subjects during controlled respiration with the 26 subjects during spontaneous respiration.
5.2	**Results and discussion**	Several circulatory variables (cardiac output, cardiac index, stroke volume, stroke index, mean arterial pressure, left ventricular stroke work, left ventricular work, forearm blood flow and heart rate) were significantly increased, while one (peripheral resistance) decreased. The slopes of the various carbon dioxide response curves were similar during controlled and spontaneous respiration (a volume-limited respirator was used to control respiration in the so called controlled respiration group, where it was not used in the spontaneous respiration group). For example, although the control cardiac output was lower during controlled respiration, the responsiveness of cardiac output to carbon dioxide administration was not different. The results to this study give an indication about how the body adjusts to increased carbon dioxide concentrations. For the purposes of the application of carbon dioxide as a biocide, it shows that the human body can tolerate atmospheres containing approximately 6.7% carbon dioxide for at least six minutes without it being lethal.

Section A6.1.3 Acute Toxicity: Inhalation (5 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
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5.3	**Conclusion**	
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes

This study gives an indication about a tolerated dose for carbon dioxide in man, rather than determining the LC_{50}.

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 gives an indication about the level of carbon dioxide that can be tolerated by man.

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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{L0} values for humans and mammals have been reported to be 90,000 ppm.)

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (6 of 14)

Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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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.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]

Section A6.1.3 Acute Toxicity: Inhalation (6 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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 fully reported. Mean age 26.5 years, mean height 179 cm, mean weight 75.9 kg and mean body surface area 193 m^2.
3.2.6	Number of animals per group	Twelve.
3.2.7	Control animals	Twelve.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other: No post-exposure period reported. **Inhalation**
3.3.8	Concentrations	2% carbon dioxide. No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Nose and mouth only.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	Gas mixture for controls contained air. Test gas contained a gas mixture producing $PICO_2$ of 15 +/- 2 mmHg. (Note 15 mmHg CO_2 is equivalent to 2% CO_2)
3.3.14	Duration of exposure	Average duration of exposure: 14 minutes.
3.3.15	Controls	Each subject was his own control, having one test on air and one on increased carbon dioxide.
3.5	**Method of determination of LD_{50}**	Other. LD_{50} not reported, but expressed as a tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	This test investigated the effects of being exposed to 2% carbon dioxide on exercise tolerance in terms of maximal aerobic power (as measured by working on an exercise bike). At increased carbon dioxide concentrations, heart rate and systolic blood pressure was higher than in the controls, but this was not statistically significant. At lower and submaximal work levels, ventilation was increased, with the difference between the control and test being 40-50%, and statistically highly significant. At maximal work, however, the difference was only 2% and not statistically significant. At intermediate work levels, mean oxygen consumption was consistently slightly higher in increased carbon dioxide concentrations, but the maximal oxygen uptake was lower in test subjects than controls reflecting the fact that subjects could not perform as much work under increased carbon dioxide. In addition, carbon dioxide elimination was consistently lower on the test mixture than the controls at comparable work levels. Subjective sensations during tests with carbon dioxide, elicited after the test had been performed varied from no difference to a feeling of acute suffocation at the end point.

Section A6.1.3 Acute Toxicity: Inhalation (6 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

4.4	**LD_{50}**	Refer to "4.3 Other"
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC. Twelve subjects worked on a bicycle ergometer at an initial brake load of 300 kpm/min at 50 rpm for the first three minutes. Subsequently, the brake load was increased by 75 kpm/min every minute until the subject was unable to maintain a pedalling rhythm given by a metronome. Each subject was given his own control, with one test on air and the other with a mixture producing a $PICO_2$ of 15 +/- 2 mmHg in random sequence. With the exception of the technician operating the blood supply, neither the test subject nor investigators knew which gas was being administered. Both gases were supplied from pressure tanks through a large humidifying bottle and buffer bag with a wide-bore tubing (id. 3.4 cm) to a low resistance breathing valve. The total resistance of the valve and collecting tubing was 2.5 cm water at a flow rate of 5.1/sec. Heart rate and blood pressure were recorded each minute, and ventilation and gas exchange were derived from expired air collected at regular intervals in neoprene bags and analysed immediately by the Scholander Technique. With two exceptions, the subjects were not habitually active physically. Their mean age was 26.5 years, mean height 179 cm, mean weight 75.9 kg and mean body surface area 1.93 m^2.
5.2	**Results and discussion**	At increased carbon dioxide concentrations, heart rate and systolic blood pressure was higher than in the controls, but this was not statistically significant. At lower and submaximal work levels, ventilation was increased, with the difference between the control and test being 40-50%, and statistically highly significant. At maximal work, however, the difference was only 2% and not statistically significant. At intermediate work levels, mean oxygen consumption was consistently slightly higher in increased carbon dioxide concentrations, but the maximal oxygen uptake was lower in test subjects than controls reflecting the fact that subjects could not perform as much work under increased carbon dioxide. In addition, carbon dioxide elimination was consistently lower on the test mixture than the controls at comparable work levels. Subjective sensations during tests with carbon dioxide, elicited after the test had been performed varied from no difference to a feeling of acute suffocation at the end point. The results to this study gives an indication about how the body adjusts to increased carbon dioxide concentrations under increasing work loads. For the purposes of the application of carbon dioxide as a biocide, it shows that the human body can tolerate atmospheres containing at least 2% carbon dioxide without it being lethal.
5.3	**Conclusion**	
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes This study gives an indication about a tolerated dose for carbon dioxide in man, rather than determining the LC_{50}. 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.

Continued....

Section A6.1.3 **Acute Toxicity: Inhalation (6 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies

Special investigation in man.

5.3.2 Deficiencies (Continued)	Despite the major reporting deficiencies in this study, it gives an indication about the level of carbon dioxide that can be tolerated by man. 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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{L0} values for humans and mammals have been reported to be 90,000 ppm.)

Section A6.1.3 **Acute Toxicity: Inhalation (6 of 14)**

Annex Point IIA6.1 Section 6: Toxicological and Metabolic Studies
Special investigation in man.

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (7 of 14)
Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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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.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 A6.1.3 Acute Toxicity: Inhalation (7 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

3.2	**Test Animals**	
3.2.1	Species	Rat.
3.2.2	Strain	Albino.
3.2.3	Source	Not reported.
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	Post exposure period	Other: No post-exposure period reported.
		Inhalation
3.3.8	Concentrations	10%, 15%, 20%, 25%, 50% carbon dioxide. No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Whole body.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	Gas mixture contains desired concentration of carbon dioxide being tested with 21% oxygen.
3.3.14	Duration of exposure	Rats were exposed until they succumbed to the effects of carbon dioxide (inferred to be a maximum of 30 days if death did not occur – refer to "4.3 other" for details).
3.3.15	Controls	No information about controls reported.
3.5	**Method of determination of LD_{50}**	Other. LD_{50} not reported, but expressed as maximum tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	The maximal tolerated concentration of carbon dioxide after sudden exposure is approximately 15%. All animals survived 10% carbon dioxide indefinitely.** An occasional animal succumbed at 15% carbon dioxide. 80% of rats placed in 20% carbon dioxide died within 4 days. No animals survived in 25% carbon dioxide for more than 36 hours. 50% carbon dioxide was uniformly lethal within 6 hours. Rats were narcotised immediately when placed in concentrations of carbon dioxide above 30%. A detectable grade of depression occurred with concentrations as low as 20% although the animals were not deeply narcotised. **The term "indefinitely" is not clarified in the report. However, Barbour and Seevers did several exposure studies to carbon dioxide at the same time as this study and reported all the results in this report. One of the studies they carried out was to expose rats to 10% carbon dioxide for 30 days. The rats were exposed to the carbon dioxide in exactly the same way as they were in the study detailed in this summary, and no adverse effects were reported. It is concluded that the statement "all animals survived 10% carbon dioxide indefinitely" can be inferred as meaning rats can survive 10% carbon dioxide for a period of at least 30 days.

4.4	**LD_{50}**	Refer to "4.3 Other"

Section A6.1.3 Acute Toxicity: Inhalation (7 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods

This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC.

The acute toxicity of carbon dioxide was determined by placing animals in a closed circuit system of 1100 litres capacity consisting of a 600 litre animal chamber, a 100 litre spirometer, and two tanks of 200 litres each. The atmosphere in this system was circulated by a small blower; the concentration of oxygen was maintained at approximately 21%.

5.2 Results and discussion

The maximal concentration of carbon dioxide for the albino rat after sudden exposure is approximately 15%. Concentrations above 20% are usually lethal within 96 hours of continuous exposure when the animals are placed immediately in these concentrations of gas from normal atmospheric levels of carbon dioxide.

5.3 Conclusion

5.3.1 Reliability: 3

5.3.2 Deficiencies: Yes

This study gives an indication about the maximal tolerated dose for carbon dioxide in rats, rather than determining the LC_{50}.

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 gives an indication about the level of carbon dioxide that can be tolerated by rats.

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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{L0} values for humans and mammals have been reported to be 90,000 ppm.)

Section A6.1.3 **Acute Toxicity: Inhalation (7 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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	

Section A6.1.3 **Acute Toxicity: Inhalation (8 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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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.2.in Annex V of Directive 67/548/EEC.
2.2	**GLP**	Not reported.
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 A6.1.3 **Acute Toxicity: Inhalation (8 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

3.2	**Test Animals**	
3.2.1	Species	Rat.
3.2.2	Strain	Sprague Dawley.
3.2.3	Source	Charles River Laboratory, Boston MA.
3.2.4	Sex	Male.
3.2.5	Age/weight at study Initiation	250g – 350g.
3.2.6	Number of animals per group	Eight.
3.2.7	Control animals	Eight.
3.3	**Administration/ Exposure**	퐂싚퐂늸
3.3.1	Post exposure period	Two hours. Note that the animals, following the two hour post-exposure observation period were returned to their cages in the animal room where body weights were recorded for at least 14 days.
		Inhalation
3.3.8	Concentrations	5% (Analytical concentration: 52200 ppm or 5.22%).
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Nose-only exposure.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	Test animals were exposed to "medical grade air" containing 5% carbon dioxide and 19.5% oxygen.
3.3.14	Duration of exposure	30 minutes.
3.3.15	Controls	Test animals were exposed to "medical grade air" containing 0.5% carbon dioxide and 20.4% oxygen for 30 minutes.
3.5	**Method of determination of LD_{50}**	Other LD_{50} not reported, but expressed as a tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	No mortalities were recorded following 30 minutes exposure to medical grade air containing 5% carbon dioxide and 19.5% oxygen.
4.4	**LD_{50}**	Refer to "4.3 Other"

Section A6.1.3 **Acute Toxicity: Inhalation (8 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods

This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC.

The purpose of the study detailed in this summary was to determine the toxicity of various concentrations of nitrogen dioxide and carbon monoxide. The animals being exposed to 5% carbon dioxide and 19.5% oxygen in "medical grade air", and animals exposed to "medical grade air" containing 0.5% carbon dioxide and 20.4% oxygen were acting as controls for this study. Despite this, this data is useful to determine the toxicity of carbon dioxide (given that the normal environmental concentration of carbon dioxide is 0.03% v/v).

Given that the aim of this study is to determine the toxicity of various concentrations of nitrogen dioxide and carbon monoxide, the method detailed below includes details about exposure to nitrogen dioxide and carbon monoxide as well as carbon dioxide (acting as the control for the CO and NO_2 exposure study).

Mature male Sprague Dawley rats weighing 250 – 350 g at time of exposure were randomised and segregated into groups of eight animals per group (identified by eartags). The animals were allowed to acclimate for one week prior to exposure housed in individual micro-isolators. Animals were weighed at least every two days and monitored for normal growth.

The exposure chamber consisted of a 134 litre plexiglas box, about 4 feet long 12 inches wide and 16 inches high. Reagent grade gases were combined at atmospheric pressure in a four-channel stainless steel mixer to the pre-selected composition and delivered to the inlet port on the chamber via 0.250" 1D Teflon tubing.

The exposure chamber contained an 8-litre chamber, which was gasketed from the exposure atmosphere. This inner box contained in the inlet portholes into which the animal restrainers were placed. These animal restrainers (Lexan, part number 70054 sleeve fitted with part number 70057 stainless steel tail tube/back plate) are designed for use in a flow-through nose only exposure chamber. Medical grade breathing air was pumped through this chamber at a flow rate of 12 to 18 cubic feet per minute while the animals were being loaded into the chamber prior to exposure. Animals were placed in restrainers as quickly as possible to minimise stress and loaded into the exposure chamber at once. The restrainers were adjusted such that the animals nose was positioned firmly into the inlet port.

The composition of the atmosphere inside the chamber was constantly monitored by gas analysers. Individual pumps on the analysers drew air samples through a six port sampling manifold made of non-reactive plastics and into the appropriate analyser cell. After analysis, the air was returned to the chamber via return ports at the top of the chamber. Excess air flow in the sampling manifold was returned to the chamber via a return inlet on the bottom of the chamber. This arrangement maintained constant mixing while simulating a closed atmosphere.

Continued.....

Section A6.1.3 **Acute Toxicity: Inhalation (8 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

5.1 Materials and Methods

(Continued)

The following gases (in the exposure chamber atmosphere) were monitored : % oxygen by electrochemical analyser, % carbon dioxide by infrared analyser, carbon monoxide, ppm levels, by non-dispersive infrared-ultraviolet IR-UV spectrophotometer and nitrogen dioxide, ppm levels by dual-beam infrared-ultraviolet (IR-UV) spectrophotometer. Atmospheres were also sampled for contamination by nitrogen monoxide (NO) using dual beam infrared-ultraviolet (IR-UV) spectrophotometer. NO was not detected as a contaminate in any of the exposures.

The chamber was fitted with a remotely operated door, which was released, dropping to the floor of the chamber and allowing the test atmosphere to contact the animals. The thirty minute exposure time started at the moment the door was dropped. The test atmosphere, as monitored by the various gas analysers equilibrated in less than 90 seconds.

Eight animals were exposed in each test. No animal was exposed more than once. Additional sets of animals were run as controls, breathing only medical grade air, or medical grade air containing 5% carbon dioxide.

On the day before the planned exposure, two of the eight test animals had been fitted with a catheter in the caudal tail artery in order to take blood samples at various times during exposure. The results of the blood analysis have not been reported, so the methodology used to obtain the blood samples is not detailed here.

Of the remaining six animals, three were selected at random and placed in restrainers which had been modified with adapters to receive a linear pneumotachometer (model 8411B non-heated pneumotachometer). The modification of the restrainer allowed it to be used as a partial body phythesmography enclosure – the deflections of the chest wall during respiration were recorded as changes in the pressure across the screens in the pneumotach housing. The inlet and outlet ports on the pneumotachs were connected to low range differential pressure transducers MP45-20-817. Pressure flow data was collected at a rate of 40 samples/second/channel and recorded using a computerised data acquisition system from DATAQ Instruments. Complete respiratory curves were recorded for the three animals throughout the 30 minutes exposure duration. Respiration rate and time of death were obtained from these recordings.

In order to determine pulmonary damage after exposure, lung weights were done on selected animals, both in controls and in exposures involving nitrogen dioxide either alone in air or combined with carbon monoxide. The results of the lung weight analysis have not been reported, so the methodology used to obtain the lung weights is not detailed here.

At the conclusion of the 30 minute exposure time, all eight animals were quickly removed from the chamber and removed to normal air. The non catheterised animals were removed from the restrainers, visually assessed for physical condition and returned to individual cages in the laboratory. The animals were closely monitored for two hours following exposures as it was noted that deaths typically occurred within or shortly after exposure. No deaths in any of the exposures occurred more than 24 hours post exposure.

(Continued....)

Section A6.1.3		**Acute Toxicity: Inhalation (8 of 14)**
Annex Point IIA, VI, 6.1.3		Section 6: Toxicological and Metabolic Studies Special investigation in rats.
5.1	**Materials and Methods** (Continued)	At the end of the two hour post-exposure observation period, the animals were returned to their microisolator cages in the animal room. Body weights were recorded daily for at least 14 days following the exposure, in some cases weights were tracked for 30 to 45 days.
5.2	**Results and discussion**	No mortalities were recorded following 30 minutes exposure to medical grade air containing 5% carbon dioxide and 19.5% oxygen.
5.3	**Conclusion**	
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes This study indicates that 5% carbon dioxide is not lethal for rats following 30 minutes exposure, rather than determining the LC_{50}. 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 gives an indication about a minimum level of carbon dioxide that can be tolerated by rats for at least 30 minutes. 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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{LO} values for humans and mammals have been reported to be 90,000 ppm).

Section A6.1.3 **Acute Toxicity: Inhalation (8 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (9 of 14)

Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

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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.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 A6.1.3 Acute Toxicity: Inhalation (9 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

3.2	**Test Animals**	
3.2.1	Species	Dog.
3.2.2	Strain	Mongrel.
3.2.3	Source	Not reported.
3.2.4	Sex	Not reported.
3.2.5	Age/weight at study Initiation	Not reported.
3.2.6	Number of animals per group	One group of 15, and one group of 2.
3.2.7	Control animals	Not reported.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other. No post-exposure period reported.
		Inhalation
3.3.8	Concentrations	10%, 15%, 20%, 25%, 50% carbon dioxide. No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Animals were exposed to test atmosphere through a tracheotomy tube connected in an open system to a 120 litre spirometer containing the gas being tested.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	30% carbon dioxide: Gas mixture contained 30 % carbon dioxide in oxygen. 40% carbon dioxide: 100% carbon dioxide gas added to a gas mixture containing 30 % carbon dioxide in oxygen, to maintain a concentration of 40% carbon dioxide.
3.3.14	Duration of exposure	2 hours exposed to 30% carbon dioxide, followed by 2 hours exposed to 40% carbon dioxide then returned to normal air.
3.3.15	Controls	No information about controls reported.
3.5	**Method of determination of LD_{50}**	Other LD_{50} not reported, but expressed as an observation of the effects of exposure to 30% carbon dioxide for 2 hours in dogs followed by exposure to 40% carbon dioxide for a further 2 hours and then returned to normal air.
3.6	**Further remarks**	None.

Section A6.1.3 **Acute Toxicity: Inhalation (9 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

4. RESULTS AND DISCUSSION

4.1 Clinical signs Refer to "4.3 Other"

4.2 Pathology Refer to "4.3 Other"

4.3 Other Of the 17 dogs used in this study 15 were changed suddenly from high carbon dioxide to air, while 2 were gradually reduced from 40% carbon dioxide to air over a period of 30 minutes.

Cardiac arrhythmias appeared within 30 seconds to 6 minutes after the change from carbon dioxide to air in all of the 15 dogs subjected to rapid change, and ventricular fibrillation and death occurred in 2.5 to 10 minutes in 11 of these dogs.

Neither of the two dogs in which the carbon dioxide concentration was gradually lowered to that of air showed any cardiac irregularities of note, and both survived the procedure.

No cardiac irregularities, except the occasional extrasystole were observed in any of the dogs during the first 5 minutes of breathing 30% carbon dioxide. Mechanical hyperventilation of an anaesthetised dog before administration of carbon dioxide also failed to produce any cardiac arrhythmias.

Arterial blood pH and plasma carbon dioxide concentration was determined on five test animals. Blood pH fell from an average of 7.36 in the anaesthetised dog breathing air, to 6.67 at the end of the four hours breathing the high concentrations of carbon dioxide. Carbon dioxide tensions in blood plasma rose from the control average of 43.2 mm Hg to 295 mm Hg on 40% carbon dioxide. Determination of blood pH at 30 second intervals following the change from carbon dioxide to air showed the pH rising rapidly and reaching a value of approximately 7.3 within 3 to 4 minutes.

4.4 LD_{50} Refer to "4.3 Other"

5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC.

Mongrel dogs were anaesthetised with 30 mg/kg body weight of Pentothal sodium, administered intravenously. Tracheotomy was performed, and if blood pressure was to be recorded, the femoral artery was cannulated. The dog was connected in an open system to a 120 litre spirometer containing 30% carbon dioxide in oxygen and weight so as to keep a 5 litre rubber anaesthesia bag, interposed between the spirometer and tracheotomy tube partially inflated. The spirometer was kept filled from a cylinder of compressed gas.

At the end of two hours on this mixture, the concentration of carbon dioxide in the spirometer was increased to approximately 40% by admitting 100% carbon dioxide along with 30% mixture at the proper rate to maintain approximately this concentration. The composition of the gas in the spirometer was checked at intervals, using a Scholander rapid gas analyser.

After two hours on 40% carbon dioxide, the dog was suddenly changed to breathing air and hyperventilated manually by means of an anaesthesia bag or mechanically by an artificial respiration pump.

Electrocardiograms were made with a direct writing instrument before the carbon dioxide breathing began, continuously during the first five minutes of 30% carbon dioxide administration, at intervals during the carbon dioxide breathing, and continuously following the change from carbon dioxide to air until ventricular fibrillation

occurred or the record appeared to be returning to normal.

Continued...

Section A6.1.3 Acute Toxicity: Inhalation (9 of 14)

Annex Point IIA, VI, 6.1.3

Section 6: Toxicological and Metabolic Studies

Special investigation in dogs.

5.1 Materials and Methods

(Continued)

When blood pH and plasma carbon dioxide content determinations were to be made, arterial blood samples were drawn after the dog was anaesthetised and breathing air, at the end of 2 hours breathing 30% carbon dioxide and just before changing from 40% carbon dioxide to air. Blood pH was determined with a specially constructed gas electrode pH meter, maintained at the temperature of the dog. Plasma carbon dioxide content was determined with the Van Slyke manometric apparatus.

In two of the experiments, the dog was connected to a closed system containing oxygen, and the carbon dioxide was allowed to accumulate slowly to approximately the same concentrations as were used in the other experiments. Oxygen was admitted to this system as needed.

5.2 Results and discussion

Ventricular fibrillation and death have been produced in 11 of the 15 dogs by a rapid reduction in alveolar carbon dioxide tension following four hours of breathing 30% to 40% carbon dioxide in oxygen. Cardiac arrhythmias appeared in the 4 dogs that survived the procedure. Two dogs subjected to the same high concentration of carbon dioxide for the same length of time showed no cardiac arrhythmias and survived the procedure when the alveolar carbon dioxide concentration was reduced slowly.

It is therefore concluded that rapid reduction in alveolar carbon dioxide tension could cause death, for example following surgery when carbon dioxide concentrations increase during anaesthesia.

5.3 Conclusion

5.3.1 Reliability: 3

5.3.2 Deficiencies: Yes

This study gives an indication about the effects of exposure to 30% carbon dioxide for 2 hours in dogs, followed by exposure to 40% carbon dioxide for a further 2 hours and then returned to normal air.

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 gives an indication about the level of carbon dioxide that can be tolerated by dogs.

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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats tolerate exposure to 100,000 ppm (10%) carbon dioxide, LC_{LO}

	values for humans and mammals have been reported to be 90,000 ppm).

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (10 of 14)

Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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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.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 A6.1.3 Acute Toxicity: Inhalation (10 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

3.2	**Test Animals**	
3.2.1	Species	Rat.
3.2.2	Strain	Sherman.
3.2.3	Source	Not reported.
3.2.4	Sex	Male.
3.2.5	Age/weight at study Initiation	Age of test animals not reported. Weight of test animals were 205g +/- 8g.
3.2.6	Number of animals per group	17 rats exposed for 3h, 15 rats exposed for 5h.
3.2.7	Control animals	16.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other: No post-exposure period reported.
		Inhalation
3.3.8	Concentrations	20% carbon dioxide. No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Whole body.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	20% carbon dioxide in 25% oxygen and 55% nitrogen.
3.3.14	Duration of exposure	3h and 5h
3.3.15	Controls	Control animals breathed room air for 3h and 5h.
3.5	**Method of determination of LD_{50}**	Other. LD_{50} not reported, but expressed as a tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	After exposure to 20% carbon dioxide for either 3h or 5h, the animals were sacrificed and their adrenal, heart, salivary glands and brain were removed. The adrenal catecholamine content of rats breathing 20% carbon dioxide for 3-5h was not significantly different from the controls. The norepinephrine content of the heart did not change significantly after exposure to 20% carbon dioxide for 3-5h, neither did the norepinephrine content of the salivary gland and brain or dopamine content of the brain.
4.4	**LD_{50}**	Refer to "4.3 Other"

Section A6.1.3 Acute Toxicity: Inhalation (10 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods

This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC.

Studies were carried out to investigate the effect of increased carbon dioxide of catecholamine stores in the body. Catecholamine is required to maintain vital physiological functions during prolonged exposure to elevated carbon dioxide levels.

Male Sherman rats, weighing 205 +/-8g were fasted for 18 hours prior to the test, and given water *ad libitum.* These animals were placed in plastic chambers having a volume of 3.3 litres. There were three rats in each chamber. Each chamber, with an inlet, an outlet and snug fitting cover was connected in parallel and attached to a specially constructed manifold which had been calibrated so that at 25 psi the gas flow through each container was 3.3.litres/minute. The manifold was equipped with a switch, which allowed a simultaneous flushing of all the chambers. Hypercapnia (exposure to increased carbon dioxide levels) was produced in rats with a mixture of 20% carbon dioxide, 25% oxygen, and 55% nitrogen. A control group of animals breathing room air for the same period of time was also studied.

Two groups of rats inhaled the test gas mixture for 3h (17 rats), and 5h (15 rats). 16 control animals breathed room air for the same period of time. The test animals were sacrificed after 3h and 5h by the use of 30mg diabutal, administered by intraperitoneal injection. The adrenal, heart, salivary glands and brain were removed and frozen until analysis.

Tissue catecholamines were extracted with 0.4N perchloric acid, absorbed, eluted from alumina and measured fluorophotometrically (as described by Neff and Costa [1]). Adrenal norepinephrine and epinephrine content were added and expressed as the total adrenal catecholamine content. The same extraction and purification procedure was used for the labelled catecholamines, and the radioactivity was assayed in a Packard tri-carb liquid scintillation spectrometer. The data was analysed for significance according to the Student "t" test.

[redacted]

5.2 Results and discussion

The adrenal catecholamine content of rats breathing 20% carbon dioxide for 3-5h was not significantly different from the controls. The control levels were 55.6 +/- 2.9 µg/kg compared to that of 54.9 +/- 2.5 µg/kg and 54.9 +/- 2.7 µg/kg in animals after 3h and 5h of hypercapnia. The control norepinephrine content of the heart was 0.80 +/- 0.05 µg/g and it did not change significantly after exposure to 20% carbon dioxide for 3-5h, neither did the norepinephrine content of the salivary gland and brain or dopamine content of the brain.

Catecholamine is required to maintain vital physiological functions during prolonged exposure to elevated carbon dioxide levels. The results to this study suggest that because catecholamine content of the adrenals was maintained in hypercapnia even though the hypercapnic state causes large releases of catecholamine into the body, synthesis of catecholamine must have increased when in the hypercapnic state in order to maintain levels in the adrenals.

These results show that 20% carbon dioxide can be tolerated for at least 5h by rats without it being lethal

Section A6.1.3 Acute Toxicity: Inhalation (10 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

5.3	**Conclusion**	
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes

This study gives an indication about a tolerated dose for carbon dioxide in rats, rather than determining the LC_{50}.

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 gives an indication about the level of carbon dioxide that can be tolerated by rats.

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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{L0} values for humans and mammals have been reported to be 90,000 ppm.).

Section A6.1.3 **Acute Toxicity: Inhalation (10 of 14)**
Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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	

Section A6.1.3 **Acute Toxicity: Inhalation (11 of 14)**
Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs..

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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.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 A6.1.3 **Acute Toxicity: Inhalation (11 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

3.2	**Test Animals**	
3.2.1	Species	Dog.
3.2.2	Strain	Beagle.
3.2.3	Source	Not reported.
3.2.4	Sex	Male and female.
3.2.5	Age/weight at study Initiation	Age of test animals not reported. Average weight of test animals: 8.4 kg
3.2.6	Number of animals per group	Six.
3.2.7	Control animals	Six.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other: No post-exposure period reported. **Inhalation**
3.3.8	Concentrations	10 % carbon dioxide. No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Via an endotracheal tube into the nose and/or mouth.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	10% carbon dioxide and 25% oxygen in nitrogen.
3.3.14	Duration of exposure	60 minutes.
3.3.15	Controls	Each test animal acted as it's own control prior to commencement of the test.
3.5	**Method of determination of LD_{50}**	Other. LD_{50} not reported, but expressed as a tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	Graph 1 at the end of this study summary shows that exposure to 10% carbon dioxide for 60 minutes did not significantly change oxygen uptake. At the lowest pH values of 7.02 there was a significant increase of blood glucose above the control value (+ 24 mg/100 ml, $P < 0.005$) whereas free fatty acids and glycerol did not change. Thirty minutes after the end of hypercapnia, when both the pCO_2 and pH had returned to control values, the oxygen uptake rose significantly (+19%, $P = 0.04$). Free fatty acids increased by 150% ($P < 0.003$) and glycerol by 190% ($P < 0.002$). Sixty minutes after the end of acidosis, oxygen uptake was still significantly increased above control (+25%, $P = 0.006$) as were free fatty acids (+140%, $P = 0.02$) and glycerol (+150%, $P = 0.05$%). Cardiovascular changes are also shown in Graph 1. During hypercapnia both heart rate and mean blood pressure fell significantly ($P = 0.01$ and 0.05, respectively). After acidosis, no significant change from control was found.
4.4	**LD_{50}**	Refer to "4.3 Other"

Section A6.1.3 **Acute Toxicity: Inhalation (11 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods

This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC.

6 pedigree, healthy young beagles of both sexes (average weight 8.4 kg) were used for this study. The dogs had fasted for 18h prior to study commencement, and at the beginning of the procedure sedimentation rate of these dogs never exceeded 2-20 mm/hr, and mid-oesophageal temperature was between 36 and 37°C.

The test animals were anaesthetised with an intravenous dose of sodium pentobarbital (30 mg/kg) intubated with a cuffed endotracheal tube and mechanically ventilated at constant rate and volume. Polyethylene catheters were inserted in the arterial and venous femoral vessels. Muscular paralysis was induced with an initial dose of 4 mg succinylcholine chloride infused by intravenous catheter and maintained throughout the procedure at a constant rate of 250 µg/min. The dogs were ventilated with room air for at least 1 h after the administration of anaesthetic before control measurements were performed. Ventilation was adjusted to maintain arterial blood pH close to 7.40. Environmental temperature was kept within 1°C during the procedure.

After the control period, hypercapnia was induced by ventilation with a gas mixture of 10% carbon dioxide and 25% oxygen in nitrogen and maintained for 60 minutes. Observations were continued for 60 minutes after the end of acidosis. Blood samples and ventilatory measurements were taken just before the acidosis, at the 30th and 60th minute of acidosis and at the 30th and 60th minutes after the end of high-carbon dioxide breathing.

Mean blood pressure and pulse rates were continuously recorded by means of a Statham pressure transducer and a grass model 5 multichannel recorder. During all experiments recordings were read every 5 minutes and the average of three measurements was calculated and used as the final value. Midesophageal temperature was measured continuously and recorded on the Grass recorder and maintained constant within 1°C by means of a heating pad.

The expired air was collected during a 9 minute period in a Tissot spirometer and the minute ventilation was calculated from the spirogram and corrected to standard temperature and pressure, dry. Two gas-collecting periods were used for each change of the procedure, i.e. two in the control period, two during acidosis and two at the 30th and 60th minute after acidosis. Samples were taken from the Tissot spirometer at the end of each collection period and oxygen and carbon dioxide concentrations were determined with a Scholander microgasometer. Each determination was made in tri- or quadruplicate and the average was calculated (the oxygen and carbon dioxide concentrations of each gas tank were measured in quadruplicate by the same procedure).

Oxygen uptake was calculated according to the formula given by the Pappenheimer Committee[1]. Each absolute value represents the average of two determinations. Results are expressed in millimetres per kilogram per minute, and changes are expressed as percentage

(Continued....)

Section A6.1.3 **Acute Toxicity: Inhalation (11 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

5.1 Materials and Methods

(Continued)

Increase from the control value. Samples of arterial blood for acid-base measurements were taken anaerobically in heparinized syringes. The samples were immediately stored in ice and the determinations were made within 3 minutes after collection. The pH was measured with an Astrup microglass electrode and a Radiometer pH meter, and Pco_2 was determined by the Astrup triple pH method and the Sigaard-Andersen nomogram[2]. In addition, an arterial blood sample, drawn immediately after, was separated in a refrigerated centrifuge. Plasma glucose was determined by the enzymatic method [3,4], free fatty acids by a colorimetric method [5]. Glycerol was measured according to the enzymatic method as modified by Vaughn [3,4].

The determinations were made in duplicate and the control values are the average of two samples. After each collection of blood, which averaged 10 ml, saline was infused to replace the blood volume taking into account the volume of the infusions. No heparin was used during the procedure except a small amount in the saline filing the catheters before their insertion at the beginning of the experiment.

Statistical evaluation of the results was made by the Student *t* test. The significance of differences between paired measurements was calculated by the method of difference between correlated pairs. *P* was evaluated within 5% limit of confidence with N – 2 degrees of freedom. Figures refer to mean values +/- SE and mean pair differences (MPD) were calculated from the control.

5.2 Results and discussion

The increase in oxygen uptake following the termination of hypercapnia indicates that when pH is returned to normal, the catecholamines endogenously released during acidosis are able to exert their full metabolic activity. The inhibition of acidosis of the metabolic effects of catecholamines endogenously released by hypercapnia might indicate the presence of a regulatory mechanism by which {H+] is one of the factors controlling the release and activity of catecholamines.

The results to this study give an indication about how the body adjusts to increased carbon dioxide concentrations. For the purposes of the application of carbon dioxide as a biocide, it shows that dogs can tolerate exposure to atmospheres containing approximately 10% carbon dioxide for at least 60 minutes without it being fatal.

5.3 Conclusion

5.3.1 Reliability 3

5.3.2 Deficiencies Yes

This study gives an indication about a tolerated dose for carbon dioxide in dogs, rather than determining the LC_{50}.

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.

(Continued.. ...)

Rentokil Initial plc	Carbon Dioxide	March 2004
Section A6.1.3 **Annex Point IIA, VI, 6.1.3**	**Acute Toxicity: Inhalation (11 of 14)** Section 6: Toxicological and Metabolic Studies Special investigation in dogs.	
5.3.2 Deficiencies (Continued)	Despite the major reporting deficiencies in this study, it gives an indication about the level of carbon dioxide that can be tolerated by dogs. 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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{L0} values for humans and mammals have been reported to be 90,000 ppm.).	

Section A6.1.3 **Acute Toxicity: Inhalation (11 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (12 of 14)
Section 6: Toxicological and Metabolic Studies
Special investigation in dogs..

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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.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 A6.1.3 **Acute Toxicity: Inhalation (12 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

3.2	**Test Animals**	
3.2.1	Species	Dog.
3.2.2	Strain	Mongrel.
3.2.3	Source	Not reported.
3.2.4	Sex	Male.
3.2.5	Age/weight at study Initiation	Age of test animals not reported. Weight of test animals ranged from 11- 19 kg.
3.2.6	Number of animals per group	10, 8 and 7.
3.2.7	Control animals	10.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other: No post-exposure period reported.
		Inhalation
3.3.8	Concentrations	2.5%, 5%, 10%, 20% and 30% carbon dioxide. No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Whole body.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	Levels of carbon dioxide as specified in question 3.3.8, in air.
3.3.14	Duration of exposure	Ten dogs were subjected to concentrations of 2.5%, 5% and 10% carbon dioxide for successive periods of one hour. Eight dogs exposed to 10%, 20% and 30% carbon dioxide successively for 1 hour each. Seven dogs were subjected to 20% carbon dioxide for four hours.
3.3.15	Controls	For control measurements, 10 animals were exposed to room air for 4 hours.
3.5	**Method of determination of LD_{50}**	Other. LD_{50} not reported, but expressed as a tolerated dose.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	Ten dogs were subjected to concentrations of 2.5%, 5% and 10% carbon dioxide for successive periods of one hour. An increase in adrenal 17-hydroxycorticosteroid secretion occurred in one animal at the 2.5% carbon dioxide level, in three additional dogs at the 5% carbon dioxide level, and in three more dogs at the 10% level (refer to graph 1, group 1 at the end of this study summary for details). Thus by the end of the 10% exposure period, 7 of the 10 animals had displayed an adrenocortical stimulation. In a second group of 8 dogs, who were exposed to 10%, 20% and 30% carbon dioxide successively for 1 hour each, increased adrenal corticosteroid occurred in six dogs at the 10% level and in all dogs at the 20% and 30% levels (refer to graph 1, group II at the end of this study

(Continued.....)

Section A6.1.3 **Acute Toxicity: Inhalation (12 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

4.3	**Other** (Continued)	summary for details). Adrenocortical stimulation, in these animals, is defined as an increase in adrenal 17-hydoxysteroid secretion over control values persisting for at least 30 minutes. The adrenocortical response occurring in dogs exposed to 10%, 20% and 30% carbon dioxide was usually maximal (as determined by comparison with the response following injection of 20 IU ACTH) and persisted throughout the exposure periods. The adrenal stimulation which occurred in 2.5% and 5% were submaximal and transitory in nature (30-45 minutes). Of interest is the finding that an equal percentage of dogs displayed an adrenocortical stimulation when subjected immediately to 10% carbon dioxide (refer to graph 1, group II at the end of this study summary for details) as did those exposed to lower concentrations of carbon dioxide (refer to graph 1, group 1 at the end of this study summary for details). The changes in arterial pH and PCO_2 which occurred in these 18 dogs during the periods of carbon dioxide exposure are illustrated in graph 2 at the end of this study summary. It was found in these experiments that the maximal alterations in arterial pH and PCO_2, induced by exposure to a given concentration of carbon dioxide for 1 hour occurred within the first 30 minutes. Exposure to increasing concentrations of carbon dioxide resulted in progressively severe respiratory acidosis. In each animal exposed to 20% carbon dioxide for 4 hours, 20 IU ACTH (a dose sufficient to induce maximal adrenocortical stimulation) was injected intravenously on the day prior to the carbon dioxide exposure in or to determine the maximal adrenal 17-hydroxycorticosteroid secretory level. On the following day, the animals were placed in the chamber, a number of arterial and adrenal venous blood samples were collected and the carbon dioxide concentration in the chamber was then increased to 20% in a period of 3-5 minutes. After 15 minutes, a marked increase in adrenal corticoid secretion had occurred and at 30 minutes maximal output was attained. This marked stimulation persisted for the entire 4 hours exposure, and at the end of the period no additional increase in adrenal 17-hydroxycorticosteroid output could be elicited by the intravenous administration of 20 IU ACTH. In each animal the adrenocortical response was accompanied by a marked decrease in arterial pH and increase in arterial carbon dioxide tension.
4.4	**LD_{50}**	Refer to "4.3 Other"
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC. Male mongrel dogs ranging in weight from 11- 19 kg were used in these studies. <u>Preparation of animals.</u> Each dog was anaesthetised with pentobarbital sodium and the right lumbo-adrenal vein was cannulated according to the procedure described by Hume and Nelson. This preparation permits either continuous or intermittent collection of the total venous effluent of the right adrenal gland. A carotid artery was also cannulated for the collection of arterial blood samples. The animals were then allowed to recover for a period of 24 hours.

Section A6.1.3 **Acute Toxicity: Inhalation (12 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

5.1 Materials and Methods

(Continued).

Exposure to carbon dioxide

On the day following surgery, 30 dogs were re-anaesthetised with pentobarbital sodium and placed in a large sealed chamber. Approximately 90 minutes after anaesthetisation control arterial and adrenal venous blood samples were collected during a period of 30 minutes, at which time the dogs were breathing room air. The animals were then exposed to various concentrations of carbon dioxide. The oxygen concentration in the chamber was kept at 21% and the chamber was kept at atmospheric pressure. Oxygen concentration in the chamber was measured by continuous sampling through a Beckman model F-3, oxygen analyser. A Liston-Becker infrared carbon dioxide analyser model 16 was used to monitor the chamber atmosphere and activate a system for maintaining the carbon dioxide at a selected level. Chamber temperatures ranged between 24°C - 25°C. Duplicate adrenal venous blood samples were collected at 15-minute intervals and single arterial blood samples at either 15 or 30 minute intervals throughout the carbon dioxide exposure periods.

Control experiments

On the day after surgery, 10 dogs were re-anaesthetised with pentobarbital and arterial and adrenal blood samples were collected intermittently for a period of 4 hours, during which the animals breathed room air.

Adrenal bloods

All adrenal blood samples were obtained by collecting the total venous effluent of the right adrenal gland for a period of either 30 or 60 seconds. Each sample was analysed for 17-hydroxycorticosteriods by the method of Nelson and Samuels as modified by Nelson and Hume[1,2].

Arterial bloods

Arterial bloods were collected under anaerobic conditions and the plasma of each sample analysed for carbon dioxide content in the Van Slyke manometric apparatus. Plasma pH was determined with a Beckman pH meter, model G.

Body temperature

As it is known that alterations in body temperature exert a marked influence upon adrenocortical function, frequent temperature readings were obtained from indwelling thermometers inserted 6 inches into the rectum of each dog. The range of rectal temperature in these animals, during the period of experimentation, was from 36.5°C to 39°C.

1. Nelson DH and LT Samuels J Clin Endocrinol 12: 519, 1952.
2. Nelson DH and DM Hume Endiocrinology 57: 184, 1955.

5.2 Results and discussion

The results from dogs exposed to 2.5%, 5%, 10%, 20% and 30% carbon dioxide indicate that the incidence and magnitude of adrenal corticortical response are directly related to the concentration of carbon dioxide. All dogs exposed to 20% and 30% carbon dioxide displayed a maximal increase in adrenal corticoid secretion. Exposure to increasing concentrations of carbon dioxide was accompanied by an increasing magnitude of respiratory acidosis. Adrenocortical stimulation occurred in all dogs with an arterial pH of 7.00 or less and an arterial PCO_2 of 100 mm Hg or greater, in 8 of 13 animals (62%) with an arterial pH of 7.00- 7.20 and PCO_2 of 70-100

	mmHg, and in 4 of the 10 dogs (40%) with an arterial pH range of 7.20-7.40 and PCO_2 range of from 40-70 mmHg. (Continued....)	

Section A6.1.3 **Annex Point IIA, VI, 6.1.3**	**Acute Toxicity: Inhalation (12 of 14)** Section 6: Toxicological and Metabolic Studies Special investigation in dogs.	
5.2 Results and discussion (Continued....)	Dogs exposed to 20% carbon dioxide for 4 hours respond with a rapid (within 15 minutes) and marked (5- to 40 fold) increase in adrenal 17-hydorxycorticosteriod secretion, which persists for the entire 4 hour exposure period. This adrenal stimulatory response is maximal as determined by comparison with adrenal corticoid output following ACTH administration, before and during carbon dioxide exposure. These findings show that pentobarbital anaesthesia does not impair the magnitude of adrenaocortical response in dogs exposed to 20% carbon dioxide. That the rate of response is similarly unaffected is borne out by the rapidity with which stimulation of the adrenal cortices of these animals occurred (within 15 minutes after the carbon dioxide concentration had reached 20%). Arterial oxygen content was determined in 6 of the 7 dogs exposed to 20% carbon dioxide (oxygen concentration 21%) for 4 hours. The mean oxygen content in arterial blood of these animals during the control period was 14.7% vol. % (11.9 - 18.6). Thirty minutes after exposure to 20% carbon dioxide in air, the arterial oxygen content actually increased slightly (0.5 to 1.3 vol. %) in all six animals. After 4 hours exposure this finding was again noted and is most likely attributable to hyperventilation during exposure to carbon dioxide. As it was found that a rapid and prolonged increase in adrenal 17-hydorxycorticosteriod output occurred in these animals, and since there was no evidence of hypoxia, it is concluded that exposure to 20% carbon dioxide (in 21% oxygen) is a potent adrenocortical stimulus in dogs, and that a concomitant decrease in arterial oxygen does not occur and is not required for this response. The results to this study give an indication about how the body adjusts to increased carbon dioxide concentrations. For the purposes of the application of carbon dioxide as a biocide, it shows that dogs can tolerate exposure to atmospheres containing up to 30% carbon dioxide for at least one hour, and 20% carbon dioxide for up to 4 hours without it being fatal.	

Section A6.1.3 Acute Toxicity: Inhalation (12 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

5.3	**Conclusion**	
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes

This study gives an indication about a tolerated dose for carbon dioxide in dogs, rather than determining the LC_{50}.

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 gives an indication about the level of carbon dioxide that can be tolerated by dogs.

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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement, giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats can tolerate exposure to 100,000 ppm carbon dioxide (10%), LC_{L0} values for humans and mammals have been reported to be 90,000 ppm.).

Section A6.1.3 **Acute Toxicity: Inhalation (12 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in dogs.

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (13 of 14)
Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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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.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 A6.1.3 Acute Toxicity: Inhalation (13 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

3.2	**Test Animals**	
3.2.1	Species	Rat.
3.2.2	Strain	Wistar Albino.
3.2.3	Source	Not reported.
3.2.4	Sex	Male.
3.2.5	Age/weight at study	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	Post exposure period	Other: No post-exposure period reported.
		Inhalation
3.3.8	Concentrations	100% carbon dioxide. No analytical concentration reported.
3.3.9	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.11	Type of exposure	Exposure is via inhalation but whether the exposure was whole body or nose only has not been specified.
3.3.12	Vehicle	Gas.
3.3.13	Concentration in vehicle	100% carbon dioxide gas.
3.3.14	Duration of exposure	30 minutes.
3.3.15	Controls	No information about controls reported.
3.5	**Method of determination of LD_{50}**	Other LD_{50} not reported, but expressed as an observation of the effects of exposure to 100% carbon dioxide gas after 30 minutes.
3.6	**Further remarks**	None.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3 Other"
4.2	**Pathology**	Refer to "4.3 Other"
4.3	**Other**	Rats inhaling carbon dioxide died between 10 to 30 minutes after exposure. Upon examination under a light and electron microscope, the rats' lungs were seen to be dark red in colour and markedly shrunk. Electron microscope observations showed the alveoli of the lungs to have an irregular wavy appearance, reflecting the shrinkage of the lung due to the decreased air in the alveoli. A large number of substances containing tubular myelin, membranous structures and fibrin fibres were found, and erythrocyte and thrombocyte fragments were seen occasionally in the in the alveolar cavity. These observations were consistent between those rats that died at the beginning of exposure, and those which died after 30 minutes exposure.
4.4	**LD_{50}**	Refer to "4.3 Other"

Section A6.1.3 Acute Toxicity: Inhalation (13 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods

This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC.

The Wistar albino male rats were put into an observation box to inhale air containing 20% oxygen, and each gas in a controlled proportion with nitrogen. All gases were controlled with a fluorometer. Gas content of the inhaled air was maintained at 20% oxygen, 30-50% carbon dioxide and 50-20% nitrogen for the first 30 minutes of the experiment. At 30 minutes from the start of the experiment, both oxygen and nitrogen were withdrawn and entirely interchanged with 100% carbon dioxide.

5.2 Results and discussion

Rats inhaling carbon dioxide died from 10 to 30 minutes after exposure.

Upon examination under a light and electron microscope, the rats' lungs were seen to be dark red in colour and markedly shrunk. Electron microscope observations showed the alveoli of the lungs to have an irregular wavy appearance, reflecting the shrinkage of the lung due to the decreased air in the alveoli. A large number of substances containing tubular myelin, membranous structures and fibrin fibres were found, and erythrocyte and thrombocyte fragments were seen occasionally in the in the alveolar cavity.

These observations were consistent between those rats that died at the beginning of exposure, and those which died after 30 minutes exposure.

20% oxygen was maintained for the first 30 minutes of the experiment to discover the specific effect carbon dioxide has on the morphological changes in rat lungs compared to those exposed to 5% oxygen, and to examine the distribution of carbon dioxide in several organs. No change in the alveolar wall attributable specifically to carbon dioxide was found, except those changes, which were similar to the 5% oxygen. It is on the basis of this these findings that it is concluded that the morphological changes in the lung tissue following inhalation of 100% carbon dioxide could not be attributed to the carbon dioxide itself, but to decreased oxygen.

Section A6.1.3 — **Acute Toxicity: Inhalation (13 of 14)**

Annex Point IIA, VI, 6.1.3 — Section 6: Toxicological and Metabolic Studies

Special investigation in rats.

5.3	**Conclusion**	
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes

This study gives an indication about the effects of exposure of rats to 100% carbon dioxide gas after 30 minutes, rather than determining the LC_{50}.

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 gives an indication about the level of carbon dioxide that cannot be tolerated by rats.

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. There is sufficient data available concerning the toxicity of carbon dioxide in various species (including rats, man and mammals) and this data is in general agreement giving an inhalation toxicity of around 90,000 ppm carbon dioxide (rats tolerate exposure to 100,000 ppm (10%) carbon dioxide, LC_{LO} values for humans and mammals have been reported to be 90,000 ppm).

Section A6.1.3 **Acute Toxicity: Inhalation (13 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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	

Section A6.1.3
Annex Point IIA, VI, 6.1.3

Acute Toxicity: Inhalation (14 of 14)

Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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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.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 Oxygen, presented in nitrogen.

[redacted]

[redacted]

The study summarised here investigates whether morphological changes in the lungs of rats, killed by asphyxia, is not caused directly by carbon dioxide, but by the decreased oxygen content in air.

3.1.1. Lot/Batch number Not reported.

3.1.2 Specification [redacted]

Section A6.1.3 Acute Toxicity: Inhalation (14 of 14)

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

3.2	**Test Animals**	
3.2.1	Species	Rat.
3.2.2	Strain	Wistar Albino.
3.2.3	Source	Not reported.
3.2.4	Sex	Male.
3.2.5	Age/weight at study Initiation	Age: not reported. Weight: 200-300g.
3.2.6	Number of animals per group	10 animals for the 5% oxygen/95% nitrogen group. 6 animals for the 10% oxygen / 90% nitrogen group. 4 animals for the control group.
3.2.7	Control animals	Yes.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Post exposure period	Other: No post-exposure period reported.
		Inhalation
3.3.8	Concentrations	5% oxygen in 95% nitrogen. 10% oxygen in 90% nitrogen. No analytical concentration reported.
3.3.9	Particle size	Not applicable – oxygen is not an aerosol.
3.3.10	Type or preparation of particles	Not applicable – oxygen is not a particulate.
3.3.11	Type of exposure	Exposure is via inhalation but whether the exposure was whole body or nose only has not been specified.
3.3.12	Vehicle	Nitrogen gas.
3.3.13	Concentration in vehicle	5% oxygen in 95% nitrogen. 10% oxygen in 90% nitrogen.
3.3.14	Duration of exposure	1 hour.
3.3.15	Controls	Control animals were used, but no specific details reported.
3.5	**Method of determination of LD_{50}**	LD_{50} not reported, but expressed as an observation of the morphology of lungs after exposure to decreased oxygen content in inhaled air.
3.6	**Further remarks**	See 3.1 for explanation about how this study relates to toxicity of carbon dioxide.
		4. RESULTS AND DISCUSSION
4.1	**Clinical signs**	Refer to "4.3: Other"
4.2	**Pathology**	Refer to "4.3: Other"

Section A6.1.3 **Acute Toxicity: Inhalation (14 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies

Special investigation in rats.

4.3 Other

5% oxygen (in 95% nitrogen)

All rats in this group became still within 1 minute of exposure, except for an occasional spasmodic movement such as jumping and scratching at the wall of the exposure chamber. They dragged their hind legs as if paralysed when moving, and could not stand in the normal position after 30 minutes from the beginning of the experiment. All animals died after 1 hour of exposure, showing convulsions in all four legs followed by cheyne-stokes-like respiration.

10% oxygen (in 90% nitrogen)

All animals in this group moved actively at the beginning of the experiment, but took a crouching position with eyes half-open, and seemed to avoid useless movement except occasional scratching, stretching and jumping at the wall of the exposure chamber (in the same way as those of the 5% box).

Morphological examination of the lung

Gross appearance:

In all cases of both 5% oxygen and 10% oxygen group, the lung was atelectatic and contained less air than normal, but there were no differences in colour.

Light microscope examination:

At higher magnification, there was an extremely faint, pinkish homogeneous substance on the surface of alveoli of the 5% oxygen group in the N&E stained section, but this substance was negative by mucicarmine, alcian blue and PAS staining. This homogeneous substance was not observed on the alveolar surface of the 10% oxygen group, and the atelectatic changes were the only remarkable findings.

Electron microscope examination

In normal rat lung, there were several great pneumocytes which were releasing a linear or thread-like component of the lamellar body trailing into the alveolar lumen. In the 5% oxygen group, the great pneumocytes with their lamellar bodies were often fused together and thus the mitochondria were occasionally surrounded by large lamellar bodies in the cytoplasm. Several great pneumocytes had been destroyed and had discharged their cytoplasm with the fused lamellar bodies into the alveolar space. Lamellar, except those mentioned above, and lattice-like structures were seen in the alveolar space with or without the homogeneous substance in various amounts, arranged randomly at an arbitrary width. They were observed attached or connected with each other.

The substance observed as homogeneous and faint y pink coloured by light microscopy in the 5% oxygen group, was observed to be of medium electron density and it either filled the recesses of the alveoli, or thinly covered the alveolar surface. The inner surface of the substance was delineated clearly as a somewhat curved line. At or near the centre of several alveoli there was seen a round encircled space through which the air was thought to pass. The lamellar, lattice formed or tubular myelin and thread-like structures were observed frequently in the homogenous substance, and the majority of lamellar and lattice like structures were found at the surface of the substance, near to the air passage. But, they were often observed very near to the cell surface as well, attached to the cell membrane.

Continued……

Section A6.1.3 **Acute Toxicity: Inhalation (14 of 14)**

Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

4.3 Other (continued)

In the typically formed lattice-like structure, there was occasionally found in each square a small medium electron-dense dot, like a core.

Another structural characteristic of the homogeneous substance was that it seemed to have some close connection with the lamellar or lattice like structure. The density of the cytoplasm of the pneumocytes lining the alveoli diminished to where it was pale and thus was ascertained with ease by the contrast to the medium density of both the homogenous substance and the basement membrane.

The membrane of the cells lining the alveoli showed bleb formation, especially in the 5% oxygen group.

The width of the lamellae and the lattice-like structure was typically 10-30 nm and the interval between each was typically 50-70 nm between the centre of each width.

In the rat lungs of the 10% oxygen group, the substance which was observed on the alveolar surface of the 5% oxygen group rats was occasionally found, but a majority of them was not dense but greyish debris-like. Lattice-like structure was also found in a groove in a rare case.

4.4 LD_{50}

Refer to "4.3 Other".

5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods

This study was not carried out to Guideline B.2.in Annex V of Directive 67/548/EEC.

The Wistar albino rats were put into an observation box to inhale air containing either 5% oxygen in 95% nitrogen, or 10% oxygen in 90% nitrogen. All gases were controlled with a fluorometer. Gas content in the observation box was checked by gas chromatography.

All animals in the 5% oxygen group died after 1h exposure. A strike on the head with a blunt instrument was given to the rats of the 10% oxygen group, after 1h exposure (the same time point at which rats in the 5% oxygen group died).

Perfusion technique
Immediately death, the abdomens of the rats were opened to expose the inferior vena cava through which flowed the fixation fluid of 4% formaldehyde and 1% glutaraldehyde in a 0.1 M cacodylate buffer solution with a pH of 7.4, from an irrigator hanging 80-100 cm higher than the plate on which the rats were laid. The blood flowed out from the cut aperture distal to the point where the fixation fluid flowed in.

Preparation of tissue samples for microscopy
Tissue samples were taken from the left lung and put into a chilled fixative (the same used as for the perfusion, above). Small pieces of the sample were processed according to the ordinary method for electron microscopy. The ultra-thin sections for the electron microscope were stained doubly with uranyl-acetate and lead citrate, and then observed with a Hitachi H-500 type microscope. Parts of the tissue blocks taken from the area near the small pieces used for electron microscopy were embedded in paraffin and thinly sliced sections were examined by H&E, PAS, alcian blue and mucicarmine staining.

Section A6.1.3 Acute Toxicity: Inhalation (14 of 14)

Annex Point IIA, VI, 6.1.3

Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

5.2 Results and discussion

For details of results, refer to "4.3 Other".

In the forensic field, there are many cases in which the cause of death is considered to be by asphyxia, whatever the mode of death should be, and almost all cases are resolved by macroscopic observations of the outside of the body and the findings in the organs. The authors of this study expected to find the morphological changes of cell components attributable to oxygen deficiency. But contrarily, the morphological changes were not so remarkable except in the destruction of the cytoplasm of the great pneumocytes, releasing cell organelles together with a large amount of the fused lamellar bodies into the alveolar lumen, and the bleb formation of both the cells lining the alveoli and endothels of the alveolar vessels.

But the unexpected findings in this experiment were the appearance of the lamellar and/or lattice-like structures and tubular myelin with a large amount of the homogeneous electron-dense manifest, especially in the 5% oxygen group. Whereas in the 10% oxygen group, the changes of cellar components were not severe and the appearance of the homogeneous substance, like that of the 5% oxygen group was not discernible but the lattice-like structure was observed occasionally in a small amount. The appearance of a large amount of lamellar, lattice and thread like structures with massive homogeneous substance in the alveoli is easily ascertainable and seems to be attributable to the reaction of lung tissue in the hypoxic state.

When the hypoxic state was produced by exposure to carbon dioxide [1] it was concluded that the morphological changes in the lung tissue following inhalation of 100% carbon dioxide could not be attributed to carbon dioxide itself, but decreased oxygen.

[redacted]

5.3 Conclusion

5.3.1 Reliability: 4

5.3.2 Deficiencies: Yes

It is duly acknowledged that there is insufficient reporting of methods and results data, and this data has not been generated in accordance with scientifically acceptable protocols.

[redacted]

Section A6.1.3 **Acute Toxicity: Inhalation (14 of 14)**
Annex Point IIA, VI, 6.1.3 Section 6: Toxicological and Metabolic Studies
Special investigation in rats.

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 6.1.4 **Annex Point IIA, VI, 6.1.3**	**Acute Dermal 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 [4]	**Other justification** [ ]	
Detailed justification:	It is not technically possible to determine the skin irritation potential of carbon dioxide. In addition, 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 acute toxicity of gases and volatile liquids should be determined by the inhalation route only. As carbon dioxide is a gas, acute dermal irritation is not required under the Biocidal Products Directive. 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. It is not only technically not possible to determine the skin irritation potential of carbon dioxide, but it is also not scientifically necessary on the basis of low exposure to carbon dioxide during it's normal use as a biocide. 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. *Refer to next page for full details of the scientific calculation, which supports this statement.* In addition, the potential for exposure to carbon dioxide when it is manufactured for use as a rodenticide is minimal. [redacted] (Continued….)	

Detailed justification:

[redacted]

Undertaking of intended data submission [ ]	Not applicable.

Section 6.1.4 **Annex Point IIA, VI, 6.1.3**	**Acute Dermal Irritation** 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 6.1.4 **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	[4]	**Other justification**	[ ]		

Detailed justification:

It is not technically possible to determine the eye irritation potential of carbon dioxide. In addition, 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 acute toxicity of gases and volatile liquids should be determined by the inhalation route only. As carbon dioxide is a gas, acute eye irritation is not required under the Biocidal Products Directive.

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.

It is not only technically not possible to determine the eye irritation potential of carbon dioxide, but it is also not scientifically necessary on the basis of low exposure to carbon dioxide during it's normal use as a biocide. 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. *Refer to next page for full details of the scientific calculation, which supports this statement.*

In addition, the potential for exposure to carbon dioxide when it is manufactured for use as a rodenticide is minimal. [redacted]

(Continued....)

Section 6.1.4 **Annex Point IIA, VI, 6.1.3**	**Acute Eye Irritation** Section 6: Toxicological and Metabolic Studies

Detailed justification:

[redacted]

[redacted]

Undertaking of intended data submission [ ]	Not applicable.

Section 6.1.4 **Annex Point IIA, VI, 6.1.3**	**Acute Eye Irritation** 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 6.1.5 **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 [ ]	**Technically not feasible** [4] **Scientifically unjustified** [ ]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	It is not technically possible to determine the skin sensitisation potential of carbon dioxide. In addition, 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 acute toxicity of gases and volatile liquids should be determined by the inhalation route only. As carbon dioxide is a gas, acute skin sensitisation is not required under the Biocidal Products Directive. 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 [4]	**Other justification** [ ]	
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 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 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]

Detailed justification:

Official use only

3. There is low exposure to carbon dioxide during its normal use as a biocide. 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.
   Refer to next page for full details of the scientific calculation, which supports this statement.
4. In addition to the above, the potential for exposure to carbon dioxide when it is manufactured for use as a rodenticide is minimal [redacted]
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.

Continued.....

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:

Official use only

[redacted]

Undertaking of intended data submission [ ]	Not applicable.

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

	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]