Section A6.4.3 **Subchronic Inhalation Toxicity Test (6 of 11)**

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3.4.7	Clinical Chemistry	Yes. Number of subjects: All Time points: Not reported. Parameters: Other: changes in serum electrolytes Total serum protein. Whole blood lactate.
3.4.8	Urinalysis	Not reported.
3.5	**Sacrifice and Pathology**	
3.5.1	Organ weights	No. No mortalities in test. No sacrifices made.
3.5.2	Gross and histopathology	No. No mortalities in test. No sacrifices made.
3.5.3	Other examinations	None
3.5.4	Statistics	Not reported.
3.6	**Further remarks**	Changes in arterial pH and PO_2 were determined.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	On admission to hospital many of the test subjects exhibited the effects of acute respiratory dyspnea, cyanosis, lethargy and confusion. Several test subjects were semi-comatose. As the acute problems were rectified by appropriate therapy, most patients became alert, ambulatory and asymptomatic except for continuing dyspnea and weakness. There appeared to be little correlation between the mental state of a given test subject and the degree of carbon dioxide retention in the compensated "steady state"
4.1.2	Mortality	No mortalities reported.
4.2	**Body weight gain**	Not reported.
4.3	**Food consumption and compound intake**	Not reported.
4.4	**Ophthalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	There was a considerable variation in the observed haematocrit values but there was no apparent correlation with changes in carbon dioxide tensions. The data does not allow correlation with chronic hypoxemia since some of the patients received intermittent oxygen therapy during the course of study.
4.5.2	Clinical chemistry	The average "steady state" serum electrolyte values are given in Table A6_3-1 at the end of this study summary. It can be seen from this data that as the carbon dioxide tension increased from the normal range, there was no appreciable change in the serum sodium and potassium concentrations. The serum sodium concentrations ranged from 137 to 151 mEq per litre but varied without relation to increasing degrees of hypercapnia. Serum potassium ranged from 4.0 to 5.2 mEq per litre, but again there was only a tendency for higher values to occur as pCO_2 increased. The serum chloride concentration progressively decreased from an average value of 103 mEq per litre in the normal volunteers to

(Continued.....)

4.5.2	Clinical chemistry (Continued)	a low of 80 mEq in the patient with an average "steady-state" pCO_2 value of 13.7%. There was a progressive increase in the average plasma bicarbonate concentration of 24.4 mEq per litre in normal controls to 45.8 mEq in the patient with the highest average "steady-state" carbon dioxide tension observed of 14.1 %. The unmeasured anions ($Na - [Cl + HCO_3]$) ranged between 8.6 and 20.4 mEq per litre in the entire group of patients, but there was no systemic change that could be related to increasing degrees of hypercapnia. Total serum protein in eight patients ranged between 5.7 – 7.5 g/100ml. In no case was the serum albumin less than 2.1 or the globulin greater than 3.9 g/100ml. Whole blood lactate determinations in four patients were not increased despite severe hypoxemia.
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No mortalities in test. No sacrifices made.
4.6.2	Gross and histopathology	No mortalities in test. No sacrifices made.
4.7	**Other**	The changes in arterial pH and PO_2 were determined, and the results are given in Table A6_3-1 at the end of this study summary. Although the number of "steady-state" values within a given PCO_2 was is small, the change in pH (or estimated hydrogen ion activity) as carbon dioxide tension increases suggests a progressive decrease in pH as PCO_2 values increase. The changes in arterial oxygen tension varied from 6.9 – 11.8%, but there was only a tendency for lower PO_2 values to be associated with the higher carbon dioxide tensions. This lack of correlation probably reflects the complexity of the cardiopulmonary abnormalities observed in a patient population with chronic pulmonary disease as well as the variations in administered oxygen during the period of observation.
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC. Patients included in the study were selected from admissions to the general medical wards at the Medical College of Virginia, Richmond, Virginia and the pulmonary ward at the McGuire Veterans Administration Hospital, Richmond Virginia. The criteria for inclusion in the study group and for the presence of a "steady-state" included the following: All patients admitted to the hospital with alveolar hypoventilation and persisting hypercapnia were considered for inclusion in the study group but those that had conditions or diseases that might alter the physiologic responses to chronic elevations of carbon dioxide were excluded. All but two patients were regarded as having "chronic obstructive pulmonary disease" or "chronic bronchitis". "Kyphoscoliotic lung disease was diagnosed in one case, and "alveolar hypoventilation associated with obesity" was diagnosed in another. About half the patients studied had tracheostomies but none were on constant assisted ventilation. Most patients received nebulized isoproterenol (Isuprel) and acetylcysteine (Mucomyst) via an intermittent positive-pressure

5.1 Materials and Methods

(Continued)

apparatus (IPPB) for intervals of 10-15 minutes three or four times daily during the period of the study.

A stable or "steady-state" period of hypercapnia for a minimum of 3 days, as determined by the retrospective analysis of serial arterial blood gas and pH determinations, was required for a patient's inclusion in the "steady-state" group.

All patients selected for study were on an unrestricted general hospital diet. Twenty-four hour urine collections were obtained daily in most cases, and aliquots were analysed for protein, creatinine, sodium, chloride, and potassium. Patients were excluded from the study if either the urinary chloride or potassium was less than 20 mEq per 24 hours. No patient was included who had an associated illness that would predictably alter the acid-base response to increases in carbon dioxide tension and all patients were essentially afebrile (temperature under 100.5°F by rectum) during the time that the "steady-state" was observed. Renal disease was excluded by appropriate studies including routine urinalysis, serum urea nitrogen (SUN), creatinine and endogenous creatinine clearances. Patients with overt diabetes mellitus were excluded from the study group. None of the patients had persistent vomiting or diarrhoea and none were on gastric suction during the "steady-state" period. Those in whom congestive heart failure was present to an extent that sodium restriction or continuous diuretic therapy was required for compensation was also excluded from this study. No patient was receiving systemic corticosteroid therapy or diuretic agents, and none were receiving salicylates regularly during the period defined as the "steady-state".

Methods for blood and urine determinations are described in Brackett et al [1]. Determinations of pH and carbon dioxide tension (PCO_2) were done with the use of either the Radiometer pH meter or blood gas analyser, or an Epsco blood parameter analyser. The arterial blood was drawn into a mercury-sealed syringe 10 to 30 minutes after percutaneous arterial puncture with the patient breathing room air. Plasma bicarbonate was calculated from the Henderson-Hasselbalch equation with the use of a pK^1 of 6.10 and a solubility coefficient of 0.0301 for blood. Whole blood lactate determinations were performed in the Clinical Research Centre Laboratory with the kit available from the Sigma Chemical Company.

[redacted]

5.2 Results and discussion

The whole-body titration curve in chronic uncompensated hypercapnia is characterised by a linear increase in estimated hydrogen ion activity as carbon dioxide tension increases. Over a range of carbon dioxide tensions from 4.53 – 13.7%, the arterial-blood hydrogen ion activity increased by 0.24 nM per litre per 0.1% increase in carbon dioxide tension. Thus the whole body defence of arterial-blood pH appears equally effective over the range of carbon dioxide tensions observed in this study.

5.3 Conclusion

5.3.1 LO(A)EL

LOEL: 13.7 % carbon dioxide.

5.3.2	NO(A)EL	Not reported.

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Subchronic Inhalation Toxicity Test (6 of 11)

5.3.1	Reliability	3
5.3.2	Deficiencies	Yes

It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of pronged exposure to various levels of carbon dioxide up to 13.7% to man. While this study was not generated to modern, scientifically accepted protocols, nor was it a full 90 day investigation, it does provide useful data on some of the parameters measured in a subchronic study.

Despite the deficiencies in this study, it does gives an indication about the level of carbon dioxide that can be tolerated by humans over a pronged period.

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

1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges.
2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal, and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions.
3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary.
4. There is sufficient data available concerning the subchronic toxicity of carbon dioxide in various species (including rats, man and mammals). However, because the occupational exposure standard for safe working conditions with carbon dioxide is well established, this value can be used for the risk assessment*.

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

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Table A6 3-1 Average Serum Electrolyte and Arterial-Blood Gas Values for Chronic Hypercapnia in Man

PCO_2 ranges %	No. of observations	Arterial blood gases		Plasma electrolytes				
		PH	PO_2 ranges %	Sodium	Chloride *mEq/litre*	Potassium	HCO_3^-	Na(Cl +HCO_3) *mEq/litre*
4.5 - 5.3 *	7	7.42	12.0 – 12.4	138	105	4.0	24.4	8.6
4.6 - 5.2 #	3	7.45	9.4 – 9.5	151	106	4.3	24.0	21.0
5.3 - 5.8	1	7.42	6.9	147	100	5.0	27.2	18.8
6.0 - 6.5	2	7.45	8.1 – 9.4	142	99	5.1	31.0	11.5
6.6 - 7.2	4	7.42	6.9 – 10.0	142	90	5.2	33.1	18.9
7.3 – 7.8	11	7.35	4.1 – 10.1	142	94	4.9	31.4	16.3
8.0 – 8.5	4	7.38	6.0 – 7.6	137	92	5.1	35.3	9.7
8.6 – 9.2	5	7.38	7.1 – 8.6	142	90	4.9	38.3	12.7
9.3 – 9.8	3	7.32	6.6 – 10.5	137	90	4.2	36.0	11.0
10.0 – 10.5	2	7.32	8.3 – 10.3	145	92	4.8	38.7	13.3
13.3 – 13.8	1	7.31	11.8	137	84	4.7	48.0	5.0
14.0 – 14.1	1	7.26	11.5	146	80	5.2	45.8	20.4

Key: * [redacted]

"Steady State" values from patients in this series.

Section A6.4.3 **Subchronic Inhalation Toxicity Test (6 of 11)**
Annex Point IIA, VI, 6.4

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

Section A6.4.3 **Subchronic Inhalation Toxicity Test (7 of 11)**
Annex Point IIA, VI, 6.4

Official use only

1. REFERENCE

1.1 Reference [redacted]

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2

1.2.3 Criteria for data protection [redacted]

2. GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No.

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

2.2 GLP No.

GLP was not compulsory at the time study was performed.

2.3 Deviations Yes.

No set guideline followed.

3. MATERIALS AND METHODS

3.1 Test material As given in section 2.

3.1.1. Lot/Batch number Not reported.

3.1.2 Specification [redacted]

[redacted]

Section A6.4.3 Subchronic Inhalation Toxicity Test (7 of 11)

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3.2	**Test Animals**	
3.2.1	Species	Rhesus monkey (*Macaca mulatta*).
3.2.2	Strain	Not reported.
3.2.3	Source	NMRI colony and NIH colony Okatie Farms and monkeys from Trefflich's Bird and Animal Company.
3.2.4	Sex	Male.
3.2.5	Age/weight at study Initiation	Age of test subjects not reported, other than the test monkeys were adults. Mean weight of test animals: 14 lb.
3.2.6	Number of animals per group	10.
3.2.7	Control animals	2 groups of 10.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of treatment	Other: 93 days.
3.3.2	Frequency of exposure	Other: Continuous.
3.3.3	Post exposure period	Other: Observations made at 28, 35 and 46 days after exposure.
3.3.5	**Inhalation**	
3.3.5.1	Concentrations	Nominal concentration 3 % carbon dioxide (+/- 0.1%). No analytical concentration reported.
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.5.4	Type of exposure	Whole body.
3.3.5.5	Vehicle	Gas.
3.3.5.6	Concentration in vehicle	Gas mixture contains 3 % carbon dioxide and 21 % oxygen (both component gases were maintained within +/- 0.5% of stated concentrations).
3.3.5.7	Duration of exposure	93 days, continual exposure.
3.3.5.8	Controls	Pre-exposure control data was obtained on the test animals. In addition, 10 monkeys were kept in test conditions, but exposed to normal atmospheric concentrations of carbon dioxide and oxygen.
3.4.	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	None reported.
3.4.1.2	Mortality	Yes. One mortality recorded on day 62.
3.4.2	Body weight	Yes. The study report includes a graph charting the measurement of weight. (Refer to graph 1 at the end of this study summary for further details). This chart shows that weight measurement was recorded regularly throughout the test period, however the exact times for determination of weight changes has not been specifically recorded.
3.4.3	Food consumption	Yes. Observed continuously throughout the test period.
3.4.4	Water consumption	Yes. Observed continuously throughout the test period.
3.4.5	Ophthalmoscopic	Not reported.

Section A6.4.3 **Subchronic Inhalation Toxicity Test (7 of 11)**

Annex Point IIA, VI, 6.4

3.4.6	Haematology	Yes.	
		Number of subjects:	All.
		Time points:	Not specifically reported, but graph of results for haematocrit levels show measurements taken prior to exposure to carbon dioxide, and then regularly throughout the entire test period. (Refer to graph 2 at the end of this study summary about time points when measurements were taken)
		Parameters:	Other: Haemoglobin, total and differential leukocyte count, haematocrit, erythrocyte sedimentation rate.
			Refer to graph 2 at the end of this study summary for details about haematocrit levels measured in the test animals prior to and during exposure to 3% carbon dioxide.
3.4.7	Clinical Chemistry	Yes	
		Number of animals:	All
		Time points:	Not specifically reported, but graph of results show measurements taken prior to exposure to carbon dioxide, and then regularly throughout the entire test period. (Refer to graphs 3, 4, and 5 at the end of this study summary for details about time points when measurements were taken).
		Parameters:	Other. Blood glucose, serum cholesterol, non-protein nitrogen, serum bilirubin, serum chloride, calcium, serum phosphorous and thymol turbidity.
			Refer to graph 3, 4 and 5 at the end of this study summary for details about blood glucose, non-protein nitrogen and serum chloride measured in the test animals prior to and during exposure to 3% carbon dioxide.
3.4.8	Urinalysis	Not reported.	
3.5	**Sacrifice and Pathology**		
3.5.1	Organ weights	Pathology studies were carried out but not fully reported other than five randomly selected animals from the group exposed to increased carbon dioxide were autopsied immediately after the 93-day exposure period, and studied for pathological changes. The remaining four monkeys were autopsied after observation for about 28, 35, 40 and 46 days, and were studied for possible tissue changes.	
		Conclusions have been drawn about possible adrenal impairment.	
3.5.2	Gross and histopathology	Pathology studies were carried out but not fully reported other than five randomly selected animals from the group exposed to increased carbon dioxide were autopsied immediately after the 93-day exposure period, and studied for pathological changes. The remaining four monkeys were autopsied after observation for about 28, 35, 40 and 46 days, and were studied for possible tissue changes.	

3.5.3	Other examinations	None reported.

3.5.4	Statistics	In statistical analysis, the following comparisons were made: 1. For the period in the chamber and for the period of the follow-up, the animals were compared by analysis of variance with their own pre-exposure control values for each factor studied. 2. Group mean values of each factor were analysed as to significant change with time (regression) over the 93-day period. 3. Values for the group in the chamber were compared by analysis of variance with the 10 control animals maintained at normal atmospheric conditions.
3.6	**Further remarks**	Carbon dioxide levels in arterial and venous blood were determined. Observations were made regarding general behaviour and sleep patterns. Respiratory rate under increased carbon dioxide levels was measured.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No clinical signs reported.
4.1.2	Mortality	The one mortality, recorded on day 62, had all of the symptoms of a gram-negative septicaemia. It died after a 5-day illness marked by diarrhoea, innanition and associated leukopenia. On autopsy, lungs were not abnormal. Liver and kidneys were very pale and the bowel was massively haemorrhagic, with small superficial mucosal ulcerations in three areas. *Shigella flexneri,* type III, was cultured from the bowel. These results are consistent with gram-negative septicaemia complicating an acute enteritis. It should be noted that the occurrence of 1 death in 10 animals over 90 days is not unusual when the spontaneous mortality of monkeys in the test house reported that of 72 monkeys, 11 died after an average of 4 months observation. (This is a similar number to that reported in the literature from other test houses).
4.2	**Body weight gain**	The weight of the experimental animals remained essentially constant over the 93 days of exposure to carbon dioxide, and the follow up period. There were no significant variations or differences between the test animals and the controls. Refer to graph 1 at the end of this study summary for further details.
4.3	**Food consumption and compound intake**	The only change in appetite and eating habits noted was a slight decrease in the consumption of bananas beginning about midway through the exposure period.
4.4	**Ophthalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	Statistical analysis shows that there was no significant, consistent variation in haemoglobin, leukocyte count or haematocrit when each test animal was compared with it's own control value, or when the group mean values were analysed for regression over the 93-day period or when the mean value of the test group were compared with the group kept in normal room air (p values were all > 0.1). The erythrocyte sedimentation rate was never significantly elevated with

	the single exception of the terminally elevated sedimentation rate in the (Continued....)	

4.5.1	Haematology (Continued)	one animal which died. It was noted that there was no significant fall in haematocrit or haemoglobin, although a volume of blood roughly equivalent to 6% of the total body weight was removed from the monkeys during the period of exposure.
4.5.2	Clinical chemistry	Statistical analysis shows that serum cholesterol, serum bilirubin and thymol turbidity were never significantly elevated and there was no significant, consistent variation in blood glucose, non-protein nitrogen, serum chloride, calcium and serum phosphorous when each test animal was compared with it's own control value or when the group mean values were analysed for regression over the 93-day period or when the mean value of the test group were compared with the group kept in normal room air (p values were all > 0.1).
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Five randomly selected animals from the group exposed to increased carbon dioxide were autopsied immediately after the 93-day exposure period, and studied for pathological changes. The remaining four monkeys were autopsied after observation for about 28, 35, 40 and 46 days, and were studied for possible tissue changes. The results to the autopsies were not fully reported, other than the only consistent finding was the presence of lung mites, which was expected. The presence of lung mites in the test animals is considered under section 5.1 Materials and Methods. There was no evidence of adrenal impairment as a result of exposure to increased carbon dioxide for 93 days, as demonstrated by the absence of cardiovascular collapse, the tolerance to the stress of blood sampling, the absence of lymphopenia, the normal serum chlorides, the maintenance of normal weight and continued good health of the exposed monkeys. Note that the 4 monkeys not selected for autopsy immediately after 93 days exposure to increased carbon dioxide survived the post-exposure period and seemed healthy.
4.6.2	Gross and histopathology	Refer to section 4.6.1 'Organ weights' (above)
4.7	**Other**	Statistical analysis of carbon dioxide levels in arterial and venous blood show that there were no significant consistent variation when each animal is compared with it's own control value, or when the group mean values were analysed for regression over the 93-day period or when the mean value of the test group were compared with the group kept in normal room air (p values were all > 0.1). It was considered that the general activity of the monkeys and their sleep patterns were unaltered during exposure to increased carbon dioxide. Respiratory rate was also not definably varied because of exposure to increased carbon dioxide. The monkeys exhibited a vigorous resistance to handling at all times.

Section A6.4.3 **Subchronic Inhalation Toxicity Test (7 of 11)**
Annex Point IIA, VI, 6.4

5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods

This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC.

The study consisted of three phases:

1. Preparation period. A colony of monkeys were screened for disease-free animals, and during this time control baseline physiologic data was obtained.

2. Exposure period. 10 monkeys were exposed for 93 days to 3% carbon dioxide and 21% oxygen in a controlled-environment chamber. During this time the animals were carefully observed while physiologic data was obtained. Such data were compared to with the pre-exposure control data and with data from 10 monkeys kept at normal atmospheric concentrations of carbon dioxide and oxygen. Five randomly selected animals from the group exposed to increased carbon dioxide were autopsied immediately after the 93-day exposure period, and studied for pathological changes.

3. Follow-up period. Post exposure data were obtained on the five remaining monkeys while the animals were observed for any post-withdrawal alterations. These monkeys were autopsied after observation for about 28, 35, 40 and 46 days, and were studied for possible tissue changes. *(Note that one animal died during the exposure period so post exposure data was obtained only on 4 animals instead of 5.)*

- Selection of animals:

A total of 72 animals were screened for selection. From these 20 animals were carefully selected on the basis of the following criteria:
Free from obvious disease.
Vigorous and well nourished.
Free from tuberculosis (confirmed by negative tests performed bimonthly)
Negative for *Shigella flexneri* (if animals were positive on arrival they were treated with chlortetracycline until tests were consistently negative).
Negative for intestinal parasites (only hookworm was found. If hookworm was found, the monkeys were treated with hexylresorcinol in doses adequate to give consistently negative specimens).

Presence of lung mite, *Pneumonyssus simicola*
The lung mite, *Pneumonyssus simicola* is ubiquitous in *Macaca mulatta* monkeys. It has been shown that animals that yield only the occasional mites on bronchial lavage have minimal, if any pulmonary parenchymal damage on autopsy, especially if the actual volumes of functional pulmonary parenchyma involved are considered. Of the animals used, approximately one-half had 1 to 5 mites on bronchial washing. The remaining half was negative on bronchial washing. As a further precaution, all animals received the recommended treatment for lung mite (tryparsamide in doses of 45 mg/kg every 2 weeks for 8 doses), but the author of this study acknowledges that this treatment is not always effective.

The 10 animals placed in the exposure chamber were randomly selected from the 20 monkeys, which met the above criteria.

(Continued...)

Section A6.4.3 Subchronic Inhalation Toxicity Test (7 of 11)

Annex Point IIA, VI, 6.4

5.1 Materials and Methods

(Continued)

- Diet

During the period of the study, the animals were fed on Dietrich and Gambrill monkey diet. Approximately one-forth of a pound of this meal, with 50 mg ascorbic acid were mixed with water to form a very heavy paste and offered to each monkey daily. In addition, each monkey was offered one banana every other day. Monkeys had free access to their food and water at all times.

- Carbon dioxide exposure chamber

A sealed chamber of approximately 350 cubic ft. was used. Monkeys were kept in separate cages. Entrance to the chamber was via a 150 cubic ft. air lock, which equilibrated with the gas concentrations of the main chamber before the hatch to the main chamber was opened. All procedures during the exposure period were carried out within the chamber, with the personnel breathing room air from a mask connected to an air line. A carbon dioxide concentration of 3% +/- 0.1% was maintained by "bleeding-in" carbon dioxide at a constant rate, and removing any excess carbon dioxide by an automatically monitored soda-lime "scrubbing system". The control of carbon dioxide concentration was achieved by continuously sampling the chamber through a Liston-Becker infrared carbon dioxide analyser. The output of the analyser was fed into a limit control relay, which, in turn, activated the scrubbing apparatus. A delay circuit with a 15-second time constant eliminated frequent stops and starts of the scrubbing mechanism for small transient fluctuations from such things as jarring of the apparatus. If a true excess of carbon dioxide existed in the chamber, the limit control relay closes which turns on a high volume carbon dioxide "scrubber". When the carbon dioxide levels fell to within the limits allowed, the opening of the relay shut off the "scrubber". The oxygen concentration of the chamber was maintained close to 21% by manually regulating the rate of a constant inflow of oxygen. Chamber gas was constantly monitored with an A.O Beckman Type F-3 O_2 analyser and it never varied more than +/- 0.5%. Figure 1, given at the end of this study summary, includes a diagram showing how carbon dioxide exposure chamber is set up. Both the carbon dioxide and oxygen analysers were calibrated every four hours, day and night, with reference gases of known concentrations. A further check was made by daily gasometric analysis of chamber gas samples. At no time during the 93 days was there any significant variation in the gas concentrations, except for the slow, steady rise of carbon dioxide from atmospheric concentrations to 3% during the first 4 hours of chamber operation. Relative humidity was maintained at 50% (+/- 5%) by a dehumidifier controlled by a humidistat. Temperature was maintained at 75°F by a thermostatically controlled York Heat exchange apparatus built Into the chamber. Circulating fans were in constant operation. A 24-hour watch was kept to ensure constant function of the apparatus.

5.2 Results and discussion

Monkeys exposed to air containing 3% carbon dioxide and 21% oxygen for a period of 93 days showed no demonstrable changes in weight, activity, haemoglobin, haematocrit, blood glucose, total leukocyte count, non-protein nitrogen, serum chloride, serum calcium, phosphorous, thymol turbidity, erythrocyte sedimentation rate, serum bilirubin, cephalin flocculation or serum cholesterol during the period

of exposure or during the follow-up period after removal from the chamber. There was no evidence of adrenal impairment as a result of

(Continued....)

5.2	**Results and discussion** (Continued).	exposure to increased carbon dioxide for 93 days, as demonstrated by the absence of cardiovascular collapse, the tolerance to the stress of blood sampling, the absence of lymphopenia, the normal serum chlorides, the maintenance of normal weight and continued good health of the exposed monkeys.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not reported.
5.3.2	NO(A)EL	NOAEL: 3 % carbon dioxide* * Despite there not being a range of carbon dioxide levels tested, the results to this study show no observed adverse effect level to monkeys when exposed to 3 % carbon dioxide.
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of pronged exposure to 3% carbon dioxide to monkeys. While this study was not generated to modern, scientifically accepted protocols, it does provide useful data on the majority of parameters measured in a subchronic study. Despite the deficiencies in this study, and the fact that test subjects were only exposed to one level of carbon dioxide, it does gives an indication about the level of carbon dioxide that can be tolerated by monkeys over a pronged period. This study, notwithstanding it's deficiencies, can be used to support the inhalation toxicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges. 2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal, and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions. 3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary. 4. There is sufficient data available concerning the subchronic toxicity of carbon dioxide in various species (including rats, man and mammals). However, because the occupational exposure standard for safe working conditions with carbon dioxide

	is well established, this value can be used for the risk assessment*. *The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period).

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

Section A6.4.3
Annex Point IIA, VI, 6.4

Subchronic Inhalation Toxicity Test (8 of 11)

Official use only

1. REFERENCE

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

2. GUIDELINES AND QUALITY ASSURANCE

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

3. MATERIALS AND METHODS

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

[redacted]

Section A6.4.3 Subchronic Inhalation Toxicity Test (8 of 11)

Annex Point IIA, VI, 6.4

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, but test animals would have included some females because young were born during the test period.	
3.2.5	Age/weight at study Initiation	Not reported.	
3.2.6	Number of animals per group	10.	
3.2.7	Control animals	Not reported.	
3.3	**Administration/ Exposure**	Inhalation.	
3.3.1	Duration of treatment	Other: 30 days	
3.3.2	Frequency of exposure	Other: Continuous	
3.3.3	Post exposure period	None reported.	
3.3.5	**Inhalation**		
3.3.5.1	Concentrations	Nominal concentration	10% carbon dioxide (note that the concentration of carbon dioxide was allowed to vary from the stated level by as much as 2-3% in a 24 hour period).
		No analytical concentration reported.	
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.	
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.	
3.3.5.4	Type of exposure	Whole body.	
3.3.5.5	Vehicle	Gas.	
3.3.5.6	Concentration in vehicle	Gas mixture contains 10% carbon dioxide and 19-21% oxygen. (note that the concentrations of carbon dioxide and oxygen were allowed to vary from the stated level by as much as 2-3% in 24 hours).	
3.3.5.7	Duration of exposure	30 days, continual exposure.	
3.3.5.8	Controls	Not reported.	
3.4.	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	None reported.	
3.4.1.2	Mortality	No mortalities reported. Time periods for observations have not been reported.	
3.4.2	Body weight	Yes. Time periods for when weight was determined has not been reported.	
3.4.3	Food consumption	Yes. The periods for when food consumption levels were determined have not been reported.	
3.4.4	Water consumption	Not reported, but note body weight observations.	
3.4.5	Ophthalmoscopic examination	Not reported.	

Section A6.4.3 **Subchronic Inhalation Toxicity Test (8 of 11)**
Annex Point IIA, VI, 6.4

3.4.6	Haematology	Yes. Number of subjects: 5 out of 10 test animals. Time points: Weekly intervals. Parameters: Other: haemoglobin concentration, erythrocyte count, leukocyte count and reticulocyte count.
3.4.7	Clinical Chemistry	Details of clinical chemistry investigations not reported.
3.4.8	Urinalysis	Details of urinalysis not reported.
3.5	**Sacrifice and Pathology**	
3.5.1	Organ weights	No mortalities in test. No sacrifices made.
3.5.2	Gross and histopathology	No mortalities in test. No sacrifices made.
3.5.3	Other examinations	None.
3.5.4	Statistics	Statistical analysis not reported.
3.6	**Further remarks**	Observations regarding the general behaviour of the test animals were made. Observations regarding respiratory rate of the test animals were made.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No clinical signs reported.
4.1.2	Mortality	No mortalities reported. An observation was made that the test animals were in good general condition at the end of the test period, and given this the test period could have been much prolonged without death of the animals.
4.2	**Body weight gain**	Considerable weight loss occurred, ranging from 14-27%. This loss of weight is believed to be due primarily to a reduction in food intake, since the animals ate sparingly.
4.3	**Food consumption and compound intake**	Animals ate sparingly during the exposure period.
4.4	**Ophthalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	There was no significant change in levels of haemoglobin or the number of leukocytes or erythrocytes. The only significant change was a marked reticulocytosis.
4.5.2	Clinical chemistry	Details of clinical chemistry investigations not reported.
4.5.3	Urinalysis	Details of urinalysis not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No mortalities in test. No sacrifices made.
4.6.2	Gross and histopathology	No mortalities in test. No sacrifices made.
4.7	**Other**	The usual respiratory response to excess carbon dioxide was the only objective sign observed, since the rats appeared normally active and the young born under these conditions were apparently normal.

Section A6.4.3 Subchronic Inhalation Toxicity Test (8 of 11)

Annex Point IIA, VI, 6.4

5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods

This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC.

The chronic 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

This study is one in a series of studies by the same authors where they compare the acute and chronic toxicity of carbon dioxide. They conclude that rats can tolerate higher levels of carbon dioxide when the level is attained gradually over several days, as demonstrated by the results described. This indicates that the rat is capable of a certain degree of acclimatisation.

5.3 Conclusion

5.3.1 LO(A)EL

LOEL: 10 % carbon dioxide*

* Despite there not being a range of carbon dioxide levels tested, the results to this study show low observable effect level to rats when exposed to 10% carbon dioxide.

5.3.2 NO(A)EL

Not reported.

5.3.3 Reliability

3

5.3.4 Deficiencies

Yes

It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of pronged exposure to 10% carbon dioxide to rats. While this study was not generated to modern, scientifically accepted protocols, nor was it a full 90 day investigation, it does provide useful data on some of the parameters measured in a subchronic study.

Despite the deficiencies in this study, and the fact that test subjects were only exposed to one level of carbon dioxide, it does gives an indication about the level of carbon dioxide that can be tolerated by rats over a pronged period.

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

1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges.
2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal, and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions.
3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary.

(Continued.....)

Section A6.4.3 Subchronic Inhalation Toxicity Test (8 of 11)

Annex Point IIA, VI, 6.4

5.3.4	Deficiencies (Continued....)	4. There is sufficient data available concerning the subchronic toxicity of carbon dioxide in various species (including rats, man and mammals). However, because the occupational exposure standard for safe working conditions with carbon dioxide is well established, this value can be used for the risk assessment*. *The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period). • • •

Section A6.4.3 **Subchronic Inhalation Toxicity Test (8 of 11)**
Annex Point IIA, VI, 6.4

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

Section A6.4.3
Annex Point IIA, VI, 6.4

Subchronic Inhalation Toxicity Test (9 of 11)

Official use only

1. REFERENCE

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

2. GUIDELINES AND QUALITY ASSURANCE

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

3. MATERIALS AND METHODS

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

[redacted]

Section A6.4.3 Subchronic Inhalation Toxicity Test (9 of 11)

Annex Point IIA, VI, 6.4

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	10.	
3.2.7	Control animals	Not reported.	
3.3	**Administration/ Exposure**	Inhalation.	
3.3.1	Duration of treatment	Other: Two experiments, one lasting 24 days and the other lasting 34 days.	
3.3.2	Frequency of exposure	Other: Continuous	
3.3.3	Post exposure period	None reported.	
3.3.5	**Inhalation**		
3.3.5.1	Concentrations	Nominal concentration:	Exposure was levels between 20-25% carbon dioxide. Refer to section 3.3.5.6 for further details. (Note that the concentration of carbon dioxide was allowed to vary from the stated level by as much as 2-3% in a 24 hour period).
		No analytical concentration reported.	
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.	
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.	
3.3.5.4	Type of exposure	Whole body.	
3.3.5.5	Vehicle	Gas.	
3.3.5.6	Concentration in vehicle	Experiment 1. Carbon dioxide was allowed to accumulate for a period of 5 days until a concentration of 20% was reached. Test animals were exposed to 20% carbon dioxide for the next 6 days after which the carbon dioxide was allowed to accumulate to 25% during the next three days. Experiment 2. Carbon dioxide was allowed to accumulate for a period of 5 days until a concentration of 20% was reached. Test animals were exposed to 20% carbon dioxide for the next 6 days after which the carbon dioxide was allowed to accumulate to 23 % during the next three days. Oxygen levels were maintained at 21% during both experiments. All carbon dioxide and oxygen levels varied from stated levels as much as 2-3% during any 24-hour period.	
3.3.5.7	Duration of exposure	Experiment 1: 24 days, continual exposure. Experiment 2: 34 days, continual exposure. (These time periods include the 5 days where carbon dioxide levels were allowed to slowly accumulate to 20% carbon dioxide.)	
3.3.5.8	Controls	Not reported.	

Section A6.4.3 **Subchronic Inhalation Toxicity Test (9 of 11)**

Annex Point IIA, VI, 6.4

3.4.	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes. Observed in experiment 1, when animals were exposed to 25% carbon dioxide. Clinical signs were observed after 4 days exposure to 25% carbon dioxide. No specific clinical signs reported in experiment 2, when animals were exposed to 23% carbon dioxide. Time periods for when clinical signs were determined have not been reported.
3.4.1.2	Mortality	One mortality reported in experiment 1, when animals were exposed to 25% carbon dioxide. Mortality reported after 4 days at 25% carbon dioxide.
3.4.2	Body weight	Yes. Reported for experiment 2, when animals were exposed to 23% carbon dioxide. Body weight observations are not reported for experiment 1 (where animals were exposed to 25% carbon dioxide). Time periods for when weight was determined has not been reported.
3.4.3	Food consumption	Yes. Time periods for when food consumption levels were determined have not been reported.
3.4.4	Water consumption	Not reported, but note body weight observations.
3.4.5	Ophthalmoscopic examination	Not reported.
3.4.6	Haematology	Details of haematology investigations not reported.
3.4.7	Clinical Chemistry	Details of clinical chemistry investigations not reported.
3.4.8	Urinalysis	Details of urinalysis not reported.
3.5	**Sacrifice and Pathology**	
3.5.1	Organ weights	No pathology investigations reported.
3.5.2	Gross and histopathology	No pathology investigations reported.
3.5.3	Other examinations	None reported.
3.5.4	Statistics	Statistical analysis not reported.
3.6	**Further remarks**	None reported.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	After 4 days exposure to 25% carbon dioxide in experiment 1, the concentration of carbon dioxide was dropped to 20% since the animals became seriously depressed and a serosanguineous exudate appeared on the exposed mucous membranes. No specific clinical signs reported in experiment 2, when animals were exposed to 23% carbon dioxide however the observation was made that the experiment could not have continued for much longer than the 34 day exposure period, without death of the animals. When this group of animals was removed to room atmosphere, they became very irritable and developed moderate tetany with episodes of mild clonic convulsions. This state lasted 12 hours but no permanent effects were

4.1.1	Clinical signs (Continued).	observed. The animals had recovered completely within a few days. The action of these animals is in direct contrast to that observed in acute toxicity studies carried out by the same authors. Limited details of these tests have been given, however the authors have reported the following results: when rats were exposed to 25% carbon dioxide they were much more depressed during exposure and no convulsive manifestations were evident on removal: in fact the depression produced by a given concentration of carbon dioxide was always greater upon sudden exposure than during pronged exposure. The toxicity of carbon dioxide is approximately the same for certain other laboratory animals, as it is for the rat. Rabbits were depressed very significantly by a concentration of 27% carbon dioxide, built up slowly over a period of 72 hours. A sanguineous exudate appeared on exposed mucous membranes, but no convulsions were noted on removal. Dogs were partially narcotised by a concentration of 23% carbon dioxide, even when this level was reached by slow accumulation over a five-day period. No convulsions occurred when the dogs were moved to room air.
4.1.2	Mortality	In experiment 1, where carbon dioxide levels were allowed to reach 25%, one mortality was reported after 4 days at 25% carbon dioxide. Following this mortality, the concentration of carbon dioxide was dropped to 20% due to concerns about the welfare of the test animals.
4.2	**Body weight gain**	No body weight observations were reported for those animals exposed to 25% carbon dioxide in experiment 1. However, it was reported that 50% of the original body weight was lost in those animals exposed to 23% carbon dioxide in experiment 2. An observation was made that experiment 2 (34 days exposure to increased levels of carbon dioxide) could not have continued for much longer without the death of the animals. It should be noted, however, that when the animals were moved to room atmosphere after 34 days exposure to increased carbon dioxide levels, they gained weight rapidly and had recovered completely within a few days.
4.3	**Food consumption and compound intake**	Rats ate sparingly at carbon dioxide above 10% and food was refused when carbon dioxide levels were above 23%
4.4	**Ophthalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	Details of haematology investigations not reported.
4.5.2	Clinical chemistry	Details of clinical chemistry investigations not reported.
4.5.3	Urinalysis	Details of urinalysis not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No pathology investigations reported.
4.6.2	Gross and histopathology	No pathology investigations reported.
4.7	**Other**	No other examinations reported.
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and**	This study was not carried out to Guideline B.29 in Annex V of

	Methods	Directive 67/548/EEC. (Continued.....)

Section A6.4.3 Subchronic Inhalation Toxicity Test (9 of 11)

Annex Point IIA, VI, 6.4

5.1	**Materials and Methods** (Continued).	The chronic 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**	Prolonged exposure of rats to 25% carbon dioxide could not be maintained. Exposure to 25% carbon dioxide for 4 days caused the death of one animal, and the others became seriously depressed and a serosanguineous exudate appeared on the exposed mucous membranes. The maximal tolerated dose of carbon dioxide under prolonged exposure was approximately 23% although due to the significant weight loss observed (around 50%), this level of exposure could not have continued for much longer than the test period of 34 days. When the animals were moved back into room atmosphere, the clinical signs and weight loss were quickly reversed, and the test animals had recovered completely within a few days. This study is one in a series of studies by the same authors where they compare the acute and chronic toxicity of carbon dioxide. They conclude that rats can tolerate higher levels of carbon dioxide when the level is attained gradually over several days indicating that the rat is capable of a certain degree of acclimatisation.
5.3	**Conclusion**	
5.3.1	LO(A)EL	LOEL: 23 % carbon dioxide.
5.3.2	NO(A)EL	Not reported.
5.3.3	Reliability	3
5.3.4	Deficiencies	Yes It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of pronged exposure to 20-25% carbon dioxide to rats. While this study was not generated to modern, scientifically accepted protocols, nor was it a full 90 day investigation, it does provide useful data on some of the parameters measured in a subchronic study. Despite the deficiencies in this study, it does gives an indication about the level of carbon dioxide that can be tolerated by rats over a pronged period. This study, notwithstanding it's deficiencies, can be used to support the inhalation toxicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric concentrations.

	(Continued.....)

Section A6.4.3 Subchronic Inhalation Toxicity Test (9 of 11)

Annex Point IIA, VI, 6.4

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

Section A6.4.3 **Subchronic Inhalation Toxicity Test (9 of 11)**
Annex Point IIA, VI, 6.4

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

Section A6.4.3
Annex Point IIA, VI, 6.4

Subchronic Inhalation Toxicity Test (10 of 11)

Official use only

1. REFERENCE

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

2. GUIDELINES AND QUALITY ASSURANCE

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

3. MATERIALS AND METHODS

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

[redacted]

Section A6.4.3 Subchronic Inhalation Toxicity Test (10 of 11)

Annex Point IIA, VI, 6.4

3.2	**Test Animals**	
3.2.1	Species	Guinea Pig.
3.2.2	Strain	Hartley.
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. Test animals weighed between 400g and 600g.
3.2.6	Number of animals per group	Groups of animals between 15-28 used for organ weight determination. Groups of animals between 8-15 used for clinical chemistry investigations. Groups of animals between 5-20 used for adrenal cholesterol content determination (refer to Table A63-1 at end of this summary for further details of grouping in this test). Groups of animals between 5-15 used for total leukocytes and total lymphocyte determination. (Refer to Table A63-2 at end of this summary for further details of grouping in this test).
3.2.7	Control animals	Test animals acted as their own controls, as well as control groups of 30, 15 and 10 animals.
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of treatment	Other: 7 days, 15 days, 20-40 days and 42 days (see details, below). Adrenal cholesterol values, total leukocytes and total lymphocytes were determined after 7, 15 and 42 days exposure. Acid-base balance, blood corticosteroids, adrenal medullary response and epinephrine-dependent effects were determined after 7 and 15 days exposure. Organ weights were determined after 7 days exposure and 20-40 days exposure to 15% carbon dioxide.
3.3.2	Frequency of exposure	Other: Continuous exposure and intermittent (8 hour daily for 7 days).
3.3.3	Post exposure period	11 days.
3.3.5	**Inhalation**	
3.3.5.1	Concentrations	Nominal concentration 15 % carbon dioxide (+/- 0.5 %) No analytical concentration reported.
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.5.4	Type of exposure	Whole body.
3.3.5.5	Vehicle	Gas.
3.3.5.6	Concentration in vehicle	Gas mixture contains 15 % carbon dioxide in air (21% oxygen). Carbon dioxide levels were maintained +/- 0.5% of the stated level, and the oxygen concentration was maintained +/- 1% of the stated level.

Section A6.4.3 Subchronic Inhalation Toxicity Test (10 of 11)

Annex Point IIA, VI, 6.4

3.3.5.7	Duration of exposure	7 days, exposure to 15% carbon dioxide, followed by 11 days in normal atmospheric levels of carbon dioxide. For some investigations, exposure to 15% carbon dioxide was extended to 15 days exposure, 20-40 days exposure and 42 days exposure.
3.3.5.8	Controls	30 animals used for organ weight determination. 15 animals used for clinical chemistry investigations. 10 animals used for adrenal cholesterol content determination. Test animals acted as their own controls for the total leukocytes and total lymphocyte determination.
3.4.	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	None reported.
3.4.1.2	Mortality	None reported. Time periods when mortality was checked has not been reported.
3.4.2	Body weight	Yes. The study report includes a graph charting the measurement of weight. (Refer to graph 1 at the end of this study summary for further details). This chart shows that weight measurement was recorded regularly throughout the exposure period, and the post exposure period (11 days in air).
3.4.3	Food consumption	Not reported, but note body weight observations.
3.4.4	Water consumption	Not reported, but note body weight observations.
3.4.5	Ophthalmoscopic examination	Not reported.
3.4.6	Haematology	Yes. Number of subjects: Refer to table A6_3.2 at end of this study summary for details. Time points: Total leukocytes and total lymphocytes measured after 1 day, then on days 2-3 and days 4-7 followed by measurements on day 15 and day 42. Parameters: Other: Total leukocytes and total lymphocytes.
3.4.7	Clinical Chemistry	Yes Number of animals: All. Time points: Not specifically reported, but graph of results, given in graph 2 at the end of this study summary, shows that measurements were taken prior to exposure to carbon dioxide, and then regularly throughout the entire test period. Parameters: Other. Acid-base balance (pH of arterial blood), adrenal cortical (blood corticosteroids) adrenal medullary response (adrenal epinephrine content), and epinephrine-dependent effects (free fatty acids). Refer to graph 2 at the end of this study summary for details about blood pH, blood corticosteroids, adrenal epinephrine and free fatty acid levels measured in the test animals prior to, during and after exposure

3.4.8	Urinalysis	Not reported.	
3.5	**Sacrifice and Pathology**		
3.5.1	Organ weights	Yes. Organs: Other: Adrenals, thymus, para-arterial nodes and spleen. Refer to graph 3 at the end of this study summary for details about organ weights of the test animals prior to, during and after exposure to 15% carbon dioxide.	
3.5.2	Gross and histopathology	Yes. Number of animals: Organs:	All. Other: Adrenal cholesterol levels were measured after 1 hour exposure to 15% carbon dioxide, and then daily between days 1-7 then on day 15 and day 42. During the 11 day recovery period, adrenal cholesterol levels were measured on day 1 and day 11. Refer to Table A6_3-1at the end of this study summary for further details about the adrenal cholesterol levels measured in the test animals.
3.5.3	Other examinations	None reported.	
3.5.4	Statistics	Yes. Standard deviation and *t* test carried out on data obtained. Refer to tables A63-1, A63-2 and A63-3, and graphs of results given at the end of study summary for details of statistics carried out on results.	
3.6	**Further remarks**	None.	
		4. RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	No clinical signs reported.	
4.1.2	Mortality	No mortalities reported.	
4.2	**Body weight gain**	Exposure of guinea pigs to 15% carbon dioxide in 21% oxygen resulted in a precipitous loss of body weight amounting to about 10% during the first 2 days, followed by a rapid gain during the subsequent exposure period. Refer to graph 1 at the end of this study summary for further details. Initial values were reached between 5 and 7 days of the exposure period. During the subsequent recovery period on air (for 11 days post-exposure), animals increased their body weight at a rate corresponding to that measured during the control period.	
4.3	**Food consumption and compound intake**	Not reported, but note body weight observations.	
4.4	**Ophthalmoscopic examination**	Not reported.	
4.5	**Blood analysis**		
4.5.1	Haematology	Total leukocytes and total lymphocytes of guinea pigs exposed to 15% carbon dioxide for various periods of time are listed in Table A63_3-2, at the end of this study summary. A marked lymphopenia indicative of adrenal cortical stimulation was found to be limited to the 3-day period of uncompensated respiratory acidosis (the first 3 days of exposure to 15% carbon dioxide, after which the respiratory acidosis	

		was practically compensated). The transition to air following a 7-day exposure to 15% carbon dioxide also resulted in a transitory lymphopenia during 1-day recovery.

4.5.2	Clinical chemistry	Refer to graph 2 at the end of this study summary for details about blood pH, blood corticosteroids, adrenal epinephrine and free fatty acid levels measured in the test animals prior to, during and after exposure to 15% carbon dioxide. The pH of arterial blood fell to it's lowest point (pH 7) after 1 hour exposure to 15% carbon dioxide, it then rose to 7.10 after 6 hours and remained at this level during the first day, but increased 0.1 pH units on each of the subsequent two days. After 3 days, the respiratory acidosis induced by the inhalation of 15% carbon dioxide was practically compensated. Both blood corticosteroid increase and adrenal epinephrine depletion were limited to the 3-days of uncompensated respiratory acidosis. The same is true for the rise in free fatty acids. Extended exposure to 15% carbon dioxide for 15 days did not produce significant changes in the values measured after 7 days of exposure. Data for pH, blood corticosteroids and adrenal epinephrine content of guinea pigs exposed intermittently to 15% carbon dioxide (in 21% oxygen) for 8 hours daily for 7 days to 15% carbon dioxide in 21% oxygen are given in table A63-3 at the end of this study summary, with control data and values obtained after 7 days of continuous exposure to 15% carbon dioxide. It is clearly evident from this data that animals exposed intermittently to 15% carbon dioxide for 7 days do neither attain a compensation of the respiratory acidosis or the associated decline of the sympathoadrenal response.
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	The effect of chronic hypercapnia on organ weights of adrenals, thymus, para-arterial nodes and spleen, expressed as percent body-weight has been shown in graph 3 at the end of this study summary. Adrenal weights were found to be significantly increased after 1 day exposure to 15% carbon dioxide, and remained elevated for 7 days. Thymus, para-arterial nodes and spleen showed a marked fall in weight during the first day and did not return to initial values during the 7-day exposure period, with the exception of the spleen, which showed a transitory decrease limited to 1 day. After an extended exposure for 20-40 days to 15% carbon dioxide, most of the organ weights (expressed in percent body-weight) had returned to approximately normal values with the exception of the thymus weight, which remained lower. Similar observations were made after the recovery period of 11 days following a 7-day exposure to 15% carbon dioxide. In this case most of the organ weights had reached initial values. However, the adrenal weight still persisted.
4.6.2	Gross and histopathology	Adrenal cholesterol values of guinea pigs exposed to 15% carbon dioxide for various periods of time are listed in table A6_3-1 at the end of this study summary. A significant decrease in adrenal cholesterol indicative of adrenal cortical stimulation was found to be limited to the 3-day period of uncompensated respiratory acidosis (the first 3 days of exposure to 15% carbon dioxide, after which the respiratory acidosis was practically compensated). The transition to air following a 7-day exposure to 15% carbon dioxide also resulted in a transitory adrenal

		cholesterol decrease during 1-day recovery.
4.7	**Other**	Not reported.

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5. APPLICANTS SUMMARY AND CONCLUSION

5.1 Materials and Methods

This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC.

Male guinea pigs of the Hartley strain, weighing between 400 and 600g were exposed to 15% carbon dioxide in air (21% oxygen). The gas mixtures were prepared in the laboratory by mixing pure carbon dioxide with compressed air and oxygen in high-pressure cylinders. These were analysed with the Scholander apparatus. A plastic chamber was employed for the experiments. The animals were carefully selected. After arrival at the laboratory, the guinea pigs were housed in individual cages and measurements of body weights were made for 3-4 days. Only animals that gained weight and had a leukocyte count below 11,000 were used in the experiments. The carbon dioxide concentrations were kept at 15% (within limits +/-0.5%) and the oxygen concentration at 21% (+/- 1%). The exposure chamber was installed in an air-conditioned room. A closed-circuit system within the chamber circulated air continuously through silica gel containers. With these means, the environmental temperature was kept at 78F (+/- 2F), and the humidity at 65-75%. Ammonia vapour was absorbed by boric acid placed in a second closed circuit within the chamber. The exposure chamber was opened every morning for a period of about 3-5 minutes to fill the water and food containers, and to take out the urine and faeces.

Prior to sacrifice, the animals received 40 mg/kg pentobarbital subcutaneously and were returned to the carbon dioxide exposure chamber within 4-5 seconds. The anaesthesia was usually effective after approximately 5 minutes, at which time the animals were taken out of the exposure chamber and immediately placed under a mask through which they breathed the same carbon dioxide mixture to which they had been exposed. Blood samples were drawn from the abdominal aorta. Blood pH was determined with an Instrumentation Laboratory blood gas and pH analysing system. Blood corticosteroids were determined using the technique of Siber, Busch and Oslapas[1].

Epinephrine content of the adrenals was measured using the method of Anton and Sayre[2]. The adrenal tissues were immediately frozen until used. Nonesterified free fatty acids were determined with the technique of Dole[3], as modified by Trout *et al.*[4] Adrenal cholesterol was measured according to the method of Kingsley and Schaffert.[5] Organ weights were determined using a Christian-Becker balance, to 0.10 mg.

[redacted]

5.2	**Results and discussion**	The stress of exposure to 15% carbon dioxide produces a drastic drop in body weight during the first two days of exposure, which must be (Continued....)

5.2	**Results and discussion** (Continued)	related to a marked decrease in food intake during this period. Organ changes associated with the stress response (adrenal enlargement and involvement of lympathic organs) are clearly expressed after 1 day of exposure. The thymus gland weight appears to be continuously depressed during carbon dioxide exposure, whereas the weight of adrenal glands and that of the periarterial nodes return to control values after 20 days of exposure, but not during the 4-days of compensated respiratory acidosis within the 7 day exposure period. This demonstrates a time-lag between functional changes expressed in blood corticosteroids, adrenal epinephrine responses and total organ weight changes. The spleen, which is known to function as a blood store, shows a significant weight decrease after 1 day of exposure to 15% carbon dioxide during the uncompensated phase of respiratory acidosis and returns to approximately normal values after 3 days exposure, associated with the compensation of the respiratory acidosis. This result suggests a pH dependent spleen contraction. The carbon dioxide-induced adrenal cortical and adrenal medullary response represents an unspecific pH-dependent effect. Compensation of the respiratory acidosis was found to be associated with an abatement of the sympathoadrenal response. Further evidence for the pH dependence of the latter is shown in the results of experiments with intermittent exposure to 15% carbon dioxide for 7 days, which failed to produce a compensation of the respiratory acidosis and resulted in a persistence of the sympatho-adrenal response. The results also demonstrate a close interaction of adrenal cortical and adrenal medullary responses. After 1 hour exposure to 15% carbon dioxide, blood corticosteroids have significantly increased and the adrenal epinephrine content has markedly declined, suggesting a release of catecholamines. However, the free fatty acid level did not change during this period of in spite of the endogenous epinephrine release. A further increase of the epinephrine release after 6-hr exposure to 15% carbon dioxide results in a rise of free fatty acid levels to twice normal. Both free fatty acid values and adrenal epinephrine content return to initial values with the compensation of the respiratory acidosis which demonstrates pH dependence of the sympathoadrenal stimulation in chronic hypercapnia. Although the recovery response following chronic hypercapnia has not been investigated in regard to such sensitive parameters as blood corticosteroid levels and adrenal epinephrine content, lymphopenia and a significant decrease found in adrenal cholesterol after 1-day recovery on air, following 7 days of exposure to 15% carbon dioxide suggests a stimulatory effect of the adrenals.
5.3	**Conclusion**	
5.3.1	LO(A)EL	LOEL: 15 % carbon dioxide* * Despite there not being a range of carbon dioxide levels tested, the results to this study show a low observable effect level to guinea pigs when exposed to 15 % carbon dioxide.
5.3.2	NO(A)EL	Not reported.

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5.3.1	Reliability	3
5.3.2	Deficiencies	Yes

It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of pronged exposure to 15% carbon dioxide to guinea pigs. While this study was not generated to modern, scientifically accepted protocols, it does provide useful data on the majority of parameters measured in a subchronic study.

Despite the deficiencies in this study, and the fact that test subjects were only exposed to one level of carbon dioxide, it does gives an indication about the level of carbon dioxide that can be tolerated by guinea pigs over a pronged period.

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

1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any elevation in the level of carbon dioxide in air, outside normal atmospheric ranges.
2. The potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal, and any exposure would be well below the established occupational exposure limits set by a number of different regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions.
3. The objective of toxicity testing is to predict the toxicological effect in humans, however as a maximum occupational exposure limit for carbon dioxide is already well established, and the limit set by a number of regulatory authorities is in general agreement, further toxicity testing is not considered scientifically necessary.
4. There is sufficient data available concerning the subchronic toxicity of carbon dioxide in various species (including rats, man and mammals). However, because the occupational exposure standard for safe working conditions with carbon dioxide is well established, this value can be used for the risk assessment*.

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

Results of Haematology

Table A63-1 Effect of prolonged exposure to 15% carbon dioxide in 21% oxygen on adrenal cholesterol content of guinea pigs

Condition	Adrenal Cholesterol, mg/100g	
	Mean +/- SD	N
Control	6.1 +/- 0.80	10
Exposure to 15% carbon dioxide in 21% oxygen		
1 hour	4.52* +/- 0.96	8
1 day	4.02* +/- 1.1	20
2 days	3.85* +/- 1.3	6
3 days	5.86 +/- 1.9	12
4 days	5.90 +/- 1.6	6
5 days	6.30 +/- 1.3	8
6 days	7.34 +/- 2.15	6
7 days	6.92 +/- 1.54	7
15 days	5.15 +/- 1.45	8
42 days	7.64 +/- 1.28	5
Recovery on air following 7 days of exposure to 15% carbon dioxide		
1 day	4.73 # +/- 1.2	17
11 days	5.28 +/- 0.82	13

Key: N Number of animals exposed
* Differences from controls statistically significant at the 1% level ($P < 0.01$)
# Differences statistically significant when compared when compared with data obtained at 7 days of exposure to 15% carbon dioxide ($P < 0.01$).

Table A63-2 Effect of prolonged exposure to 15% carbon dioxide in 21% oxygen on leukocyte count and number of total lymphocytes of guinea pigs.

Conditions	Leukocytes (cells.mm^2)		Lymphocytes (cells.mm^2)		
	Mean +/- SD	N	Mean +/- SD	N	*P*
Exposure to 15% carbon dioxide in 21% oxygen					
Control	5,840 +/- 2,033	15	3,820 +/- 1,047	15	
1 day	6,715 +/- 2,669	15	2,240* +/- 1,500	15	0.01
Control	5,667 +/- 2,225	15	3,490 +/- 597	15	
2-3 days	4,647 +/- 1,513	15	2,296* +/- 634	15	0.001
Control	5,400 +/- 1,713	20	3,188 +/- 1,097	15	
4-7 days	6,257 +/- 1,221	20	3461 +/- 1,165	15	
Control	5,800 +/- 1,296	7	4,019 +/- 1,137	5	
15 days	5,471 +/- 1,247	7	4,055 +/- 1,109	5	
Control	7,900 +/- 1,380	5	5,495 +/- 1,175	5	
42 days	7,270 +/- 1,128	5	4,825 +/- 825	5	
Recovery on air following 7 days exposure to 15% carbon dioxide					
Control	5,707 +/- 1,716	14	4,871 +/- 1,027	14	

1-day recovery	4,185 +/- 1,215	14	2,742* +/- 796	14	0.05
Control	5,100 +/- 1,100	7	3,815 +/- 1,071	7	
11-days recovery	5,300 +/- 1,350	7	3,151 +/- 881	7	

Key: N Number of animals exposed
* Differences from controls statistically significant at the 5% level and better. Each experimental group served as it's own control.

Results of Haematology

Table A63-3 Stress effect of intermittent 8-hr exposure to 15% carbon dioxide in 21% oxygen for 7 days as compared with that of continuous 7-day exposure.

Conditions		pH	Blood corticosteroids mg/l	Adrenal epinephrine µg/g
Control	Mean +/- SD N	7.410 +/- 0.025 15	29.0 +/- 12.8 11	179.3 +/- 42.0 10
15% carbon dioxide 7 days continuous exposure	Mean +/- SD N	7.37 +/-0.035 8	32.3 +/- 13.9 8	150.9 +/- 73.0 8
7 days intermittent exposure, sacrificed end of 8 hour carbon dioxide exposure.	Mean +/- SD N	7.111* +/- 0.07 5	72.1* +/- 31.5 5	107.4* +/- 38.3 (5)
7 days intermittent exposure sacrificed end of 16h on air	Mean +/- SD N	7.396 +/- 0.130 5	67.6* +/-35.4 (5)	106.4* +/- 20.1 5

Key: N Number of animals exposed
* Differences from controls statistically different at the 5% level and better (*t* test)

Section A6.4.3 Subchronic Inhalation Toxicity Test (10 of 11)

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

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Official use only

1. REFERENCE

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

2. GUIDELINES AND QUALITY ASSURANCE

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

3. MATERIALS AND METHODS

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

[redacted]

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3.2	**Test Animals**	
3.2.1	Species	Rat and guinea pigs.
3.2.2	Strain	Rats: Albino. Guinea pigs: Connaught.
3.2.3	Source	Rats were from the colony of the Harvard Biological Laboratories. Source of guinea pigs has not been reported.
3.2.4	Sex	Male rats. Male guinea pigs.
3.2.5	Age/weight at study Initiation	Ages of rats were between 75 and 120 days old. Weights of rats have not been reported. Guinea pigs weighed between 500g and 750g. Age of guinea pigs have not been reported, other than mature guinea pigs were used.
3.2.6	Number of animals per group	Test Group II: 32 animals Test group III and IV: 18 animals (each). Note that Group I was the control group.
3.2.7	Control animals	10 normal animals (those animals with their pituitary gland intact), and 5 hypophysectomised animals (animals with their pituitary gland removed).
3.3	**Administration/ Exposure**	Inhalation.
3.3.1	Duration of treatment	Other: Up to 42 days exposure to 1.5% carbon dioxide, followed by a 10-day post exposure period.
3.3.2	Frequency of exposure	Other: Continuous.
3.3.3	Post exposure period	Not reported.
3.3.5	**Inhalation**	
3.3.5.1	Concentrations	Nominal concentration: 1.5% carbon dioxide. No analytical concentration reported.
3.3.5.2	Particle size	Not applicable – carbon dioxide is not an aerosol.
3.3.5.3	Type or preparation of particles	Not applicable – carbon dioxide is not a particulate.
3.3.5.4	Type of exposure	Not reported.
3.3.5.5	Vehicle	Gas.
3.3.5.6	Concentration in vehicle	Not reported.
3.3.5.7	Duration of exposure	Exposure was between 1-42 days exposure.
3.3.15	Controls	Control group consisted of 10 normal animals (those animals with their pituitary gland intact), and 5 hypophysectomised animals (animals with their pituitary gland removed). Control subjects were exposed to normal air.
3.4.	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Clinical signs have not been reported.
3.4.1.2	Mortality	No mortalities reported. Timescales for observation of mortality is not reported.
3.4.2	Body weight	Not reported.
3.4.3	Food consumption	Not reported.

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3.4.4	Water consumption	Not reported.
3.4.5	Ophthalmoscopic examination	Not reported.
3.4.6	Haematology	Yes. Number of subjects: All. Time points: Not reported. Parameters: Other: Leukocyte count, eosinophil content and blood sugar content.
3.4.7	Clinical Chemistry	Not reported.
3.4.8	Urinalysis	Not reported.
3.5	**Sacrifice and Pathology**	
3.5.1	Organ weights	Not reported.
3.5.2	Gross and histopathology	Yes. Number of test subjects: All, unless otherwise specified below. Organs: For rats: "cholesterol" content of the adrenal glands, liver glycogen content and muscle glycogen. Adrenal ascorbic acid content for normal test animals only (those animals with their pituitary gland intact). For guinea pigs: "cholesterol" content of the adrenal glands, liver glycogen content and muscle glycogen.
3.5.3	Other examinations	None
3.5.4	Statistics	Refer to Table A6_3-1, A6_3-2, and A6_3-3, A6_3-4, A6_3-5, A6_3-6 and A6_3-7 for details of statistical analysis.
3.6	**Further remarks**	Blood carbon dioxide tension in rats was determined. Blood carbon dioxide tension and blood pH was determined in guinea pigs.
		4. RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Clinical signs have not been reported.
4.1.2	Mortality	No mortalities reported.
4.2	**Body weight gain**	Not reported.
4.3	**Food consumption and compound intake**	Not reported.
4.4	**Ophthalmoscopic examination**	Not reported.
4.5	**Blood analysis**	
4.5.1	Haematology	White blood cells and lymphocytes: Rats Graph 1 and Table A6_3-1 at the end of this study summary shows the level of white blood cells and lymphocytes measured for the test animals. The mean value of leukocytes of the normal rats (those animals with their pituitary gland intact) under air was 20,219 cells/mm^3 which decreased progressively when exposed to 1.5% carbon dioxide to 9697 cells/mm^3 for the first period (day 1-15) and

4.5.1	Haematology (Continued)	7083 cells/mm^3 for the second period of exposure to carbon dioxide (day 29-42). During the ran parallel to those of the normal animals (those animals with their post-exposure control period on air (10 days), the mean value of leukocytes remained low (7958 cells/mm^3). The leukocyte counts in the hypophysectomised rats (those animals with their pituitary gland removed) ran parallel to those of the normal animals. The mean control value on air was 16,565 cells/mm^3, for the first period of exposure to carbon dioxide (day 1-15) it was 12,793 cells/mm^3 and for the second period of carbon dioxide exposure (day 29-42) it was 3683 cells/mm^3. During the post-exposure period on air the value rose slightly to 5766 cells/mm^3. In both the normal rats (with their pituitary gland intact) and the hypophysectomised rats (those with their pituitary gland removed), the changes in the white blood cell count were of statistical significance according to the Mood median test. The absolute number of lymphocytes paralleled the leukocyte count of the normal rats (those with their pituitary gland intact) and the hypophysectomised rats (those with their pituitary gland removed) (refer to Table A6_3-1 at the end of this study summary for details). The hypophysectomised animals reacted in a similar manner, and differences were not statistically significant Lymphocytes: Guinea pigs Table A6_3-5 at the end of this study summary shows the level of lymphocytes measured for the test animals. The absolute counts of lymphocytes exhibited a decrease during exposure to carbon dioxide and returned to approximately the initial levels within a 10-day recovery period. Eosinophils : Rats Graph 1 and Table A6_3-1 at the end of this study summary shows the level of eosinophils measured for the test animals. The normal rats (those with their pituitary gland intact) and the hypophysectomised rats (those with their pituitary gland removed) developed a marked and significant eosinopenia during the exposure period, and this eosinopenia continued during the recovery period of 10 days. Eosinophils: Guinea pigs Table A6_3-5 at the end of this study summary shows the level of eosinophils measured for the test animals. The absolute counts of eosinophils exhibited a decrease during exposure to carbon dioxide and returned to approximately the initial levels within the 10-day recovery period. Blood Sugar Level: Rats Graph 2 and Table A6_3-2 at the end of this study summary shows the blood sugar levels measured for the test animals. The blood sugar level in both the normal rats (those with their pituitary gland intact) and the hypophysectomised rats (those with their pituitary gland removed) did not change significantly during or after exposure to 1.5% carbon dioxide. Blood Sugar Level: Guinea pigs Table A6_3-6 at the end of this study summary shows the blood sugar levels measured for the test animals. These results show that the blood sugar levels in guinea pigs did not vary markedly throughout the experiment.

4.5.2	Clinical chemistry	Not reported.
4.5.3	Urinalysis	Not reported.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Not reported.
4.6.2	Gross and histopathology	Adrenal Ascorbic Acid Content: Rats The adrenal ascorbic acid content was determined for normal rats only (those animals with their pituitary gland intact). Results are given in graph 1 and Table A6_3-1 at the end of this study summary. The results show that the mean value fell significantly from 470 mg/100g of tissue in the pre-exposure control period on air to 291.44 and 277.28 mg/100g tissue during the first period (day 1-15) and second period (day 29-42) of exposure to 1.5% carbon dioxide. The values obtained during the recovery period did not quite return to normal (363.4 mg). "Cholesterol" Content of the Adrenal Glands: Rats The "cholesterol" content of the adrenal glands of the normal rats (those animals with their pituitary gland intact) and the hypophysectomised rats (those animals with their pituitary gland removed) was determined histochemically by the Schultz (II) test. Refer to section 5.1 Materials and Methods for further details about the Schultz (II) test for determination of cholesterol. Results of the determination of cholesterol content of the adrenal glands are given in Table A6_3-3 at the end of this study summary. An exact quantitative determination was not attempted. Slides were evaluated in the following way: the adrenal glands that had a full green reaction to the Schultz reagent were considered to have a 4+ reaction, and a gradient (depending on the colour of the reaction) was standardised from a 4+ to a 0 value. In order to minimise variations, all the glands were processed by the same laboratory technician, at the same time using the same reagents and they were read by the same observer. The adrenal cortices of the normal animals (those with their pituitary gland intact) in the pre-exposure (control) period on air gave a uniform reaction of 3+/- to 4+/- to the reagent. The medulla, as would be expected, did not react in any instance. During exposure to 1.5% carbon dioxide the adrenal Schultz-demonstrable lipid decreased significantly in the zona fasciculata and was absent, in most cases, from the reticularis. The "cholesterol" content of the adrenal cortex did not return to a normal level during the recovery period of 10 days. During the pre-exposure period on air, the adrenal cortices of the hypophysectomised rats (those animals with their pituitary gland removed) gave a 2 to 3+ positive Schultz test; and during exposure to carbon dioxide, the Schultz-reacting material disappeared in most cases from the fasciculata and reticularis, and it's presence was detected only in the glomerulosa. As in the normal rats (those animals with their pituitary gland intact), the Schultz-positive material did not return to the pre-exposure level during the post exposure period of 10 days. "Cholesterol" Content of the Adrenal Glands: Guinea pigs Table A6_3-5 at the end of this study summary gives details of the adrenal cholesterol levels of the test animals. During the first period (day 1-15) and second period (day 29-42) of exposure to 1.5% carbon dioxide and during the 10-day recovery period on air after this carbon dioxide exposure, adrenal cholesterol values in the test animals were

lower than the control values.

(Continued.....)

4.6.2	Gross and histopathology (Continued)	Liver Glycogen: Rats Graph 2 and Table A6_3-2 at the end of this study summary gives details of the average liver glycogen of the test animals. These results show that the average liver glycogen values decreased during exposure to 1.5% carbon dioxide, and returned to initial levels within a 10-day recovery period on air in both normal rats (those animals with their pituitary gland intact) and the hypophysectomised rats (those animals with their pituitary gland removed). Liver Glycogen: Guinea pigs Table A6_3-6 at the end of this study summary gives details of liver glycogen content of the test animals. These results show that liver glycogen decreased significantly during carbon dioxide exposure. Liver glycogen returned to the initial level within the 10-day recovery period on air after the carbon dioxide exposure. Muscle Glycogen: Rats Graph 2 and Table A6_3-2 at the end of this study summary gives details of glycogen content of muscles of the test animals. These results show the muscle glycogen in normal rats (those with their pituitary gland intact) and hypophysectomised rats (those with their pituitary gland removed) decreased significantly during the first period of exposure to carbon dioxide (day 1-15) and the second period of carbon dioxide as well (day 29-42). In contrast to liver glycogen content, muscle glycogen did not return to the initial level during the post-exposure period. Muscle Glycogen: Guinea pigs Table A6_3-6 at the end of this study summary gives details of glycogen content of muscles of the test animals. These results show that skeletal muscle glycogen decreased significantly during carbon dioxide exposure. In contrast to liver glycogen content, muscle glycogen remained low during the post-exposure period.
4.7	**Other**	Blood carbon dioxide content in normal rats (those animals with their pituitary gland intact) was determined. Results are given in Table A6_3-4 at the end of this study summary. The plasma carbon dioxide content of normal rats, as determined with the Kopp-Natelson microgasometer, did not show any significant changes during exposure to 1.5% carbon dioxide. Blood carbon dioxide tension and pH of guinea pigs are presented in Table A6_3-7 at the end of this study summary. Guinea pigs show a significant increase in carbon dioxide tension and a slight drop in pH during exposure to carbon dioxide. Both values did not return to the initial levels within a 10 day period of recovery on air.
		5. APPLICANTS SUMMARY AND CONCLUSION
5.1	**Materials and Methods**	This study was not carried out to Guideline B.29 in Annex V of Directive 67/548/EEC. The animals used in these experiments were mature male albino rats from the colony of the Harvard Biological Laboratories, and mature male guinea pigs of the Connaught strain. The rats varied in age from 75-120 days, and the guinea pigs weighed between 500 and 750g. All animals in any particular group were of the same age.

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

Annex Point IIA, VI, 6.4

5.1 Materials and Methods

(Continued...)

Twenty-nine animals were hypophysectomised (their pituitary gland removed) via the parapharyngeal approach 60 days before the experiment was started, and they were placed in a closed chamber with 83 normal rats (those with their pituitary gland intact). The hypophysectomised rats were fed 5% glucose in their drinking water, and one ounce of evaporated milk daily. All of the test animals were fed Purina Laboratory Chow, oranges and greens.

The experimental animals were divided into four groups. The first group consisted of 10 normal animals (with their pituitary gland intact) and 5 hypophysectomised animals (with their pituitary gland removed), killed during a pre-exposure period of 9 days on air in the chamber. The second group was made up of 21 normal and 11 hypophysectomised animals, killed at intervals during 15 days exposure to 1.5% carbon dioxide. The third group (12 normal and 6 hypophysectomised animals) was killed from 1-10 days during recovery following 42 days exposure to carbon dioxide.

The animals were killed by a blow to the head and immediate exsanguination by cardiac puncture. From each sample of blood, a complete blood count was made, including an absolute eosinophil determination using the method of Randolf[1]. The blood plasma carbon dioxide content in rats was determined by the Kopp-Natelson microgasometer method[2]. In guinea pigs, blood carbon dioxide and oxygen capacity were measured in the Van Slyke apparatus and pH by a Beckman pH meter. Haematocrit was also obtained in guinea pigs. Carbon dioxide tension of the blood in guinea pigs was determined indirectly using the nomogram of Van Slyke and Sendroy[3]. The blood sugar was determined by Folin's micro method[4].

Tissue specimens from each group of experimental animals were sent to the Armed Forces Institute of Pathology for morphological investigation. Supplemental data on the carbohydrate metabolism were collected, such as liver glycogen, muscle glycogen and blood sugar, and carbon dioxide content of the blood were measured as the necessary frame of reference. At the time of autopsy, pieces of liver and muscle were frozen and glycogen determination was made later by the procedure of Good, Kramer and Somogyi[5]. The adrenal ascorbic acid determinations on normal male rats (those with the pituitary gland intact) were made according to Roe and Kuether's method[6] for determination of ascorbic acid in tissue extracts. The remaining adrenal gland of each normal and hypophysectomised rat (with their pituitary gland removed) was placed in 10% buffered formalin and prepared for histochemical cholesterol examination by the technique of Schultz[7]. Adrenal cholesterol in guinea pigs was determined by the technique of Kingsley and Schaffert[8].

The statistical analysis of the values obtained during this experiment was performed according to the Mood Median Test[9] and the criterion of 5% confidence level was used for rejection of null hypothesis

[redacted]

5.2	**Results and discussion**	The adrenal-pituitary interrelationship was investigated during prolonged exposure to 1.5% carbon dioxide for 42 days in normal rats (with their pituitary gland intact) and hypophysectomised rats (with their pituitary gland removed), and in normal guinea pigs. During exposure to carbon dioxide, animals were killed during two periods 11-15 days and 28-42 days of exposure to 1.5% carbon dioxide. In normal and hypophysectomised rats adrenal cortical activity was found increased during both experimental periods of carbon dioxide exposure and in the following recovery period on air. This was indicated by a significant decrease of adrenal cholesterol and a significant eosinopenia and lymphopenia in normal and hypophysectomised rats. Adrenal ascorbic acid content, studied only in normal rats (those animals with their pituitary gland intact) was significantly reduced during exposure to carbon dioxide, but returned approximately to the initial level during a 10-day recovery period on normal air. In guinea pigs adrenal cortical activity was found increased only by during the 28-42 day period of exposure to 1.5% carbon dioxide as shown by a significant eosinopenia and lymphopenia as well as in a decrease of the adrenal cholesterol content. In both rats and guinea pigs the blood sugar was maintained at a normal level, apparently at the expense of the liver and muscle glycogen stores. Liver glycogen returned to pre-exposure levels during a 10-day recovery period, in contrast to muscle glycogen, which remained at a lower level.
5.3	**Conclusion**	
5.3.1	LO(A)EL	LOEL: 1.5 % carbon dioxide.* *Despite there not being a range of carbon dioxide levels tested, the results to this study show low observable effect level to rats or guinea pigs when exposed to 1.5% carbon dioxide.
5.3.2	NO(A)EL	Not reported.
5.3.1	Reliability	3
5.3.2	Deficiencies	Yes It is duly acknowledged that this study has major methodological and reporting deficiencies and this data has not been generated in accordance with scientifically acceptable protocols. Notwithstanding this, this study determines the effects of pronged exposure to 1.5% carbon dioxide to rats and guinea pigs. While this study was not generated to modern, scientifically accepted protocols, nor was it a full 90 day investigation, it does provide useful data on some of the parameters measured in a subchronic study. Despite the deficiencies in this study, it does gives an indication about the level of carbon dioxide that can be tolerated by rats and guinea pigs over a pronged period. This study, notwithstanding it's deficiencies, can be used to support the inhalation toxicity of carbon dioxide because: 1. Under normal conditions of use, the use of carbon dioxide in Rentokil Initial's rodenticide (PT14) products will not cause any

	elevation in the level of carbon dioxide in air, outside normal atmospheric ranges. (Continued....)	

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

TABLE A6_3-1 TO TABLE A6_3-4 DATA FOR RATS

Table A6_3-1 Effect of Prolonged Exposure to 1.5% Carbon Dioxide Upon Adrenal Cortical Activity in Normal and hypophysectomised rats

Chemical Determination and Statistic	Control period on air Group I	Exposure to 1.5% carbon dioxide		Post exposure on air (1-10 days) Group IV
		1-15 days exposure Group II	28-42 days exposure Group III	
Adrenal ascorbic acid Normal rats				
Mean, mg/100g	470.82	291.4	277.28	363.44
SD mg/100g	160.65	110.94	58.21	63.68
No. of rats	8	22	11	10
P		0.01 *	0.01 *	0.1
Eosinophils Normal rats				
Mean, cells/mm^2	186.7	74.9	24.2	31.7
SD cells/mm^2	114.0	90.4	23.4	33.7
No of rats	29	24	12	12
P		< 0.001 *	< 0.001 *	< 0.001 *
Eosinophils Hypophysectomised rats				
Mean, cells/mm^2	139.3	62.6	10.5	21.8
SD cells/mm^2	53.4	44.0	7.1	25.7
No of rats	7	8	6	6
P		< 0.02 *	< 0.001*	<0.001*
Lymphocytes Normal rats				
Mean, cells/mm^2	16,956	8,430	7,663	6,924
SD cells/mm^2	7,020	3,031.6	3,242.0	4,137.1
No. of rats	35	24	12	12
P		<0.001 *	< 0.001 *	< 0.001 *
Lymphocytes Hypophysectomised rats				
Mean, cells/mm^2	13,433	11,266	3,194	5,028
SD cells/mm^2	6,669	5,034.1	1,087.8	730.3
No of rats	9	8	6	6
P		> 0.1	< 0.01 *	< 0.02 #

Key: * Statistically significant difference from mean control on air at 1% level.
Statistically significant difference from mean control on air at 5% level.

Normal rats: rats with their pituitary gland intact.
Hypophysectomised rats: rats with their pituitary gland removed.

Table A6 3-2 Effect of Prolonged Exposure to 1.5% Carbon Dioxide Upon Carbohydrate Metabolism in Normal and hypophysectomised Rats

Chemical Determination and Statistic	Control period on air Group I	Exposure to 1.5% carbon dioxide		Post exposure on air (1-10 days) Group IV
		1-15 days exposure Group II	28-42 days exposure Group III	
Blood sugar Normal rats				
Mean mg %	86.8	94.8	78.0	84.4
SD mg %	27.4	20.1	9.7	14.0
No. of rats	34	22	12	8
P		> 0.1	> 0.1	> 0.1
Blood sugar Hypophysectomised rats				
Mean mg %	91.6	86.5	85.4	96.8
SD mg %	17.6	27.4	8.9	15.1
No. of rats	8	10	5	5
P		> 0.1	> 0.1	> 0.1
Liver glycogen Normal rats				
Mean g %	2.93	2.00	1.36	3.45
SD g %	1.49	1.20	1.06	1.27
No. of rats	10	18	12	11
P		0.1	< 0.02 #	> 0.01
Liver glycogen Hypophysectomised rats				
Mean g %	2.51	1.05	1.67	2.50
SD g %	0.23	0.77	1.07	1.51
No. of rats	5	9	6	6
P		< 0.01 *	> 0.1	> 0.1
Muscle glycogen Normal rats				
Mean g %	0.21	0.09	0.13	0.06
SD g %	0.11	0.11	0.23	0.05
No. of rats	10	22	11	11
P		< 0.02 #	> 0.1	0.001*
Muscle glycogen Hypophysectomised rats				
Mean g %	0.37	0.11	0.12	0.12
SD g %	0.06	0.07	0.05	0.08

No. of rats	5	8	6	2
P		< 0.001 *	< 0.001 *	< 0.01 *

Key: * Statistically significant difference from mean control on air at 1% level.
Statistically significant difference from mean control on air at 5% level.
Normal rats: rats with their pituitary gland intact.
Hypophysectomised rats: rats with their pituitary gland removed

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

Table A6 3-3 Effect of Prolonged Exposure to 1.5% Carbon Dioxide Upon Adrenal Cholesterol (+) in Normal and hypophysectomised Rats

Zone of Cortex and Statistic	Control period on air Group I	Exposure to 1.5% carbon dioxide		Post exposure on air (1-10 days) Group IV
		1-15 days exposure Group II	28-42 days exposure Group III	
Glomerulosa Normal rats				
Mean	3.5 #	2.76	2.6	2.5
SD	0.67	0.69	0.58	0.67
No. of rats	10	21	12	12
t *		2.7 *	3.3	3.2
P		< 0.02	< 0.01	< 0.01
Glomerulosa Hypophysectomised rats				
Mean	2.5	1.8	2.0	2.2
SD	0.5	1.2	0	0.75
No. of rats	4	5	6	5
t		0.97	2.2	0.61
P		> 0.1	< 0.1	> 0.1
Fasciculata Normal rats				
Mean	3.3	2.28	2.5	2.2
SD	0.64	0.48	0.22	0.24
No. of rats	10	21	12	12
t		4.8	4.2	5.0
P		< 0.001	< 0.001	< 0.001
Fasciculata Hypophysectomised rats				
Mean	2.5	0.8	1.8	1.4
SD	0.5	0.4	0.51	0.2
No. of rats	4	5	6	5
t		5.0	1.9	3.79
p		< 0.01	< 0.1	< 0.01
Reticularis Normal rats				
Mean	3.3	1.00	1.1	0.73
SD	0.64	1.23	1.2	1.05
No. of rats	10	21	12	12
t		5.3	5.0	6.4
P		< 0.001	< 0.001	< 0.001
Reticularis Hypophysectomised rats				

Mean	2.25	0	0	0.6
SD	0.43	0	0	0.8
No. of rats	4	5	6	5
t		10.32	11.0	5.0
P		< 0.001	< 0.001	< 0.01

Key: (+) Values describe the degree of colour reaction in arbitrary units (zero to four plus).
* t-ratio with control means (group I)
Normal rats: rats with their pituitary gland intact.
Hypophysectomised rats: rats with their pituitary gland removed.

Table A6 3-4 Effect of Prolonged Exposure to 1.5% Carbon Dioxide Over a Period of 42 Days on the Carbon Dioxide Content of Blood Plasma in Normal Rats

	Control period on air Group I	Exposure to 1.5% carbon dioxide		Post exposure on air (1-10 days) Group IV
		1-15 days exposure Group II	28-42 days exposure Group III	
Blood plasma carbon dioxide in Normal rats				
Mean, vol. %	57.46	58.50	55.50	51.30
SD, vol. %	5.28	9.50	3.01	1.08
No. of rats	10	22	12	5

TABLE A6_3-5 TO TABLE A6_3-7 DATA FOR GUINEA PIGS

Table A6 3-5 Effect of Prolonged Exposure to 1.5% Carbon Dioxide Upon Adrenal Cortical Activity in Guinea Pigs

Chemical Determination and Statistic	Control period on air Group I	Exposure to 1.5% carbon dioxide		Post exposure on air (1-10 days) Group IV
		1-15 days exposure Group II	28-42 days exposure Group III	
Adrenal cholesterol				
Mean, mg %	6.02	5.15	5.34	4.44 #
SD mg %	1.57	0.89	0.85	0.47
No. of animals	9	10	12	7
P		0.1	0.1	0.05
Lymphocytes				
Mean, cells/mm^2	5500	3488 #	3909 #	4792
SD cells/mm^2	2184	1587	1226	1557.3
No. of animals	20	11	11	7
P		0.02	0.05	0.1
Eosinophils				
Mean, cells/mm^2	75.6	76.5	37.34 #	94.6
SD cells/mm^2	39.9	73.5	24.10	65.8
No of animals	20	10	12	7
P		0.1	0.001	0.1
White blood cells				
Mean, cells/mm^2	6120	4386 #	5259	5657
SD cells/mm^2	2004	2140	2064	1730
No of animals	20	11	11	7
P		0.02	0.1	0.1

Key: # Difference from control period statistically significant at 5% level or less.

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

Table A6 3-6 Effect of Prolonged Exposure to 1.5% Carbon Dioxide Upon Carbohydrate Metabolism of Guinea Pigs

Chemical Determination and Statistic	Control period on air Group I	Exposure to 1.5% carbon dioxide		Post exposure on air (1-10 days) Group IV
		1-15 days exposure Group II	28-42 days exposure Group III	
Blood sugar				
Mean mg %	102.3	105.5	90.71	98.9
SD mg %	16.5	17.19	24.15	19.18
No. of animals	19	11	12	7
P		0.1	0.1	0.1
Liver glycogen				
Mean g %	3.32	4.00	1.32 #	3.66
SD g %	1.05	2.54	1.18	1.65
No. of animals	15	9	11	7
P		0.1	<0.001	0.1
Muscle glycogen				
Mean g %	0.708	0.29 #	0.37 #	0.46 #
SD g %	0.26	0.17	0.09	0.11
No. of animals	13	6	9	6
P		0.01	0.01	0.05

Key: # Difference from controls statistically significant at 5% level or less.

Table A6 3-7 Effect of Prolonged Exposure to 1.5% Carbon Dioxide Over a Period of 42 Days on the Blood Carbon Dioxide Tension and pH in Guinea Pigs

Chemical Determination and Statistic	Control period on air Group I	Exposure to 1.5% carbon dioxide		Post exposure on air (1-10 days) Group IV
		1-15 days exposure Group II	28-42 days exposure Group III	
Blood carbon-dioxide tension				
Mean mm Hg	39.7	45.2	47.0 #	45.7
SD mmHg	4.9	8.7	8.7	9.0
No. of guinea pigs	13	9	10	6

Blood pH				
Mean	7.42	7.37	7.38	7.38
SD	0.04	0.19	0.05	0.04
No. of guinea pigs	13	9	11	6

Key: # statistically significantly different from controls at the 5% level

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

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

Reliability	*Discuss if deviating from view of rapporteur member state.*
Acceptability	*Discuss if deviating from view of rapporteur member state.*
Remarks	

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

Section 6.5 **Annex Point IIA, VI, 6.5**	**Chronic Toxicity** 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** [4] **Scientifically unjustified** [4]	
Limited exposure [4]	**Other justification** [ ]	
Detailed justification:	It is not considered scientifically necessary to carry out a chronic toxicity study for carbon dioxide on the basis of the findings of the 90-day subchronic toxicity test (A6.4.3). All effects found in the subchronic 90-day toxicity test were found to be reversible. 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 data on the long term toxicity of the active substance may not be required if the subchronic toxicity test demonstrates reversibility. Even though this is the case for carbon dioxide, and it forms part of the justification for not submitting data on the long-term toxicity of this compound, data on the chronic toxicity of carbon dioxide is not considered scientifically necessary for the following additional reasons: **<u>Introduction</u>** The Biocidal Products Directive (98/8/EEC 'the Directive) requires long-term testing in rodents as part of the suite of toxicology tests in order to assess the possible adverse consequences of chronic exposure (i.e., chronic toxicity and carcinogenicity) to the biocidal active substance. It is a unique feature of the rodenticides that the test species used in long-term toxicity and carcinogenicity studies is also the target species. This gives rise to several questions: Is it relevant to consider the possible use of long term rodent studies to predict possible effects of rodenticides in humans, and is it scientifically feasible? Are there other data that demonstrate the potential, or lack of potential, carcinogenic properties of active substances used as rodenticides? The Directive states in Article 8 (5) that "*information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information. In such cases, a justification, acceptable to the competent authority must be submitted…*". A more detailed waiver concept is given in the TNsG on data requirements. The TNsG gives the strong recommendation "*to minimise testing on vertebrate animals or to avoid unnecessary suffering of experimental animals the data should not be generated*". (Continued……)	

Detailed justification:
(Continued)

Behind this background, the waiver concept outlined in the TNsG on data requirements is considered applicable for (at least some types of) rodenticides with regard to rodent long-term toxicity / carcinogenicity studies and therefore, a scientific justification for waiving these studies is presented below.

Rodenticides fall broadly into three types: anticoagulants, CO_2 and general chemicals. Anticoagulants inhibit the production pathways of Vitamin K and cause death through fatal haemorrhage, CO_2 causes cellular acidosis, and general chemicals typically affect cellular and/or neural function.

Scientific necessity

It is not considered necessary to determine the chronic toxicity of carbon dioxide for a number of reasons, including:

- [redacted]It is not scientifically necessary on the basis of low exposure to carbon dioxide during its normal use as a biocide. The use of carbon dioxide as a rodenticide, under normal conditions of use, will not cause any elevation in the level of carbon dioxide found in air, outside normal atmospheric ranges. [redacted]

  For details of the scientific calculation, which supports this statement, refer to Annex 1.

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

(Continued.....)

Detailed justification:
(Continued)

[redacted]

- Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers[7]. Such data have been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement, for example the US OSHA permissible exposure level (PEL) is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm.[5] The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period)[8]. As explained above, the use of carbon dioxide as a rodenticide does not increase atmospheric carbon dioxide levels, and this is well below these agreed maximum exposure limits for safe working conditions. As the objective of an animal test is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value.
- There is a substantial volume of information available for carbon dioxide, and while there are no studies available which consider chronic toxicity, carcinogenicity, or genotoxicity specifically nor was the data generated to modern scientifically acceptable protocols, it does cover all the major biological considerations. Given the large volume of data available for carbon dioxide, only the typical findings have been summarised below with regards to the chronic toxicity and carcinogenic potential of carbon dioxide. A number of reviews have been carried out by different regulatory authorities including the EPA[5] and FDA, who considered the health aspects of carbon dioxide as a food additive[6]. Both the EPA and FDA consider that the amount of data that is available on carbon dioxide, and the levels of exposure which occur when carbon dioxide is being used as a biocide means that the margin of safety is acceptable. As a result they have not asked for any new studies to be generated, even though there is no specific data regarding the chronic toxicity, carcinogenic or geneotoxic potential of carbon dioxide.

Technical feasibility

While it is possible to carry out a chronic toxicity study on carbon dioxide, it will be technically very difficult, full of constraints and expensive. The data given below shows how the body's metabolism and physiology are extremely sensitive to carbon dioxide levels, and will adjust to any atmospheric changes. This effects the body metabolism making it difficult to differentiate any observations on the test animal as a toxic effect of carbon dioxide itself, or as a secondary effect of the body's change in metabolism as it adjusts to the change in atmospheric

(Continued.....)

Detailed justification:
(Continued)

carbon dioxide levels. Because of this, even if the chronic toxicity study was carried out, it is not going to provide any useful data for the risk assessment.

Exposure to increasing concentrations of carbon dioxide: Effects and Observations

Carbon dioxide is a natural substance, produced by cellular breakdown of carbon-based materials. It is excreted by exhaling. Toxicity is acute, by cellular acidosis disrupting enzyme activities and reducing cellular respiration beyond the point where the organism as a whole can survive [1].

Carbon dioxide is naturally produced by the body, and is effectively regulated by a series of homeostatic mechanisms designed to maximise the carbon dioxide-carrying capacity of the blood. Cells produce carbon dioxide as part of the normal catabolic process. This carbon dioxide diffuses in solution from the cell to the blood plasma and thence to the red cells. Under normal circumstances, in the resting human, the dissolved concentration of carbon dioxide in the blood is between 48 (arterial) and 52 (venous) ml/100 ml blood. Very low levels of carbon dioxide may lead to failure to stimulate inspiration. Vigorous exercise increases the amount of carbon dioxide carried and exhaled (mainly by increased heart rate and respiratory rate), but as the excretion of the gas depends on a diffusion gradient across the alveolar wall, the amount of carbon dioxide already present in the air will govern the efficiency of excretion. Normal alveolar partial pressure of carbon dioxide is approximately 5-6% carbon dioxide. Typically, normal air contains 0.03% carbon dioxide. If extra carbon dioxide is added such that alveolar concentration increases by just 0.2%, the resting pulmonary ventilation is doubled [2]. If the concentration of carbon dioxide is so high that the organism cannot cope by further increasing respiratory rate, death occurs when the diffusion gradient between the cells of the body and the blood no longer functions.

Exposure to increasing levels of carbon dioxide produces respiratory distress, as the animal attempts to exhale the increasing amounts accumulating in the body [2]. Breathing rate increases to a maximum, followed by loss of consciousness and death. When guinea pigs were exposed to 15% carbon dioxide in 21% oxygen continuously for seven days, blood pH initially fell after 1 hour of exposure, and then rose to 7.10 after 6 hours, and continued to rise back to the initial pH value. Blood corticosteriods rose markedly, and adrenal epinephrine fell. Levels of free fatty acids in the arterial blood rose, and lymphocytes and adrenal cholesterol decreased. These changes occurred only during the first three days of exposure. After this, corticosteriods, adrenal epinephrine, free fatty acids, lymphocytes and adrenal cholesterol content all returned to initial levels, as the body's metabolism compensated for the increase in carbon dioxide [3]. There is also data available to show this effect in man, when 23 subjects were exposed to 1.5% carbon dioxide in 21% oxygen for 42 days. The body began to compensate for the increased level of carbon dioxide after 23 days exposure [4]. The compensation effect does not appear to occur when animals are exposed to increased levels of carbon dioxide for intermittent periods [3]. An occupational exposure study on brewery workers, over

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Detailed justification:
(Continued)

five days where the time weighted average concentrations of carbon dioxide ranged from 0.5 to 1.95% (with a mean of 1.08 % but momentary concentrations reached 8%), concluded that there were no significant physiological effect of chronic intermittent exposure to these levels of carbon dioxide [7].

Conclusion

On the basis of exposure alone, it is not scientifically necessary to conduct a chronic toxicity study for carbon dioxide. The use of carbon dioxide as a rodenticide does not increase carbon dioxide above levels found naturally in the atmosphere, and this is well below established maximum occupational exposure limits for safe working conditions. A chronic toxicity study is technically feasible, but difficult, and given the body's metabolic and physiological sensitivity to changes in carbon dioxide levels it is unlikely to provide any useful data for the risk assessment. The toxicological profile of carbon dioxide is well established with a substantial amount of data. Although this information has it's limitations and it does not address the issue of chronic toxicity, carcinogenicity or genotoxicity specifically, it is considered sufficient to address the toxicity of carbon dioxide particularly given the low level of exposure expected from it's use as a rodenticide.

References for chronic toxicity data waiver for carbon dioxide

Section 6.5 **Annex Point IIA, VI, 6.5**	**Chronic Toxicity** Section 6: Toxicological and Metabolic Studies

Detailed justification:

	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 6.6.1 **Annex Point IIA, VI, 6.6.1**	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in bacteria	
	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**	[4]
Limited exposure	[4]	**Other justification**	[ ]		

Detailed justification:

An *in vitro* gene mutation study in bacteria for carbon dioxide is not considered necessary for a number of reasons including:

[redacted]It is not scientifically necessary on the basis of low exposure. The use of carbon dioxide as a rodenticide, under normal conditions of use will not cause any elevation in the level of carbon dioxide found in air, outside normal atmospheric ranges. [redacted]

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

[redacted]The use of carbon dioxide in an aerosol means that there is no primary exposure to the operator - the only potential exposure to the operator occurs when the rodenticide unit is tripped by a rodent, and the carbon dioxide is released from the aerosol canister to kill the animal. [redacted]

(Continued……)

Section 6.6.1 **Annex Point IIA, VI, 6.6.1**	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in bacteria

Detailed justification:
(Continued)

[redacted]

- Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers [3]. Such data have been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement, for example the US OSHA permissible exposure level (PEL) is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm [5]. The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period)[4]. As explained above, the use of carbon dioxide as a rodenticide does not increase atmospheric carbon dioxide levels, and this is well below these agreed maximum exposure limits for safe working conditions. As the objective of a laboratory test on bacteria is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value.

- There is a substantial volume of information available for carbon dioxide, and while there are no studies available which consider Genotoxicity specifically nor was the data generated to modern scientifically acceptable protocols, it does cover all the major biological considerations. These are considered elsewhere in the toxicity section of this dossier (Section 6 Toxicological and Metabolic Studies). A number of reviews have been carried out by different regulatory authorities including the EPA[5] and FDA, who considered the health aspects of carbon dioxide as a food additive[6]. Both the EPA and FDA consider that the amount of data that is available on carbon dioxide, and the levels of exposure which occur when carbon dioxide is being used as a biocide means that the margin of safety is acceptable. As a result they have not asked for any new studies to be generated, even though there is no specific data regarding the genotoxic potential of carbon dioxide.

(Continued....)

Section 6.6.1 **Annex Point IIA, VI, 6.6.1**	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in bacteria

Detailed justification:
(Continued)

- Technical feasibility
  While it is possible to carry out an *in vitro* gene mutation study in bacteria for carbon dioxide, it will be technically very difficult, full of constraints and expensive. Some of the problems include the fact carbon dioxide is naturally produced by all aerobic cells as a by-product of respiration. This makes it impossible to remove carbon dioxide from the negative controls. Even if the natural atmospheric concentrations of carbon dioxide were taken into account when doing the test, the fact the test cells on both the treated and untreated plates are continually producing carbon dioxide as a by-product of

respiration means that there will be variable concentrations of carbon dioxide at a cellular level. The design of the test could address the various complicating factors such as background carbon dioxide levels, possible pH effects and low oxygen before concluding whether the effect was due to the toxic effects of carbon dioxide, but given the other factors outlined in this data waiver, carrying out an *in vitro* gene mutation study on carbon dioxide is not going to provide any useful data for the risk assessment.

Conclusion

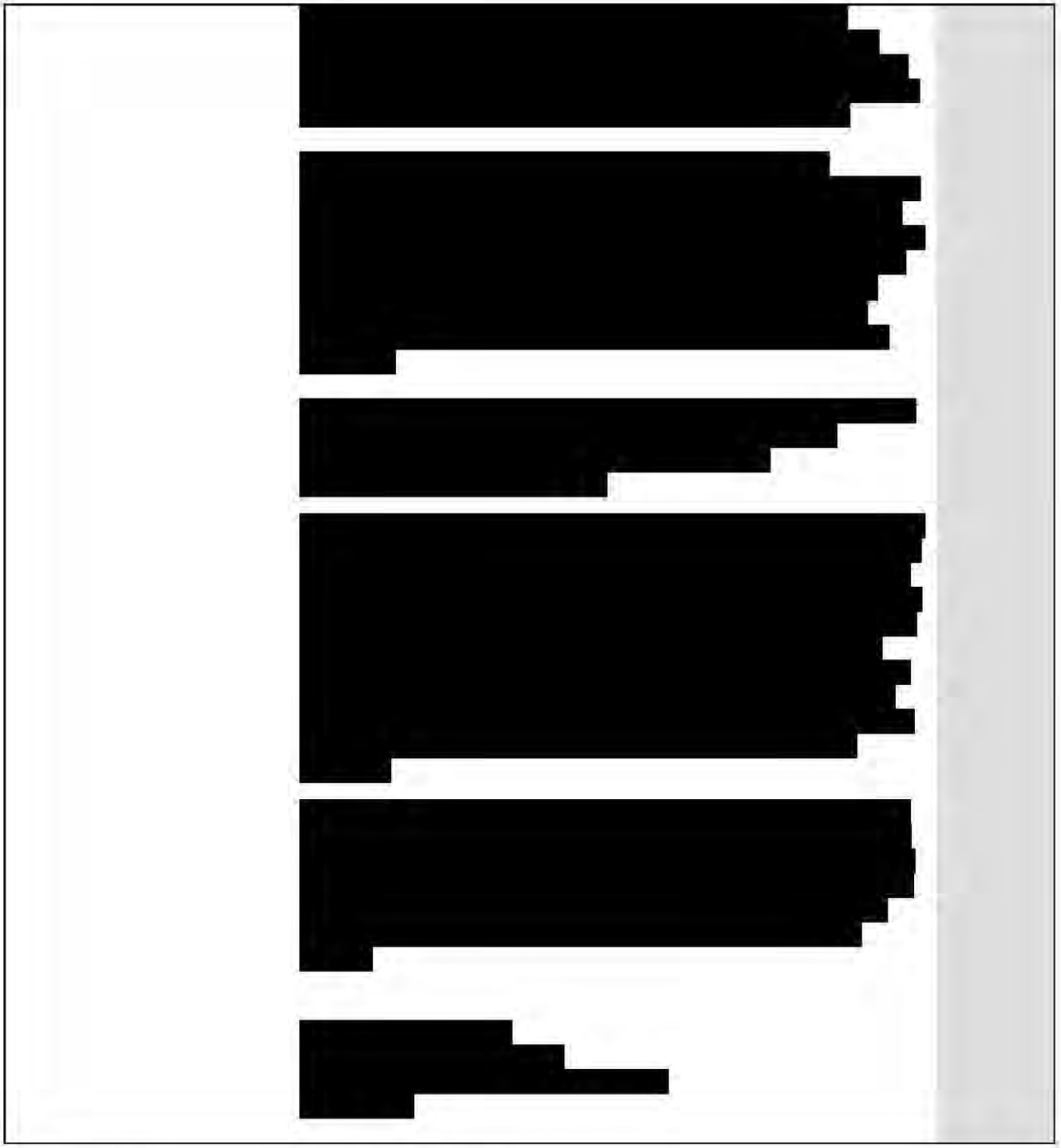
On the basis of exposure alone, it is not scientifically necessary to conduct an *in vitro* gene mutation study in bacteria for carbon dioxide. The use of carbon dioxide as a rodenticide does not increase carbon dioxide above levels found naturally in the atmosphere, and this is well below established maximum occupational exposure limits for safe working conditions. A gene mutation study, while technically possible, will be very difficult, and given the points outlined in this data waiver, will not provide any useful data for the risk assessment.

[redacted]

Section 6.6.1 **Annex Point IIA, VI, 6.6.1**	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in bacteria

Detailed justification:

[redacted]



Section 6.6.1 **Annex Point IIA, VI, 6.6.1**	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* gene mutation study in bacteria

	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 6.6.2 **Annex Point IIA, VI, 6.6.2**	**Genotoxicity in vitro** Section 6: Toxicological and Metabolic Studies *In vitro* cytogenicity study in mammalian cells	
	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:	An *in vitro* cytogenicity study in mammalian cells is not considered necessary for a number of reasons, including: [redacted] It is not scientifically necessary on the basis of low exposure. The use of carbon dioxide as a rodenticide, under normal conditions of use will not cause any elevation in the level of carbon dioxide found naturally in air, outside normal atmospheric ranges. [redacted] [redacted] In addition to the above, the potential for exposure to carbon dioxide when it is manufactured and used as a rodenticide is minimal. [redacted] (Continued……)	

Detailed justification:
(Continued)

- Occupational exposure work has been carried out in humans exposed to an environment with high $paCO_2$ values such as brewery workers [3]. Such data have been used previously by a number of regulatory authorities to set national, international and supranational maximum exposure limits for safe working conditions, and all of these exposure limits are in general agreement, for example the US OSHA permissible exposure level (PEL) is 10,000 ppm and short term exposure limit (STEL) is 30,000 ppm [5]. The long-term occupational exposure limit for carbon dioxide set in the UK is 5,000 ppm (8 hour time weighted average) while the short term occupational exposure limit is 15,000 ppm (15 minutes reference period)[4]. As explained above, the use of carbon dioxide as a rodenticide does not increase atmospheric carbon dioxide levels, and this is well below these agreed maximum exposure limits for safe working conditions. As the objective of a laboratory test on bacteria is to predict the toxicological effect in humans, then an established safe exposure level based on human data takes precedence over animal data generated for the approximation of a theoretical safe value.

- There is a substantial volume of information available for carbon dioxide, and while there are no studies available which consider Genotoxicity specifically nor was the data generated to modern scientifically acceptable protocols, it does cover all the major biological considerations. These are considered elsewhere in the toxicity section of this dossier (Section 6 Toxicological and Metabolic Studies). A number of reviews have been carried out by different regulatory authorities including the EPA[5] and FDA, who considered the health aspects of carbon dioxide as a food additive[6]. Both the EPA and FDA consider that the amount of data that is available on carbon dioxide, and the levels of exposure which occur when carbon dioxide is being used as a biocide means that the margin of safety is acceptable. As a result they have not asked for any new studies to be generated, even though there is no specific data regarding the genotoxic potential of carbon dioxide.

- Technical feasibility
While it is possible to carry out an *in vitro* cytogenicity study in mammalian cells for carbon dioxide, it will be technically very difficult, full of constraints and expensive. Some of the problems include the fact carbon dioxide is naturally produced by all mammalian cells as a by-product of respiration. This makes it

(Continued...)

Detailed justification:
(Continued)

impossible to remove it from negative controls. Even if the natural atmospheric concentrations of carbon dioxide were taken into account when doing the test, the fact that the test cells in both the treated and untreated medium are continually producing carbon dioxide as a by-product of respiration means that there will be variable concentrations of carbon dioxide at a cellular level. The design of the test could address the various complicating factors such as background carbon dioxide levels, possible pH effects and low oxygen before concluding whether the effect was due to the toxic effects of carbon dioxide, but given the other factors outlined in this data waiver, carrying out an *in vitro* cytogenicity study in mammalian cells for carbon dioxide is not going to provide any useful data for the risk assessment.

Conclusion

On the basis of exposure alone, it is not scientifically necessary to conduct an *in vitro* cytogenicity study in mammalian cells for carbon dioxide. The use of carbon dioxide as a rodenticide does not increase carbon dioxide above levels found naturally in the atmosphere, and this is well below established maximum occupational exposure limits for safe working conditions. A cytogenicity study, while technically possible, will be very difficult, and given the points outlined in this data waiver, will not provide any useful data for the risk assessment.

Detailed justification: