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Dear Sir or Madam:

This submission is provided on behalf of CONDEA Vista Company (CONDEA Vista) in accordance with section 8(e) of the Toxic Substances Control Act. It presents the results of a developmental toxicity study conducted on 2-butyl octanoic acid (CAS No. 27610-92-0) also referred to as ISOCARB 12 in this submission. CONDEA Vista has received a draft report of the study which provides details of its conduct and findings. Key findings of the study are summarized below.

2-butyl octanoic acid was administered to mated female rats which had been divided into four treatment groups, each containing 25 animals. These animals were dosed orally by gavage during days 6-19 inclusive of gestation at dose levels of 0, 25, 200 and 400 mg/kg of the test material. On day 20, the animals were killed and the conceptuses were evaluated. The live fetuses were subsequently examined for developmental abnormalities and variants of the viscera and skeleton, including the state of ossification.

At 400 mg/kg/day, maternal toxicity was manifest as clinical observation in all animals, including altered respiration pattern, piloerection, hunched appearance, red/brown staining of coat, and salivation. It was necessary to discontinue dosing for 4 animals, and 2 of these were subsequently killed because of their condition. Decreases in body weight gain and food consumption were also observed. At 200 mg/Kg/ day, maternal toxicity was limited to clinical observations including altered respiration pattern, hunched appearance and piloerection. There was no indication of maternal toxicity at 25 mg/Kg/day.

At 400 mg/Kg/day mean fetal weight was slightly lower than control, and there was an increased incidence of (mainly vestigial) supernumerary ribs. Fetal ossification at 400 mg/Kg/day was slightly reduced, as indicated by an increased incidence of incomplete ossification affecting the sternbrae and digital bones, probably associated with the slight decrease in fetal weight. There were no obvious fetal effects at 25 or 200 mg/Kg/day.

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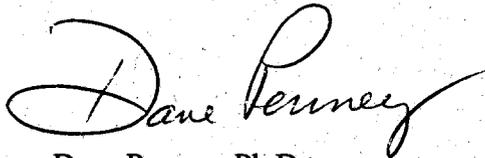
TSCA 8(e) notification

The maternal NOAEL was considered to be 25 mg/kg/day and the fetal NOAEL was considered to be 200 mg/Kg/day.

Limited quantities of ISOCARB 12 have been imported by CONDEA Vista. ISOCARB 12 is currently considered a developmental product whose distribution has been limited to providing small quantity samples of the product to potential customers.

Any questions about this submission should be directed to the undersigned as indicated.

Respectfully submitted,



Dave Penney, Ph.D.
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Inveresk Report No. 15104

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ISOCARB 12
DEVELOPMENTAL TOXICITY STUDY IN RATS

Inveresk Project No. 491713

DRAFT

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Total Number of Pages: 67

AUTHENTICATION

'I, the undersigned, hereby declare that this work was performed under my direction and in accordance with the principles of Good Laboratory Practice. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.'

S J Barton BA MSc DABT
Study Director

Date:

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DRAFT REPORT No. 15104

On receipt of approval or amendments, or 16 weeks from today's date if no amendments have been requested, Inveresk reserves the right to despatch the final report.

Inveresk reserves the right to make additional charges for a review of data, amendments or for corrections of minor errors following issue of the final report.

For the final report, this page will be replaced by the Quality Assurance Statement.

Inveresk Research

ISSUED

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SUMMARY

The test material Isocarb 12 (2-butyl octanoic acid), was accepted from Condea Chemie, Hamburg, Germany for developmental toxicity testing in rats.

Mated female Sprague-Dawley rats were randomised into 4 treatment groups, each containing 25 animals. These animals were dosed orally by gavage once daily over Days 6-19 inclusive of gestation, where Day 0 was the day of detection of mating. The dose levels applied were as follows:

	<u>mg Isocarb 12.kg⁻¹.day⁻¹</u>
Control	0
Low dose	25
Intermediate dose	200
High dose	400

The animals were monitored during gestation for clinical signs of toxicity and for body weight, food and water consumption performance. They were killed on Day 20 of gestation for evaluation of pregnancy outcome. The live foetuses were subsequently examined for abnormalities and variants of the viscera and skeleton.

Maternal toxicity at 400 mg Isocarb 12.kg⁻¹.day⁻¹ was manifest as clinical observations in all animals, including altered respiration pattern, piloerection, hunched appearance, red/brown staining of coat, and salivation. It was necessary to discontinue dosing for 4 animals, and 2 of these were subsequently killed because of their condition. At 200 mg Isocarb 12.kg⁻¹.day⁻¹ maternal toxicity was limited to clinical observations including altered respiration pattern, hunched appearance and piloerection. There was no indication of maternal toxicity at 25 mg Isocarb 12.kg⁻¹.day⁻¹.

PERSONNEL INVOLVED IN PROJECT 491713

Study Director:	S J Barton BA MSc DABT
Project Leader:	S K Clubb BSc
Assistance with Report Preparation:	K Brockie BSc MSc
Animal Services Manager:	A Dick FIAT
Senior Animal Technicians:	R J Orvis E Owenson
Test Material Formulation and Analysis under the Direction of:	K Fisher BSc
Foetal Pathology:	S Wilcox BSc S K Clubb BSc
Head of Pathology:	J Finch BVM&S MSc DLAS MRCPATH MRCVS
Quality Assurance:	J Wood BSc FIBMS G Birnie BSc GIBiol

At 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ there were decreases in body weight gain and food consumption during the middle part of the dosing period. There were no obvious effects on body weight or food consumption at the lower levels.

At 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ mean foetal weight was slightly lower than Control, and there was an increased incidence of supernumerary ribs. Foetal ossification at 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ was slightly reduced, as indicated by an increased incidence of incomplete ossification affecting the sternbrae and digital bones, probably associated with the slight decrease in foetal weight. There were no obvious foetal effects at 25 or 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$.

In conclusion, under the conditions of this study, the maternal No Effect Level was considered to be 25 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ and the foetal No Effect Level was considered to be 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$.

INTRODUCTION

The test material Isocarb 12 (2-butyl octanoic acid) was accepted from Condea Chemie, Hamburg, Germany for developmental toxicity testing in rats. This report describes the methods used and the results obtained in the study, which was carried out at Inveresk Research according to the following schedule:

Protocol Signed by Study Director:	14 November 1996
Animal Arrival:	21 March 1997
First Day of Dosing:	24-26 March 1997
Terminal Necropsy on Day 20 of Gestation:	7-9 April 1997
Study Completion Date:	See Authentication page for date of Study Director's signature

This developmental toxicity study in rats is part of a programme of experiments designed to evaluate the toxicity of the test material to reproduction in experimental animals. The rat is a standard rodent species for the toxicological testing in animals required by Regulatory Authorities and this study was designed to be acceptable to the EC, USA and Japanese authorities. The normal processes of gestation in the rat are well documented in this laboratory.

The test material was administered orally (by gavage) because this is a likely route of exposure in man. Dosing was performed once daily at approximately the same time each day.

All data generated and recorded during this study, including a copy of the final report, will be stored in the Scientific Archives of Inveresk Research for 5 years after issue of the final report. At the end of the 5 year period the Sponsor will be consulted regarding the disposal or continued storage of raw data.

EXPERIMENTAL PROCEDURE

Test Material

A delivery of 1 litre of Isocarb 12, Batch Number 51165 was received at Inveresk on 13 December 1996. The material, a colourless liquid, was stored in the dark at ambient temperature, in the Inveresk Dispensary. A copy of the certificate of analysis is reproduced in Appendix 1.

Animals

One batch of 105 female Sprague-Dawley rats of the Charles River CD strain (outbred albino) was obtained from Charles River (UK) Limited, Margate, Kent, England on 21 March 1997. These animals were *ca* 9 weeks of age on arrival. On the day of delivery, sub-batches of 32, 36 and 37 animals were on Day 3, 2 and 1 of gestation respectively (Day 0 = day of detection of mating). No more than 2 females were inseminated by the same male, and the identity of each male was provided.

All animals were clinically examined on arrival for signs of abnormality or disease. No such signs were found and the animals were accepted for use in the study.

The animals were acclimatised in the Inveresk animal room for 3-5 days prior to commencement of treatment.

From the 105 animals supplied, 100 were initially allocated to the study. The remaining 5 animals were provided as spares; of these, one was used to replace an animal that was killed prematurely, but the remaining spares were not needed and not regarded as part of the study.

ANIMAL MANAGEMENT

Room Environment and Sanitation

The study was conducted in Room N111 of the rodent toxicology accommodation at the Elphinstone Research Centre of Inveresk Research.

Light cycle, temperature and humidity were automatically controlled. Light hours were 0700-1900 h. Target ranges for temperature and relative humidity were $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $50\% \pm 15\%$ respectively, with 15-20 air changes per hour. Daily monitoring showed both temperature and humidity to be within the stated target ranges.

Each day, on completion of all other work, the floor was swept and then mopped with a 0.5% solution of Tego[®] 2000 (Th. Goldschmidt Limited, Ruislip, Middlesex, UK), an amphoteric biocide/cleanser. The room was washed with this solution once during the study.

Caging and Cage Sanitation

The females were housed singly in polypropylene cages with stainless steel grid bottoms and mesh tops, measuring 42 x 27 x 20 cm. A separate stainless steel food hopper and a polycarbonate water bottle were provided for each cage.

Excreta were collected on a tray, lined with absorbent paper, suspended beneath each cage. The cages were suspended on racks, each full rack containing 6 rows of 4 cages.

Cages, tray paper and water bottles were changed as required.

Diet

Food

Rat and Mouse Breeder Diet No. 3 (Expanded) SQC, was supplied by Special Diets Services (SDS) Limited, Stepfield, Witham, Essex, UK, and was available to the rats *ad libitum*. The diet was supplied with a batch analysis for nutritive constituents and a range of significant contaminants. A typical analysis, of a batch used during the study, is reproduced in Appendix 2.

Water

The animals had access to domestic mains water *ad libitum*. The supply is analysed regularly for dissolved and suspended materials, including a range of significant contaminants. A typical analysis is reproduced in Appendix 3.

None of the contaminants revealed by the analyses of food and water were considered to have been present in sufficient quantity to have affected the outcome of the study.

TREATMENT

Allocation of Animals to Treatment Groups

On arrival, the animals were allocated to the treatment groups using a computer generated series of randomly sequenced numbers, such that the treatment groups were evenly distributed throughout the caging system. It was ensured that females inseminated by the same male did not appear in the same treatment group.

After allocation to treatment group, each female received a unique ear mark which identified it individually within the study and corresponded to that animal's number. Each animal was ascribed a cage card which was colour coded for treatment group and

marked with the project, cage and animal numbers, sex and the relevant treatment group.

The treatment groups and animal numbers were arranged as follows:

Group Number	Treatment and Dose Level (mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$)	Animal Numbers
1	Control 0	1-25, 101
2	Low dose 25	26-50
3	Intermediate dose 200	51-75
4	High dose 400	76-79, 188, 81-100

Animal 80 was renumbered as 188 due to an ear marking error
Animal 3 was killed due to its condition; Animal 101 was added to this group

Selection of Dose Levels

The dose levels were agreed with the Sponsor after evaluation of the results of a preliminary study (Inveresk Project No. 491708, Report No. 14810), which indicated that dose levels of $500\text{ mg Isocarb }12.\text{kg}^{-1}.\text{day}^{-1}$ or higher would be unsuitable for this study.

Treatment Regime

The animals were dosed orally by gavage at a volume of 10 ml dosing formulation per kg of body weight, using a steel dosing cannula. The volume to be administered to each animal was determined each day by the weight of that animal as measured at the time of administration.

The animals were dosed once daily at approximately the same time each day over Days 6-19 (inclusive) of gestation.

Preparation of Dosing Formulations

The dosing solutions were prepared at approximately weekly intervals and then dispensed into appropriate volumes sufficient for one day of dosing.

The highest concentration of dose formulation was prepared by mixing equimolar amounts of test material and sodium hydroxide (diluted in water for irrigation). Lower dose concentrations were then prepared by serial dilutions in water for irrigation and adjusted to approximately pH 9.5 with sodium hydroxide.

All solutions were mixed by manual inversion until visibly homogenous.

The Control animals received water for irrigation.

Analysis of Dosing Formulations

Triplicate 3 ml samples were taken from each formulation containing test material, and one 3 ml samples were taken from the Control formulation immediately after preparation of the stock to be used for dosing during the first and second weeks of treatment. The samples were analysed for concentration and homogeneity in the Inveresk Analytical Chemistry Laboratory by Method Number 7630 developed under the provisions of Inveresk Project No. 376300.

Triplicate 3 ml samples from each formulation containing test material and one 3 ml sample from the Control formulation were taken immediately after preparation and stored at 2-8°C in the dark until despatch to the Sponsor 4 days later.

OBSERVATIONS

Clinical Observations

All the animals were checked for viability at the beginning of each day and again as late as possible on each day.

All the animals were examined for reaction to treatment on each day. The nature, onset, duration and intensity of any signs were recorded, a specific examination being made 1-2 h after dosing.

Body Weight

Individual body weights were recorded on Days 4 and 6-20 of gestation, but for clarity/brevity only the weights on Days 4, 6, 9-13, 17 and 20 of gestation are presented in this report, although all weights are retained in the archive.

Food Consumption

The weight of the food consumed by each animal was recorded daily, commencing on Day 4 of gestation (weighed quantity first offered on Day 3, residue recorded on Day 4).

TERMINAL STUDIES

The females were killed by carbon dioxide asphyxiation on Day 20 of gestation. Foetuses were killed by chilling at *ca* 4°C for approximately 10 min.

Termination on Day 20 of Gestation

The adults were subjected to a gross necropsy, involving an external examination following by macroscopic examination of the thoracic and abdominal contents. Any findings were recorded as appropriate.

The reproductive tract was removed and weight intact. The uterus was then opened and the contents were examined. The number and position of all implantation sites in the uterus were recorded. Each implant was classified as being live, a foetal death (death judged to have occurred during the foetal period after *ca* Day 16 of gestation), a late embryonic death (embryonic remains visible), or an early embryonic death (only early placental remains or a decidual scar were visible). The number of *corpora lutea graviditatis* in each ovary was recorded.

Each live foetus was individually identified within the litter and its weight was recorded. The foetuses were examined for externally visible abnormalities. Approximately one half of the foetuses from each uterus were then fixed in methylated ethyl alcohol, and the remaining half in Bouin's fluid.

The foetuses fixed in alcohol were subsequently examined for abnormalities of the thoracic and abdominal viscera. The eviscerated foetuses were then macerated in potassium hydroxide, the skeletons stained with Alizarin Red S, then the foetuses were cleared with aqueous glycerol solutions. Skeletal structures were examined for abnormalities and variants, including extent of ossification.

The foetuses fixed in Bouin's fluid were examined for soft tissue abnormalities by a free-hand serial sectioning technique derived from that of Wilson (1965).

The sex of each foetus was determined during the visceral examination.

STATISTICAL ANALYSIS OF RESULTS

In evaluating and interpreting data from this study it was not considered useful to conduct any formal statistical analysis. Interpretation was based on inspection of the individual and group values.

RESULTS

Analysis of Dosing Formulations (Appendix 4)

The analysed concentrations of the dose formulations were within 4% of the nominal indicating accurate formulation of the test material; the low coefficients of variation indicated that the formulations were homogenous.

Clinical Observations (Table 1, Appendix 5)

At 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ clinical reaction to treatment was observed for all animals, and included altered respiration pattern, piloerection, hunched appearance, red/brown staining of coat and salivation. These findings were first apparent in a few animals on Day 7 of gestation, but most findings were observed during the second half of the gestation period. The condition of 4 animals at this level required dosing to be suspended for 1 or 2 days, and 2 of these animals were killed prematurely.

At 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ altered respiration pattern, hunched appearance and piloerection were observed in 10 out of 24 animals at this level.

At 25 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ clinical observations were essentially similar to those of the Controls.

Animal 3 (Control) was killed on Day 7 of gestation due to poor condition. This animal was killed and replaced, and excluded from group summaries.

Body Weight (Table 2, Appendix 6)

At 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ a decrease in body weight gain was apparent between Days 9 and 13 of gestation. Weight gain over Days 13 to 20 of gestation was similar to

that of the Controls, although the overall weight gain over Days 6 to 20 remained lower than the Control value.

Body weight gain at 25 and 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ was similar to that of the Controls.

Food Consumption (Table 3, Appendix 7)

At 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ there was a decrease in group mean food consumption over Days 10 - 14 of gestation inclusive. At other times, consumption was similar to that of the Controls.

Food consumption at 25 and 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ was comparable with that of the Controls throughout the treatment period.

Pregnancy Performance and Foetal Weight (Table 4, Appendix 8)

There were no obvious effects of treatment on pregnancy performance (including the incidence and survival of implants) at any of the dose levels applied.

At 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ there was a slight decrease in mean foetal weight. There were no obvious effects of treatment observed at 25 or 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$.

Foetal Abnormalities, Variants and Skeletal Ossification Parameters (Table 5-7, Appendices 9-11)

The incidence and type of major foetal abnormalities did not indicate any effect of treatment.

Among the minor foetal abnormalities, the incidence of supernumerary (14th) ribs at 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ was greater than Control. The incidences of supernumerary ribs at 25 and 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ were slightly greater than Control, but these incidences were within the recent background range and were not obviously associated with treatment.

The incidences of the other minor foetal abnormalities did not indicate any effect of treatment.

At 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ there was a slight increase in the number of foetuses with unossified 5th metacarpals and the number of sternebrae incompletely ossified. These findings were considered to reflect the slight decrease in foetal weight noted at this dose level. The slight changes in the foetal ossification parameters at the lower levels were considered too small to be associated with treatment.

DISCUSSION AND CONCLUSIONS

Maternal toxicity at 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ was manifest as clinical observations in all animals, including altered respiration pattern, piloerection, hunched appearance, red/brown staining of coat, and salivation. It was necessary to discontinue dosing for 4 animals, and 2 of these were subsequently killed because of their condition. Decreases in body weight gain and food consumption were also observed. At 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ maternal toxicity was limited to clinical observations including altered respiration pattern, hunched appearance and piloerection. There was no indication of maternal toxicity at 25 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$.

At 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ mean foetal weight was slightly lower than Control, and there was an increased incidence of (mainly vestigial) supernumerary ribs. Foetal ossification at 400 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ was slightly reduced, as indicated by an increased incidence of incomplete ossification affecting the sternebrae and digital bones, probably associated with the slight decrease in foetal weight. There were no obvious foetal effects at 25 or 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$.

In conclusion, under the conditions of this study, the maternal No Effect Level was considered to be 25 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$ and the foetal No Effect Level was considered to be 200 mg Isocarb $12.\text{kg}^{-1}.\text{day}^{-1}$.

REFERENCES

Wilson, J G (1965). Teratology: Principles and Techniques, J G Wilson and J Warkany, Eds, 267-277. The University of Chicago Press, Chicago, Illinois.

TABLE 1
 Isocarb 12
 Developmental Toxicity Study in Rats
 Group Incidence of Clinical Observations and Necropsy Findings

Observation/Finding	Group/Dose Level (mg Isocarb 12.kg ⁻¹ .day ⁻¹)			
	1 (0)	2 (25)	3 (200)	4 (400)
Total Number Examined	25 ^a	25	25	25
Subdued behaviour	1	0	0	3
Altered respiration pattern	0	0	6	16
Piloerection	0	0	4	17
Hunched appearance	0	0	3	8
Salivation	0	0	0	24
Discharge or wet staining around vagina	0	0	0	2
Skin cold to touch	0	0	0	2
Pale ears	0	0	0	1
Body appears dark	0	0	0	1
Dark eyes	0	0	1	1
Swollen eyelids	0	0	1	0
Bulging eyes	0	0	1	2
Hairloss	3	6	1	3
Staining	0	0	0	3
Unscheduled kill	0	0	0	2

a = Excludes Animal 3

TABLE 2

Isocarb 12
Developmental Toxicity Study in Rats
Group Mean Body Weight (g) \pm Standard Deviation
(Pregnant Animals Only)

Day of Gestation	Group/Dose Level (mg Isocarb 12.kg ⁻¹ .day ⁻¹)			
	1 (0)	2 (25)	3 (200)	4 (400)
4	230 \pm 17	233 \pm 16	235 \pm 15	238 \pm 13
6	246 \pm 19	249 \pm 17	250 \pm 18	255 \pm 14
9	261 \pm 21	264 \pm 18	263 \pm 16	264 \pm 16
10	268 \pm 21	271 \pm 20	269 \pm 17	263 \pm 23
11	274 \pm 23	277 \pm 22	273 \pm 17	264 \pm 24
12	281 \pm 23	282 \pm 21	279 \pm 18	271 \pm 24
13	286 \pm 23	288 \pm 21	286 \pm 17	275 \pm 25
16	325 \pm 28	328 \pm 26	325 \pm 22	315 \pm 27
20	360 \pm 28	364 \pm 33	368 \pm 25	355 \pm 35
Gain Days 6-20	114 \pm 14	115 \pm 19	118 \pm 17	100 \pm 28
% of Control	-	101	104	88

TABLE 3

Isocarb 12
Developmental Toxicity Study in Rats
Group Mean Food Consumption (g) \pm Standard Deviation
(Pregnant Animals Only)

Day of Gestation	Group/Dose Level (mg Isocarb 12.kg ⁻¹ .day ⁻¹)			
	1 (0)	2 (25)	3 (200)	4 (400)
4	28	29	30	31
5	28	27	29	30
6	27	28	29	29
7	28	28	27	27
8	28	30	30	30
9	29	28	30	29
10	29	30	29	25
11	30	30	29	23
12	32	31	28	26
13	32	31	31	28
14	31	31	30	26
15	31	32	32	31
16	35	33	34	31
17	35	34	35	34
18	34	35	35	33
19	33	34	35	32
20	27	28	31	31
Days 7-20	434	435	436	406
% of Control	-	100	100	94

TABLE 4
Isocarb 12
Developmental Toxicity Study in Rats
Pregnancy Performance and Foetal Weight

	Group/Dose Level (mg Isocarb 12.kg ⁻¹ .day ⁻¹)			
	1 (0)	2 (25)	3 (200)	4 (400)
Number of animals mated	25 ^a	25	25	25
Number pregnant	25	25	24	25
Number of premature decedents	1 ^b	0	0	2
Number of decedents pregnant	1 ^b	0	0	2
Number pregnant at Day 20 necropsy	24	25	24	23
Pregnancy frequency as %	100	100	96	100
<hr/>				
Total corpora lutea graviditatis	323	357	345	325
Total number of implants	306	329	307	307
Pre-implantation loss as %	5	8	11	6
<hr/>				
Total live implants (%)	292 (95)	314 (95)	290 (94)	298 (97)
Total dead implants (%)	14 (5)	15 (5)	17 (6)	9 (3)
Total early embryonic deaths (%)	13 (4)	13 (4)	17 (6)	9 (3)
Total late embryonic deaths (%)	1 (0.3)	2 (1)	0	0
Total foetal deaths (%)	0	0	0	0
<hr/>				
Mean corpora lutea graviditatis	13.5 ± 2.1	14.3 ± 1.9	14.4 ± 3.0	14.1 ± 1.8
Mean implants	12.8 ± 1.9	13.2 ± 1.9	12.8 ± 2.1	13.3 ± 1.8
Mean live implants	12.2 ± 2.1	12.6 ± 2.3	12.1 ± 2.8	13.0 ± 1.7
Mean dead implants	0.6 ± 0.8	0.6 ± 1.0	0.7 ± 1.3	0.4 ± 0.6
Mean early embryonic deaths	0.5 ± 0.8	0.5 ± 0.8	0.7 ± 1.3	0.4 ± 0.6
Mean late embryonic deaths	0.04 ± 0.2	0.1 ± 0.3	0	0
Mean foetal deaths	0	0	0	0
<hr/>				
Total live male foetuses (%)	155 (53)	162 (52)	154 (53)	148 (50)
Total live female foetuses (%)	137 (47)	152 (48)	136 (47)	150 (50)
Live foetal sex ratio (♂:♀)	1:0.88	1:0.94	1:0.88	1:1.01
<hr/>				
Mean total uterus weight (g)	75 ± 12	77 ± 14	75 ± 16	76 ± 12
Mean litter mean foetal weight (g)	3.89 ± 0.22	3.78 ± 0.29	3.79 ± 0.21	3.60 ± 0.32

Means are given ± Standard Deviation
 Note: Premature decedents excluded below double line
 a = Excludes Animal 3
 b = Animal 101

TABLE 5
Isocarb 12
Developmental Toxicity Study in Rats
Group Incidence of Major Foetal Abnormalities

Abnormality	Group/Dose Level (mg Isocarb 12.kg ⁻¹ .day ⁻¹)			
	1 (0)	2 (25)	3 (200)	4 (400)
	Incidence of Foetuses (Litters)			
Anophthalmia/microphthalmia	0	0	0	1 (1)
Small eye	1 (1)	0	0	0
Internal hydrocephaly	1 (1)	1 (1)	0	0
Retro oesophageal aortic arch	0	1 (1)	0	0
Interventricular septal defect	2 (1)	1 (1)	0	0
Kinked ribs with/without in complete ossification	0	1 (1)	2 (2)	0
Marked protrusion of median liver lobe with thinning of diaphragm	2 (2)	0	0	0
Termination of normal vertebral column at 13th thoracic vertebra	0	1 (1)	0	0
Subcutaneous oedema	2 (1)	0	0	0
Number with major abnormality	4 (3)	4 (3)	2 (2)	1 (1)
Total number examined	292 (24)	314 (25)	290 (24)	298 (23)

TABLE 6

Isocarb 12
Developmental Toxicity Study in Rats
Group Incidence of Minor Foetal Abnormalities and Variants

Abnormality/Variant	Group/Dose Level (mg Isocarb 12.kg ⁻¹ .day ⁻¹)			
	1 (0)	2 (25)	3 (200)	4 (400)
	Incidence of Foetuses (Litters)			
Visceral				
Reduced thyroid	0	1 (1)	1 (1)	1 (1)
Haemorrhage affecting eye (and surrounding tissue)	0	1 (1)	1 (1)	0
Subcutaneous haemorrhage affecting:				
Head	3 (2)	1 (1)	1 (1)	6 (4)
Trunk/limbs	0	2 (2)	2 (2)	1 (1)
Absent innominate artery	0	1 (1)	0	0
Small interventricular septal defect	2 (2)	0	0	0
Intra abdominal haemorrhage	2 (2)	2 (2)	2 (1)	0
Hepatic haemorrhage	2 (2)	0	1 (1)	1 (1)
Protrusion(s) of median liver lobe with/without thinning of diaphragm	4 (4)	3 (2)	1 (1)	0
Additional liver lobe in median cleft	0	0	0	1 (1)
Bilateral/unilateral increased renal pelvic cavitation	1 (1)	3 (3)	1 (1)	3 (2)
Dilated ureter(s)	1 (1)	2 (2)	2 (1)	3 (2)
Displaced testis(es)	2 (2)	1 (1)	0	1 (1)
Number with minor visceral abnormality/variant	19 (15)	17 (10)	16 (13)	17 (10)
Number examined by Wilson sectioning	146 (24)	155 (25)	144 (24)	148 (23)
Total number examined visceraally	146 (24)	159 (25)	146 (24)	150 (23)
Skeletal				
Additional area of ossification in cranium	0	1 (1)	1 (1)	0
Irregular ossification of sternebra	1 (1)	0	1 (1)	0
Sacral centra unossified	0	0	0	1 (1)
Number with minor skeletal abnormality/variant	1 (1)	1 (1)	2 (2)	1 (1)
Number of Ribs:				
13 complete ribs	133 (24)	128 (25)	114 (23)	96 (21)
14th vestigial supernumary rib(s)	13 (8)	27 (14)	28 (12)	50 (16)
14th reduced supernumary rib(s)	0	0	1 (1)	1 (1)
14 complete ribs	0	0	0	1 (1)

TABLE 7
Isocarb 12
Developmental Toxicity Study in Rats
Group Incidence of Incomplete Ossification Parameters

Parameter	Group/Dose Level (mg Isocarb 12.kg ⁻¹ .day ⁻¹)			
	1 (0)	2 (25)	3 (200)	4 (400)
	Incidence of Foetuses (litters)			
<u>Incomplete ossification affecting:</u>				
≥ than 4 skull bones	1 (1)	3 (2)	11 (8)	5 (4)
≤ than 3 skull bones	10 (5)	12 (6)	22 (13)	18 (13)
Cervical vertebral arches	0	0	1 (1)	1 (1)
Thoracic vertebral centrum/a	2 (2)	9 (8)	7 (7)	13 (10)
2nd to 4th metacarpal(s)	0	0	3 (2)	1 (1)
2nd to 4th metatarsal(s)	0	0	0	1 (1)
Pubis/es	0	2 (2)	5 (4)	5 (4)
Ischium/a	0	0	3 (3)	1 (1)
Sacral vertebral arches	2 (2)	3 (3)	14 (10)	6 (4)
<u>Unossified:</u>				
5th metacarpal(s)	32 (13)	41 (15)	66 (18)	88 (22)
5th metatarsal(s)	0	1 (1)	1 (1)	2 (2)
<u>Number of sternebrae with incomplete ossification:</u>				
0	98 (24)	85 (21)	58 (19)	32 (13)
1	32 (18)	51 (21)	55 (22)	84 (22)
2	16 (12)	16 (11)	28 (11)	25 (13)
3	0	2 (2)	2 (2)	4 (3)
>3	0	1 (1)	0	3 (3)
Total number examined skeletally	146 (24)	155 (25)	143 (24)	148 (23)

APPENDIX 1

Isocarb 12

Developmental Toxicity Study in Rats
Certificate of Analysis of the Test Material

APPENDIX 2

Isocarb 12
Developmental Toxicity Study in Rats
Analysis of Diet

SPECIAL DIETS SERVICES

*Special Quality Control
Certificate of Analysis*

PRODUCT: RM3 (E) SQC

BATCH NO: 2804

PREMIX BATCH NO: 397

DATE OF MANUFACTURE: 10-JUL-96

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	9.4	%	Fluoride	22	mg/kg	1.0 mg/kg
Crude Fat	5.5	%	Nitrate as NaNO3	39	mg/kg	1.0 mg/kg
Crude Protein	20.0	%	Nitrite as NaNO2	1.9	mg/kg	1.0 mg/kg
Crude Fibre	4.9	%	Lead	1.25	mg/kg	0.25 mg/kg
Ash	6.3	%	Arsenic	0.25	mg/kg	0.2 mg/kg
Calcium	1.04	%	Cadmium	0.19	mg/kg	0.05 mg/kg
Phosphorus	0.74	%	Mercury	Non Detected	mg/kg	0.01 mg/kg
Sodium	0.42	%	Selenium	0.23	mg/kg	0.05 mg/kg
Chloride	0.67	%				
Potassium	0.95	%				
Magnesium	0.16	%	Total Aflatoxins	Non Detected	mcg/kg	1 mcg/kg each of B1, B2, G1, G2
Iron	284	mg/kg				
Copper	21	mg/kg	Total P.C.B	Non Detected	mcg/kg	10.0 mcg/kg
Manganese	90	mg/kg	Total D.D.T	Non Detected	mcg/kg	10.0 mcg/kg
Zinc	76	mg/kg	Dieldrin	Non Detected	mcg/kg	10.0 mcg/kg
			Lindane	Non Detected	mcg/kg	10.0 mcg/kg
			Heptachlor	Non Detected	mcg/kg	10.0 mcg/kg
			Malathion	Non Detected	mcg/kg	20.0 mcg/kg
Vitamin A	35.7	iu/g	Total Viable Organisms x 1000	Non Detected	per gram	1000/g
Vitamin E	139	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores x 100	Non Detected	per gram	100/g
			Salmonellae Species	Non Detected	per gram	Absent in 20 gram
			Presumptive E.coli	Non Detected	per gram	Absent in 20 gram
			E.coli Type 1	Non Detected	per gram	Absent in 20 gram
			Fungal Units	375	per gram	Absent in 20 gram
			Antibiotic Activity	Non Detected		

Signed *J. Rickett*
Dated *2-8-96*



APPENDIX 3

**Isocarb 12
Developmental Toxicity Study in Rats
Analysis of Water**



Water Quality Directorate

Edinburgh Laboratory
4 Marine Esplanade
Edinburgh
EH6 7LU

Tel: 0131 555 7963
Fax: 0131 555 7979



East of Scotland Water

TEST REPORT

Inveresk Research Int. Ltd.
Environmental Chemistry
Tranent
EH33 2NE

Date of Report: 15/07/97
Order No.: None.
Lab. Ref.: WM/15416
Cust. Ref.: None.
Taken on: 28/05/97
Received on: 28/05/97
Taken by: A.Barclay
Analysis Started: 29/05/97
Page: 1 of 2

F.A.O.: Alison Barclay

Description: Inveresk Research Int. Ltd., (Block L clean side)

Comments: Block L clean side

Test Results¹⁻⁴:

pH	8.4	Volume filtered	2.5 litres
Chloride (as Cl)	11 mg/l	C.Perf	None detected in 100ml
Fluoride (as F)	<100 ug/l	F.Strap	None detected in 100ml
Nitrate (as NO3)	3.26 mg/l	Ammonia (as NH4)	<0.02 mg/l
Sulphate (as SO4)	18 mg/l	*Nickel (as Ni)	0.8 ug/l
Alkalinity (as HCO3)	63 mg/l	Nitrite (as NO2)	<0.01 mg/l
Conductivity	165 uS/cm	Heptachlor	<0.010 ug/l
Silver (as Ag)	<0.18 ug/l	Tributalin	<0.010 ug/l
Arsenic (as As)	<2.5 ug/l	alpha endosulphan	<0.010 ug/l
Boron (as B)	<17 ug/l	beta endosulphan	<0.010 ug/l
Cadmium (as Cd)	<0.14 ug/L	opDDE	<0.010 ug/l
*COD	<30 mg/l	opDDT	<0.010 ug/l
Chromium (as Cr)	<1.7 ug/l	opTDE	<0.010 ug/l
Dry Residues	101.5 mg/l	Aldrin	<0.010 ug/l
Total Halocarbons	15 ug/l	Alpha HCH	<0.010 ug/l
bromodichloromethane	3.3 ug/l	Dieldrin	<0.010 ug/l
bromoform	<2.0 ug/l	Endrin	<0.010 ug/l
chloroform	12.0 ug/l	Gamma HCH	<0.010 ug/l
dibromochloromethane	<2.0 ug/l	HCB	<0.010 ug/l
Mercury (as Hg)	<0.05 ug/l	ppDDE	<0.010 ug/l
Coliform organisms	None detected in 100ml	ppDDT	<0.010 ug/l
E.coli	None detected in 100ml	ppTDE	<0.010 ug/l
Plate count at 22C	None detected in 1ml	Carbophenothion	<0.010 ug/l
Plate count at 37C	None detected in 1ml	Ethyl parathion	<0.010 ug/l
Salmonella spp.	Absent	Methyl parathion	<0.010 ug/l

Authorised By: *A Dick*
Name: **A DICK
CHEMIST**
Date: **15 JUL 1997**

NOTES:(1) Sampling is outside the scope of NAMAS Accreditation for this laboratory. (2) Tests marked with an asterisk (*) in this report are not included in the NAMAS Accreditation Schedule for this laboratory. (3) Analytical instruments, opinions and interpretations are outside the scope of NAMAS Accreditation for this laboratory. (4) Methods used are documented in-house based on the "Methods for the Examination of Water and Associated Materials" published by IRMSO. Further details and performance characteristics are available on request.

APPENDIX 3 (continued)

Lab. Ref.: WM15416

Page 2 of 2

Azinphos ethyl	<0.010 ug/l	m Chlorophenol	<0.07 ug/l
Chlorfenvinphos	<0.010 ug/l	o Chlorophenol	<0.06 ug/l
Fenitrothion	<0.010 ug/l	*Phosphorus SR (as P)	<65 ug/l
Malathion	<0.010 ug/l	Oxidizability (as O)	0.67 mg/l
Methyl azinphos	<0.010 ug/l	Selenium (as Se)	<1 ug/l
Benzo(a)pyrene	<0.001 ug/l	*Suspended solids	<2.0 mg/l
Benzo(b)fluoranthene	<0.004 ug/l	*Sulphide (as S)	0.2 mg/l
Benzo(ghi)perylene	<0.004 ug/l	Total Organic Carbon (as C)	14.6 mg/l
Benzo(k)fluoranthene	<0.004 ug/l	Atrazine	<0.010 ug/l
Fluoranthene	0.008 ug/l	Propazine	<0.010 ug/l
Indeno(123cd)pyrene	<0.004 ug/l	Simazine	<0.010 ug/l
Total PAHs	0.008 ug/l	Trietazine	<0.010 ug/l
Lead (as Pb)	<0.8 ug/l	Turbidity	0.2 NTU
CB 101	<0.010 ugs/l	1,2 dichloroethene	<1.0 ug/l
CB 105	<0.010 ugs/l	1,2,4 trichlorobenzene	<1.0 ug/l
CB 118	<0.010 ugs/l	3-chlorotoluene	<1.0 ug/l
CB 138	<0.010 ugs/l	Tetrachloroethene	<1.0 ug/l
CB 149	<0.010 ugs/l	Tetrachloromethane	<0.6 ug/l
CB 153	<0.010 ugs/l	Trichloroethene	<3.0 ug/l
CB 180	<0.010 ugs/l	Aluminium (as Al)	64 ug/l
CB 28	<0.010 ugs/l	Barium (as Ba)	22.8 ug/l
CB 31	<0.010 ugs/l	Calcium (as Ca)	17.9 mg/l
CB 52	<0.010 ugs/l	Copper (as Cu)	14.4 ug/l
TOTAL	0.000 ugs/l	Iron (as Fe)	20 ug/l
2,4,6 Tri Chlorophenol	<0.05 ug/l	Magnesium (as Mg)	6.3 mg/l
Pentachlorophenol	<0.07 ug/l	Manganese (as Mn)	2.6 mg/l
Phenol	<0.05 ug/l	Potassium (as K)	0.54 mg/l
Total Cresols (m,p,o)	<0.15 ug/l	Sodium (as Na)	6.4 mg/l
Total Phenols	<0.05 ug/l	Zinc (as Zn)	5.0 ug/l

Analysis Comments(3):

Signature:  Date:
 A. DICK
 CHEMIST
 15 JUL 1997

APPENDIX 4

Isocarb 12
Developmental Toxicity in Rats
Analysis of Dosing Formulations

Sampled and analysed 21 March 1997

Dose Group	Nominal Concentration (mg.ml ⁻¹)	Found Concentration (mg.ml ⁻¹)	Mean Found	Coefficient of Variation (%)	% Difference from Nominal
1	0	0 0 0	0	-	-
2	2.50	2.44 2.41 2.49	2.45	1.6	-2.0
3	20.0	19.8 19.6 18.8	19.4	2.7	-3.0
4	40.0	39.5 40.0 39.4	39.6	0.8	-1.0

Sampled and analysed 2 April 1997

Dose Group	Nominal Concentration (mg.ml ⁻¹)	Found Concentration (mg.ml ⁻¹)	Mean Found	Coefficient of Variation (%)	% Difference from Nominal
1	0	0 0 0	0	-	-
2	2.50	2.49 2.52 2.45	2.49	1.4	-0.4
3	20.0	18.6 19.5 19.6	19.2	2.9	-4.0
4	40.0	39.8 39.9 36.3	38.7	5.3	-3.3

APPENDIX 5

Isocarb 12
Developmental Toxicity Study in Rats
Individual Clinical Observations and Necropsy Findings

Group/ Dose Level (mg Isocarb 12. kg ⁻¹ .day ⁻¹)	Animal Number	Clinical Observations	Day(s) of Gestation Recorded	Necropsy Findings
1 (0)	3a	Muzzle swollen, nose stained and bleeding, red staining around eyes, ears pale, hunched, piloerection, laboured respiration. Unscheduled kill	7	Red staining on muzzle
	7	Partial hairloss dorsal abdomen	6-20	NAD
	10	Partial hairloss dorsal surface	2-20	NAD
	13	Partial hairloss upper hindlimb	2-20	NAD
	24	Subdued behaviour	18-20	NAD
2 (25)	26	Partial hairloss dorsal abdomen	12-20	NAD
	34	Partial hairloss dorsal cervical region	2-20	Mild hairloss dorsal cervical region
	36	Bald area right sacral region	12-20	Hairloss right hindlimb
	38	Partial hairloss dorsal abdomen	10-20	Hairloss dorsal abdomen
	41	Partial hairloss lower dorsal abdomen	2-20	NAD
	43	Partial hairloss lower dorsal abdomen	1-20	NAD
3 (200)	51	Wheezing respiration	14,15,19	NAD
	53	Eyelids swollen	8,9	NAD
	54	Bulging eyes	7-9	NAD
		Wheezing	18	
	55	Wheezing respiration, hunched appearance	14,15	NAD
	56	Piloerection	14-20	NAD
	65	Hairloss lower right dorsal abdomen	2,6-20	NAD
	66	Wheezing respiration	16,17	NAD
	68	Piloerection	14	NAD
		Hunched appearance	14-16	
		Crackling respiration	16	
	70	Hunched appearance	16-20	NAD
		Piloerection	17-20	
74	Wheezing respiration	14-19	NAD	
	Piloerection	16-19		

a = Findings not included in group values
NAD = No abnormalities detected

APPENDIX 5 (continued)

Individual Clinical Observations and Necropsy Findings

Group/ Dose Level (mg Isocarb 12. kg ⁻¹ .day ⁻¹)	Animal Number	Clinical Observations	Day(s) of Gestation Recorded	Necropsy Findings
4 (400)	76	Salivation	15,18	NAD
	77	Salivation	9,15	NAD
		Piloerection	11,13	
		Wheezing	14-17	
		Red/ brown staining around mouth	14	
		Red/ brown salivation	16	
		78	Salivation	9,19
	79	Wheezing respiration	15,16,18-20	
		Fast respiration	15-17	
		Red/ brown salivation	16	
		Laboured respiration	18	
		Salivation	9,14,15	Partial hairloss right shoulder
	188	Partial hairloss on right shoulder	11-20	
		Piloerection	15-17	
		Red/ brown salivation	16,17	
	81	Brown salivation	18	
Red/ brown salivation and wheezing respiration		17	NAD	
Salivation		19		
Salivation		7	Brown staining perigenital region	
Fast respiration		7,8		
82	Hunched appearance, piloerection, gasping respiration and subdued behaviour. Not dosed	13,14		
	Red/ brown discharge from vagina, pale ears, red/ brown staining ventral surface and both forelimbs, skin cold to touch, laboured respiration, unscheduled kill	14		
	Wheezing respiration	14-16	NAD	
	Red/ brown salivation	16,17		
		Piloerection	17-20	
		Salivation	19	

NAD = No abnormalities detected

APPENDIX 5 (continued)

Individual Clinical Observations and Necropsy Findings

Group/ Dose Level (mg Isocarb 12. kg ⁻¹ .day ⁻¹)	Animal Number	Clinical Observations	Day(s) of Gestation Recorded	Necropsy Findings
4 (400)	83	Red/ brown salivation	16	NAD
	84	Piloerection	11,12,17, 18	NAD
		Wheezing respiration	12,17,18	
		Red/ brown salivation	15,19	
		Laboured respiration	17,18	
		Salivation and hunched appearance	18	
	85	Red/ brown salivation	16	NAD
		Salivation	18	
	86	Red/ brown salivation	16	NAD
		Piloerection	16-19	
		Salivation	17	
	87	Partial hairloss lower dorsal abdomen	2-20	NAD
		Wheezing respiration	13,14	
		Eyelids swollen	13-16	
		Salivation	14	
		Red/ brown salivation	19	
	88	Wheezing respiration	14-18,20	NAD
		Salivation	14-18	
		Red/ brown salivation	15,19	
		Brown salivation	17	
89	Salivation	8,19	NAD	
	Wheezing respiration	13-16		
	Subdued behaviour	14-18		
	Piloerection	16-20		
	Laboured respiration	16,17		
	Brown salivation	17		
	Irregular respiration	18-20		

NAD = No abnormalities detected

APPENDIX 5 (continued)

Individual Clinical Observations and Necropsy Findings

Group/ Dose Level (mg Isocarb 12. kg ⁻¹ .day ⁻¹)	Animal Number	Clinical Observations	Day(s) of Gestation Recorded	Necropsy Findings	
4 (400)	90	Wheezing respiration	8,13-16	NAD	
		Piloerection	11-13, 15-20		
		Hunched appearance	13-18		
		Laboured respiration. Not dosed	13,14		
		Subdued behaviour	13		
		Red/ brown salivation	15,16,19		
		Shallow respiration	16-20		
	91	Salivation	8,14,18		NAD
		Wheezing, irregular, crackling and fast respiration	15-19		
		Piloerection	18-20		
		Red/ brown salivation	15,16,19		
	92	Salivation	8,18		NAD
		Piloerection	11-20		
		Hunched appearance	13-16		
		Wheezing respiration	18-20		
	93	Piloerection	10,11,16-20		NAD
		Salivation	13,16,17		
		Red/ brown salivation	15		
		Wheezing respiration	15,16		
		Crackling respiration	17		
94	Salivation	7,13,16,17	NAD		
	Piloerection	9-11,13-20			
	Hunched appearance	13-15			
	Red/ brown salivation	14,15,18,19			
95	Salivation	12	NAD		
	Red/ brown salivation	14			

NAD = No abnormalities detected

APPENDIX 5 (continued)

Individual Clinical Observations and Necropsy Findings

Group/ Dose Level (mg Isocarb 12. kg ⁻¹ .day ⁻¹)	Animal Number	Clinical Observations	Day(s) of Gestation Recorded	Necropsy Findings
4 (400)	96	Piloerection	10,11	NAD
		Salivation	12,16,17	
		Red/ brown salivation	14,15,18, 19	
	97	Salivation	7,13,17	NAD
		Piloerection	12,16-20	
		Wheezing respiration	12-16,20	
		Hunched appearance	12-20	
		Laboured respiration	12-14	
		Wet, yellow staining of coat around vagina. Not dosed	12	
		Irregular respiration	13-20	
		Red/ brown salivation	14,18	
		Brown salivation	16	
	98	Piloerection	10-13	NAD
		Hunched appearance	10-13	
		Laboured respiration. Not dosed	12,13	
		Wheezing respiration	12	
		Whole body appears dark	12,13	
		Skin cold to touch	12,13	
		Dark eyes, unscheduled kill	13	
	99	Piloerection	10,11,16- 20	NAD
Wheezing respiration		11,13,14		
Salivation		12,17		
Hunched appearance		13-20		
Severe red/ brown salivation, red/ brown staining around mouth, laboured respiration		13		
Red/ brown staining on both forelimbs		18,19		
Red/ brown salivation		20		
100	Salivation	12,16,17	NAD	
	Piloerection	16-20		

NAD = No abnormalities detected

APPENDIX 6

Isocarb 12
Developmental Toxicity Study in Rats
Individual Body Weight Data (g)

Group 1, Control: 0 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Day of Gestation								
	4	6	9	10	11	12	13	17	20
1	210	232	242	251	255	264	267	304	347
2	236	251	271	279	283	293	292	332	367
4	234	247	259	269	280	286	291	334	362
5	226	244	257	261	271	283	284	316	356
6	211	233	243	250	258	271	269	313	360
7	234	229	266	266	276	288	284	332	371
8	232	237	260	267	274	277	279	321	340
9	247	265	280	282	298	302	311	350	392
10	258	281	295	301	311	311	321	365	396
11	222	239	251	259	265	271	276	312	347
12	226	247	261	275	281	283	287	329	374
13	220	242	256	261	271	276	283	318	355
14	231	251	268	272	276	278	288	320	355
15	247	268	289	302	306	316	320	364	378
16	229	240	251	258	264	268	281	313	338
17	232	255	264	276	281	289	292	329	373
18	242	258	275	278	286	294	297	345	375
19	249	269	281	287	289	301	304	355	390
20	214	235	248	248	246	260	267	304	344
21	267	283	305	316	322	336	342	393	432
22	247	256	265	272	271	281	287	316	349
23	206	217	226	233	237	242	250	288	326
24	196	209	223	228	229	235	237	271	305
25	213	221	230	240	244	244	254	280	310
101a	206	218	233	222	233	239	262	297	-

a = Animal killed Day 18 in error, excluded from group values

APPENDIX 6 (continued)
Individual Body Weight Data (g)

Group 2, Low dose: 25 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Day of Gestation								
	4	6	9	10	11	12	13	17	20
26	255	273	289	298	306	313	314	356	399
27	203	213	227	233	237	246	249	285	312
28	236	248	263	268	273	282	281	313	348
29	235	252	267	274	288	299	299	343	383
30	226	250	263	269	278	287	283	335	369
31	221	234	252	255	265	267	275	312	345
32	265	275	290	303	311	318	326	377	428
33	228	244	254	260	268	271	276	309	334
34	244	264	281	291	298	302	309	353	404
35	242	259	272	280	288	292	295	330	375
36	241	254	265	274	280	281	291	337	379
37	250	266	272	290	290	291	295	338	377
38	227	240	255	263	274	272	280	313	340
39	226	244	259	266	276	278	287	322	366
40	228	251	275	285	297	296	308	355	394
41	235	251	265	278	283	281	291	333	367
42	252	268	284	293	298	303	316	354	406
43	219	235	243	253	253	267	270	316	333
44	215	225	236	238	239	240	251	279	295
45	255	275	294	303	306	314	320	366	407
46	209	224	242	247	248	255	261	293	328
47	230	241	254	263	262	268	269	313	344
48	256	270	290	296	300	304	311	355	390
49	220	236	250	250	253	263	267	301	335
50	213	227	248	254	254	267	270	302	341

APPENDIX 6 (continued)

Individual Body Weight Data (g)

Group 3, Intermediate dose: 200 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Day of Gestation								
	4	6	9	10	11	12	13	17	20
51	207	227	237	246	253	265	267	286	328
52	228	251	260	269	254	251	280	330	379
(53)	256	270	281	287	292	300	300	288	290
54	221	239	245	253	259	249	258	294	336
55	234	252	266	273	281	287	289	332	377
56	246	259	268	276	275	278	280	316	347
57	240	266	276	287	295	275	278	334	381
58	246	264	272	279	288	293	300	338	373
59	234	251	265	252	263	272	283	316	356
60	241	256	269	278	283	287	300	344	399
61	215	225	244	253	261	265	272	310	349
62	247	264	275	283	285	292	302	352	396
64	221	238	251	263	268	271	280	320	372
65	255	266	283	283	293	301	311	355	393
66	234	248	266	276	274	274	291	331	384
67	231	249	265	272	278	284	295	338	384
68	248	268	241	241	258	278	278	308	339
69	230	248	257	262	266	278	278	325	364
70	225	206	253	257	256	265	268	299	329
71	242	253	264	270	273	283	290	325	369
72	241	254	270	277	286	295	302	343	391
73	239	253	272	278	277	288	295	337	364
74	202	216	230	234	237	244	254	279	319
75	235	254	269	273	276	283	291	331	380
167	269	291	303	315	318	328	328	367	418

() = Animal not pregnant

APPENDIX 6 (continued)
Individual Body Weight Data (g)

Group 4, High dose: 400 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Day of Gestation								
	4	6	9	10	11	12	13	17	20
76	232	249	253	260	268	278	280	318	364
77	245	261	268	279	289	291	297	338	386
78	245	262	276	284	284	284	300	310	343
79	274	293	308	317	328	338	339	386	439
81b	206	223	238	247	253	263	212	-	-
82	256	278	288	299	278	278	288	338	380
83	238	259	265	270	274	284	286	324	366
84	246	267	258	270	260	271	280	337	377
85	236	251	268	270	279	282	294	334	386
86	230	244	264	266	266	278	271	303	340
87	244	263	277	283	289	293	284	334	353
88	220	236	243	244	234	237	251	298	333
89	214	235	248	232	226	248	263	249	260
90	248	268	278	247	249	263	235	290	330
91	247	265	271	255	249	243	260	309	349
92	229	249	258	228	246	247	255	309	336
93	227	239	232	232	245	255	248	292	330
94	232	239	249	255	258	264	271	306	338
95	240	247	256	258	260	269	278	311	356
96	240	254	267	274	278	286	292	333	383
97	237	253	257	247	224	228	248	288	314
98b	213	234	240	220	215	188	181	-	-
99	239	248	254	230	234	248	230	276	331
100	247	262	266	276	280	292	297	336	386
188	216	238	259	277	278	285	278	319	375

b = Premature decedent

APPENDIX 7

Isocarb 12
Developmental Toxicity Study in Rats
Individual Food Consumption Data (g)Group 1, Control: 0 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Day of Gestation																
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	23	21	24	22	23	24	25	27	27	30	28	28	27	31	29	27	24
2	32	29	31	26	27	35	30	31	34	35	36	34	35	32	36	37	28
4	35	28	35	29	31	26	32	30	35	34	32	31	35	37	36	34	29
5	36	28	28	26	22	27	27	29	30	29	30	30	30	33	31	29	24
6	8	25	31	28	24	26	29	26	30	32	31	27	33	34	31	37	24
7	26	27	16	24	28	31	34	29	34	30	33	25	34	38	38	31	28
8	30	30	31	29	28	32	32	29	31	35	33	24	34	35	35	34	14
9	28	36	26	23	29	29	27	33	32	32	34	33	38	37	36	38	30
10	27	31	26	28	33	33	30	31	31	33	25	32	37	33	38	30	22
11	26	27	25	25	29	28	27	27	31	29	26	29	37	28	28	34	29
12	31	29	25	26	28	27	30	35	30	33	31	31	35	38	34	35	33
13	29	28	31	29	30	30	29	33	33	32	31	34	33	38	35	32	29
14	29	29	28	25	27	28	19	28	31	28	27	32	31	27	35	32	26
15	28	28	30	34	35	35	31	35	40	33	35	32	36	40	30	32	10
16	26	29	25	24	30	29	30	31	33	35	31	35	37	37	42	31	23
17	28	33	28	28	32	38	36	34	37	31	31	37	35	35	41	38	31
18	30	29	31	34	33	27	27	32	31	28	31	35	38	40	31	35	24
19	29	33	29	31	30	31	33	32	32	34	35	38	42	40	34	32	30
20	28	27	28	27	27	29	28	27	31	32	32	31	33	35	30	34	30
21	32	32	33	35	34	34	39	36	39	34	39	39	44	45	38	41	35
22	28	32	26	27	27	27	28	26	29	31	33	31	34	33	31	33	30
23	27	24	25	26	25	25	28	25	29	30	32	32	35	35	31	34	29
24	30	20	22	30	28	25	25	25	28	27	31	28	31	33	29	30	29
25	28	27	21	28	21	23	27	25	23	31	25	25	24	30	27	27	26
101a	27	26	23	29	27	28	24	28	31	30	31	30	28	33	31	-	-

a = Animal killed Day 18 in error, excluded from group values

APPENDIX 7 (continued)
Individual Food Consumption Data (g)

Group 2, Low dose: 25 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Day of Gestation																
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
26	31	27	33	24	37	28	42	37	34	37	29	33	35	37	43	43	27
27	25	21	26	24	22	13	25	18	25	24	24	29	24	30	28	29	22
28	31	31	27	28	27	22	29	25	27	28	24	33	28	29	30	30	24
29	47	28	31	28	26	27	29	32	36	35	22	35	34	34	35	38	27
30	34	31	27	27	30	27	28	29	34	27	32	34	33	34	36	36	20
31	24	26	24	23	23	30	27	26	29	33	28	31	30	32	35	35	24
32	32	19	31	26	29	32	33	30	35	32	35	34	36	39	40	37	26
33	22	24	25	23	21	25	26	27	28	29	27	30	26	28	33	29	25
34	31	29	32	34	36	33	33	32	36	33	37	33	37	36	38	39	33
35	27	24	30	28	32	35	28	31	31	30	36	33	33	33	38	30	27
36	27	27	30	28	35	33	30	32	33	26	34	35	38	38	36	35	27
37	29	29	29	25	31	27	32	29	28	28	30	30	33	34	35	25	26
38	25	30	27	25	30	28	26	31	29	30	30	30	35	29	36	28	32
39	24	28	27	25	34	30	32	30	33	32	35	30	37	32	39	31	32
40	29	27	29	28	32	30	32	35	34	29	37	39	38	38	42	39	33
41	31	32	31	31	35	32	35	33	35	30	34	34	34	36	37	30	27
42	26	30	27	29	36	31	32	30	33	31	33	32	34	34	37	34	34
43	28	27	26	33	30	26	25	29	28	34	30	33	35	37	32	37	25
44	28	24	25	31	25	28	25	26	26	32	27	27	32	32	30	32	14
45	34	27	35	32	33	33	35	35	35	35	36	40	35	43	36	38	38
46	28	27	26	27	30	28	27	30	27	31	30	28	30	30	29	32	32
47	26	26	24	28	26	28	23	29	26	30	28	32	30	35	36	32	20
48	31	27	30	27	28	28	30	32	28	33	32	32	38	37	31	36	24
49	29	27	28	27	28	28	31	27	28	35	32	31	30	37	28	28	35
50	31	27	24	30	27	28	29	31	31	33	31	33	34	35	42	39	35

APPENDIX 7 (continued)

Individual Food Consumption Data (g)

Group 3, Intermediate dose: 200 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Day of Gestation																
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
51	25	26	31	27	27	29	33	32	37	39	22	32	29	34	36	39	29
52	36	35	34	29	29	30	33	19	16	42	26	39	33	42	41	40	26
(53)	47	30	30	24	27	28	32	29	33	31	19	23	26	27	23	28	21
54	33	30	31	29	30	27	32	31	22	29	25	28	30	33	27	31	24
55	40	27	33	29	27	29	29	31	31	32	20	31	31	38	31	37	27
56	31	26	27	23	25	28	28	23	22	27	25	31	32	33	29	30	28
57	26	33	32	23	31	31	30	29	19	27	30	35	34	33	34	34	27
58	24	28	31	30	27	31	35	32	31	34	31	33	32	37	36	35	30
59	27	29	28	25	30	29	15	27	26	29	33	30	35	35	34	32	34
60	30	30	29	26	34	29	29	31	35	32	31	30	33	38	36	33	33
61	25	26	24	24	27	28	27	32	34	30	33	32	35	37	39	36	29
62	31	37	29	28	31	32	29	34	38	32	34	38	39	42	39	41	28
64	26	31	25	26	32	30	33	29	31	30	37	32	39	36	40	34	33
65	31	28	27	27	29	28	26	34	32	23	35	35	36	39	38	36	33
66	26	30	27	25	33	31	32	30	22	32	36	34	35	36	41	37	35
67	37	31	32	27	33	32	25	26	34	24	33	35	33	42	38	34	36
68	31	26	30	27	30	52	31	29	37	32	25	27	37	33	36	37	39
69	31	30	27	25	27	25	29	24	27	28	27	29	31	29	31	31	25
70	28	26	18	30	28	27	26	21	27	31	27	27	28	28	29	30	28
71	30	25	27	26	30	28	29	25	29	32	29	29	37	28	30	33	33
72	31	30	32	33	35	30	31	31	23	38	32	40	38	38	39	38	36
73	33	31	27	31	30	31	31	28	20	31	37	33	34	31	35	35	30
74	27	24	24	29	25	26	27	26	25	31	29	27	33	28	28	33	30
75	32	35	33	29	32	32	35	30	27	36	34	35	41	38	38	37	40
167	25	32	31	27	35	33	28	35	31	31	32	35	35	38	39	38	31

() = Animal not pregnant

APPENDIX 7 (continued)

Individual Food Consumption Data (g)

APPENDIX 8 (continued)

Individual Pregnancy Data

Group 2, Low dose: 25 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Corpora Lutea Graviditatis	Total Implant Sites	Early Deaths	Late Deaths	Foetal Deaths	Live Implants	Uterus Weight (g)	Mean Foetal Weight (g) ± SD
26	18	13	0	0	0	13	76	3.45 ± 0.28
27	12	11	1	0	0	10	61	3.83 ± 0.21
28	14	13	1	0	0	12	71	3.62 ± 0.45
29	13	13	1	0	0	12	75	3.73 ± 0.22
30	15	14	0	0	0	14	86	3.88 ± 0.17
31	13	12	0	0	0	12	73	3.73 ± 0.36
32	18	17	0	0	0	17	107	3.77 ± 0.15
33	14	12	0	0	0	12	69	3.89 ± 0.17
34	16	16	0	0	0	16	97	3.61 ± 0.36
35	14	13	0	0	0	13	78	3.89 ± 0.18
36	17	15	0	0	0	15	91	3.82 ± 0.21
37	16	15	0	0	0	15	88	3.71 ± 0.29
38	13	13	3	1	0	9	57	3.62 ± 0.65
39	14	13	0	0	0	13	80	3.94 ± 0.13
40	13	11	0	0	0	11	73	4.14 ± 0.16
41	15	14	0	0	0	14	88	4.00 ± 0.19
42	14	14	0	0	0	14	94	4.34 ± 0.32
43	12	12	1	0	0	11	69	3.94 ± 0.17
44	13	11	0	0	0	11	61	3.66 ± 0.13
45	15	15	2	1	0	12	75	3.48 ± 0.18
46	13	10	1	0	0	9	54	3.59 ± 0.14
47	15	15	0	0	0	15	83	3.58 ± 0.23
48	17	16	1	0	0	15	90	3.73 ± 0.20
49	12	11	1	0	0	10	53	3.03 ± 0.61
50	11	10	1	0	0	9	67	4.52 ± 0.27

SD = Standard deviation.

APPENDIX 8 (continued)
Individual Pregnancy Data

Group 3, Intermediate dose: 200 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Corpora Lutea Graviditatis	Total Implant Sites	Early Deaths	Late Deaths	Foetal Deaths	Live Implants	Uterus Weight (g)	Mean Foetal Weight (g) ± SD
51	14	12	0	0	0	12	69	3.71 ± 0.18
52	14	14	0	0	0	14	88	3.86 ± 0.35
53	0	0	-	-	-	-	-	-
54	15	14	0	0	0	14	84	3.79 ± 0.23
55	14	14	0	0	0	14	81	3.56 ± 0.35
56	15	10	4	0	0	6	39	3.96 ± 0.13
57	15	15	0	0	0	15	84	3.58 ± 0.27
58	20	14	2	0	0	12	73	3.71 ± 0.34
59	13	12	3	0	0	9	58	3.70 ± 0.29
60	15	14	0	0	0	14	86	3.65 ± 0.19
61	14	14	2	0	0	12	77	3.85 ± 0.23
62	13	13	0	0	0	13	92	4.39 ± 0.20
167	24	16	0	0	0	16	89	3.53 ± 0.25
64	12	12	0	0	0	12	75	3.82 ± 0.26
65	14	12	0	0	0	12	77	4.06 ± 0.20
66	13	11	1	0	0	10	64	3.88 ± 0.54
67	15	13	0	0	0	13	82	4.16 ± 0.21
68	9	6	1	0	0	5	34	3.74 ± 0.09
69	14	14	0	0	0	14	85	3.74 ± 0.15
70	13	13	4	0	0	9	55	3.55 ± 0.30
71	14	14	0	0	0	14	79	3.46 ± 0.17
72	15	13	0	0	0	13	82	3.87 ± 0.31
73	16	14	0	0	0	14	90	3.80 ± 0.22
74	9	9	0	0	0	9	59	3.70 ± 0.28
75	15	14	0	0	0	14	89	4.00 ± 0.21

SD = Standard deviation.

APPENDIX 8 (continued)
Individual Pregnancy Data

Group 4, High dose: 400 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Corpora Lutea Graviditatis	Total Implant Sites	Early Deaths	Late Deaths	Foetal Deaths	Live Implants	Uterus Weight (g)	Mean Foetal Weight (g) ± SD
76	14	14	0	0	0	14	80	3.33 ± 0.46
77	15	15	2	0	0	13	76	3.65 ± 0.26
78	13	13	0	0	0	13	65	3.10 ± 0.26
79	16	15	0	0	0	15	95	3.72 ± 0.26
188	16	16	1	0	0	15	93	3.78 ± 0.26 ^d
81b	15	13	-	-	-	-	-	-
82	14	14	1	0	0	13	75	3.52 ± 0.16
83	14	14	0	0	0	14	87	3.97 ± 0.23
84	16	14	0	0	0	14	79	3.74 ± 0.25
85	14	14	1	0	0	13	88	4.16 ± 0.23
86	12	10	0	0	0	10	69	4.00 ± 0.25
87	13	13	0	0	0	13	78	3.83 ± 0.22
88	15	13	0	0	0	13	76	3.56 ± 0.20
89	11	10	0	0	0	10	49	3.05 ± 0.19
90	14	13	0	0	0	13	71	3.39 ± 0.23
91	17	12	1	0	0	11	63	3.64 ± 0.24
92	14	14	0	0	0	14	82	3.63 ± 0.19
93	15	13	1	0	0	12	68	3.43 ± 0.27
94	9	9	0	0	0	9	58	3.84 ± 0.24
95	16	14	0	0	0	14	83	3.63 ± 0.21
96	12	12	1	0	0	11	71	3.79 ± 0.16
97	15	15	1	0	0	14	69	2.98 ± 0.21
98b	14	11	-	-	-	-	-	-
99	15	15	0	0	0	15	75	3.12 ± 0.29
100	15	15	0	0	0	15	95	3.88 ± 0.27

SD = Standard deviation
b = Premature decedent
d = One foetus not weighed in error

APPENDIX 9

Isocarb 12

Developmental Toxicity Study in Rats

Individual Incidence of Foetal Abnormalities and Variants

Notes:

Only those foetuses/litters with abnormalities/variants are listed. All other foetuses/litters examined in the study had no abnormalities detected.

Major, deleterious (or potentially deleterious) abnormalities appear in capital letters.

A dash (-) in the 'skeletal' and 'ribs' column indicates that the foetus was preserved in Bouin's fluid and was not examined skeletally.

Foetuses with supernumerary rib(s) are categorised according to the length of the longest supernumerary rib.

Abbreviations used:

NAD = No abnormality detected

a = 13 complete ribs

b = 14th vestigial supernumerary rib(s)

c = 14th reduced supernumerary rib(s)

d = 14th complete rib

APPENDIX 9 (continued)

Individual Incidence of Foetal Abnormalities and Variants

Group 1, Control: 0 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Foetus Number /Sex/ Weight (g)	Abnormality		
		Visceral	Skeletal	Ribs
4	1σ 2.27	INTERNAL HYDROCEPHALY INTERVENTRICULAR SEPTAL DEFECT SUBCUTANEOUS OEDEMA	-	-
	3σ 1.89	SMALL RIGHT EYE INTERVENTRICULAR SEPTAL DEFECT SUBCUTANEOUS OEDEMA	-	-
5	4σ 4.29	Left testis not fully descended to pelvic position	-	-
6	11♀ 3.65	Protrusion of median liver lobe with thinning of diaphragm	-	-
7	2σ 4.32	Protrusion of median liver lobe	-	-
8	5σ 3.66	Right testis not fully descended to pelvic position	-	-
	7σ 3.52	Increased right renal pelvic cavitation	-	-
	10♀ 3.19	Subcutaneous haemorrhage on lower jaw	NAD	a
	15σ 3.81	Subcutaneous haemorrhage on lower jaw	-	-
9	3σ 3.77	Subcutaneous haemorrhage on lower jaw	NAD	a
	11♀ 3.77	NAD	Additional area of ossification on 5th sternebra	a
10	3σ 4.19	Intra abdominal haemorrhage	-	-
13	4♀ 4.04	Intra abdominal haemorrhage	-	-
14	7σ 3.77	Small interventricular septal defect	-	-
19	14σ 3.76	Dilated ureters	-	-

APPENDIX 9 (continued)

Individual Incidence of Foetal Abnormalities and Variants

Group 1, Control: 0 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Foetus Number /Sex/ Weight (g)	Abnormality		
		Visceral	Skeletal	Ribs
20	5♀ Not weighed in error	Hepatic haemorrhage within right median liver lobe	-	-
21	4♀ 4.29	Hepatic haemorrhage within median liver lobe	-	-
23	2♀ 4.30	Protrusion of median liver lobe with thinning of diaphragm	-	-
	8♂ 3.78	MARKED PROTRUSION OF MEDIAN LIVER LOBE WITH THINNING OF DIAPHRAGM	-	-
24	3♂ 3.89	Small interventricular septal defect Protrusion of median liver lobe with thinning of diaphragm	-	-
25	2♂ 4.53	MARKED PROTRUSION OF MEDIAN LIVER LOBE WITH THINNING OF DIAPHRAGM	-	-

APPENDIX 9 (continued)

Individual Incidence of Foetal Abnormalities and Variants

Group 2, Low dose: 25 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Foetus Number /Sex/ Weight (g)	Abnormality		
		Visceral	Skeletal	Ribs
26	7♀ 3.50	Dilated ureters and increased right renal pelvic cavitation	-	-
27	6♂ 3.85	Absent innominate artery	-	-
28	6♀ 3.71	Increased right renal pelvic cavitation	NAD	a
	10♂ 3.80	NAD	KINKED 11th RIGHT AND 12th BILATERAL RIBS; IRREGULAR OSSIFICATION OF 11th BILATERAL RIBS, 12th LEFT RIB AND 13th RIGHT VERTEBRAL ARCH KINKED 6th AND 13th BILATERAL RIBS, MINIMAL	a
	11♂ 3.79	RETRO OESOPHAGEAL AORTIC ARCH	-	-
31	3♀ 3.23	-	TERMINATION OF NORMAL VERTEBRAL COLUMN AT 13th THORACIC VERTEBRA	a
	4♀ 3.47	Haemorrhagic left orbital sinus	-	-
	8♂ 4.26	Intra-abdominal haemorrhage	-	-
32	1♂ 3.75	Subcutaneous haemorrhage dorsal thoracic region	-	-
	15♂ 3.54	Subcutaneous haemorrhage lower jaw	-	-
37	4♀ 3.48	Protrusion of median liver lobe with thinning of diaphragm	-	-
	8♂ 3.89	Protrusion of median liver lobe with thinning of diaphragm	-	-
38	9♂ 1.98	INTERNAL HYDROCEPHALY INTERVENTRICULAR SEPTAL DEFECT Reduced right side of thyroid	-	-

APPENDIX 9 (continued)

Individual Incidence of Foetal Abnormalities and Variants

Group 2, Low dose: 25 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Foetus Number /Sex/ Weight (g)	Abnormality		
		Visceral	Skeletal	Ribs
39	2♀ 4.09	Intra abdominal haemorrhage	-	-
46	1♀ 3.59	Protrusion of median liver lobe with thinning of diaphragm	-	-
49	3♂ 3.57	NAD	Additional area of ossification between interparietal and right parietal	a
	6♂ 3.86	Dilated ureters and increased right renal pelvic cavitation	-	-
	8♂ 1.82	Subcutaneous haemorrhage on left hindlimb	-	-
	10♂ 2.76	Medial displacement of right testis	-	-

APPENDIX 9 (continued)

Individual Incidence of Foetal Abnormalities and Variants

Group 3, Intermediate dose: 200 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Foetus Number /Sex/ Weight (g)	Abnormality		
		Visceral	Skeletal	Ribs
52	3♂ 4.06	Minimal haemorrhage on posterior chamber of right eye	-	-
54	9♀ 3.97	Hepatic haemorrhage within median liver lobe	-	-
55	10♀ 3.64	Dilated ureters	-	-
	14♂ 3.77	Dilated ureters	-	-
57	2♀ 3.32	Subcutaneous haemorrhage in dorsal thoracic region	-	-
60	1♂ 3.63	Marked subcutaneous haemorrhage on cranium	-	-
62	5♂ 4.53	Protrusion of median liver lobe	-	-
	10♂ 4.43	NAD	Irregular ossification of sixth sternebra	b
167	8♂ 3.49	Reduced right side of thyroid	-	-
65	1♂ 4.27	NAD	KINKED 6th-9th RIGHT RIBS; INCOMPLETE OSSIFICATION OF 10th-12th RIGHT RIBS KINKED 10th-12th RIGHT RIBS, MINIMAL	b
66	6♀ 3.90	NAD	Area of ossification between parietals	a
	7♀ 2.63	Subcutaneous haemorrhage left hindlimbs Intra-abdominal haemorrhage	-	-
67	2♀ 4.15	Intra-abdominal haemorrhage	-	-
74	8♂ 3.92	NAD	IRREGULAR OSSIFICATION OF KINKED 4-12th BILATERAL RIBS	b
75	14♂ 4.28	Increased right renal pelvic cavitation	-	-

APPENDIX 9 (continued)

Individual Incidence of Foetal Abnormalities and Variants

Group 4, High dose: 400 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Foetus Number /Sex/ Weight (g)	Abnormality		
		Visceral	Skeletal	Ribs
76	2♂ 1.87	-	Third and fourth sacral centrum unossified	b
	7♀ 2.96	Subcutaneous haemorrhage on lower jaw	-	-
	11♂ 3.44	Subcutaneous haemorrhage on lower jaw	-	-
78	1♀ 3.21	Subcutaneous haemorrhage on lower jaw	-	-
79	6♀ 3.41	Dilated left ureter	-	-
83	2♂ 4.00	Subcutaneous haemorrhage in ventral cervical region	-	-
	4♂ 3.48	Subcutaneous haemorrhage on lower lip	-	-
87	13♂ 4.21	Increased right renal pelvic cavitation	NAD	a
90	1♀ 3.52	Increased right renal pelvic cavitation	-	-
	3♂ 2.77	LEFT ANOPHTHALMIA, RIGHT MICROPHTHALMIA Reduced left side of thyroid Increased right renal pelvic cavitation Dilated left ureter	-	-
	7♂ 3.59	Dilated left ureter	-	-
94	1♀ 3.77	Hepatic haemorrhage within left liver lobe	-	-
95	10♂ 3.59	Left testis not fully descended to pelvic position (marked)	-	-
97	3♀ 2.92	Subcutaneous haemorrhage in nasal region	-	-
	13♂ 2.61	Subcutaneous haemorrhage on cranium	-	-
99	10♀ 3.16	Additional lobe of liver within median cleft	-	-

APPENDIX 10

Isocarb 12

Developmental Toxicity Study in Rats

Litter Incidences of Number of Ribs

Number of Ribs

a = 13 complete rib(s)

b = 14th vestigial supernumerary rib(s)

c = 14th reduced supernumerary rib(s)

d = 14th complete rib

Foetuses with supernumerary rib(s) are categorised according to the length of the longest supernumerary rib present

APPENDIX 10 (continued)

Litter Incidences of Number of Ribs

Group 1: 0 mg Isocarb 12.kg⁻¹.day⁻¹

Group 2: 25 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Number of Foetuses Examined	Number of Ribs			
		a	b	c	d
1	6	6	0	0	0
2	5	5	0	0	0
4	5	5	0	0	0
5	6	6	0	0	0
6	7	6	1	0	0
7	6	6	0	0	0
8	7	6	1	0	0
9	7	7	0	0	0
10	7	7	0	0	0
11	5	5	0	0	0
12	6	6	0	0	0
13	6	6	0	0	0
14	6	6	0	0	0
15	8	6	2	0	0
16	6	6	0	0	0
17	7	5	2	0	0
18	6	4	2	0	0
19	8	8	0	0	0
20	6	6	0	0	0
21	7	4	3	0	0
22	5	5	0	0	0
23	6	6	0	0	0
24	4	3	1	0	0
25	4	3	1	0	0

Animal Number	Number of Foetuses Examined	Number of Ribs			
		a	b	c	d
26	6	6	0	0	0
27	5	3	2	0	0
28	6	4	2	0	0
29	6	6	0	0	0
30	7	6	1	0	0
31	6	6	0	0	0
32	8	7	1	0	0
33	6	6	0	0	0
34	8	2	6	0	0
35	7	7	0	0	0
36	7	6	1	0	0
37	8	8	0	0	0
38	4	2	2	0	0
39	7	7	0	0	0
40	5	3	2	0	0
41	7	6	1	0	0
42	7	6	1	0	0
43	6	6	0	0	0
44	5	5	0	0	0
45	6	4	2	0	0
46	4	4	0	0	0
47	8	6	2	0	0
48	7	4	3	0	0
49	5	4	1	0	0
50	4	4	0	0	0

APPENDIX 10 (continued)

Litter Incidences of Number of Ribs

Group 3: 200 mg Isocarb 12.kg⁻¹.day⁻¹

Group 4: 400 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Number of Foetuses Examined	Number of Ribs			
		a	b	c	d
51	6	5	1	0	0
52	7	6	0	1	0
54	7	5	2	0	0
55	7	4	3	0	0
56	3	3	0	0	0
57	8	8	0	0	0
58	6	4	2	0	0
59	5	5	0	0	0
60	7	7	0	0	0
61	6	3	3	0	0
62	6	2	4	0	0
167 ^a	7 ^a	7	0	0	0
64	6	0	6	0	0
65	6	4	2	0	0
66	5	5	0	0	0
67	7	6	1	0	0
68	2	2	0	0	0
69	7	7	0	0	0
70	4	4	0	0	0
71	7	6	1	0	0
72	6	6	0	0	0
73	7	4	3	0	0
74	4	2	2	0	0
75	7	7	0	0	0

Animal Number	Number of Foetuses Examined	Number of Ribs			
		a	b	c	d
76	7	4	3	0	0
77	7	4	3	0	0
78	6	6	0	0	0
79	8	4	4	0	0
188	7	7	0	0	0
82	6	6	0	0	0
83	7	0	7	0	0
84	7	6	1	0	0
85	7	0	6	0	1
86	5	4	1	0	0
87	7	5	2	0	0
88	6	6	0	0	0
89	5	4	1	0	0
90	6	6	0	0	0
91	6	2	4	0	0
92	7	1	6	0	0
93	6	4	2	0	0
94	4	2	1	1	0
95	7	2	5	0	0
96	5	5	0	0	0
97	7	7	0	0	0
99	8	6	2	0	0
100	7	5	2	0	0

a = One foetus missing

APPENDIX 11

Isocarb 12

Developmental Toxicity Study in Rats

Litter Incidences of Incomplete Ossification Parameters

Key to Parameter Numbers

Incomplete ossification affecting:

1. ≥ 4 skull bones
2. ≤ 3 skull bones
3. Cervical vertebral arch(es)
4. Thoracic vertebral centrum/a
5. 2nd to 4th metacarpal(s)
6. 2nd to 4th metatarsal(s)
7. Pubis/es
8. Ischium/a
9. Sacral vertebral arch(es)

Unossified:

10. 5th metacarpal(s)
11. 5th metatarsal(s)

APPENDIX 11 (continued)

Litter Incidences of Incomplete Ossification Parameters

Group 2, Low dose: 25 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Number of Foetuses Examined	Number of Sternebrae with Incomplete Ossification					Parameter number										
		0	1	2	3	>3	1	2	3	4	5	6	7	8	9	10	11
26	6	2	3	1	0	0	0	0	0	0	0	0	0	0	0	5	0
27	5	1	3	0	1	0	0	0	0	1	0	0	0	0	0	1	0
28	6	4	0	2	0	0	1	2	0	1	0	0	1	0	0	3	1
29	6	1	3	2	0	0	0	0	0	0	0	0	0	0	0	2	0
30	7	4	3	0	0	0	0	0	0	0	0	0	0	0	0	2	0
31	6	5	1	0	0	0	0	2	0	2	0	0	0	0	0	1	0
32	8	7	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
33	6	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	8	3	2	3	0	0	0	0	0	0	0	0	0	0	0	3	0
35	7	6	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0
36	7	6	1	0	0	0	0	0	0	0	0	0	0	0	0	5	0
37	8	3	5	0	0	0	2	3	0	0	0	0	0	0	0	1	0
38	4	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
39	7	3	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
40	5	0	5	0	0	0	0	0	0	0	0	0	0	0	0	3	0
41	7	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	7	5	2	0	0	0	0	1	0	0	0	0	1	0	0	0	0
43	6	4	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
44	5	0	4	1	0	0	0	0	0	1	0	0	0	0	0	2	0
45	6	3	3	0	0	0	0	0	0	0	0	0	0	0	1	6	0
46	4	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
47	8	4	3	1	0	0	0	2	0	1	0	0	0	0	0	0	0
48	7	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	5	0	2	1	1	1	0	2	0	1	0	0	0	0	1	5	0
50	4	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

APPENDIX 11 (continued)

Litter Incidences of Incomplete Ossification Parameters

Group 3, Intermediate dose: 200 mg Isocarb 12.kg⁻¹.day⁻¹

Animal Number	Number of Foetuses Examined	Number of Sternebrae with Incomplete Ossification					Parameter number										
		0	1	2	3	>3	1	2	3	4	5	6	7	8	9	10	11
51	6	2	2	2	0	0	2	2	0	0	1	0	1	1	2	5	0
52	7	4	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0
54	7	2	4	0	1	0	1	0	0	0	0	0	0	0	0	2	0
55	7	6	0	0	1	0	0	1	0	1	0	0	0	0	0	2	0
56	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	8	0	4	4	0	0	1	1	0	0	0	0	0	0	1	8	0
58	6	1	3	2	0	0	0	0	0	1	0	0	0	0	1	3	0
59	5	2	2	1	0	0	0	1	0	0	0	0	0	0	0	5	0
60	7	2	1	4	0	0	0	5	0	0	0	0	0	0	0	5	0
61	6	1	5	0	0	0	1	1	0	0	0	0	0	0	0	3	0
62	6	5	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0
167a	7a	0	3	4	0	0	0	0	0	1	0	0	0	0	0	5	0
64	6	2	3	1	0	0	3	2	0	1	2	0	2	1	2	5	1
65	6	6	0	0	0	0	1	1	0	0	0	0	1	0	2	3	0
66	5	0	5	0	0	0	0	2	0	0	0	0	0	0	1	1	0
67	7	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	2	0	1	1	0	0	1	0	0	0	0	0	0	0	1	2	0
69	7	2	2	3	0	0	0	0	0	0	0	0	0	0	0	6	0
70	4	0	2	2	0	0	0	0	0	0	0	0	0	0	0	2	0
71	7	1	2	4	0	0	0	0	0	0	0	0	0	0	1	4	0
72	6	2	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0
73	7	5	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0
74	4	2	2	0	0	0	1	1	1	0	0	0	1	1	1	3	0
75	7	5	2	0	0	0	0	2	0	1	0	0	0	0	2	2	0

a = One foetus missing

APPENDIX 12

Isocarb 12

Developmental Toxicity Study in Rats

Experimental Protocol

(To be included at final)