STUDY TITLE

The detection of dominant lethal mutations and foetal malformations in the offspring of male rats treated sub-chronically with 1,3-butadiene by inhalation

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STUDY COMPLETED ON

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TESTING FACILITY

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SPONSORS

CEFIC (European Chemical Industry Council)
IISRP (International Institute of Synthetic Rubber Producers)

LABORATORY PROJECT IDENTITY

CONTENTS

GOOD LABORATORY PRACTICE

Acceptance of report

I hereby declare that the study described in this report was conducted under my supervision and that to the best of my knowledge and belief the study was conducted in compliance with accepted international standards of Good Laboratory Practice. However, the critical phases of the study and the final report have not been subject to QA inspection or audit.

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Jane A. Hughes, BSc, MPhil.

Signature ..... Date ..12th July 1996.....

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This report is approved by BIBRA Management.

Director:
S.E. Jaggers, BSc, PhD, FIBiol.

Signature ..... Date ..16th July 1996.....

Critical dates

Start date	:	10 July 1995
Receipt, check weighing and randomisation of animals		
- Males	:	27 June 1995
- Females	:	5 September 1995
Weighing males	:	10 July - 25 September 1995
Treatment of males	:	10 July - 15 September 1995
Animal mating	:	18 September - 28 September 1995
Necropsy of males	:	27 September - 28 September 1995
Dominant lethal necropsies	:	9 October - 18 October 1995
Weighing females	:	9 October - 18 October 1995
Report date	:	31 July 1996

Responsible staff

Supervisor	:	Diana Anderson, BSc, MSc, PhD, DipEd, FIBiol, FRCPath, FATS, FIFST.
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Archives

All raw data, documentation, any relevant specimens and a copy of the protocol and final report will be retained, for a period of 10 years, in the Department of Genetic and Reproductive Toxicology at BIBRA International under the appropriate reference. Specimens will be retained as long as they afford evaluation.

1. SUMMARY

The study reported was conducted as part of a programme at BIBRA International to determine the toxicity of butadiene to the male reproductive system in rodents, the present study being to examine dominant lethality in rats.

Male Sprague-Dawley rats were given sub-chronic doses of butadiene by inhalation exposure for approximately 6 hours per day, 5 days per week, for 10 weeks at 65, 400 and 1250 ppm or vehicle (air) control. A further group was untreated (room control). They were mated to untreated females 3 days later. The pregnant females were taken for dominant lethal examination, where possible, just before full-term.

Only one male, treated with 65 ppm butadiene died (cause unknown); no animals in any of the other treatment groups died. Butadiene treatment did not cause a persistent decrease in body weight in any treatment group.

Mating frequency and pregnancy rate were not significantly reduced as a result of treatment. The period to coition was also unaffected by treatment.

There was no significant reduction in comparison with the appropriate controls in the number of corpora lutea in any treatment group indicating that there had been no effect on pre-implantation loss. The number of implantation sites was significantly reduced in the 65 ppm group but this was not considered to represent a genetic effect since it was not accompanied by a significant increase in post-implantation losses and it was not dose-related. There was no significant reduction in any other group.

Dominant lethal data were analysed using three statistical tests: t-tests after double arc-sine transformation (Freeman and Tukey, 1950), t-tests after square root transformation and Fisher's exact test. Neither post-

implantation losses (early deaths, late deaths or late deaths including dead foetuses) nor abnormal foetuses were significantly increased in any treatment group using any statistical test.

In conclusion, all male rats survived sub-chronic butadiene treatment at 65, 400 or 1250 ppm. Reproductive behaviour was not affected and the success of pregnancy was also unaffected by treatment. [redacted]

2. OBJECTIVE AND INTRODUCTION

2.1. Objective

The objective of the study was to determine the mutagenic potential of butadiene by measuring genetic damage in the germ cells of male rats after sub-chronic treatment.

2.2. Dominant lethal mutation assay

The test system used involved treatment of the male animal and subsequent mating to untreated females.

A dominant lethal mutation is one which kills a heterozygous cell carrying the mutation as a single copy. The mutation arises in the male germ cell and may kill the zygotes formed (after fertilization with normal ova) at any time in their development. In practice, these events are limited to a portion of zygote development and can be recognised from mid-term pregnancy examination as dominant lethal effects. There may be a reduced number of implantations (pre-implantation losses) arising from either genetic effects, a reduction in the quality or quantity of sperm, or from lack of libido. Alternatively there may be a reduced proportion of viable implantations (post-implantation losses) arising from early or late deaths. An early death is one in which a deciduum developed only from the uterine tissue (no embryonic tissue) is present: the upper part of the deciduum necroses forming a brown mass (the deciduum capsularis) which is easily recognisable. A late death is one in which embryonic tissue is also present; a foetus of subnormal size is seen which is pale or white in colour, indicating haemopoietic failure (Bateman, 1977).

2.3. Foetal malformations

Non-lethal mutations may also arise in the germ-cells of treated males. These could lead to foetal malformations of the type seen in teratology studies. An additional category of post-implantation loss can also be defined: a dead foetus is the same size as a live foetus (or slightly smaller),

different in colour, may or may not be slightly shrivelled and does not respond to touch. Thus, a modification of the standard dominant lethal assay, in which pregnant females are examined at immediately prior to full-term instead of mid-term (Knudsen *et al.*, 1977), has been used to assess male-mediated foetal malformations.

2.4. Previous work

Morrissey *et al.* (1990) found a dominant lethal effect in Swiss CD-1 mice one week after exposure of males to 200, 1000 or 5000 ppm butadiene for 6 hours per day for 5 days. The effect persisted to week two in the 200 and 1000 ppm groups. Adler *et al.* (1994) found a dominant lethal effect in weeks 2 and 3 after male (102/F1 x C3H/E1)F_1 mice were exposed to 1300 ppm butadiene for 6 hours per day for 5 days.

Work in our laboratory (Anderson *et al.* 1993; BIBRA Report No.1060/5) confirmed this dominant lethal effect of butadiene in male CD-1 mice at concentrations of 12.5 and 1250 ppm for 6 hours per day, 5 days per week for 10 weeks. There was also an increase in the number of abnormal foetuses from the treated animals, although adult offspring were not found to be significantly different from controls and no karyotype abnormalities were observed. In a follow-up study (BIBRA Report No.1542/1) exposure to 65 and 130 ppm for 4 weeks only, induced a dominant-lethal effect but not foetal abnormalities and 12.5 ppm had no effect. A similar study (Anderson *et al.* 1993; BIBRA Report No.1060/4) where male mice were exposed for a single 6 h period to 1250 or 6250 ppm butadiene found no dominant lethal effect.

Other work in our laboratory has shown that acute treatment of male mice with cyclophosphamide (CP) caused dominant lethal mutations and foetal malformations (Jenkinson *et al.*, 1987) but that a much higher incidence could be achieved with a low-dose, sub-chronic treatment regimen in male rats (Jenkinson and Anderson, 1990). In the latter study, karyotype

abnormalities were found in the offspring of the treated males (Jenkinson and Anderson, 1990) and some of these persisted into adulthood (Francis *et al.*, 1990).

2.5. Basis of the study

Male rats were treated sub-chronically at a range of three concentrations of butadiene (6 hours exposure for 5 days per week for 10 weeks to 65, 400 and 1250 ppm) and a vehicle (air) control. An additional group was maintained as a room (untreated) control. The current UK maximum exposure limit (MEL) for butadiene is 10 ppm (HSE, 1996) giving a theoretical highest daily exposure of 75 ppm (10 ppm for 7.5 h per day). To achieve this level in animals exposed for 6 h, a concentration of 12.5 ppm would be required and the highest concentration was chosen to be equivalent to a one-hundred-fold increase as used in the first study with mice (Anderson *et al.*, 1993; BIBRA Report No.1060/5). The two lower concentrations were chosen to characterise the dose response pattern at lower levels, 65 ppm being based on the lowest dose used by Thornton-Manning *et al.* (1995), who showed that 62.5 ppm was the lowest level at which metabolites could be detected in rat tissue. Exposure time was 10 weeks in order to cover the complete spermatogenic cycle (Dym and Clermont, 1970).

Each of the surviving treated males were allowed to mate with two untreated females for 10 days, 3 days after the end of treatment. After the end of the mating period, the treated males were humanely killed and a gross necropsy performed.

The pregnant females were humanely killed immediately prior to full-term (day 20) and the uterine contents examined. For all females, the number of resulting implantations (living or dead) was recorded, foetal sex was determined and foetal abnormalities noted. The foetuses were humanely killed and selected individuals were photographed and stored in 70% alcohol.

3. TEST AND CONTROL ARTICLES

3.1. Test article

3.1.1. Definition

The test article, defined as butadiene, was supplied by ICI Chemicals & Polymers Limited, Wilton, UK.

3.1.2. Description

Information supplied by manufacturer:-

Alternative names	:	Biethylene, Bivinyl, Butadieen (Dutch), Buta-1,3-dieen (Dutch), Butadien (Polish), Buta-1,3-dien (German), Butadiene, Buta-1,3-diene, Alpha-gamma butadiene, Butadiene (OSHA), Divinyl, Erythrene, NCI-C50602, Pyrrolylene, Vinyl-ethylene, butadiene, 1,3-butadiene
Molecular formula	:	$CH_2=CHCH=CH_2$
Formula weight	:	54
Boiling point	:	-4.4°C
Purity	:	≥99.5%
Appearance	:	Colourless gas
Hazards	:	Cancer suspect agent; toxic; harmful by inhalation; low acute toxicity; vapour may be respiratory irritant at high exposures; liquid splashes or spray on eyes or skin may cause freeze burns.

3.1.3. Supply

Cylinders of butadiene were obtained at the start of the study and weekly thereafter, having been transported in dry ice. They were stored at -20°C when not in use.

A certificate of analysis was provided by the supplier for each batch of cylinders, defining purity and the concentrations of contaminants.

3.2. Control articles

3.2.1. Negative control

The negative control was the vehicle used for the test article, i.e. normal atmosphere (air). A room (untreated) control group was also included.

3.2.2. Positive control

No positive control was used since the previous mouse study (Anderson *et al.* 1993; BIBRA Report No.1060/5) had shown a positive response with butadiene. It has been shown in this laboratory using cyclophosphamide (Jenkinson & Anderson, 1990) that a positive response can be induced in rats of the Sprague-Dawley strain.

3.3. Safety procedures

3.3.1. Handling

Butadiene exposure of animals was performed in a closed inhalation exposure chamber located in a room with extract units of high face velocity ($0.6\ ms^{-1}$).

3.3.2. Disposal

Waste gases from the inhalation system were trapped on carbon filters and the air vented to the atmosphere via the fume cupboard's exhaust stack. Used filters were incinerated.

Empty cylinders were returned to the supplier.

3.3.3. Monitoring

The atmosphere in the room was continuously sampled and analysed for butadiene content by infra-red spectrophotometry (Miran).

4. ANIMALS

4.1. Description

Rats of the Sprague-Dawley strain were obtained from Harlan Olac, Bicester, UK at 8 weeks of age. This strain was chosen since it was known to have a low background incidence of dominant lethality and to be sensitive to known mutagens (Jenkinson and Anderson, 1990).

150 Male rats of unproven fertility arrived at the start of the study and 300 nulliparous female rats arrived ten weeks later. These were designated the F_0 generation and were mated as part of the experimental design to produce the F_1 generation.

4.2. Maintenance

Males and females were housed individually up until the mating week. During mating, one male was housed with up to two females. After mating, males and females were housed individually.

Animals were housed in polypropylene cages with stainless steel tops and grid floors. All cages were suspended in racks over paper to collect excreta which were removed twice weekly.

The cages were kept in ventilated rooms (15 air changes per hour, with no recirculation, using high efficiency filters) at 19-23°C, 45-65% humidity with artificial light for 12 h per day (06.00-18.00 GMT).

Animals had unlimited access to a nutritionally adequate, pelleted diet (R&M No.3 for females; R&M No.1 for males; both from Special Diet Services, Waltham, UK) and tap water except during the 6 h exposure periods when neither food nor water was available. Manufacturer's certificates of analysis of the diet and the supplying authority's analysis of the water are held on a central file at BIBRA.

4.3. Identification

Animals were given ear punch codes as follows:-

Male : 1-150

Female : 151-450

Cages were labelled with the following information:-

Date of arrival

Date of start of treatment (males)

Date of start of mating period (females)

Strain of rat

Sex

Treatment group (males and females (only males treated))

Route of treatment (males)

Cage number

Individual number

BIBRA Project licence number : 90/00378

BIBRA Project number : 1542/2

UK Home Office Licensee : Jane Hughes

Personal licence number : 90/01278

Diet

Cage labels were colour-coded for treatment group separately for males and females.

4.4. Health

All animals were in good health on arrival.

4.5. Weighing and observations

Each animal was weighed as part of a health check on arrival. Ear numbers and groups were allocated randomly to the animals.

All animals were examined twice daily on exposure days (a.m. and p.m.) (approximately 8.30 a.m. on loading and 3.15 p.m. on unloading) and constantly during treatment (5 days per week) and any deviation from the normal reported to the Study Director.

Males were weighed on the first day of exposure and weekly thereafter during the treatment and mating period. Females were weighed at necropsy.

5. PROCEDURES

5.1. Treatment of males

5.1.1. Treatment groups

Males were allocated to treatment groups as follows:-

Treatment group (males)	Cage label colour code	Concentration of butadiene	Number of males	Identification No.
1	White	0 ppm Vehicle control	25	1-25
2	Yellow	65 ppm	25	26-50
3	Green	400 ppm	25	51-75
4	Orange	1250 ppm	25	76-100
5	White	0 ppm Room control	50[a]	101-150

[a] The group size for the room control was twice that of the other groups.

5.1.2. Concentration of atmospheres and administration

Samples of butadiene were obtained weekly in cylinders each with a certificate of analysis from the supplier. Using rotameters, butadiene was metered at a known and constant flow rate into a diluting air stream of clean, dry air to achieve the desired concentration. The atmospheres were analysed by infra-red spectrophotometry (Mirans and Fourier Transform Infra Red (FTIR)) at an appropriate wavelength and path length and monitored continuously during animal exposure. The atmosphere in the inhalation room was similarly monitored. The concentration of butadiene in each chamber was considered acceptable if kept within 15% of the required concentration, according to the technical specification of the Mirans. Contamination of the butadiene with the dimerized form (4-Vinyl-1-Cyclohexene) was monitored by gas chromatography and recorded after the end of use of each cylinder to confirm that the dimer level had not risen above the 1000 ppm limit defined in the protocol, as in the previous mouse study (Anderson *et al.* 1993; BIBRA Report No.1060/5). As a precaution against such a rise, the butadiene cylinders were transported in dry ice and stored at -20°C prior to use. The cylinders were

stored at 4°C between inhalation exposure periods and used at room temperature.

Exposed animals and controls were placed in individual segments of stainless steel grid cages, which were placed in sealed glass chambers attached to the appropriate gas stream. Room control animals were placed in small polypropylene cages with stainless steel tops and grid floors, suspended in racks. The racks were placed in the inhalation room during the exposure period.

5.1.3. **Temperature and relative humidity**

Temperature and relative humidity were measured using a thermo-hygrometer (ATP Instrumentation Ltd, Ashby-de-la-Zouch, UK), up to study day 7; after this a separate solid state hygrometer (Pastorelli and Rapkin) and thermometer were used. Measurements were made in one of the four inhalation chambers, selected at random, on each study day. Temperature and relative humidity were recorded every 15 minutes during the exposure period, as far as possible.

5.1.4. **Time of exposure**

Males were first exposed 13 days after arrival.

5.2. **Mating of animals**

5.2.1. **Time of mating**

The mating period began 3 days after the end of exposure of the males and 13 days after the untreated females arrived.

5.2.2. Animals

Each surviving male was housed with two untreated virgin females for up to 10 days.

Females were mated to treated males as follows:

Treatment group (males)	Cage label colour code[a]	Concentration of butadiene	Number of females[b]
1	Pink stripe	0 ppm Vehicle control	50
2	Yellow stripe	65 ppm	48[c]
3	Green stripe	400 ppm	50
4	Blue stripe	1250 ppm	50
5	Blue stripe and pink stripe	0 ppm Room control	100

[a] Stripe(s) on white label; [b] two females were allocated to each male in random order using computer-generated random numbers, [c]one male died during the treatment period.

5.2.3. Vaginal plugs

Females were checked daily for the presence of a vaginal plug and vaginal smears taken. Those in which a plug and/or a sperm positive vaginal smear was found were removed and re-housed in separate cages, this being designated day 0 of pregnancy. Those without a plug or sperm in a vaginal smear remained in the cage with the male and a record was made of the stage of the oestrus cycle, determined from the smear according to cellular appearance. After 10 days, males and remaining females were separated and re-housed in their original separate cages.

5.2.4. Weighing females

All females were weighed at necropsy.

5.3. Necropsies

5.3.1. Males

The males were humanely killed after mating and a gross necropsy examination carried out.

5.3.2. Females

Females were taken for necropsy on day 20 of pregnancy. Animals for which there was no indication of pregnancy were taken for necropsy 20 days after the end of the mating period.

For necropsy examination for the dominant lethal assay (Section 5.4), females were humanely killed and their uterine contents examined. The uteri of those with no apparent implantation sites were stained using ammonium sulphide to enable the number of implantation sites to be counted (e.g. Chapin *et al.*, 1985).

5.4. Dominant lethal assay

5.4.1. Necropsy of pregnant females

The females were humanely killed by gassing with CO_2 followed by cervical dislocation; their abdomens were opened. They were checked for gross malformations, the number of corpora lutea were counted in each ovary and the uterine contents were examined.

5.4.2. Uterine examination

The intact uterus was examined for the numbers of live implantations, early deaths, late deaths and dead foetuses. The uterus and embryonic sac was opened for each live implantation and the foetus examined for malformations. The following definitions were used:

Implantation	Description
Live foetus	Pink-coloured, relatively large size
Early death	Small, discrete, round black/dark brown area representing a necrotic mass of a fairly well-formed placenta with no foetal tissue
Late death	Pale/whitish-coloured foetus, generally smaller in size than the live implants
Dead foetus[a]	As large (or slightly smaller) and developed as live foetus but with no circulation and not responsive to touch
Malformed foetus[a]	Live foetus with a recognisable gross malformation. Runts were included as malformed foetuses. A runt was defined as a foetus whose body weight was 75% or less of the mean body weight of the remainder of the litter[b]

[a] As detailed in teratology studies, according to MARTA glossary of Foetal Alterations for Studies of Development and Reproductive Toxicology (DART) (October, 1993).

[b] Kirk and Lyon, 1984.

5.4.3. Selection of foetuses pending further studies

Each malformed foetus and a normal litter-mate was stored pending further studies. In addition, for each malformed foetus, one foetus from a control mating necropsied on the same day was also kept.

5.4.4. Processing foetuses

Each live foetus was placed on ice immediately after removal from the embryonic sac. The sex was determined and the foetus dried and weighed. Selected foetuses were photographed and stored pending further studies; the remainder were humanely killed and discarded. Each selected foetus was placed in 70% alcohol.

5.5. Evaluation and statistics

Butadiene-exposed groups were compared with the vehicle control group. The vehicle group was compared with the room control group.

5.5.1. Body weights

Mean body weights of males on specific days of the study were compared using a two-sided least significant difference test (Snedecor & Cochran, 1968a) for treated groups *vs.* vehicle control group and using a two-sided pooled two-sample t-test (Snedecor and Cochran, 1968b) for room *vs.* vehicle control group. Data were untransformed.

5.5.2. Temperature and relative humidity

An analysis of variance was performed to test the difference between chambers.

5.5.3. Mating and fertility data

Two statistics were used to measure fertility: a) the proportion of males or females mated (either plug found in female, sperm positive vaginal smear, plug under cage housing one female only (or assigned retrospectively if two females present) or female known to have been pregnant) of those paired; b) the proportion of males or females with a pregnancy of those paired. These were tested for any significant reduction by comparison with the appropriate control group using Fisher's exact test (Siegel, 1956).

Pregnancy rate was measured as the proportion of females pregnant of those mated and tested for any significant reduction by comparison with the appropriate control group using Fisher's exact test. Period to coition (untransformed) was tested with an analysis of variance (Snedecor & Cochran, 1968c). Tests for reduction by comparison with appropriate control values (untransformed data) were made using a two-sided two-sample t-test.

5.5.4. Dominant lethal data

The number of corpora lutea and the number of implantation sites were expressed per pregnant female and tested with an analysis of variance.

Tests for reduction by comparison with the appropriate control group were made using the one-sided two-sample t-test.

The number of post-implantation losses or foetal abnormalities was expressed per implantation per pregnancy. The statistic used was a double arc sine transformation (Freeman & Tukey, 1950) of this value:-

$$\sin^{-1}\sqrt{\frac{E}{T+1}} + \sin^{-1}\sqrt{\frac{E+1}{T+1}}$$

where E = number of post-implantation losses and T = number of implantations. This was tested with an analysis of variance and for any significant increase by comparison with the appropriate control group using a two-sample t-test. The results of these t-tests are presented in the table (Table 5). The data for abnormal foetuses were also subject to this transformation where T = number of live implantations, and the same statistical analyses performed.

In addition, analysis of variance and two-sample t-tests for post-implantation losses and abnormal foetuses were performed on the data using a square root transformation, according to the formula $\sqrt{E + 1}$.

A further statistic was obtained: the proportion of pregnant females in each treatment group with at least one early death, late death, late death or dead foetus, or abnormal foetus. This was tested for any significant increase by comparison with the appropriate control group using Fisher's exact test.

6. RESULTS

6.1. Treated males

6.1.1. Survival

The numbers of males which survived the inhalation treatments were as follows:-

Treatment group	Number of males				
	Treated	Died during treatment	Survived	Used for mating	Died during mating period
Vehicle control	25	0	25 (100%)	25	0
65 ppm butadiene	25	1*	24 (96%)	24	0
400 ppm butadiene	25	0	25 (100%)	25	0
1250 ppm butadiene	25	0	25 (100%)	25	0
Room control	50	0	50 (100%)	50	0

* Cause of death could not be established at necropsy

6.1.2. Body weights

Body weights of males on the day of treatment and up until the end of the mating period are shown in Table 1 and Appendix 1. Mean body weights were not significantly different from the vehicle control group in any group on any of the twelve occasions on which the animals were weighed.

6.1.3. Butadiene

The supplier's certificates of analysis (Appendix 2) indicated that the purity of the butadiene was ≥99.5% and that no contaminant was present at a concentration outside the normal product specification except for cylinders 17 and 18. For these two cylinders (in use on study days 32, 35-37) methyl acetylene was present at 12 ppm and butenes at 0.41%; all other contaminants were within the normal product specification. The concentrations of butadiene recorded during the ten week exposure period

are shown in Table 2 and Appendix 3. The daily exposure period (nominally 6 h) approximately comprised a 13 minute build up period (range 3-38 min) from 0 ppm to the required concentration, a 5¾ h period (range 5 h 10 min-6 h 20 min) of constant concentration and a 16 minute period (range 4-45 min) in which the concentration returned to 0 ppm prior to removing the animals from the cages. Occasional fluctuations in butadiene concentrations outside the designated 15% limits were due to e.g. liquid butadiene outflow from an overfull cylinder or interruption of gas flow due to misconnected tubing or an empty cylinder. Such variations outside the designated limits were corrected as quickly as possible to minimize their effect. Any shut down period e.g. due to a change of cylinder, was compensated by a suitable extension of the exposure period.

A sample of butadiene was taken from each cylinder used at the end of each exposure period. Appendix 4 shows its dimer content which varied from 10 to 678 ppm but was only above 500 ppm for study days 39, 42, 43, 58 and 59.

6.1.4. Temperature and relative humidity

Temperature and relative humidity data are shown in Table 3 and Appendix 5. After one week of the butadiene exposure period it became apparent that the thermohygrometer had been adversely affected by the butadiene. The accuracy of the relative humidity measurements using this instrument is therefore in doubt and a separate hygrometer was used from study day 1. Temperature readings were not affected and a separate thermometer was not introduced until study day 8. (Temperature was recorded on both instruments from study days 1-8.)

There appeared to be no concentration-related change in temperature or relative humidity, although there was considerable variation (23.4-30.0°C and 42-78% respectively) between study days and chambers.

An analysis of variance performed to test the difference between chambers was not statistically significant for temperature. Measurements were made on separate days, hence variability between days was included as a factor in the model but this resulted in unsatisfactory assumptions for normality and for homogeneity between the variances, so the p-value of 0.861 obtained from the analysis of variance is rather inexact. Usually when the above assumptions do not hold a transformation is carried out or a non-parametric analysis is performed, but in this case there were no suitable transformation and there is no suitable non-parametric method which includes the variability between days. There was little difference in the mean temperature for chambers 1 to 4 which were 27.5, 27.7, 27.5 and 27.5 respectively, and the p-value is so high that the statistical non-significance of the result can be relied upon.

An analysis of variance performed to test the difference between chambers was highly statistically significant ($p \leq 0.001$) for relative humidity. For this endpoint, variances were equal but again the distribution of data was not normal and no suitable transformation could be found. There were large differences in the means for relative humidity in chambers 1 to 4 which were 59.6, 49.9, 48.8 and 57.9 respectively. In this case, the p-value was so low that the statistically significance of the results can be relied on.

6.2. Mating success and fertility

Mating and fertility data are presented in Table 4 and Appendix 6.

6.2.1. Mating

Males were recorded as successfully mated if at least one of the two females was found to have a vaginal plug, a sperm positive vaginal smear or to have been pregnant. Females were recorded as successfully mated if any of the following were found: vaginal plug in the female, sperm positive vaginal smear, plug under cage (plug assigned to female retrospectively if two females present) or pregnancy. In a few instances, no pregnancy

resulted, despite a plug or sperm having been found, presumably due to a lack of viable sperm.

Females were recorded as pregnant if a litter was seen at dominant lethal examination or if at least one implantation site was counted either at dominant lethal examination or after ammonium sulphide staining.

Neither the proportion of males mated (of those paired) nor the proportion of females mated (of those paired) was significantly decreased at 65, 400 or 1250 ppm butadiene by comparison with the vehicle control group, or in the vehicle control group by comparison with the room control group. Pregnancy rate (proportion of females pregnant of those mated) was not significantly decreased at any exposure concentration.

The period to coition was calculated for those animals for which either a vaginal plug or a sperm positive vaginal smear had been found. Analysis of variance was not significant and there were no significant changes at any concentration by comparison with the appropriate control group using a two-sided two-sample t-test.

6.2.2. Fertility

Two measures of fertility were made and comparisons between exposure groups and the appropriate control were made by Fisher's exact test. Neither the proportion of males with at least one pregnant female (of those paired) nor the proportion of pregnant females (of those paired) was significantly reduced at 65, 400 or 1250 ppm butadiene by comparison with the vehicle control group or in the vehicle control group by comparison with the room control group.

6.3. Dominant lethal assay

Dominant lethal data are presented in Table 5 and Appendix 7.

6.3.1. Corpora lutea

Analysis of variance for the mean number of corpora lutea per pregnant female was not statistically significant. There was no statistically significant decrease at 65, 400 or 1250 ppm butadiene by comparison with the vehicle control group, nor in the vehicle control group by comparison with the room control group using a two-sample t-test.

6.3.2. Implantations

Analysis of variance for the mean number of implantations per pregnant female was not statistically significant. There was a statistically significant decrease ($p \leq 0.05$) in the 65 ppm butadiene group in comparison with the vehicle control group using a two-sample t-test but this was not confirmed by a least significant difference test. There was no statistically significant decrease at 400 or 1250 ppm butadiene by comparison with the vehicle control group and the vehicle control group showed no significance decrease by comparison with the room control group using a two-sample t-test.

6.3.3. Early deaths

6.3.3.1. Early deaths per implantation per pregnancy

Analysis of variance for the mean number of early deaths per implantation per pregnancy (arc-sine transformation) was not statistically significant. There was no statistically significant increase at 65, 400 or 1250 ppm butadiene by comparison with the vehicle control group nor in the vehicle control group by comparison with the room control group using a two-sample t-test.

6.3.3.2. Early deaths per pregnancy

Analysis of variance and two-sample t-tests were used to compare the mean number of early deaths per pregnancy (square root transformation): statistical significance was not achieved in any comparison.

6.3.3.3. Proportion of females with an early death

The proportion of pregnant females with at least one early death was not significantly increased in the 65, 400 or 1250 ppm butadiene-exposed groups by comparison with the vehicle control group or in the vehicle control group by comparison with the room control group, using Fisher's exact test.

6.3.4. Late deaths

6.3.4.1. Late deaths per implantation per pregnancy

Analysis of variance for the mean number of late deaths per implantation per pregnancy (arc-sine transformation) was not statistically significant. There was no statistically significant increase at 65, 400 or 1250 ppm butadiene by comparison with the vehicle control group nor in the vehicle control group by comparison with the room control group using a two-sample t-test.

6.3.4.2. Late deaths per pregnancy

Analysis of variance and two-sample t-tests were used to compare the mean number of late deaths per pregnancy (square root transformation): statistical significance was not achieved in any comparison.

6.3.4.3. Proportion of females with an late death

The proportion of pregnant females with at least one late death was not significantly increased in the 65, 400 or 1250 ppm butadiene-exposed groups by comparison with the vehicle control group or in the vehicle control group by comparison with the room control group, using Fisher's exact test.

6.3.5. Late deaths including dead foetuses

6.3.5.1. Late deaths including dead foetuses per implantation per pregnancy

Since the dominant lethal assay is normally performed at mid-term, dead foetuses are not seen. However, since the assessment has been made in this study immediately prior to full-term, this additional categorisation is

possible and data are also presented as a total for late deaths including dead foetuses.

Analysis of variance for the mean number of late deaths including dead foetuses per implantation per pregnancy (arc-sine transformation) was not statistically significant. There was no statistically significant increase at any exposure concentration by comparison with the vehicle control group nor in the vehicle control group by comparison with the room control group using a two-sample t-test.

6.3.5.2. Late deaths including dead foetuses per pregnancy

Analysis of variance and two-sample t-tests were used to compare the mean number of late deaths or dead foetus per pregnancy (square root transformation): statistical significance was not achieved in any comparison.

6.3.5.3. Proportion of females with a late death or dead foetus

The proportion of pregnant females with at least one late death or dead foetuses was not significantly increased in the 65, 400 or 1250 ppm butadiene-exposed groups by comparison with the vehicle control group or in the vehicle control group by comparison with the room control group, using Fisher's exact test.

6.3.6. Abnormal foetuses

In the population of foetuses from non exposed males in the vehicle control group, there were five runts in five litters. There were four runts and one foetus with gastroschisis in five litters from males in each of the groups treated with 65 ppm and 400 ppm butadiene. From males treated with 1250 ppm butadiene there were two runts in two litters and from males maintained as room controls there were nine runts and one foetus with scoliosis in ten litters.

6.3.6.1. Abnormal foetuses per implantation per pregnancy

Analysis of variance for the mean number of abnormal foetuses per implantation per pregnancy (arc-sine transformation) was not statistically significant. There was no statistically significant increase at any exposure concentration in comparison with the vehicle control group nor in the vehicle control group by comparison with the room control group using a two-sample t-test.

6.3.6.2. Abnormal foetuses per live implantation per pregnancy

Analysis of variance and two-sample t-tests were used to compare the mean number of abnormal foetuses per live implantation per pregnancy (arc-sine transformation): statistical significance was not achieved in any comparison.

6.3.6.3. Abnormal foetuses per pregnancy

Analysis of variance and two-sample t-tests were used to compare the mean number of abnormal foetuses (square root transformation): statistical significance was not achieved in any comparison.

6.3.6.4. Proportion of females with an abnormal foetus

There were no females with more than one abnormal foetus so no analysis was performed on the proportion of females with at least one abnormal foetus.

7. DISCUSSION

Butadiene concentrations occasionally fluctuated outside the defined 15% limits but these deviations were not considered long enough to have affected the outcome of the study. The butadiene supplied was at least 99.5% pure throughout the study and on all but four study days it was more than 99.7% pure. During four days of exposure, two contaminants were present at slightly higher than the normal product specification: methyl acetylene 12 ppm (limit = 10 ppm) and butenes 0.41% (limit = 0.30%). This deviation from the normal specification was not considered to have affected the outcome of the study. The dimer content of the butadiene measured at the end of use of each cylinder varied from 10 to 678 ppm, which was within the designated limit (1000 ppm) for contamination. Temperature did not vary between the inhalation exposure chambers but relative humidity varied due to differences in the dryness of diluting air-streams attached to each one.

Only one male rat (in the 65 ppm butadiene exposed group) died during sub-chronic dosing by inhalation exposure with 0, 65, 400 or 1250 ppm butadiene. None of 50 room control animals died. Mean body weights of the vehicle control group, the treated groups and the room control group were not significantly different throughout the treatment and mating periods.

Neither mating frequency nor pregnancy rate was significantly decreased as a result of treatment. For those animals for which a vaginal plug or a sperm positive vaginal smear was found, the mean period to coition was not significantly affected by treatment. It would therefore appear that the reproductive systems of the treated males have not been affected by sub-chronic butadiene exposure nor by the exposure system itself and that they were able to reproduce normally.

Investigation of an effect of sub-chronic butadiene treatment on male germ cells was carried out by means of a modified dominant lethal assay. There was no significant difference in the number of corpora lutea between the treatment groups indicating that there had been no effect on pre-implantation loss. The statistically significant decrease in the number of implantation sites after paternal exposure to 65 ppm butadiene was not considered to represent a genetic effect since it was not accompanied by a significant increase in post-implantation losses and was not found in other treatment groups. All the other parameters measured in the dominant lethal assay and in the foetuses (early deaths, late deaths, including or excluding dead foetuses and foetal abnormalities) remained unchanged in all treatment groups. Thus, there has not been a genetic effect of sub-chronic butadiene treatment at 65, 400 or 1250 ppm on the germ cells of male rats as measured by the dominant lethal assay.

Statistical analysis was performed on the dominant lethal data (i.e. post-implantation losses and foetal abnormalities) by three different methods: t-tests using a double arc-sine transformation (Freeman and Tukey, 1950), t-test using a square root transformation and Fisher's exact tests. None of the differences between the exposed and control groups were statistically significant by any of these methods.

These results show that mutation of the male germ cells cannot be induced by butadiene given sub-chronically by inhalation exposure under these conditions to rats of the Sprague-Dawley strain. They are in contrast to those obtained for male mice (Anderson *et al.* 1993) (BIBRA Report No. 1060/5). This species difference may be explained by differences in butadiene metabolism as suggested by e.g. Henderson *et al.*, 1993 and Bolt *et al.*, 1993.

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Table 1. Summary of body weights of male rats treated sub-chronically with butadiene

Treatment group	Number of exposed males	Body weight (g) (mean and standard deviation) on study days: 0	7	14	21	28	35	42	49	56	63	70	77
Vehicle control[b]	25	270.5 ±16.90	296.3 19.49	314.4 ±23.32	334.0 ±26.21	347.0 ±29.7	365.0 ±31.77	378.8 ±33.70	388.5 ±34.36	399.2 ±34.62	411.1 ±37.22	417.6 ±39.38	413.9 ±38.19
65 ppm butadiene[b]	25	272.0 ±14.04	293.5[ns] ±18.36	310.9[ns] ±20.21	331.9[ns] ±21.65	345.2[ns] ±23.05	360.8[*,ns] ±24.12	373.8[*,ns] ±29.41	385.0[*,ns] ±27.70	395.0[*,ns] ±28.16	403.9[*,ns] ±25.99	412.3[*,ns] ±28.23	409.4[*,ns] ±27.11
400 ppm butadiene[b]	25	271.7 ±10.78	294.9[ns] ±15.38	313.0[ns] ±19.38	334.4[ns] ±21.15	349.4[ns] ±23.05	363.6[ns] ±24.38	376.9[ns] ±25.53	390.9[ns] ±26.76	402.9[ns] ±27.85	412.0[ns] ±28.64	419.0[ns] ±28.97	416.1[ns] ±30.20
1250 ppm butadiene[b]	25	271.2 ±14.07	291.8[ns] ±19.45	307.7[ns] ±18.63	326.3[ns] ±22.23	341.8[ns] ±22.22	354.5[ns] ±23.05	367.1[ns] ±25.44	377.5[ns] ±27.29	387.6[ns] ±27.75	398.4[ns] ±28.05	405.4[ns] ±29.00	403.1[ns] ±29.69
Room control[c]	50	266.4 ±13.87	292.8[ns] ±17.75	314.2[ns] ±20.81	335.5[ns] ±23.75	352.4[ns] ±26.07	367.6[ns] ±28.72	383.0[ns] ±30.72	393.8[ns] ±32.07	404.3[ns] ±31.96	413.3[ns] ±33.41	417.6[ns] ±35.91	412.6[ns] ±36.08

Data for body weights for individual male rats are shown in Appendix 1.

[a] Number of exposed males = 24 due to death of animal number 37 on day 30 (cause unknown).

[b] Levels of significance resulting from comparing vehicle control group against other groups using two-sided least significant difference test.
[ns] = not significantly different from the vehicle control group, $p > 0.05$.

[c] Levels of significance resulting from comparing vehicle control group against room control group using two-sided pooled two sample t-test.
[ns] = not significantly different from vehicle control group, $p > 0.05$.

Table 2. Summary of concentration of butadiene in atmospheres generated for sub-chronic exposure of male rats

Treatment week[a]	Concentration of butadiene (ppm)	Length of time to reach required concentration (min) ($\bar{x}$ ± SD)	Length of time at required concentration ($\bar{x}$ ± SD)	Range of concentration of butadiene during main exposure period (ppm)	Length of time to return to 0 ppm (min) ($\bar{x}$ ± SD)
1	65	10 ± 6	5 h 49 min ± 6 min	9-79	8 ± 3
	400	15 ± 11	5 h 44 min ± 11 min	40-580	14 ± 2
	1250	8 ± 3	5 h 48 min ± 4 min	112-2174	[b]
2	65	10 ± 3	5 h 50 min ± 3 min	14-81	10 ± 1
	400	10 ± 4	5 h 49 min ± 4 min	70-540	16 ± 1
	1250	11 ± 11	5 h 48 min ± 11 min	282-1612	[b]
3	65	20 ± 8	5 h 40 min ± 8 min	42-85	11 ± 1
	400	17 ± 9	5 h 43 min ± 9 min	290-520	16 ± 2
	1250	15 ± 11[c]	5 h 39 min ± 13 min	979-1627	[b]
4	65	18 ± 6	5 h 41 min ± 6 min	29-83	9 ± 1
	400	18 ± 6	5 h 42 min ± 6 min	160-520	15 ± 2
	1250	16 ± 4	5 h 45 min ± 4 min	530-1412	[b]
5	65	18 ± 13	5 h 48 min ± 20 min	9-87	10 ± 1
	400	18 ± 13	5 h 49 min ± 20 min	30-590	13 ± 2
	1250	8 ± 3[d]	5 h 44 min ± 27 min	70-1594	32 ± 8[d]
6	65	12 ± 2	5 h 43 min ± 17 min	9-89	10 ± 1
	400	9 ± 4	5 h 46 min ± 17 min	40-580	13 ± 2
	1250	14 ± 7	5 h 41 min ± 18 min	255-1481	32 ± 8

continued....

Table 2. continued.

Treatment week[a]	Concentration of butadiene (ppm)	Length of time to reach required concentration (min) ($\bar{x}$ ± SD)	Length of time at required concentration ($\bar{x}$ ± SD)	Range of concentration of butadiene during main exposure period (ppm)	Length of time to return to 0 ppm (min) ($\bar{x}$ ± SD)
7	65	11 ± 3	5 h 49 min ± 3 min	49-78	10 ± 1
	400	10 ± 3	5 h 49 min ± 3 min	270-470	14 ± 1
	1250	19 ± 4	5 h 40 min ± 7 min	877-1435	30 ± 5
8	65	13 ± 7	5 h 47 min ± 7 min	55-78	8 ± 2
	400	8 ± 2	5 h 51 min ± 2 min	350-470	14 ± 2
	1250	13 ± 3	5 h 46 min ± 4 min	1068-1488	36 ± 4
9	65	9 ± 7	5 h 50 min ± 7 min	46-76	11 ± 1
	400	8 ± 4	5 h 52 min ± 4 min	280-1506	17 ± 2
	1250	17 ± 4	5 h 43 min ± 4 min	60-1659	39 ± 2
10	65	8 ± 3	5 h 52 min ± 3 min	19-126	10 ± 1
	400	8 ± 3	5 h 52 min ± 3 min	120-910	18 ± 3
	1250	20 ± 10	5 h 46 min ± 7 min	618-1700	34 ± 2

Individual daily butadiene exposure data are shown in Appendix 3.

[a] Monday to Friday; n=5.
[b] Missing data due to 6 h time schedule for Fourier Transform Infra Red (FTIR) used to measure butadiene concentration in chamber 4 (1250 ppm).
[c] Mean value from 4 days exposure data only (see Appendix 3).
[d] Mean value from 3 days exposure data only (see Appendix 3).

Table 3. Summary of temperature and relative humidity readings for inhalation chambers during sub-chronic exposure of male rats to butadiene

Study day	Chamber number[a]	Concentration of butadiene (ppm)	Temperature (°C)[b]				Relative humidity (%)[b]			
			Start	End	Max.	Min.	Start	End	Max.	Min.
0	1	0	23.4[c]	24.1[c]	24.1[c]	23.4[c]	64[c]	67[c]	69[c]	53[c]
1	4	1250	25.8[c]	25.9[c]	26.7[c]	25.8[c]	49[c]	30[c]/58[d]	49[c]	29[c]/56[d]
2	1	0	26.0[c]	27.5[c]	27.5[c]	26.3[c]	48[c]/68	44[c]/60	48[c]/68	38[c]/60
3	1	0	26.9[c]	27.1[c]	27.4[c]	26.8[c]	72[c]/59	52[c]/65	72[c]/59	41[c]/62
4	3	400	25.6[c]	27.1[c]	27.1[c]	25.6[c]	51[c]/64	32[c]/50	51[c]/64	21[c]/48
7	2	65	26.3[c]	27.5[c]	27.5[c]	26.3[c]	50[c]/56	31[c]/44	50[c]/56	31[c]/44
8	2	65	26.6	27.2	27.2	26.6	54	48	54	47
9	4	1250	25.8	26.2	26.2	25.8	70	52	70	49
10	2	65	27.6	28.8	28.8	27.6	54	53	54	48
11	3	400	26.0	28.0	28.2	26.0	60	47	60	46
14	2	65	27.4	28.6	28.6	27.4	61	50	61	48
15	2	65	26.4	29.2	29.4	26.4	62	52	62	50
16	1	0	26.8	29.0	29.0	26.8	72	66	72	56
17	2	65	26.4	28.0	28.0	26.4	60	52	60	47
18	4	1250	24.6	26.8	27.0	24.6	66	57	66	54
21	4	1250	24.0	29.6	29.6	24.0	70	54	70	53
22	1	0	28.0	30.0	30.0	27.8	60	64	64	55
23	3	400	27.0	29.8	29.8	27.0	66	54	66	49
24	1	0	26.2	30.0	30.0	26.2	60	66	66	54
25	1	0	26.4	29.3	29.3	26.4	68	58	68	56
28	4	1250	27.0	29.0	29.0	27.0	65	50	65	49
29	2	65	27.4	28.8	28.8	27.4	64	49	64	44
30	3	400	27.0	28.8	28.8	26.4	57	46	57	42
31	2	65	25.8	29.0	29.0	25.8	62	51	62	46
32	4	1250	27.0	29.0	29.0	27.0	58	58	58	52
35	4	1250	27.4	28.4	29.0	27.4	62	60	62	55
36	1	0	24.4	28.6	28.6	24.4	66	56	66	52
37	4	1250	24.0	28.0	28.2	24.0	68	58	68	54

continued....

Table 3. continued.

Study day	Chamber number[a]	Concentration of butadiene (ppm)	Temperature (°C)[b]				Relative humidity (%)[b]			
			Start	End	Max.	Min.	Start	End	Max.	Min.
38	2	65	27.0	28.0	28.0	27.0	56	54	56	46
39	4	1250	24.8	27.2	27.2	24.8	64	58	64	56
42	2	65	26.2	28.4	28.4	26.2	64	50	64	44
43	3	400	25.2	27.2	27.2	25.2	63	46	63	44
44	1	0	27.4	28.2	28.4	27.2	78	68	78	55
45	4	1250	26.4	27.6	28.0	26.4	64	61	64	58
46	4	1250	27.2	27.4	27.8	27.0	62	65	65	58
49	2	65	27.2	28.2	28.2	27.2	61	52	61	47
50	3	400	27.2	28.4	28.4	27.2	56	54	56	46
51	3	400	27.4	28.4	28.4	27.4	56	52	56	45
52	4	1250	25.8	28.0	28.6	25.8	65	61	65	58
53	4	1250	26.6	28.0	28.6	26.6	66	62	66	56
56	2	65	26.6	27.8	28.0	26.6	57	56	57	48
57	4	1250	24.4	27.4	28.0	24.4	60	60	64	59
58	3	400	26.0	28.2	28.4	26.0	60	48	60	44
59	1	0	25.0	27.0	27.2	25.0	68	65	68	56
60	1	0	26.0	27.6	27.6	26.0	64	64	64	54
63	3	400	25.4	26.8	26.8	25.4	58	46	58	46
64	3	400	24.8	27.7	27.7	24.8	62	49	62	46
65	3	400	27.2	28.0	28.3	27.0	56	46	56	43
66	4	1250	27.0	27.6	27.6	27.0	64	59	64	57
67	3	400	26.0	27.4	27.4	26.0	58	49	58	46

Individual temperature and relative humidity readings are shown in Appendix 5.

[a] Temperature and relative humidity were measured in one chamber, selected at random, on each study day.

[b] Temperature and relative humidity were recorded every 15 minutes, where possible, during the exposure periods; separate thermometer and solid state hygrometer used except for readings marked [c].

[c] Thermohygrometer used for temperature and relative humidity recordings: probably inaccurate for relative humidity (see text Section 6.1.4. for explanation).

[d] First 5 readings missing.

Table 4. Summary of mating and fertility data for male rats treated sub-chronically with butadiene

Treatment group	Vehicle control	65 ppm butadiene	400 ppm butadiene	1250 ppm butadiene	Room control
No. of treated males paired with untreated females	25	24[a]	25	25	50
No. of untreated females paired with treated males[b]	50	48	50	50	100
No. of males mated[c,d] (% of number paired)	25 (100%)	24[ns] (100%)	25[ns] (100%)	25[ns] (100%)	50[ns] (100%)
No. of females mated[d,e] (% of number paired)	49 (98%)	46[ns] (96%)	48[ns] (96%)	49[ns] (98%)	94[ns] (94%)
Period to coition[f,e,h] (days) (mean ± SD)	4.25±2.39	3.96[ns]±2.54	3.92[ns]±2.41	4.02[ns]±1.98	4.12[ns]±2.25
No. of pregnant females[d,h,i] (% of number mated)	46 (94%)	45[ns] (98%)	44[ns] (92%)	46[ns] (94%)	92[ns] (98%)
No. of males with at least one pregnant female[d,h,i] (% of number paired)	25 (100%)	24[ns] (100%)	25[ns] (100%)	25[ns] (100%)	50[ns] (100%)
No. of pregnant females[d,h,i] (% of number paired)	46 (92%)	45[ns] (94%)	44[ns] (88%)	46[ns] (92%)	92[ns] (92%)

Mating and fertility data for individual male rats are shown in Appendix 6.

[a] One male rat died during the exposure period due to an unknown cause.
[b] Two females per male for up to 9 days.
[c] Either : at least one plug found
or : at least one female with sperm positive vaginal smear
or : at least one female known to have been pregnant.
[d] Statistical analysis by Fisher's exact test; [ns] treated not significantly lower than vehicle control, or room control not significantly higher than vehicle control.
[e] Either : plug found in female
or : sperm positive vaginal smear
or : plug found under cage housing one female only or assigned retrospectively if two females present.
or : female known to have been pregnant
[f] Number of days from housing males and females together to finding plug in female, sperm positive vaginal smear or plug under cage (date of mating determined retrospectively if two females present).
[g] Statistical analysis by two-sided two-sample t-test, [ns] not significantly different from vehicle control.
[h] Some females were found not to be pregnant despite a plug or a sperm positive vaginal smear having been found.
[i] Pregnant female defined as female in which foetus(es) were seen at dominant lethal necropsy examination.

Table 5. Summary of dominant lethal data for male rats treated sub-chronically with butadiene

Treatment group	Vehicle control	65 ppm butadiene	400 ppm butadiene	1250 ppm butadiene	Room control
No. of pregnant females[a]	46	45	44	46	92
Corpora lutea[b]	857	812	770	814	1632
Total number	18.63±3.52	18.04[ns]±3.62	17.91[ns]±3.14	18.09±3.51	18.55[ns]±3.92
Mean[c] ± SD			[n=43][d]	[n=45][d]	[n=88][d]
Implantations[b]					
Number	695	627	650	655	1370
Mean[c] ± SD	15.11±3.16	13.93*±3.33	14.78[ns]±3.32	14.24[ns]±3.53	14.90[ns]±3.24
Early deaths[e,f]					
Total number	19	26	16	31	53
Mean[g] ± SD	0.030±0.074	0.055[ns]±0.131	0.022[ns]±0.043	0.045[ns]±0.078	0.040[ns]±0.087
No. of pregnant females with ≥ 1 early death[h] (% of number pregnant)	13 (28%)	12[ns] (27%) [n=44][d]	11[ns] (25%)	17[ns] (38%) [n=45][d]	33[ns] (36%)
Late deaths[e,f]					
Total number	5	7	3	14	15
Mean[g] ± SD	0.009±0.029	0.014[ns]±0.047	0.004[ns]±0.015	0.019[ns]±0.064	0.011[ns]±0.040
No. of pregnant females with ≥ 1 late death[h] (% of number pregnant)	5 (11%)	5[ns] (11%) [n=44][d]	3[ns] (1%)	6[ns] (13%) [n=45][d]	10[ns] (11%)
Late deaths including dead foetuses[e,f]					
Total number	9	9	6	16	17
Mean[g] ± SD	0.014±0.037	0.020[ns]±0.055	0.009[ns]±0.026	0.042[ns]±0.159	0.012[ns]±0.040
No. of pregnant females with ≥ 1 late death or dead foetus[h] (% of number pregnant)	7 (15%)	7[ns] (16%) [n=44][d]	5[ns] (11%)	8[ns] (18%) [n=45][d]	12[ns] (13%)
Abnormal foetuses[e,f]					
Total number	5[i]	5[j]	5[k]	2[l]	10[m]
Mean[g] ± SD	0.007±0.020	0.010[ns]±0.034	0.008[ns]±0.022	0.003[ns]±0.014	0.007[ns]±0.020
No. of pregnant females with ≥ 1 abnormal foetuses[n] (% of number pregnant)	5 (11%)	5 (11%) [n=44][d]	5 (11%)	2 (4%) [n=45][d]	10 (11%)

Table 5. continued.

Footnotes

Dominant lethal data for individual male rats are shown in Appendix 7.

[a] Data from Table 4.
[b] Statistical analysis by two-sample t-test; [ns], treated not significantly lower than vehicle control; room control not significantly higher than vehicle control; *, treated significantly lower than vehicle control ($p \leq 0.05$).
[c] Per pregnant female.
[d] Square brackets shows number of pregnant females when different to that reported.
[e] Statistical analysis on transformed data - see text section 5.5.4. for details.
[f] Statistical analysis by two-sample t-test; [ns], treated not significantly higher than vehicle control; room control not significantly lower than vehicle control.
[g] Per implantation per pregnancy.
[h] Statistical analysis by Fisher's exact test; [ns] treated not significantly higher than vehicle control; room control not significantly lower than vehicle control.
[i] 5 runts (67, 67, 71, 71 and 57% of mean body weight of others in litter; total litter sizes 16, 14, 12, 15 and 15 respectively).
[j] 1 gastroschisis; 4 runts (67, 41, 74 and 72% of mean body weight of others in litter; total litter sizes 17, 18, 12 and 14 respectively).
[k] 1 gastroschisis; 4 runts (75, 73, 64 and 73% of mean body weight of others in litter; total litter sizes 17, 15, 14 and 11 respectively).
[l] 2 runts (64 and 52% of mean body weight of others in litter; total litter sizes 14 and 15 respectively).
[m] 1 scoliosis in lumber and sacral spinal column; 9 runts (62, 68, 69, 71, 68, 55, 73, 70 and 71% of mean body weight of others in litter; total litter sizes 16, 12, 11, 10, 13, 14, 18, 16 and 18 respectively).
[n] Fisher's exact test not performed since no litter contained >1 abnormal foetus: see two-sample t-test.

Appendix 1. Body weights of individual male rats treated sub-chronically with butadiene

Treatment group	Male number	Body weight (g) on study days:-											
		0	7	14	21	28	35	42	49	56	63	70	77
Vehicle control	1	290	320	340	369	379	405	416	429	440	455	470	464
	2	280	309	325	339	356	372	382	388	402	410	414	410
	3	264	287	305	323	340	355	371	375	390	397	405	405
	4	263	299	310	339	348	370	384	395	398	405	415	418
	5	311	344	376	403	430	444	468	480	490	509	518	504
	6	270	295	318	345	362	380	396	400	419	430	437	439
	7	265	292	314	335	352	375	388	398	405	420	422	405
	8	281	298	311	329	343	356	369	383	393	405	411	405
	9	268	289	307	324	327	339	345	359	365	371	377	382
	10	277	300	317	351	336	350	361	371	381	395	399	389
	11	284	318	337	360	372	395	406	418	425	448	453	448
	12	244	266	278	292	297	320	330	342	360	372	377	378
	13	263	286	301	321	333	342	361	369	375	391	399	387
	14	290	319	340	361	382	404	420	429	439	450	463	460

continued....

Appendix 1. continued.

Treatment group	Male number	Body weight (g) on study days:-											
		0	7	14	21	28	35	42	49	56	63	70	77
Vehicle control	15	266	288	313	336	352	371	389	400	415	430	446	450
	16	262	303	324	345	357	380	397	410	422	435	443	433
	17	247	269	282	303	321	332	351	357	369	380	382	373
	18	283	320	342	363	387	405	422	433	448	465	473	470
	19	268	289	311	322	331	342	358	370	377	399	399	396
	20	239	254	266	278	296	305	315	321	330	340	342	337
	21	272	289	301	316	324	340	350	359	362	370	377	374
	22	262	279	294	316	329	347	358	370	384	385	387	387
	23	282	309	331	351	374	390	400	405	415	421	426	422
	24	263	285	294	307	319	331	342	350	366	370	375	380
	25	279	301	323	333	327	376	392	402	410	425	432	431
65 ppm butadiene	26	281	305	319	341	354	369	384	398	404	410	428	428
	27	269	285	316	330	340	349	372	384	389	397	407	411
	28	264	292	311	328	343	363	381	383	397	410	416	414
	29	268	291	304	326	333	351	361	371	381	394	403	401
	30	267	288	297	317	334	350	362	366	380	392	406	403
	31	266	291	305	326	340	357	377	387	395	406	418	414
	32	299	325	341	365	380	396	415	426	432	440	452	444

continued....

Appendix 1. continued.

Treatment group	Male number	Body weight (g) on study days:-											
		0	7	14	21	28	35	42	49	56	63	70	77
65 ppm butadiene	33	254	264	277	295	310	321	334	339	350	362	367	375
	34	278	296	311	336	347	362	376	382	384	395	402	394
	35	273	297	308	333	348	363	370	384	400	416	419	424
	36	247	263	274	292	302	320	328	329	340	360	365	365
	37	291	309	328	342	352	-	-	-	-	-	-	-
	38	263	272	287	303	317	332	345	348	360	364	365	366
	39	282	302	329	317	370	391	409	413	424	430	456	448
	40	271	286	298	316	331	350	311	362	375	389	390	392
	41	285	304	332	349	372	389	413	417	429	432	440	437
	42	273	294	306	328	332	351	366	383	383	390	402	400
	43	289	330	346	372	385	404	416	432	442	442	452	450
	44	251	269	288	315	321	340	351	364	372	382	390	382
	45	255	280	297	322	336	351	361	374	383	390	391	379
	46	291	318	340	364	385	405	422	434	450	460	463	456
	47	269	288	302	322	339	345	368	380	391	397	403	400
	48	278	300	320	344	346	363	380	393	405	412	420	421
	49	254	272	295	352	330	347	358	375	384	390	396	385
	50	288	321	342	368	382	392	410	415	430	434	441	437

continued....

Appendix 1. continued.

Treatment group	Male number	Body weight (g) on study days:-											
		0	7	14	21	28	35	42	49	56	63	70	77
400 ppm butadiene	51	254	272	282	308	324	341	355	364	380	388	401	404
	52	269	290	310	330	355	361	382	398	411	420	434	436
	53	284	316	339	360	381	392	409	425	440	452	455	447
	54	277	306	324	346	364	379	390	406	418	420	421	419
	55	267	294	311	336	350	364	379	396	405	419	420	422
	56	261	281	303	316	322	339	349	360	371	375	384	384
	57	275	293	304	320	333	344	358	366	382	394	402	405
	58	286	309	331	357	360	379	399	414	439	442	451	451
	59	252	263	269	285	295	309	319	332	345	356	356	353
	60	280	307	314	340	355	369	376	391	403	410	418	415
	61	275	322	344	371	389	407	422	435	445	456	463	463
	62	283	289	312	323	342	349	370	386	396	410	418	417
	63	275	285	299	314	325	338	343	355	365	374	382	379
	64	263	280	300	325	343	351	365	379	388	400	405	396
	65	287	296	319	349	372	385	402	418	432	445	459	457
	66	289	309	333	352	367	386	394	405	416	430	435	436
	67	251	299	317	336	353	364	371	389	400	406	416	419
	68	277	300	324	342	352	362	382	395	402	415	421	412

continued....

Appendix 1. continued.

Treatment group	Male number	Body weight (g) on study days:-											
		0	7	14	21	28	35	42	49	56	63	70	77
400 ppm butadiene	69	269	288	310	332	343	362	375	392	419	416	426	421
	70	265	311	329	359	365	378	393	411	420	430	435	432
	71	266	312	336	353	372	382	398	414	421	424	437	434
	72	273	268	272	295	306	313	327	338	345	350	358	346
	73	261	284	304	331	354	362	385	398	409	421	427	421
	74	279	309	331	357	377	397	412	426	440	455	456	451
	75	275	290	308	324	336	358	367	380	386	393	394	382
1250 ppm butadiene	76	256	274	291	320	324	333	334	347	361	375	381	372
	77	268	285	303	306	336	354	361	375	385	400	402	407
	78	284	309	329	359	371	382	402	412	417	428	437	440
	79	260	246	262	279	295	302	314	325	333	340	347	349
	80	291	321	334	364	376	387	402	411	424	442	450	447
	81	279	306	323	350	369	386	404	423	432	445	453	446
	82	285	314	327	346	358	352	380	388	407	415	418	425
	83	269	291	306	330	351	357	374	386	390	400	409	409
	84	280	307	324	350	369	374	394	410	414	428	438	438
	85	292	316	334	352	371	380	391	405	415	420	422	425

continued....

Appendix 1. continued.

Treatment group	Male number	Body weight (g) on study days:-											
		0	7	14	21	28	35	42	49	56	63	70	77
1250 ppm butadiene	86	289	316	324	336	348	358	371	391	396	395	398	406
	87	276	293	302	320	336	351	354	367	375	380	381	373
	88	274	304	318	342	363	380	387	402	416	422	429	421
	89	253	281	302	320	340	351	362	370	380	395	402	389
	90	268	310	328	347	361	375	390	394	414	426	435	442
	91	249	264	281	300	315	328	340	353	360	370	382	384
	92	261	275	298	296	311	324	330	336	341	357	358	354
	93	270	289	302	322	335	352	368	377	382	396	402	403
	94	288	309	321	336	341	369	377	381	392	400	411	402
	95	279	300	315	335	352	372	384	397	410	425	434	420
	96	245	270	267	299	309	315	327	331	341	351	354	351
	97	270	282	292	317	332	349	362	365	378	390	402	396
	98	267	281	299	313	329	342	353	361	376	389	395	397
	99	269	281	307	320	347	360	376	387	395	405	417	411
	100	248	265	284	298	312	329	340	343	355	367	378	371
Room control	101	279	308	333	355	372	391	410	422	431	435	448	438
	102	252	276	297	315	335	346	355	369	376	385	392	382

continued....

Appendix 1. continued.

Treatment group	Male number	Body weight (g) on study days:-											
		0	7	14	21	28	35	42	49	56	63	70	77
Room control	103	290	320	341	367	382	400	410	424	429	438	438	433
	104	254	280	304	325	347	360	377	390	411	414	424	421
	105	269	296	311	334	349	367	383	395	409	422	417	409
	106	269	289	300	322	332	348	356	364	376	385	393	387
	107	269	298	323	341	356	365	380	394	402	415	418	417
	108	260	296	316	343	361	380	393	405	415	425	432	435
	109	256	270	292	309	326	333	344	343	361	368	375	372
	110	278	305	330	358	379	395	413	421	430	445	447	439
	111	240	263	278	296	311	320	339	347	355	365	368	357
	112	270	291	304	329	351	365	380	395	410	415	416	408
	113	260	289	306	323	341	352	363	374	384	395	396	393
	114	255	286	308	333	346	366	384	399	409	420	425	427
	115	265	288	304	320	333	340	351	364	384	378	380	377
	116	288	317	344	365	382	398	415	427	436	450	453	444
	117	273	306	336	362	381	410	423	442	451	466	476	464
	118	268	287	308	325	341	355	371	378	393	395	399	387
	119	270	301	335	364	385	402	415	427	440	452	460	465
	120	236	252	271	290	303	309	321	331	345	350	347	340

continued....

Appendix 1. continued.

Treatment group	Male number	Body weight (g) on study days:-											
		0	7	14	21	28	35	42	49	56	63	70	77
Room control	121	268	292	314	335	348	368	376	384	387	405	411	412
	122	283	319	349	376	391	412	435	455	463	470	477	466
	123	291	329	354	378	399	421	435	444	461	465	473	476
	124	253	280	301	319	334	349	364	375	385	392	400	392
	125	267	295	320	342	359	377	393	399	419	422	434	435
	126	260	287	314	325	335	361	365	374	383	400	405	399
	127	261	298	325	350	370	392	405	423	436	445	447	445
	128	273	310	334	358	375	390	422	431	441	454	469	448
	129	247	271	289	302	316	330	348	352	366	372	372	359
	130	291	321	341	369	389	407	424	436	449	460	461	460
	131	252	284	305	323	339	352	365	373	376	390	398	392
	132	280	317	341	366	393	410	428	443	451	465	483	477
	133	289	324	337	368	387	402	435	428	437	445	453	444
	134	266	293	313	339	354	364	381	392	397	402	406	404
	135	243	264	284	305	319	333	344	349	357	370	372	370
	136	280	308	334	353	373	389	406	421	430	435	443	429
	137	254	281	301	320	334	352	365	374	390	395	396	385
	138	280	305	326	351	376	389	407	414	420	437	441	439

continued....

Appendix 1. continued.

Treatment group	Male number	Body weight (g) on study days:-											
		0	7	14	21	28	35	42	49	56	63	70	77
Room control	139	285	311	342	372	393	409	422	429	440	455	457	447
	140	261	275	295	313	332	343	358	367	380	385	385	382
	141	274	293	311	332	351	364	379	389	400	410	407	404
	142	255	274	307	327	347	365	388	400	410	419	423	428
	143	254	281	297	319	330	343	364	377	380	392	389	380
	144	260	281	294	307	312	331	341	353	363	364	370	366
	145	254	275	291	307	323	339	348	361	367	375	372	372
	146	280	274	289	306	321	336	349	360	370	375	371	367
	147	274	299	322	338	357	375	390	408	420	425	435	435
	148	282	312	342	362	385	405	421	441	450	466	471	473
	149	254	269	279	296	311	321	335	346	357	365	366	372
	150	279	302	321	340	355	364	375	382	385	392	387	379

- = missing data (animal died on study day 80; cause unknown).

Appendix 2. Manufacturer's certificates of analysis for butadiene

See Appendix 4 for period of use for each sample cylinder.

Rec'd 30/6/95
Bomb delivered 30/6/95
<1H SAMPLE CYLINDER

- A11 - Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		28-6-95
Bomb No.		— WPV853
Appearance	Clr H_2O white	CLEAR WATER WHITE
Butadiene 1:3	min 99.7 % w/w	99.94
Butadiene 1:2	max 20 ppm w/w	<1
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	19
Non vol res	max 200 ppm w/w	<10
NMP	max 5 ppm w/w	1
TBC	100 - 150 ppm	150
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	<1
Vinyl acetylene	max 10 ppm w/w	7
Ethyl acetylene	max 10 ppm w/w	4
Total acetylenes	max 20 ppm w/w	13
Methanol	max 10 ppm w/w	<1
Total butenes	max 0.30 % w/w	0.06
Signature		J.E. Fawcett

Accepted by ATM	Date
	19.5.95

Fax To 0181-661-7029

Project 1542/2

SAMPLE CYLINDER No. - A12 -

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		4/7/95
Bomb No.		WPV 265
Appearance	Clr H_2O white	CLEAR WATER WHITE
Butadiene 1:3	min 99.7 % w/w	99.80
Butadiene 1:2	max 20 ppm w/w	3
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	1.0
Peroxide as H_2O_2	max 2 ppm w/w	<1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	11
Non vol res	max 200 ppm w/w	<0.01
NMP	max 5 ppm w/w	3
TBC	100 - 150 ppm	130
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	2
Vinyl acetylene	max 10 ppm w/w	3
Ethyl acetylene	max 10 ppm w/w	1
Total acetylenes	max 20 ppm w/w	6
Methanol	max 10 ppm w/w	<1
Total butenes	max 0.30 % w/w	0.20
Signature		

Accepted by ATM	Date
	19.5.95

PROJECT 1542/2

-1-/95

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		7/7/95
Bomb No.		WPV 2bb
Appearance	Clr H,O white	clear water white
Butadiene 1:3	min 99.7 % w/w	99.83
Butadiene 1:2	max 20 ppm w/w	7
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	<1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	17
Non vol res	max 200 ppm w/w	
NMP	max 5 ppm w/w	<1
TBC	100 - 150 ppm	160
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	2
Vinyl acetylene	max 10 ppm w/w	4
Ethyl acetylene	max 10 ppm w/w	2
Total acetylenes	max 20 ppm w/w	8
Methanol	max 10 ppm w/w	2
Total butenes	max 0.30 % w/w	0.17
Signature		[signature]

Accepted by ATM	Date
[signature]	19.5.95

PROJECT 1522/2

[signature] 8/7/95

8/7/95

END

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		7/7/95
Bomb No.		WPV 1077
Appearance	Clr H_2O white	CLEAR WATER WHITE
Butadiene 1:3	min 99.7 % w/w	99.83
Butadiene 1:2	max 20 ppm w/w	7
C 5	max 0.05 % w/w	< 0.01
Sulphur	max 2 ppm w/w	< 1
Peroxide as H_2O_2	max 2 ppm w/w	< 1
Carbonyl as acetaldehyde	max 10 ppm w/w	< 10
Butadiene dimer	max 100 ppm w/w	17
Non vol res	max 200 ppm w/w	
NMP	max 5 ppm w/w	< 1
TBC	100 - 150 ppm	160
Allene	max 5 ppm w/w	< 1
Methyl acetylene	max 10 ppm w/w	2
Vinyl acetylene	max 10 ppm w/w	4
Ethyl acetylene	max 10 ppm w/w	2
Total acetylenes	max 20 ppm w/w	8
Methanol	max 10 ppm w/w	2
Total butenes	max 0.30 % w/w	0.17
Signature		

Accepted by ATM	Date
	11.5.95

PROJECT 1562R

8.7 -95 8 JPhillips 8/7/95

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		18.7.95
Bomb No.		WIA 5362/WIE 0903
Appearance	Clr H_2O white	CLEAR WATER WHITE
Butadiene 1:3	min 99.7 % w/w	99.79
Butadiene 1:2	max 20 ppm w/w	7
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	<1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	20
Non vol res	max 200 ppm w/w	<10
NMP	max 5 ppm w/w	1
TBC	100 - 150 ppm	125
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	6
Vinyl acetylene	max 10 ppm w/w	3
Ethyl acetylene	max 10 ppm w/w	5
Total acetylenes	max 20 ppm w/w	14
Methanol	max 10 ppm w/w	<1 mm
Total butenes	max 0.30 % w/w	0.2
Signature		

PROJECT 1542R
19.7.95
19/7/95

Accepted by ATM	Date
	19.5.95

11th + 12th Samples shipped
Delivered 25-7-95 - A16 -

Teesside Operations Analytical Services
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		25/7/95
Bomb No.	2 Bombs	WPV 853 WPV265
Appearance	Clr H_2O white	clear water white
Butadiene 1:3	min 99.7 % w/w	99.84
Butadiene 1:2	max 20 ppm w/w	6
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	<1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	21
Non vol res	max 200 ppm w/w	<10
NMP	max 5 ppm w/w	<1
TBC	100 - 150 ppm	140
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	<1
Vinyl acetylene	max 10 ppm w/w	4
Ethyl acetylene	max 10 ppm w/w	3
Total acetylenes	max 20 ppm w/w	8
Methanol	max 10 ppm w/w	5 → 1 *
Total butenes	max 0.30 % w/w	0.16
Signature		

* Telephone call to request change from 5 → 1 ppm on 25.7.95.

Accepted by ATM	Date
	19.5.95

PROJECT 1542/2

13th, 14th samples by limbo.
Delivered 1.8.95

- A17 -

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		28/7/95
Bomb No.		WPV264 WPV 1077
Appearance	Clr H_2O white	Clear water white
Butadiene 1:3	min 99.7 % w/w	99.85
Butadiene 1:2	max 20 ppm w/w	6
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	<1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	36
Non vol res	max 200 ppm w/w	<10
NMP	max 5 ppm w/w	<1
TBC	100 - 150 ppm	100
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	2
Vinyl acetylene	max 10 ppm w/w	1
Ethyl acetylene	max 10 ppm w/w	<1
Total acetylenes	max 20 ppm w/w	4
Methanol	max 10 ppm w/w	2
Total butenes	max 0.30 % w/w	0.15
Signature		R.S. Heppenstall

Accepted by ATM	Date
	19.5.95

PROJECT 1542/2

1.8.95.

B.J. Phillips 1/8/95

15th. 16th Cylinders
Delivered 8.8.95

- A18 -

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		7 AUG 1995
Bomb No.		W1E 0903, W1A 5362
Appearance	Clr H_2O white	CLEAR WATER WHITE
Butadiene 1:3	min 99.7 % w/w	99.78
Butadiene 1:2	max 20 ppm w/w	5
C 5	max 0.05 % w/w	< 0.01
Sulphur	max 2 ppm w/w	< 1
Peroxide as H_2O_2	max 2 ppm w/w	1
Carbonyl as acetaldehyde	max 10 ppm w/w	< 10
Butadiene dimer	max 100 ppm w/w	18
Non vol res	max 200 ppm w/w	< 10
NMP	max 5 ppm w/w	3
TBC	100 - 150 ppm	135
Allene	max 5 ppm w/w	< 1
Methyl acetylene	max 10 ppm w/w	2
Vinyl acetylene	max 10 ppm w/w	< 1
Ethyl acetylene	max 10 ppm w/w	1
Total acetylenes	max 20 ppm w/w	5
Methanol	max 10 ppm w/w	< 1
Total butenes	max 0.30 % w/w	0.22
Signature		

Accepted by ATM	Date
	17.5.95

PROJECT 1522/2

8/8/95

8.8.95

17th. 18th cylinders
received 11.8.95

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		10 Aug 1995
Bomb No.		VBN 853 VBN 1077
Appearance	Clr H,O white	Clear water white
Butadiene 1:3	min 99.7 % w/w	99.59 *
Butadiene 1:2	max 20 ppm w/w	6
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	< 1
Peroxide as H_2O_2	max 2 ppm w/w	1
Carbonyl as acetaldehyde	max 10 ppm w/w	< 10
Butadiene dimer	max 100 ppm w/w	13
Non vol res	max 200 ppm w/w	< 10
NMP	max 5 ppm w/w	< 1
TBC	100 - 150 ppm	100
Allene	max 5 ppm w/w	< 1
Methyl acetylene	max 10 ppm w/w	12 *
Vinyl acetylene	max 10 ppm w/w	< 1
Ethyl acetylene	max 10 ppm w/w	4
Total acetylenes	max 20 ppm w/w	17
Methanol	max 10 ppm w/w	< 1
Total butenes	max 0.30 % w/w	0.41 *
Signature		[signature]

Accepted by ATM	Date
[signature]	19.5.95

PROJECT
1542/2

[signature] 11/8/95
11.8.95

END

19 & 20 Cylinders
WOP 265, 265
Delivered 16/8/95

- A20 -

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date	15-8-95	
Bomb No.	LPV 264 + 265	
Appearance	Clr H_2O white	CLEAR WATER WHITE
Butadiene 1:3	min 99.7 % w/w	99.82
Butadiene 1:2	max 20 ppm w/w	2
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	26
Non vol res	max 200 ppm w/w	<10
NMP	max 5 ppm w/w	<1
TBC	100 - 150 ppm	100
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	<1
Vinyl acetylene	max 10 ppm w/w	<1
Ethyl acetylene	max 10 ppm w/w	<1
Total acetylenes	max 20 ppm w/w	3
Methanol	max 10 ppm w/w	<1
Total butenes	max 0.30 % w/w	0.18
Signature		

Accepted by ATM	Date
	18.5.95

PROJECT SUPR

FROM

24 x 22 cylinders

Delivered 28/8/95

23/8/95

08.23.1995 13:39

- A21 -

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		22/8/95
Bomb No.		UN 5362 / 853
Appearance	Clr H,O white	CLEAR WATER WHITE
Butadiene 1:3	min 99.7 % w/w	99.76
Butadiene 1:2	max 20 ppm w/w	7
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	<1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	22
Non vol res	max 200 ppm w/w	<10
NMP	max 5 ppm w/w	<1
TBC	100 - 150 ppm	110
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	3
Vinyl acetylene	max 10 ppm w/w	<1
Ethyl acetylene	max 10 ppm w/w	8
Total acetylenes	max 20 ppm w/w	12
Methanol	max 10 ppm w/w	<1
Total butenes	max 0.30 % w/w	0.24
Signature		

PROJECT 1542/2

23.8.95

Accepted by ATM	Date
	19.5.95

Delivery 2 cylinders WPV1077 (23kg)
WIF 0903 (25kg)

Delivered 30/8/95

A22

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		29/8/95
Bomb No.		WPV 1077 / WIF 0903
Appearance	Clr H_2O white	CLEAR WATER WHITE
Butadiene 1:3	min 99.7 % w/w	99.71
Butadiene 1:2	max 20 ppm w/w	14 ppm
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	14 ppm
Non vol res	max 200 ppm w/w	<10
NMP	max 5 ppm w/w	<1
TBC	100 - 150 ppm	130
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	<1
Vinyl acetylene	max 10 ppm w/w	5
Ethyl acetylene	max 10 ppm w/w	17 *
Total acetylenes	max 20 ppm w/w	23 *
Methanol	max 10 ppm w/w	<1
Total butenes	max 0.30 % w/w	0.29
Signature		

PROJECT 1542/2

Accepted by ATM	Date
	19.5.95

20/9/95

25?, 26? cylinders

Teesside Operations Analytical Services.
Olefines 6 Laboratory.
Wilton Site
Tel 01642 434695

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		4/9/95
Bomb No.		WPU 264/265
Appearance	Clr H_2O white	CLEAR WATER WHITE
Butadiene 1:3	min 99.7 % w/w	99.76
Butadiene 1:2	max 20 ppm w/w	1
C 5	max 0.05 % w/w	<0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	<1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	14
Non vol res	max 200 ppm w/w	<10
NMP	max 5 ppm w/w	<1
TBC	100 - 150 ppm	110
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	<1
Vinyl acetylene	max 10 ppm w/w	<1
Ethyl acetylene	max 10 ppm w/w	1
Total acetylenes	max 20 ppm w/w	3
Methanol	max 10 ppm w/w	<1
Total butenes	max 0.30 % w/w	0.24
Signature		[signature]

Accepted by ATM	Date
[signature]	19.5.95

PROJECT 1542/2

[signature]
JAM 4.9.95
4.9.95
[signature]

Delivered 9-9-95

- A24 -
Olefines 6 Laboratory
Wilton Site
Tel 01642 434695

27th, 28th, 29th cylinders.

Certificate of Analysis

1,3-Butadiene for BIBRA International

Date		8/9/95
Bomb No.		UPV653 / W1A5362 / WF 0983
Appearance	Clr H_2O white	clear water white
Butadiene 1:3	min 99.7 % w/w	99.75
Butadiene 1:2	max 20 ppm w/w	7
C 5	max 0.05 % w/w	< 0.01
Sulphur	max 2 ppm w/w	<1
Peroxide as H_2O_2	max 2 ppm w/w	1
Carbonyl as acetaldehyde	max 10 ppm w/w	<10
Butadiene dimer	max 100 ppm w/w	86
Non vol res	max 200 ppm w/w	<10
NMP	max 5 ppm w/w	1
TBC	100 - 150 ppm	136
Allene	max 5 ppm w/w	<1
Methyl acetylene	max 10 ppm w/w	3
Vinyl acetylene	max 10 ppm w/w	3
Ethyl acetylene	max 10 ppm w/w	8
Total acetylenes	max 20 ppm w/w	14
Methanol	max 10 ppm w/w	<1
Total butenes	max 0.30 % w/w	0.25
Signature		

Accepted by ATM	Date
	19.5.95

PROJECT 1542/2

9.9.95

Appendix 8. Concentration of butadiene in atmospheres generated for sub-chronic exposure of male rats

Treatment week	Study day	Concentration of butadiene (ppm)	Initial exposure period: Length of time to reach required concentration (min)	Initial exposure period: Maximum concentration (ppm)	Main exposure period: Length of time at required concentration	Main exposure period: Range of concentration (ppm)	Final exposure period: Length of time to return to 0 ppm (min)	Final exposure period: Maximum concentration (ppm)
1	0	65	9	66	5 h 51 min	36-74	4	67
		400	33	380	6 h 27 min	190-440	11	400
		1250	5	2174	5 h 45 min	780-2174	[a]	[a]
	1	65	10	57	5 h 50 min	57-73	10	70
		400	11	350	5 h 49 min	350-450	14	430
		1250	10	1262	5 h 45 min	1262-1436	[a]	[a]
	2	65	5	57	5 h 55 min	14-74	11	64
		400	5	430	5 h 55 min	70-530	14	410
		1250	5	1091	5 h 55 min	361-1615	[a]	[a]
	3	65	8	54	5 h 52 min	9-79	9	11
		400	7	390	5 h 53 min	40-490	17	410
		1250	10	1110	5 h 45 min	112-1405	[a]	[a]
	4	65	20	48	5 h 40 min	9-77	8	70
		400	20	370	5 h 40 min	60-490	15	450
		1250	10	1177	5 h 50 min	173-1363	[a]	[a]
2	7	65	11	56	5 h 49 min	55-75	9	66
		400	8	340	5 h 52 min	350-430	16	420
		1250	10	1146	5 h 50 min	1081-1491	[a]	[a]
	8	65	8	61	5 h 52 min	57-73	9	63
		400	8	390	5 h 52 min	380-470	16	400
		1250	5	1156	5 h 55 min[b]	1081[b]-1403[b]	[a]	[a]

continued....

Appendix 3. continued.

Treatment week	Study day	Concentration of butadiene (ppm)	Initial exposure period: Length of time to reach required concentration (min)	Initial exposure period: Maximum concentration (ppm)	Main exposure period: Length of time at required concentration	Main exposure period: Range of concentration (ppm)	Final exposure period: Length of time to return to 0 ppm (min)	Final exposure period: Maximum concentration (ppm)
2	9	65	6	29	5 h 54 min	14-75	11	67
		400	7	410	5 h 53 min	70-450	15	440
		1250	5	1083	5 h 55 min	282-1413	[a]	[a]
	10	65	12	70	5 h 48 min	53-72	10	57
		400	15	470	5 h 46 min	380-500	15	420
		1250	30	5128	5 h 29 min[c]	484[c]-1207[c]	[a]	[a]
	11	65	13	53	5 h 47 min	47-61	10	68
		400	13	400	5 h 47 min	320-540	17	450
		1250	5	1109	5 h 55 min	941-1612	[a]	[a]
3	14	65	18	66	5 h 42 min	42-65	9	61
		400	16	420	5 h 44 min	230-520	16	420
		1250	[a]	[a]	5 h 20 min	979-1530	20	1310
	15	65	17	62	5 h 43 min	47-74	12	66
		400	17	430	5 h 43 min	300-480	18	430
		1250	5	1276	5 h 50 min	1082-1636	[a]	[a]
	16	65	33	68	5 h 27 min	55-74	10	57
		400	32	440	5 h 28 min	330-480	13	350
		1250	15	1068	5 h 45 min	1040-1627	[a]	[a]
	17	65	14	64	5 h 46 min	45-74	11	63
		400	8	330	5 h 52 min	260-470	17	380
		1250	10	1162	5 h 50 min	1038-1501	[a]	[a]
	18	65	16	66	5 h 44 min	50-79	11	70
		400	10	340	5 h 50 min	390-490	15	440
		1250	30	1072	5 h 30 min	989-1383	[a]	[a]

continued....

Appendix 3. continued.

Treatment week	Study day	Concentration of butadiene (ppm)	Initial exposure period: Length of time to reach required concentration (min)	Initial exposure period: Maximum concentration (ppm)	Main exposure period: Length of time at required concentration	Main exposure period: Range of concentration (ppm)	Final exposure period: Length of time to return to 0 ppm (min)	Final exposure period: Maximum concentration (ppm)
4	21	65	21	64	5 h 39 min	29-73	10	68
		400	21	470	5 h 39 min	160-470	18	450
		1250	15	1096	5 h 45 min	530-1298	[a]	[a]
	22	65	17	72	5 h 43 min	40-92	9	66
		400	14	440	5 h 46 min	220-510	15	410
		1250	10	1076	5 h 50 min	770-1412	[a]	[a]
	23	65	16	64	5 h 44 min	43-78	9	59
		400	16	410	5 h 44 min	240-480	13	360
		1250	20	1167	5 h 40 min	744-1268	[a]	[a]
	24	65	11	70	5 h 49 min	50-83	9	74
		400	11	440	5 h 49 min	270-520	16	460
		1250	15	1120	5 h 45 min	860-1321	[a]	[a]
	25	65	27	67	5 h 33 min	62-83	9	61
		400	27	430	5 h 33 min	370-520	14	400
		1250	15	1099	5 h 45 min	1021-1370	[a]	[a]
5	28	65	9	57	5 h 51 min	57-73	10	70
		400	9	420	5 h 51 min	350-440	16	410
		1250	6	1241	5 h 55 min	1285-1594	[a]	[a]
	29	65	12	67	6 h 03 min[a]	9-77	9	61
		400	12	380	6 h 03 min[a]	30-470	11	370
		1250	10	1111	5 h 50 min[a]	70-1392	[a]	[a]
	30	65	38	70	5 h 22 min	55-73	10	63
		400	38	450	5 h 22 min	360-460	15	400
		1250	[f]	1906[f]	5 h 10 min[f]	1148-1326[f]	40	1227

continued....

Appendix 8. continued.

Treatment week	Study day	Concentration of butadiene (ppm)	Initial exposure period: Length of time to reach required concentration (min)	Initial exposure period: Maximum concentration (ppm)	Main exposure period: Length of time at required concentration	Main exposure period: Range of concentration (ppm)	Final exposure period: Length of time to return to 0 ppm (min)	Final exposure period: Maximum concentration (ppm)
5	31	65	25	70	5 h 35 min	63-73	10	66
		400	22	400	5 h 38 min	380-430	12	390
		1250	J	1402[f]	5 h 25 min[f]	1145[f]-1402[f]	25	1152
	32	65	8	72	6 h 12 min[g]	9-87	10	74
		400	8	460	6 h 12 min[g]	40-530	13	460
		1250	10	1201	6 h 20 min[g]	149-1366	30	1278
6	35	65	14	64	5 h 46 min	18-83	10	68
		400	6	340	5 h 54 min	230-580	11	410
		1250	15	1081	5 h 60 min	738-1481	25	1371
	36	65	14	64	5 h 46 min	62-77	10	268
		400	14	410	5 h 46 min	370-480	16	420
		1250	20	1139	5 h 40 min	1021-1228	30	1112
	37	65	9	61	5 h 51 min	59-77	10	70
		400	4	380	5 h 56 min	390-460	11	430
		1250	5	1206	5 h 55 min	1206-1470	30	1453
	38	65	12	73	5 h 58 min[h]	11-78	11	73
		400	12	410	5 h 58 min[h]	70-470	15	440
		1250	10	1109	5 h 50 min[h]	338-1348	45	1168
	39	65	12	62	6 h 13 min	9-73	9	61
		400	7	370	6 h 18 min	40-460	13	380
		1250	20	1198	6 h 10 min	255-1460	30	1201
7	42	65	15	66	5 h 45 min	49-75	10	68
		400	15	440	5 h 45 min	270-470	14	350
		1250	15	1064	5 h 45 min	932-1314	25	1039

continued....

Appendix 3. continued.

Treatment week	Study day	Concentration of butadiene (ppm)	Initial exposure period: Length of time to reach required concentration (min)	Initial exposure period: Maximum concentration (ppm)	Main exposure period: Length of time at required concentration	Main exposure period: Range of concentration (ppm)	Final exposure period: Length of time to return to 0 ppm (min)	Final exposure period: Maximum concentration (ppm)
7	43	65	10	61	5 h 50 min	52-74	10[1]	64
		400	10	440	5 h 50 min	280-450	14	410
		1250	25	1111	5 h 30 min	877-1221	[m]	1174
	44	65	8	56	5 h 52 min	54-77	11	72
		400	6	360	5 h 54 min	320-460	12	420
		1250	20	1114	J	J	J	J
	45	65	11	68	5 h 49 min	53-78	10	68
		400	11	440	5 h 49 min	350-470	14	460
		1250	15	1074	5 h 45 min	1061-1435	30	1411
	46	65	10	74	5 h 50 min	53-74	11	73
		400	10	470	5 h 50 min	340-470	15	460
		1250	20	1103	5 h 40 min	1037-1319	35	1319
8	49	65	12	73	5 h 48 min	58-74	9	67
		400	8	410	5 h 52 min	370-470	17	410
		1250	15	1184	5 h 45 min	1161-1340	35	1182
	50	65	11	61	5 h 49 min	60-70	10[1]	66
		400	8	450	5 h 52 min	380-470	15	410
		1250	10	1131	5 h 50 min	1131-1486	40	1251
	51	65	24	72	5 h 36 min	55-76	5	61
		400	10	430	5 h 50 min	360-460	12	400
		1250	15	1182	5 h 40 min	1141-1337	40	1169
	52	65	6	91	5 h 54 min	57-74	8	73
		400	6	430	5 h 54 min	350-450	12	440
		1250	15	1068	5 h 45 min	1068-1353	30	1075

continued....

Appendix 3. continued.

Treatment week	Study day	Concentration of butadiene (ppm)	Initial exposure period: Length of time to reach required concentration (min)	Initial exposure period: Maximum concentration (ppm)	Main exposure period: Length of time at required concentration	Main exposure period: Range of concentration (ppm)	Final exposure period: Length of time to return to 0 ppm (min)	Final exposure period: Maximum concentration (ppm)
8	53	65	10	68	5 h 50 min	59-78	10	62
		400	10	410	5 h 50 min	380-450	15	390
		1250	10	1077	5 h 50 min	1077-1486	35	1248
9	56	65	22	64	5 h 38 min	55-74	11	68
		400	14	350	5 h 46 min	380-430	16	420
		1250	20	1104	5 h 40 min	1104-1506	40	1328
	57	65	7	68	5 h 53 min	60-73	10	67
		400	7	430	5 h 53 min	380-450	17	410
		1250	15	1099	5 h 45 min	1099-1567	40	1234
	58	65	8	74	5 h 52 min	46-75	10	70
		400	9	450	5 h 51 min	280-500	15	420
		1250	20	1111	5 h 40 min	1111-1618	40	1181
	59	65	4	62	5 h 56 min	58-75	12	62
		400	3	420	5 h 57 min	370-450	19	400
		1250	10	1109	5 h 50 min	1096-1659	40	1096
	60	65	5	70	5 h 55 min	55-74	10	68
		400	5	520	5 h 55 min	340-510	15	420
		1250	20	1138	5 h 40 min	1046-1355	35	1197
10	63	65	12	70	5 h 48 min	54-74	9	62
		400	12	500	5 h 48 min	380-510	14	380
		1250	25	1124	5 h 35 min	887-1260	35	1105
	64	65	8	57	5 h 52 min	48-73	9	63
		400	8	380	5 h 52 min	280-450	10	390
		1250	10	1063	5 h 50 min	977-1365	35	1341

continued....

Appendix 3. continued.

Treatment week	Study day	Concentration of butadiene (ppm)	Initial exposure period: Length of time to reach required concentration (min)	Initial exposure period: Maximum concentration (ppm)	Main exposure period: Length of time at required concentration	Main exposure period: Range of concentration (ppm)	Final exposure period: Length of time to return to 0 ppm (min)	Final exposure period: Maximum concentration (ppm)
10	65	65	8	64	6 h 52 min	54-72	10	64
		400	8	420	5 h 52 min	330-450	17	410
		1250	15	1215	5 h 45 min	1111-1372	35	1127
	66	65	5	53	5 h 55 min	19-126[b]	11	68
		400	5	370	5 h 55 min	120-610[b]	12	450
		1250	35	1076	5 h 55 min	618-1700[b]	35	1205
	67	65	6	59	5 h 55 min	48-67	10	64
		400	5	440	5 h 55 min	230-530	11	380
		1250	15	1078	5 h 45 min	860-1442	30	1038

- Missing data.

[a] Data not available for final exposure period from study days 0-30 due to 6 h time schedule for Fourier Transform Infra Red (FTIR) used to measure the butadiene concentration in chamber 4 (1250 ppm).

[b] Data not available for final 30 min of main exposure period due to delayed start time and limitation described in note [a].

[c] Data not available for final 1 h 10 min of main exposure period due to extended initial exposure period and limitation described in note [i].

[d] Data not available for initial exposure period due to delayed start up of FTIR.

[e] Additional 15 minutes for main exposure period to compensate for temporary fall in butadiene concentration; cylinder changed during main exposure period.

[f] Data not available for initial exposure period or beginning of main exposure period due to FTIR malfunction.

[g] Additional 20 minutes for main exposure period to compensate for temporary fall in butadiene concentration; cylinder changed during main exposure period.

[h] Additional 10 minutes for main exposure period to compensate for temporary fall in butadiene concentration; equipment malfunction.

[i] Final exposure period estimated due to flat bed recorder malfunction.

[j] Data not available for main or final exposure periods due to FTIR malfunction.

[k] High maximum concentrations of butadiene during main exposure period possibly due to over-filled new cylinder of butadiene.

[m] Data not available for final exposure period due to delayed start time and limitation described in note [a].

Appendix 4. Dimer content and cylinder use for butadiene atmospheres generated for sub-chronic exposure of male rats

Butadiene sample	Manufacturer's cylinder ID	Delivery date	In use: From Date	From Study day	Until Date	Until Study day	Dimer* content (w/w) (ppm)
5	WPV 853	30 June 95	10 July 95	0	12 July 95	2	-
6	WPV 265	5 July 95	13 July 95	3	17 July 95	7	118
7	WPV 264	8 July 95	18 July 95	8	19 July 95	9	379
8	WPV 1077	8 July 95	20 July 95	10	23 July 95	13	140
9	WIF 0903	19 July 95	24 July 95	14	25 July 95	15	370
10	WIA 5362	19 July 95	26 July 95	16	27 July 95	17	219
11	WPV 853	26 July 95	28 July 95	18	31 July 95	21	197
12	WPV 265	26 July 95	1 Aug 95	22	2 Aug 95	23	10
13	WPV 264	1 Aug 95	3 Aug 95	24	6 Aug 95	27	307
14	WPV 1077	1 Aug 95	7 Aug 95	28	7 Aug 95	28	-
15	WIA 5362	8 Aug 95	8 Aug 95	29	10 Aug 95	31	207
17	WPV 853	11 Aug 95	11 Aug 95	32	14 Aug 95	35	376
18	WPV 1077	11 Aug 95	15 Aug 95	36	16 Aug 95	37	140
16	WIF 0903	8 Aug 95	17 Aug 95	38	17 Aug 95	38	146
20	WPV 264	16 Aug 95	18 Aug 95	39	22 Aug 95	43	678
19	WPV 265	16 Aug 95	23 Aug 95	44	24 Aug 95	45	251
22	WPV 853	24 Aug 95	25 Aug 95	46	28 Aug 95	49	475
21	WIA 5362	24 Aug 95	29 Aug 95	50	30 Aug 95	51	262
24	WIF 0903	30 Aug 95	31 Aug 95	52	3 Sept 95	55	169
23	WPV 1077	30 Aug 95	4 Sept 95	56	5 Sept 95	57	201
25	WPV 265	5 Sept 95	6 Sept 95	58	7 Sept 95	59	617
26	WPV 264	5 Sept 95	8 Sept 95	60	11 Sept 95	63	413
27	WIF 0903	9 Sept 95	12 Sept 95	64	13 Sept 95	65	337
29	WIA 5362	9 Sept 95	14 Sept 95	66	14 Sept 95	66	348
28	WPV 853	9 Sept 95	14 Sept 95	66	15 Sept 95	67	248

- Dimer content not measured (insufficient butadiene at end of exposure period)

* Butadiene dimer = 4-vinyl-1-cyclohexane.

Appendix 5. Individual temperature and relative humidity readings for inhalation chambers during sub-chronic exposure of male rats to butadiene

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
0	1	0 ppm	23.4[c]	64[c]	10.46
			23.8[c]	61[c]	11.01
			23.5[c]	69[c]	11.16
			23.6[c]	64[c]	11.31
			23.6[c]	59[c]	11.46
			23.6[c]	54[c]	12.01
			23.7[c]	53[c]	12.16
			23.8[c]	53[c]	12.31
			23.8[c]	56[c]	12.46
			23.8[c]	54[c]	13.01
			23.8[c]	54[c]	13.16
			23.8[c]	54[c]	13.31
			23.9[c]	58[c]	13.46
			23.9[c]	55[c]	14.01
			23.9[c]	55[c]	14.16
			23.9[c]	59[c]	14.31
			23.9[c]	58[c]	14.46
			23.9[c]	54[c]	15.01
			24.1[c]	58[c]	15.16
			24.0[c]	55[c]	15.31
			24.0[c]	60[c]	15.46
			24.1[c]	61[c]	16.01
			24.1[c]	62[c]	16.16
			24.1[c]	67[c]	16.31
1	4	1250 ppm	25.8[c]	49[c]	9.44
			26.1[c]	42[c]	9.59
			26.3[c]	40[c]	10.14
			26.5[c]	37[c]	10.29
			26.6[c]	37[c]	10.44
			26.7[c]	39[c]/61	10.59
			26.7[c]	37[c]/60	11.14
			26.5[c]	34[c]/60	11.29
			26.1[c]	31[c]/59	11.44
			26.5[c]	31[c]/58	11.59
			26.5[c]	31[c]/59	12.14
			26.4[c]	30[c]/58	12.29
			26.5[c]	30[c]/56	12.44
			26.2[c]	29[c]/56	12.59
			26.1[c]	29[c]/56	13.14
			26.0[c]	29[c]/56	13.29
			25.9[c]	29[c]/56	13.44
			25.9[c]	29[c]/56	13.59

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
1	4	1250 ppm	25.9[c]	29[c]/56	14.14
			26.0[c]	29[c]/56	14.29
			26.1[c]	34[c]/58	14.44
			26.1[c]	31[c]/58	14.59
			25.9[c]	29[c]/58	15.14
			25.9[c]	30[c]/58	14.29
2	1	0 ppm	26.0[c]	48[c]/68	9.48
			26.3[c]	58[c]/66	9.58
			26.9[c]	45[c]/64	10.13
			27.1[c]	43[c]/62	10.28
			27.4[c]	41[c]/62	10.43
			27.4[c]	40[c]/61	10.58
			27.5[c]	40[c]/61	11.13
			27.5[c]	39[c]/61	11.28
			27.5[c]	39[c]/61	11.43
			27.5[c]	39[c]/61	11.58
			27.5[c]	38[c]/61	12.13
			27.5[c]	40[c]/60	12.20
			27.5[c]	41[c]/60	12.43
			27.5[c]	42[c]/60	12.58
			27.5[c]	41[c]/60	13.13
			27.3[c]	40[c]/60	13.28
			27.4[c]	42[c]/60	13.43
			27.4[c]	40[c]/60	13.58
			27.4[c]	41[c]/60	14.13
			27.4[c]	41[c]/60	14.37
			27.5[c]	41[c]/60	14.54
			27.5[c]	47[c]/60	15.09
			27.5[c]	44[c]/60	15.24
3	1	0 ppm	26.9[c]	72[c]/59	10.18
			27.1[c]	54[c]/70	10.33
			27.1[c]	50[c]/69	10.48
			27.2[c]	47[c]/68	11.03
			27.2[c]	47[c]/68	11.18
			27.2[c]	48[c]/66	11.33
			27.2[c]	45[c]/65	11.48
			27.3[c]	44[c]/64	12.03
			27.4[c]	44[c]/64	12.18
			27.4[c]	43[c]/63	12.33
			27.3[c]	42[c]/62	12.48
			27.1[c]	41[c]/62	13.03
			27.0[c]	44[c]/62	13.18
			26.9[c]	42[c]/62	13.33
			27.0[c]	43[c]/62	13.48
			26.9[c]	43[c]/63	14.03

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
3	1	0 ppm	26.8°	43°/63	14.18
			26.7°	42°/62	14.33
			26.7°	42°/62	14.48
			26.9°	44°/62	15.03
			27.0°	45°/63	15.18
			26.9°	45°/63	15.33
			26.8°	45°/63	15.48
			27.1°	52°/65	16.03
4	3	400 ppm	25.6°	51°/64	9.34
			26.0°	39°/58	9.49
			26.0°	35°/56	10.04
			26.3°	38°/56	10.19
			26.3°	32°/54	10.34
			26.2°	30°/53	10.49
			26.3°	28°/52	11.04
			26.5°	26°/52	11.19
			26.7°	25°/52	11.34
			26.6°	24°/50	11.49
			26.6°	24°/50	12.04
			26.7°	22°/48	12.19
			26.7°	21°/48	12.34
			26.7°	21°/48	12.49
			26.7°	24°/48	13.04
			26.7°	23°/50	13.19
			26.8°	28°/50	13.34
			26.7°	29°/50	13.49
			26.9°	28°/50	14.04
			26.8°	29°/50	14.19
			26.7°	29°/50	14.34
			26.9°	29°/50	14.49
			26.6°	28°/50	15.04
			27.0°	29°/50	15.19
			27.1°	32°/50	15.34
7	2	65 ppm	26.3°	50°/56	9.56
			26.5°	45°/56	10.11
			26.4°	42°/52	10.26
			26.7°	40°/51	10.41
			26.8°	35°/50	11.17[a]
			26.8°	35°/50	11.19
			26.9°	34°/50	11.43
			27.0°	32°/49	11.58
			27.0°	31°/49	12.13
			27.0°	32°/44	12.28
			27.2°	34°/44	13.01
			27.2°	40°/48	13.28

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
7	2	65 ppm	27.3[c]	39[c]/46	13.43
			27.3[c]	35[c]/44	13.58
			27.5[c]	39[c]/46	14.13
			27.4[c]	39[c]/46	14.28
			27.3[c]	34[c]/44	14.43
			27.5[c]	34[c]/44	14.58
			27.5[c]	33[c]/44	15.13
			27.5[c]	31[c]/44	15.28
8	2	65 ppm	26.6	54	10.04
			26.6	52	10.19
			26.8	51	10.34
			26.8	50	10.49
			26.8	50	11.04
			27.0	49	11.20
			27.0	49	11.35
			27.0	48	11.50
			27.0	47	12.05
			27.0	47	12.20
			27.0	48	12.35
			27.0	48	12.50
			27.0	48	13.10
			27.0	47	13.20
			27.0	47	13.35
			27.0	47	13.55
			27.0	48	14.10
			27.0	48	14.25
			27.0	47	14.40
			27.0	47	14.55
			27.2	47	15.10
			27.2	48	15.25
			27.2	48	15.40
9	4	1250 ppm	25.8	70	9.25
			26.2	68	9.40
			26.2	68	9.55
			26.2	58	10.10
			26.2	56	10.25
			26.0	56	10.40
			25.8	54	10.55
			26.0	53	11.10
			26.0	52	11.25
			26.0	52	11.40
			26.0	51	11.55
			26.0	51	12.10
			25.8	51	12.25
			25.8	50	12.40

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
9	4	1250 ppm	25.8	49	12.55
			26.0	50	13.10
			26.0	50	13.25
			26.2	50	13.40
			26.2	51	13.55
			26.2	51	14.15
			26.2	51	14.30
			26.2	51	14.45
			26.2	51	15.00
			26.2	52	15.15
10	2	65 ppm	27.6	54	10.25
			27.6	52	10.40
			27.8	52	10.55
			27.4	52	11.10
			27.8	52	11.25
			27.8	50	11.40
			27.8	50	11.55
			27.8	50	12.10
			27.8	50	12.25
			27.8	50	12.40
			28.0	50	12.55
			28.0	48	13.10
			28.0	48	13.25
			28.0	48	13.40
			28.0	48	13.55
			28.0	48	14.10
			28.0	48	14.25
			28.0	48	14.40
			28.0	48	14.55
			28.4	48	15.10
			28.4	50	15.25
			28.4	50	15.40
			28.6	52	15.55
			28.8	53	16.10
			28.8	53	16.25
11	3	400 ppm	26.0	60	9.15
			26.0	58	9.30
			26.4	55	9.45
			26.4	53	10.00
			26.8	52	10.15
			26.4	50	10.30
			26.4	50	10.45
			26.8	48	11.00
			27.0	48	11.15
			27.0	46	11.30

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
11	3	400 ppm	27.0	47	11.45
			27.2	47	12.00
			27.0	46	12.15
			27.2	46	12.30
			27.2	46	12.45
			27.4	47	13.00
			27.4	48	13.15
			27.8	48	13.30
			27.8	47	13.45
			27.8	46	14.05
			28.2	47	14.20
			28.0	47	14.35
			28.0	46	14.50
			28.0	47	15.05
14	2	65 ppm	27.4	61	9.00
			27.4	59	9.15
			28.0	56	9.30
			28.0	55	9.45
			28.0	52	10.00
			28.0	52	10.15
			28.0	50	10.30
			28.0	50	10.45
			28.0	49	11.00
			28.0	49	11.15
			28.0	49	11.30
			28.2	49	11.45
			28.2	49	12.00
			28.2	49	12.15
			28.5	49	12.30
			28.5	48	12.45
			28.5	50	13.00
			28.4	50	13.15
			28.4	50	13.30
			28.6	50	13.45
			28.6	50	14.00
			28.6	50	14.15
			28.4	50	14.30
			28.6	50	14.45
15	2	65 ppm	26.4	62	9.30
			27.0	58	9.45
			27.6	56	10.00
			27.5	53	10.15
			27.6	52	10.30
			27.8	51	10.45
			27.8	50	11.00

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
15	2	65 ppm	27.9	51	11.15
			27.9	51	11.30
			28.0	50	11.45
			28.0	50	12.00
			28.0	50	12.15
			28.0	50	12.30
			28.0	50	12.45
			28.0	50	13.00
			28.1	50	13.15
			28.4	50	13.30
			28.8	50	13.45
			29.0	50	14.00
			28.7	50	14.15
			28.7	50	14.30
			29.4	52	14.45
			29.2	52	15.00
			29.2	52	15.15
16	1	0 ppm	26.8	72	9.25
			27.4	68	9.40
			28.8	64	9.55
			28.7	62	10.10
			28.8	61	10.30
			29.0	59	10.45
			28.7	58	11.00
			28.8	58	11.15
			28.8	56	11.30
			28.8	56	11.45
			28.8	56	12.00
			28.6	56	12.15
			28.2	56	15.30
			28.4	56	12.45
			28.2	56	13.00
			28.4	56	13.15
			28.4	56	13.30
			28.4	62	13.45
			28.2	62	14.00
			28.0	62	14.15
			28.4	60	14.30
			28.4	60	14.45
			28.4	60	15.00
			28.8	62	15.15
			29.0	66	15.30
17	2	65 ppm	26.4	60	9.20
			27.0	58	9.35
			27.4	56	9.50

continued

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
17	2	65 ppm	27.1	54	10.05
			28.2	53	10.20
			27.4	52	10.35
			27.4	50	10.50
			27.6	50	11.05
			27.8	49	11.20
			27.7	49	11.35
			27.9	49	11.50
			27.9	47	12.05
			27.8	48	12.20
			27.8	48	12.35
			27.8	48	12.50
			28.0	48	13.05
			27.8	48	13.20
			27.8	50	13.25
			27.8	50	13.50
			27.9	50	14.05
			28.0	50	14.20
			27.8	50	14.35
			28.0	51	14.50
			28.0	52	15.05
18	4	1250 ppm	24.6	66	9.20
			25.9	65	9.35
			26.4	63	9.50
			26.8	60	10.05
			26.8	58	10.20
			26.8	56	10.35
			27.0	55	10.50
			27.0	55	11.05
			26.8	54	11.20
			26.8	54	11.35
			26.8	54	11.50
			26.8	54	12.05
			26.8	54	12.20
			26.8	54	12.35
			26.8	54	12.50
			26.7	54	13.05
			26.8	54	13.20
			26.8	54	13.35
			26.9	54	13.50
			26.8	55	14.05
			26.8	55	14.20
			26.8	55	14.35
			26.8	56	14.50
			26.8	57	15.05

Appendix 5. *continued.*

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
21	4	1250 ppm	24.0	70	9.50
			26.8	68	10.05
			27.8	64	10.20
			27.8	61	10.35
			28.0	59	10.50
			28.1	58	11.05
			28.1	58	11.20
			28.1	56	11.35
			28.1	56	11.50
			28.2	56	12.05
			28.2	54	12.20
			28.2	54	12.35
			28.0	54	12.50
			27.9	53	13.05
			27.9	53	13.20
			28.2	53	13.35
			28.2	54	13.50
			28.4	54	14.05
			28.8	54	14.20
			29.4	54	14.35
			29.2	54	14.50
			29.2	56	15.05
			29.2	56	15.20
			29.6	54	15.35
			29.6	54	15.50
22	1	0 ppm	26.0	60	9.35
			27.8	59	9.50
			27.8	58	10.05
			28.0	56	10.20
			28.2	56	10.35
			28.2	56	10.50
			28.0	56	11.05
			28.0	56	11.20
			28.2	58	11.35
			28.6	56	11.50
			28.6	56	12.05
			28.8	55	12.20
			28.6	56	12.35
			28.6	56	12.50
			28.9	56	13.05
			29.2	56	13.20
			29.2	57	13.35
			29.2	58	13.50
			29.4	57	14.05
			29.4	58	14.20
			29.6	56	14.35

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
22	1	0 ppm	29.8	56	14.50
			29.8	58	15.05
			30.0	64	15.20
23	3	400 ppm	27.0	66	9.20
			27.8	58	9.35
			28.0	56	9.50
			28.0	52	10.05
			28.0	52	10.20
			28.2	50	10.35
			28.4	50	10.50
			28.5	50	11.05
			28.6	50	11.20
			28.7	50	11.35
			28.8	50	11.50
			29.0	50	12.05
			29.0	50	12.20
			29.0	50	12.35
			28.8	50	12.50
			28.8	50	13.05
			29.1	50	13.20
			29.3	50	13.50
			29.2	49	13.50
			29.2	49	14.05
			29.3	49	14.20
			29.5	50	14.35
			29.8	54	14.50
			29.8	54	15.05
24	1	0 ppm	26.2	60	9.15
			26.6	60	9.30
			27.8	60	9.45
			27.8	60	10.00
			28.0	62	10.15
			28.2	57	10.30
			28.5	56	10.45
			28.8	56	11.00
			28.8	56	11.15
			29.1	55	11.30
			29.2	55	11.45
			29.2	55	12.00
			29.0	54	12.15
			29.0	54	12.30
			29.2	56	12.45
			29.2	56	13.00
			29.1	57	13.15
			29.0	58	13.30

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
24	1	0 ppm	29.0	58	13.45
			29.2	58	14.00
			29.2	58	14.15
			29.0	60	14.30
			29.0	62	14.45
			29.6	65	15.00
25	1	0 ppmn	30.0	66	15.15
			26.4	68	9.10
			27.6	64	9.25
			28.0	64	9.40
			28.3	62	9.55
			28.0	62	10.10
			28.0	62	10.25
			28.3	60	10.40
			28.4	60	10.55
			28.3	58	11.10
			28.3	58	11.25
			28.4	58	11.40
			28.6	58	11.55
			28.6	62	12.10
			28.4	63	12.25
			28.4	64	12.40
			28.6	62	12.55
			28.8	60	13.10
			29.0	59	13.25
			28.8	58	13.40
			29.0	56	13.55
			29.0	56	14.10
			29.2	56	14.25
			29.2	56	14.40
			29.3	58	14.55
28	4	1250 ppm	27.0	65	9.50
			27.8	62	10.05
			28.0	60	10.20
			28.8	57	10.35
			28.8	56	10.50
			28.8	56	11.05
			28.8	55	11.20
			28.8	55	11.35
			28.8	54	11.50
			29.0	54	12.05
			29.0	52	12.20
			28.8	52	12.32
			28.8	51	12.50
			28.8	51	13.05

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
28	4	1250 ppm	28.8	51	13.20
			28.8	51	13.35
			29.0	51	13.50
			29.0	50	14.05
			28.8	50	14.20
			28.6	50	14.35
			28.4	49	14.50
			28.4	49	15.05
29	2	65 ppm	28.8	49	15.20
			28.8	49	15.35
			29.0	50	15.50
			27.4	64	9.45
			27.8	61	9.40
			28.8	56	9.55
			28.4	53	10.10
			28.2	50	10.25
			28.2	50	10.40
			28.2	48	10.55
			28.0	48	11.10
			28.0	46	11.25
			28.0	46	11.40
			28.0	45	11.55
			28.2	44	12.10
			28.4	44	12.25
			28.0	44	12.40
			28.0	44	12.55
			28.2	44	13.10
			28.4	44	13.25
			28.4	44	13.40
			28.2	44	13.55
			28.6	46	14.10
			28.6	48	14.25
			28.6	49	14.40
			28.6	49	14.55
			28.8	49	15.10
			28.8	49	15.25
30	3	400 ppm	27.0	57	9.10
			26.4	52	9.25
			27.8	50	9.40
			27.8	48	9.55
			28.0	48	10.10
			28.0	48	10.25
			28.4	46	10.40
			28.4	45	10.55
			28.6	44	11.10

continued

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
30	3	400 ppm	28.6	44	11.25
			28.6	42	11.40
			28.6	42	11.55
			28.6	42	12.10
			28.6	42	12.25
			28.4	42	12.40
			28.4	42	12.55
			28.6	42	13.10
			28.6	42	13.25
			28.6	42	13.40
			28.4	48	13.55
			28.6	46	14.10
			28.6	46	14.25
			28.8	46	14.40
			28.8	46	14.55
31	2	65 ppm	25.8	62	9.30
			26.4	58	9.45
			26.8	55	10.00
			27.0	53	10.15
			27.5	51	10.30
			27.4	50	10.45
			27.4	49	11.00
			27.8	48	11.15
			27.8	48	11.30
			27.8	48	11.45
			28.0	46	12.00
			28.0	46	12.15
			28.0	47	12.30
			28.2	46	12.45
			28.2	46	13.00
			28.5	46	13.15
			28.5	47	13.30
			28.6	47	13.45
			28.6	48	14.00
			28.6	50	14.15
			28.6	50	14.30
			28.6	50	14.45
			28.6	50	15.00
			29.0	51	15.15
			29.0	51	15.30
32	4	1250 ppm	27.0	58	9.45
			27.6	58	10.00
			27.6	58	10.15
			27.8	58	10.30
			27.8	56	10.45

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
32	4	1250 ppm	27.8	55	11.00
			28.0	54	11.15
			28.4	54	11.30
			28.7	55	11.45
			28.6	54	12.00
			28.6	54	12.15
			28.4	54	12.30
			28.2	53	12.45
			28.2	53	13.00
			28.4	52	13.15
			28.4	52	13.30
			28.4	52	13.45
			28.2	53	14.00
			28.4	53	14.15
			28.4	53	14.30
			28.5	53	14.45
			28.5	53	15.00
			28.8	58	15.15
			28.8	58	15.30
			28.8	58	15.45
			29.0	58	16.00
35	4	1250 ppm	27.4	62	9.40
			27.8	62	9.55
			28.4	62	10.10
			29.0	60	10.25
			29.0	60	10.40
			28.8	60	10.55
			29.0	58	11.10
			28.8	59	11.25
			28.8	59	11.40
			28.6	59	11.55
			29.0	58	12.10
			29.0	58	12.25
			29.0	57	12.40
			28.6	57	12.55
			28.2	57	13.10
			28.2	57	13.25
			28.2	56	13.40
			28.2	56	13.55
			28.2	55	14.10
			27.8	55	14.25
			27.8	55	14.40
			27.8	55	14.55
			28.0	56	15.10
			28.0	58	15.25

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
35	4	1250 ppm	28.4	60	15.40
36	1	0 ppm	24.4	66	9.05
			26.4	66	9.20
			27.0	62	9.35
			27.0	60	9.50
			27.8	57	10.05
			27.4	56	10.20
			27.6	54	10.35
			27.8	54	10.55
			27.6	54	11.05
			27.8	53	11.20
			27.1	53	11.35
			27.9	53	11.50
			28.0	52	12.05
			28.0	52	12.20
			28.0	52	12.35
			28.2	52	12.50
			28.2	52	13.05
			28.4	54	13.20
			28.4	54	13.35
			28.4	56	13.50
			28.4	56	14.05
			28.4	56	14.20
			28.4	56	14.35
			28.6	56	14.50
37	4	1250 ppm	24.0	68	9.15
			27.0	66	9.30
			27.4	62	9.45
			27.8	61	10.00
			27.8	60	10.15
			28.0	60	10.30
			28.0	58	10.45
			28.2	58	11.00
			28.2	58	11.15
			28.0	58	11.30
			28.0	58	11.45
			28.0	56	12.00
			28.0	54	12.15
			27.8	56	12.30
			27.8	56	12.45
			27.8	56	13.00
			28.0	56	13.15
			27.8	56	13.30
			27.8	56	13.45
			28.2	56	14.00

continued...

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
37	4	1250 ppm	28.0	57	14.15
			28.2	58	14.30
			28.2	58	14.45
			28.2	58	15.00
			28.0	58	15.15
38	2	65 ppm	27.0	56	9.35
			27.2	52	9.50
			27.2	50	10.05
			27.2	48	10.20
			27.4	48	10.35
			27.6	48	10.50
			27.6	46	11.05
			27.6	46	11.20
			27.6	46	11.35
			27.6	48	11.50
			27.6	47	12.05
			27.6	46	12.20
			27.6	46	12.35
			27.6	46	12.50
			27.6	46	13.05
			28.0	48	13.20
			27.8	47	13.35
			27.8	47	13.50
			27.8	49	14.05
			27.8	54	14.20
			27.6	52	14.35
			27.4	53	14.50
			27.6	52	15.05
			27.8	52	15.20
			28.0	54	15.35
39	4	1250 ppm	24.8	64	9.10
			25.8	63	9.25
			26.0	62	9.40
			26.2	60	9.55
			27.0	60	10.10
			26.6	60	10.25
			26.6	60	10.40
			26.2	59	10.55
			26.6	59	11.10
			26.6	58	11.25
			26.6	57	11.40
			26.6	56	11.55
			26.6	56	12.10
			26.8	56	12.25
			26.8	56	

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
39	4	1250 ppm	26.6	56	12.55
			26.6	56	13.10
			26.6	56	13.25
			26.8	56	13.40
			26.8	56	13.55
			26.4	56	14.10
			26.8	56	14.25
			27.2	57	14.40
			27.0	57	14.55
			27.0	58	15.10
			27.2	58	15.25
42	2	65 ppm	26.2	64	9.35
			26.8	58	9.50
			27.0	55	10.05
			27.2	52	10.20
			27.4	50	10.35
			27.2	50	10.50
			27.4	48	11.05
			27.6	48	11.20
			27.6	48	11.35
			27.6	46	11.50
			27.6	44	12.05
			27.8	44	12.20
			27.8	45	12.35
			27.8	46	12.50
			27.8	46	13.05
			27.8	46	13.20
			27.8	46	13.35
			27.8	48	13.50
			28.0	48	14.05
			28.0	48	14.20
			28.0	48	14.35
			28.2	48	14.50
			28.2	50	15.10
			28.4	50	15.20
43	3	400 ppm	25.5	63	9.10
			26.2	58	9.25
			26.4	55	9.40
			26.6	50	9.55
			26.8	50	10.10
			26.8	48	10.25
			26.8	48	10.40
			26.8	46	10.55
			26.8	46	11.10
			26.8	44	11.25

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
43	3	400 ppm	26.8	44	11.40
			27.0	44	11.55
			27.0	44	12.10
			27.0	46	12.25
			27.0	46	12.40
			26.8	46	12.55
			27.0	48	13.10
			27.0	46	13.25
			27.0	46	13.40
			27.0	46	13.55
			27.2	47	14.10
			27.0	47	14.25
			27.0	47	14.40
			27.2	46	14.55
44	1	0 ppm	27.4	78	9.25
			27.2	78	9.40
			27.4	68	9.55
			27.8	66	10.10
			27.8	62	10.25
			28.0	60	10.40
			27.8	58	10.55
			27.8	58	11.10
			27.8	56	11.25
			28.0	56	11.40
			28.0	56	11.55
			28.0	56	12.10
			27.8	55	12.25
			27.8	55	12.40
			27.8	56	12.55
			27.8	56	13.10
			27.8	56	13.25
			27.8	56	13.40
			27.8	56	13.55
			27.8	56	14.10
			28.2	58	14.25
			28.0	58	14.40
			28.0	60	14.55
			28.4	62	15.10
			28.2	68	15.25
45	4	1250 ppm	26.4	64	9.20
			27.2	63	9.35
			27.2	62	9.50
			27.2	60	10.05
			28.0	60	10.20
			27.4	60	10.35

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
45	4	1250 ppm	27.0	58	10.50
			27.8	58	11.05
			28.0	58	11.20
			27.6	58	11.35
			27.4	58	11.50
			27.6	58	12.05
			27.6	58	12.20
			27.4	58	12.35
			27.4	58	12.50
			27.4	58	13.05
			27.4	58	13.20
			27.4	58	13.35
			27.4	59	13.50
			27.4	59	14.05
			27.6	59	14.20
			27.6	59	14.35
			27.6	59	14.50
			27.6	61	15.05
46	4	1250 ppm	27.2	62	9.25
			27.4	62	9.40
			27.4	60	9.55
			27.4	60	10.10
			27.6	60	10.25
			27.8	60	10.40
			27.8	60	10.55
			27.6	60	11.10
			27.4	60	11.25
			27.2	60	11.40
			27.4	60	11.55
			27.4	59	12.10
			27.2	59	12.25
			27.2	59	12.40
			27.4	59	12.55
			27.2	59	13.10
			27.4	59	13.25
			27.2	58	13.40
			27.0	59	13.55
			27.0	60	14.10
			27.1	60	14.25
			27.4	60	14.40
			27.2	60	14.55
			27.3	60	15.10
			27.4	65	15.25
49	2	65 ppm	27.2	61	9.20
			27.4	58	9.35

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
49	2	65 ppm	27.6	56	9.50
			27.8	54	10.05
			27.8	52	10.20
			27.8	53	10.35
			28.0	50	10.50
			28.0	49	11.05
			28.0	48	11.20
			28.0	47	11.35
			28.0	47	11.50
			28.0	47	12.05
			28.2	47	12.20
			28.0	47	12.35
			28.0	48	12.50
			28.2	48	13.05
			28.2	48	13.20
			28.2	48	13.35
			28.2	48	13.50
			28.0	48	14.05
			28.0	50	14.20
			28.0	52	14.35
			28.2	52	14.50
			28.2	52	15.05
			28.2	52	15.20
50	3	400 ppm	27.2	56	9.15
			28.0	54	9.30
			28.2	52	9.45
			28.2	50	10.00
			28.0	52	10.15
			28.2	48	10.30
			28.2	48	10.45
			28.2	46	11.00
			28.0	46	11.15
			28.0	46	11.30
			28.2	46	11.45
			28.2	46	12.00
			28.0	46	12.15
			28.0	46	12.30
			27.8	46	12.45
			27.4	46	13.00
			27.4	48	13.15
			27.6	49	13.30
			27.6	49	13.45
			27.8	48	14.00
			27.8	50	14.15
			27.8	49	14.30
			28.0	48	14.45

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
50	3	400 ppm	28.0	49	15.00
			28.4	54	15.15
51	3	400 ppm	24.4	56	9.00
			27.6	54	9.15
			27.8	50	9.30
			27.8	49	9.45
			27.8	48	10.00
			28.0	48	10.15
			28.0	48	10.30
			28.0	47	10.45
			27.8	46	11.00
			28.0	45	11.15
			28.2	46	11.30
			28.0	46	11.45
			28.0	45	12.00
			28.2	45	12.15
			28.0	47	12.30
			28.0	47	12.45
			27.8	47	13.00
			28.0	48	13.15
			28.2	48	13.30
			28.0	48	13.45
			28.0	47	14.00
			28.0	47	14.15
			28.2	47	14.30
			28.4	52	14.45
			28.4	52	15.00
52	4	1250 ppm	25.8	65	9.10
			27.8	65	9.25
			28.2	65	9.40
			28.4	62	9.55
			28.4	61	10.10
			28.4	60	10.25
			28.4	60	10.40
			28.6	59	10.55
			28.4	62	11.05
			28.2	62	11.25
			28.0	62	11.40
			28.3	62	11.55
			28.4	62	12.10
			28.2	62	12.25
			28.2	62	12.40
			27.8	58	12.55
			27.8	58	13.05
			28.2	62	13.25

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
52	4	1250 ppm	28.2	62	13.40
			28.2	62	13.55
			28.2	62	14.10
			28.3	63	14.25
			28.4	63	14.40
			28.6	63	14.55
			28.0	61	15.10
53	4	1250 ppm	26.6	66	9.00
			27.6	65	9.15
			28.3	63	9.30
			28.4	61	9.45
			28.4	61	10.00
			28.2	61	10.15
			28.5	61	10.30
			28.4	61	10.45
			28.5	60	11.00
			28.7	59	11.15
			28.8	59	11.30
			28.4	58	11.45
			28.4	57	12.00
			28.2	58	12.15
			28.2	58	12.30
			28.0	58	12.45
			28.0	57	13.00
			28.0	56	13.15
			28.0	58	13.30
			28.0	58	13.45
			27.8	58	14.00
			28.0	58	14.15
			27.8	60	14.30
			28.0	60	14.45
			28.0	62	15.00
56	2	65 ppm	26.6	57	9.45
			26.8	54	10.00
			27.2	52	10.15
			27.2	52	10.30
			27.2	50	10.45
			27.2	50	11.00
			27.2	50	11.15
			27.4	50	11.30
			27.4	50	11.45
			27.2	49	12.00
			27.4	48	12.15
			27.2	48	12.30
			27.2	48	12.45

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
56	2	65 ppm	27.4	50	13.00
			27.4	50	13.15
			27.4	52	13.30
			27.2	54	13.45
			27.2	54	14.00
			27.4	53	14.15
			27.4	52	14.30
			27.4	53	14.45
			27.8	56	15.00
			28.0	56	15.15
			27.8	56	15.30
57	4	1250 ppm	24.4	60	8.55
			25.0	64	9.10
			27.0	64	9.25
			27.4	64	9.40
			27.4	64	9.55
			27.4	64	10.10
			27.6	64	10.25
			27.8	64	10.40
			27.6	64	10.55
			28.0	61	11.10
			27.6	61	11.25
			27.6	61	11.40
			27.4	61	11.55
			27.4	60	12.10
			27.4	59	12.25
			27.4	59	12.40
			27.4	59	12.55
			27.4	60	13.10
			27.6	60	13.25
			27.6	60	13.40
			27.7	60	13.55
			27.4	60	14.14
			27.4	60	14.25
			27.4	60	14.40
58	3	400 ppm	26.0	60	9.05
			26.8	56	9.20
			27.2	52	9.35
			27.6	50	9.50
			27.6	49	10.05
			27.8	48	10.20
			27.8	48	10.35
			27.8	48	10.50
			27.8	49	11.05
			28.0	47	11.20

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
58	3	400 ppm	28.0	47	11.35
			28.0	46	11.50
			28.0	46	12.05
			27.8	44	12.20
			28.0	45	12.35
			28.0	45	12.50
			28.0	45	13.05
			28.0	45	13.20
			28.0	45	13.35
			28.0	45	13.50
			28.2	46	14.05
			28.2	50	14.20
			28.4	50	14.35
			28.4	49	14.50
			28.2	48	15.05
59	1	0 ppm	25.0	68	9.00
			25.8	66	9.15
			26.4	63	9.30
			26.8	61	9.45
			27.0	60	10.00
			27.2	60	10.15
			27.0	58	10.30
			27.0	58	10.45
			27.2	58	11.00
			27.2	57	11.15
			27.2	57	11.30
			27.2	57	11.45
			27.2	57	12.00
			26.8	56	12.15
			26.8	57	12.30
			27.0	58	12.45
			27.0	60	13.00
			27.0	60	13.15
			27.0	62	13.30
			27.0	62	13.45
			26.8	61	14.00
			27.0	64	14.15
			26.8	65	14.30
			27.0	65	14.45
			27.0	65	15.00
60	1	0 ppm	26.0	64	9.10
			26.0	62	9.25
			26.4	60	9.40
			26.6	60	9.55
			26.8	58	10.10

continued

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
60	1	0 ppm	27.0	58	10.25
			27.3	54	10.40
			27.2	54	10.55
			27.2	56	11.10
			27.4	57	11.25
			27.2	56	11.40
			27.2	56	11.55
			27.2	55	12.10
			27.2	55	12.25
			27.4	56	12.40
			27.2	56	12.55
			27.2	56	13.10
			27.2	57	13.25
			27.4	60	13.40
			27.4	61	13.55
			27.4	62	14.10
			27.4	64	14.25
			27.6	64	14.40
			27.6	64	14.55
68	3	400 ppm	25.4	58	9.30
			25.8	54	9.45
			26.2	54	10.00
			26.2	51	10.15
			26.2	50	10.30
			26.4	50	10.45
			26.4	50	11.00
			26.2	48	11.15
			26.2	50	11.30
			26.2	48	11.45
			26.2	48	12.00
			26.2	47	12.15
			26.4	48	12.30
			26.2	48	12.45
			26.2	47	13.00
			26.2	47	13.15
			26.2	47	13.30
			26.2	46	13.45
			26.2	46	14.00
			26.2	46	14.15
			26.2	46	14.30
			26.4	46	14.45
			26.4	46	15.00
			26.8	46	15.15
			26.8	46	15.30

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
64	3	400 ppm	24.8	62	9.05
			26.2	58	9.20
			26.6	56	9.35
			27.7	53	9.50
			27.0	52	10.05
			27.2	52	10.20
			27.4	50	10.35
			27.4	48	10.50
			27.4	48	11.05
			27.4	48	11.20
			27.4	48	11.35
			27.6	47	11.50
			27.6	46	12.05
			27.5	47	12.20
			27.6	47	12.35
			27.7	46	12.50
			27.7	46	13.05
			27.6	46	13.20
			27.4	48	13.35
			27.7	49	13.50
			27.7	48	14.05
			27.7	48	14.20
			27.7	49	14.35
			27.7	49	14.50
65	3	400 ppm	27.2	56	9.10
			27.0	54	9.25
			27.7	53	9.40
			27.3	51	9.55
			28.0	50	10.10
			28.0	48	10.25
			27.6	48	10.40
			27.8	48	10.55
			28.3	46	11.10
			28.3	45	11.25
			27.6	45	11.40
			28.2	45	11.55
			28.4	43	12.10
			27.2	45	12.25
			28.0	46	12.40
			27.8	46	12.55
			27.6	46	13.10
			27.8	46	13.25
			27.4	46	13.40
			27.4	47	13.55
			27.6	47	14.10

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
65	3	400 ppm	27.8	48	14.25
			28.0	47	14.40
			28.0	46	14.55
66	4	1250 ppm	27.0	64	9.30
			27.2	63	9.45
			27.2	62	10.00
			27.4	61	10.15
			27.6	61	10.30
			27.6	60	10.45
			27.6	60	11.00
			27.4	60	11.15
			27.2	60	11.30
			27.4	60	11.45
			27.4	58	12.00
			27.4	58	12.15
			27.6	58	12.30
			27.5	57	12.45
			27.4	57	13.00
			27.2	57	13.15
			27.2	58	13.30
			27.2	58	13.45
			27.4	58	14.00
			27.2	58	14.15
			27.2	57	14.30
			27.2	58	14.45
			27.0	58	15.00
			27.2	58	15.15
			27.6	59	15.30
67	3	400 ppm	26.0	58	9.05
			26.6	52	9.20
			27.0	52	9.35
			27.0	52	9.50
			27.2	52	10.05
			27.2	52	10.20
			27.4	51	10.35
			27.2	50	10.50
			27.4	51	11.05
			27.4	48	11.20
			27.4	48	11.35
			27.4	47	11.50
			27.4	47	12.05
			27.4	47	12.20
			27.4	46	12.35
			27.4	47	12.55
			27.4	47	13.05

continued....

Appendix 5. continued.

Study day	Chamber number[a]	Concentration of butadiene	Temperature[b] (°C)	Relative humidity[b] (%)	Time
67	3	400 ppm	27.4	48	13.20
			27.4	49	13.35
			27.4	49	13.50
			27.4	48	14.05
			27.4	48	14.20
			27.4	49	14.35
			24.4	49	14.50
			24.4	49	15.05

[a] Temperature and relative humidity were measured in one chamber, selected at random, on each study day.

[b] Separate thermometer and solid state hygrometer used except for readings marked[c].

[c] Thermohygrometer used for temperature and relative humidity recordings, probably inaccurate for relative humidity (see text Section 6.1.4. for explanation).

[d] Readings delayed due to equipment malfunction.

Appendix 6. Mating and fertility data for individual male rats treated sub-chronically with butadiene

Male number	Female number	Period to coition (days)[a]	Pregnant/Not pregnant
Vehicle control			
1	273	3	Pregnant
	350	1	Pregnant
2	374	2	Pregnant
	341	4	Pregnant
3	207	2	Pregnant
	394	7	Pregnant
4	182	5	Not pregnant
	419	4	Pregnant
5	192	Not mated	Not pregnant
	339	1	Pregnant
6	389	3	Pregnant
	420	1	Pregnant
7	198	6	Pregnant
	362	3	Pregnant
8	376	6	Pregnant
	292	6	Not pregnant
9	416	3	Pregnant
	430	1	Pregnant
10	397	1	Pregnant
	241	7	Pregnant
11	349	5	Pregnant
	250	1	Pregnant
12	434	5	Not pregnant
	433	4	Pregnant
13	312	5	Pregnant
	346	3	Pregnant
14	191	3	Pregnant
	314	1	Pregnant
15	197	6	Pregnant
	412	4	Pregnant
16	205	5	Pregnant
	157	6	Pregnant
17	370	7	Pregnant
	328	3	Pregnant

Appendix 6. continued.

Male number	Female number	Period to coition (days)*	Pregnant/Not pregnant
Vehicle control			
18	321	4	Pregnant
	425	7	Pregnant
19	167	1	Pregnant
	291	2	Pregnant
20	280	2	Pregnant
	327	9	Pregnant
21	303	7	Pregnant
	284	1	Pregnant
22	221	10	Pregnant
	183	5	Pregnant
23	448	6	Pregnant
	421	5	Pregnant
24	396	5	Pregnant
	391	4	Pregnant
25	320	9	Pregnant
	247	7	Pregnant
65 ppm butadiene			
26	264	10	Pregnant
	160	6	Pregnant
27	293	4	Pregnant
	257	4	Pregnant
28	304	1	Pregnant
	201	1	Pregnant
29	371	4	Pregnant
	154	6	Pregnant
30	265	2	Pregnant
	242	3	Pregnant
31	359	Not mated	Not pregnant
	225	6	Pregnant
32	208	1	Pregnant
	395	4	Pregnant
33	214	7	Pregnant
	230	1	Pregnant
34	186	7	Not pregnant
	343	4	Pregnant
35	165	2	Pregnant
	271	6	Pregnant

Appendix 6. continued.

Male number	Female number	Period to coition (days)[a]	Pregnant/Not pregnant
65 ppm butadiene			
36	323	6	Pregnant
	281	4	Pregnant
37	331	Male died during butadiene exposure period (cause unknown)	
	369		
38	240	10	Pregnant
	278	5	Pregnant
39	228	4	Pregnant
	386	1	Pregnant
40	443	5	Pregnant
	363	4	Pregnant
41	344	1	Pregnant
	267	2	Pregnant
42	277	2	Pregnant
	315	1	Pregnant
43	164	1	Pregnant
	276	6	Pregnant
44	290	1	Pregnant
	187	3	Pregnant
45	429	7	Pregnant
	211	Not mated	Not pregnant
46	309	1	Pregnant
	180	1	Pregnant
47	332	7	Pregnant
	360	4	Pregnant
48	196	5	Pregnant
	224	8	Pregnant
49	189	2	Pregnant
	236	7	Pregnant
50	256	1	Pregnant
	156	4	Pregnant
400 ppm butadiene			
51	325	6	Pregnant
	310	8	Pregnant
52	246	Not mated	Not pregnant
	215	6	Pregnant
53	408	6	Not pregnant
	361	5	Pregnant

Appendix 6. continued.

Male number	Female number	Period to coition (days)*	Pregnant/Not pregnant
400 ppm butadiene			
54	174	3	Pregnant
	392	1	Pregnant
55	378	2	Pregnant
	410	2	Pregnant
56	161	4	Pregnant
	422	2	Pregnant
57	296	4	Pregnant
	405	2	Pregnant
58	407	2	Pregnant
	261	1	Pregnant
59	334	6	Pregnant
	342	3	Pregnant
60	234	6	Pregnant
	417	4	Pregnant
61	170	6	Pregnant
	199	2	Not pregnant
62	235	5	Pregnant
	194	1	Pregnant
63	383	9	Pregnant
	333	4	Pregnant
64	260	6	Pregnant
	169	3	Pregnant
65	178	5	Pregnant
	175	1	Pregnant
66	388	4	Pregnant
	212	4	Pregnant
67	355	1	Pregnant
	248	2	Pregnant
68	206	8	Pregnant
	330	8	Pregnant
69	179	2	Pregnant
	216	3	Not pregnant
70	377	1	Pregnant
	316	1	Pregnant
71	437	1	Pregnant
	404	3	Pregnant

Appendix 6. continued.

Male number	Female number	Period to coition (days)[1]	Pregnant/Not pregnant
400 ppm butadiene			
72	381	Not mated	Not pregnant
	289	9	Pregnant
73	311	8	Pregnant
	441	3	Pregnant
74	435	1	Pregnant
	418	4	Not pregnant
75	294	3	Pregnant
	152	7	Pregnant
1250 ppm butadiene			
76	329	6	Not pregnant
	319	5	Pregnant
77	288	7	Not pregnant
	399	4	Pregnant
78	283	4	Pregnant
	153	4	Pregnant
79	286	1	Not pregnant
	305	3	Pregnant
80	297	7	Pregnant
	171	4	Pregnant
81	365	7	Pregnant
	226	2	Pregnant
82	379	5	Pregnant
	336	1	Pregnant
83	450	8	Pregnant
	428	4	Pregnant
84	272	4	Pregnant
	384	4	Pregnant
85	227	4	Pregnant
	409	1	Pregnant
86	353	2	Pregnant
	406	4	Pregnant
87	354	6	Pregnant
	318	6	Pregnant
88	245	7	Pregnant
	173	4	Pregnant
89	426	5	Pregnant
	188	2	Pregnant

Appendix 6. continued. 1542/2

Male number	Female number	Period to coition (days)[a]	Pregnant/Not pregnant
1250 ppm butadiene			
90	203	1	Pregnant
	231	3	Pregnant
91	193	2	Pregnant
	258	4	Pregnant
92	162	7	Pregnant
	184	4	Pregnant
93	298	4	Pregnant
	274	4	Pregnant
94	364	8	Pregnant
	220	2	Pregnant
95	275	5	Pregnant
	335	2	Pregnant
96	223	5	Pregnant
	210	1	Pregnant
97	436	Not mated	Not pregnant
	431	6	Pregnant
98	181	3	Pregnant
	213	3	Pregnant
99	255	3	Pregnant
	375	6	Pregnant
100	307	1	Pregnant
	295	2	Pregnant
Room control			
101	322	4	Pregnant
	168	3	Pregnant
102	177	Not mated	Not pregnant
	163	4	Pregnant
103	387	Not mated	Not pregnant
	229	4	Pregnant
104	440	5	Pregnant
	252	3	Pregnant
105	302	8	Pregnant
	423	4	Pregnant
106	262	5	Pregnant
	202	5	Pregnant
107	358	7	Pregnant
	373	1	Pregnant

Appendix 6. continued.

Male number	Female number	Period to coition (days)*	Pregnant/Not pregnant
Room control			
108	357	6	Pregnant
	366	5	Pregnant
109	427	3	Pregnant
	172	4	Pregnant
110	166	1	Pregnant
	338	1	Pregnant
111	217	7	Pregnant
	442	3	Pregnant
112	401	Not mated	Not pregnant
	449	7	Pregnant
113	195	8	Pregnant
	268	3	Pregnant
114	445	4	Pregnant
	367	10	Pregnant
115	326	2	Pregnant
	400	2	Pregnant
116	287	4	Pregnant
	266	7	Pregnant
117	414	3	Pregnant
	415	6	Pregnant
118	447	7	Pregnant
	239	2	Pregnant
119	398	7	Pregnant
	249	1	Pregnant
120	393	2	Pregnant
	299	4	Pregnant
121	308	1	Pregnant
	270	1	Pregnant
122	218	5	Pregnant
	159	1	Pregnant
123	244	3	Not pregnant
	438	2	Pregnant
124	155	7	Pregnant
	222	3	Pregnant
125	411	1	Pregnant
	301	1	Pregnant

Appendix 6. continued.

Male number	Female number	Period to coition (days)*	Pregnant/Not pregnant
Room control			
126	352	5	Pregnant
	248	9	Pregnant
127	282	3	Pregnant
	337	4	Pregnant
128	324	6	Pregnant
	347	1	Pregnant
129	200	Not mated	Not pregnant
	209	7	Pregnant
130	269	3	Pregnant
	317	3	Pregnant
131	444	3	Pregnant
	151	4	Pregnant
132	340	5	Pregnant
	232	1	Pregnant
133	413	2	Pregnant
	233	2	Pregnant
134	351	4	Pregnant
	356	4	Pregnant
135	238	4	Pregnant
	253	1	Pregnant
136	390	6	Pregnant
	176	7	Pregnant
137	446	5	Pregnant
	237	1	Pregnant
138	259	5	Pregnant
	380	1	Pregnant
139	348	1	Pregnant
	439	1	Pregnant
140	263	Not mated	Not pregnant
	345	4	Pregnant
141	306	5	Pregnant
	382	4	Pregnant
142	372	7	Pregnant
	190	3	Pregnant
143	408	7	Pregnant
	219	4	Pregnant

Appendix 6. continued.

Male number	Female number	Period to coition (days)*	Pregnant/Not pregnant
Room control			
144	254	5	Pregnant
	385	6	Not pregnant
154	368	Not mated	Not pregnant
	424	6	Pregnant
146	158	5	Pregnant
	279	8	Pregnant
147	251	9	Pregnant
	204	4	Pregnant
148	285	7	Pregnant
	313	1	Pregnant
149	402	4	Pregnant
	300	4	Pregnant
150	185	4	Pregnant
	432	5	Pregnant

* Number of days from housing males and females together to finding of plug or a sperm positive vaginal smear.

Appendix 7. Dominant lethal data for individual male rats treated sub-chronically with butadiene

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
Vehicle control								
1	278	17	16	0	0	0	16	0
1	350	21	7	3	1	1	3	0
2	374	18	16	0	0	0	16	0
2	341	14	13	0	0	0	13	0
3	207	15	15	0	0	0	15	0
3	394	17	9	0	0	0	9	0
4	182					Not pregnant		
4	419	20	16	0	0	0	16	0
5	192					Not pregnant		
5	339	16	12	0	0	0	12	0
6	389	14	14	0	0	0	14	0
6	420	19	11	0	0	0	11	0
7	198	16	16	1	0	0	15	0
7	362	24	18	1	0	0	17	0
8	376	17	15	0	0	0	15	0
8	292					Not pregnant		
9	416	20	16	0	0	0	16	1*
9	430	21	17	0	0	0	17	0
10	397	21	18	0	0	0	18	0
10	241	17	17	0	0	0	17	0
11	349	20	17	1	0	0	16	0
11	250	21	21	0	0	0	21	0
12	434					Not pregnant		
12	433	15	15	1	0	0	14	0
13	312	20	19	0	0	0	19	0
13	346	21	12	1	0	0	11	0
14	191	16	15	0	0	0	15	0

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
Vehicle control								
14	314	21	15	0	1	1	14	1
15	197	18	17	1	0	0	16	0
15	412	15	12	0	1	1	11	0
16	205	25	14	1	0	0	13	0
16	157	16	16	1	0	0	15	0
17	370	13	9	0	0	0	9	0
17	328	29	17	4	0	1	12	1[b]
18	321	17	15	1	0	0	14	0
18	425	17	17	0	0	0	17	0
19	167	22	13	0	1	1	12	0
19	291	17	15	0	0	0	15	1[b]
20	280	22	18	0	0	3	15	0
20	327	23	19	1	0	0	18	0
21	303	12	10	0	0	0	10	0
21	284	21	18	0	1	1	17	0
22	221	24	24	2	0	0	22	0
22	183	15	14	0	0	0	14	0
23	448	22	15	0	0	0	15	0
23	421	21	11	0	0	0	11	0
24	396	15	14	0	0	0	14	0
24	391	16	16	0	0	0	16	0
25	320	20	15	0	0	0	15	1[c]
25	247	16	16	0	0	0	16	0
65 ppm butadiene								
26	264	15	11	1	0	0	10	0
26	160	16	15	0	0	0	15	0
27	293	15	14	-	-	-	11	-
27	257	24	14	0	0	0	14	0
28	304	24	13	1	3	3	9	0
28	201	14	14	0	0	0	14	0

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
65 ppm butadiene								
29	371	22	17	0	0	0	17	1[a]
29	154	23	19	0	1	1	18	1[d]
30	265	16	14	2	0	0	12	1[e]
30	242	18	18	0	0	0	18	0
31	359					Not pregnant		
31	225	25	16	0	0	0	16	0
32	208	16	15	0	0	0	15	0
32	395	14	13	1	0	0	12	0
33	214	15	15	1	0	0	14	0
33	230	19	14	9	1	1	4	0
34	186					Not pregnant		
34	343	14	14	0	0	0	14	0
35	165	17	10	2	0	0	8	0
35	271	18	16	2	0	0	14	1[f]
36	323	23	14	0	0	0	14	0
36	281	15	13	1	0	0	12	0
37						Male died during butadiene exposure period (cause unknown)		
38	240	21	16	0	1	1	15	0
38	278	27	14	0	0	0	14	0
39	228	14	14	0	0	0	14	0
39	336	14	5	0	0	1	4	0
40	443	16	16	0	0	0	16	0
40	363	18	15	0	0	0	15	0
41	344	17	8	0	0	0	8	0
41	267	21	17	0	0	0	17	0
42	277	24	20	0	0	0	20	0
42	315	20	5	2	1	1	2	1[g]
43	164	16	16	0	0	0	16	0
43	276	22	14	0	0	1	13	0
44	290	17	15	0	0	0	15	0

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
65 ppm butadiene								
44	187	19	19	0	0	0	19	0
45	211	16	15	0	0	0	15	0
45	429					Not pregnant		
46	309	22	7	3	0	0	4	0
46	180	17	16	0	0	0	16	0
47	332	13	13	0	0	0	13	0
47	360	15	11	0	0	0	11	0
48	196	20	15	0	0	0	15	0
48	224	13	12	1	0	0	11	0
49	189	18	15	0	0	0	15	0
49	236	16	15	0	0	0	15	0
50	256	16	8	0	0	0	8	0
50	156	17	17	0	0	0	17	0
400 ppm butadiene								
51	325	18	16	0	0	0	16	1[g]
51	310	19	17	0	0	0	17	1[h]
52	246					Not pregnant		
52	215	12	12	0	0	0	12	0
53	408					Not pregnant		
53	361	23	17	2	1	2	13	0
54	174	21	19	0	0	0	19	0
54	392	18	18	0	0	0	18	0
55	378	21	17	0	0	0	17	0
55	410	14	12	0	0	0	12	0
56	161	16	15	0	0	0	15	0
56	422	18	16	1	0	0	15	1[i]
57	296	18	15	0	0	0	15	0
57	405	16	14	0	0	0	14	0
58	407	20	12	1	0	0	11	0
58	261	19	16	2	0	0	14	0

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
400 ppm butadiene								
59	334	28	16	0	0	1	15	0
59	342	24	12	0	0	0	12	0
60	234	18	15	1	0	0	14	1[1]
60	417	17	13	1	0	0	12	0
61	170	18	16	0	1	1	15	0
61	199					Not pregnant		
62	285	17	12	0	0	1	11	1[1]
62	194	19	17	0	0	0	17	0
63	383	21	16	1	0	0	15	0
63	338	15	15	1	0	0	14	0
64	260	15	15	0	0	0	15	0
64	169	17	16	0	0	0	16	0
65	178	15	15	0	0	0	15	0
65	175	17	13	1	0	0	12	0
66	388	17	16	0	0	0	16	0
66	212	12	11	0	0	0	11	0
67	355	18	17	0	1	1	16	0
67	243	20	17	0	0	0	17	0
68	206	18	15	0	0	0	15	0
68	330	12	1	0	0	0	1	0
69	179	15	14	0	0	0	14	0
69	216					Not pregnant		
70	377	17	14	0	0	0	14	0
70	316	17	14	0	0	0	14	0
71	487	[illegible]	22	4	0	0	18	0
71	404	21	17	0	0	0	17	0
72	381					Not pregnant		
72	289	21	8	0	0	0	8	0
73	311	18	18	1	0	0	17	0
73	441	18	18	0	0	0	18	0

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
400 ppm butadiene								
74	435	15	14	0	0	0	14	0
74	418					Not pregnant		
75	294	16	10	0	0	0	10	0
75	152	21	17	0	0	0	17	0
1250 ppm butadiene								
76	329					Not pregnant		
76	319	16	15	0	0	0	15	0
77	288					Not pregnant		
77	399	15	15	0	0	0	15	0
78	283	13	13	1	0	0	12	0
78	153	19	17	0	0	0	17	0
79	286					Not pregnant		
79	305	17	14	1	0	0	13	0
80	297	20	15	1	0	0	14	1
80	171	16	16	0	0	0	16	0
81	365	21	19	0	7	7	12	0
81	226	22	17	4	0	0	13	0
82	379	21	11	0	0	0	11	0
82	336	17	16	0	0	0	16	0
83	450	20	15	3	3	3	9	0
83	428	23	16	1	1	1	14	0
84	272	23	17	0	0	0	17	0
84	384	15	15	0	0	0	15	0
85	227	23	18	0	0	0	18	0
85	409	17	12	0	1	1	11	0
86	353	27	15	1	0	0	14	0
86	406	17	15	0	0	0	15	0
87	354	19	16	1	0	1	14	0
87	318	14	13	0	1	1	12	0
88	245	14	13	2	0	0	11	0

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
1250 ppm butadiene								
88	173	19	13	1	0	0	12	0
89	426	16	1	0	0	1	0	0
89	188	13	11	0	0	0	11	0
90	203	16	5	0	0	0	5	0
90	231	16	16	0	0	0	16	0
91	193	15	14	0	0	0	14	0
91	258	20	19	0	0	0	19	0
92	162	15	13	0	0	0	13	0
92	184	14	13	1	0	0	12	0
93	298	20	15	0	0	0	15	1*
93	274	12	11	1	0	0	10	0
94	364	18	16	2	0	0	14	0
94	220	17	17	0	0	0	17	0
95	275	16	15	1	0	0	14	0
95	335	22	17	4	0	0	13	0
96	223	19	12	0	0	0	12	0
96	210	18	18	0	0	0	18	0
97	436					Not pregnant		
97	431	19	16	1	0	0	15	0
98	181	24	13	0	0	0	13	0
98	213	23	5	0	0	0	5	0
99	255	17	14	0	0	0	14	0
99	375	21	17	-	-	-	16	-
100	307	15	13	0	0	0	13	0
100	295	20	18	5	1	1	12	0
Room control								
101	322	17	14	0	0	0	14	0
101	168	15	15	0	0	0	15	0
102	177					Not pregnant		

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
Room control								
102	163	19	17	1	0	0	16	1[l]
103	387					Not pregnant		
103	229	18	17	0	0	0	17	0
104	440	15	13	1	0	0	12	1[m]
104	252	19	13	1	0	0	12	0
105	303	18	17	0	0	0	17	0
105	423	20	18	0	0	0	18	0
106	262	16	16	1	0	0	15	0
106	202	19	19	0	0	0	19	0
107	358	20	16	0	5	5	11	1[n]
107	373	17	12	1	1	1	10	0
108	357	25	18	0	0	0	18	0
108	366	16	11	0	0	0	11	0
109	427	17	16	0	1	1	15	0
109	172	14	12	0	0	0	12	0
110	166	17	10	5	0	0	5	0
110	338	-	11	1	0	0	10	1[b]
111	217	19	19	1	0	0	18	0
111	442	38	14	6	2	2	6	0
112	401					Not pregnant		
112	449	17	15	2	0	0	13	0
113	195	18	14	0	1	1	13	1[m]
113	268	18	18	2	1	1	15	0
114	445	15	14	0	0	0	14	0
114	367	20	17	2	0	0	15	0
115	326	21	14	1	0	0	13	0
115	400	23	12	2	0	0	10	0
116	287	21	16	1	0	0	15	0
116	266	15	14	0	1	1	13	0
117	414	28	16	0	0	0	16	0

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
Room control								
117	415	20	18	0	0	0	18	0
118	447	20	17	0	0	0	17	0
118	239	19	19	0	0	0	19	0
119	398	15	15	0	0	0	15	0
119	249	19	18	0	1	1	17	0
120	393	21	17	1	0	0	16	0
120	299	16	15	0	0	0	15	0
121	308	23	18	0	0	0	18	0
121	270	17	8	0	0	0	8	0
122	218	17	16	0	0	0	16	0
122	159	[illegible]	20	4	0	0	20	0
123	244					Not pregnant		
123	488	17	15	0	0	0	15	0
124	155	12	8	1	0	0	7	0
124	222	13	12	0	0	0	12	0
125	411	16	16	0	0	1	15	0
125	301	23	13	0	0	0	13	0
126	352	21	13	0	0	0	13	0
126	248	[illegible]	19	3	0	0	16	0
127	232	20	17	0	0	0	17	0
127	337	21	15	1	0	0	14	0
128	324	15	14	0	0	0	14	0
128	347	18	17	0	0	0	17	0
129	200					Not pregnant		
129	209	18	15	0	0	0	15	0
130	269	18	16	0	0	0	16	0
130	317	17	16	1	1	1	14	0
131	444	15	14	0	0	0	14	0
131	151	14	14	0	0	0	14	0
132	340	17	8	1	0	0	7	0

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
Room control								
132	232	17	15	1	0	0	14	0
133	413	19	19	1	0	0	18	0
133	233	21	20	1	0	0	19	0
134	351	17	16	1	1	1	14	1[e]
134	356	19	10	0	0	0	10	0
135	238	19	18	0	0	0	18	1[b]
135	253	18	14	0	0	0	14	0
136	390	16	16	1	0	0	15	0
136	176	21	8	0	0	0	8	0
137	446	19	16	0	0	0	16	0
137	237	26	18	0	0	0	18	1[f]
138	259	-	17	2	0	0	15	0
138	380	17	14	0	0	0	14	0
139	348	14	12	0	0	0	12	0
139	439	12	6	0	0	0	6	0
140	268					Not pregnant		
140	345	15	13	0	0	0	13	0
141	306	29	5	2	0	0	3	0
141	382	16	15	0	0	0	15	0
142	372	17	17	1	0	0	16	0
142	190	29	18	1	0	0	17	0
143	408	18	16	0	0	0	16	0
143	219	16	15	0	0	0	15	0
144	219					Not pregnant		
144	254	19	17	0	0	0	17	0
145	424	16	15	0	0	0	15	0
145	368					Not pregnant		
146	279	20	18	1	0	1	16	1[b]
146	158	21	19	1	0	0	18	1[c]
147	251	14	14	0	0	0	14	0

Appendix 7. continued.

Male number	Female number	No. of corpora lutea	No. of implantation sites	No. of early deaths	No. of late deaths	No. of late deaths including dead foetuses	No. of live implants	No. of abnormal foetuses
Room control								
147	204	19	19	0	0	0	19	0
148	285	16	8	0	0	0	8	0
148	313	20	18	0	0	0	18	0
149	402	19	17	0	0	0	17	0
149	300	16	14	0	0	0	14	0
150	185	17	14	0	0	0	14	0
150	432	23	8	0	0	0	8	0

Key:

\- Missing data due to female having delivered before date due for necropsy.
[a] runt (67% of mean body weight of others in litter)
[b] runt (71% of mean body weight of others in litter)
[c] runt (57% of mean body weight of others in litter)
[d] runt (41% of mean body weight of others in litter)
[e] runt (74% of mean body weight of others in litter)
[f] runt (72% of mean body weight of others in litter)
[g] gastroschisis
[h] runt (75% of mean body weight of others in litter)
[i] runt (73% of mean body weight of others in litter)
[j] runt (64% of mean body weight of others in litter)
[k] runt (52% of mean body weight of others in litter)
[l] runt (62% of mean body weight of others in litter)
[m] runt (68% of mean body weight of others in litter)
[n] runt (69% of mean body weight of others in litter)
[o] runt (55% of mean body weight of others in litter)
[p] scoliosis in lumbar and sacral spinal column
[q] runt (75% of mean body weight of others in litter)
[r] Missing data due to technical error.