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**Health and Environmental
Sciences Department**

A Preliminary Study of the Effect of Toluene on Pregnancy of the Rat (Inhalation Exposure)

JUNE 1993

TOXICOLOGY REPORT NUMBER TR400

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The full study, utilizing dose levels based on this study, can be found in report TR401, "Toluene: The Effect on Pregnancy of the Rat (Inhalation Exposure)."

APT 1/91309

A PRELIMINARY STUDY OF THE EFFECT
OF TOLUENE ON
PREGNANCY OF THE RAT
(INHALATION EXPOSURE)

Addressee:

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AUTHORS' SIGNATURE PAGE

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.



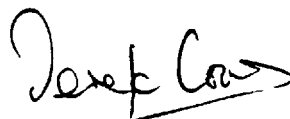
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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

HRC Report No. APT 1/91309

To the best of my knowledge and belief the Study described in this Report was conducted in compliance with the following Good Laboratory Practice Standards and I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health, 1989.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

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3.9.92

Date

DEPARTMENT OF QUALITY ASSURANCE

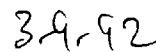
REPORT AUDIT STATEMENT

HRC REPORT No. APT 1/91309

This report has been audited by HRC Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

Date of reporting Audit Findings to
the Study Director and HRC Management

16.10.91



Peter H.C.V. Richold, B.Sc.,
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P.R. QA.3 (1)

DEPARTMENT OF QUALITY ASSURANCE

DATES OF STUDY INSPECTIONS

HRC REPORT No. APT 1/91309

Inspections were made by the Quality Assurance Department of the various phases of the study described in this report. The dates on which the inspections were made and the dates on which the findings were reported to the Study Director and to HRC Management are given below.

Phase of Study	Date of Inspection*	Date of Reporting
Protocol Review	-	16.08.90
Pre-experimental Period		
Experimental Period	15.08.90	16.08.90
	31.08.90	31.08.90
	07.09.90	10.09.90

 3.9.92

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*Week beginning. This has been adopted to avoid the presentation of excessive dates in instances of an inspection being conducted over several days.

Dates on which any 'process-based' inspections were made while the study was in progress are not included in the above listing.

P.R. Q.A.3 (2)

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SUMMARY

1. In this assessment of the effect of Toluene, a solvent, on the pregnancy and in utero development of the rat, dosages of 0, 500, 1000, 2000, 3500 and 5000 ppm were administered by inhalation for a period of 6 hours a day from Day 6 to 15 of pregnancy inclusive. On Day 20, surviving females were sacrificed, subjected to post mortem examination with liver weights being recorded, litter values were determined and fetuses were examined externally.
2. Exposure to Toluene was associated with the following maternal effects which were generally dosage-related in degree:

5000 ppm

- one mortality after first exposure.
- marked signs indicative of a gradual narcosis mainly confined to the period of direct exposure.
- marked increase in water consumption and reduction in food consumption.
- initial bodyweight loss followed by retarded weight gains.

3500 ppm

- moderate signs indicative of a gradual narcosis mainly confined to the period of direct exposure.
- marked increase in water consumption and reduction in food consumption.
- reductions in bodyweight gains.

2000 ppm

- an initial reduction in food intake and increase in water intake.
- retardations in bodyweight gains.

1000 ppm

- slight initial retardation in bodyweight gain.

500 ppm

- slight initial retardation in bodyweight gain.

3. Examination of the litter data revealed:

6 animals at 5000 ppm with total resorptions.

Among litters surviving to termination, a marked increase in post implantation losses at 5000 ppm, a marked reduction in litter and mean foetal weights at 5000 and, to a lesser extent, 3500 ppm. Litter parameters at 500, 1000 and 2000 ppm were generally comparable to controls.

Conclusion

Within the confines of the study, exposure to Toluene was associated with clear signs of maternal toxicity at 2000 ppm and above. Signs of embryofoetal toxicity were observed at 5000 and, to a lesser extent, 3500 ppm.

In view of these effects it can be concluded that a suitable high dosage for an ensuing embryofoetal toxicity study should not exceed 3500 ppm.

INTRODUCTION

This report describes a preliminary experiment performed in the pregnant rat to determine appropriate exposure levels of Toluene, a solvent, for future investigation of embryofetal toxicity in the rat when administered via the inhalation route from Days 6 to 15 of pregnancy inclusive.

The inhalation route of administration, was chosen by the Sponsor as the most likely route of exposure to man. The exposure levels were chosen by the Sponsor following a review of currently available information.

Toluene was supplied from BDH Ltd., in fifty-seven 2.5 litre bottles. It was supplied as a clear colourless liquid, purity 99.9%, batch number 2212770L and was received in these laboratories on 16 July 1990, and was stored in the dark under ambient temperature. This batch was used throughout the study.

Key dates of the study were as follows:

Protocol approval by:

Study Director:	9 August 1990
HRC Management:	9 August 1990
Sponsor (by fax):	13 August 1990

Arrival of animals:	19 July 1990
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Mating

First animals:	6/7 August 1990
Last animals	20/21 August 1990

Commencement of treatment:	13 August 1990
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End of treatment:	5 September 1990
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Terminal sacrifice

First animals:	27 August 1990
Last animals:	10 September 1990

PROCEDURE

Animal management and accommodation

Sexually mature (10 - 13 weeks old, weight range 194 - 268 g) Specific Pathogen Free female rats (Cr1: CD R (SD) BR VAF/Plus strain) which were time-mated to identified males of the same strain, in the Reproductive Toxicology facility were forwarded to the Inhalation Toxicity facility. The day of mating, as judged by the appearance of sperm in the vaginal smear or by the presence of a vaginal plug, was considered as Day 0 of pregnancy.

Time-mated animals were assigned to six groups on Day 0 of pregnancy taking into account, where possible, the distribution of males to which the females were mated and the bodyweight of the animals. Following allocation, the animals were ear tattooed to give individual identification. Prior to the commencement of treatment, all animals were inspected by a Veterinary Officer.

Animal room controls for temperature and relative humidity were set at $21 \pm 3^{\circ}\text{C}$ and $55 \pm 15\%$ respectively. During the course of the study recorded values for temperature ranged from $21 \pm 3^{\circ}\text{C}$ and for relative humidity $61 \pm 17\%$. Lighting was controlled to give 12 hours light (8 am to 8 pm) and 12 hours dark per 24 hours.

The animals were individually housed in suspended stainless steel cages equipped with solid and mesh sides and mesh floor. The cages constituting each treatment group were held on separate batteries, each in a separate ventilated cabinet in order to minimise the possibility of inhalation of test substance vapour from the fur of, or exhaled by, rats in other test groups.

Throughout the study, each cage was identified by a label coloured according to the group and recording the study schedule number, animal numbers, details of treatment and the name of the Study Supervisor. The dates treatment commenced and terminated were identified on each cage label.

All animals were given free access to Biosure Laboratory Animal Diet No. 1 and to tap water other than during inhalation exposure when food and water were withheld. (Quality Assurance Aspects for Food and Water are presented in Addenda 4 and 5).

Experimental design

The experimental design was as follows:

Group/ colour code	Exposure level Toluene (ppm)	No. of rats ♀	Animal numbers
1: White	Control	12	1 - 12
2: Yellow	500	12	13 - 24
3: Blue	1000	12	25 - 36
4: Green	2000	12	37 - 48
5: Orange	3500	12	49 - 60
6: Red	5000	12	61 - 72

Exposure of rats to the test substanceDosing regimen

A 6-hour daily whole-body exposure to the test substance commenced on Day 6 of pregnancy and continued up to and including Day 15 of pregnancy. The 6-hour daily exposure was timed from the time taken for the concentration of Toluene in the chamber to reach 90% of the theoretical equilibrium concentrations. This time to 90% (T_{90}) was estimated as follows:

$$T_{90} = \frac{V}{Q} \times \text{Logn} \left\{ \frac{100}{100-90} \right\}$$

where V = chamber volume (litres)

Q = chamber air flow rate (litres/min)

Observation

The following observations were made during the study:

1. Parent animals

(a) Signs

All animals were regularly handled and observed daily for obvious changes or signs of reaction to treatment. During the treatment period signs were recorded prior to exposure and post exposure. All animals were also observed where possible at intervals during exposure, any adverse signs, together with responses to tapping on the inhalation chamber walls were reported. The times of observations were recorded.

(b) Mortalities

All animals that died were weighed and subjected to post mortem examination. Pregnancy status was assessed.

(c) Food and water consumption

Food consumption was measured from weighday to weighday.

Water consumption was measured daily from Day 0 of pregnancy through to termination.

(d) Bodyweights

All animals were weighed initially (= Day 0 of pregnancy) and on Days 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

2. Litter data and foetal examinations

On Day 20 of pregnancy the animals were killed by CO₂ asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The livers of all animals were weighed and preserved in formalin. Gravid uterine weights were recorded for all animals. The ovaries and uteri were examined immediately to determine:

- (a) number of corpora lutea
- (b) number and distribution of live young
- (c) number and distribution of embryofoetal deaths
- (d) individual foetal weight from which the litter weight was calculated
- (e) foetal abnormalities

Embryofoetal deaths were classified as:

Early: only placenta visible at termination.

Late: both placental and embryonic remnants visible at termination. Uteri or individual uterine horns without visible implantations were subjected to the Salewski test to reveal evidence of embryonic death at very early stages of implantation.^a

Live young were examined externally, weighed and preserved against the contingency of further examinations. The foetuses were processed, sex ratios determined, but not examined.

Assessment of results

Individual litter values

In assessing litter parameters, pre-implantation loss was calculated as a percentage from the formula:

$$\frac{(\text{No. of corpora lutea} - \text{no. of implantations})}{\text{No. of corpora lutea}} \times 100$$

Post implantation loss was similarly calculated from the formula:

$$\frac{(\text{No. of implantations} - \text{no. of live young})}{\text{No. of implantations}} \times 100$$

Litter weight and mean foetal weight were calculated from individual foetal weight.

Group values

Group mean values calculated from individual litter values were presented where appropriate in two ways:

Mean A: included all valid data from surviving animals that provided evidence of pregnancy including those showing total resorption.

Mean B: included valid data from any animals with viable young at termination.

This approach allows assessment in terms of an overall effect of treatment as well as the effect of treatment on surviving litters.

For litter and mean pup weights only Mean B values or the equivalent were calculated.

Statistical analyses were not performed in view of the low group size.

Location of study records

All specimens, raw data and other documents generated at HRC during the course of this study together with a copy of the final report, are lodged in the Huntingdon Research Centre Ltd., Archives, Huntingdon, England.

Any such material arising from investigations made by the Sponsor, the findings of which are included in the final report, will be retained by the Sponsor.

All study-related specimens and raw data lodged in the Huntingdon Research Centre Ltd., Archives will be kept for ten years after the issue of the final report and then discarded. The Sponsor will be notified of this date and given the option of receiving this material into their own archives.

A hard copy of the final bound report will be kept in the Huntingdon Research Centre, Archives.

References

a Salewski, E. 1964

Farbemethode zum makroskopischen
Nachweis von Implantationsstellen am
Uterus der Ratte. Naunyn-Schmiedebergs
Arch. exp. Pathol. Pharmacol. 247:367

RESULTS

1. PARENT ANIMALS

Exposure concentrations: 0, 500, 1000, 2000, 3500, 5000 ppm
Toluene vapour (Addendum 1).

Treatment period: Days 6 to 15 of pregnancy inclusive.

Assessment of effects at 5000 ppm include all pregnant animals (including 6 females showing total resorption of litters). Assessment at lower concentrations include females with live young at termination.

(a) Clinical signs and mortalities (Table 1, Appendices 1 and 2, Addendum 1)

Clinical signs associated with treatment were apparent at 3500 and 5000 ppm and were mainly confined to or immediately after the 6 hour daily exposure period. These signs consisted mainly of those indicative of a gradual narcosis with a degree of anaesthesia and increased in severity and duration with dosages. Related signs persisted through to initial examination the following day in 3 animals (1 at 3500 ppm and 2 at 5000 ppm for 2 and 1 days respectively). There were no signs considered related to treatment at 500, 1000 and 2000 ppm.

There was one mortality at 5000 ppm. A female (No. 71) was found dead after the first exposure. Post mortem examination did not reveal any macroscopic changes that were clearly attributable to treatment and confirmed the pregnancy status.

(b) Water consumption (Figure 1, Table 2, Appendix 3)

There was a dosage-related increase in water consumption apparent at 2000 ppm and above from Day 8 of pregnancy. This response, which was considered marked at 3500 and 5000 ppm, generally increased in magnitude throughout the exposure period and persisted for two days after treatment was terminated (Day 17). Thereafter the degree of response lessened, with intake at 2000 ppm, attaining parity with controls by termination. Water consumption at 500 and 1000 ppm was generally comparable to controls.

(c) Food consumption (Figure 2, Table 3, Appendix 4)

There was an initial marked reduction in food consumption at 3500 and 5000 ppm during the first 2 days of treatment (Days 6 - 8 of pregnancy). Thereafter, although the magnitude of the response gradually lessened, intake remained lower than controls until Day 15. Food intake during the post treatment period was not overtly affected at 3500 and 5000 ppm. Food intake at 2000 ppm was reduced from Day 6 of pregnancy through to Day 11. Food intake at 500 and 1000 ppm was generally comparable to controls.

(d) Bodyweights (Figure 3, Table 4, Appendix 5)

At 5000 ppm, bodyweight loss persisted following the start of treatment through to Day 10 of pregnancy. Thereafter, the rate of weight gain was lower than controls with a further period of weight loss between Days 14 and 16 of pregnancy. This effect on weight change is probably attributable to the high incidence of embryonic losses occurring in these animals.

At 3500 ppm, weight loss was similarly evident following the onset of treatment and although weight gain was apparent from Day 8 of pregnancy, this was reduced throughout the remainder of the exposure period when compared with the controls. After the exposure period had ended, the rate of weight gain was comparable with the controls.

At 2000 ppm, no weight gain was apparent during the first two days of exposure; thereafter the pattern of response was similar to that at 3500 ppm.

At lower exposures, there was a slight reduction in weight gain following the onset of treatment; from Day 8/10 however, weight gains were comparable with the controls.

(e) Terminal post mortem examination (Appendix 1)

No gross macroscopic findings indicative of an effect of treatment were noted at post mortem examination.

There were no consistent treatment-related effects on absolute liver weights at termination, especially when the differing pregnancy status in the groups is accounted for.

2. LITTER DATA (Tables 6 and 7, Appendices 7 - 9)

At 5000 ppm, there was an increase in both pre- and post implantation loss which culminated in 6/9 pregnant animals losing their entire litters and mean post implantation loss of 52% in the remaining 3 litters. Litter size was approximately half that of the controls at Day 20 sacrifice. Litter and mean foetal weights in surviving litters were markedly reduced. Gross macroscopic examination of the 20 fetuses at termination revealed marked subcutaneous oedema in 1 fetus.

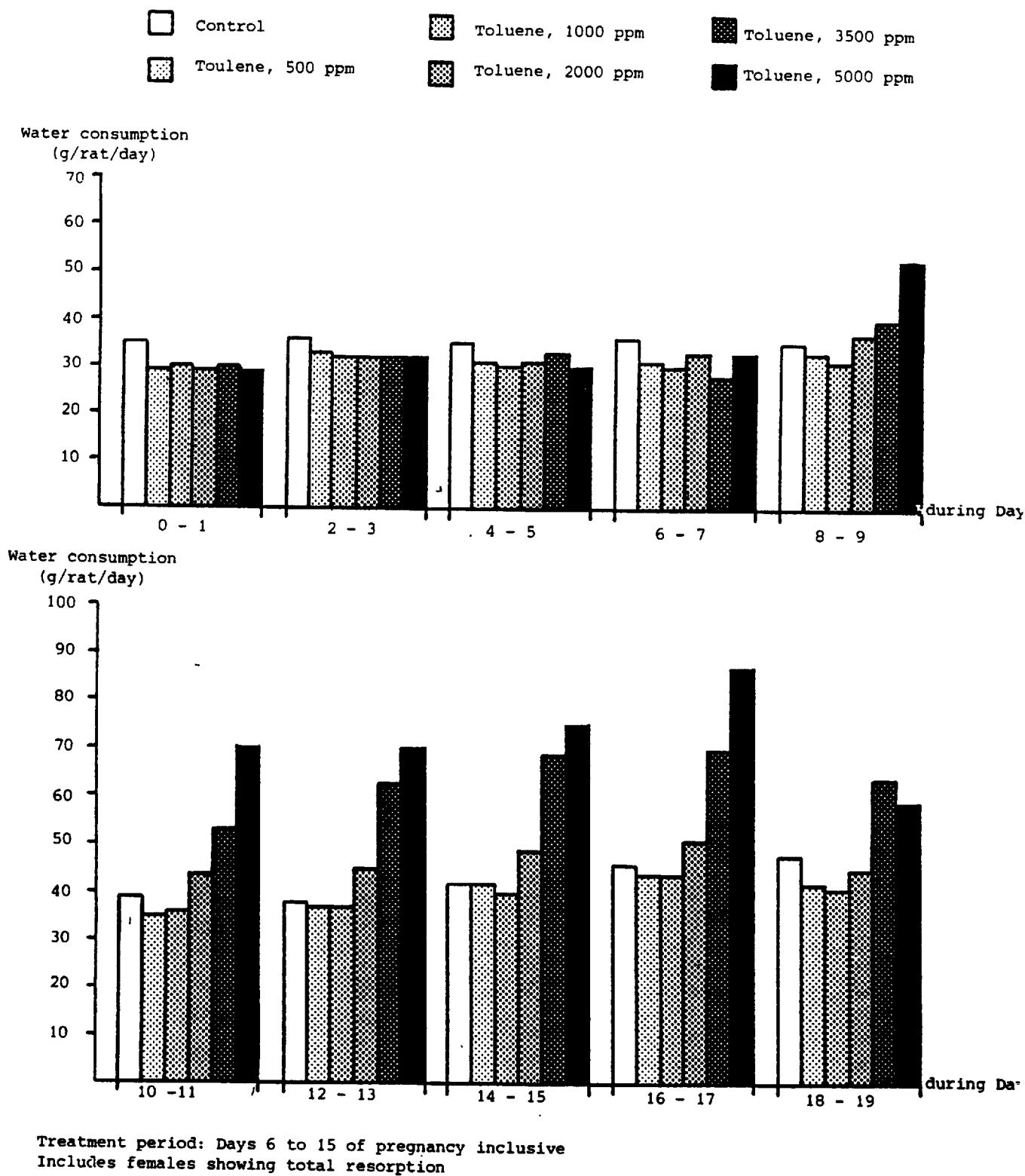
At 3500 ppm and, to a lesser extent 2000 ppm, there was a slight increase in embryonic losses (with subsequent increase in post implantation loss) resulting in a marginally lower litter size. Litter weight was lower than controls at both concentrations; mean foetal weight was reduced at 3500 ppm only when compared with the controls. 14/15 fetuses in one litter and 1/15 fetuses in a second litter at 3500 ppm had craniofacial abnormalities.

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At 500 and 1000 ppm, there were no adverse effects of exposure to Toluene on embryonic development as assessed in this study. 2/13 fetuses in one litter at 1000 ppm had craniofacial abnormalities.

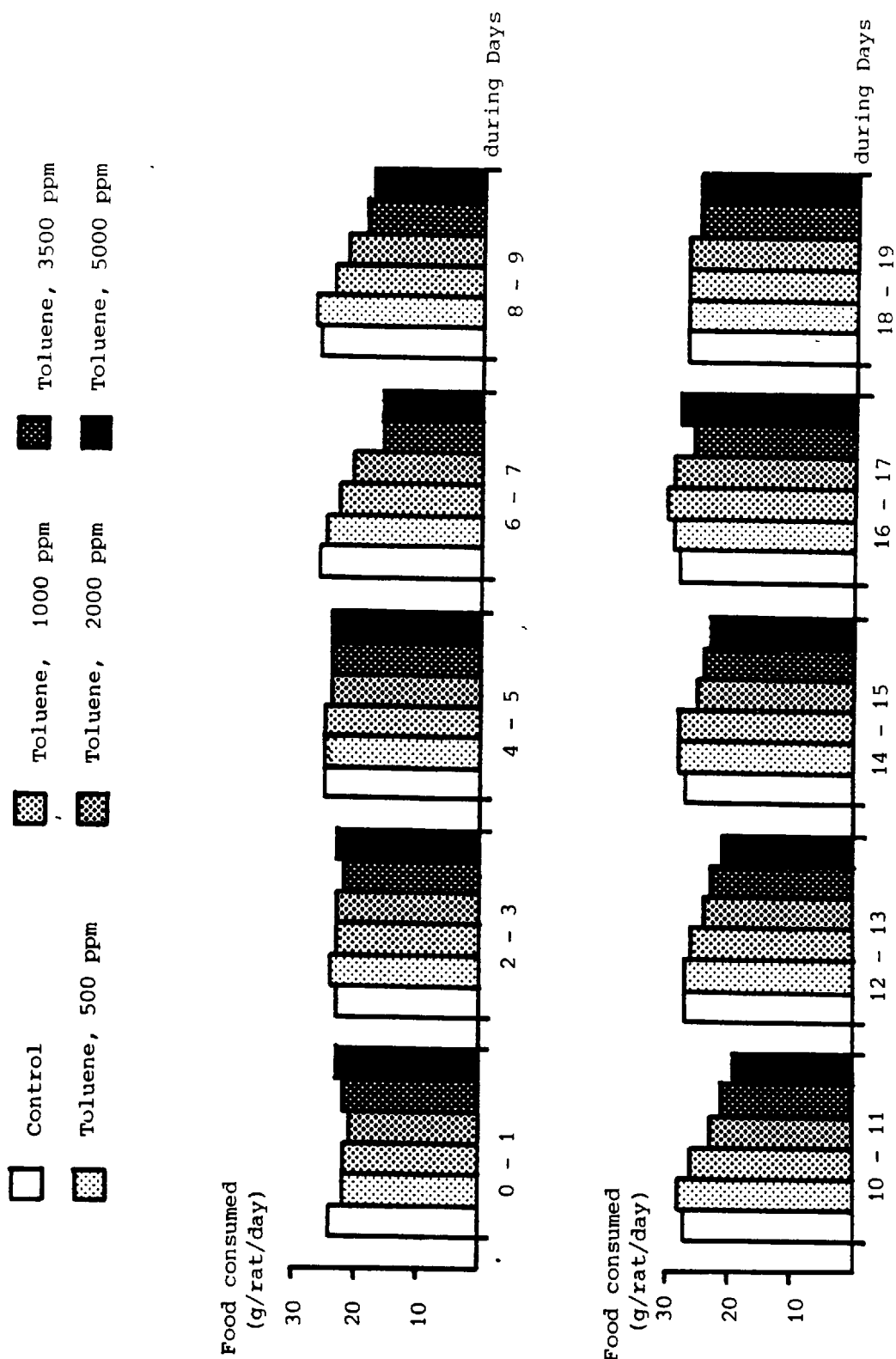
FIGURE 1

Water consumption of pregnant females - group mean values



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FIGURE 2
Food consumption of pregnant females - group mean values

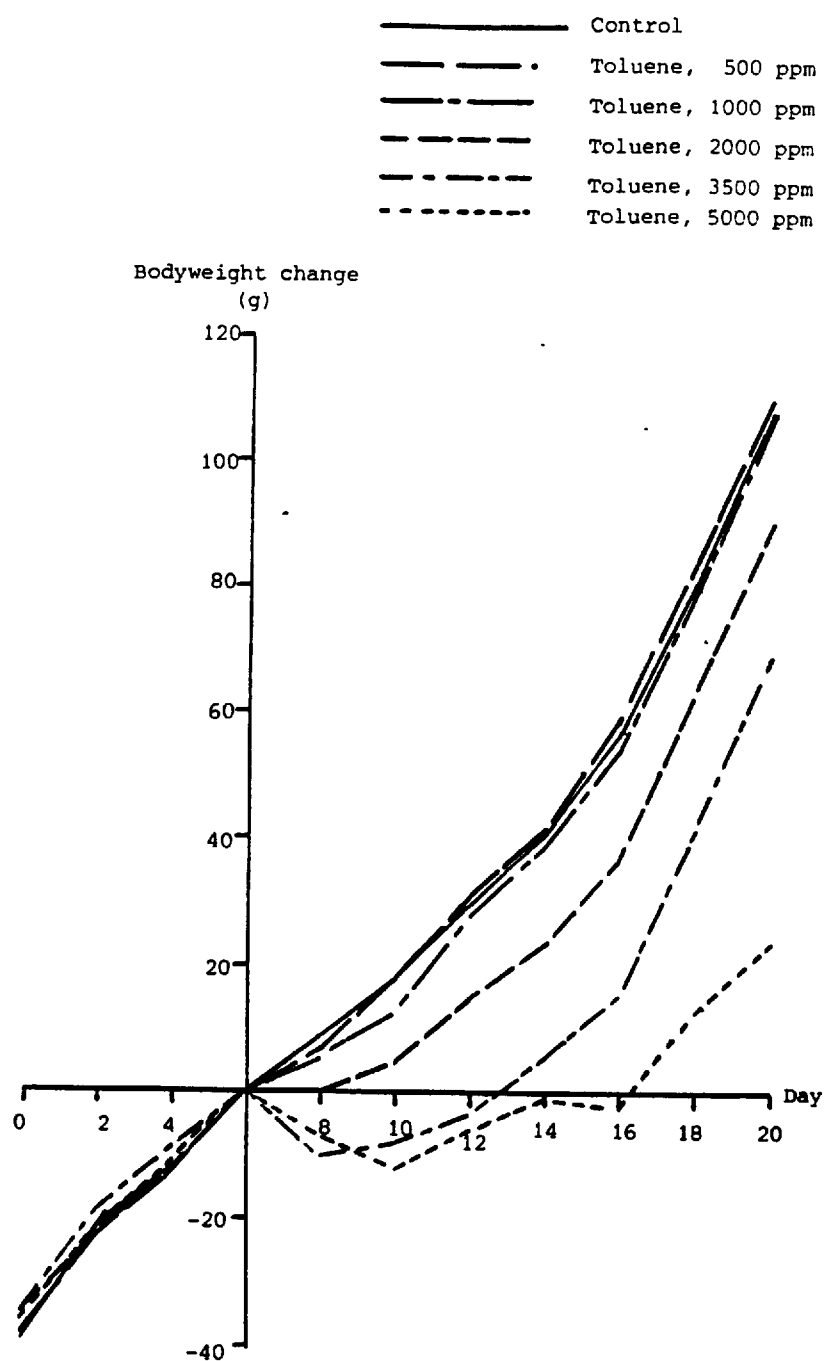


Treatment period Days 6 to 15 of pregnancy inclusive
Includes females showing total resorption

APT/1

FIGURE 3

Bodyweight change of pregnant females - group mean values



Treatment period Days 6 to 15 inclusive
Includes females showing total resorption

APT/1

TABLE 1
Adult performance - summary

Group:	1	2	3	4	5	6
Compound:	Control			Toluene		
Dosage (ppm):	-	500	1000	2000	3500	5000

Category	Number of animals in group					
	1	2	3	4	5	6
Animals mated	12	12	12	12	12	12
Died	-	-	-	-	-	1
Total resorption	-	-	-	-	-	6
Non-pregnant	1	2	1	-	2	2
With live young Day 20	11	10	11	12	10	3

TABLE 2

Water consumption - animals with live young - group mean values

Group:	1	2	3	4	5	6
Compound:	Control			Toluene		
Dosage (ppm):	-	500	1000	2000	3500	5000

Group	Number of animals	Water consumption (g/rat/day) during Days of gestation									
		0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19
1	11	35	36 ²	35 ²	36 ²	35 ²	39 ²	38	42	46	48
2	10	29	33	31	31	33	35	37	42	44	42
3	11	30	32	30	30	31	36 ¹	37	40	44	41
4	12	29	32	31	33	37	44	45	49	51	45
5	10	30	32	33	28	40	53	63	69	70	64
6	A=9	29	32	30	33	53	70	70	75	87	59
	B=3	28	33	33	36	62	80	70	80	99	65

Superscript indicates the number of values excluded
Treatment period Days 6 to 15 of pregnancy inclusive

TABLE 3

Food consumption - animals with live young - group mean values

Group:	1	2	3	4	5	6
Compound:	Control			Toluene		
Dosage (ppm):	-	500	1000	2000	3500	5000

Group	Number of animals	Food consumption (g/rat/day) during Days of gestation									
		0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19
1	11	24	23	25	26	26	27	27	27	28	27
2	10	22	24	25	25	27	28	27	28	29	27
3	11	22	23	25	23	24	26	26	28	30	27
4	12	21	23	24	21	22	23	24	25	29	27
5	10	22	22	24	16	19	21	23	24	26	25
6	A=9	23	23	24	16	18	19	21	23	28	25
	B=3	22	22	24	17	19	21	21	24	28	25

Treatment period Days 6 to 15 of pregnancy inclusive

TABLE 4

Bodyweights - animals with live young - group mean values

Group:	1	2	3	4	5	6
Compound:	Control			Toluene		
Dosage (ppm):	-	500	1000	2000	3500	5000

Group	Number of animals	Bodyweight (g) at Day of gestation											
		0	2	4	6	8	10	12	14	16	18	20	
1	11	226	244	253	265	274	283	295	306	322	346	374	
2	10	226	241	251	264	271	282	295	306	323	348	375	
3	11	226	241	251	264	270	279 ¹	292	303	318	344	371	
4	12	224	237	247	259	259	264	274	283	296	323	349	
5	10	223	239	249	258	248	250	255	264	274	300	327	
6	A=9	225	239	250	261	254	249	255	259 ¹	259	274	285	
	B=3	221	234	244	255	251	247	256	262 ¹	264	285	299	

¹ Excludes one animal with spurious weight
Treatment period Days 6 to 15 of pregnancy inclusive

APT/1

TABLE 5

Liver weights - group mean values

Group:	1	2	3	4	5	6
Compound:	Control			Toluene		
Dosage (ppm):	-	500	1000	2000	3500	5000

Group	Number of animals	Weight at Day 20	
		Bodyweight (g)	Liver weight (g)
1	11	374	16.54
2	10	375	16.50
3	11	371	16.15
4	12	349	15.83
5	10	327	14.89
6	A=9	285	15.02
	B=3	299	15.66

TABLE 6

Litter data - group mean values

Group:	1	2	3	4	5	6
Compound:	Toluene					
Dosage (ppm):	-	500	1000	2000	3500	5000

Group	Number of animals	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths		Post implant loss %	Total live young	Gravid uterine weight (g)	Litter weight (g)	Mean foetal weight (g)	
					Early	Late	Total					
1	11	16.1	14.3	10.0	0.5	0.1	0.6	4.4	13.6	70.96	45.46	3.33
2	10	16.8	15.1	8.9	0.6	0.0	0.6	3.9	14.5	73.43	47.51	3.28
3	11	15.9	13.5	13.0	0.5	0.0	0.5	3.5	13.0	66.02	42.12	3.23
4	12	14.8	13.3	11.7	0.5	0.5	1.0	8.6	12.3	60.47	39.79	3.29
5	10	16.1	14.1	12.4	0.6	0.9	1.5	10.4	12.6	53.78	33.98	2.70
6	A=9 B=3	14.6 ¹ 16.3	11.3 13.7	22.4 ¹ 13.9	8.8 6.0	0.3 1.0	9.1 7.0	84.0 52.0	- 6.7	9.10 22.78	- 12.13	- 1.87

Superscript indicates number of values excluded

..
16
..

APT/1

TABLE 7

Sex ratios - group mean values

Group:	1	2	3	4	5	6
Compound:	Control			Toluene		
Dosage (ppm):	-	500	1000	2000	3500	5000

Group	Number of litters	Number of males	Number of females	Total	% males/ litter
1	11	7.6	6.0	13.6	56.2
2	10	6.9	7.6	14.5	47.1
3	11	6.2	6.8	13.0	44.8
4	12	5.9	6.3	12.2 ^a	48.8
5	10	6.2	6.4	12.6	47.2
6	3	4.0	2.3	6.3	42.3

^a Excludes one pup, sex not recorded
in error

APPENDICES

APPENDIX 1

Signs and autopsy findings - individual incidence post exposure

Group 1: Control

Animal number	Signs Days inclusive										Autopsy findings
	Post-dose examination Days 6 to 15										
	Daily examination (Days 0-20)	Loss of body tone	Unsteady	Limb tremors	Rales	No righting reflex	Rapid respiration	Cold to touch	Lacrimation	Eyelids half-closed	
	<u>With live young at Day 20</u>										
1											N
2											N
3											N
5											N
6											N
7											N
8											N
9											N
10											N
11											N
12											N
	<u>Non-pregnant</u>										
4										N	N

N No abnormal signs or autopsy findings observed

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APPENDIX 1
(Signs and autopsy findings - continued)

Group 2: Toluene, 500 ppm

Animal number	Signs Days inclusive										Autopsy findings
	Daily examination (Days 0-20)	Post-dose examination Days 6 to 15									
		Loss of body tone	Limb tremors	Rales	No righting reflex	Rapid respiration	Cold to touch	Lacrimation	Eyelids half-closed	Other	
13	N	With live young at Day 20									Kidneys: bilateral, minimal increased pelvic dilatation
14	N										N
15	N										N
16	N										N
18	N										N
19	N										N
20	N										N
21	N										N
23	N										V
24	N										N
	Non-pregnant										N
17	N										N
22	N										N

N No abnormal signs or autopsy findings observed
V Dark red discharge from vagina Day 15

APPENDIX 1
(Signs and autopsy findings - continued)

Group 3: Toluene, 1000 ppm

Animal number	Signs Days inclusive										Autopsy findings
	Daily examination (Days 0-20)	Post-dose examination Days 6 to 15									
		Loss of body tone	Limb tremors	Rales	No righting reflex	Rapid respiration	Cold to touch	Lacrimation	Eyelids half-closed	Other	
	<u>With live young at Day 20</u>										
25	N									Salivation 6	
26	N									N	
28	N									N	
29	N									N	
30	N									N	
31	N									N	
32	N									N	
33	N									N	
34	N									N	
35	N									N	
36	N									N	
	<u>Non-pregnant</u>										
27	N									N	

N No abnormal signs or autopsy findings observed

APPENDIX 1
(Signs and autopsy findings - continued)

Group 4: Toluene, 2000 ppm

Animal number	Signs Days inclusive										Autopsy findings
	Post-dose examination Days 6 to 15										
	Daily examination (Days 0-20)	Loss of body tone	Unsteady	Limb tremors	Râles	No righting reflex	Rapid respiration	Cold to touch	Lacrimation	Eyelids half-closed	
	<u>With live young at Day 20</u>										
37	N										N
38	N										Hair on face red/brown stained 6
39u	N										N
40u	N										N
41	N										N
42	N										Muzzle swollen 13
43	N										N
44	N										N
45	N										N
46	N										N
47	N										N
48	N										N

N No abnormal signs or autopsy findings observed
u Unilateral implantation

APPENDIX 1
(Signs and autopsy findings - continued)

Group 5: Toluene, 3500 ppm

Animal number	Signs Days inclusive											Autopsy findings
	Daily examination (Days 0-20)	Post-dose examination Days 6 to 15										
		Loss of body tone	Unsteady	Limb tremors	Râles	No righting reflex	Rapid respiration	Cold to touch	Lacrimation	Eyelids half-closed	Other	
	With live young at Day 20											
49	N	6-8	6-8	6							Dark red discharge from vagina 15	N
50	Râles (7,8)	10-15	11,13,14		6-8							N
51	N	12-15				15					Salivation 14	N
52	N	6,9,10	6,14		6,11,14,15	14		14			Salivation 14	N
		6-13	6,7,14								Dark red discharge from vagina 15	N
53	N	6-12	6,7,9,11,12,14,15		6,7			14			Salivation 15	N
54	N	6-12,14	8,12-15		6,8		12,14,15	12			Hair on face red/brown stained 14	N
57	N				6	6					Hair on face red/brown stained 7	N
58	N					6		6			Upper incisors broken 14,15	N
59	N				12						-	N
60a	N		15		6,7						Salivation 15	N
	Non-pregnant											
55	N	6,7	6		6,7	10					Hair on face red/brown stained 10	N
56	N	6	6		6						Salivation 11	N

N No abnormal signs or autopsy findings observed
u Unilateral implantation

APPENDIX 1
(Signs and autopsy findings - continued)

Group 6: Toluene, 5000 ppm

Animal number	Signs Days inclusive											Autopsy findings
	Daily examination (Days 0-20)	Post-dose examination Days 6 to 15										
		Loss of body tone	Unsteady	Limb tremors	Râles	No righting reflex	Rapid respiration	Cold to touch	Lacrimation	Eyelids half-closed	Other	
	With live young at Day 20											
63	Râles (7)	6-8-13 15	6,8-15	9-12	6-10 12-13		9-11,14	9	9-12, 14	12,14	Salivation 14	N
66	N	6-15	6-15	7,8,11	6-8 10-12,15	12	7,8,12	12	6-12, 14	13	Hair on face red/ brown stained 12	N
68	N	6-15	6-15	6,15	6,11-15		8-10		6,8,10-13, 15	9-12	-	N
	Total resorption											
61	Râles (7)	6-15	7-15	6,10-13	6-8 10-15	6	11,12	7,8, 10,11	11-14		-	N
62	N	6-15	7-15	10-15	6-12 14,15	10	6,11,12	7,8, 10,11	10-15	15	Salivation 15	N
67	N	6-15	6-15	6,8,13, 14,15	6-8 10-15	6,10-15	6,10-12	12		8	Salivation 10,11	N
69	N	6-15	6-15	6,11-15	6-8 11-15	6	8-10	6	6,8,10,13	6,8-13	-	N
70		6-15	6-15	9-11, 14,15	6-11 13-15	6	7-10	8	6		-	N
72	N	6-15	6-15	7-11, 13-15	6-15		6,9,10,14		6,9,10, 13,14	9	-	N
	Non-pregnant											
64	Left hind limb swollen (14)	6-15	6-15	6,9-14	8-10	6,14	9,10	8,9	8-14	13	Salivation 14,15 Left hind limb swollen 13	N
65	N	6-13	6-15	9-12	6,8-10		9,10,14	6,11	6,8-12,14	11,12	Salivation 14,15	N
71	-										-	-
	Dead - see Appendix 2											

N No abnormal signs or autopsy findings observed

APPENDIX 2

Mortalities - individual findings

Animal number	Signs (Days) inclusive	Autopsy findings
Group 6: Toluene, 5000 ppm		
71	Found dead Day 6 after exposure	Oral cavity: lower incisors pale. Lymph nodes: cervical, enlarged. Lungs: firm, not collapsed. Uterus: 15 implantation sites. Liver weight: 11.08 g.

APPENDIX 3

Water consumption - individual values

Group 1: Control

Animal number	Water consumption (g/rat) on Days:																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	<u>With live young at Day 20</u>																			
1	27	31	38	42	36	41	37	50	43	43	44	46	34	38	38	37	39	44	41	37
2	29	32	23	33	26	35	22	30	26	29	26	34	25	23	29	30	32	36	34	35
3	25	28	35	35	30	33	30	30	27	32	30	35	32	33	31	35	35	35	40	37
5	26	81	116*	167*	107*	118*	191*	183*	201*	237*	240*	122*	36	46	38	59	62	67	83	101
6 .	25	28	29	27	26	29	29	28	24	29	26	28	28	34	40	37	48	43	52	45
7	28	34	33	36	37	37	40	37	31	36	34	39	36	41	37	40	39	40	43	39
8	34	42	40	40	37	39	40	40	42	40	44	45	43	45	56	56	48	56	53	50
9	37	36	39	27	36	37	34	32	34	34	38	35	43	39	45	44	43	45	40	36
10	35	38	35	38	29	32	33	30	30	31	38	40	43	40	35	40	43	43	42	39
11	34	34	38	42	35	36	37	38	35	43	44	48	48	48	44	52	52	50	59	52
12	39	46	39	50	39	44	53	48	43	47	44	54	20	56	48	57	54	52	51	45
	<u>Non-pregnant</u>																			
4																				

* Excessive wastage value excluded

APPENDIX 3

(Water consumption - continued)

Group 2: Toluene, 500 ppm

Animal number	Water consumption (g/rat) on Days:																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	<u>With live young at Day 20</u>																			
13	27	33	32	39	31	39	40	32	40	40	36	42	37	38	41	53	47	48	44	42
14	29	29	29	28	26	27	28	29	30	31	34	30	29	31	33	35	34	33	30	30
15	26	31	30	36	33	30	37	37	36	39	37	38	39	38	44	48	41	47	38	43
16	26	29	26	32	27	37	29	33	30	33	29	32	31	35	31	36	28	33	30	34
18	27	41	39	51	34	33	34	32	33	34	38	36	40	53	43	52	60	59	50	56
19	34	29	33	32	32	33	32	32	32	35	40	38	43	44	46	56	56	63	56	59
20	13	40	31	32	31	34	31	35	35	34	37	37	39	42	42	46	39	43	47	43
21	20	27	24	28	26	27	25	24	27	27	29	30	31	30	33	34	34	36	38	31
23	29	30	27	32	27	26	23	27	27	32	25	32	37	33	34	38	52	37	37	32
24	24	26	34	39	29	28	31	33	36	34	35	40	34	40	45	51	34	52	48	45
	<u>Non-pregnant</u>																			
17																				
22																				

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APPENDIX 3
(Water consumption - continued)

Group 3: Toluene, 1000 ppm

Animal number	Water consumption (g/rat) on Days:																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	<u>With live young at Day 20</u>																			
25	27	32	24	29	25	32	29	29	32	31	59	47	35	39	40	42	44	51	39	45
26	32	36	35	38	37	34	34	32	33	33	34	35	35	40	36	38	40	41	36	37
28	28	28	28	30	26	27	32	33	32	32	31	40	37	38	40	44	40	42	37	41
29	22	30	31	29	29	27	25	28	26	30	31	9#	45	40	42	39	49	49	45	39
30	31	30	35	34	34	35	32	32	33	35	34	39	36	39	38	45	42	43	41	38
31	24	23	27	28	25	27	25	29	30	30	32	33	30	30	35	40	38	42	38	39
32	40	39	38	37	33	33	33	34	33	34	36	36	40	46	40	45	52	57	55	46
33	26	36	35	37	35	26	29	31	27	32	36	40	38	48	39	48	49	49	52	44
34	24	27	29	25	27	35	24	26	25	25	29	32	33	33	33	39	41	42	40	35
35	31	28	34	34	31	32	30	35	34	39	36	44	39	42	39	42	45	40	43	43
36	25	31	28	30	26	25	26	26	26	28	28	29	26	31	33	35	32	35	36	30
	<u>Non-pregnant</u>																			

27

Value excluded - water withheld from animal in error

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APPENDIX 3
(Water consumption - continued)

Group 4: Toluene, 2000 ppm

Animal number	Water consumption (g/rat) on Days:																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	<u>With live young at Day 20</u>																			
37	25	29	31	33	25	29	34	37	44	44	47	47	40	43	46	51	42	38	36	36
38	23	27	30	29	25	29	30	30	33	34	33	36	36	37	40	42	35	37	34	32
39	23	26	22	30	26	25	25	24	24	29	31	30	29	29	28	31	30	34	27	30
40	25	32	29	33	32	30	36	35	34	35	34	37	42	43	38	47	47	51	42	45
41	28	34	28	31	29	30	32	40	43	50	57	58	70	62	72	68	62	78	65	68
42	26	37	31	34	36	44	32	40	41	39	44	48	56	59	60	70	72	78	67	55
43	23	24	25	25	21	23	23	21	28	25	29	34	30	27	32	34	36	39	34	34
44	40	41	41	43	35	38	43	45	45	46	46	46	46	47	44	59	47	50	47	46
45	23	24	27	25	26	27	26	25	24	25	26	29	31	36	37	39	37	34	33	32
46	27	34	33	39	35	34	35	34	32	39	47	42	58	63	59	55	52	50	50	48
47	21	28	30	27	28	28	23	26	33	37	40	46	42	41	44	47	58	51	48	38
48	38	46	51	51	37	46	37	53	53	60	63	100	55	59	68	70	82	79	76	62

APPENDIX 3

(Water consumption - continued)

Group 5: Toluene, 3500 ppm

Animal number	Water consumption (g/rat) on Days:																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	<u>With live young at Day 20</u>																			
49	27	32	30	35	29	34	28	27	29	35	38	43	50	49	48	60	57	52	48	44
50	27	25	28	30	26	30	26	30	35	43	48	46	55	55	49	60	54	63	52	46
51	24	31	26	30	28	30	23	31	39	46	61	63	46	55	61	62	67	62	48	43
52	28	30	27	27	31	29	23	28	37	44	58	55	53	65	66	63	64	67	59	51
53	26	32	37	40	37	38	24	29	38	41	60	64	57	75	76	75	84	72	79	83
54	26	34	29	28	28	46	27	32	37	38	40	49	57	62	65	61	69	73	52	51
57	27	32	33	30	28	40	24	27	34	39	45	57	59	77	80	79	82	83	79	67
58	41	47	41	40	38	42	43	41	46	55	60	79	95	114	106	116	103	107	131	107
59	25	28	31	29	31	29	22	27	37	32	38	38	49	57	57	60	57	53	51	48
60	31	33	33	32	31	36	23	32	42	60	55	54	63	64	74	59	71	69	70	65
	<u>Non-pregnant</u>																			
55																				
56																				

APPENDIX 3

(Water consumption - continued)

Group 6: Toluene, 5000 ppm

Animal number	Water consumption (g/rat) on Days:																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<u>With live young at Day 20</u>																				
63	25	35	29	33	32	31	38	37	42	60	144	71	83	69	71	80	90	71	62	54
66	16	29	33	32	34	35	27	41	56	78	79	83	83	55	77	96	118	90	71	65
68	30	35	32	37	35	32	35	36	50	83	54	51	62	69	73	83	109	115	73	64
<u>Total resorption</u>																				
61	28	27	25	30	21	29	28	29	40	53	50	55	69	64	72	74	87	73	51	44
62	22	27	28	31	24	31	37	36	54	71	75	89	88	85	80	86	88	42	36	37
67	30	30	34	36	34	32	3	29	45	53	57	50	60	57	57	68	85	86	65	54
69	28	25	24	22	19	22	16	17	26	35	41	43	46	44	44	50	74	47	43	34
70	21	27	28	28	24	25	25	30	35	33	42	39	43	48	48	51	67	58	51	40
72	44	48	43	47	39	39	53	48	60	80	104	130	122	119	118	127	133	126	124	101
<u>Non-pregnant</u>																				
64																				
65																				
<u>Died</u>																				
71	31	32	30	29	25	25														

APPENDIX 4

Food consumption - individual values

Group 1: Control

Animal number	Food consumption (g/rat) during Days:									
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19
	<u>With live young at Day 20</u>									
1	48	47	53	52	62	57	57	56	55	53
2	41	36	46	42	47	52	47	51	51	46
3	40	47	46	48	47	47	52	50	54	52
5	53	53	52	57	52	53	53	53	55	56
6	40	40	46	45	47	49	44	45	48	49
7	45	48	52	54	50	49	52	50	53	53
8	46	56	60	54	59	58	61	59	61	59
9	50	40	55	56	56	59	64	64	66	60
10	43	39	49	40	39	45	47	48	53	45
11	57	51	39	50	54	54	55	56	59	56
12	57	58	57	64	62	63	66	64	64	71
	<u>Non-pregnant</u>									
4										

APPENDIX 4

(Food consumption - continued)

Group 2: Toluene, 500 ppm

Animal number	Food consumption (g/rat) during Days:									
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19
<u>With live young at Day 20</u>										
13	41	45	46	38	45	41	49	37	52	48
14	44	48	53	50	62	62	58	59	58	52
15	40	48	47	51	55	60	53	54	60	51
16	41	44	44	56	56	59	53	54	46	46
18	46	46	59	55	59	63	60	67	67	56
19	41	45	47	49	47	51	55	53	60	51
20	43	58	58	54	58	59	62	63	67	63
21	46	48	49	48	54	54	58	65	64	57
23	49	47	47	48	50	53	51	56	62	56
24	40	41	43	46	49	48	44	51	53	56
<u>Non-pregnant</u>										
17										
22										

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APPENDIX 4

(Food consumption - continued)

Group 3: Toluene, 1000 ppm

Animal number	Food consumption (g/rat) during Days:									
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19
	<u>With live young at Day 20</u>									
25	41	37	48	41	42	57	51	53	59	49
26	46	45	50	46	48	50	56	46	50	49
28	42	50	44	46	51	53	46	53	60	51
29	39	43	45	43	44	37	44	57	55	51
30	42	52	56	51	51	57	54	60	69	59
31	43	53	52	50	52	54	54	57	66	65
32	46	49	47	44	49	48	56	53	60	52
33	52	51	58	54	54	64	68	61	65	59
34	47	44	48	43	41	47	51	55	51	53
35	43	43	46	44	50	59	45	53	60	51
36	48	49	51	48	53	56	57	61	65	61
	<u>Non-pregnant</u>									
27										

APPENDIX 4

(Food consumption - continued)

Group 4: Toluene, 2000 ppm

Animal number	Food consumption (g/rat) during Days:									
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19
	<u>With live young at Day 20</u>									
37	39	42	45	36	40	41	45	44	48	43
38	48	51	55	47	53	56	56	56	62	57
39	39	47	48	45	46	47	44	45	52	48
40	42	48	46	45	44	49	49	44	54	45
41	40	46	47	46	45	59	50	53	56	54
42	39	41	51	38	35	35	39	43	49	50
43	43	54	50	43	41	45	42	46	63	62
44	47	56	53	44	45	40	48	47	67	54
45	45	42	47	40	42	44	49	57	52	53
46	48	37	44	34	35	38	48	51	47	48
47	40	43	46	36	42	45	50	52	62	62
48	45	54	55	47	61	61	56	62	73	73

APPENDIX 4

(Food consumption - continued)

Group 5: Toluene, 3500 ppm

Animal number	Food consumption (g/rat) during Days:									
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19
	<u>With live young at Day 20</u>									
49	43	44	50	34	45	47	59	55	55	52
50	43	42	47	30	38	38	45	46	44	46
51	39	46	46	39	42	48	46	47	58	50
52	38	37	46	30	40	41	40	45	52	48
53	42	50	45	38	43	45	45	47	46	46
54	42	38	46	35	31	33	36	40	48	43
57	48	51	46	23	33	43	47	60	60	55
58	46	44	46	33	40	48	54	49	47	54
59	47	48	49	29	36	39	43	45	53	50
60	46	47	50	30	38	42	48	49	64	63
	<u>Non-pregnant</u>									
55										
56										

APPENDIX 4

(Food consumption - continued)

Group 6: Toluene, 5000 ppm

Animal number	Food consumption (g/rat) during Days:									
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19
<u>With live young at Day 20</u>										
63	37	41	44	37	40	40	40	43	48	46
66	41	45	53	33	35	43	39	47	65	62
68	54	47	49	34	41	42	46	51	54	44
<u>Total resorption</u>										
61	49	48	50	24	37	36	41	44	55	52
62	41	42	44	30	41	33	40	42	38	34
67	42	52	52	33	36	33	41	42	60	54
69	51	48	40	25	26	40	44	48	65	50
70	44	45	46	33	33	41	45	49	54	52
72	48	52	50	40	26	39	49	48	60	62
<u>Non-pregnant</u>										
64										
65										
<u>Died</u>										
71	47	46	45							

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APPENDIX 5

Bodyweights - individual values

Group 1: Control

Animal number	Bodyweights (g) at Day of gestation										
	0	2	4	6	8	10	12	14	16	18	20
	<u>With live young at Day 20</u>										
1	217	234	246	260	269	282	294	301	317	348	378
2	216	231	232	242	253	261	276	277	300	323	346
3	222	231	242	253	262	269	280	293	312	340	370
5	238	256	269	280	287	291	304	312	327	345	371
6	198	214	220	230	234	247	257	270	275	295	319
7	223	244	253	266	274	287	295	308	326	342	367
8	229	244	257	272	282	294	308	323	335	362	397
9	241	258	257	281	295	304	320	333	356	385	411
10	205	224	228	234	240	246	252	267	284	306	332
11	234	262	270	279	288	300	308	321	337	362	394
12	268	290	308	317	327	337	347	357	376	403	434
	<u>Non-pregnant</u>										
4											

APPENDIX 5

(Bodyweights - continued)

Group 2: Toluene, 500 ppm

Animal number	Bodyweights (g) at Day of gestation										
	0	2	4	6	8	10	12	14	16	18	20
	<u>With live young at Day 20</u>										
13	233	239	249	261	260	271	279	293	301	334	362
14	199	215	228	245	252	265	287	295	314	340	367
15	215	227	240	248	256	269	278	288	306	336	359
16	215	229	244	251	262	274	286	295	312	327	347
18	243	256	263	280	294	299	318	334	355	382	408
19	201	219	227	241	251	262	277	292	302	334	361
20	260	270	279	297	302	311	327	343	357	387	421
21	255	274	280	288	295	308	319	328	350	373	402
23	215	242	251	263	271	281	293	304	326	348	378
24	221	241	249	262	268	277	284	285	305	322	348
	<u>Non-pregnant</u>										
17											
22											

APPENDIX 5

(Bodyweights - continued)

Group 3: Toluene, 1000 ppm

Animal number	Bodyweights (g) at Day of gestation										
	0	2	4	6	8	10	12	14	16	18	20
	<u>With live young at Day 20</u>										
25	236	247	256	270	272	255	297	303	317	345	371
26	206	221	231	245	251	256	273	284	288	317	349
28	208	237	248	254	260	276	285	290	313	339	361
29	207	223	229	240	247	253	248	277	289	316	349
30	234	248	258	280	287	296	316	326	337	374	398
31	238	255	266	284	291	302	321	332	347	377	407
32	209	225	240	249	255	270	279	291	304	323	341
33	252	276	282	296	303	316	328	345	363	382	421
34	216	235	241	250	259	263	274	282	297	320	345
35	255	245	256	269	271	282	298	301	319	343	364
36	227	244	254	266	269	279	293	303	319	347	373
	<u>Non-pregnant</u>										
27											

APPENDIX 5

(Bodyweights - continued)

Group 4: Toluene, 2000 ppm

Animal number	Bodyweights (g) at Day of gestation										
	0	2	4	6	8	10	12	14	16	18	20
	<u>With live young at Day 20</u>										
37	194	201	212	224	221	224	240	244	260	287	318
38	236	250	263	279	281	285	305	314	328	359	393
39	222	237	247	256	254	266	271	278	287	305	308
40	230	241	252	259	259	268	274	283	291	302	311
41	191	206	217	230	235	245	255	266	288	308	332
42	226	237	244	253	255	256	261	269	284	314	348
43	237	249	256	270	266	273	284	291	302	339	370
44	221	241	252	269	266	269	277	291	299	331	354
45	241	254	259	270	271	276	285	295	307	330	357
46	205	224	229	240	241	242	242	253	267	290	316
47	244	248	260	274	271	273	284	293	310	348	377
48	235	256	272	285	282	294	304	315	331	367	409

APPENDIX 5

(Bodyweights - continued)

Group 5: Toluene, 3500 ppm

Animal number	Bodyweights (g) at Day of gestation										
	0	2	4	6	8	10	12	14	16	18	20
	<u>With live young at Day 20</u>										
49	229	240	252	253	255	255	258	274	284	302	323
50	206	226	237	250	241	236	243	248	257	284	309
51	199	212	226	237	227	231	248	255	269	298	321
52	206	222	228	241	224	233	240	251	259	290	314
53	244	256	265	268	262	267	265	279	287	307	334
54	205	217	225	235	232	227	230	237	245	267	293
57	224	245	256	265	249	253	260	264	284	316	348
58	234	254	260	268	259	261	263	276	285	314	346
59	250	270	279	289	275	274	278	282	291	320	349
60	232	250	262	273	259	261	267	271	282	306	329
	<u>Non-pregnant</u>										
55											
56											

APPENDIX 5

(Bodyweights - continued)

Group 6: Toluene, 5000 ppm

Animal number	Bodyweights (g) at Day of gestation										
	0	2	4	6	8	10	12	14	16	18	20
	<u>With live young at Day 20</u>										
63	204	216	227	238	232	238	245	248	245	260	265
66	218	232	242	258	252	247	250	(273)	264	297	326
68	241	253	262	270	268	256	272	276	282	297	307
	<u>Total resorption</u>										
61	236	252	265	273	255	250	257	253	256	273	286
62	200	209	220	232	225	223	226	223	215	214	221
67	227	237	248	266	254	254	255	261	257	269	284
69	214	235	248	251	245	245	253	257	254	276	282
70	231	247	254	263	260	255	256	257	259	271	277
72	257	266	286	295	293	276	283	293	298	305	316
	<u>Non-pregnant</u>										
64											
65											
	<u>Died</u>										
71	223	244	253	262							

() Spurious weight excluded from calculation

APPENDIX 6

Liver weights - individual values

Group 1: Control		Group 2: Toluene, 500 ppm		Group 3: Toluene, 1000 ppm	
Animal number	Liver weight (g)	Animal number	Liver weight (g)	Animal number	Liver weight (g)
	<u>With live young</u>		<u>With live young</u>		<u>With live young</u>
1	17.68	13	14.91	25	17.01
2	15.04	14	17.67	26	14.01*
3	16.08	15	16.41	28	16.37
5	16.67	16	16.61	29	13.78
6	14.20	18	17.41	30	17.81
7	17.92	19	16.89	31	19.13
8	16.81	20	19.19	32	16.15
9	17.19	21	17.21	33	17.06
10	14.46	23	15.08	34	14.66
11	18.09	24	13.59	35	16.20
12	17.78			36	15.51
	<u>Non-pregnant</u>		<u>Non-pregnant</u>		<u>Non-pregnant</u>
4	9.74	17	11.06	27	12.59
		22	11.73		

* Extra small lobe within median cleft

APPENDIX 6

(Liver weights - continued)

Group 4: Toluene, 2000 ppm		Group 5: Toluene, 3500 ppm		Group 6: Toluene, 5000 ppm	
Animal number	Liver weight (g)	Animal number	Liver weight (g)	Animal number	Liver weight (g)
	<u>With live young</u>		<u>With live young</u>		<u>With live young</u>
37	14.29	49	17.04	63	15.27
38	17.21	50	13.68	66	16.73
39	16.41	51	14.20	68	14.97
40	14.44	52	14.61		
41	16.40	53	15.05		<u>Total resorption</u>
42	14.66	54	12.58		
43	17.34	57	16.19	61	15.39
44	15.63	58	15.36	62	11.44
45	15.45	59	15.64	67	13.90
46	13.04	60	14.56	69	15.52
47	16.01			70	13.48
48	19.04			72	18.48
			<u>Non-pregnant</u>		<u>Non-pregnant</u>
		55	13.09	64	13.07
		56	12.11	65	14.31
					<u>Died</u>
				71	11.08

APPENDIX 7

Litter data - individual values

Group 1: Control

Female number	Corpora lutea	Implants	Pre- implant loss %	Embryonic deaths			Post implant loss %	Total live young	Litter weight (g)	Mean foetal weight (g)
				Early	Late	Total				
1	16	15	6.3	0	0	0	0.0	15	52.98	3.53
2	14	14	0.0	1	0	1	7.1	13	42.77	3.29
3	16	14	12.5	1	0	1	7.1	13	42.03	3.23
5	16	13	18.8	1	1	2	15.4	11	35.84	3.26
6	12	12	0.0	0	0	0	0.0	12	38.22	3.19
7	15	14	6.7	0	0	0	0.0	14	46.44	3.32
8	16	16	0.0	0	0	0	0.0	16	50.96	3.19
9	16	16	0.0	2	0	2	12.5	14	51.73	3.70
10	22	14	36.4	0	0	0	0.0	14	42.26	3.02
11	17	13	23.5	0	0	0	0.0	13	47.40	3.65
12	17	16	5.9	1	0	1	6.3	15	49.44	3.30
	<u>Non-pregnant</u>									
4										

APPENDIX 7

(Litter data - continued)

Group 2: Toluene, 500 ppm

Female number	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths			Post implant loss %	Total live young	Litter weight (g)	Mean foetal weight (g)
				Early	Late	Total				
13	19	16	15.8	1	0	1	6.3	15	47.72	3.18
14	15	15	0.0	1	0	1	6.7	14	44.81	3.20
15	14	14	0.0	0	0	0	0.0	14	47.07	3.36
16	19	15	21.1	0	0	0	0.0	15	48.23	3.22
18	21	15	28.6	0	0	0	0.0	15	52.10	3.47
19	17	14	17.6	0	0	0	0.0	14	47.64	3.40
20	16	16	0.0	1	0	1	6.3	15	50.43	3.36
21	16	16	0.0	1	0	1	6.3	15	47.13	3.14
23	17	16	5.9	1	0	1	6.3	15	48.55	3.24
24	14	14	0.0	1	0	1	7.1	13	41.41	3.19
	<u>Non-pregnant</u>									
17										
22										

APPENDIX 7

(Litter data - continued)

Group 3: Toluene, 1000 ppm

Female number	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths			Post implant loss %	Total live young	Litter weight (g)	Mean foetal weight (g)
				Early	Late	Total				
25	19	15	21.1	1	0	1	6.7	14	43.84	3.13
26	13	13	0.0	0	0	0	0.0	13	41.92	3.22
28	12	12	0.0	0	0	0	0.0	12	38.29	3.19
29	16	14	12.5	0	0	0	0.0	14	44.80	3.20
30	17	15	11.8	0	0	0	0.0	15	49.93	3.33
31	19	14	26.3	2	0	2	9.5	12	36.21	3.02
32	12	9	25.0	0	0	0	0.0	9	28.50	3.17
33	23	15	34.8	0	0	0	0.0	15	49.21	3.28
34	13	13	0.0	2	0	2	15.4	11	34.79	3.16
35	14	14	0.0	1	0	1	7.1	13	42.93	3.30
36	17	15	11.8	0	0	0	0.0	15	52.94	3.53
	<u>Non-pregnant</u>									
27										

APPENDIX 7

(Litter data - continued)

Group 4: Toluene, 2000 ppm

Female number	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths			Post implant loss %	Total live young	Litter weight (g)	Mean foetal weight (g)
				Early	Late	Total				
37	17	15	11.8	0	0	0	0.0	15	47.29	3.15
38	14	14	0.0	1	0	1	7.1	13	44.85	3.45
39	15	10	33.3	0	6	6	60.0	4	15.04	3.76
40	12	4	66.7	0	0	0	0.0	4	14.89	3.72
41	14	14	0.0	0	0	0	0.0	14	44.58	3.18
42	15	15	0.0	0	0	0	0.0	15	50.81	3.39
43	15	15	0.0	0	0	0	0.0	15	47.60	3.17
44	14	12	14.3	1	0	1	8.3	11	30.85	2.80
45	14	12	14.3	0	0	0	0.0	12	38.85	3.24
46	12	12	0.0	2	0	2	16.7	10	30.73	3.07
47	17	17	0.0	0	0	0	0.0	17	54.54	3.21
48	19	19	0.0	2	0	2	10.5	17	57.50	3.38

APT/1

APPENDIX 7

(Litter data - continued)

Group 5: Toluene, 3500 ppm

Female number	Corpora lutea	Implants	Pre- implant loss %	Embryonic deaths			Post implant loss %	Total live young	Litter weight (g)	Mean foetal weight (g)
				Early	Late	Total				
49	14	13	7.1	1	6	7	53.8	6	14.89	2.48
50	16	15	6.3	1	0	1	6.7	14	36.48	2.61
51	19	14	26.3	0	0	0	0.0	14	37.57	2.68
52	17	15	11.8	0	1	1	6.7	14	37.08	2.65
53	18	18	0.0	3	0	3	16.7	15	29.90	1.99
54	18	14	22.2	0	1	1	7.1	13	32.99	2.54
57	14	13	7.1	0	0	0	0.0	13	40.81	3.14
58	16	15	6.3	0	0	0	0.0	15	43.65	2.91
59	17	15	11.8	1	1	2	13.3	13	41.43	3.19
60	12	9	25.0	0	0	0	0.0	9	24.96	2.77
	<u>Non-pregnant</u>									
55										
56										

APPENDIX 7

(Litter data - continued)

Group 6: Toluene, 5000 ppm

Female number	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths			Post implant loss %	Total live young	Litter weight (g)	Mean foetal weight (g)
				Early	Late	Total				
63	20	13	35.0	8	3	11	84.6	2	4.29	2.15
66	15	14	6.7	0	0	0	0.0	14	25.54	1.82
68	14	14	0.0	10	0	10	71.4	4	6.57	1.64
<u>Total resorption</u>										
61	14	12	14.3	12	0	12	100.0	-	-	-
62	N/C	13	-	13	0	13	100.0	-	-	-
67	14	12	14.3	12	0	12	100.0	-	-	-
69	20	10	50.0	10	0	10	100.0	-	-	-
70	11	7	36.4	7	0	7	100.0	-	-	-
72	9	7	22.2	7	0	7	100.0	-	-	-
<u>Non-pregnant</u>										
64										
65										

N/C Not counted

APPENDIX 8

Sex ratios - individual values

Animal number	Number of males	Number of females	Total	% males/litter
Group 1: Control				
1	8	7	15	53.3
2	6	7	13	46.2
3	7	6	13	53.8
5	7	4	11	63.6
6	7	5	12	58.3
7	6	8	14	42.9
8	9	7	16	56.3
9	8	6	14	57.1
10	8	6	14	57.1
11	9	4	13	69.2
12	9	6	15	60.0
Group 2: Toluene, 500 ppm				
13	11	4	15	73.3
14	7	7	14	50.0
15	4	10	14	28.6
16	10	5	15	66.6
18	4	11	15	26.7
19	3	11	14	21.4
20	8	7	15	53.3
21	9	6	15	60.0
23	9	6	15	60.0
24	4	9	13	30.8
Group 3: Toluene, 1000 ppm				
25	5	9	14	35.7
26	7	6	13	53.8
28	8	4	12	66.6
29	5	9	14	35.7
30	8	7	15	53.3
31	3	9	12	25.0
32	6	3	9	33.3
33	7	8	15	46.7
34	3	8	11	21.3
35	9	4	13	69.2
36	7	8	15	46.7

Animal number	Number of males	Number of females	Total	% males/litter
Group 4: Toluene, 2000 ppm				
37	8	7	15	53.3
38	6	7	13	46.2
39	3	1	4	75.0
40	1	3	4	25.0
41	8	6	14	57.1
42	8	7	15	53.3
43	7	8	15	46.7
44	3	8	11	27.3
45	8	4	12	66.6
46	5	5	10	50.0
47	6	10	16 ^a	37.5
48	8	9	17	47.1
Group 5: Toluene, 3500 ppm				
49	1	5	6	16.7
50	9	5	14	64.3
51	2	12	14	14.3
52	5	9	14	35.7
53	11	4	15	73.3
54	7	6	13	53.8
57	7	6	13	53.8
58	11	4	15	73.3
59	4	9	13	30.8
60	5	4	9	55.5
Group 6: Toluene, 5000 ppm				
63	0	2	2	0.0
66	10	3	13	76.9
68	2	2	4	50.0

^a Excludes one pup, sex not recorded in error

APPENDIX 9

Foetal examination - individual gross findings

Animal number	Number of fetuses	Probable malformations#
Group 1: Control		
7	14	1 fetus with thread-like tail
Group 3: Toluene, 1000 ppm		
26	13	2 fetuses with shortened upper and lower jaw also with slightly protruding tongue
Group 5: Toluene, 3500 ppm		
53	15	14 fetuses with brachymelia/brachydactyly
58	15	1 fetus with bilateral microphthalmia and misshapen lower jaw
Group 6: Toluene, 5000 ppm		
66	14	1 fetus with marked subcutaneous oedema

Only fetuses with macroscopic changes are reported

ADDENDUM 1

DETAILS OF THE INHALATION EXPOSURE
SYSTEM, METHODOLOGY AND
RESULTS

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ADDENDUM 1

(continued)

Test substance

Name: Toluene ('HiPerSolv' for HPLC).
Batch no.: 221227 OL.
Appearance: Colourless liquid.
Received from: BDH Limited.
Receipt date: 16 July 1990.
Purity: 99.9% minimum assay (GLC).
Expiry date: August 1995.
Storage: Ambient temperature.
Stability: Stable for duration of study.

Exposure system

The animals were exposed whole-body to the vapour of Toluene in the manner described below:

Generation of test atmosphere (Figures 1 and 2)

The vapour was produced by metering the test substance from a central pressurised reservoir to a sintered glass disc contained within a glass vessel, through which dried, filtered air was passed. The resulting vapour was swept into the inlet duct of the exposure chamber.

Exposure chambers (Figure 3)

The exposure chambers were constructed from stainless steel and glass and had an internal volume of approximately 750 litres. The chambers were of square cross-section with a pyramidal base and top.

Incoming air, monitored continuously using tapered-tube flow meters, entered through the sintered glass disc in the glass jar and carried the vapour into an elutriation column at a flow rate of 150 litres/min.

ADDENDUM 1

(continued)

The chamber atmosphere was extracted by means of individual handling units, each fitted with filters. The extract line of each chamber was fitted with a gate valve in order to maintain a chamber pressure of 10 mm water below ambient.

Each chamber was fitted with sampling ports for the withdrawal of atmosphere samples for analysis. Routinely a left upper-centre port of the chamber wall was used for sampling.

The test animals were individually housed in stainless steel wire-mesh cages suspended from stainless steel racks in the chambers.

Procedure

The test animals were placed in the exposure cages and loaded into the exposure chambers. The chamber doors were closed and the chambers sealed. The air supply was turned on and the air flow rate set to 150 litres/min. The chamber pressure was set to 10 mm water gauge.

Exposure commenced when the test substance supply valve was opened. The drip rate of test substance to each sintered glass disc was regulated by in-line flow control needle valves.

All exposure parameters i.e. air flow, pressure, drip rate, temperature and relative humidity, were recorded every 30 minutes for the duration of the exposure - a period of 6 hours.

After a period of 6 hours the test substance supply valve was closed and the chamber air supply allowed to clear the chamber for a period of 20 minutes. The chambers were then opened and the test animals removed to their holding cages.

The air control chamber was run similarly to the test chambers except that there was no Toluene supplied to the sintered glass disc.

ADDENDUM 1

(continued)

MethodologyExperimental groups and exposure levels

Test animals were placed in experimental groups which were exposed to the following target chamber concentrations of Toluene:

Group	Target concentration	
	(ppm)*	(mg/litre)
1 (Air control)	air only	
2 (Low dose Toluene)	500	1.88
3 (Low int. dose Toluene)	1000	3.77
4 (Int. dose Toluene)	2000	7.54
5 (High int. dose Toluene)	3500	13.19
6 (High dose Toluene)	5000	18.84

* ppm = (24450 x mg/litre)/molecular weight (92.14) at 25 degrees Celsius

Duration of exposures

Exposures were of 6 hours and 11.5 minutes duration. Eleven and a half minutes was the theoretical time taken, given the chamber size and air flow rate, for the chamber atmosphere to reach 90% of the target concentration (T_{90}). This was calculated from the following expression:

$$*T_{90} = \frac{\text{Chamber volume (litres)} \times \ln (100/100 - 90)}{\text{Chamber airflow rate (litres/min)}}$$

* Ref: G. O. Nelson, (1971). Controlled Test Atmospheres. Chapter 5, Dynamic systems for producing gas mixtures; page 100. Ann Arbor Scientific Publications.

Rats were exposed from Day 6 to Day 15 of pregnancy inclusive, each rat receiving a total of 10 exposures. The animals were assigned to batches according to impregnation times. A total of 8 batches were delivered for the study arriving on alternate days over a 15-day period.

Due to the overlap between batches a total of 24 exposures were performed.

ADDENDUM 1

(continued)

Chamber atmosphere concentration of Toluene

The vapour concentration and spatial distribution of the test atmosphere in each chamber were determined during the preliminary work, the results of which are presented in Table 1.

Test substance usage

As an indication of the efficiency of the generation system, the analysed study mean values were expressed as a percentage of the nominal study mean values.

Drop nominal concentrations (mg/litre) were calculated from mean drip rates, drop weights (established during preliminary work) and chamber air flow.

Analysed concentration

The concentration of Toluene in the test atmosphere at each exposure level was determined regularly during each exposure. Samples were taken at approximately 60-minute intervals. Chamber air was withdrawn using a gas-tight syringe through adsorption tubes, 10 cm in length packed with approximately 2 cm of Chromosorb 102, 60 - 80 mesh on which vapour was adsorbed.

The samples were analysed by gas chromatography (flame ionisation detector), after thermal desorption into the column, according to the method detailed in Appendix 1.

Chamber air temperature and relative humidity

Chamber air temperature and relative humidity were recorded at 30-minute intervals during exposures using a wet and dry bulb hygrometer placed in each chamber before the start of the exposure. Relative humidity was calculated from the wet and dry bulb readings using standard conversion tables supplied with the instrument.

Clinical signs

Signs of reaction during exposure were recorded at half-hourly intervals. The time at which a particular sign was first seen and the time at which the sign was no longer observed were recorded.

ADDENDUM 1

(continued)

RESULTS

CHAMBER ATMOSPHERE CONDITIONSAnalytical levels (Table 2)

The batch/study mean analysed concentrations of Toluene are presented as follows:

Batch (Exp.)	Group				
	2 (Low dose Toluene) (ppm)	3 (Low int. dose Toluene) (ppm)	4 (Int. dose Toluene) (ppm)	5 (High int. dose Toluene) (ppm)	6 (High dose Toluene) (ppm)
A (1-10)	503	1029	2014	3513	4963
C (3-12)	505	1031	1992	3529	4964
E (5-14)	504	1025	1979	3553	4985
G (7-16)	504	1019	1974	3591	4985
I (9-18)	507	1017	1972	3551	4994
K (11-20)	505	1022	1976	3547	5010
M (13-22)	504	1017	1990	3516	5007
O (15-24)	502	1018	1991	3480	**
Overall mean: (1-24)	502	1024	1993	3517	4980

** No rats in Group 6 (High dose Toluene) were assigned to Batch 'O'.

Study mean analysed chamber concentrations were in good agreement with target concentrations.

Generation efficiency (Table 3)

The generation efficiency was measured as the percentage analysed concentration of the nominal concentration. The study means were as follows:

2 (Low dose Toluene)	3 (Low int. dose Toluene)	Group			6 (High dose Toluene)
		4 (Int. dose Toluene)	5 (High int. dose Toluene)		
98.6	101.3	99.2	100.7		99.1

There was good agreement between analysed and nominal concentrations.

ADDENDUM 1

(continued)

Chamber temperature and relative humidity (Table 4)

The study mean temperature and relative humidity were as follows:

Group	T(°C)	RH(%)
1 (Air control)	21.9	36
2 (Low dose Toluene)	19.7	43
3 (Low int. dose Toluene)	20.5	46
4 (Int. dose Toluene)	20.4	46
5 (High int. dose Toluene)	20.3	44
6 (High dose Toluene)	20.1	38

Differences between groups were small and were considered not to have influenced the outcome of the study.

Clinical signs during exposure (Table 5)

Signs observed that were considered related to exposure to Toluene vapour were confined to Groups 4 (2000 ppm Toluene), 5 (3500 ppm Toluene) and 6 (5000 ppm Toluene).

Signs indicating irritant properties of a non-specific nature included:

Aware posture with licking the inside of the mouth.

Signs indicative of gradual narcosis with a degree of anaesthesia included:

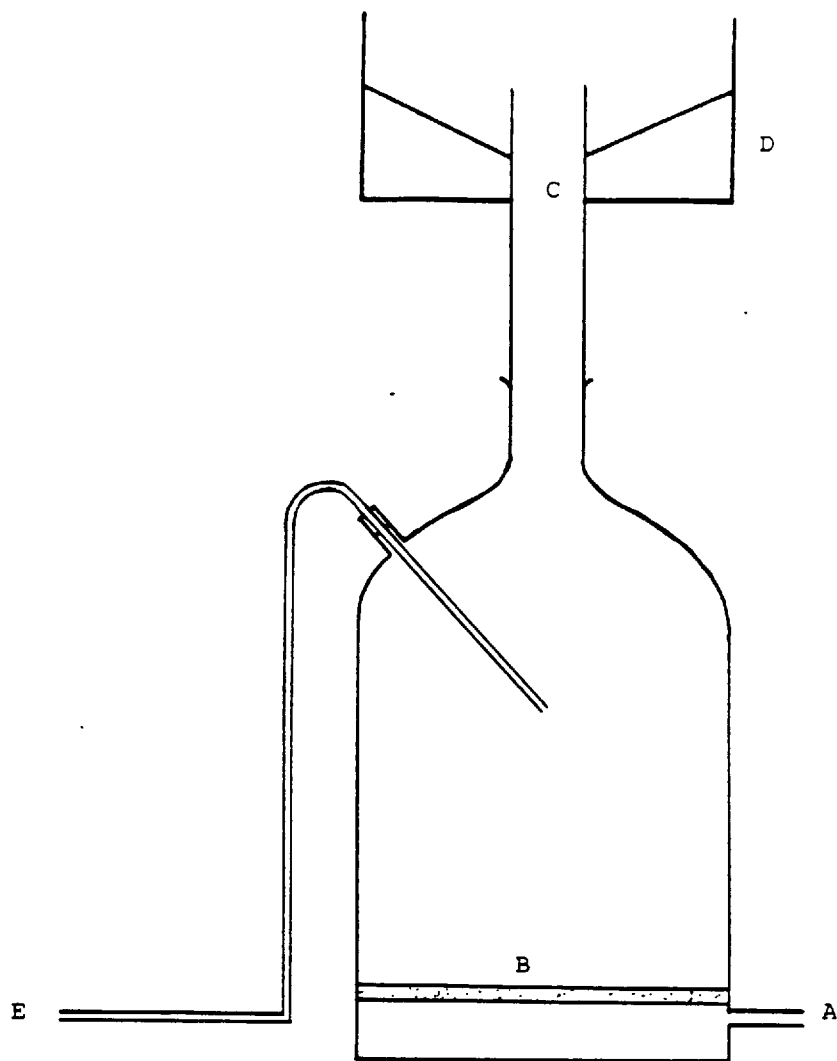
Unsteady gait, pronounced breathing and lateral recumbency. Limb tremor, uncontrolled limb movements, rapid breathing and welling of tears in the eyes were considered associated with the degree of anaesthesia observed.

ADDENDUM 1

(continued)

FIGURE 1

Vapour generator



Key

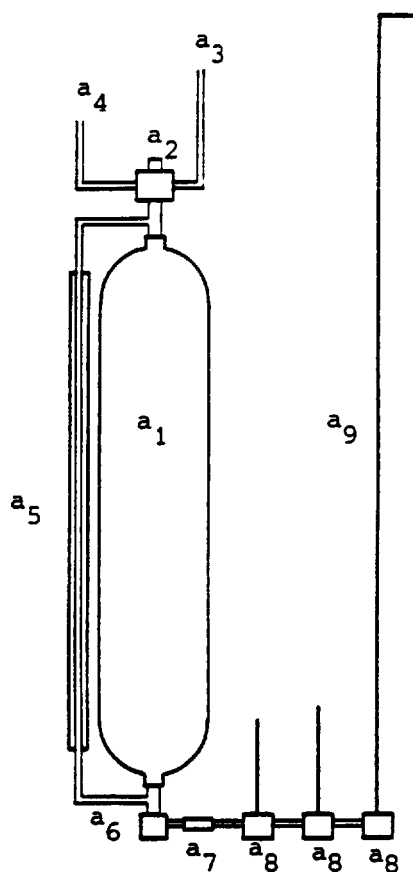
- A. Air line
- B. Fritted glass disc
- C. Vapour inlet
- D. Stainless steel and glass elutriator
- E. Test substance supply line

ADDENDUM 1

(continued)

FIGURE 2

Schematic of generation system



- a
- 1 Pressure reservoir
 - 2 3-way valve
 - 3 Nitrogen line (10 psig)
 - 4 Pressure relief
 - 5 Sight glass
 - 6 Toggle valve
 - 7 7 μ m sinter
 - 8 Metering valves
 - 9 Liquid feed line

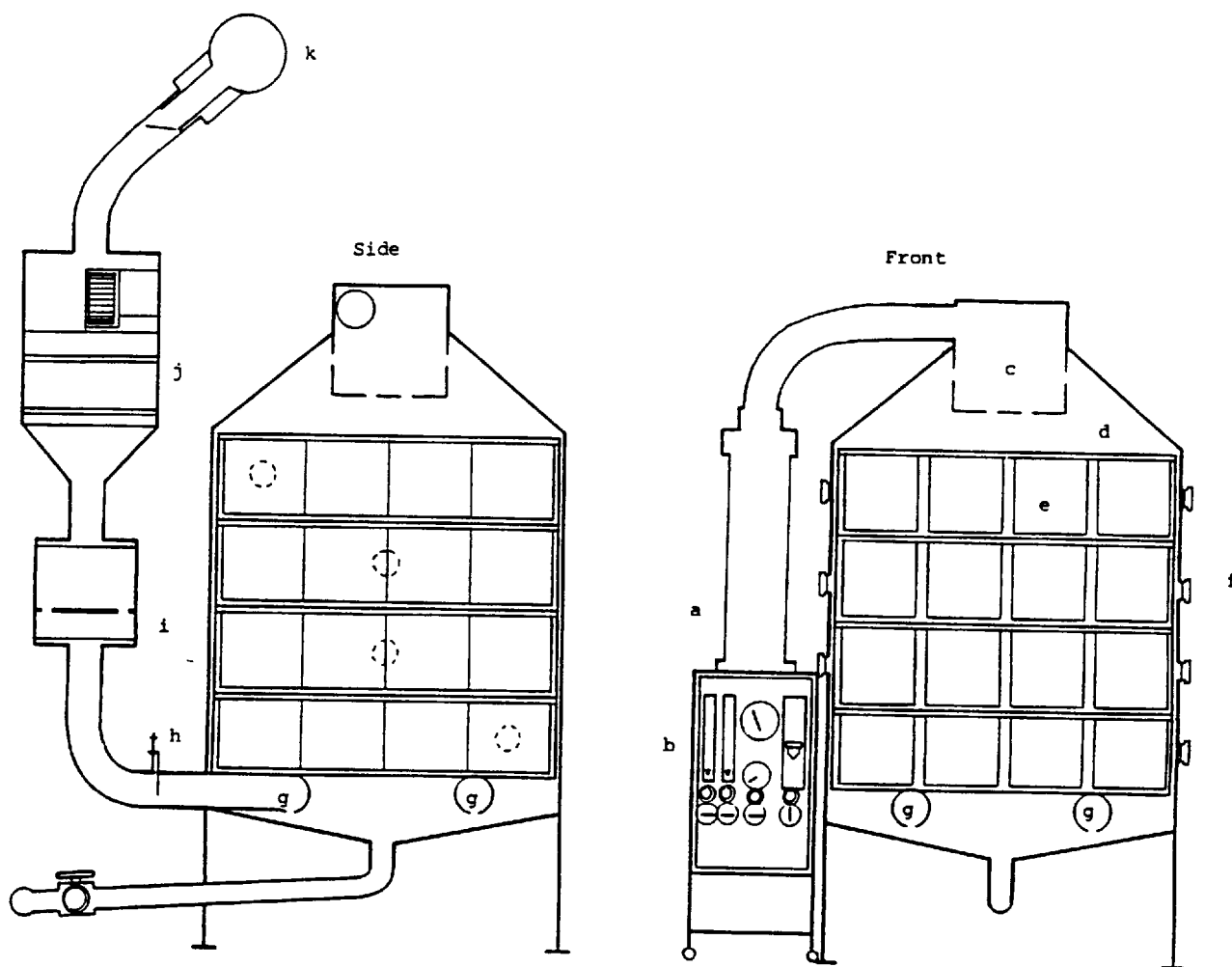
APT/1

ADDENDUM 1

(continued)

FIGURE 3

Exposure system



a Glass elutriation column.

b Airflow control and chamber pressure monitoring.

c Dispersion device.

d Exposure chamber.

e Animal exposure cages.

f Sampling port.

g Exhaust plenum.

h Gate valve.

i Pre-filter.

j Powered extract filter.

k Main exhaust.

ADDENDUM 1

(continued)

TABLE 1

Preliminary generation to preliminary study
Chamber analysed concentration of Toluene

Preliminary generation no.	Preliminary sample no.	Group (ppm)				
		2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
1	10	409	982	1919	3917	5023
	11	467	950	1807	3659	4718
	12	483	1030	1964	3609	5525
	13	483	1038	1844	3585	5612
	14	494	1125	1876	3619	4880
	15	464	1146	1884	3625	4970
	Mean Variation (%)	467 18	1045 19	1882 8	3669 9	5121 18
2	16	488	889	1945	3670	5034
	17	488	1104	1934	3450	5050
	18	488	1059	1966	3479	4946
	19	491	1019	1953	3487	4941
	20	491	1000	1969	3489	4891
	21	488	963	1950	3466	4970
	Mean Variation (%)	488 0.5	1006 21	1953 2	3508 6	4973 3
3	22	502	942	2104	3612	5036
	23	504	1008	2128	3598	5495 ^a
	24	512	987	2104	3561	4702
	25	502	1003	2059	3596	4742
	26	504	966	1977	3540	4798
	27	486	950	1937	3431	5084
	Mean Variation (%)	502 5	977 7	2051 9	3556 5	4975 16
4	28	523	984	2075	4004	5549
	29	520	1022	2059	3864	5082
	30	517	982	2035	3593	5084
	31	509	977	2033	3622	5068
	32	520	992	2033	3657	5090
	33	525	971	2030	3617	5113
	Mean Variation (%)	520 3	987 5	2043 2	3726 10	5163 9

Variation = (range/mean) x 100%

^a Run after sample 27

APT/1

ADDENDUM 1

(continued)

TABLE 1

(continued)

Preliminary generation no.	Sample port *	Group (ppm)				
		2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
5	LUM	502	961	1974	3487	4946
	RL	491	969	1966	3463	4869
	LU	486	971	1961	3471	4944
	LLM	494	955	1966	3500	4848
	RUM	494	982	1948	3487	4893
	^a RU	459/462	969	1937	3442	4896
	^a RLM	470/486	969	1961	3399	4511
	LL	512	979	1961	3434	4877
	Mean	486	969	1958	3460	4869
	Variation (%)	11	3	2	3	11

^a Second sample taken to check chamber port concentration

* Key: LUM Left upper middle
 RL Right lower
 LU Left upper
 LLM Left lower middle
 RUM Right upper middle
 RU Right upper
 RLM Right lower middle
 LL Left lower

Variation = (range/mean) x 100%

ADDENDUM 1

(continued)

TABLE 2a
Chamber concentration of Toluene

Exposure no.	Sample no.	Group (ppm)				
		2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
1	1	552	926	2099	3524	5270
	2	531	923	1993	3524	4909
	3	446	1117	2067	3550	4623
	4	483	1091	2034	3513	4837
	5	464	1088	2001	3423	4776
	6	467	1083	2003	3410	4981
	Mean Variation (%)	491 22	1038 19	2033 5	3492 4	4898 13
2	7	504	1099	2144	3715	5294
	8	496	1022	2038	3593	4840
	9	496	1022	2051	3572	5021
	10	502	1008	2017	3572	4981
	11	478	990	1993	3439	4880
	12	488	992	1966	3466	4718
	Mean Variation (%)	494 5	1022 11	2035 9	3558 8	4957 12
3	13	504	987	1972	3312	5087
	14	536	1091	2064	3383	4991
	15	544	1059	2072	3718	5143
	16	512	1067	2041	3644	4965
	17	504	1027	2035	3593	5013
	18	502	1048	2011	3596	4904
	Mean Variation (%)	517 8	1046 10	2033 5	3540 11	5018 5
4	19	480	1061	2062	3447	4654
	19a		1056			
	20	480	1043	2030	3481	4952
	21	486	1035	2049	3569	5021
	22	525	1038	2009	3519	4978
	23	525	1030	2027	3471	4787
	24	515	1043	1980	3458	4766
	Mean Variation (%)	502 9	1043 3	2027 4	3492 3	4859 8

Variation = (range/mean) x 100%

a Sample retaken to check concentration

Means (ppm) calculated from raw data (mg/ml) recorded in analytical data book

ADDENDUM 1
(continued)

TABLE 2a
(continued)

Exposure no.	Sample no.	Group (ppm)				
		2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
5	25	536	1059	2014	3535	4962
	25a	512				
	26	504	1030	2022	3444	4861
	27	491	1032	2006	3484	5286
	28	499	1040	1995	3389	4989
	28a				3532	
	29	504	1024	1995	3426	4957
	30	515	1053	1990	3402	5023
	Mean	509	1040	2003	3458	5013
	Variation (%)	9	3	2	4	8
6	31	491	1048	2030	3277	4662
	31a				3306	4726
	32	507	1008	2035	3330	5198
	33	504	1043	2030	3601	5007
	34	509	1032	2075	3598	5021
	35	491	1016	2025	3412	4909
	36	502	1008	2033	3444	5042
	Mean	502	1027	2038	3423	4938
	Variation (%)	4	4	2	9	11
7	37	467	1022	1982	3328	4938
	37a				3301	
	38	480	1006	1972	3588	5039
	39	451	987	1932	3455	4928
	40	499	979	1937	3415	4920
	41	491	1035	2035	3651	5111
	42	494	1024	2022	3535	5095
	42a				3558	
	Mean	480	1008	1980	3479	5005
	Variation (%)	7	5	5	10	4
8	43	496	963	2009	3309	4920
	43a			1903	3471	
	44	480	990	1964	4174	4747
	44a			1908	3757	4986
	45	544	979	2051	3872	4835
	46	536	1014	2059	3736	5350
	47	528	1030	2070	3723	5058
	48	528	1003	1993b	3678	5304
	Mean	520	998	1995	3715	5029
	Variation (%)	12	7	8	23	12

Variation = (range/mean) x 100%

a Sample retaken to check concentration

Means (ppm) calculated from raw data (mg/ml) recorded in analytical data book

b Run before sample 43

ADDENDUM 1

(continued)

TABLE 2a

(continued)

Exposure no.	Sample no.	Group (ppm)				
		2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
9	49	499	998	2006	3673	5087
	50	494	1022	1934	3726	4975
	51	504	1006	2009	3519	4933
	51a				3479	
	52	496	1035	1958	3487	4909
	53	494	1008	2038	3471	5079
	54	496	1022	1972	3566	4893
	Mean Variation (%)	496 2	1016 4	1988 5	3561 7	4981 4
10	55	515	1011	2057	3349	4673
	55a					4994
	56	520	1061	2003	3375	4973
	56a				3463	
	57	504	1048	1953	3412	4861
	58	525	1072	2006	3588	5105
	59	523	1043	2046	3317	5087
	60	512	1067	1958	3381	4816
	Mean Variation (%)	517 4	1051 6	2003 5	3412 8	4930 9
11	61	472	1048	1948	3521	5002
	62	528	995	1993	3317	5060
	63	509	1038	1868	3550	4673
	64	555	1038	1988	3598	5079
	65	507	1059	1881	3643	4681
	66	512	1014	1911	3617	4973
	Mean Variation (%)	515 16	1032 6	1932 6	3543 9	4912 8
12	67	515	1069	1932	3712	5023
	68	509	1091	1887	3765	4869
	69	488	1022	1956	3460	4845
	70	475	1051	1913	3665	4893
	71	491	1043	1958	3665	5127
	72	472	1027	1865	3720	4912
	Mean Variation (%)	491 9	1051 7	1919 5	3665 8	4946 6

Variation = (range/mean) x 100%

a Sample retaken to check concentration

Means (ppm) calculated from raw data (mg/ml) recorded in analytical data book

ADDENDUM 1
(continued)

TABLE 2a
(continued)

Exposure no.	Sample no.	Group (ppm)				
		2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
13	73	488	1069	2033	3588	5031
	74	499	1014	1966	3999	6164
	75	523	984	1948	3667	5005
	76	478	958	1980	3410	4848
	77	528	963	1958	3564	4692
	78	504	982	1884	3492	4737
	Mean	504	995	1961	3619	5079
	Variation (%)	10	11	8	16	29
14	79	454	982	1980	3635	5164
	80	525	947	1953	3569	5209
	81	528	1080	1916	3818	4912
	82	472	1061	2038	3625	4901
	83	525	1088	1980	3673	4946
	84	539	1051	1980	3604	4946
	Mean	507	1035	1974	3654	5013
	Variation (%)	17	14	6	7	6
15	85	568	1085	2027	3651	5007
	86	504	1038	2035	3699	4917
	87	496	1024	2046	3439	4851
	88	496	1067	1974	3736	4814
	89	486	998	2022	3606	5204
	90	488	1027	2014	3678	4636
	Mean	507	1040	2019	3635	4904
	Variation (%)	16	8	4	8	12
16	91	499	1008	1961	3420	4994
	92	547	1030	1985	3779	4994
	93	494	854	2017	3901	4912
	94	512	913	1961	3580	5222
	95	509	984	1990	3474	5105
	96	475	1000	1903	3582	5047
	Mean	507	966	1969	3622	5047
	Variation (%)	14	18	6	13	6

Variation = (range/mean) x 100%

Means (ppm) calculated from raw data (mg/ml) recorded in analytical data book

ADDENDUM 1
(continued)

TABLE 2a
(continued)

Exposure no.	Sample no.	Group (ppm)				
		2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
17	97	520	995	2011	3633	5058
	98	520	987	2006	3381	5265
	99	512	958	1950	3375	5105
	100	520	977	1980	3272	5127
	101	525	969	1942	3317	5021
	102	536	955	1961	3375	4997
	Mean	523	974	1974	3391	5095
	Variation (%)	5	4	3	11	5
18	103	533	977	2038	3277	4944
	104	502	958	1988	3625	5050
	105	472	1022	1961	3351	5007
	106	504	1075	1985	3463	5060
	107	507	963	1993	3320	4997
	108	496	1051	1934	3426	5119 ^b
	Mean	502	1008	1982	3410	5029
	Variation (%)	12	12	5	10	3
19	109	517	1125	2144	3365	4392
	109 ^a					5318
	110	512	1061	2022	3426	5148
	111	446	1053	1958	3513	4994
	112	502	1035	1998	3394	4936
	113	512	1088	1911	3466	4944
	114	512	1067	2070	3604	5281
	Mean	502	1072	2017	3460	5002
	Variation (%)	14	8	12	7	19
20	115	486	1120	2070	3543	4991
	116	507	1043	2070	3535	5350
	117	488	1038	1942	3508	4914
	118	507	1011	1990	3336	5092
	119	494	1040	1990	3503	4978
	120	488	1003	2006	3391	5087
	Mean	496	1043	2011	3468	5068
	Variation (%)	4	11	6	6	9

Variation = (range/mean) x 100%

^a Sample retaken to check concentration

Means (ppm) calculated from raw data (mg/ml) recorded in analytical data book

^b Run before sample 107

ADDENDUM 1
(continued)

TABLE 2a
(continued)

Exposure no.	Sample no.	Group (ppm)				
		2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
21	121	509	1059	2011	3566	4920
	122	517	1032	2083	3463	5235
	123	496	1006	2051	3691	5095
	124	491	1022	1953	3484	4952
	125	504	1040	2051	3402	5068
	126	507	1064	2030	3489	5063
	Mean Variation (%)	504 5	1038 6	2030 7	3516 8	5055 6
22	127	496	971	1980	3343	4859
	128	502	1003	1913	3304	4630
	129	512	1011	2041	3532	5023
	130	486	1022	1953	3455	4726
	131	488	979	1974	3259	4758
	132	462	992	1903	3375	4654
	Mean Variation (%)	491 10	998 5	1961 7	3378 8	4776 8
23	133	464	969	1826	3304	
	134	472	1030	1942	3527	
	135	475	1006	1887	3290	
	136	475	1000	1905	3328	
	137	509	1032	1913	3439	
	138	494	1061	2022	3643	
	Mean Variation (%)	483 9	1016 9	1916 10	3423 10	
24	139	472	1051	2025	3609	
	140	523	1043	1956	3519	
	141	520	1011	2099	3423	
	142	520	992	2035	3394	
	143	475	992	2094	3426	
	143a			2064		
	144	491	1032	1972	3590	
	Mean Variation (%)	502 10	1022 6	2035 7	3495 6	

Variation = (range/mean) x 100%

a Sample retaken to check concentration

Means (ppm) calculated from raw data (mg/ml) recorded in analytical data book

ADDENDUM 1

(continued)

TABLE 2b

Study mean analysed concentrations

Batch no.	Exposure numbers	Group (ppm)				
		2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
A	(1 - 10)	503	1029	2014	3513	4963
C	(3 - 12)	505	1031	1992	3529	4964
E	(5 - 14)	504	1025	1979	3553	4985
G	(7 - 16)	504	1019	1974	3591	4985
I	(9 - 18)	507	1017	1972	3551	4994
K	(11 - 20)	505	1022	1976	3547	5010
M	(13 - 22)	504	1017	1990	3516	5007
O	(15 - 24)	502	1018	1991	3480	-
Overall		502	1024	1993	3517	4980

ADDENDUM 1

(continued)

TABLE 3

Analysed/nominal concentration of Toluene (A/N x 100)%

Exposure no.	Group (ppm)				
	2 (Low dose)	3 (Low int. dose)	4 (Int. dose)	5 (High int. dose)	6 (High dose)
1	96.4	102.6	101.2	100.0	96.4
2	96.9	101.0	101.3	101.9	98.7
3	101.6	103.4	101.2	101.4	99.9
4	98.4	103.1	100.9	100.0	96.7
5	100.0	102.9	99.7	99.0	99.8
6	98.4	101.6	101.5	98.0	98.3
7	94.3	99.7	98.5	99.6	99.6
8	101.2	98.7	99.3	106.4	100.1
9	97.4	100.5	98.9	102.0	99.2
10	101.6	103.9	99.7	97.7	98.2
11	101.0	102.1	96.2	101.4	97.8
12	96.4	103.9	95.5	104.9	98.5
13	99.0	98.4	97.6	103.6	101.1
14	99.5	102.4	98.3	104.6	99.8
15	99.5	102.9	100.5	104.1	97.6
16	99.5	95.5	98.0	103.7	100.5
17	102.6	96.3	98.3	97.1	101.4
18	98.4	99.7	98.7	97.6	100.1
19	98.4	106.0	100.4	99.1	99.6
20	97.4	103.1	100.1	99.3	100.9
21	99.0	102.6	101.1	100.7	100.6
22	96.4	98.7	97.6	96.7	95.1
23	94.8	100.5	95.4	98.0	
24	98.4	101.0	101.3	100.1	
Study mean	98.6	101.3	99.2	100.7	99.1

ADDENDUM 1

(continued)

TABLE 4

Temperature and relative humidity during exposure

Exposure no.	Group											
	1 (Air control)		2 (Low dose Toluene)		3 (Low int. dose Toluene)		4 (Int. dose Toluene)		5 (High int. dose Toluene)		6 (High dose Toluene)	
	T(°C)	RH(%)	T(°C)	RH(%)	T(°C)	RH(%)	T(°C)	RH(%)	T(°C)	RH(%)	T(°C)	RH(%)
1	23.3	41	20.3	35	20.8	37	21.0	36	21.0	39	20.6	37
2	22.0	37	19.8	33	20.3	37	20.2	35	20.2	38	20.0	36
3	22.0	44	19.3	47	19.9	52	19.9	51	20.1	47	19.6	39
4	21.6	36	19.3	41	20.1	45	20.1	40	20.1	42	19.5	37
5	21.5	40	19.1	42	19.7	49	19.7	42	19.5	42	19.2	41
6	21.7	42	19.7	35	19.9	47	19.8	47	19.8	40	19.5	34
7	22.2	44	19.9	45	20.5	49	20.4	46	20.3	51	19.9	40
8	22.1	40	19.3	36	20.4	47	20.5	38	20.2	46	20.0	32
9	22.5	39	19.7	47	20.7	49	20.4	49	20.3	45	20.2	38
10	22.4	46	19.2	59	20.5	53	20.1	61	20.1	54	20.1	43
11	23.4	44	20.2	54	21.3	50	21.0	53	21.2	46	21.0	38
12	23.1	42	20.0	60	20.9	60	20.8	58	20.9	53	20.8	43
13	22.3	36	19.6	58	20.8	56	20.8	58	20.7	53	20.7	42
14	21.6	33	19.9	50	20.7	51	20.5	50	20.4	51	20.2	39
15	21.6	32	20.0	41	21.0	41	20.8	47	20.7	47	20.4	38
16	22.5	29	20.5	36	21.3	39	21.2	40	21.0	40	20.7	39
17	22.3	37	20.3	44	21.2	46	21.0	47	21.1	41	20.8	40
18	22.0	25	20.0	37	20.9	44	20.5	44	20.7	39	20.2	42
19	21.5	25	19.7	31	20.8	36	20.5	41	20.5	40	20.0	38
20	21.0	54	19.2	42	20.3	45	20.0	49	20.0	44	19.7	37
21	20.9	30	19.6	39	20.7	39	20.4	39	20.2	41	19.9	37
22	21.0	29	19.5	33	20.5	37	20.4	37	20.2	38	20.0	35
23	20.5	25	19.0	41	19.9	44	19.5	46	19.5	42		
24	20.2	24	18.5	37	19.5	43	19.1	43	19.2	43		
Study mean	21.9	36	19.7	43	20.5	46	20.4	46	20.3	44	20.1	38

ADDENDUM 1
(continued)

TABLE 5
Clinical signs during exposure

Group	Observation	Exposure																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 (Air control)	NAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2 (Low dose Toluene)	NAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3 (Low int. dose Toluene)	NAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4 (Int. dose Toluene)	Aware posture with licking inside of mouth Hunched posture Unsteady on limbs Pronounced breathing Hyper-responsive to knock on chamber wall	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

NAD Nothing abnormal detected
✓ Indicates presence of sign

ADDENDUM 1
(continued)

TABLE 5
(continued)

Group	Observation	Exposure																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
5 (High int. dose Toluene)	Aware posture with licking inside of mouth	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Unsteady on limbs	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Pronounced breathing	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Limb tremor	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Hyperventilation/ tachypnoea	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Uncontrolled limb movement	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Prone/laterally recumbent	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Lachrymation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Hyper-responsive to knock on chamber wall	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Indicates presence of sign

ADDENDUM 1
(continued)

TABLE 5
(continued)

Group	Observation	Exposure																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
6 (High dose Toluene)	Aware posture with licking inside of mouth	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Unsteady on limbs	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Pronounced breathing	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Limb tremor	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Hyperventilation/ tachypnoea	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Uncontrolled limb movement	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Prone/laterally recumbent	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Lachrymation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Hyper-responsive to knock on chamber wall	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Indicates presence of sign

ADDENDUM 1

(continued)

APPENDIX 1

Method of analysis for Toluene

1. Instrumentation and apparatus

Gas chromatograph: Pye Unicam series 304 chromatograph fitted with a flame ionisation detector.

Integrator: Spectra Physics SP4200.

Apparatus: Pyrex glass adsorption tubes, approximately 100 mm long and 2 mm i.d., packed with 20 mm of Chromosorb 102.

2. Reagents

Carbon disulphide: A.R. grade. FSA Laboratory Supplies.

Toluene: 'Hipersolv', BDH Ltd.

3. Gas chromatograph operating conditions

Column: 1.0 m x 3 mm i.d. glass packed with 20% Carbowax 20M on DCLQ 80 - 100 mesh.

Temperature: Column 100°C
 Injector 200°C
 Detector 250°C

Gases/flow rate: Helium (carrier) 30 ml/min
 Hydrogen 33 ml/min
 Air 240 ml/min

4. Analysis of samples

Samples of the test atmosphere were withdrawn through adsorption tubes. The tubes were connected to the helium line of the gas chromatograph and inserted into the modified injection port where the sample was desorbed into the column.

ADDENDUM 1

(continued)

APPENDIX 1

(continued)

The concentration of Toluene was evaluated using the external standard method. Known standard amounts of Toluene in CS₂ were injected into sample tubes. Using a Dräger pump the standard was drawn into the tube and the Toluene adsorbed on the stationary phase. The standards were treated in the same manner as the exposure samples (see above).

The concentrations of Toluene in the samples was evaluated from the expression:

$$C_x = A_x/A_s$$

where C_x = concentration of Toluene in the sample
 A_x = integrated peak area due to Toluene
 A_s = response factor ($\mu\text{g}/\text{area}$) for Toluene
(derived from standard curve)

5. Standardisation

Approximately 1.5 g of Toluene was accurately weighed into a volumetric flask and made up to 50 ml volume with carbon disulphide. Aliquots of this solution were diluted with further carbon disulphide to give standard solutions containing Toluene at concentrations of 5.9944 and 14.986 mg/ml.

These were stored in a refrigerator at 4°C when not in use, and were allowed to equilibrate to room temperature before use.

Aliquots of these standard solutions were injected into adsorption tubes, adsorbed on the stationary phase present in the tube using a Dräger pump, and thermally desorbed into the column of the GC. The resulting peak areas for each standard amount injected were used to calculate the mean response factor by regression analysis using linear least squares.

The standard amounts injected were: 11.99, 17.98 and 29.97 μg of Toluene.

ADDENDUM 2

Procedure for time-mating of animals

One hundred and fifty-four sexually mature Specific Pathogen Free male and female rats (27 males and 127 females) (Cr1: CD[®] (SD) BR VAF/Plus strain) were ordered from Charles River Portage, Michigan, USA. An additional five males and five females were ordered for health check purposes.

On arrival, all animals were examined for abnormalities and for signs of overt ill health. Those designated as health check animals were killed within 24 hours after arrival at HRC and subjected to routine macroscopic examination. Any abnormalities seen were processed immediately and examined microscopically. Lungs, liver, kidneys, spleen and heart were preserved in fixative, but not processed further. Their health status was acceptable (see Addendum 7).

The remaining animals were weighed on arrival, marked on the paw by a tattoo line to indicate that they were involved in the study and were marked individually by a temporary number written on the tail. The animals were time-mated within the Department of Reproductive Toxicology and supplied to the Department of Inhalation Toxicology. The day of mating, as judged by the appearance of sperm in the vaginal smear or by the presence of a vaginal plug, was considered Day 0 of pregnancy.

Males and females were weighed and examined for signs of ill health on a weekly basis. Those females with a positive indication of mating were also weighed on each day of mating (i.e. the day the male and female were separated).

Animals were gang-housed with no more than five to a cage prior to mating in suspended galvanised metal cages (Bowman[®]) equipped with solid sides and back, wire mesh front, floor and top. Cages containing females were interspersed between cages containing males, where practical, to promote development of regular oestrous cycles.

During the mating period, animals were housed in plastic breeding cages (North Kent Plastics, RC-1 type), on the basis of one male to three females. Suitable nesting material was provided - see Addendum 3. Twenty males were paired with sixty females on each night of mating. On the morning following mating, vaginal smears were taken from all paired females and examined for the appearance of sperm in the vaginal smear or the presence of a vaginal plug. Females with this "positive indication of mating" were formally allocated to the study as detailed in the procedure, and transported, in filtered boxes, to the Department of Inhalation Toxicology. Females without a "positive indication of mating" were rehoused in metal cages. A rest day was allowed before the apparently non-pregnant females were re-paired with the males. The 1 male : 3 female pairings were maintained by the inclusion, into the mating procedures, of previously naive females to replace females with a "positive indication of mating".

A schematic representation is presented in Figure 1.

ADDENDUM 2

(continued)

FIGURE 1

Day of mating procedure	Details	Outcome (number of animals)	
		Positive indication of mating	No indication of mating
1	20♂ x 60♀	A	B
2	REST		
3	20♂ x 60♀ (B + A 'n' extra)	C	D
4	REST		
5	20♂ x 60♀ (D + C 'n' extra)	E	F
6	REST		
7	20♂ x 60♀ (F + E 'n' extra)	G	H
8	REST		
9	20♂ x 60♀ (H + G 'n' extra)	I	J
10	REST		
11	20♂ x 60♀ (J + I 'n' extra)	K	L
12	REST		
13	20♂ x 60♀ (L + K 'n' extra)	M	N
14	REST		
15	20♂ x 60♀ (N + M 'n' extra)	O	

'n' Number

NOTES

1. Batches A + C + E + G + I + K + M + O provided 72 time-mated females.
2. There were occasions when females were mated to different males, see Appendix A.

APT/1

ADDENDUM 2

(continued)

APPENDIX A

Group allocation of females

Male number	Temporary tailmark of female paired with male at mating								Group allocation of female from mate							
	A	C	E	G	I	K	M	O	A	C	E	G	I	K	M	O
201	1*	61	61	61	61	61	61	61	5							
	2	2	74	74	74	74	74	74								
	3*	62	75	75	75	75	75	75	1							
202	4	4	76	76	76	76	76	76								
	5*	63*	77	77	77	77*	4	4*	1	5				6		2
	6	6	6	6	6	6	6	6								
203	7	7	7	7*	113	113*	127	127				5		1		
	8	8*	78	78	78	78	78	78		6						
	9	9*	79	79	79*	123*	2*	62	2				3	3	4	
204	10	10*	80	80	80	80	80	80		5						
	11	11	11*	105	105*	124	124	124			2		6			
	12*	64	64	64*	114*	125*	17	17	3			4	1	5		
205	13	13*	81	81	81*	126	126	126		5			6			
	14	14	14	14	14	14	14	14								
	15	15	15	15*	115	115*	42	42				3		4		
206	16	16*	82	82	82	82	82	82		4						
	17	17	83	83	83	83	83	83*								1
	18	18	18	18	18	18	18	18								
207	19	19*	84	84	84	84*	65	65		4					2	
	20*	65	65	65	65	65	21	21	1							
	21	21	21	21	21	21	46	46								
208	22*	66	85	85	85	85	85	85	6							
	23*	67*	86	86	86	86	86	86	5	4						
	24	24*	87	87	87	87	87	87		1						
209	25	25	25	25	25	25	25	25								
	26	26*	88	88	88	88	88	88		1						
	27	27	27*	106*	116	116*	48	48*				4	3		2	5
210	28	28*	89	89*	117	117*	68	68		6			2		3	
	29*	68	90	90	90	90	90	90	4							
	30	30	30	30	30	30	30	30								
211	31	31	91	91*	118	118*	31	31*					5		1	4
	32*	69	69	69	69	69	69	69	3							
	33	33	92*	107*	119	119*	33	33*				6	2		4	5
212	34	34														
	35	35														
	36	36														
213	37	37	37	37	37	37	37	37								
	38	38	38*	108	108	108	108	108*				1				3
	39	39*	93	93	93	93	93	93		6						

* Positive indication of mating - assigned to study

ADDENDUM 2

(continued)

APPENDIX A

(Group allocation of females - continued)

Male number	Temporary tailmark of female paired with male at mating								Group allocation of female from mate							
	A	C	E	G	I	K	M	O	A	C	E	G	I	K	M	O
214	40*	70	70*	109	109	109	109	109	4		5					
	41	41	94	94	94	94*	41	41*						6		2
	42	42	95	95	95	95	95	95*								3
215	43	43	43*	110	110	110	110	110			3					
	44	44	44	44	44	44	44	44								
	45*	71	96	96*	120	120	120	120	6			4				
216	46	46	97*	111	111	111	111	111			1					
	47*	72	72	72	72	72	72	72	2							
	48	48	98	98	98	98	98	98								
217	49	49*	99	99	99	99	99	99		3						
	50	50	50	50	50	50	50	50								
	51*	73	73	73	73	73	73	73	2							
218	52	52*	100	100	100	100	100	100		2						
	53	53*	101	101*	121	121*	122	122		3		1		5		
	54	54	102	102*	122	122	54	54				6				
219	55	55														
	56	56														
	57	57														
220	58	58														
	59	59														
	60	60														
221			34	34	34											
			35	35	35											
			36	36	36											
222			55	55	55	55	55	55			2					
			56*	112	112	112	112	112								
			57	57	57	57	57	57								
223			58	58	58	58	58	58								
			103	103	103	103	103*	66							6	
			104	104	104	104	104	104								
224																
225						34	59	59								
						35	35	35								
						36	60	60								

* Positive indication of mating - assigned to study

ADDENDUM 3

Quality assurance aspects of nesting material

The nesting material used, designated Goldchips sawdust grade 6, was produced by Biosure Ltd. The sawdust was principally derived from UK grown Norway Spruce, Picea abies, the addition of small amounts of UK grown Scots Pine, Pinus sylvestris is permitted. Combination with hardwood species or imported wood is not permitted. No chemical preservative is applied to timber during processing or storage. The standards of production adopted by the manufacturers have been approved by the Quality Assurance Unit.

As a precautionary measure a batch of sawdust was analysed for chemical contaminants every 3 months at a laboratory approved by HRC.

The maximum permitted levels of contaminants are:

Polychlorinated biphenyls	10 ppm
Pentachlorophenols	2.0 ppm
Dieldrin	0.1 ppm

The certificate of analysis was made immediately available to HRC.

ADDENDUM 4

Composition and quality assurance aspects of rodent diet

Biosure LAD was a closed formula diet suitable for normal health, growth and reproduction of laboratory rats and mice. The standards of production adopted by the manufacturers have been approved by the HRC Quality Assurance Department.

Analyses are made of all batches of diet for most nutrients and for specified substances and micro-organisms likely to be present in feed ingredients or the finished diet and which, if in excess of specified amounts, might have had an undesirable effect on the test system. Although occasional slight deviation may be permitted, batches of diet used conformed with the acceptable standards agreed by the Study Director and Quality Assurance HRC at detailed below.

(A) <u>Nutrients</u>		<u>Target level</u>	<u>Maximum Tolerance (%)</u>
Crude fat	%	3.7	±15
Crude protein	%	21.5	±10
Crude fibre	%	2.0	±40
Ash	%	5.5	±15
Moisture	%	9.5	10.5 Max
Calcium	%	1.0	±20
Phosphorus	%	0.9	±20
Sodium	%	0.3	+100-50
Chloride	%	0.5	+100-50
Potassium	%	0.8	+100-50
Magnesium	%	0.15	±50
Manganese	mg/kg	70	±50
Iron	mg/kg	220	±50
Copper	mg/kg	15	±50
Zinc	mg/kg	60	±50
Vitamin A	iu/kg	12000	+50-20
Vitamin E	mg/kg	3500	+150-20

(B) <u>Contaminants</u>		<u>Maximum allowable concentration</u> (mg/kg except where stated)
Lead		2.5
Cadmium		0.5
Arsenic		1.5
Mercury		0.1
Selenium		0.6
Fluoride		40
Nitrates (as sodium nitrate)		200
Nitrites (as sodium nitrite)		10
PCBs		0.05
Total DDT		0.15
Dieldrin		0.05
Lindane		0.15
Heptachlor		0.05
Malathion		5.0
Total Aflatoxins (B ₁ , B ₂ , G ₁ , G ₂)		5.0 µg/kg

ADDENDUM 4

(continued)

(C) Microbiological content

Maximum count/g diet (at time of manufacture)

	<u>LAD 1</u>
Total viable organisms	10,000
Mesophilic spores	30,000
Presumptive <u>E. coli</u>	0
<u>E. coli</u> Type 1	0
<u>Salmonella</u> spp.	0
Fungal units	1,000
Antibiotic activity	0

ADDENDUM 5

Quality assurance aspects of water

Results of the routine physical and chemical examination of drinking water at source (Grafham Final Water) as conducted usually weekly by the supplier - Anglian Water Authority, were made available to HRC as quarterly summaries. Additionally, levels of specified substances known to be present from time to time in local water and which, if present at levels in excess of the maxima (for humans) might have had undesirable effects on the test system, were determined in HRC tap water at approximately 6-monthly intervals.

Quarterly summary analyses of source water normally included levels of nitrites, nitrates, Ca, Mg, Na, K, P, Cl, Si, Fe.

Six monthly analyses of HRC tap water currently include levels of As, Se, Ba, Ag, Sb, organophosphorus, organochlorine and other pesticides, haloforms, chlorophenols, polychlorinated biphenyls and polycyclic aromatic hydrocarbons.

ADDENDUM 6

Certificate of analysis of test compound

CERTIFICATE OF ANALYSIS

REFERENCE NO. 3471/90



CHIEF ANALYST: Dr. K. H. Scheider

PRODUCT: 15295 TOLUENE HIPERSOLV

QC BATCH No. A04795

DATE TESTED: 2.7.90

LABELLING NUMBER: 2212770L

QUANTITY: 60 x 2.5 L

CUSTOMER: HUNTINGDON RESEARCH CENTRE, DEPARTMENT OF INHALATION,
 WOOLLEY ROAD, ALCONBURY, HUNTINGDON PE18 6ES
 Order No 192749.

Description	Clear, colourless liquid
Assay (GLC)	99.9 %
Weight per ml at 20°C	0.8659 g
Water	300 ppm maximum
Acidity (HCl)	5 ppm maximum
Non-volatile Matter	10 ppm maximum
Benzene content	< 100 ppm
transmission, after purging with nitrogen for not less than 10 minutes, in a 10mm cuvette at:	
290nm	84.4 %
310nm	97.5 %
320nm	98.7 %
340nm	99.7 %

GFL/TDS
 3rd August 1990

G F Lewis, C.Chem, FRSC
 Deputy Chief Analyst

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ADDENDUM 7

Post mortem findings of health check animals

On arrival, 5 males and 5 females were sacrificed for purposes of determining health status. These animals were killed within 24 hours.

The following macroscopic/microscopic changes were observed:

- | | |
|-------------------------|---|
| One male rat with: | Skin, stained. Cervical lymph nodes enlarged (microscopic examination revealed unilateral plasmacytosis). Stomach, nodules (microscopic examination revealed focus of ectopic squamous epithelium). |
| Second male rat with: | Cervical lymph nodes, enlarged (microscopic examination revealed bilateral plasmacytosis). |
| Third male rat with: | Stomach, nodules (microscopic examination revealed focus of ectopic squamous epithelium). |
| Fourth female rat with: | Kidneys, increased pelvic dilatation (microscopic examination revealed moderate bilateral renal pelvic dilatation). |

These changes were not considered related to the presence of infectious disease.

ADDENDUM 8

Deviations from protocol

During the course of this study the following deviations from protocol were noted:

Appendix 3, Water consumption - individual values

Animal number 29 on Day 11 of pregnancy had water bottle withheld in error.

Appendix 7, Litter data - individual values

Animal number 62, corpora lutea not countable as unable to see, macroscopically, due to early timing of embryonic deaths.

Due to an oversight, fetuses were not individually tagged at terminal autopsy therefore we were unable to record and summarise foetal weights by sex.

Appendix 8, Sex ratios - individual values

Animal number 47, the sex of one pup was not recorded in error at terminal autopsy.

Procedure, Animal management and accommodation

Owing to a temporary failure of the humidity control unit, the relative humidity recorded exceeded the limit set by 7% for a period of not more than 12 hours (the failure was rectified when discovered).

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