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Health and Environmental
Sciences Department

Toluene

The Effect on Pregnancy of the Rat (Inhalation Exposure)

JUNE 1993

**TOXICOLOGY REPORT NUMBER TR401
CAIS ABSTRACT NUMBER 40-31150**

American Petroleum Institute
Health and Environmental Sciences Department

QUALITY ASSURANCE/GLP COMPLIANCE STATEMENT

Study Title: The Effect of Toluene on Pregnancy of the Rat
(Inhalation Exposure)


Testing Facility: Huntingdon Research Centre Ltd.

Testing Facility Number: APT 1/91309 : APT 2/91279

This study was reviewed by API Quality Assurance personnel under the direction of API Management on the dates indicated below for compliance with EPA TSCA Good Laboratory Practice (GLP) regulations. This study was conducted in accordance with EPA TSCA GLP regulations, with the exception of the item listed on page 2 of this statement.

Copies of reports by API Quality Assurance personnel are available upon written request to the Director of the Health and Environmental Sciences Department of the American Petroleum Institute or his designee.

<u>Date(s) of Inspection/Review</u>	<u>Type of Inspection</u>	<u>Date of Report To Management</u>
4/6/90	Proposal Review	4/6/90
5/31/90	SOP Review	5/31/90
6/26/90	Prel. Protocol Review	6/26/90
7/5/90	Prel. Protocol Review	7/5/90
7/9/90	Report Format Review	7/9/90
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7/31/90	Prel. Protocol Review	7/31/90
9/19-20/90	Audit Preliminary Data	10/11/90
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6/3/92	3rd Draft Report Audit	6/3/92
7/13/92	Preliminary Report Audit	7/13/92
9/15/92	Final Report Acceptance	9/15/92


Christine Sexsmith
Quality Assurance Coordinator

9/15/92
Date

FOREWORD

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The range-finding study conducted to determine dose levels for this study can be found in report TR400, "A Preliminary Study of the Effect of Toluene on Pregnancy of the Rat."

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TOLUENE
THE EFFECT ON
PREGNANCY OF THE RAT
(Inhalation exposure)

Study completed:

Regulations: EPA (TSCA) 40 CFR 798.4350

Addressee:

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Report issued: 10 September 1992

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CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by AMERICAN PETROLEUM INSTITUTE. These results may not be published, either wholly or in part, or reviewed or quoted in any other publication without the prior authorisation of the Sponsor.

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

HRC Report No. APT 2/91279

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.

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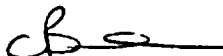
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We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.



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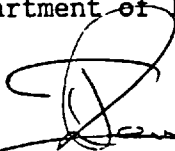
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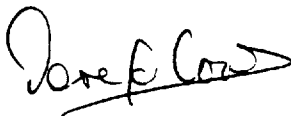
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QUALITY ASSURANCE STATEMENT

This report has been audited by the 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

09.09.92



K.P. de-Salis, B.A.,
Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

10 September 92


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QUALITY ASSURANCE STATEMENT

DATES OF STUDY INSPECTIONS

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	-	27.09.90
Pre-experimental Period	26.09.90	26.09.90
Experimental Period	04.10.90	04.10.90
	16.10.90	16.10.90


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10 September 92

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SUMMARY

1. In this assessment of the effect of the solvent, Toluene, on pregnancy and in utero development of the rat, dosages of 0, 250, 750, 1500 and 3000 ppm were administered by inhalation for a period of 6 hours a day from Days 6 to 15 of pregnancy inclusive. On Day 20 surviving females were sacrificed and subjected to post mortem examination. Litter values were determined and fetuses subsequently examined for skeletal and visceral changes.
2. Exposure to Toluene was associated with a dosage-related maternal response at 750 ppm and above. At 750 ppm, reaction was confined to awareness to exposure and closed/half-closed eyelids; this is a non-specific response to exposure and is not considered to be biologically meaningful.

Additional responses at 1500 ppm were uncoordinated gait, ataxia and hypersensitivity to knock on chamber wall. Bodyweight gain was lower than controls during the first two days of treatment. At the highest dosage of 3000 ppm, similar signs were observed, as well as abnormal limb movements, lachrymation, increased respiration and, on isolated occasions, salivation and nystagmus of the eyeball. Water consumption was increased and food consumption reduced during the treatment period. Initial bodyweight loss was recorded during the first two days of treatment.

3. Treatment-related effects of maternal exposure on embryofetal development were confined to 1500 and 3000 ppm and included an exposure-related, but minimal reduction in litter and mean foetal weights, and an increase in fetuses with reduced or unossified sternebrae.

Conclusion

Within the context of this study into the effect of inhaled Toluene on pregnancy and in utero development of the rat, exposures of 1500 and 3000 ppm were associated with obvious signs of maternal and embryofetal toxicity.

The no-effect level for specific maternal toxicity and embryofetal development (to Day 20) was considered to be 750 ppm.

It is concluded that exposure to Toluene via the inhalation route does not show any selective effects on embryofetal development.

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INTRODUCTION

This report describes an experiment performed to assess the effect of the solvent, Toluene, on the pregnancy of the rat and in utero development of offspring when administered by inhalation, 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 in manufacture and use. The exposure levels were based on the results of a preliminary study (APT/1-R) performed in these laboratories and reported separately.

Toluene was supplied from BDH (British Drug Houses Ltd., Poole, Dorset, U.K.) in sixty 2.5 litre bottles as a clear colourless liquid, (purity 99.9%, batch no. 2212770L, expiry date 1st August 1995). It was received in these laboratories on 16 July 1990 and was stored in the dark under ambient temperature.

Key dates of the study were as follows:

Protocol approval by:

Study Director:	24 September 1990.
HRC Management:	24 September 1990.
Sponsor:	25 September 1990.
	(Sponsor confirmed protocol details by telephone call)*.

Arrival of animals:	13 September 1990.
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Mating - first mating set:	24 September 1990.
- last mating set:	28 September 1990.

Commencement of treatment	
- first mating set:	1 October 1990.

Last day of treatment	
- last mating set:	14 October 1990.

Terminal sacrifice	
- first mating set:	15 October 1990.
- last mating set:	19 October 1990.

* Written protocol approval:	19 October 1990.
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METHODS

Animal management and non-exposure accommodation

Sexually mature (9 - 10 weeks old, weight range 170 - 229 g) Specific Pathogen Free female rats (CrI: CD® (SD) BR VAF/Plus strain) which were time-mated to identified males of the same strain in the Department of Reproductive Toxicology Facility (details are presented in Addendum 2), were forwarded to the Inhalation Toxicology 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, within each batch, assigned to five 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. A random allocation was used for animals from the second day of mating. 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°C and 55% respectively. During the course of the study recorded values for temperature and relative humidity ranged from 20°C ± 2°C and 62% ± 20% respectively. Lighting was controlled to give 12 hours light (8 am to 8 pm) and 12 hours dark per 24 hours.

The animals were housed individually in suspended stainless steel cages, equipped with solid and mesh sides and mesh floors. During exposure the animals were housed individually in suspended stainless steel mesh cages. 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 and Director.

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 was withheld. (Quality Assurance aspects for food and water are presented in Addenda 4 and 5).

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Experimental design

The experimental design was as follows:

Group/ colour code	Exposure level Toluene (ppm)	Number of rats ♀	Animal numbers in batch				
			I	II	III	IV	V
1:White	Control	25	1-5	6-10	11-15	16-20	21-25
2:Yellow	250	25	26-31	32-36	37-40	41-45	46-50
3:Blue	750	25	51-55	56-60	61-65	66-71	72-75
4:Green	1500	25	76-81	82-86	87-90	91-96	97-100
5:Red	3000	25	101-105	106-110	111-115	116-121	122-125

Exposure of rats to the test substance

Rats were exposed in whole-body exposure chambers for 6 hours per day from Days 6 to 15 of pregnancy inclusive. All details relating to the inhalation exposure system, methodology and results are presented in Addendum 1.

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 for all animals. A maximum of 8 animals were observed, at half-hourly intervals during exposure. Any adverse signs together with responses to tapping on the inhalation chamber walls, were reported. The times and duration of observations were recorded.

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(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 commencing on Day 0 of pregnancy.

Water consumption was measured daily, by weight, 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. Bodyweights on Day 20 corrected for gravid uterine weights are also reported.

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, but one animal in error. 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 is 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 examined for evidence of implantation using a modified Salewski technique^a.

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Live young were examined externally and weighed. Half the fetuses in each litter were preserved in Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities (Wilson technique^b); the remainder were fixed in 74 OP industrial methylated spirit for subsequent macroscopic examination, evisceration, clearing and alizarin staining (modified Dawson technique^c) for skeletal examination. Young showing suspected abnormalities were processed by the more appropriate technique for clarification of initial observations. All further foetal, visceral and skeletal examinations were conducted blind i.e. without prior knowledge of the treatment conditions. Details are maintained with the raw data.

All fetuses were sexed by gonadal inspection following preservation.

Fetuses were uniquely identified to allow correlation of initial with subsequent findings.

Structural changes were presented as:

- Malformations: rare and/or probably lethal, e.g. exencephaly, anury
- Anomalies: minor differences from 'normal' that are detected relatively frequently either by free-hand sectioning, e.g. increased renal pelvic dilatation, or at skeletal examination, e.g. bipartite centrum.
- Variants: alternative structures occurring regularly in the control population are classified as variants e.g. unossified sternbra(e).

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. Mean foetal weight was also calculated by sex to detect any sex specific effects.

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Group values

Group mean values calculated from individual values were presented for all animals with live young at Day 20.

Statistical analysis

The statistical analyses performed are summarised as follows:

Data type	Statistical test	Intergroup comparisons made by:
<u>Maternal data</u>		
Clinical signs	Not analysed	
Water consumption	AOV on daily mean values	Williams' test
Food consumption	AOV on 2-day mean values	Williams' test
Bodyweight	AOV on bodyweight change from Day 6	Williams' test
	KW on adjusted change at Day 20 (-gravid uterine weight)	Shirley's test
Liver weights	AOV on absolute weights	Williams' test
	KW on liver weights adjusted for terminal bodyweight	Shirley's test
<u>Litter data - mean values</u>		
Corpora lutea, implantations, pre- and post implantation loss, early and total embryonic deaths, live young, litter weight, mean foetal weight, gravid uterine weight, sex ratio.	Kruskal-Wallis	Shirley's or 't' test
Late embryonic deaths	Fisher's test	Fisher's test
Malformations, anomalies	Not analysed	
Sternebral variants:		
- normal, unossified, reduced ossification, total variant	Kruskal-Wallis	Shirley's or 't' test
- asymmetric/bipartite	Fisher's test	Fisher's test

Key to references: AOV, analysis of variance (or covariance) - reference 4
 Williams' test - reference 5
 Kruskal-Wallis and 't' test - reference 1
 Shirley's test - reference 2
 Fisher's test - reference 3

All intergroup comparisons (trend tests) are reported at the 5% level of significance

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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, are 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 is kept in the Huntingdon Research Centre Ltd. Archives.

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RESULTS

1. ACHIEVED EXPOSURE CONCENTRATIONS (Addendum 1)

The overall mean exposure concentrations throughout the study were within 1% of intended exposure concentrations of Toluene.

2. ADULT FEMALES

Exposure: 0, 250, 750, 1500 and 3000 ppm Toluene from Days 6 to 15 of pregnancy inclusive.

(a) Signs and mortalities (Tables 1a and 1b, Appendix 1, Addendum 1)

Signs observed during exposure were generally restricted to exposure at 750, 1500 and 3000 ppm and included at 750 ppm and above, awareness of exposure and closed/half-closed eyelids. This is a non-specific response to exposure and is not considered to be biologically meaningful. At 1500 and 3000 ppm, abnormal gait/ataxia, and hyperresponsivity to 'knock on chamber wall' were observed. Further signs of reaction to exposure at 3000 ppm only included limb tremors/uncontrolled movements, lachrymation, increased respiration and salivation and nystagmus of the eyeball were noted on isolated occasions.

There were no obvious signs of 'accommodation' as the treatment period progressed, although nystagmus of the eyeball (noted at 3000 ppm), was only observed in one animal in the earlier stages of the treatment period.

No signs of reaction to exposure to Toluene at 250 ppm were noted.

One animal at 3000 ppm (number 120) was found dead after exposure on Day 14 of pregnancy (after 9 exposures - Day 12 of the total exposure period). No obvious cause for death was established.

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

Water consumption at 3000 ppm was markedly increased from Day 8 of pregnancy and remained higher than controls ($P < 0.01$) throughout the treatment period. By sacrifice on Day 20, however, although water consumption was still greater than controls the magnitude of the response had lessened.

There were no marked treatment-related effects on water intake at lower exposure concentrations. A slight increase was noted towards the end of the treatment period at all exposures. (Difference from control $P < 0.05$).

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

Food consumption at 3000 ppm was markedly reduced during the first two days of treatment and although the magnitude of the response lessened, remained lower than controls throughout the remainder of the exposure period ($P < 0.01$).

There were not considered to be any adverse or significant effects of exposure to Toluene at lower concentrations on food consumption.

(d) Bodyweight change (Figure 3, Table 4, Appendix 4)

At 3000 ppm mean bodyweight loss occurred during the first two days of exposure and the rate of weight gain remained lower than that of the controls through to Day 14. Once treatment was terminated weight gain was slightly superior to controls, although parity was not attained by Day 20. (Difference from control for bodyweight minus gravid uterine weight, $P < 0.01$).

At 1500 ppm there was a reduction in bodyweight gain during the first two days of treatment. Thereafter, apart from a slight reduction in weight gain towards the end of the exposure period, weight gain was similar to controls to termination. (Difference from control at Day 16, $P < 0.05$).

At 750 ppm, there was a marginal non-significant difference in bodyweight gain by Day 10; thereafter gains were essentially comparable with the controls.

There were no apparent adverse effects of treatment on the pattern of bodyweight change at 250 ppm.

(e) Terminal autopsy (Table 5, Appendices 1 and 5)

There were no macroscopic changes attributable to exposure to Toluene at terminal autopsy on Day 20.

There were no dosage-related effects on liver weights of females on Day 20 of pregnancy when analysed taking terminal bodyweights into account. However, if the terminal bodyweights are corrected for the gravid uterine weight then a slight increase becomes apparent at 750 ppm and above (difference from controls $P < 0.05$ at 750 and 1500 ppm, $P < 0.01$ at 3000 ppm), although no dosage response is apparent.

3. LITTER DATA

A total of 24, 22, 20, 19 and 22 females had live young at Day 20 (and 293, 272, 233, 236 and 277 fetuses) in Groups 1 to 5 respectively and have been included in this assessment.

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- (a) Litter size, embryonic losses and sex ratios (Table 6, Appendices 6 and 7).

Intergroup differences in the corpora lutea count and implantation rate were slight (these events are usually considered to be established prior to the commencement of treatment), and did not adversely influence interpretation of later events.

There were no adverse effects of treatment on the incidence of embryonic deaths (and post implantation loss), or on resultant litter size.

There was no evidence that exposure to Toluene adversely affected in utero survival of the fetuses; the mean sex ratio was similar in all groups.

- (b) Litter, mean foetal and gravid uterine weights (Tables 6 and 7, Appendices 6 and 7)

At 3000 ppm litter and mean foetal weights were significantly reduced when compared to controls. This trend was apparent, albeit to a lesser degree, at 1500 ppm (litter weights, however, were not significantly different from controls). There were not considered to be any treatment-related effects on litter and mean foetal weights at 250 and 750 ppm. Mean foetal weight at 250 ppm were significantly lower than the controls, however, since there was no dosage relationship this finding is considered to be of no biological significance.

There was no evidence of any selective effect of Toluene on in utero development of either sex - any differences observed amongst combined litters were seen when assessing litter and mean foetal weights by sex.

Intergroup differences in gravid uterine weight tended to follow litter weights, as expected, with differences from controls attaining statistical significance at 3000 ppm.

- (c) Malformations, anomalies and variants (Tables 8 - 12, Appendices 8 - 10)

The total number of malformed fetuses and litters was higher in 3 of the 4 treated groups than in the control group, with the difference from control values attaining statistical significance. However, since the types of changes seen fall within background incidence, there was no increase in anomalies (usually expected with a real event) and no dosage relationship was apparent (no fetuses were malformed at 750 ppm - Group 3) it is concluded that these results are of no biological or toxicological significance.

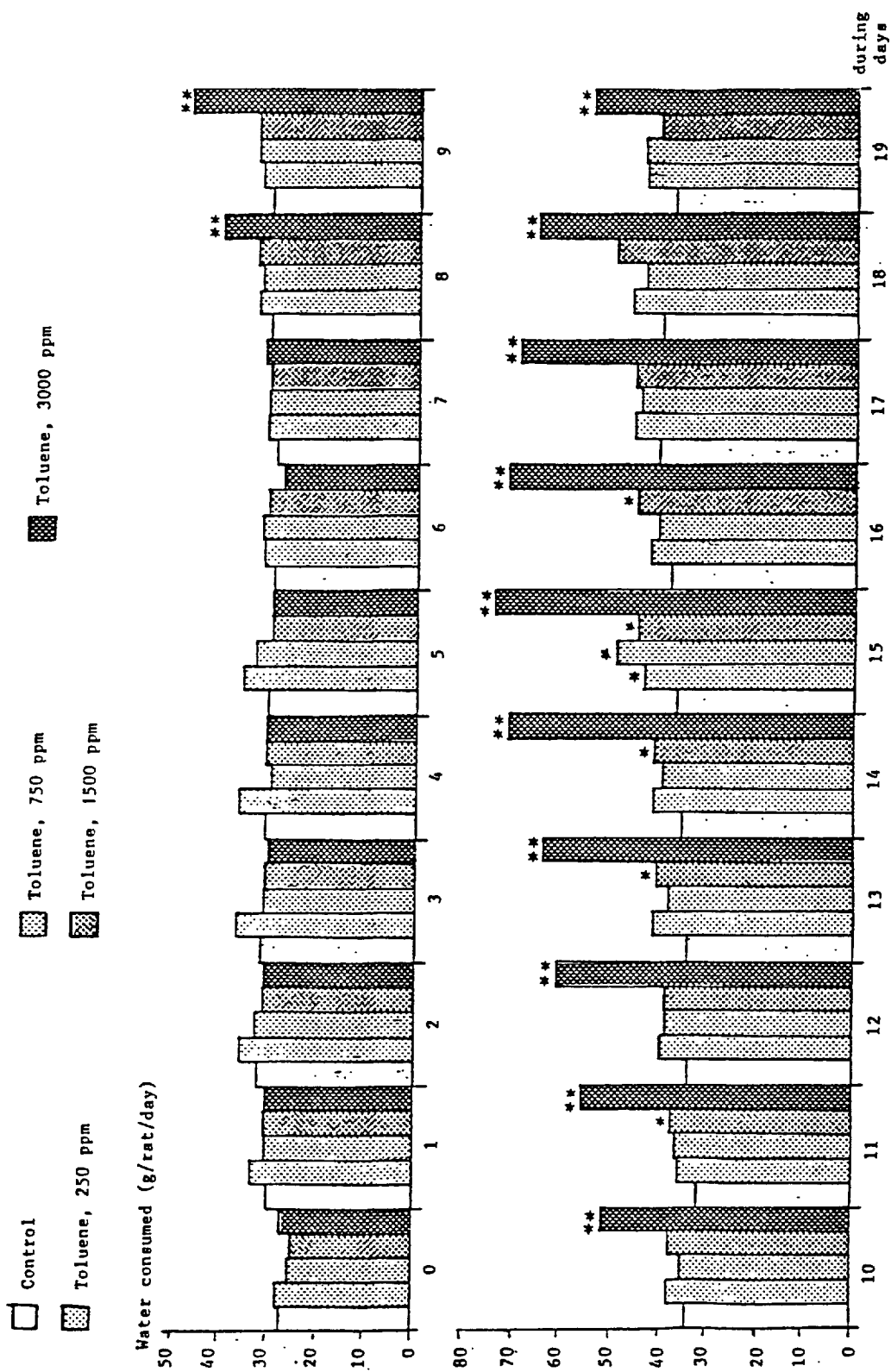
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The number of fetuses showing visceral or skeletal anomalies was similar among the groups (intergroup comparison with the control did not attain statistical significance).

There was an increase at 1500 and 3000 ppm in the incidence of fetuses with skeletal variants (reduced or unossified sternebrae), with the differences from control values attaining statistical significance. It is considered that these delays in ossification are consistent with the reduction in foetal weight at these concentrations. At 250 ppm there was a significant difference (increase) in fetuses with reduced ossification of sternebrae when compared with the controls, however, since there was no dosage relationship this finding is considered to be of no biological significance.

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FIGURE 1
Water consumption - animals with live young - group mean values



Treatment period Days 6 to 15 of pregnancy inclusive

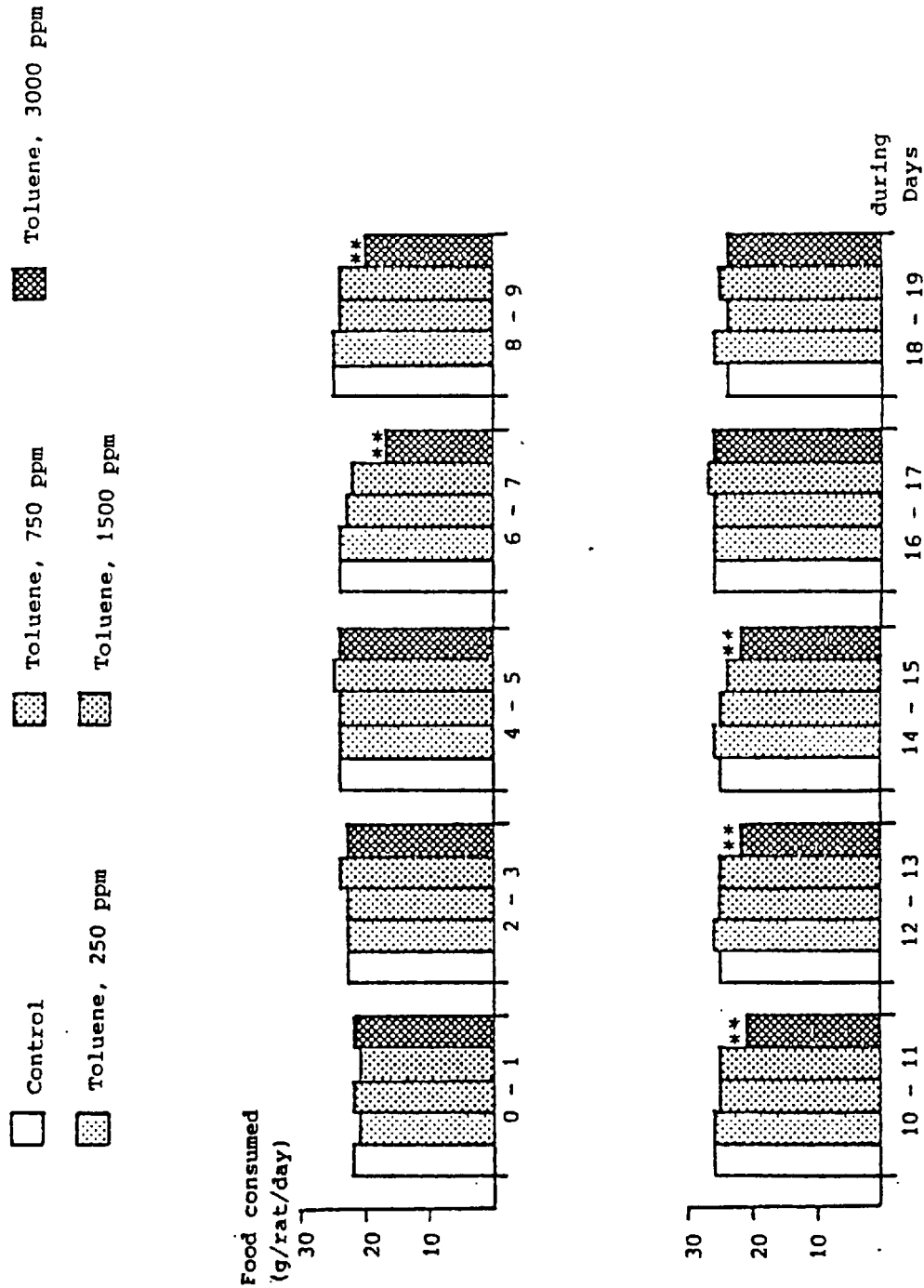
Statistical significance

* P<0.05, ** P<0.01

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FIGURE 2

Food consumption - animals with live young - group mean values



Treatment period Days 6 to 15 of pregnancy inclusive

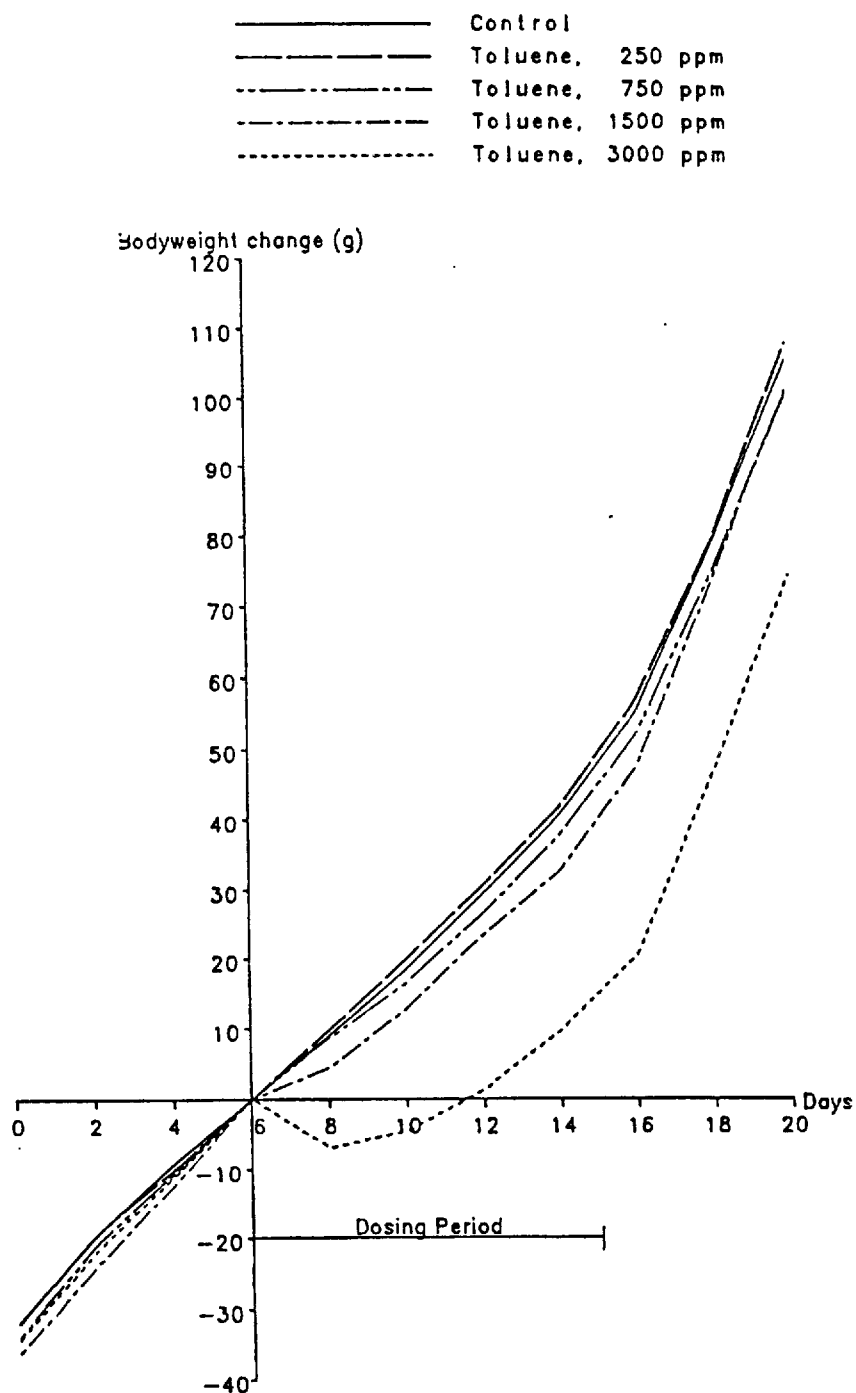
Statistical significance

** P<0.01

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FIGURE 3

Bodyweight change of animals with live young at Day 20 - group mean values



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TABLE 1a

Adult performance and clinical signs - summary

Group:	1	2	3	4	5
Compound:	Control			Toluene	
Exposure (ppm):	-	250	750	1500	3000

Category	Number of animals in Group:				
	1	2	3	4	5
Mated	25	25	25	25	25
Died	-	-	-	-	1
Non-pregnant	1	3	5	6	2
With live young at Day 20	24	22	20	19	22
Signs noted during exposure (x = presence)					
Hunched 'aware' posture			x	x	x
Eyelids closed/half-closed			x	x	x
Uncoordinated gait/ hyperexploratory behaviour				x	x
Limb tremors					x
Hyperresponsive to 'knock on chamber wall'				x	x
Lateral recumbency/ uncontrolled limb movements					x
Nystagmus					x
Lachrymation					x
Increased respiratory rate					x
Salivation					x

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TABLE 1b

Clinical signs during exposure - group incidence on each day of exposure

Group 3: Toluene, 750 ppm

Observation	Day of exposure													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Number of females exposed	5	10	15	21	25	25	25	25	25	25	20	15	10	4
Number of females observed	4	8	8	8	8	8	8	8	8	8	8	8	8	4
Hunched 'aware' posture	4	1	1	1	1	1	2	1	2	2	1	3	1	4
Eyelids closed/half-closed	0	0	1	1	1	1	2	2	4	2	1	5	1	1
Uncoordinated gait/ataxia/ hyperexploratory behaviour	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hyperresponsive to knock on chamber door	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limb tremors	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lateral recumbency with uncontrolled limb movements	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lachrymation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Increased respiratory rate	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salivation (wet chin) associated with rubbing of chin on grid floor of cage	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nystagmus of the eyeball - lateral regular oscillation of eyeball	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A Number affected, refers to number of animals affected when sign first noted

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TABLE 1b

(Clinical signs during exposure - continued)

Group 4: Toluene, 1500 ppm

Observation	Day of exposure													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Number of females exposed	6	11	15	21	25	25	25	25	25	25	19	14	10	4
Number of females observed	4	8	8	8	8	8	8	8	8	8	8	8	8	4
Hunched 'aware' posture	2	2	3	3	3	1	2	3	4	3	8	8	8	4
Eyelids closed/half-closed	4	8	4	3	3	1	2	8	8	8	8	8	8	4
Uncoordinated gait/ataxia/ hyperexploratory behaviour	1	2	1	1	1	1	2	0	0	1	2	1	0	0
Hyperresponsive to knock on chamber door	4	8	8	8	8	8	8	8	8	8	0	8	8	4
Limb tremors	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lateral recumbency with uncontrolled limb movements	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lachrymation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Increased respiratory rate	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salivation (wet chin) associated with rubbing of chin on grid floor of cage	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nystagnus of the eyeball - lateral regular oscillation of eyeball	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A Number affected, refers to number of animals affected when sign first noted

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TABLE 1b

(Clinical signs during exposure - continued)

Group 5: Toluene, 3000 ppm

Observation	Day of exposure													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Number of females exposed	5	10	15	21	25	25	25	25	25	25	20	15	10	4
Number of females observed	4	8	8	8	8	8	8	8	8	8	8	8	8	4
Hunched 'aware' posture	4	2	5	8	8	8	8	2	8	8	8	8	8	4
Eyelids closed/half-closed	4	3	8	1	1	8	8	8	8	8	8	8	8	4
Uncoordinated gait/ataxia/ hyperexploratory behaviour	1	5	2	2	3	5	1	2	1	8	2	1	3	2
Hyperresponsive to knock on chamber door	4	8	8	4	0	8	8	8	8	8	8	8	8	4
Limb tremors	1	3	3	2	3	1	3	1	2	2	2	1	1	1
Lateral recumbency with uncontrolled limb movements	1	1	1	1	2	1	1	2	0	1	1	2	1	1
Lachrymation	3	4	3	1	4	8	3	3	3	3	2	2	1	2
Increased respiratory rate	3	2	1	1	0	1	0	2	1	1	1	2	1	1
Salivation (wet chin) associated with rubbing of chin on grid floor of cage	0	0	0	0	1	0	1	1	2	2	4	2	1	2
Nystagnus of the eyeball - lateral regular oscillation of eyeball	1	1	0	0	0	0	0	0	0	0	0	0	0	0

A Number affected, refers to number of animals affected when sign first noted

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TABLE 2

Water consumption - animals with live young - group mean values

Group:	1	2	3	4	5
Compound:	Control		Toluene		
Exposure (ppm):	-	250	750	1500	3000

Day	Group				
	1♀	2♀	3♀	4♀	5♀
0	27.2	28.2	25.7	25.6	27.5
1	30.2	33.7	30.4	30.7	30.5
2	32.7	36.1	33.0	31.7	31.5
3	32.1	37.4	31.8	31.6	31.1
4	31.5	37.1	30.3	31.6	31.6
5	31.3	36.3	33.9	30.6	30.6
6	30.6	32.6	32.9	31.5	28.4
7	30.0	32.0	31.9	31.7	32.5
8	31.5	34.0	33.3	34.2	41.8**
9	31.5	33.7	34.3	34.4	48.1**
10	34.0	38.0	34.8	37.6	51.7**
11	35.1	36.0	36.2	37.2	56.0**
12	34.0	40.0	38.8	39.0	61.3**
13	39.4	41.2	38.4	40.6	64.3**
14	35.4	41.8	39.4	41.6	71.1**
15	36.9	43.6*	49.1*	44.9*	74.6**
16	38.5	42.5	41.0	45.2	71.5**
17	40.9	45.8	45.0	45.7	69.7**
18	40.5	50.6	44.0	50.1	66.1**
19	37.9	43.9	44.2	41.0	54.9

Statistical analysis

Analysis of variance followed by intergroup comparison with the control (Williams' test) significant at:

* P<0.05

** P<0.01

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TABLE 3

Food consumption - animals with live young - group mean values

Group:	1	2	3	4	5
Compound:	Control		Toluene		
Exposure (ppm):	-	250	750	1500	3000

Group	Number of animals	Food consumed (g/rat/day) during Days of pregnancy									
		0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19
1	24	22	23	24	24	25	26	25	25	26	24
2	22	21	23	24	24	25	26	26	26	26	26
3	20	22	23	24	23	24	25	25	25	26	24
4	19	21	24	25	22	24	25	25	24	28	25
5	22	22	23	24	** 17	** 20	** 21	** 22	** 22	26	24

Treatment period Days 6 to 15 of pregnancy inclusive

Statistical analysis

Analysis of variance followed by intergroup comparison with the control (Williams' test) significant at: ** P<0.01

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TABLE 4a

Bodyweights - animals with live young - group mean values

Group: 1 2 3 4 5

Compound: Control Toluene

Exposure (ppm): - 250 750 1500 3000

Group	No. of animals	Bodyweight (g) at pregnancy Day											
		0	2	4	6	8	10	12	14	16	18	20	#
1	24	199.5	211.8	222.0	231.1	240.1	249.5	260.6	271.9	286.3	309.6	336.6	269.92
2	22	198.0	210.5	219.8	229.9	239.6	249.7	260.6	271.8	286.7	309.1	337.6	272.95
3	20	198.1	211.1	221.3	231.8	240.4	248.3	258.4	269.7	283.8	306.5	332.2	270.65
4	19	200.0	212.2	223.8	236.1	240.4	248.8	259.5	268.9	283.5	309.4	337.1	274.22
5	22	198.4	210.8	221.5	232.5	225.6	228.0	233.8	242.3	253.1	279.3	306.9	248.50

Treatment period Days 6 to 15 of pregnancy inclusive

● Excludes 1 animal - gravid uterine weight not recorded in error

Adjusted bodyweight (Day 20 bodyweight - gravid uterine weight); statistical analysis:

Kruskal-Wallis 'H' statistic followed by Shirley's test significant at ** P<0.01

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TABLE 4b
Bodyweight change - animals with live young - group mean values

Group:	1	2	3	4	5							
Compound:	Control											
Exposure (ppm):	-	250	750	1500	3000							
	Toluene											
Group	No. of animals	Bodyweight change ^Ø (g) at pregnancy Day										
		0	2	4	6	8	10	12	14	16	18	20
1	24	-31.6	-19.4	-9.1	0.0	9.0	18.4	29.5	40.8	55.1	78.5	105.5
2	22	-31.8	-19.3	-10.0	0.0	9.8	19.9	30.7	41.9	56.9	79.2	107.8
3	20	-33.8	-20.7	-10.6	0.0	8.6	16.5	26.6	37.9	52.0	74.7	100.4
4	19	-36.1	-23.8	-12.3	0.0	4.4	12.8	23.5	32.8	47.4	73.4	101.1
5	22	-34.1	-21.7	-11.0	0.0	-6.9	-4.5	1.3	9.8	20.6	46.8	74.4

Ø Relative to Day 6 of pregnancy

Statistical analysis

Analysis of variance followed by intergroup comparison with the control (Williams' test)
significant at: * P<0.05
** P<0.01

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TABLE 5

Liver weights - group mean values

Group:	1	2	3	4	5
Compound:	Control		Toluene		
Exposure (ppm):	-	250	750	1500	3000

Group	Number of animals	Day 20			
		Bodyweight (g)	Liver weight (g)	Bodyweight adjusted for gravid uterine weight (g)	Relative # liver weight (g)
1	24	337	14.30	269.92	5.29
2	20†	338	15.01	272.95	5.48
3	20	332	15.20	270.45	5.63*
4	19	337	14.94	274.22@	5.45*
5	22	307	14.07	248.50** K	5.65** A

Calculated as $\frac{\text{Liver weight (g)}}{\text{Bodyweight adjusted for gravid uterine weight (g)}} \times 100$

† Excludes 1 animal - liver weight not recorded in error

@ Excludes 1 animal - gravid uterine weight not recorded

Statistical analysis

A Analysis of variance followed by Williams' test significant at: * P<0.05
** P<0.01

K Kruskal-Wallis 'H' statistic followed by Shirley's test significant at:
** P<0.01

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TABLE 6

Litter data - group mean values

Group:	1	2	3	4	5							
Compound:	Control			Toluene								
Exposure (ppm):	-	250	750	1500	3000							
Group	No. of animals	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths	Post-implant loss %	Live young wt. (g)	Litter Mean foetal wt. (g)	Gravid uterine wt. (g)			
1	24	14.0	12.8	8.3	0.5	0.0	0.6	5.3	12.2	42.41	3.47	66.69
2	22	14.4	13.3	7.5	0.8	0.1	0.9	7.0	12.4	41.01	3.32*	64.67
3	20	14.6	12.2	16.6*	0.5	0.1	0.5	4.7	11.7	39.80	3.44	61.49
4	19	14.9	13.4	9.5	0.8	0.2	1.0	7.6	12.4	39.72	3.20**	62.54
5	22	14.8	13.6	8.1	0.8	0.2	1.0	7.2	12.6	37.91(*)	3.02**	58.55

Statistical analysis

Kruskal-Wallis 'H' statistic followed, if significant, by intergroup comparison, with the control (Shirley's test or 't' test) significant at * P<0.05
 ** P<0.01

() Unsupported by 'H' statistic

F Fisher's exact test applied, not significant (P>0.05)

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TABLE 7

Sex ratios and litter weight by sex - group mean values

Group:	1	2	3	4	5
Compound:	Control		Toluene		
Exposure (ppm):	-	250	750	1500	3000

Group	Number of litters	Number		Total	% males/litter	Litter wt. (g) ♂	Mean foetal wt. (g) ♂	Litter wt. (g) ♀	Mean foetal wt. (g) ♀
		♂	♀						
1	24	5.8	6.5	12.2	47.6	21.49 ¹	3.59 ¹	21.82	3.39
2	22	6.2	6.2	12.4	50.5	21.12	3.41 [*]	19.89	3.23 [*]
3	20	5.3	6.4	11.7	45.2	18.45	3.50	21.35	3.38
4	19	5.6	6.8	12.4	45.3	18.41	3.29 ^{**}	21.31	3.13 ^{**}
5	22	6.0	6.6	12.6	48.4	18.80	3.10 ^{**}	19.11	2.93 ^{**}
					K		K		K

¹ One litter excluded as no males in litter

Statistical analysis:

K Kruskal-Wallis 'H' statistic followed by intergroup comparison with the control (Shirley's test or 't' test) significant at ** P<0.01

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TABLE 8

Malformations and anomalies - group incidences

Group:	1	2	3	4	5
Compound:	Control		Toluene		
Exposure (ppm):	-	250	750	1500	3000

Group	Number with								
	Malformations			Anomalies†					
				Visceral (Wilson technique)			Skeletal		
	Examined	Total no.	Mean %	Examined	Total no.	Mean %	Examined	Total no.	Mean %
	<u>Litters#</u>								
1	24	F 0	0.0	24	11	45.8	24	13	54.2
2	22	* 4	18.2	22	8	36.4	22	14	63.6
3	20	0	0.0	20	6	30.0	20	15	75.0
4	19	** 5	26.3	19	7	36.8	19	15	78.9
5	22	* 5	22.7	22	9	40.9	22	15	68.2
	<u>Foetuses</u>								
1	293	F 0	0.0	147	17	K 10.7	146	23	K 15.5
2	272	* 5	1.7	136	11	7.7	131	32	24.3
3	233	0	0.0	117	7	6.4	116	25	21.7
4	236	** 6	2.5	114	10	9.5	116	23	21.2
5	277	* 5	1.9	134	11	8.0	138	26	20.1

† Malformed fetuses are excluded

Mean % calculated as $\frac{\text{no. affected litters}}{\text{no. examined litters}} \times 100$ Statistical analysis:K Kruskal-Wallis 'H' statistic followed by intergroup comparison with the control not significant ($P > 0.05$)F Fisher's exact test intergroup comparison with the control significant at * $P < 0.05$, ** $P < 0.01$

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TABLE 9

Malformations - incidence by type

Group:	1	2	3	4	5
Compound:	Control			Toluene	
Exposure (ppm):	-	250	750	1500	3000

Group	Foetuses					Litters					Historical control range of foetuses
	1	2	3	4	5	1	2	3	4	5	
Number examined	293	272	233	236	277	24	22	20	19	22	Examined
Number with malformations	0	5	0	6	5	0	4	0	5	5	2348#
Description	Incidence*					Incidence*					Incidence*
CRANIAL											
Microphthalmia/anophthalmia	-	1	-	1	-	-	1	-	1	-	3
Rhinencephaly	-	-	-	-	1 ^c	-	-	-	-	1	0
Agnathia	-	-	-	-	1 ^c	-	-	-	-	1	0
Hydrocephaly	-	-	-	-	1 ^c	-	-	-	-	1	0
Cyclopia	-	-	-	-	1 ^c	-	-	-	-	1	0
Ablepharia	-	-	-	-	1 ^c	-	-	-	-	1	0
Ankyloglossia	-	1	-	-	1	-	1	-	-	1	0
CERVICAL											
Multiple vertebral irregularities	-	-	-	-	1 ^c	-	-	-	-	1	0
Termination at 3rd vertebra	-	-	-	1 ^b	-	-	-	-	1	-	0
Fused vertebral elements	-	-	-	1 ^a	-	-	-	-	1	-	0
THORACIC											
Malformed cervico-thoracic arteries	-	-	-	1	1 ^d	-	-	-	1	1	5
Interventricular septal defect	-	-	-	-	1 ^d	-	-	-	-	1	6
Multiple vertebral irregularities	-	-	-	1 ^a	-	-	-	-	1	-	3
Distortions/ossification											
irregularities ribs	-	3	-	-	-	-	2	-	-	-	1
LUMBAR/ABDOMINAL											
Duplicated inferior vena cava	-	-	-	1	-	-	-	-	1	-	0
Diaphragmatic hernia	-	-	-	-	1	-	-	-	-	1	4
Unbilical hernia	-	-	-	1 ^b	-	-	-	-	1	-	0
SACRO-CAUDAL											
No tail apparent	-	-	-	1	-	-	-	-	1	-	1
APPENDICULAR											
Forelimb flexures/malrotated hind limbs	-	-	-	1 ^b	-	-	-	-	1	-	1

* Individual foetuses may occur in more than one category

From 9 studies performed 1990 - 91, using time-mated animals of the same strain and source
Superscripts indicate same foetus

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TABLE 10

Visceral anomalies - incidence by type

Group:	1	2	3	4	5
Compound:	Control			Toluene	
Exposure (ppm):	-	250	750	1500	3000

Group	Foetuses					Litters					Historical control range of foetuses
	1	2	3	4	5	1	2	3	4	5	Examined
Number examined	147	136	117	114	134	24	22	20	19	22	1159#
Number with anomaly	17	11	7	10	11	11	8	6	7	9	
Description	Incidence*					Incidence*					Incidence*
Subcutaneous haemorrhage:											
cranium	1	1	-	-	-	1	1	-	-	-	1
trunk, tail, limbs	-	-	-	-	1	-	-	-	-	1	4
CRANIUM											
Haemorrhages affecting:											
brain/spinal cord	2	1	1	1	2	2	1	1	1	2	25
eyes/surrounding tissue	-	-	-	-	1	-	-	-	-	1	7
Small eye	-	-	-	-	1	-	-	-	-	1	3
CERVICAL											
Reduced thyroid	-	1	-	-	-	-	1	-	-	-	2
THORACIC											
Anomalous cervico-thoracic arteries	1	1	-	-	1	1	1	-	-	1	8
Interventricular septal defect (small)	-	-	-	1	-	-	-	-	1	-	4
ABDOMINAL											
Thinning of diaphragm/protrusion liver	3	1	-	1	1	3	1	-	1	1	2
Liver - abnormal lobation	4	4	-	1	1	3	3	-	1	1	24
haemorrhage within lobe	1	-	2	-	-	1	-	2	-	-	4
Intra-abdominal haemorrhage	2	-	1	-	1	2	-	1	-	1	1
Increased dilatation renal											
pelvis/ureter	4	3	3	3	2	3	3	3	2	2	27
Displaced testis(es)	-	-	-	3	-	-	-	-	2	-	11

* Individual foetuses may occur in more than one category

From 9 studies performed 1990 - 91, using time-mated animals of the same strain and source
Malformed foetuses are excluded

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TABLE 11

Skeletal anomalies - incidence by type

Group:	1	2	3	4	5
Compound:	Control			Toluene	
Exposure (ppm):	-	250	750	1500	3000

Group	Foetuses					Litters					Historical control range of foetuses
	1	2	3	4	5	1	2	3	4	5	
Number examined	146	131	116	116	138	24	22	20	19	22	Examined
Number with anomaly	23	32	24	22	26	13	14	15	15	15	1163#
Description	Incidence*					Incidence*					Incidence*
Reduced ossification of:											
one or more cranial centres	3	10	6	2	1	3	7	5	2	1	57
cervical vertebral arches	1	1	1	2	-	1	1	1	2	-	13
sacro-caudal vertebral arches	7	16	12	3	2	6	9	7	2	2	156
one or more centres pelvic girdle	2	5	2	2	-	2	5	2	2	-	42
digital centres	-	1	1	2	6	-	1	1	2	4	24
CERVICAL											
Cervical rib(s)	2	1	-	-	1	2	1	-	-	1	9
THORACIC											
Irregular ossification vertebral											
centra	5	7	5	9	13	3	6	5	7	9	31
Minimal distortions ribs	3	1	-	1	1	1	1	-	1	1	3
Shortened/absent 13th rib(s)	3	6	7	3	-	2	4	5	2	-	30
Asymmetric costal cartilage elements	-	-	1	-	-	-	-	1	-	-	0
LUMBAR											
Lumbar rib(s)	2	1	1	3	8	2	1	1	3	5	18
One less thoraco-lumbar vertebra	-	1	2	-	-	-	1	2	-	-	9

* Individual foetuses may occur in more than one category

From 9 studies performed 1990 - 91, using time-mated animals of the same strain and source
Malformed foetuses are excluded

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TABLE 12

Sternebral variants - group values

Group: 1 2 3 4 5

Compound: Control Toluene

Exposure (ppm): - 250 750 1500 3000

Group	Number of litters	Foetuses examined	Foetuses with					
			Normal sternebrae	Unossified sternebrae	Reduced sternebrae	Asym./bip. sternebrae	Total variant sternebrae	
			no. mean %	no. mean %	no. mean %	no. mean %	no. mean %	
1	24	146	69 44.7	55 37.0	37 28.5	0 0.0	77 55.3	
2	22	131	37 28.1	57 44.4	60 45.4*	0 0.0	94 71.9	
3	20	116	47 43.5	48 37.2	36 30.6	2 1.5	69 56.5	
4	19	116	27 25.3*	65 52.7	49 44.3*	3 2.1	89 74.7*	
5	22	138	32 23.1*	83 60.1(*)	61 44.9*	1 0.9	106 76.9*	K

Statistical analysis:

K Kruskal-Wallis 'H' statistic followed, if significant, by intergroup comparison with the control (Shirley's test or 't' test) significant at * P<0.05

F Fisher's exact test applied, not significant

() Unsupported by 'H' statistic

Malformed foetuses excluded

APPENDICES

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APPENDIX 1

Signs and autopsy findings - individual incidence -
animals surviving to termination

Group:	1	2	3	4	5
Compound:	Control		Toluene		
Exposure (ppm):	-	250	750	1500	3000

Observation	Animal numbers affected in Group:				
	1	2	3	4	5
Number surviving	25	25	25	25	24
<u>Signs post exposure</u> (Day post coitum)					
Unsteady gait post exposure Day 6					105
Râles post exposure Day 6					113
Urogenital or ventral region: yellow stained Day 14/15 or Day 15					111,112, 113
Coat: face, red/brown stained Day 6					119,120
No abnormal signs observed	1-25	26-50	51-75	76-100	101-104, 106-110, 114-118, 121-125
<u>Autopsy findings</u>					
Kidney(s): increased pelvic dilatation		34			125
No abnormal autopsy findings observed	1-25	26-33, 35-50	51-75	76-100	101-119, 121-124
Died Day 14 post exposure, (exposure Day 12) post mortem findings: coat moist peri-oral region, grey/blue contents in jejunum, haemorrhagic depressions and water contents in stomach, pregnant					120

For signs observed during exposure period - refer to Addendum 1

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APPENDIX 2

Water consumption - individual values

Group 1: Control

Day post coitum	Animal number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
0	25.0	36.0	23.0	36.0	32.0	25.0	29.0	25.0	24.0	31.0	37.0	21.0	43.0
1	25.0	32.0	29.0	36.0	42.0	27.0	40.0	30.0	24.0	37.0	36.0	21.0	48.0
2	31.0	32.0	35.0	42.0	39.0	27.0	34.0	34.0	24.0	33.0	37.0	24.0	61.0
3	34.0	31.0	30.0	44.0	35.0	27.0	37.0	28.0	23.0	38.0	36.0	22.0	58.0
4	32.0	30.0	26.0	43.0	33.0	24.0	31.0	35.0	23.0	49.0	32.0	23.0	57.0
5	32.0	28.0	28.0	37.0	35.0	25.0	30.0	33.0	22.0	55.0	33.0	21.0	58.0
6	28.0	30.0	27.0	39.0	32.0	23.0	28.0	31.0	22.0	62.0	32.0	27.0	53.0
7	28.0	29.0	29.0	39.0	32.0	26.0	27.0	31.0	22.0	46.0	33.0	19.0	48.0
8	41.0	31.0	29.0	37.0	35.0	26.0	27.0	32.0	21.0	80.0	33.0	22.0	42.0
9	41.0	28.0	27.0	36.0	33.0	27.0	27.0	29.0	24.0	66.0	32.0	20.0	44.0
10	33.0	23.0	30.0	38.0	38.0	29.0	32.0	37.0	23.0	83.0	39.0	22.0	43.0
11	32.0	31.0	27.0	29.0	25.0	27.0	33.0	35.0	29.0	113.0	40.0	23.0	50.0
12	35.0	33.0	33.0	43.0	45.0	31.0	34.0	35.0	30.0	39.0	38.0	25.0	46.0
13	41.0	35.0	33.0	44.0	43.0	28.0	32.0	38.0	27.0	151.0	40.0	23.0	51.0
14	35.0	33.0	34.0	39.0	40.0	29.0	30.0	37.0	29.0	44.0	40.0	23.0	53.0
15	35.0	36.0	33.0	42.0	46.0	32.0	34.0	43.0	32.0	40.0	43.0	26.0	57.0
16	40.0	42.0	37.0	44.0	46.0	37.0	36.0	41.0	32.0	42.0	46.0	28.0	55.0
17	52.0	45.0	33.0	48.0	50.0	35.0	39.0	42.0	32.0	45.0	43.0	18.0	60.0
18	43.0	44.0	38.0	46.0	47.0	32.0	38.0	40.0	30.0	44.0	50.0	25.0	61.0
19	39.0	46.0	34.0	46.0	43.0	33.0	29.0	42.0	26.0	40.0	52.0	25.0	55.0

Treatment period Days 6 to 15 post coitum inclusive

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APPENDIX 2

(Water consumption - continued)

Group 1: Control (continued)

Day post coitum	Animal number												Mean	SD
	14	15	16	17	18	19	20	21	22	23	24	25		
0	27.0	28.0	32.0	19.0	29.0	25.0	20.0	23.0	(43.0)	21.0	17.0	25.0	27.2	6.39
1	26.0	31.0	37.0	17.0	29.0	29.0	24.0	25.0	(48.0)	27.0	22.0	31.0	30.2	7.27
2	29.0	31.0	40.0	20.0	29.0	31.0	26.0	31.0	(52.0)	32.0	26.0	36.0	32.7	8.06
3	26.0	34.0	41.0	23.0	30.0	30.0	27.0	30.0	(54.0)	30.0	24.0	33.0	32.1	7.93
4	28.0	29.0	39.0	20.0	27.0	29.0	25.0	27.0	(49.0)	30.0	29.0	34.0	31.5	8.47
5	27.0	30.0	38.0	20.0	28.0	29.0	26.0	29.0	(48.0)	31.0	23.0	32.0	31.3	9.08
6	26.0	32.0	36.0	21.0	24.0	28.0	28.0	24.0	(43.0)	29.0	21.0	31.0	30.6	9.50
7	36.0	29.0	36.0	19.0	24.0	30.0	26.0	26.0	(44.0)	30.0	27.0	28.0	30.0	7.12
8	25.0	29.0	39.0	19.0	23.0	24.0	24.0	27.0	(44.0)	31.0	29.0	30.0	31.5	12.03
9	25.0	31.0	46.0	23.0	25.0	28.0	29.0	29.0	(45.0)	31.0	23.0	32.0	31.5	9.80
10	30.0	33.0	40.0	23.0	28.0	32.0	36.0	29.0	(43.0)	30.0	32.0	33.0	34.0	11.88
11	31.0	36.0	43.0	24.0	25.0	32.0	33.0	29.0	(45.0)	29.0	32.0	34.0	35.1	17.70
12	28.0	36.0	40.0	21.0	27.0	29.0	38.0	28.0	(33.0)	32.0	32.0	37.0	34.0	6.20
13	32.0	33.0	40.0	25.0	31.0	30.0	37.0	27.0	(24.0)	33.0	37.0	34.0	39.4	24.70
14	34.0	41.0	44.0	25.0	31.0	32.0	40.0	30.0	(45.0)	32.0	39.0	36.0	35.4	6.71
15	34.0	44.0	39.0	25.0	32.0	31.0	41.0	31.0	(39.0)	36.0	36.0	38.0	36.9	6.95
16	38.0	38.0	43.0	30.0	32.0	36.0	38.0	30.0	(33.0)	35.0	37.0	40.0	38.5	6.05
17	37.0	45.0	42.0	30.0	37.0	38.0	49.0	33.0	(25.0)	38.0	43.0	48.0	40.9	8.68
18	37.0	43.0	44.0	28.0	37.0	41.0	49.0	32.0	(35.0)	38.0	37.0	47.0	40.5	7.95
19	19.0	41.0	39.0	27.0	33.0	49.0	48.0	28.0	(39.0)	34.0	34.0	48.0	37.9	9.47

() Value excluded from means and statistical analysis, non-pregnant
Treatment period Days 6 to 15 post coitum inclusive
SD Standard deviation

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APPENDIX 2

(Water consumption - continued)

Group 2: Toluene, 250 ppm

Day post coitum	Animal number												
	26	27	28	29	30	31	32	33	34	35	36	37	38
0	31.0	29.0	(22.0)	24.0	21.0	33.0	27.0	30.0	27.0	28.0	25.0	28.0	(30.0)
1	45.0	32.0	(27.0)	27.0	25.0	37.0	28.0	35.0	32.0	46.0	22.0	30.0	(24.0)
2	48.0	35.0	(28.0)	32.0	26.0	43.0	29.0	36.0	29.0	52.0	26.0	33.0	(18.0)
3	43.0	32.0	(30.0)	32.0	75.0	38.0	26.0	39.0	29.0	46.0	25.0	32.0	(24.0)
4	37.0	32.0	(26.0)	29.0	24.0	38.0	27.0	35.0	31.0	48.0	26.0	32.0	(28.0)
5	36.0	31.0	(31.0)	31.0	25.0	38.0	26.0	34.0	58.0	41.0	22.0	35.0	(21.0)
6	37.0	35.0	(42.0)	280.0*	25.0	33.0	26.0	33.0	28.0	33.0	22.0	37.0	(24.0)
7	40.0	30.0	(35.0)	24.0	24.0	35.0	28.0	36.0	31.0	36.0	23.0	35.0	(27.0)
8	44.0	32.0	(30.0)	41.0	27.0	58.0	25.0	32.0	28.0	27.0	24.0	34.0	(25.0)
9	41.0	33.0	(31.0)	32.0	24.0	34.0	27.0	34.0	30.0	36.0	24.0	37.0	(20.0)
10	45.0	34.0	(27.0)	58.0	26.0	37.0	30.0	37.0	31.0	40.0	25.0	43.0	(21.0)
11	42.0	33.0	(26.0)	9.0	28.0	39.0	31.0	39.0	38.0	41.0	28.0	41.0	(25.0)
12	44.0	35.0	(19.0)	80.0	27.0	40.0	30.0	37.0	37.0	36.0	28.0	43.0	(27.0)
13	46.0	38.0	(21.0)	62.0	27.0	40.0	36.0	40.0	37.0	37.0	28.0	40.0	(21.0)
14	47.0	41.0	(23.0)	33.0	29.0	39.0	33.0	40.0	37.0	46.0	21.0	46.0	(20.0)
15	48.0	42.0	(22.0)	35.0	30.0	38.0	42.0	40.0	43.0	45.0	31.0	49.0	(25.0)
16	55.0	45.0	(23.0)	35.0	31.0	48.0	38.0	40.0	46.0	58.0	28.0	57.0	(21.0)
17	50.0	46.0	(15.0)	37.0	31.0	51.0	46.0	40.0	50.0	55.0	32.0	62.0	(26.0)
18	54.0	48.0	(21.0)	50.0	25.0	46.0	134.0	39.0	54.0	56.0	28.0	56.0	(16.0)
19	47.0	41.0	(23.0)	30.0	31.0	48.0	97.0	35.0	45.0	48.0	27.0	47.0	(27.0)

() Value excluded from means and statistical analysis, non-pregnant

* Value excluded from means - bottle leakage

Treatment period Days 6 to 15 post coitum inclusive

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APPENDIX 2

(Water consumption - continued)

Group 2: Toluene, 250 ppm (continued)

Day post coitum	Animal number											Mean	SD
	39	40	41	42	43	44	45	46	47	48	49	50	
0	31.0	23.0	26.0	41.0	26.0	30.0	31.0	25.0	(21.0)	26.0	26.0	33.0	4.25
1	34.0	24.0	37.0	49.0	34.0	37.0	35.0	28.0	(30.0)	28.0	38.0	39.0	7.12
2	38.0	27.0	41.0	51.0	36.0	35.0	32.0	33.0	(41.0)	31.0	41.0	40.0	7.56
3	40.0	27.0	37.0	50.0	36.0	38.0	35.0	31.0	(34.0)	27.0	49.0	34.0	11.00
4	43.0	56.0	34.0	60.0	36.0	33.0	35.0	30.0	(29.0)	30.0	65.0	35.0	10.93
5	41.0	22.0	35.0	53.0	38.0	38.0	41.0	28.0	(40.0)	31.0	59.0	35.0	10.11
6	39.0	28.0	39.0	49.0	31.0	39.0	35.0	29.0	(39.0)	26.0	31.0	30.0	6.19
7	36.0	26.0	30.0	44.0	35.0	39.0	34.0	28.0	(33.0)	29.0	35.0	27.0	5.63
8	38.0	24.0	31.0	51.0	34.0	39.0	39.0	26.0	(33.0)	27.0	32.0	35.0	8.81
9	42.0	26.0	29.0	48.0	36.0	42.0	35.0	29.0	(32.0)	29.0	43.0	30.0	6.53
10	46.0	31.0	32.0	56.0	38.0	44.0	43.0	27.0	(32.0)	29.0	45.0	38.0	9.02
11	46.0	28.0	29.0	55.0	36.0	44.0	40.0	29.0	(31.0)	30.0	51.0	34.0	9.68
12	46.0	30.0	32.0	53.0	47.0	45.0	40.0	32.0	(24.0)	32.0	50.0	37.0	11.50
13	47.0	31.0	34.0	61.0	42.0	51.0	45.0	33.0	(30.0)	32.0	65.0	35.0	10.60
14	50.0	33.0	36.0	57.0	42.0	50.0	44.0	44.0	(33.0)	38.0	71.0	42.0	10.24
15	49.0	32.0	41.0	46.0	54.0	54.0	50.0	37.0	(30.0)	35.0	77.0	42.0	10.21
16	50.0	31.0	36.0	56.0	39.0	47.0	50.0	35.0	(23.0)	33.0	34.0	43.0	9.26
17	51.0	23.0	41.0	55.0	51.0	54.0	60.0	43.0	(32.0)	35.0	55.0	39.0	10.09
18	53.0	31.0	36.0	63.0	48.0	57.0	68.0	45.0	(32.0)	34.0	47.0	42.0	21.68
19	49.0	30.0	46.0	53.0	44.0	55.0	53.0	38.0	(28.0)	33.0	30.0	39.0	14.62

() Value excluded from means and statistical analysis, non-pregnant
 Treatment period Days 6 to 15 post coitum inclusive
 SD Standard deviation

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APPENDIX 2

(Water consumption - continued)

Group 3: Toluene, 750 ppm

Day post coitum	Animal number													
	51	52	53	54	55	56	57	58	59	60	61	62	63	
0	17.0	25.0	(42.0)	23.0	26.0	37.0	26.0	22.0	25.0	31.0	20.0	(31.0)	25.0	
1	28.0	25.0	(72.0)	26.0	29.0	44.0	33.0	23.0	27.0	31.0	25.0	(28.0)	28.0	
2	28.0	33.0	(44.0)	25.0	34.0	54.0	31.0	23.0	28.0	30.0	28.0	(27.0)	31.0	
3	28.0	28.0	(60.0)	26.0	29.0	50.0	31.0	20.0	27.0	30.0	27.0	(18.0)	31.0	
4	25.0	27.0	(80.0)	24.0	27.0	36.0	31.0	20.0	26.0	29.0	30.0	(31.0)	35.0	
5	25.0	32.0	(39.0)	25.0	26.0	45.0	32.0	20.0	29.0	31.0	31.0	(29.0)	32.0	
6	30.0	40.0	(40.0)	26.0	33.0	36.0	34.0	21.0	29.0	25.0	42.0	(24.0)	33.0	
7	27.0	36.0	(50.0)	26.0	28.0	49.0	32.0	20.0	32.0	31.0	30.0	(16.0)	32.0	
8	31.0	35.0	(96.0)	30.0	34.0	51.0	33.0	29.0	40.0	30.0	26.0	(25.0)	30.0	
9	28.0	37.0	(46.0)	28.0	31.0	67.0	32.0	19.0	33.0	24.0	30.0	(26.0)	32.0	
10	31.0	39.0	(70.0)	28.0	32.0	56.0	39.0	22.0	34.0	31.0	47.0	(25.0)	34.0	
11	31.0	44.0	(50.0)	31.0	31.0	47.0	39.0	24.0	33.0	32.0	37.0	(17.0)	32.0	
12	33.0	54.0	(77.0)	37.0	34.0	47.0	37.0	24.0	32.0	37.0	38.0	(29.0)	39.0	
13	31.0	51.0	(86.0)	83.0	34.0	45.0	37.0	25.0	34.0	37.0	45.0	(32.0)	35.0	
14	36.0	44.0	(114.0)	30.0	35.0	45.0	43.0	25.0	35.0	39.0	38.0	(29.0)	37.0	
15	39.0	45.0	(109.0)	134.0	39.0	53.0	46.0	31.0	36.0	42.0	34.0	(21.0)	37.0	
16	34.0	35.0	(44.0)	34.0	35.0	42.0	47.0	26.0	37.0	40.0	37.0	(41.0)	42.0	
17	36.0	38.0	(52.0)	33.0	37.0	117.0	50.0	31.0	41.0	42.0	38.0	(47.0)	44.0	
18	32.0	34.0	(92.0)	29.0	35.0	113.0	51.0	29.0	44.0	42.0	42.0	(32.0)	49.0	
19	26.0	38.0	(83.0)	28.0	39.0	137.0	40.0	22.0	40.0	75.0	36.0	(22.0)	35.0	

() Value excluded from means and statistical analysis, non-pregnant
Treatment period Days 6 to 15 post coitum inclusive

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APPENDIX 2
(Water consumption - continued)

Group 3: Toluene, 750 ppm (continued)

Day post coitum	Animal number													Mean	SD
	64	65	66	67	68	69	70	71	72	73	74	75			
0	(33.0)	26.0	23.0	19.0	28.0	29.0	32.0	(33.0)	23.0	28.0	(35.0)	28.0	25.7	4.66	
1	(32.0)	25.0	37.0	24.0	45.0	36.0	36.0	(59.0)	30.0	24.0	(33.0)	32.0	30.4	6.42	
2	(35.0)	25.0	38.0	27.0	39.0	48.0	37.0	(33.0)	34.0	33.0	(37.0)	33.0	33.0	7.61	
3	(31.0)	26.0	31.0	31.0	47.0	36.0	32.0	(50.0)	45.0	27.0	(33.0)	33.0	31.8	7.52	
4	(30.0)	28.0	27.0	28.0	48.0	39.0	33.0	(71.0)	37.0	29.0	(31.0)	26.0	30.3	6.30	
5	(30.0)	25.0	25.0	31.0	93.0	57.0	33.0	(29.0)	29.0	28.0	(39.0)	29.0	33.9	16.00	
6	(34.0)	26.0	26.0	27.0	82.0	35.0	36.0	(29.0)	27.0	25.0	(36.0)	25.0	32.9	12.84	
7	(31.0)	27.0	28.0	25.0	73.0	32.0	29.0	(22.0)	27.0	25.0	(34.0)	29.0	31.9	11.23	
8	(29.0)	24.0	27.0	27.0	61.0	33.0	30.0	(30.0)	37.0	28.0	(36.0)	30.0	33.3	8.79	
9	(30.0)	25.0	30.0	28.0	96.0	33.0	30.0	(34.0)	26.0	26.0	(35.0)	30.0	34.3	17.27	
10	(33.0)	29.0	40.0	32.0	43.0	37.0	35.0	(29.0)	28.0	28.0	(35.0)	31.0	34.8	7.71	
11	(32.0)	31.0	32.0	33.0	76.0	35.0	33.0	(20.0)	40.0	30.0	(30.0)	33.0	36.2	10.70	
12	(35.0)	30.0	42.0	35.0	40.0	44.0	59.0	(40.0)	46.0	33.0	(17.0)	34.0	38.8	8.20	
13	(28.0)	33.0	37.0	33.0	37.0	35.0	36.0	(49.0)	37.0	29.0	(27.0)	34.0	38.4	11.95	
14	(21.0)	33.0	43.0	36.0	79.0	37.0	38.0	(40.0)	47.0	33.0	(25.0)	35.0	39.4	10.71	
15	(26.0)	37.0	46.0	36.0	101.0	48.0	44.0	(22.0)	60.0	34.0	(24.0)	40.0	49.1	24.98	
16	(34.0)	35.0	50.0	43.0	88.0	42.0	47.0	(27.0)	36.0	31.0	(14.0)	39.0	41.0	12.49	
17	(26.0)	39.0	42.0	44.0	74.0	38.0	42.0	(30.0)	37.0	35.0	(31.0)	41.0	45.0	19.12	
18	(19.0)	36.0	41.0	38.0	61.0	47.0	42.0	(24.0)	38.0	37.0	(23.0)	40.0	44.0	17.96	
19	(25.0)	33.0	41.0	33.0	69.0	48.0	41.0	(25.0)	35.0	27.0	(20.0)	40.0	44.2	25.34	

() Value excluded from means and statistical analysis, non-pregnant
Treatment period Days 6 to 15 post coitum inclusive
SD Standard deviation

APT 2/91279

APPENDIX 2

(Water consumption - continued)

Group 4: Toluene, 1500 ppm

Day post coitum	Animal number												
	76	77	78	79	80	81	82	83	84	85	86	87	88
0	23.0	21.0	36.0	(27.0)	24.0	22.0	23.0	22.0	(27.0)	27.0	26.0	(20.0)	(35.0)
1	29.0	25.0	37.0	(31.0)	32.0	26.0	27.0	26.0	(33.0)	38.0	36.0	(26.0)	(39.0)
2	30.0	27.0	44.0	(31.0)	40.0	31.0	24.0	21.0	(29.0)	36.0	34.0	(29.0)	(43.0)
3	28.0	28.0	41.0	(32.0)	36.0	29.0	24.0	27.0	(29.0)	37.0	34.0	(31.0)	(42.0)
4	31.0	28.0	34.0	(32.0)	30.0	28.0	35.0	22.0	(24.0)	40.0	32.0	(36.0)	(45.0)
5	31.0	27.0	33.0	(32.0)	30.0	26.0	24.0	27.0	(29.0)	39.0	35.0	(26.0)	(38.0)
6	31.0	36.0	51.0	(36.0)	34.0	33.0	24.0	23.0	(22.0)	31.0	32.0	(34.0)	(41.0)
7	34.0	36.0	44.0	(33.0)	33.0	31.0	28.0	27.0	(27.0)	35.0	31.0	(30.0)	(40.0)
8	36.0	41.0	41.0	(35.0)	41.0	33.0	26.0	26.0	(24.0)	36.0	30.0	(32.0)	(29.0)
9	34.0	44.0	49.0	(31.0)	39.0	31.0	30.0	27.0	(26.0)	34.0	32.0	(31.0)	(39.0)
10	41.0	44.0	41.0	(33.0)	45.0	37.0	31.0	32.0	(23.0)	37.0	37.0	(31.0)	(37.0)
11	42.0	42.0	43.0	(28.0)	46.0	33.0	28.0	28.0	(18.0)	39.0	37.0	(29.0)	(42.0)
12	44.0	40.0	43.0	(22.0)	50.0	35.0	29.0	31.0	(15.0)	41.0	46.0	(26.0)	(46.0)
13	46.0	45.0	40.0	(25.0)	50.0	37.0	34.0	34.0	(20.0)	40.0	46.0	(17.0)	(34.0)
14	47.0	49.0	42.0	(24.0)	56.0	36.0	35.0	32.0	(23.0)	43.0	44.0	(18.0)	(26.0)
15	61.0	53.0	60.0	(25.0)	55.0	39.0	34.0	36.0	(17.0)	41.0	45.0	(23.0)	(30.0)
16	49.0	50.0	45.0	(21.0)	53.0	36.0	34.0	34.0	(18.0)	45.0	51.0	(23.0)	(38.0)
17	59.0	53.0	47.0	(25.0)	56.0	40.0	35.0	36.0	(25.0)	45.0	51.0	(22.0)	(27.0)
18	50.0	52.0	140.0	(26.0)	58.0	38.0	33.0	40.0	(24.0)	40.0	60.0	(17.0)	(25.0)
19	48.0	46.0	49.0	(23.0)	56.0	36.0	33.0	33.0	(22.0)	36.0	46.0	(23.0)	(30.0)

() Value excluded from means and statistical analysis, non-pregnant
Treatment period Days 6 to 15 post coitum inclusive

APT 2/91279

APPENDIX 2

(Water consumption - continued)

Group 4: Toluene, 1500 ppm (continued)

Day post coitum	Animal number										Mean	SD		
	89	90	91	92	93	94	95	96	97	98			99	100
0	25.0	27.0	23.0	28.0	27.0	23.0	33.0	22.0	29.0	(27.0)	(33.0)	25.0	25.6	3.92
1	31.0	33.0	28.0	26.0	34.0	28.0	39.0	28.0	35.0	(40.0)	(31.0)	26.0	30.7	4.63
2	28.0	33.0	26.0	27.0	34.0	27.0	38.0	26.0	43.0	(38.0)	(42.0)	34.0	31.7	6.44
3	29.0	31.0	28.0	31.0	36.0	28.0	37.0	30.0	34.0	(38.0)	(36.0)	33.0	31.6	4.39
4	29.0	32.0	31.0	30.0	37.0	28.0	37.0	30.0	37.0	(32.0)	(33.0)	29.0	31.6	4.27
5	28.0	28.0	29.0	29.0	33.0	26.0	39.0	28.0	39.0	(52.0)	(46.0)	30.0	30.6	4.60
6	32.0	32.0	26.0	33.0	35.0	27.0	35.0	29.0	26.0	(25.0)	(33.0)	29.0	31.5	6.05
7	37.0	30.0	19.0	32.0	35.0	24.0	34.0	30.0	36.0	(36.0)	(32.0)	27.0	31.7	5.46
8	40.0	29.0	28.0	33.0	45.0	31.0	32.0	30.0	37.0	(46.0)	(38.0)	34.0	34.2	5.56
9	33.0	29.0	28.0	30.0	44.0	24.0	42.0	29.0	42.0	(64.0)	(45.0)	33.0	34.4	6.90
10	36.0	32.0	30.0	36.0	51.0	26.0	43.0	32.0	42.0	(30.0)	(49.0)	41.0	37.6	6.21
11	37.0	31.0	30.0	38.0	46.0	29.0	43.0	32.0	46.0	(32.0)	(23.0)	37.0	37.2	6.33
12	34.0	34.0	30.0	36.0	53.0	32.0	40.0	36.0	46.0	(33.0)	(24.0)	41.0	39.0	6.85
13	39.0	37.0	33.0	40.0	51.0	36.0	43.0	33.0	47.0	(41.0)	(33.0)	41.0	40.6	5.65
14	40.0	38.0	32.0	40.0	50.0	37.0	45.0	37.0	50.0	(47.0)	(32.0)	38.0	41.6	6.60
15	55.0	16.0	37.0	40.0	58.0	39.0	50.0	35.0	54.0	(41.0)	(29.0)	46.0	44.9	11.40
16	44.0	70.0	34.0	39.0	57.0	39.0	54.0	39.0	54.0	(30.0)	(24.0)	32.0	45.2	9.98
17	51.0	45.0	37.0	37.0	50.0	42.0	51.0	36.0	62.0	(56.0)	(33.0)	36.0	45.7	8.50
18	47.0	45.0	33.0	37.0	48.0	41.0	49.0	37.0	60.0	(57.0)	(36.0)	43.0	50.1	23.33
19	42.0	39.0	31.0	33.0	41.0	43.0	44.0	35.0	53.0	(39.0)	(29.0)	35.0	41.0	7.32

() Value excluded from means and statistical analysis, non-pregnant
Treatment period Days 6 to 15 post coitum inclusive
SD Standard deviation

APT 2/91279

APPENDIX 2

(Water consumption - continued)

Group 5: Toluene, 3000 ppm

Day post coitum	Animal number												
	101	102	103	104	105	106	107	108	109	110	111	112	113
0	27.0	30.0	25.0	21.0	34.0	25.0	(28.0)	24.0	26.0	22.0	28.0	31.0	23.0
1	25.0	39.0	31.0	25.0	40.0	29.0	(34.0)	28.0	24.0	23.0	31.0	30.0	23.0
2	28.0	40.0	32.0	28.0	38.0	29.0	(31.0)	25.0	24.0	22.0	34.0	29.0	21.0
3	25.0	35.0	26.0	25.0	37.0	29.0	(31.0)	27.0	27.0	18.0	32.0	29.0	23.0
4	25.0	34.0	29.0	26.0	37.0	27.0	(27.0)	26.0	24.0	20.0	36.0	33.0	25.0
5	28.0	34.0	28.0	25.0	36.0	29.0	(30.0)	30.0	27.0	22.0	30.0	28.0	23.0
6	28.0	26.0	29.0	21.0	41.0	25.0	(25.0)	25.0	21.0	18.0	24.0	26.0	20.0
7	30.0	30.0	33.0	20.0	40.0	30.0	(29.0)	29.0	24.0	22.0	30.0	33.0	19.0
8	33.0	42.0	46.0	30.0	52.0	36.0	(25.0)	32.0	29.0	29.0	38.0	41.0	23.0
9	34.0	62.0	59.0	32.0	60.0	40.0	(33.0)	37.0	34.0	41.0	44.0	48.0	32.0
10	37.0	65.0	65.0	33.0	62.0	37.0	(36.0)	43.0	34.0	31.0	45.0	60.0	36.0
11	42.0	67.0	72.0	36.0	66.0	40.0	(36.0)	40.0	34.0	35.0	51.0	64.0	47.0
12	48.0	77.0	66.0	35.0	70.0	42.0	(44.0)	52.0	49.0	42.0	53.0	75.0	61.0
13	46.0	75.0	73.0	41.0	68.0	49.0	(41.0)	55.0	44.0	42.0	60.0	79.0	90.0
14	316.0*	72.0	82.0	51.0	64.0	57.0	(42.0)	57.0	51.0	47.0	65.0	76.0	94.0
15	56.0	84.0	96.0	51.0	79.0	58.0	(58.0)	63.0	47.0	48.0	75.0	93.0	100.0
16	64.0	87.0	74.0	51.0	88.0	71.0	(36.0)	53.0	43.0	49.0	64.0	67.0	67.0
17	66.0	81.0	69.0	42.0	86.0	62.0	(43.0)	56.0	41.0	46.0	66.0	81.0	81.0
18	65.0	76.0	73.0	36.0	89.0	59.0	(38.0)	59.0	37.0	40.0	61.0	77.0	77.0
19	52.0	64.0	55.0	36.0	70.0	49.0	(30.0)	46.0	33.0	35.0	50.0	64.0	51.0

() Value excluded from means and statistical analysis, non-pregnant

* Value excluded from means - bottle leakage
Treatment period Days 6 to 15 post coitum inclusive

APT 2/91279

APPENDIX 2

(Water consumption - continued)

Group 5: Toluene, 3000 ppm (continued)

Day post coitum	Animal number												Mean	SD
	114	115	116	117	118	119	120D	121	122	123	124	125		
0	(24.0)	34.0	30.0	21.0	27.0	32.0	(30.0)	30.0	33.0	24.0	36.0	23.0	27.5	4.54
1	(28.0)	40.0	32.0	25.0	33.0	32.0	(32.0)	37.0	38.0	26.0	34.0	26.0	30.5	5.65
2	(38.0)	44.0	32.0	25.0	36.0	30.0	(36.0)	37.0	39.0	33.0	39.0	27.0	31.5	6.34
3	(36.0)	47.0	35.0	27.0	36.0	40.0	(32.0)	38.0	31.0	36.0	36.0	26.0	31.1	6.69
4	(39.0)	51.0	29.0	24.0	34.0	31.0	(32.0)	32.0	33.0	56.0	35.0	28.0	31.6	8.44
5	(29.0)	45.0	33.0	24.0	37.0	35.0	(37.0)	33.0	32.0	28.0	37.0	29.0	30.6	5.41
6	(42.0)	48.0	9.0	20.0	40.0	42.0	(29.0)	31.0	23.0	50.0	22.0	36.0	28.4	10.36
7	(47.0)	52.0	29.0	20.0	30.0	38.0	(40.0)	48.0	46.0	41.0	30.0	40.0	32.5	9.15
8	(61.0)	69.0	38.0	26.0	38.0	42.0	(29.0)	58.0	56.0	79.0	31.0	51.0	41.8	14.20
9	(72.0)	76.0	43.0	29.0	48.0	46.0	(49.0)	78.0	54.0	58.0	44.0	59.0	48.1	13.74
10	(82.0)	83.0	50.0	32.0	42.0	51.0	(60.0)	72.0	60.0	78.0	46.0	76.0	51.7	16.45
11	(71.0)	92.0	49.0	33.0	41.0	55.0	(98.0)	88.0	64.0	90.0	50.0	76.0	56.0	18.85
12	(91.0)	101.0	55.0	43.0	48.0	68.0	(103.0)	92.0	77.0	80.0	49.0	66.0	61.3	17.37
13	(99.0)	101.0	54.0	42.0	47.0	69.0	(73.0)	95.0	85.0	80.0	51.0	68.0	64.3	18.63
14	(72.0)	126.0	59.0	99.0	54.0	79.0	.	56.0	94.0	86.0	57.0	68.0	71.1	20.02
15	(73.0)	123.0	56.0	105.0	64.0	76.0	.	57.0	87.0	102.0	58.0	64.0	74.6	21.45
16	(63.0)	112.0	64.0	54.0	61.0	81.0	.	76.0	75.0	112.0	67.0	94.0	71.5	18.46
17	(41.0)	111.0	59.0	51.0	64.0	74.0	.	77.0	71.0	104.0	68.0	77.0	69.7	17.70
18	(27.0)	111.0	66.0	44.0	60.0	70.0	.	78.0	68.0	83.0	56.0	70.0	66.1	17.69
19	(29.0)	84.0	62.0	36.0	54.0	61.0	.	72.0	56.0	69.0	50.0	59.0	54.9	13.13

() Value excluded from means and statistical analysis, non-pregnant

D Found dead

Treatment period Days 6 to 15 post coitum inclusive

SD Standard deviation

APT 2/91279

APPENDIX 3

Food consumption - individual values

Group 1: Control

Animal no.	Food consumed (g/rat) during Days post coitum									
	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	46	48	50	46	47	56	54	50	49	43
2	40	37	39	40	40	40	45	41	46	40
3	32	34	31	38	38	38	43	40	41	44
4	44	46	47	53	52	53	56	52	56	51
5	51	56	54	51	52	56	56	47	57	49
6	42	42	46	44	46	49	48	47	49	47
7	48	46	48	51	49	53	49	49	52	48
8	36	39	42	42	41	47	50	49	45	44
9	44	43	47	47	44	51	54	52	53	48
10	44	49	58	56	53	57	56	53	53	56
11	50	53	52	54	56	57	56	53	65	59
12	37	43	43	42	45	47	48	46	48	45
13	52	58	55	58	59	61	58	53	60	52
14	39	39	42	45	40	46	43	43	52	39
15	48	53	52	57	55	59	51	59	53	57
16	44	56	49	48	52	48	47	53	58	48
17	43	48	44	43	46	48	48	50	53	46
18	40	46	43	39	41	43	46	48	51	46
19	41	44	46	42	49	44	48	44	54	47
20	41	46	47	48	47	56	53	52	55	53
21	38	46	45	42	50	49	44	48	44	41
23	46	49	55	53	56	54	56	54	56	57
24	48	53	56	58	60	63	58	63	64	58
25	44	45	54	52	59	58	54	55	55	53
Mean	43.3	46.6	47.7	47.9	49.0	51.4	50.9	50.0	52.9	48.8
SD	4.95	6.15	6.24	6.29	6.52	6.61	4.88	5.31	5.83	5.81
	<u>Non-pregnant</u>									
22	43	49	52	50	50	48	39	44	38	42

SD Standard deviation

APT 2/91279

APPENDIX 3

(Food consumption - continued)

Group 2: Toluene, 250 ppm

Animal no.	Food consumed (g/rat) during Days post coitum									
	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>									
26	45	50	48	47	53	55	58	57	61	54
27	43	47	45	47	49	51	49	52	50	48
29	38	42	42	41	46	43	51	44	46	40
30	45	49	48	47	49	50	53	50	54	47
31	42	44	48	47	51	50	53	51	49	49
32	47	34	49	46	43	53	50	54	52	54
33	48	47	54	57	54	63	55	59	61	54
34	39	37	46	43	43	48	48	47	45	49
35	39	44	50	49	40	57	49	47	50	52
36	49	50	55	59	59	63	64	54	56	54
37	43	48	51	55	55	61	56	57	58	56
39	51	54	63	60	62	60	63	65	62	64
40	47	48	50	58	52	60	52	54	51	51
41	41	51	48	44	48	49	45	47	48	47
42	42	48	46	49	52	54	51	50	54	50
43	32	39	41	40	44	41	44	41	47	41
44	39	51	54	51	55	52	57	56	56	54
45	37	44	50	40	46	43	45	44	48	49
46	42	51	48	52	49	52	55	52	56	60
48	40	45	46	45	49	50	45	51	50	50
49	34	37	43	40	45	44	43	46	47	48
50	48	46	47	44	54	44	47	52	52	54
Mean	42.3	45.7	48.7	48.2	49.9	52.0	51.5	51.4	52.4	51.1
SD	4.91	5.24	4.82	6.31	5.45	6.69	5.83	5.59	5.07	5.41
	<u>Non-pregnant</u>									
28	39	42	41	45	49	41	26	29	58	32
38	41	33	42	40	44	40	43	35	44	34
47	20	26	44	47	57	49	43	45	41	42

SD Standard deviation

APT 2/91279

APPENDIX 3

(Food consumption - continued)

Group 3: Toluene, 750 ppm

Animal no.	Food consumed (g/rat) during Days post coitum									
	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>									
51	38	44	41	43	48	47	49	46	48	45
52	43	47	47	49	53	52	54	52	57	49
54	44	45	49	48	50	51	54	50	49	44
55	45	40	46	45	47	46	53	47	52	46
56	53	51	55	52	49	57	52	55	56	55
57	42	40	39	42	40	48	45	45	49	44
58	35	39	43	42	37	42	42	40	38	42
59	47	44	49	47	44	53	49	50	54	49
60	48	46	49	48	42	49	49	50	49	52
61	42	45	48	46	48	51	49	51	56	52
63	42	45	50	49	45	50	45	46	49	47
65	49	47	51	49	46	55	49	51	53	45
66	35	48	49	43	54	46	45	49	55	51
67	35	46	46	43	47	53	46	48	56	47
68	49	57	57	55	56	57	52	52	58	54
69	45	56	57	51	56	53	53	51	54	50
70	47	54	59	51	56	56	56	54	66	58
72	43	49	48	46	54	52	52	50	54	51
73	38	40	49	41	46	38	45	47	49	44
75	42	44	46	41	49	46	47	47	56	52
Mean	43.1	46.4	48.9	46.6	48.4	50.1	49.3	49.1	52.9	48.9
SD	5.05	5.10	5.14	4.02	5.37	4.95	3.85	3.43	5.54	4.30
	<u>Non-pregnant</u>									
53	50	52	51	51	54	51	50	36	31	39
62	43	38	44	36	43	38	41	34	43	39
64	45	48	49	43	41	42	34	29	40	30
71	37	37	35	34	46	36	45	38	42	34
74	41	49	49	45	51	40	28	35	29	32

SD Standard deviation

APT 2/91279

APPENDIX 3

(Food consumption - continued)

Group 4: Toluene, 1500 ppm

Animal no.	Food consumed (g/rat) during Days post coitum									
	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>									
76	47	49	51	47	50	48	54	50	64	55
77	38	41	44	39	39	41	44	41	48	43
78	45	46	51	47	54	57	58	59	55	54
80	39	47	44	42	49	53	52	54	58	56
81	42	41	45	42	48	42	46	41	48	46
82	40	40	46	42	35	45	46	40	50	45
83	41	48	45	45	43	55	55	52	56	53
85	47	47	57	53	57	61	57	58	66	55
86	46	49	55	44	42	50	46	45	50	45
89	44	47	45	49	47	49	45	46	54	52
90	47	50	50	49	58	58	52	40	53	45
91	44	49	54	42	51	50	49	53	56	52
92	49	57	60	51	58	60	55	54	62	59
93	38	47	48	42	48	45	46	45	56	51
94	38	44	48	46	51	50	50	50	52	46
95	38	51	52	41	50	50	50	48	56	49
96	47	59	59	48	55	50	52	51	62	55
97	45	50	53	45	49	44	42	42	62	56
100	40	45	50	36	41	37	39	44	45	44
Mean	42.9	47.7	50.4	44.7	48.7	49.7	49.4	48.1	55.4	50.6
SD	3.78	4.81	5.02	4.27	6.44	6.55	5.24	6.01	5.87	4.99
	<u>Non-pregnant</u>									
79	47	51	55	52	55	52	43	42	39	42
84	40	40	42	40	35	38	27	35	29	35
87	44	49	53	52	50	48	35	31	41	40
88	46	54	54	46	47	48	44	32	46	39
98	42	44	45	42	39	39	31	38	36	35
99	51	52	56	44	50	42	34	40	37	39

SD Standard deviation

APT 2/91279

APPENDIX 3

(Food consumption - continued)

Group 5: Toluene, 3000 ppm

Animal no.	Food consumed (g/rat) during Days post coitum									
	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>									
101	44	46	49	35	45	46	53	54	55	46
102	44	45	48	21	31	36	42	35	50	47
103	43	41	45	36	46	47	45	45	52	46
104	46	44	51	35	42	42	46	41	56	47
105	46	48	52	32	39	38	38	38	46	49
106	48	47	54	42	38	39	41	40	54	51
108	41	48	49	39	43	44	44	45	56	46
109	48	43	51	37	42	41	50	47	51	50
110	40	37	40	31	30	30	33	38	41	43
111	42	45	46	37	41	45	38	39	46	42
112	52	52	56	46	40	46	45	48	52	49
113	40	42	43	26	32	41	42	37	48	43
115	47	50	48	36	36	41	46	52	60	55
116	42	50	44	27	33	38	45	44	60	51
117	39	43	43	33	37	35	40	53	46	39
118	35	41	41	29	34	36	36	37	47	43
119	44	50	52	41	49	46	46	44	50	44
121	42	51	53	37	49	50	49	38	66	62
122	40	52	43	32	39	43	44	45	51	39
123	44	50	51	37	42	44	42	44	50	46
124	47	51	53	35	42	41	45	51	54	49
125	39	42	46	24	30	33	38	42	43	48
Mean	43.3	46.3	48.1	34.0	39.1	41.0	43.1	43.5	51.5	47.0
SD	3.86	4.26	4.53	5.99	5.74	5.00	4.73	5.60	5.91	5.15
	<u>Died</u>									
120	35	43	42	30	27	37	41			
	<u>Non-pregnant</u>									
107	40	43	46	38	39	43	44	52	68	74
114	35	44	43	36	30	38	34	33	38	30

SD Standard deviation

APT 2/91279

APPENDIX 4

Bodyweights - individual values (g)

Group 1: Control

Animal number	Bodyweight (g) at Day																		
	post coitum																		
	0	2	4	6	8	10	12	14	16	18	20	#							
<u>Live young at Day 20</u>													271	342	313	291	274	266	274
1	199	209	225	234	242	248	266	274	291	313	342	271	271	342	313	291	274	266	274
2	176	186	199	201	213	214	223	232	242	266	290	234	234	290	266	242	232	223	232
3	183	185	197	200	207	212	218	225	234	249	268	236	236	268	249	234	225	218	225
4	198	205	221	231	242	249	262	275	285	312	336	278	278	336	312	285	275	262	275
5	197	209	228	238	245	253	267	277	289	315	340	273	273	340	315	289	277	267	277
6	187	200	205	222	221	231	237	249	262	286	312	248	248	312	286	262	249	237	249
7	209	224	230	239	248	260	270	282	299	323	351	272	272	351	323	299	282	270	282
8	191	204	207	218	223	233	244	255	273	292	318	256	256	318	292	273	255	244	255
9	209	223	228	238	245	256	264	277	294	320	345	271	271	345	320	294	277	264	277
10	186	202	212	226	238	255	262	271	287	309	340	272	272	340	309	287	271	262	271
11	200	218	233	243	256	271	283	302	316	350	391	310	310	391	350	316	302	283	302
12	206	217	225	231	237	249	254	265	271	278	291	272	272	291	278	271	265	254	265
13	218	234	251	257	264	285	292	303	314	335	354	308	308	354	335	314	303	292	303
14	187	199	204	213	224	230	242	255	273	300	320	238	238	320	300	273	255	242	255
15	201	213	226	235	243	256	267	279	302	322	355	280	280	355	322	302	279	267	279
16	212	225	239	248	257	267	277	289	309	334	360	282	282	360	334	309	289	277	289
17	196	209	221	228	236	245	261	272	283	302	325	256	256	325	302	283	272	261	272
18	203	213	222	225	231	235	242	253	272	292	321	251	251	321	292	272	253	242	253
19	207	221	228	236	246	253	259	269	282	308	335	266	266	335	308	282	269	259	269
20	210	222	229	238	249	253	269	277	299	316	348	277	277	348	316	299	277	269	277
21	201	211	220	228	236	245	257	266	276	299	325	256	256	325	299	276	266	257	266
23	184	198	208	221	259	242	257	271	280	308	343	267	267	343	308	280	271	257	271
24	213	227	236	251	266	278	296	310	328	363	398	310	310	398	363	328	310	296	310
25	215	228	235	246	234	269	285	297	309	339	371	294	294	371	339	309	297	285	297
Mean	199.5	211.8	222.0	231.1	240.1	249.5	260.6	271.9	286.3	309.6	336.6	269.9	269.9	336.6	309.6	286.3	271.9	260.6	271.9
SD	11.38	12.85	13.46	14.16	15.17	18.00	19.47	20.67	22.20	25.65	29.71	21.40	21.40	29.71	25.65	22.20	20.67	19.47	20.67
<u>Non-pregnant</u>													234	245	234	238	235	246	245
22	186	203	211	226	233	238	246	235	238	234	245								

Adjusted bodyweight: Day 20 bodyweight minus gravid uterine weight
SD Standard deviation

APT 2/91279

APPENDIX 4
(Bodyweights - continued)

Group 2: Toluene, 250 ppm													
Animal number	Bodyweight (g) at Day												
	post coitum												
	0	2	4	6	8	10	12	14	16	18	20	#	
<u>Live young at Day 20</u>													
26	197	207	223	235	239	255	269	280	296	321	353	280	
27	192	201	214	227	236	244	260	265	279	303	339	270	
29	190	200	213	222	226	239	236	260	270	290	312	250	
30	202	211	227	240	245	255	267	274	291	313	337	275	
31	193	204	216	230	238	252	263	272	287	312	341	270	
32	193	211	217	225	236	248	258	268	285	305	333	275	
33	196	213	221	232	249	265	282	290	312	343	367	297	
34	196	212	219	228	234	245	251	263	276	298	324	269	
35	196	205	212	225	237	240	257	267	276	302	328	262	
36	209	224	228	233	247	263	270	281	292	320	344	278	
37	192	206	219	231	246	258	268	281	298	321	349	284	
39	216	229	241	257	268	284	297	312	333	357	388	321	
40	195	210	220	230	244	255	272	285	304	324	358	282	
41	207	217	226	236	244	252	264	273	289	309	336	271	
42	190	206	212	220	232	239	250	263	279	300	336	264	
43	191	201	204	210	222	224	231	244	256	272	290	247	
44	203	216	228	242	253	261	271	286	306	329	363	288	
45	205	214	218	229	232	240	245	255	271	286	314	250	
46	212	225	238	242	258	267	282	294	306	331	370	299	
48	193	206	215	220	231	238	249	255	272	294	322	257	
49	194	204	209	219	226	234	245	254	260	280	298	263	
50	195	210	216	224	229	236	246	257	270	290	326	253	
Mean	198.0	210.5	219.8	229.9	239.6	249.7	260.6	271.8	286.7	309.1	337.6	273.0	
SD	7.51	7.89	8.86	9.94	11.18	13.60	15.87	15.90	18.41	20.69	23.57	17.99	
<u>Non-pregnant</u>													
28	172	181	191	198	209	217	216	202	206	200	208		
38	212	217	222	225	229	230	234	238	243	244	246		
47	203	200	200	221	234	245	250	252	250	253	262		

Adjusted bodyweight: Day 20 bodyweight minus gravid uterine weight
SD Standard deviation

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APPENDIX 4
(Bodyweights - continued)

Group 3: Toluene, 750 ppm

Animal number	Bodyweight (g) at Day												
	post coitum												
	0	2	4	6	8	10	12	14	16	18	20	#	
<u>Live young at Day 20</u>													
51	187	194	212	217	225	233	244	250	265	283	304	259	
52	199	207	221	235	245	257	267	280	293	319	347	269	
54	178	191	206	221	228	239	253	262	277	290	305	264	
55	183	196	206	220	226	234	242	259	275	297	321	256	
56	220	238	241	251	259	268	282	291	305	332	361	291	
57	201	210	218	225	233	242	249	258	276	301	328	252	
58	170	182	189	194	203	210	215	224	237	258	281	219	
59	178	191	203	215	223	232	244	252	265	290	317	251	
60	200	216	225	236	240	245	254	264	277	301	329	272	
61	196	208	218	224	232	236	244	256	264	284	301	266	
63	213	220	229	239	247	255	265	277	286	305	327	275	
65	202	220	228	241	250	258	268	284	300	322	350	280	
66	207	213	225	236	248	258	267	281	300	327	365	289	
67	195	205	214	223	235	242	256	265	282	310	336	262	
68	211	231	242	254	265	273	285	295	312	332	357	304	
69	229	245	255	270	280	284	295	306	318	329	353	316	
70	192	212	222	236	246	254	265	281	299	332	367	279	
72	202	217	228	236	246	257	268	283	300	326	355	277	
73	200	212	223	235	239	245	249	262	272	298	321	258	
75	198	214	220	228	237	244	256	264	273	294	318	274	
Mean	198.1	211.1	221.3	231.8	240.4	248.3	258.4	269.7	283.8	306.5	332.2	270.7	
SD	14.42	15.79	14.76	16.11	16.65	16.57	17.82	18.70	19.60	20.52	24.17	20.78	
<u>Non-pregnant</u>													
53	210	218	233	244	253	262	268	257	260	248	255		
62	186	196	197	208	200	215	208	216	216	223	226		
64	204	217	222	234	234	237	242	239	234	239	239		
71	195	204	202	215	213	225	220	228	228	235	235		
74	198	212	226	232	241	245	243	239	237	240	240		

Adjusted bodyweight: Day 20 bodyweight minus gravid uterine weight
SD Standard deviation

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APPENDIX 4
(Bodyweights - continued)

Group 4: Toluene, 1500 ppm													
Animal number	Bodyweight (g) at Day												
	post coitum												
	0	2	4	6	8	10	12	14	16	18	20	#	
<u>Live young at Day 20</u>													
76	206	217	232	247	252	259	271	281	293	324	358	290	
77	182	188	202	214	216	220	232	245	261	288	316	236	
78	181	192	205	223	227	241	259	269	295	314	345	271	
80	185	191	210	219	226	236	256	264	287	314	343	NC	
81	190	199	209	223	226	233	239	249	263	291	320	248	
82	197	211	211	222	225	228	238	242	248	272	287	246	
83	190	205	216	228	233	243	257	272	286	311	338	273	
85	198	214	223	239	243	258	265	273	291	319	352	282	
86	207	223	233	247	249	256	262	268	279	300	319	275	
89	205	220	231	239	246	257	263	274	287	311	338	277	
90	216	229	243	252	254	270	281	298	290	332	365	290	
91	223	236	247	261	263	271	283	291	313	340	371	298	
92	221	236	249	261	267	274	288	300	313	334	362	314	
93	193	206	218	228	234	244	248	257	269	291	312	265	
94	195	200	207	219	228	238	246	249	278	298	327	258	
95	185	201	211	225	227	234	244	254	267	293	319	263	
96	222	236	254	266	274	280	292	304	321	356	392	318	
97	201	214	228	237	242	247	259	266	277	306	337	280	
100	203	214	223	235	236	239	248	253	268	285	304	252	
Mean	200.0	212.2	223.8	236.1	240.4	248.8	259.5	268.9	283.5	309.4	337.1	274.2	
SD	13.44	15.23	16.02	15.83	16.08	16.81	17.34	18.96	19.00	21.35	25.87	22.48	
<u>Non-pregnant</u>													
79	186	201	221	236	243	252	260	252	249	251	253		
84	205	215	219	228	233	235	232	229	229	235	236		
87	210	216	228	235	246	253	253	247	247	248	252		
88	196	209	223	230	234	242	244	245	237	238	248		
98	189	200	206	217	220	225	227	224	224	227	231		
99	197	217	232	243	247	253	250	248	245	248	248		

Adjusted bodyweight: Day 20 bodyweight minus gravid uterine weight
SD Standard deviation

APT 2/91279

APPENDIX 4

(Bodyweights - continued)

Group 5: Toluene, 3000 ppm

Animal number	Bodyweight (g) at Day												
	post coitum												
	0	2	4	6	8	10	12	14	16	18	20	#	
	<u>Live young at Day 20</u>												
101	183	194	211	230	221	225	232	239	247	274	302	240	
102	190	199	210	221	206	206	214	223	230	256	284	230	
103	188	199	209	226	215	223	234	241	254	275	309	241	
104	185	198	211	225	217	225	234	240	254	286	315	244	
105	199	211	228	243	232	232	238	243	253	280	314	246	
106	209	222	231	242	237	245	247	254	261	292	318	258	
108	203	215	225	239	233	240	246	258	271	300	326	262	
109	207	223	226	241	232	239	240	252	258	283	309	258	
110	182	197	201	209	206	204	204	208	221	236	252	220	
111	192	199	210	217	211	216	221	227	239	270	304	236	
112	216	230	247	257	251	249	255	266	278	302	335	272	
113	195	205	215	223	214	214	221	228	233	258	286	229	
115	206	220	231	236	231	240	246	262	277	311	344	270	
116	192	209	221	230	226	224	227	243	252	284	312	251	
117	192	204	211	219	215	212	216	226	250	263	290	222	
118	193	200	205	213	205	210	218	223	230	253	281	229	
119	227	237	246	256	252	252	254	257	267	290	322	265	
121	202	214	229	242	240	244	250	265	280	306	338	282	
122	206	219	225	236	230	228	235	244	253	282	293	251	
123	198	216	228	238	234	231	241	245	254	283	306	252	
124	211	225	240	251	244	248	253	259	272	306	332	276	
125	188	201	213	221	211	208	217	227	235	254	280	233	
Mean	198.4	210.8	221.5	232.5	225.6	228.0	233.8	242.3	253.1	279.3	306.9	248.5	
SD	11.46	12.13	12.94	13.41	14.39	15.17	14.76	15.93	16.83	19.45	22.37	17.89	
	<u>Non-pregnant</u>												
107	181	192	201	215	205	206	207	202	201	206	208		
114	180	188	204	213	207	207	207	196	202	200	203		
	<u>Died</u>												
120	211	222	226	233	225	218	224	235					

Adjusted bodyweight: Day 20 bodyweight minus gravid uterine weight
SD Standard deviation

APT 2/91279

APPENDIX 5
Liver weights - individual values

Group 1: Control			Group 2: Toluene, 250 ppm			Group 3: Toluene, 750 ppm			Group 4: Toluene, 1500 ppm			Group 5: Toluene, 3000 ppm		
Anim. no.	Liver wt. (g)	Relative Liver wt.*	Anim. no.	Liver wt. (g)	Relative Liver wt.*	Anim. no.	Liver wt. (g)	Relative Liver wt.*	Anim. no.	Liver wt. (g)	Relative Liver wt.*	Anim. no.	Liver wt. (g)	Relative Liver wt.*
	<u>With live young at Day 20</u>			<u>With live young at Day 20</u>			<u>With live young at Day 20</u>			<u>With live young at Day 20</u>			<u>With live young at Day 20</u>	
1	14.65	5.41	26	15.63	5.58	51	13.52	5.22	76	15.69	5.41	101	14.16	5.90
2	12.38	5.29	27	13.92	5.16	52	14.71	5.47	77	14.81	6.28	102	11.83	5.14
3	13.52	5.73	29	12.10	4.84	54	13.83	5.24	78	15.80	5.83	103	12.50	5.19
4	14.00	5.04	30	14.42	5.24	55	14.64	5.72	80	15.48	NC	104	14.18	5.81
5	14.49	5.31	31	NR	-	56	17.51	6.02	81	12.66	5.10	105	13.68	5.56
6	12.41	5.00	32	15.25	5.54	57	13.30	5.28	82	13.78	5.60	106	16.22	6.29
7	14.02	5.15	33	17.40	5.86	58	14.10	6.44	83	14.73	5.40	108	13.27	5.07
8	14.65	5.72	34	14.35	5.33	59	14.35	5.72	85	16.30	5.78	109	14.66	5.68
9	13.92	5.14	35	14.03	5.35	60	13.74	5.05	86	14.61	5.31	110	12.15	5.52
10	15.93	5.86	36	15.04	5.41	61	16.83	6.33	89	16.27	5.87	111	13.60	5.76
11	17.35	5.60	37	16.39	5.77	63	15.89	5.78	90	14.60	5.03	112	15.50	5.70
12	14.19	5.22	39	17.32	5.40	65	15.14	5.41	91	14.99	5.03	113	12.25	5.35
13	17.33	5.63	40	15.56	5.52	66	15.96	5.52	92	16.59	5.28	115	17.19	6.37
14	11.93	5.01	41	14.96	5.52	67	14.11	5.39	93	14.86	5.61	116	14.37	5.73
15	15.66	5.59	42	NR	-	68	16.41	5.40	94	14.75	5.72	117	12.65	5.70
16	14.14	5.01	43	12.94	5.24	69	15.69	4.97	95	13.64	5.19	118	12.62	5.51
17	13.22	5.16	44	17.45	6.06	70	17.22	6.17	96	16.54	5.20	119	14.92	5.63
18	13.38	5.33	45	15.18	6.07	72	16.54	5.97	97	15.19	5.43	121	17.21	6.10
19	13.19	4.96	46	17.11	5.72	73	14.01	5.43	100	12.53	4.97	122	12.25	4.88
20	14.71	5.31	48	13.92	5.42	75	16.40	5.99				123	14.04	5.57
21	12.20	4.77	49	13.34	5.07							124	15.97	5.79
23	15.25	5.71	50	13.91	5.50							125	14.22	6.10
24	15.63	5.04												
25	14.94	5.08												
Mean	14.30	5.29		15.01	5.48		15.20	5.63		14.94	5.45		14.07	5.65
SD	1.44	0.30		1.54	0.31		1.33	0.42		1.18	0.35		1.60	0.38
22	<u>Non-pregnant</u>		28	<u>Non-pregnant</u>		53	<u>Non-pregnant</u>		79	<u>Non-pregnant</u>		120	<u>Dead</u>	
	11.51		38	9.78		62	10.47		84	12.30		107	11.96	
			47	10.95		64	10.07		87	11.73		114	Non-pregnant	
				11.42		71	12.08		88	12.57			9.16	
						74	11.21		98	14.21			10.69	
							11.57		99	11.57				
										12.31				

NR Not recorded in error
* Liver weight adjusted according to the formula: $\frac{\text{SD Standard deviation}}{\text{SD Standard deviation}} \times 100$
SD Standard deviation
NC Gravid uterine weight not recorded in error
Liver weight
Day 20 bodyweight - gravid uterine weight

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APPENDIX 6

Litter data - individual values

Group 1: Control

Animal number	Corpora lutea	Pre-implant loss %	Embryonic deaths			Post implant loss %	Live young	Litter wt.(g)	Mean foetal wt.(g)	Gravid uterine wt.(g)
			Early	Late	Total					
<u>With live young</u>										
1	18	16.7	1	0	1	6.7	14	44.98	3.21	70.92
2	11	0.0	0	0	0	0.0	11	34.38	3.13	55.93
3	15	33.3	4	0	4	40.0	6	17.76	2.96	32.28
4	11	0.0	0	0	0	0.0	11	38.05	3.46	58.21
5	13	0.0	1	0	1	7.7	12	43.34	3.61	66.64
6	12	0.0	0	0	0	0.0	12	43.01	3.58	63.56
7	15	0.0	0	0	0	0.0	15	50.46	3.36	78.60
8	12	0.0	1	0	1	8.3	11	39.84	3.62	62.23
9	14	0.0	1	0	1	7.1	13	47.38	3.64	73.66
10	15	0.0	2	1	3	20.0	12	43.93	3.66	68.48
11	17	5.9	1	0	1	6.3	15	50.77	3.38	80.62
12a	12	66.7	1	0	1	25.0	3	11.37	3.79	18.85
13	9	11.1	0	0	0	0.0	8	27.69	3.46	46.18
14	15	0.0	0	0	0	0.0	15	54.46	3.63	82.30
15	13	13.3	0	0	0	0.0	13	45.67	3.51	75.15
16	16	0.0	1	0	1	6.3	15	50.56	3.37	78.34
17	15	20.0	0	0	0	0.0	12	40.49	3.37	68.99
18	16	18.8	0	0	0	0.0	13	48.07	3.70	69.67
19	13	0.0	0	0	0	0.0	13	43.25	3.33	68.63
20	14	7.1	0	0	0	0.0	13	46.76	3.60	71.32
21	13	0.0	0	0	0	0.0	13	43.49	3.35	68.84
23	13	0.0	0	0	0	0.0	13	45.74	3.52	75.98
24	17	5.9	0	0	0	0.0	16	56.48	3.53	87.66
25	14	0.0	0	0	0	0.0	14	49.94	3.57	77.46
Mean	14.0	8.3	0.5	0.0	0.6	5.3	12.2	42.41	3.47	66.69
SD	2.16	15.27	0.93	0.20	1.02	9.89	2.96	10.61	0.19	15.68
<u>Non-pregnant</u>										
22										

a Unilateral implantation
SD Standard deviation

APT 2/91279

APPENDIX 6

(Litter data - continued)

Group 2: Toluene, 250 ppm

Animal number	Corpora lutea	Pre-implant loss %	Embryonic deaths		Post implant loss %	Live young	Litter wt.(g)	Mean foetal wt.(g)	Gravid uterine wt.(g)
			Early	Late	Total				
	<u>With live young</u>								
26	14	0.0	1	0	1	13	45.82	3.52	72.81
27	13	0.0	0	0	0	13	44.24	3.40	69.31
29	14	6.7	1	0	1	13	39.85	3.07	62.49
30	13	0.0	0	0	0	13	40.41	3.11	62.47
31	14	6.7	0	0	0	14	42.36	3.03	70.67
32	14	7.1	2	0	2	11	35.99	3.27	57.78
33	13	7.1	1	0	1	12	42.82	3.57	69.51
34	13	0.0	2	0	2	11	34.67	3.15	54.62
35	12	7.7	0	0	0	12	44.07	3.67	66.16
36	14	0.0	0	0	0	14	39.49	2.82	66.48
37	14	17.6	3	0	3	11	41.57	3.78	64.95
39	14	6.7	1	0	1	13	44.13	3.39	67.17
40	15	0.0	0	0	0	15	49.05	3.27	75.69
41	12	0.0	0	0	0	12	39.87	3.32	64.55
42	13	7.1	0	0	0	13	46.06	3.54	71.51
43a	9	25.0	0	0	0	9	27.75	3.08	43.11
44	15	6.3	0	0	0	15	49.04	3.27	75.20
45	13	0.0	1	0	1	12	42.27	3.52	63.84
46	14	6.7	1	0	1	13	46.69	3.59	70.71
48	15	16.7	1	1	2	13	40.11	3.09	65.43
49	11	31.3	4	1	5	6	20.39	3.40	35.29
50	14	12.5	0	0	0	14	45.48	3.25	72.87
Mean	14.4	7.5	0.8	0.1	0.9	12.4	41.01	3.32	64.67
SD	1.56	1.39	1.10	0.29	1.27	1.99	6.69	0.24	9.83
	<u>Non-pregnant</u>								
28									
38									
47									

a Unilateral implantation
SD Standard deviation

APT 2/91279

APPENDIX 6

(Litter data - continued)

Group 3: Toluene, 750 ppm

Animal number	Corpora lutea	Pre-implant loss %	Embryonic deaths		Post implant loss %	Live young	Litter wt.(g)	Mean foetal wt.(g)	Gravid uterine wt.(g)
			Early	Late	Total				
<u>With live young</u>									
51	11	27.3	0	0	0	8	29.23	3.65	45.27
52	18	16.7	0	0	0	15	55.39	3.69	78.32
54	15	53.3	0	0	0	7	27.07	3.87	40.92
55	13	0.0	1	0	1	12	39.27	3.27	64.94
56	16	18.8	0	0	0	13	45.02	3.46	70.20
57	17	11.8	0	0	0	15	49.02	3.27	75.53
58	14	0.0	1	0	1	13	40.83	3.14	61.69
59	13	0.0	1	0	1	12	43.98	3.67	65.61
60	14	21.4	0	0	0	11	34.53	3.14	56.64
61	13	46.2	1	0	1	6	23.10	3.85	35.11
63	10	0.0	1	0	1	9	32.17	3.57	51.95
65	15	13.3	0	0	0	13	43.58	3.35	69.55
66	16	6.3	0	0	0	15	49.09	3.27	76.20
67	15	0.0	0	0	0	15	48.20	3.21	74.16
68	15	33.3	0	0	0	10	32.92	3.29	52.92
69a	13	30.8	1	0	1	8	23.24	2.91	37.31
70	18	5.3	0	0	0	18	60.23	3.35	88.21
72	16	12.5	0	0	0	14	50.66	3.62	77.52
73	14	14.3	0	0	0	12	42.37	3.53	63.35
75	14	21.4	3	1	4	7	26.13	3.73	44.39
Mean	14.6	16.6	0.5	0.1	0.5	11.7	39.80	3.44	61.49
SD	2.16	15.45	0.76	0.22	0.95	3.31	10.84	0.26	15.30
<u>Non-pregnant</u>									
53									
62									
64									
71									
74									

a Unilateral implantation
SD Standard deviation

APT 2/91279

APPENDIX 6

(Litter data - continued)

Group 4: Toluene, 1500 ppm

Animal number	Corpora lutea	Pre-implant loss %	Embryonic deaths		Post implant loss %	Live young	Litter wt.(g)	Mean foetal wt.(g)	Gravid uterine wt.(g)
			Early	Late					
<u>With live young</u>									
76	21	23.8	3	0	3	13	45.53	3.50	68.23
77	16	0.0	1	0	1	15	51.43	3.43	79.94
78	15	6.7	0	0	0	14	48.76	3.48	73.94
80	14	14.3	0	0	0	12	39.63	3.30	NR
81	17	5.9	0	0	0	16	45.83	2.86	72.21
82	9	0.0	0	1	1	8	25.31	3.16	40.76
83	14	0.0	0	0	0	14	39.60	2.83	64.96
85	18	22.2	1	0	1	13	40.60	3.12	70.08
86	16	37.5	2	0	2	8	26.94	3.37	44.19
89	13	15.4	0	0	0	11	36.53	3.32	60.71
90	16	0.0	2	0	2	14	46.75	3.34	74.95
91	15	0.0	0	0	0	15	47.16	3.14	72.97
92	14	7.1	2	2	4	9	29.54	3.28	47.99
93	16	18.8	3	0	3	10	30.68	3.07	47.31
94	15	0.0	1	0	1	14	44.34	3.17	68.86
95	14	14.3	0	0	0	12	37.63	3.14	56.43
96	15	0.0	0	0	0	15	48.44	3.23	73.95
97	14	14.3	0	0	0	12	37.87	3.16	56.56
100	12	0.0	1	0	1	11	32.09	2.92	51.73
Mean	14.9	9.5	0.8	0.2	1.0	12.4	39.72	3.20	62.54
SD	2.44	2.12	1.07	0.50	1.25	2.41	7.91	0.19	12.13
<u>Non-pregnant</u>									
79									
84									
87									
88									
98									
99									

NR Not recorded in error
SD Standard deviation

APT 2/91279

APPENDIX 6

(Litter data - continued)

Group 5: Toluene, 3000 ppm

Animal number	Corpora lutea	Implants	Pre-implant loss %	Embryonic deaths		Post implant loss %	Live young	Litter wt.(g)	Mean foetal wt.(g)	Gravid uterine wt.(g)
				Early	Late	Total				
<u>With live young</u>										
101	15	14	6.7	0	1	1	13	41.00	3.15	62.38
102	13	13	0.0	2	0	2	11	37.37	3.40	54.41
103	14	14	0.0	0	0	0	14	45.84	3.27	68.07
104	14	14	0.0	0	0	0	14	48.37	3.46	71.09
105	16	16	0.0	1	0	1	15	45.96	3.06	68.08
106	16	14	12.5	0	2	2	12	38.37	3.20	60.09
108	15	14	6.7	1	0	1	13	44.08	3.39	64.48
109	13	13	0.0	2	0	2	11	32.28	2.93	50.97
110a	11	6	45.5	0	0	0	6	20.25	3.38	31.64
111	17	15	11.8	0	0	0	15	42.70	2.85	68.11
112	18	15	16.7	0	0	0	15	40.93	2.73	63.44
113	15	14	6.7	0	0	0	14	37.33	2.67	57.21
115	17	16	5.9	0	0	0	16	48.13	3.01	74.16
116	15	15	0.0	2	0	2	13	39.65	3.05	60.73
117	17	15	11.8	0	1	1	14	42.41	3.03	68.14
118	15	14	6.7	2	0	2	12	32.44	2.70	51.96
119	14	12	14.3	0	0	0	12	37.83	3.15	57.44
121	16	14	12.5	2	0	2	12	37.98	3.17	56.28
122	16	14	12.5	4	0	4	10	25.21	2.52	42.39
123	12	12	0.0	1	0	1	11	34.18	3.11	53.75
124	13	12	7.7	0	0	0	12	34.87	2.91	56.12
125	13	13	0.0	1	0	1	12	26.74	2.23	47.16
Mean	14.8	13.6	8.1	0.8	0.2	1.0	12.6	37.91	3.02	58.55
SD	1.80	2.04	10.08	1.10	0.50	1.07	2.15	7.33	0.31	10.04
<u>Non-pregnant</u>										
<u>Died</u>										
107										
114										
120										

a Unilateral implantation
SD Standard deviation

APT 2/91279

APPENDIX 7

Sex ratios and litter weight by sex - individual litter values

Group 1: Control

Animal no.	Number		Total	% males per litter	Litter wt. (g) ♂	Mean foetal wt. (g) ♂	Litter wt. (g) ♀	Mean foetal wt. (g) ♀
	♂	♀						
	<u>With live young at Day 20</u>							
1	9	5	14	64.3	30.26	3.36	14.72	2.94
2	3	8	11	27.3	9.78	3.26	24.60	3.08
3	0	6	6	0.0	-	-	17.76	2.96
4	6	5	11	54.5	21.53	3.59	16.52	3.30
5	7	5	12	58.3	25.78	3.68	17.56	3.51
6	3	9	12	25.0	11.13	3.71	31.88	3.54
7	4	11	15	26.7	14.41	3.60	36.05	3.28
8	9	2	11	81.8	32.28	3.59	7.56	3.78
9	6	7	13	46.2	22.00	3.67	25.38	3.63
10	6	6	12	50.0	22.12	3.69	21.81	3.64
11	8	7	15	53.3	29.37	3.67	21.40	3.06
12	2	1	3	66.7	7.90	3.95	3.47	3.47
13	6	2	8	75.0	21.11	3.52	6.58	3.29
14	7	8	15	46.7	26.20	3.74	28.26	3.53
15	7	6	13	53.8	25.39	3.63	20.28	3.38
16	6	9	15	40.0	20.19	3.37	30.37	3.37
17	7	5	12	58.3	23.95	3.42	16.54	3.31
18	8	5	13	61.5	30.13	3.77	17.94	3.59
19	5	8	13	38.5	17.44	3.49	25.81	3.23
20	9	4	13	69.2	32.69	3.63	14.07	3.52
21	5	8	13	38.5	15.63	3.13	27.86	3.48
23	4	9	13	30.8	14.66	3.67	31.08	3.45
24	4	12	16	25.0	14.87	3.72	41.61	3.47
25	7	7	14	50.0	25.39	3.63	24.55	3.51
Mean	5.8	6.5	12.2	47.56	21.49	3.59	21.82	3.39
SD	2.31	2.70	2.96	18.91	7.25	0.18	9.25	0.22

SD Standard deviation

APT 2/91279

APPENDIX 7

(Sex ratios and litter weight by sex - continued)

Group 2: Toluene, 250 ppm

Animal no.	Number		Total	% males per litter	Litter wt. (g) ♂	Mean foetal wt. (g) ♂	Litter wt. (g) ♀	Mean foetal wt. (g) ♀
	♂	♀						
	<u>With live young at Day 20</u>							
26	4	9	13	30.8	14.80	3.70	31.02	3.45
27	9	4	13	69.2	31.16	3.46	13.08	3.27
29	7	6	13	53.8	21.56	3.08	18.29	3.05
30	4	9	13	30.8	13.41	3.35	27.00	3.00
31	6	8	14	42.9	18.83	3.14	23.53	2.94
32	9	2	11	81.8	30.05	3.34	5.94	2.97
33	6	6	12	50.0	21.80	3.63	21.02	3.50
34	5	6	11	45.5	17.11	3.42	17.56	2.93
35	9	3	12	75.0	33.39	3.71	10.68	3.56
36	7	7	14	50.0	20.42	2.92	19.07	2.72
37	5	6	11	45.5	19.78	3.96	21.79	3.63
39	7	6	13	53.8	24.13	3.45	20.00	3.33
40	7	8	15	46.7	24.02	3.43	25.03	3.13
41	9	3	12	75.0	30.31	3.37	9.56	3.19
42	7	6	13	53.8	25.86	3.69	20.20	3.37
43 ^a	2	7	9	22.2	6.21	3.11	21.54	3.08
44	7	8	15	46.7	25.40	3.34	25.64	3.21
45	8	4	12	66.7	28.52	3.57	13.75	3.44
46	4	9	13	30.8	14.59	3.65	32.10	3.57
48	4	9	13	30.8	12.12	3.03	27.99	3.11
49	4	2	6	66.7	13.81	3.45	6.58	3.29
50	6	8	14	42.9	19.38	3.23	26.10	3.26
Mean	6.2	6.2	12.4	50.52	21.12	3.41	19.89	3.23
SD	1.99	2.30	1.99	16.38	7.04	0.26	7.45	0.24

a Unilateral implantation

SD Standard deviation

APT 2/91279

APPENDIX 7

(Sex ratios and litter weight by sex - continued)

Group 3: Toluene, 750 ppm

Animal no.	Number		Total	% males per litter	Litter wt. (g) ♂	Mean foetal wt. (g) ♂	Litter wt. (g) ♀	Mean foetal wt. (g) ♀
	♂	♀						
	<u>With live young at Day 20</u>							
51	4	4	8	50.0	15.46	3.87	13.77	3.44
52	8	7	15	53.3	30.47	3.81	24.92	3.56
54	1	6	7	14.3	3.73	3.73	23.34	3.89
55	7	5	12	58.3	23.11	3.30	16.16	3.23
56	4	9	13	30.8	14.68	3.67	30.34	3.37
57	6	9	15	40.0	20.61	3.44	28.41	3.16
58	3	10	13	23.1	9.53	3.18	31.30	3.13
59	6	6	12	50.0	22.50	3.75	21.48	3.58
60	8	3	11	72.7	25.48	3.19	9.05	3.02
61	2	4	6	33.3	8.27	4.14	14.83	3.71
63	5	4	9	55.6	18.14	3.63	14.03	3.51
65	10	3	13	76.9	33.71	3.37	9.87	3.29
66	9	6	15	60.0	30.07	3.34	19.02	3.17
67	5	10	15	33.3	16.79	3.36	31.41	3.14
68	7	3	10	70.0	23.05	3.29	9.87	3.29
69 ^a	2	6	8	25.0	4.92	2.46	18.32	3.05
70	5	13	18	27.8	17.62	3.52	42.61	3.28
72	5	9	14	35.7	18.59	3.72	32.07	3.56
73	6	6	12	50.0	21.40	3.57	20.97	3.50
75	3	4	7	42.9	10.91	3.64	15.22	3.81
Mean	5.3	6.4	11.7	45.15	18.45	3.50	21.35	3.38
SD	2.43	2.81	3.31	17.44	8.27	0.35	9.07	0.25

^a Unilateral implantation

SD Standard deviation

APT 2/91279

APPENDIX 7

(Sex ratios and litter weight by sex - continued)

Group 4: Toluene, 1500 ppm

Animal no.	Number		Total	% males per litter	Litter wt. (g) ♂	Mean foetal wt. (g) ♂	Litter wt. (g) ♀	Mean foetal wt. (g) ♀
	♂	♀						
	<u>With live young at Day 20</u>							
76	6	7	13	46.2	21.69	3.62	23.84	3.41
77	6	9	15	40.0	21.57	3.60	29.86	3.32
78	4	10	14	28.6	14.46	3.62	34.30	3.43
80	4	8	12	33.3	13.16	3.29	26.47	3.31
81	7	9	16	43.8	20.38	2.91	25.45	2.83
82	2	6	8	25.0	6.42	3.21	18.89	3.15
83	6	8	14	42.9	17.84	2.97	21.76	2.72
85	6	7	13	46.2	19.47	3.25	21.13	3.02
86	6	2	8	75.0	20.13	3.36	6.81	3.41
89	7	4	11	63.6	23.52	3.36	13.01	3.25
90	10	4	14	71.4	33.93	3.39	12.82	3.21
91	6	9	15	40.0	20.23	3.37	26.93	2.99
92	4	5	9	44.4	13.86	3.47	15.68	3.14
93	3	7	10	30.0	9.38	3.13	21.30	3.04
94	8	6	14	57.1	25.92	3.24	18.42	3.07
95	3	9	12	33.3	9.56	3.19	28.07	3.12
96	7	8	15	46.7	23.84	3.41	24.60	3.08
97	8	4	12	66.7	25.34	3.17	12.53	3.13
100	3	8	11	27.3	9.12	3.04	22.97	2.87
Mean	5.6	6.8	12.4	45.34	18.41	3.29	21.31	3.13
SD	2.09	2.19	2.41	15.09	7.02	0.21	6.90	0.20

SD Standard deviation

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APPENDIX 7

(Sex ratios and litter weight by sex - continued)

Group 5: Toluene, 3000 ppm

Animal no.	Number		Total	% males per litter	Litter wt. (g) ♂	Mean foetal wt. (g) ♂	Litter wt. (g) ♀	Mean foetal wt. (g) ♀
	♂	♀						
	<u>With live young at Day 20</u>							
101	7	6	13	53.8	22.48	3.21	18.52	3.09
102	7	4	11	63.6	24.19	3.46	13.18	3.30
103	8	6	14	57.1	26.50	3.31	19.34	3.22
104	8	6	14	57.1	28.07	3.51	20.30	3.38
105	6	9	15	40.0	18.01	3.00	27.95	3.11
106	7	5	12	58.3	23.40	3.34	14.97	2.99
108	6	7	13	46.2	21.19	3.53	22.89	3.27
109	4	7	11	36.4	11.98	3.00	20.30	2.90
110 ^a	4	2	6	66.7	13.93	3.48	6.32	3.16
111	2	13	15	13.3	5.91	2.96	36.79	2.83
112	5	10	15	33.3	14.24	2.85	26.69	2.67
113	6	8	14	42.9	16.61	2.77	20.72	2.59
115	11	5	16	68.8	33.68	3.06	14.45	2.89
116	6	7	13	46.2	18.95	3.16	20.70	2.96
117	5	9	14	35.7	15.69	3.14	26.72	2.97
118	5	7	12	41.7	13.79	2.76	18.65	2.66
119	9	3	12	75.0	28.66	3.18	9.17	3.06
121	4	8	12	33.3	13.25	3.31	24.73	3.09
122	5	5	10	50.0	13.18	2.64	12.03	2.41
123	6	5	11	54.5	19.41	3.24	14.77	2.95
124	7	5	12	58.3	20.75	2.96	14.12	2.82
125	4	8	12	33.3	9.66	2.42	17.08	2.14
Mean	6.0	6.6	12.6	48.43	18.80	3.10	19.11	2.93
SD	2.00	2.44	2.15	14.61	6.83	0.30	6.88	0.30

^a Unilateral implantation

SD Standard deviation

APT 2/91279

APPENDIX 8

Malformations and anomalies - individual incidence

Group 1: Control

Litter no.	Malformations			Anomalies†					
	Foetuses examined	Foetuses affected	%	Allocated for visceral examination			Allocated for skeletal examination		
				Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
1	14	0	0.0	7	1	14.3	7	2	28.6
2	11	0	0.0	6	2	33.3	5	0	0.0
3	6	0	0.0	3	0	0.0	3	0	0.0
4	11	0	0.0	6	0	0.0	5	1	20.0
5	12	0	0.0	6	0	0.0	6	0	0.0
6	12	0	0.0	6	0	0.0	6	0	0.0
7	15	0	0.0	7	1	14.3	8	0	0.0
8	11	0	0.0	6	0	0.0	5	1	20.0
9	13	0	0.0	6	0	0.0	7	3	42.9
10	12	0	0.0	6	2	33.3	6	1	16.7
11	15	0	0.0	7	2	28.6	8	2	25.0
12	3	0	0.0	2	0	0.0	1	0	0.0
13	8	0	0.0	4	1	25.0	4	3	75.0
14	15	0	0.0	8	0	0.0	7	0	0.0
15	13	0	0.0	6	0	0.0	7	1	14.3
16	15	0	0.0	8	4	50.0	7	0	0.0
17	12	0	0.0	6	1	16.7	6	2	33.3
18	13	0	0.0	7	0	0.0	6	0	0.0
19	13	0	0.0	6	0	0.0	7	1	14.3
20	13	0	0.0	7	1	14.3	6	0	0.0
21	13	0	0.0	6	0	0.0	7	0	0.0
23	13	0	0.0	6	0	0.0	7	1	14.3
24	16	0	0.0	8	1	12.5	8	2	25.0
25	14	0	0.0	7	1	14.3	7	3	42.9
Mean	12.2	0		6.1		10.7	6.1		15.5
SD	2.96	-		1.4		14.25	1.6		19.11

† Malformed foetuses are excluded

SD Standard deviation

APT 2/91279

APPENDIX 8

(Malformations and anomalies - continued)

Group 2: Toluene, 250 ppm

Litter no.	Malformations			Anomalies!					
	Foetuses examined	Foetuses affected	%	Allocated for visceral examination			Allocated for skeletal examination		
				Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
26	13	0	0.0	7	0	0.0	6	0	0.0
27	13	0	0.0	6	0	0.0	7	1	14.3
29	13	0	0.0	6	1	16.7	7	0	0.0
30	13	0	0.0	7	0	0.0	6	2	33.3
31	14	0	0.0	7	1	14.3	7	3	42.9
32	11	0	0.0	6	0	0.0	5	0	0.0
33	12	0	0.0	6	2	33.3	6	2	33.3
34	11	0	0.0	6	1	16.7	5	3	60.0
35	12	0	0.0	6	0	0.0	6	0	0.0
36	14	1	7.1	6	2	33.3	7	4	57.1
37	11	0	0.0	5	0	0.0	6	2	33.3
39	13	2	15.4	6	0	0.0	5	4	80.0
40	15	0	0.0	8	2	12.5	7	3	42.9
41	12	0	0.0	6	0	0.0	6	1	16.7
42	13	1	7.7	6	1	16.7	6	1	16.7
43	9	0	0.0	4	0	0.0	5	1	20.0
44	15	1	6.7	8	1	12.5	6	2	33.3
45	12	0	0.0	6	0	0.0	6	0	0.0
46	13	0	0.0	7	0	0.0	6	3	50.0
48	13	0	0.0	7	0	0.0	6	0	0.0
49	6	0	0.0	3	0	0.0	3	0	0.0
50	14	0	0.0	7	0	0.0	7	0	0.0
Mean	12.4		1.7	6.2		7.7	6.0		24.3
SD	1.99		3.96	1.14		11.44	0.95		23.99

! Malformed foetuses are excluded

SD Standard deviation

APT 2/91279

APPENDIX 8

(Malformations and anomalies - continued)

Group 3: Toluene, 750 ppm

Litter no.	Malformations			Anomalies†					
	Foetuses examined	Foetuses affected	%	Allocated for visceral examination			Allocated for skeletal examination		
				Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
51	8	0	0.0	4	0	0.0	4	0	0.0
52	15	0	0.0	8	0	0.0	7	1	14.3
54	7	0	0.0	4	0	0.0	3	1	33.3
55	12	0	0.0	6	0	0.0	6	0	0.0
56	13	0	0.0	7	0	0.0	6	1	16.7
57	15	0	0.0	7	0	0.0	8	5	62.5
58	13	0	0.0	7	0	0.0	6	0	0.0
59	12	0	0.0	6	1	16.7	6	2	33.3
60	11	0	0.0	6	0	0.0	5	1	20.0
61	6	0	0.0	3	1	33.3	3	1	33.3
63	9	0	0.0	4	0	0.0	5	3	60.0
65	13	0	0.0	6	0	0.0	7	1	14.3
66	15	0	0.0	8	1	12.5	7	2	28.6
67	15	0	0.0	7	2	28.6	8	3	37.5
68	10	0	0.0	5	0	0.0	5	0	0.0
69	8	0	0.0	4	1	25.0	4	1	25.0
70	18	0	0.0	9	1	11.1	9	0	0.0
72	14	0	0.0	7	0	0.0	7	1	14.3
73	12	0	0.0	6	0	0.0	6	1	16.7
75	7	0	0.0	3	0	0.0	4	1	25.0
Mean	11.7	0		5.9		6.4	5.8		21.7
SD	3.31	-		1.73		11.01	1.67		18.39

† Malformed foetuses are excluded

SD Standard deviation

APT 2/91279

APPENDIX 8

(Malformations and anomalies - continued)

Group 4: Toluene, 1500 ppm

Litter no.	Malformations			Anomalies†					
	Foetuses examined	Foetuses affected	%	Allocated for visceral examination			Allocated for skeletal examination		
				Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
76	13	0	0.0	7	0	0.0	6	2	33.3
77	15	0	0.0	7	1	14.3	8	1	12.5
78	14	0	0.0	7	0	0.0	7	1	14.3
80	12	0	0.0	6	0	0.0	6	2	33.3
81	16	0	0.0	8	1	12.5	8	1	12.5
82	8	0	0.0	4	2	50.0	4	2	50.0
83	14	2	14.3	5	0	0.0	7	0	0.0
85	13	0	0.0	6	2	33.3	7	1	14.3
86	8	1	12.5	4	1	25.0	3	1	33.3
89	11	0	0.0	5	0	0.0	6	2	33.3
90	14	1	7.1	6	0	0.0	7	0	0.0
91	15	1	6.7	7	0	0.0	7	1	14.3
92	9	0	0.0	5	0	0.0	4	1	25.0
93	10	0	0.0	5	0	0.0	5	0	0.0
94	14	0	0.0	7	2	28.6	7	2	28.6
95	12	0	0.0	6	0	0.0	6	3	50.0
96	15	1	6.7	7	0	0.0	7	2	28.6
97	12	0	0.0	6	0	0.0	6	0	0.0
100	11	0	0.0	6	1	16.7	5	1	20.0
Mean	12.4		2.5	6.0		9.5	6.1		21.2
SD	2.41		4.61	1.11		14.87	1.37		15.74

† Malformed foetuses are excluded

SD Standard deviation

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APPENDIX 8

(Malformations and anomalies - continued)

Group 5: Toluene, 3000 ppm

Litter no.	Malformations			Anomalies†					
	Foetuses examined	Foetuses affected	%	Allocated for visceral examination			Allocated for skeletal examination		
				Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
101	13	0	0.0	6	1	16.7	7	1	14.3
102	11	0	0.0	6	0	0.0	5	0	0.0
103	14	0	0.0	7	0	0.0	7	1	14.3
104	14	0	0.0	7	0	0.0	7	0	0.0
105	15	0	0.0	7	1	14.3	8	0	0.0
106	12	1	8.3	6	0	0.0	5	1	20.0
108	13	0	0.0	7	0	0.0	6	0	0.0
109	11	0	0.0	5	0	0.0	6	0	0.0
110	6	0	0.0	3	0	0.0	3	2	66.7
111	15	1	6.7	6	0	0.0	8	2	25.0
112	15	0	0.0	8	1	12.5	7	1	14.3
113	14	0	0.0	7	1	14.3	7	4	57.1
115	16	0	0.0	8	1	12.5	8	1	12.5
116	13	0	0.0	7	0	0.0	6	1	16.7
117	14	0	0.0	7	2	28.6	7	1	14.3
118	12	0	0.0	6	0	0.0	6	1	16.7
119	12	1	8.3	5	1	20.0	6	0	0.0
121	12	0	0.0	6	1	16.7	6	0	0.0
122	10	1	10.0	4	0	0.0	5	1	20.0
123	11	0	0.0	5	0	0.0	6	3	50.0
124	12	0	0.0	6	0	0.0	6	1	16.7
125	12	1	8.3	5	2	40.0	6	5	83.3
Mean	12.6		1.9	6.1		8.0	6.3		20.1
SD	2.15		3.60	1.23		11.3	1.16		23.41

† Malformed foetuses are excluded

SD Standard deviation

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APPENDIX 9

Malformations and anomalies - individual descriptions

Group 1: Control

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
	M	A	Foetus No.	Code	Description	Foetus No.	Code	Description
1	0	3	14	A	Minimal subdural haemorrhage left cerebral region (brain)	9	A	Reduced ossification of sacro-caudal vertebral arches
2	0	2	5	A	Abnormal lobation of liver	13	A	Reduced ossification of sacro-caudal vertebral arches
3	0	0	7	A	Abnormal lobation of liver	-	-	
4	0	0	-	-		-	-	
5	0	0	-	-		6	A	Reduced ossification of occipital and sacro-caudal vertebral arches
6	0	0	-	-		-	-	
7	0	0	-	-		-	-	
8	0	1	14	A	Abnormal lobation of liver	-	-	
9	0	1	-	-		8	A	Shortened 13th right rib
	0	3	-	-		7	A	Right cervical rib
			-	-		3	A	Butterfly-shaped 11th thoracic centrum
			-	-		11	A	Reduced ossification of sacro-caudal vertebral arches

M Malformation
A Anomaly

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APPENDIX 9
(Malformations and anomalies - continued)

Group 1: Control (continued)

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
	M	A	Foetus No. Code	Description	Foetus No. Code	Description
10	0	3	9 A 3 A	Intra-abdominal haemorrhage Increased dilatation left renal pelvis	6 A	Right lumbar rib
11	0	4	9 A 8 A	Subcutaneous haemorrhage mandible region Area of haemorrhage left liver lobe	15 A 3 A	Left cervical rib, bipartite 12th thoracic centrum, reduced ossification of left pubis Bipartite 4th, 11th and 13th, butterfly-shaped 12th thoracic centra, reduced ossification of cervical vertebral arches
12	0	0	-		-	
13	0	4	6 A	Abnormal lobation of liver	5 A 1 A 7 A	Minimal distortions affecting 11th and 12th left, 6th to 12th right ribs Minimal distortions affecting 11th right rib Minimal distortions affecting 11th right rib
14	0	0	-		-	
15	0	1	-		5 A	Reduced ossification of sacro-caudal vertebral arches

M Malformation
A Anomaly

APT 2/91279

APPENDIX 9
(Malformations and anomalies - continued)

Group 1: Control (continued)

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
	M	A	Foetus No. Code	Description	Foetus No. Code	Description
16	0	4	15 A	Minimal subdural haemorrhage between hypothalamus and right cerebral region (brain)	-	-
			7 A	Intra-abdominal haemorrhage		
			9 A	Increased dilatation bilateral renal pelvis		
			3 A	Increased dilatation left renal pelvis		
17	0	3	10 A	Absent innominate artery: increased dilatation of bilateral renal pelvis and ureters	1 A	Shortened 13th bilateral rib
18	0	0	-		9 A	Shortened 13th right rib
19	0	1	-		-	-
			-		5 A	Reduced ossification of interparietal and occipital
20	0	1	9 A	Thinning of diaphragm with protrusion of median liver lobe	-	-
21	0	0	-		-	-

M Malformation
A Anomaly

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APPENDIX 9
(Malformations and anomalies - continued)

Group 1: Control (continued)

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
	M	A	Foetus		Foetus	Description
			No.	Code		
23	0	1	-	-	11	A Reduced ossification of sacro-caudal vertebral arches
24	0	3	5	A	4 10	A A Right lumbar rib Reduced ossification of interparietal and right pubis
25	0	4	4	A	11 9 13	A A A Butterfly-shaped 11th thoracic centrum Bipartite 12th thoracic centrum Reduced ossification of sacro-caudal vertebral arches

M Malformation
A Anomaly

APT 2/91279

APPENDIX 9
(Malformations and anomalies - continued)

Group 2: Toluene, 250 ppm

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
	M	A	Foetus		Description	Foetus		Description
			No.	Code		No.	Code	
26	0	0	-	-	Minimal subdural haemorrhage olfactory lobes and cerebral hemispheres (brain), increased dilatation right renal pelvis	-	-	Shortened 13th left rib
27	0	1	-	-		7	A	
29	0	1	8	A		-	-	
30	0	2	-	-	Left cervical rib Small (2.24 g) with reduced ossification of sacro-caudal vertebral arches, unossified 5th metatarsals	2	A	
						6	A	
31	0	4	14	A	Bipartite 10th thoracic centrum Bipartite 11th thoracic centrum, reduced ossification of sacro-caudal vertebral arches and pubes Reduced ossification of sacro-caudal vertebral arches	5	A	
						11	A	
						13	A	
32	0	0	-	-		-	-	

Shortened 13th left rib

Left cervical rib
Small (2.24 g) with reduced ossification of sacro-caudal vertebral arches, unossified 5th metatarsals

Bipartite 10th thoracic centrum
Bipartite 11th thoracic centrum, reduced ossification of sacro-caudal vertebral arches and pubes
Reduced ossification of sacro-caudal vertebral arches

M Malformation
A Anomaly

APT 2/91279

APPENDIX 9
(Malformations and anomalies - continued)

Group 2: Toluene, 250 ppm (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
	M	A	Foetus		Description	Foetus		Description
			No.	Code		No.	Code	
33	0	4	4	A	Abnormal lobation of liver Abnormal lobation of liver	9	A	Butterfly-shaped 12th thoracic centrum Reduced ossification of cranial centres, cervical and sacro-caudal vertebral arches right ischium
		8	A	7		A		
34	0	4	5	A	Increased dilation right renal pelvis	10	A	Bilateral lumbar rib Reduced ossification of occipital, sacro-caudal vertebral arches and left pubis
				6		A		
35	0	0	-	-		4	A	Small (2.28 g) with reduced ossification of sacro-caudal vertebral arches
				-		-		
36	1	6	1	M	Left microphthalmia Subcutaneous haemorrhage nasal region Absent innominate artery	10	A	Bipartite 5th thoracic centrum Shortened 13th bilateral rib, reduced ossification of sacro-caudal vertebral arches
			6	A		14	A	
37	0	2	-	-		12	A	Shortened 13th right rib Reduced ossification of cranial centres and sacro-caudal vertebral arches
				7		A		
	0	2	-	-		1	A	Shortened 13th bilateral rib, reduced ossification of cranial centres (minimal) and sacro-caudal vertebral arches Shortened 13th right rib; one less thoracic lumbar vertebra
				7		A		

M Malformation
A Anomaly

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APPENDIX 9

(Malformations and anomalies - continued)

Group 2: Toluene, 250 ppm (continued)

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
	M	A	Foetus No.	Description	Foetus No.	Description
39	2	4	-		1	M Distortions/ossification irregularities affecting 4th to 13th right and 6th to 12th left ribs. Also reduced ossification of cranial centres (marked) cervical lumbar and sacro-caudal vertebral arches, pelvic girdle and metacarpals
					5	M Ossification irregularity affecting 9th right rib. Also reduced ossification of cranial centres, sacro-caudal vertebral arches and pelvic girdle
					13	A Minimal distortions affecting 6th and 7th bilateral ribs, reduced ossification of cranial centres and sacro-caudal vertebral arches
					7	A Reduced ossification of interparietal, occipital, sacro-caudal vertebral arches and pubes
					9	A Reduced ossification of interparietal
					3	A Reduced ossification of sacro-caudal vertebral arches

M Malformation
A Anomaly

APT 2/91279

APPENDIX 9

(Malformations and anomalies - continued)

Group 2: Toluene, 250 ppm (continued)

Litter no.	Foetuses affected		Foetus		Description		Foetus		Description	
	M	A	No.	Code			No.	Code		
40	0	5	5	A	Thinning of diaphragm with protrusion of median liver lobe	Increased dilatation left ureter	10	A	Reduced ossification of sacro-caudal vertebral arches	Reduced ossification of right ischium
			13	A			14	A	Reduced ossification of sacro-caudal vertebral arches	
							12	A	Reduced ossification of right ischium	
41	0	1	-	-		Ankyloglossia Abnormal lobation of liver	7	A	Reduced ossification of interparietal	Reduced ossification of interparietal
42	1	2	9	M			4	A	Bipartite 12th thoracic centrum	
			3	A						
43	0	1	-	-		Abnormal lobation of liver	3	A	Butterfly-shaped 11th thoracic centrum	Distortions/ossification irregularities affecting 8th to 11th right ribs. Also reduced ossification of cranial centres
44	1	3	7	A			10	M		
							6	A	Reduced ossification of sacro-caudal vertebral arches	Reduced ossification of sacro-caudal vertebral arches
45	0	0	-	-			12	A	Reduced ossification of sacro-caudal vertebral arches	
							-	-		

M Malformation
A Anomaly

APT 2/91279

APPENDIX 9
(Malformations and anomalies - continued)

Group 2: Toluene, 250 ppm (continued)

Litter no.	Foetuses affected	Allocated for visceral examination	Allocated for skeletal examination		
	M	A	Foetus	No.	Description
				Code	
46	0	3	-	-	
48	0	0	-	-	
49	0	0	-	-	
50	0	0	-	-	
			10	A	Bipartite 9th thoracic centrum
			12	A	Shortened 13th bilateral rib, reduced ossification of interparietal
			8	A	Reduced ossification of interparietal
			-	-	
			-	-	
			-	-	

M Malformation
A Anomaly

APT 2/91279

APPENDIX 9
(Malformations and anomalies - continued)

Group 3: Toluene, 750 ppm

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
			Foetus No.	Description	Foetus No.	Description
	M	A				
51	0	0	-	-	-	-
52	0	1	-	-	2	Butterfly-shaped 11th thoracic centrum
54	0	1	-	-	6	Reduced ossification of sacro-caudal vertebral arches
55	0	0	-	-	-	-
56	0	1	-	-	4	Shortened 13th right rib
57	0	5	-	-	11	Right lumbar rib
					15	Reduced ossification of cranial centres, sacro-caudal vertebral arches and pelvic girdle
					3	Reduced ossification of cervical and sacro-caudal vertebral arches
					5	Reduced ossification of sacro-caudal vertebral arches
					7	Reduced ossification of sacro-caudal vertebral arches
58	0	0	-	-	-	-

M Malformation
A Anomaly

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APPENDIX 9

(Malformations and anomalies - continued)

Group 3: Toluene, 750 ppm (continued)

Litter no.	Foetuses affected	Allocated for visceral examination			Allocated for skeletal examination		
		M	A	No. Code	Description	Foetus	Description
59	0	2	8	A	Intra-abdominal haemorrhage	3 A	Shortened 13th right rib, one less thoraco-lumbar vertebra
						5 A	Reduced ossification of cranial centres (minimal)
60	0	1	-	-		4 A	Butterfly-shaped 12th thoracic centrum, reduced ossification of interparietal and sacro-caudal vertebral arches
61	0	2	6	A	Area of haemorrhage left liver lobe	5 A	Bipartite 11th thoracic centrum
63	0	3	-	-		3 A	Bipartite 10th thoracic centrum
						1 A	Reduced ossification of sacro-caudal vertebral arches
						5 A	Reduced ossification of sacro-caudal arches
65	0	1	-	-		7 A	Reduced ossification of sacro-caudal vertebral arches
66	0	3	7	A	Increased dilatation left renal pelvis	10 A	Reduced ossification of cranial centres and sacro-caudal vertebral arches
						4 A	Reduced ossification of occipital and sacro-caudal vertebral arches
						[2]	[Break in caudal vertebra-haemorrhagic ring observed at autopsy]Ø

M Malformation

A Anomaly

Ø Observed during processing for skeletal examination therefore not included in calculation of means

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APPENDIX 9

(Malformations and anomalies - continued)

Group 3: Toluene, 750 ppm (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
	M	A	Foetus		Description	Foetus		Description
			No.	Code		No.	Code	
67	0	5	14	A	Area of haemorrhage within left liver lobe	13	A	Absent 13th right, shortened 13th left rib, one less thoraco-lumbar vertebra
			8	A	Increased dilatation bilateral renal pelvis	5	A	Shortened 13th bilateral rib
						11	A	Absent 13th right, shortened 13th left rib
68	0	0	-	-		-	-	
69	0	2	6	A	Increased dilatation right renal pelvis	3	A	Small (1.71 g) with asymmetric alignment of costal cartilage elements, bipartite 11th thoracic centrum, reduced ossification of occipital, sacro-caudal vertebral arches and pubes, unossified prespheroide and 5th metatarsals
70	0	1	13	A	Subdural haemorrhage right cerebral region, between hypothalamus and right cerebral hemisphere (brain)	-	-	
72	0	1	-	-		6	A	Shortened 13th right rib
73	0	1	-	-		5	A	Reduced ossification of interparietal
75	0	1	-	-		7	A	Shortened 13th left rib

M Malformation
A Anomaly

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APPENDIX 9

(Malformations and anomalies - continued)

Group 4: Toluene, 1500 ppm

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
			Description	Foetus No. Code	Description	Foetus No. Code
	M	A				
76	0	2	-	-	Misshapen 13th thoracic centrum Left lumbar rib	2 A 10 A
77	0	2	2	A	Butterfly-shaped 11th thoracic centrum	11 A
78	0	1	-	-	Left lumbar rib Misshapen 8th and 9th thoracic centra	2 A 10 A
80	0	1	-	-	Butterfly-shaped 11th thoracic centrum	12 A
81	0	2	4	A	Misshapen 13th thoracic centrum	1 A
82	0	4	7	A	Bipartite 12th thoracic centrum Butterfly-shaped 12th thoracic centrum	2 A 4 A
83	2	0	2	M	-	-
			8	M	-	-

M Malformation
A Anomaly

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APPENDIX 9
(Malformations and anomalies - continued)

Group 4: Toluene, 1500 ppm (continued)

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
	M	A	Foetus No.	Description	Foetus No.	Description
85	0	3	8 A	Increased dilatation left renal pelvis and ureter	13 A	Small (2.21 g) with reduced ossification of cervical vertebral arches, unossified 5th metatarsals
			6 A	Increased dilatation right renal pelvis		
86	1	2	1 A	Medial displacement of right testis	6 M	Fused occipital condyle to 1st right cervical vertebral arch, additional thoracic vertebral arch/rib between 11th and 12th right vertebral arches with additional rib fused to 11th, branched 11th left rib.
						Also bipartite 11th thoracic centrum
89	0	2	-		4 A	Right lumbar rib
					[8]	[Marked displacement of left testis]*
90	1	0	7 M	No tail seen	3 A	Shortened 13th left rib
					11 A	Reduced ossification of cranial centres and sacro-caudal vertebral arches
					-	-

M Malformation

A Anomaly

* Found during processing for skeletal examination not included in totals or means

APT 2/91279

APPENDIX 9
(Malformations and anomalies - continued)

Group 4: Toluene, 1500 ppm (continued)

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
	M	A	Foetus No.	Description	Foetus No.	Description
91	1	1	-		7	M Small (2.05 g) with termination of normal vertebral column at 3rd cervical vertebra, bilateral forelimb flexure, malrotated hind limbs, umbilical hernia. Also reduced ossification of occipital and pelvic girdle
					11	A Minimal distortions affecting 7th and 10th right, 11th bilateral ribs, reduced ossification of cranial centres and pubes
92	0	1	-		2	A Small (2.43 g) with reduced ossification of cervical vertebral arches, unossified 5th metatarsals
93	0	0	-		-	-
94	0	4	3	Anterior displacement right testis	2	A Reduced ossification of sacro-caudal vertebral arches
			5	Medial displacement left testis	4	A Reduced ossification of sacro-caudal vertebral arches

M Malformation
A Anomaly

APT 2/91279

APPENDIX 9
(Malformations and anomalies - continued)

Group 4: Toluene, 1500 ppm (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			M	A	No.	Description	Foetus No.	Code
95	0	3	-	-	-		1 7 9	A A A
96	1	2	13	M		Duplicated inferior vena cava	12 14	A A
97	0	0	-	-	-		-	-
100	0	2	7	A		Increased dilatation right renal pelvis	10	A

M Malformation
A Anomaly

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APPENDIX 9

(Malformations and anomalies - continued)

Group 5: Toluene, 3000 ppm

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
	M	A	Foetus		Description	Foetus
			No.	Code		No. Code
101	0	2	10	A	Moderate haemorrhage anterior chamber right eye	3 A [5]
102	0	0	-	-		-
103	0	1	-	-		9 A
104	0	0	-	-		[12] -
105	0	1	8	A	Marked intra-abdominal haemorrhage	-
106	1	1	-	-		9 M
108	0	0	-	-		4 A
109	0	0	-	-		-

M Malformation
A Anomaly
Ø Observed during processing for skeletal examination therefore not included in calculation of means

APT 2/91279

APPENDIX 9

(Malformations and anomalies - continued)

Group 5: Toluene, 3000 ppm (continued)

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
	M	A	Foetus No.	Description	Foetus No.	Description
110	0	2	-	-	2	A Minimal distortions affecting 11th bilateral rib
111	1	2	4	M Ankyloglossia	4	A Butterfly-shaped 12th thoracic centrum
112	0	2	12	A Small (1.70 g) with small left eye	1	A Butterfly-shaped 9th thoracic centrum
					15	A Small (2.29 g) with left lumbar rib
113	0	5	6	A Subcutaneous haemorrhage caudal region	11	A Bipartite 12th thoracic centrum, reduced ossification of sacro-caudal vertebral arches
					13	A Bipartite 12th thoracic centrum, bilateral lumbar rib
115	0	2	4	A Increased dilatation right renal pelvis	5	A Small (2.38 g) with bilateral lumbar rib, unossified 5th metatarsals
116	0	1	-	-	9	A Left lumbar rib
					11	A Left lumbar rib
					13	A Right lumbar rib
					12	A Right lumbar rib

M Malformation
A Anomaly

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APPENDIX 9
(Malformations and anomalies - continued)

Group 5: Toluene, 3000 ppm (continued)

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
	M	A	Foetus No.	Description	Foetus No.	Description
117	0	3	6 A 14 A	Increased dilatation left renal pelvis Minimal haemorrhage 4th ventricle (brain)	11 A	Unossified 5th metatarsals
118	0	1	-		10 A	Bipartite 12th thoracic centrum
119	1	1	4 M 12 A	Hydrocephaly Minimal subdural haemorrhage right cerebral region (brain)	-	
121	0	1	12 A	Thinning of diaphragm with protrusion of median liver lobe	-	
122	1	1	5 M	Small (2.18 g) with retro-oesophageal right subclavian artery; interventricular septal defect	10 A	Small (2.25 g) with reduced ossification of occipital and sacro-caudal vertebral arches, unossified 5th metatarsals
123	0	3	-		5 A 1 A 11 A	Bipartite 11th thoracic centrum Butterfly-shaped 12th thoracic centrum Butterfly-shaped 13th thoracic centrum

M Malformation
A Anomaly

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APPENDIX 9
(Malformations and anomalies - continued)

Group 5: Toluene, 3000 ppm (continued)

Litter no.	Foetuses affected		Allocated for visceral examination		Allocated for skeletal examination	
			Description		Description	
	M	A	Foetus No.	Code	Foetus No.	Code
124	0	1	-	-	2	A
125	1	7	10	M	3	A
				A	5	A
				A	7	A
				A	1	A
				A	9	A

M Malformation
A Anomaly

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APPENDIX 10

Skeletal variants - individual values

Group 1: Control

Rat no.	Foetuses examined	Foetuses with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	7	1	14.3	4	57.1	2	28.6	0	0.0	6	85.7
2	5	1	20.0	4	80.0	2	40.0	0	0.0	4	80.0
3	3	1	33.3	1	33.3	1	33.3	0	0.0	2	66.7
4	5	1	20.0	2	40.0	3	60.0	0	0.0	4	80.0
5	6	4	66.7	2	33.3	0	0.0	0	0.0	2	33.3
6	6	2	33.3	4	66.7	2	33.3	0	0.0	4	66.7
7	8	6	75.0	1	12.5	1	12.5	0	0.0	2	25.0
8	5	4	80.0	0	0.0	1	20.0	0	0.0	1	20.0
9	7	6	85.7	1	14.3	0	0.0	0	0.0	1	14.3
10	6	0	0.0	5	83.3	3	50.0	0	0.0	6	100.0
11	8	2	25.0	6	75.0	1	12.5	0	0.0	6	75.0
12	1	0	0.0	0	0.0	1	100.0	0	0.0	1	100.0
13	4	0	0.0	4	100.0	1	25.0	0	0.0	4	100.0
14	7	6	85.7	1	14.3	0	0.0	0	0.0	1	14.3
15	7	3	42.9	2	28.6	2	28.6	0	0.0	4	57.1
16	7	2	28.6	2	28.6	5	71.4	0	0.0	5	71.4
17	6	2	33.3	2	33.3	2	33.3	0	0.0	4	66.7
18	6	6	100.0	0	0.0	0	0.0	0	0.0	0	0.0
19	7	2	28.6	3	42.9	2	28.6	0	0.0	5	71.4
20	6	6	100.0	0	0.0	0	0.0	0	0.0	0	0.0
21	7	5	71.4	1	14.3	2	28.6	0	0.0	2	28.6
23	7	6	85.7	0	0.0	1	14.3	0	0.0	1	14.3
24	8	0	0.0	7	87.5	4	50.0	0	0.0	8	100.0
25	7	3	42.9	3	42.9	1	14.3	0	0.0	4	57.1
Mean		44.7		37.0		28.5		0.0		55.3	
SD		33.6		31.3		24.8		-		33.6	

Malformed foetuses are excluded
SD Standard deviation

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APPENDIX 10

(Skeletal variants - continued)

Group 2: Toluene, 250 ppm

Rat no.	Foetuses examined	Foetuses with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
26	6	6	100.0	0	0.0	0	0.0	0	0.0	0	0.0
27	7	0	0.0	1	14.3	6	85.7	0	0.0	7	100.0
29	7	1	14.3	1	14.3	5	71.4	0	0.0	6	85.7
30	6	2	33.3	4	66.7	1	16.7	0	0.0	4	66.7
31	7	1	14.3	4	57.1	4	57.1	0	0.0	6	85.7
32	5	1	20.0	2	40.0	3	60.0	0	0.0	4	80.0
33	6	1	16.7	4	66.7	1	16.7	0	0.0	5	83.3
34	5	0	0.0	5	100.0	3	60.0	0	0.0	5	100.0
35	6	4	66.7	1	16.7	1	16.7	0	0.0	2	33.3
36	7	1	14.3	4	57.1	3	42.9	0	0.0	6	85.7
37	6	1	16.7	3	50.0	4	66.7	0	0.0	5	83.3
39	5	0	0.0	3	60.0	3	60.0	0	0.0	5	100.0
40	7	0	0.0	7	100.0	3	42.9	0	0.0	7	100.0
41	6	3	50.0	1	16.7	2	33.3	0	0.0	3	50.0
42	6	1	16.7	3	50.0	5	83.3	0	0.0	5	83.3
43	5	0	0.0	5	100.0	2	40.0	0	0.0	5	100.0
44	6	1	16.7	2	33.3	4	66.7	0	0.0	5	83.3
45	6	5	83.3	0	0.0	1	16.7	0	0.0	1	16.7
46	6	2	33.3	4	66.7	2	33.3	0	0.0	4	66.7
48	6	1	16.7	2	33.3	4	66.7	0	0.0	5	83.3
49	3	1	33.3	1	33.3	1	33.3	0	0.0	2	66.7
50	7	5	71.4	0	0.0	2	28.6	0	0.0	2	28.6
Mean		28.1		44.4		45.4		0.0		71.9	
SD		28.8		31.7		23.9		-		28.8	

Malformed foetuses are excluded

SD Standard deviation

: 100 :

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APPENDIX 10

(Skeletal variants - continued)

Group 3: Toluene, 750 ppm

Rat no.	Foetuses examined	Foetuses with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
51	4	3	75.0	0	0.0	1	25.0	0	0.0	1	25.0
52	7	6	85.7	0	0.0	1	14.3	0	0.0	1	14.3
54	3	2	66.7	0	0.0	1	33.3	0	0.0	1	33.3
55	6	4	66.7	1	16.7	1	16.7	0	0.0	2	33.3
56	6	1	16.7	4	66.7	3	50.0	0	0.0	5	83.3
57	8	0	0.0	7	87.5	3	37.5	0	0.0	8	100.0
58	6	1	16.7	1	16.7	5	83.3	1	16.7	5	83.3
59	6	3	50.0	3	50.0	0	0.0	0	0.0	3	50.0
60	5	0	0.0	4	80.0	1	20.0	0	0.0	5	100.0
61	3	1	33.3	1	33.3	2	66.7	0	0.0	2	66.7
63	5	4	80.0	1	20.0	0	0.0	0	0.0	1	20.0
65	7	1	14.3	4	57.1	3	42.9	0	0.0	6	85.7
66	7	3	42.9	2	28.6	3	42.9	0	0.0	4	57.1
67	8	1	12.5	6	75.0	2	25.0	1	12.5	7	87.5
68	5	3	60.0	2	40.0	1	20.0	0	0.0	2	40.0
69	4	2	50.0	2	50.0	0	0.0	0	0.0	2	50.0
70	9	1	11.1	8	88.9	5	55.6	0	0.0	8	88.9
72	7	5	71.4	0	0.0	2	28.6	0	0.0	2	28.6
73	6	4	66.7	2	33.3	0	0.0	0	0.0	2	33.3
75	4	2	50.0	0	0.0	2	50.0	0	0.0	2	50.0
Mean		43.5		37.2		30.6		1.5		56.5	
SD		28.2		31.7		23.3		4.5		28.2	

Malformed foetuses are excluded

SD Standard deviation

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APPENDIX 10

(Skeletal variants - continued)

Group 4: Toluene, 1500 ppm

Rat no.	Foetuses examined	Foetuses with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
76	6	5	83.3	1	16.7	1	16.7	0	0.0	1	16.7
77	8	0	0.0	6	75.0	3	37.5	1	12.5	8	100.0
78	7	2	28.6	4	57.1	2	28.6	1	14.3	5	71.4
80	6	1	16.7	3	50.0	2	33.3	0	0.0	5	83.3
81	8	0	0.0	8	100.0	0	0.0	1	12.5	8	100.0
82	4	2	50.0	1	25.0	2	50.0	0	0.0	2	50.0
83	7	1	14.3	6	85.7	4	57.1	0	0.0	6	85.7
85	7	0	0.0	5	71.4	6	85.7	0	0.0	7	100.0
86	3	2	66.7	0	0.0	1	33.3	0	0.0	1	33.3
89	6	1	16.7	3	50.0	4	66.7	0	0.0	5	83.3
90	7	5	71.4	1	14.3	1	14.3	0	0.0	2	28.6
91	7	2	28.6	5	71.4	1	14.3	0	0.0	5	71.4
92	4	0	0.0	3	75.0	4	100.0	0	0.0	4	100.0
93	5	1	20.0	2	40.0	3	60.0	0	0.0	4	80.0
94	7	0	0.0	2	28.6	5	71.4	0	0.0	7	100.0
95	6	0	0.0	6	100.0	3	50.0	0	0.0	6	100.0
96	7	1	14.3	5	71.4	2	28.6	0	0.0	6	85.7
97	6	3	50.0	3	50.0	2	33.3	0	0.0	3	50.0
100	5	1	20.0	1	20.0	3	60.0	0	0.0	4	80.0
Mean		25.3		52.7		44.3		2.1		74.7	
SD		26.6		29.6		26.0		4.9		26.6	

Malformed foetuses are excluded

SD Standard deviation

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APPENDIX 10

(Skeletal variants - continued)

Group 5: Toluene, 3000 ppm

Rat no.	Foetuses examined	Foetuses with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
101	7	1	14.3	5	71.4	5	71.4	0	0.0	6	85.7
102	5	2	40.0	2	40.0	2	40.0	1	20.0	3	60.0
103	7	6	85.7	0	0.0	1	14.3	0	0.0	1	14.3
104	7	7	100.0	0	0.0	0	0.0	0	0.0	0	0.0
105	8	0	0.0	6	75.0	7	87.5	0	0.0	8	100.0
106	5	0	0.0	4	80.0	1	20.0	0	0.0	5	100.0
108	6	4	66.7	2	33.3	1	16.7	0	0.0	2	33.3
109	6	0	0.0	4	66.7	3	50.0	0	0.0	6	100.0
110	3	1	33.3	1	33.3	2	66.7	0	0.0	2	66.7
111	8	1	12.5	5	62.5	2	25.0	0	0.0	7	87.5
112	7	1	14.3	6	85.7	3	42.9	0	0.0	6	85.7
113	7	0	0.0	6	85.7	3	42.9	0	0.0	7	100.0
115	8	1	12.5	5	62.5	3	37.5	0	0.0	7	87.5
116	6	1	16.7	3	50.0	3	50.0	0	0.0	5	83.3
117	7	2	28.6	3	42.9	2	28.6	0	0.0	5	71.4
118	6	1	16.7	5	83.3	4	66.7	0	0.0	5	83.3
119	6	2	33.3	2	33.3	3	50.0	0	0.0	4	66.7
121	6	0	0.0	6	100.0	4	66.7	0	0.0	6	100.0
122	5	0	0.0	5	100.0	3	60.0	0	0.0	5	100.0
123	6	1	16.7	3	50.0	2	33.3	0	0.0	5	83.3
124	6	1	16.7	4	66.7	3	50.0	0	0.0	5	83.3
125	6	0	0.0	6	100.0	4	66.7	0	0.0	6	100.0
Mean		23.1		60.1		44.9		0.9		76.9	
SD		28.1		29.1		21.8		4.3		28.1	

Malformed foetuses are excluded

SD Standard deviation

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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.: 221277 OL.

Appearance: Colourless liquid with typical aromatic hydrocarbon odour.

Received from: BDH Ltd.

Receipt date: 16 July 1990.

Purity: 99.9% minimum assay (GLC).

Expiry date: 1 August 1995.

Storage: Flammable solvent. Store ambient temperature in the dark.

Stability: Stable for duration of study.

Stability of the Toluene batch used on the study was confirmed by infra-red spectroscopy on 28 September, 8 and 16 October and 13 November 1990.

Analysis was performed using a Pye Unicam SP3-200S infra-red spectrophotometer by liquid film spectrum (sodium chloride plates), calibrated using polystyrene film. No differences between spectra were seen.

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, onto the 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.

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ADDENDUM 1

(continued)

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 vapour generator and carried the vapour into a glass column at a flow rate of 150 l/min.

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 test atmosphere samples for analysis. Routinely an 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.

During the series of exposure the rats were alternated each day so that clinical observations of all rats were made during exposure at least once during the study.

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 l/min. The chamber pressure was set to 10 mm water gauge below ambient.

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 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.

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(continued)

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

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/l)
1 (Air control)	air only	
2 (Low dose Toluene)	250	0.94
3 (Low intermediate dose Toluene)	750	2.83
4 (High intermediate dose Toluene)	1500	5.65
5 (High dose Toluene)	3000	11.31

* ppm = (24450 x mg/l)/molecular weight (92.14) at 25°C and 760 mmHg

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}).

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

* 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.

Five batches arrived for the main study, arriving on 5 consecutive days. Due to the overlap between batches a total of 14 exposures were performed for the main study.

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(continued)

Chamber atmosphere concentration of Toluene

The vapour concentration and spatial distribution of the test atmosphere in each chamber was determined in 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.

Nominal concentrations (mg/l) were calculated from mean drip rates, drop weights (established during preliminary work) and chamber airflow.

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 absorption tubes 10 cm in length packed with approximately 2 cm Chromosorb 102, 60-80 mesh onto which the 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.

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ADDENDUM 1

(continued)

RESULTS

Chamber atmosphere conditions

Analytical levels are presented in Table 2 and are summarised as follows:

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

Batch (Exp.)	Group			
	2 (Low dose Toluene) (ppm)	3 (Low int. Toluene) (ppm)	4 (High int. Toluene) (ppm)	5 (High dose Toluene) (ppm)
A (1-10)	251	751	1513	2986
C (2-11)	252	750	1522	2987
E (3-12)	250	748	1524	3014
G (4-13)	250	748	1525	3014
I (5-14)	250	748	1524	3031
Overall mean: (1-14)	250	748	1519	3009

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

Chamber concentrations of Toluene were determined quantitatively as mg/l. Conversions to ppm values were performed using mg/l values, both individual and mean figures throughout this Addendum using the accepted conversion detailed on page 100 of this report.

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ADDENDUM 1

(continued)

Generation efficiency are presented in Table 3 and are summarised as below:

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. Toluene)	4 (High int. Toluene)	5 (High dose Toluene)
96.7	97.6	101.4	103.8

There was good agreement between analysed and nominal concentration.

Chamber temperature and relative humidity are presented in Table 4 and are summarised below:

The study mean temperature and relative humidity were as follows:

Group	T(°C)	RH(%)
1 (Air control)	20.5	40
2 (Low dose Toluene)	21.3	45
3 (Low intermediate dose Toluene)	21.3	42
4 (High intermediate dose Toluene)	21.1	43
5 (High dose Toluene)	20.3	42

Differences in mean temperatures and relative humidity between groups were small and were considered not to have influenced the outcome of the study.

Mortality

Rat 1209, Group 5 (3000 ppm) died after removal from the exposure chamber following its ninth exposure. This was an isolated incident and considered not related to treatment with Toluene.

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ADDENDUM 1

(continued)

Clinical signs during exposure (Tables 5 and 6)

Signs observed that were considered related to exposure to the vapour were confined to Groups 3 (750 ppm), 4 (1500 ppm) and 5 (3000 ppm), and are summarised below:

Clinical observation	750 ppm		1500 ppm		3000 ppm	
	N	F	N	F	N	F
Relatively non-specific indications (not considered markedly adverse):						
hunched 'aware' posture	14	1-8	14	1-8	14	2-8
eyelids closed or half-closed	12	0-5	14	1-8	14	1-8
Signs indicating irritation and partial narcosis:						
unsteady gait			10	0-2	14	1-8
hyperexploratory behaviour			13	0-8	13	0-8
lateral recumbency/uncontrolled limb movements					13	0-2
limb tremor					14	1-8
welling of tears in the eyes					14	1-8
wet chins					9	0-4
nystagmus					2	0-2

Key:

N Number of exposures noted (14 total exposure)

F Minimum to maximum number of observed animals affected on any one day (8 animals observed) [where only 4 animals observed, the number affected has been doubled]

Signs seen, common to Groups 4 and 5 were, in general, observed earlier during exposure to the higher concentration of vapour.

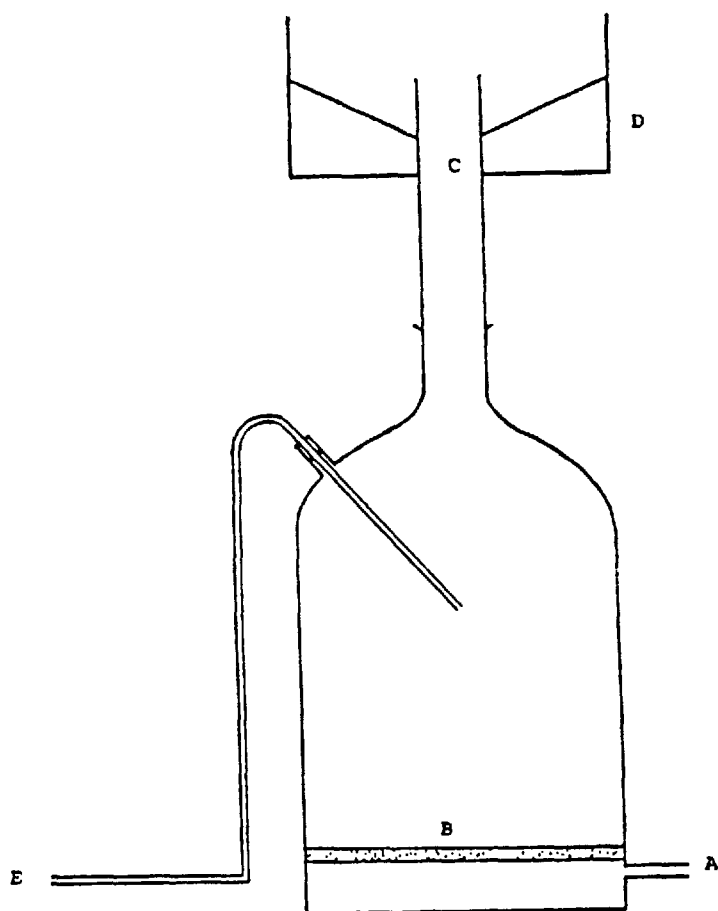
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ADDENDUM 1

(continued)

FIGURE 1

Vapour generator



Key

- A. Air line
- B. Fitted glass disc
- C. Vapour outlet
- D. Stainless steel and glass column
- E. Test substance supply line

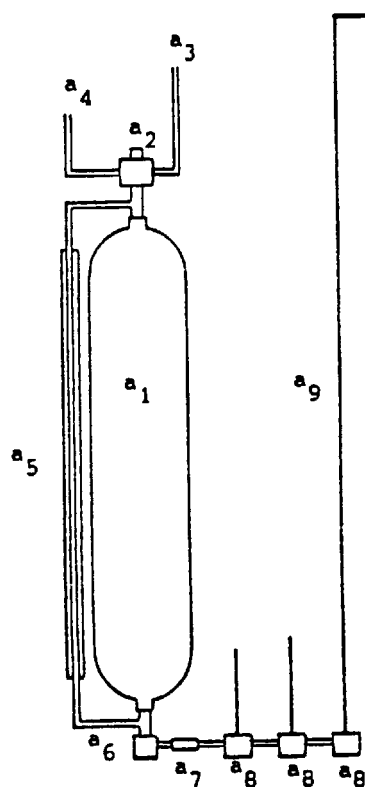
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ADDENDUM 1

(continued)

FIGURE 2

Schematic of generation system



- a1 Pressurised 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 (to vapour generator)

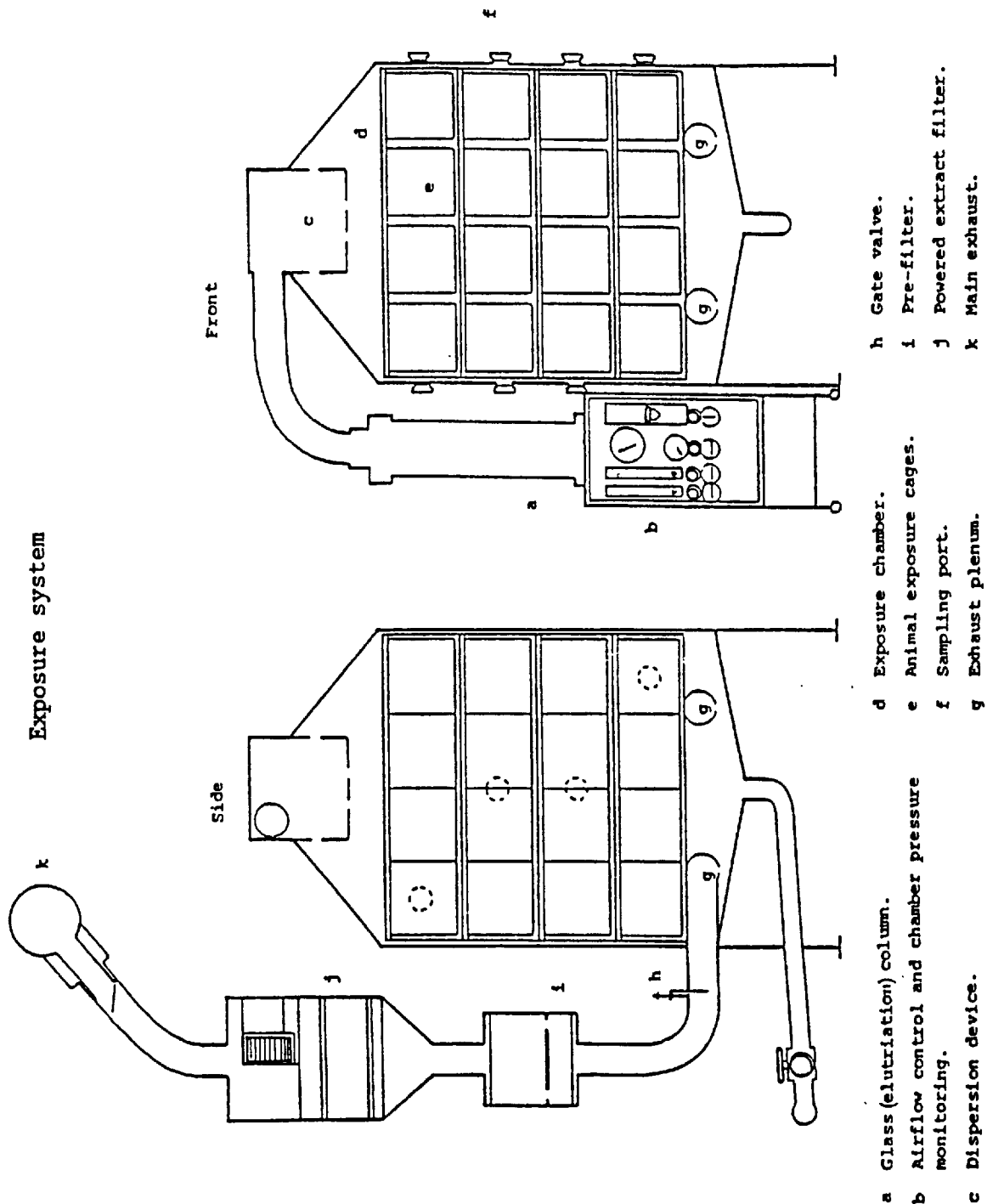
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ADDENDUM 1

(continued)

FIGURE 3

Exposure system



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ADDENDUM 1

(continued)

TABLE 1

Preliminary generation to main study
Chamber analysed concentration of Toluene

Preliminary generation no.	Preliminary sample no.	Group				
		1 (Air control)	2 (Low dose) (ppm)	3 (Low int. dose) (ppm)	4 (High int. dose) (ppm)	5 (High dose) (ppm)
1	1	ND	228	605	1425	3224
	2	ND	257	820	1592	3325
	3	ND	281	730	1510	2754
	Mean		255	718	1509	3101
	Variation %		21	30	11	18
2	7	ND	252	852	1563	2887
	8	ND	239	788	1422	3033
	9	ND	236	746	1420	3001
	10	ND	273	738	1520	2845
	Mean		249	780	1481	2943
	Variation %		15	15	10	6
3 (Spatial distribution)*	LLM	ND	231	735	1489	3099
	LL	ND	220	759	1510	3038
	RL	ND	228	762	1470	3062
	RUM	ND	223	735	1459	2956
	LUM	ND	220	751	1510	3030
	RU	ND	228	751	1510	3052
	LU	ND	239	792	1505	3012
	Mean		228	754	1494	3036
	Variation %		8	5	3	5

ND No peaks detected

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

$$\text{Variation} = (\text{Range}/\text{Mean}) \times 100\%$$

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ADDENDUM 1

(continued)

TABLE 2

Chamber analysed concentration of Toluene

Exposure no.	Sample no.	Group				
		1 (Air control)	2 (Low dose) (ppm)	3 (Low int. dose) (ppm)	4 (High int. dose) (ppm)	5 (High dose) (ppm)
1	1	ND	276	884	1467	3073
	2	ND	220	793	1422	3213
	3	ND	244	799	1513	2999
	4	ND	231	716	1481	3099
	5	ND	257	671	1515	2975
	6	ND	(204)e	724	1467	3078
	6r		241			
	Mean Variation %		244 22.8	764 27.8	1478 6.3	3073 7.8
2	7	ND	255	785	1459	2953
	8	ND	252	693	1520	2821
	9	ND	244	767	1510	3006
	10	ND	265	746	1566	2911
	11	ND	257	775	1513	3158
	12	ND	268	748	1552	2969
	Mean Variation %		257 9.3	752 12.3	1520 7.0	2969 11.3
3	13	ND	241	674	1459	3001
	14	ND	244	690	1507	2882
	15	ND	263	748	1563	2850
	16	ND	260	748	1536	2916
	17	ND	244	a	a	3014
	17r			738	1539	
	18	ND	234	780	1486	3105
	Mean Variation %		247 11.8	730 14.5	1515 6.8	2961 8.6

ND = No peaks detected

r = Repeated samples - included in mean

() = Value not included in mean

a = Value not available as 17HID taken on top of 17LID resulting in combined value

e = Sampling technique error

Variation = (Range/Mean) x 100%

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ADDENDUM 1

(continued)

TABLE 2

(continued)

Exposure no.	Sample no.	Group				
		1 (Air control)	2 (Low dose) (ppm)	3 (Low int. dose) (ppm)	4 (High int. dose) (ppm)	5 (High dose) (ppm)
4	19	ND	249	(239)*	1497	2404
	19r			762		2420b
	20	ND	236	738	1412	2805
	21	ND	265	714	1566	2330
	21r					2725b
	22	ND	247	775	1510	3500
	23	ND	247	791	1520	3458
	24	ND	244	722	1571	2884
	Mean		249	751	1513	2815
	Variation %		11.7	10.2	10.5	41.6
5	25	ND	(226)c	801	1538	3046
	25r		244			
	26	ND	273	(666)c	1502	2850
	26r			815		
	27	ND	234	(586)d	1478	2800
	27r			706		
	28	ND	236	817	1497	3097
	29	ND	268	743	(1152)e	(2331)e
	29r				(1234)f	2667
	30	ND	(141)f	846	1600	3312
	30r		(202)f			
	Mean		252	788	1523	2961
	Variation %		15.8	17.8	8.0	21.8

ND = No peaks detected

r = Repeated samples - included in mean

() = Value not included in mean

b = Repeated samples - value included in mean

c = Absorption tube interfered with by rat in chamber

d = Unconditioned sample tube used

e = Sampling technique error

f = Column loose at injector - new sealing ring fitted at end of exposure

* = Wrong chamber sampled

$$\text{Variation} = (\text{Range}/\text{Mean}) \times 100\%$$

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ADDENDUM 1

(continued)

TABLE 2

(continued)

Exposure no.	Sample no.	Group				
		1 (Air control)	2 (Low dose) (ppm)	3 (Low int. dose) (ppm)	4 (High int. dose) (ppm)	5 (High dose) (ppm)
6	31	ND	268	730	1589	3158
	32	ND	252	772	1491	3105
	33	ND	252	(708)e	1513	2916
	33r			716		
	34	ND	(228)e	(624)e	1433	2898
	34r		249	666		
	35	ND	255	772	1510	3206
	36	ND	(226)e	764	1587	3001
	36r		252			
	Mean Variation %		255 7.3	738 14.4	1520 10.3	3046 10.1
7	37	ND	244	801	1664	3293
	38	ND	279	695	1444	3288
	39	ND	247	722	1499	2945
	40	ND	245	748	1433	2935
	40r		(226)e			
	40rr		257			
	41	ND	255	711	1473	2863
	42	ND	234	788	1406	3052
	42r				1398	
	Mean Variation %		252 17.9	746 14.2	1473 18.0	3062 14.0

ND = No peaks detected

r = Repeated samples - not included in mean

rr = Re-repeated sample - value included in mean

() = Value not included in mean

e = Sampling technique error

Variation = (Range/Mean) x 100%

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ADDENDUM 1

(continued)

TABLE 2

(continued)

Exposure no.	Sample no.	Group				
		1 (Air control)	2 (Low dose) (ppm)	3 (Low int. dose) (ppm)	4 (High int. dose) (ppm)	5 (High dose) (ppm)
8	43	ND	255	788	1539	3256
	44	ND	276	730	1563	3121
	45	ND	220	748	1473	3187
	46	ND	241	724	1555	3091
	47	ND	(210)e	727	1452	2943
	47r		247			
	48	ND	244	796	1449	3171
	Mean Variation %		247 22.6	754 9.5	1505 7.6	3129 10.0
9	49	ND	239	796	1473	3235
	50	ND	268	722	1613	2741
	51	ND	231	785	1446	3229
	52	ND	226	791	1491	3057
	53	ND	263	(674)e	1566	2773
	53r			775		
	54	ND	268	714	1619	2850
	Mean Variation %		249 17.0	764 10.8	1534 11.2	2980 16.6

ND = No peaks detected

r = Repeated samples - included in mean

() = Value not included in mean

e = Sampling technique error

$$\text{Variation} = (\text{Range}/\text{Mean}) \times 100\%$$

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ADDENDUM 1

(continued)

TABLE 2

(continued)

Exposure no.	Sample no.	Group				
		1 (Air control)	2 (Low dose) (ppm)	3 (Low int. dose) (ppm)	4 (High int. dose) (ppm)	5 (High dose) (ppm)
10	55	ND	268	(695)h	1571	2701
	55r			695h		
	56	ND	257	690	1542	(2701)h
	56r					2823
	57	ND	263	732	1560	2887
	58	ND	260	732	1568	2940
	59	ND	255	732	1536	2903
	60	ND	249	727	1542	2938
Mean			260	719	1552	2866
Variation %			7.1	5.9	2.2	8.3
11	61	ND	260	791	1600	2887
	62	ND	263	788	1576	2794
	63	ND	239	788	1539	3293
	64	ND	244	788	1542	3046
	65	ND	241	687	1587	3184
	66	ND	244	695	1536	3264
Mean			249	746	1563	3078
Variation %			9.6	13.7	4.1	16.2

ND = No peaks detected

r = Repeated samples - included in mean

() = Value not included in mean

h = Initial chamber concentration low - system corrected

$$\text{Variation} = (\text{Range}/\text{Mean}) \times 100\%$$

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ADDENDUM 1

(continued)

TABLE 2

(continued)

Exposure no.	Sample no.	Group				
		1 (Air control)	2 (Low dose) (ppm)	3 (Low int. dose) (ppm)	4 (High int. dose) (ppm)	5 (High dose) (ppm)
12	67	ND	226	655	1558	3378
	68	ND	228	714	1547	3179
	69	ND	249	788	1510	3312
	70	ND	257	751	1568	3174
	71	ND	247	762	1520	3259
	72	ND	252	732	1550	3155
	Mean Variation %		244 13.0	735 18.1	1542 3.8	3243 6.9
13	73	ND	231	677	1589	2930
	74	ND	226	738	1494	3006
	75	ND	244	709	1486	2916
	76	ND	236	743	1467	2999
	77	ND	249	724	1560	2890
	78	ND	249	756	1539	2999
	Mean Variation %		239 10.0	724 11.0	1523 8.0	2956 3.9
14	79	ND	273	764	1616	3060
	80	ND	239	730	1290	3073
	81	ND	252	735	1619	2776
	82	ND	252	791	1467	2983
	83	ND	255	748	1534	2964
	84	ND	247	767	1497	3033
	Mean Variation %		252 13.7	756 8.1	1505 21.9	2983 10.0

ND = No peaks detected

$$\text{Variation} = (\text{Range}/\text{Mean}) \times 100\%$$

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ADDENDUM 1

(continued)

TABLE 2

(continued)

Batch/Study mean analysed concentrations of Toluene

Batch no.	Exposure numbers	Group			
		2 (Low dose) (ppm)	3 (Low int. dose) (ppm)	4 (High int. dose) (ppm)	5 (High dose) (ppm)
A	(1 - 10)	251	751	1513	2986
C	(2 - 11)	252	749	1522	2987
E	(3 - 12)	250	747	1524	3014
G	(4 - 13)	250	747	1525	3014
I	(5 - 14)	250	747	1524	3031
Overall	(1 - 14)	250	748	1519	3009
Target concentration		250	750	1500	3000

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ADDENDUM 1

(continued)

TABLE 3

Analysed/nominal concentration of Toluene (%)

Exposure no.	Group			
	2 (Low dose) (ppm)	3 (Low int. dose) (ppm)	4 (High int. dose) (ppm)	5 (High dose) (ppm)
1	89.3	91.4	104.3	112.9
2	100.0	99.0	107.3	102.8
3	95.9	95.8	100.2	101.6
4	96.9	98.6	100.0	96.5
5	97.9	103.5	100.7	101.5
6	99.0	96.9	100.5	104.5
7	97.9	97.9	97.4	105.0
8	95.9	99.0	99.5	107.3
9	96.9	100.4	101.4	102.2
10	101.0	94.4	102.6	98.3
11	96.9	99.3	103.3	105.6
12	94.8	96.5	101.9	111.2
13	92.8	95.1	100.7	101.4
14	97.9	99.3	99.5	102.3
Study mean	96.7	97.6	101.4	103.8

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ADDENDUM 1

(continued)

TABLE 4

Temperature and relative humidity during exposures to Toluene
(exposure mean values)

Exposure no.	Group									
	1 (Air control)		2 (Low dose)		3 (Low int. dose)		4 (High int. dose)		5 (High dose)	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
1	19.5	31	20.4	37	20.1	37	20.0	39	19.5	35
2	19.8	36	20.7	45	20.6	42	20.7	40	20.1	35
3	20.6	39	21.5	44	21.5	43	21.3	37	20.7	37
4	20.3	45	21.3	45	21.5	43	21.3	40	20.4	38
5	21.2	40	22.2	39	22.2	39	22.0	38	20.8	41
6	20.9	48	21.7	48	21.7	47	21.8	47	20.7	49
7	21.1	36	22.1	43	21.8	39	21.7	38	20.7	40
8	20.7	34	21.3	39	21.4	36	21.2	39	19.9	46
9	21.4	36	22.3	38	22.1	41	22.2	40	20.8	46
10	21.2	42	22.0	44	22.2	39	21.8	41	20.8	47
11	20.3	43	21.8	42	21.7	43	21.5	46	20.3	43
12	20.3	42	20.2	68	20.8	48	19.6	52	19.8	48
13	20.1	38	20.6	54	20.4	47	20.2	48	19.7	44
14	18.9	54	20.2	48	19.8	50	19.8	50	19.6	43
Study mean	20.5	40	21.3	45	21.3	42	21.1	43	20.3	42

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ADDENDUM 1

(continued)

TABLE 5

Clinical signs during exposure to Toluene

Group	Observation	Exposure													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 (Air control)	Nothing abnormal detected	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2 (250 ppm)	Nothing abnormal detected	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3 (750 ppm)	Hunched 'aware' posture	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Eyelids closed/half-closed	0	0	+	+	+	+	+	+	+	+	+	+	+	+
4 (1500 ppm)	Hunched 'aware' posture	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Eyelids closed/half-closed	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Uncoordinated gait/ataxia/hyperexploratory behaviour	+	+	+	+	+	+	+	0	0	+	+	+	0	0
	Hyper responsive to knock on chamber wall	+	+	+	+	+	+	+	+	+	0	+	+	+	+
5 (3000 ppm)	Hunched 'aware' posture	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Eyelids closed/half-closed	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Uncoordinated gait/ataxia/hyperexploratory behaviour	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Hyperresponsive to knock on chamber wall	+	+	+	+	0	+	+	+	+	+	+	+	+	+
	Limb tremors	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Lateral recumbency with uncontrolled limb movements	+	+	+	+	+	+	+	0	+	+	+	+	+	+
	Lachrymation (welling of tears in the eyes)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Increased respiratory rate	+	+	+	+	0	+	0	+	+	+	+	+	+	+
	Salivation (wet chin) associated with occasional rubbing of chin on grid floor of cage	0	0	0	0	+	0	+	+	+	+	+	+	+	+
	Nystagmus of the eyeball - lateral regular oscillation of the eyeball	+	+	0	0	0	0	0	0	0	0	0	0	0	0

+ Presence

0 Absence

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ADDENDUM 1

(continued)

TABLE 6

Clinical signs during exposure to Toluene - average time
of first record to last record

Group	Observation	Hours												
		0	½	1	1½	2	2½	3	3½	4	4½	5	5½	6
1 (Air control)	Nothing abnormal detected	*****												
2 (250 ppm)	Nothing abnormal detected	*****												
3 (750 ppm)	Hunched 'aware' posture Eyelids closed/half-closed	*****												
4 (1500 ppm)	Hunched 'aware' posture Eyelids closed/half-closed Uncoordinated gait/ataxia/hyperexploratory behaviour Hyperresponsive to knock on chamber wall	*****												
5 (3000 ppm)	Hunched 'aware' posture Eyelids closed/half-closed Uncoordinated gait/ataxia/hyperexploratory behaviour Hyperresponsive to knock on chamber wall Limb tremors Lateral recumbency with uncontrolled limb movements Lachrymation (welling of tears in the eyes) Increased respiratory rate Salivation (wet chin) associated with occasional rubbing of chin on grid floor of cage Nystagmus of the eyeball - lateral regular oscillation of the eyeball	*****												

* Presence

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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 (Serial no. 301301).

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: AR 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 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 onto the column.

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ADDENDUM 1

(continued)

APPENDIX 1

(continued)

The concentration of Toluene was evaluated using the external standards method. Known standard amounts of Toluene in CS₂ were injected into sample tubes. Using a Draeger pump the standard was drawn into the tube and the Toluene adsorbed on the stationary phase of Chromosorb 102. The standards were treated in the same manner as the exposure samples (see above).

The concentration 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 were accurately weighed into a volumetric flask and made up to volume with carbon disulphide. This resulted in standard solutions as follows:

6.3424 mg/ml
 15.856 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 onto the stationary phase present in the tube using a Draeger pump, and thermally desorbed onto the column of the gas chromatograph. The resulting peak areas for each standard amount injected were used to calculate the mean response factor using linear least squares.

The standard amounts injected were: 12.685, 19.027 and 31.712 μg of Toluene.

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ADDENDUM 2

Procedure for time-mating of animals

Three hundred and seventeen sexually mature Specific Pathogen Free male (12 - 13 weeks of age) and female rats (7 - 8 weeks of age) (65 males and 252 females) Crl: 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 (weight range males 386 - 429 g and females 128 - 168 g), marked on the paw by a tattoo line to indicate that they were involved in the study and 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).

Females were housed in fives and males were housed in twos 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.

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ADDENDUM 2

(continued)

During the mating period, animals were housed in plastic breeding cages (North Kent Plastics, RC-1 type), on the basis of, when mating, one male to two females. Females not being mated were gang-housed with not more than 5 to a cage, males not being mated were housed individually. Suitable nesting material was provided - see Addendum 3. One of four batches of 15 males be paired with 30 females on each night of mating. Pre-mating smears were taken from all females for seven days prior to the first mating and thereafter smears were recorded throughout the mating period where applicable. Only females that appeared to be in anticipated oestrus stage (by examination of daily smears), were mated. 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 to the Department of Inhalation Toxicology in filtered boxes. Females without a 'positive indication of mating' were returned to their original pre-mating cages. The 1 male : 2 female pairing was 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.

FIGURE 1

Day of mating procedure	Details		Batch designation of animals with a positive indication of mating
1	Batch 1♂	15♂ x 30♀	A
2	Batch 2♂	15♂ x 30♀	C
3	Batch 3♂	15♂ x 30♀	E
4	Batch 4♂	15♂ x 30♀	G
5	Batch 1♂	15♂ x 30♀	I

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ADDENDUM 2

(continued)

Allocation of animals on study

Male number	Temporary tail mark of female paired with male at mating					Group allocation (animal number) of female from mate				
	A	C	E	G	I	A	C	E	G	I
1	70*				75*	1(1)				5(124)
	72*				76*	2(26)				4(97)
2	82*				96*	3(51)				2(47)
	86*				105*	4(76)				1(22)
3	106*				116*	5(101)				4(99)
	69*				126	1(2)				
4	125*				142	2(27)				
	141*				152	3(52)				
5	145				160*					3(71)
	154*				161*	5(105)				2(46)
6	155*				166*	4(77)				3(72)
	162*				170*	5(102)				2(48)
7	167*				176*	1(3)				5(122)
	174*				213	2(28)				
8	177*				217*	3(53)				1(23)
	179*				220*	4(78)				5(123)
9	187				226*					4(100)
	189*				249*	2(31)				1(25)
10	194*				253	5(103)				
	195*				272	1(4)				
11	210*				273*	2(29)				4(98)
	215*				274*	3(54)				5(125)
12	233*				276*	4(79)				2(49)
	251*				279*	5(104)				1(21)
13	275*				280*	1(5)				3(73)
	277*				281	2(30)				
14	282*				288	3(55)				
	287*				289*	4(80)				1(24)
15	294				292*					2(50)
	300*				79*	4(81)				3(74)
16		77*					3(56)			
		85*					4(82)			
17		110*					1(6)			
		112								
18		122*					1(7)			
		144*					4(83)			
19		146*					1(8)			
		150*					5(106)			
20		101*					3(57)			
		163*					4(84)			
21		165*					3(58)			
		172*					2(32)			

* Positive indication of mating - female assigned to study

() Indicates permanent identification of animal on study

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ADDENDUM 2

(continued)

(Allocation of animals on study - continued)

Male number	Temporary tail mark of female paired with male at mating					Group allocation (animal number) of female from mate				
	A	C	E	G	I	A	C	E	G	I
22		180*					1(9)			
		181*					2(33)			
23		182*					1(10)			
		186*					5(107)			
24		201*					2(34)			
		214								
25		218*					3(59)			
		232*					5(108)			
26		242								
		244*					5(109)			
27		245								
		256*					4(85)			
28		264*					4(86)			
		278*					2(35)			
29		299*					2(36)			
		301*					5(110)			
30		312*					3(60)			
		314								
31			78*					5(112)		
			81*					3(64)		
32			90*					5(113)		
			95*					3(61)		
33			115*					4(87)		
			117							
34			119*					3(62)		
			121*					2(37)		
35			123*					2(38)		
			128*					1(11)		
36			135							
			147							
37			168*					2(39)		
			178							
38			188*					5(114)		
			190							
39			200*					1(12)		
			204*					4(88)		
40			209*					1(13)		
			216*					4(89)		
41			222							
			223*					4(90)		

* Positive indication of mating - female assigned to study

() Indicates permanent identification of animal on study

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ADDENDUM 2

(continued)

(Allocation of animals on study - continued)

Male number	Temporary tail mark of female paired with male at mating					Group allocation (animal number) of female from mate				
	A	C	E	G	I	A	C	E	G	I
42			225*					1(14)		
			247*					3(63)		
43			258							
			270*					2(40)		
44			298*					1(15)		
			305*					5(111)		
45			317*					3(65)		
			311*					5(115)		
46				67*					1(17)	
				68*					3(67)	
47				80*					2(41)	
				109*					3(68)	
48				111*					3(69)	
				118						
61				120*					4(91)	
				132*					5(116)	
50				138*					4(92)	
				149*					2(42)	
51				158*					1(16)	
				164*					3(66)	
52				169*					4(94)	
				171*					5(117)	
53				198*					3(71)	
				203*					2(43)	
54				207*					1(18)	
				219*					5(118)	
55				230*					2(44)	
				237*					5(119)	
56				241*					2(45)	
				254*					5(120)	
57				259*					1(19)	
				265*					4(93)	
58				266						
				267*					1(20)	
59				271*					3(70)	
				284*					4(95)	
60				291*					5(121)	
				297*					4(96)	

* Positive indication of mating - female assigned to study

() Indicates permanent identification of animal on study

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ADDENDUM 3

Quality assurance aspects of nesting material

The nesting material used, designated Goldchips sawdust grade 6, was produced by Biosure Ltd. The sawdust is 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 Department.

As a precautionary measure a batch of sawdust is 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 is made immediately available to HRC.

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ADDENDUM 4

Biosure Laboratory Animal Diet No. 1

Composition and quality assurance aspects of diet

Biosure LAD is a fixed formula diet suitable for normal health, growth and reproduction of laboratory rats and mice. Each batch of diet was analysed for nutrients, possible contaminants and micro-organisms, likely to be present in the diet, and which, if in excess, may have had an undesirable effect on the test system.

Prior to release of diet for use HRC Quality Assurance Department checks each certificate of analysis for conformity with the specification detailed below. Occasional slight deviations to this specification may be permitted.

<u>Nutrients</u>	<u>Target level</u>	<u>Tolerance %</u>	<u>Acceptable range</u>	
Moisture	9.5	+10	3.1	10.5 % max
Crude fat	3.7	±15	4.3	%
Crude protein	21.5	±10	19.4	23.7 %
Crude fibre	2.0	±40	1.2	2.8 %
Ash	5.5	±15	4.7	6.3 %
Calcium	1.0	±20	0.8	1.2 %
Phosphorus	0.9	±20	0.7	1.1 %
Sodium	0.3	+100-50	0.15	0.60 %
Chloride	0.5	+100-50	0.25	1.0 %
Potassium	0.8	+100-50	0.4	1.6 %
Magnesium	0.15	±50	0.08	0.23 %
Iron	220	±50	110.0	330 mg/kg
Copper	15	±50	8.0	23 mg/kg
Manganese	70	±50	35.0	105 mg/kg
Zinc	60	±50	30.0	90 mg/kg
Vitamin A	12	+50-20	9.5	18 iu/g
Vitamin E	35	+150-20	28	88 mg/kg

<u>Contaminants</u>	<u>Maximum concentration</u>
Fluoride	40 mg/kg
Nitrate (as NaNO ₃)	200 mg/kg
Nitrite (as NaNO ₂)	10 mg/kg
Lead	2.5 mg/kg
Arsenic	1.5 mg/kg
Cadmium	0.5 mg/kg
Mercury	0.1 mg/kg
Selenium	0.6 mg/kg
Total Aflatoxins	5 mcg/kg
Total P.C.B.	50 mcg/kg
Total D.D.T.	150 mcg/kg
Dieldrin	50 mcg/kg
Lindane	150 mcg/kg
Heptachlor	50 mcg/kg
Malathion	5000 mcg/kg

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ADDENDUM 4

(continued)

Microbiological contentsMaximum concentration

	LAD 1 (nuts)
Total viable organisms	10,000
Mesophilic spores	30,000
Salmonellae species	0
Presumptive E. coli	0
E. coli type 1	0
Fungal units	1,000
Antibiotic activity	0

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ADDENDUM 5

Quality assurance aspects of water

Results of the routine physical and chemical examination of drinking water at source (Grafham Final Water - Huntingdon North supply zone) as conducted usually weekly by the supplier - Anglian Water Services Ltd. - were made available to HRC as quarterly summaries.

These summaries of source water included levels of:

Nitrites		Potassium	(K)
Nitrates		Phosphorus	(P)
Calcium	(Ca)	Chlorine	(Cl)
Magnesium	(Mg)	Silicon	(Si)
Sodium	(Na)	Iron	(Fe)

Additionally, levels of substances which are known to be present occasionally in local water and which, if present at levels in excess of recommended maxima (for humans) might have had undesirable effects on the test system, were determined in HRC tap water at approximately 6-monthly intervals.

Six-monthly analyses of HRC tap water included levels of:

Arsenic	(As)	Barium	(Ba)
Selenium	(Se)	Silver	(Ag)
Antimony	(Sb)		

organophosphorus, organochlorine and other pesticides, haloforms, chlorophenols, polychlorinated biphenyls and polycyclic aromatic hydrocarbons (including benzene and toluene).

It was not considered that any concentrations of contaminations has had any detrimental effect on the study.

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ADDENDUM 6

Certificate of analysis

CERTIFICATE OF ANALYSIS

REFERENCE NO. 3471/90



CHIEF ANALYST: Dr. K. H. Schelder

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, ALCONSBURY, 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

BDH Limited

Broom Road, Poole, BH12 4NN, England.
 Telephone: National (0202) 745520 International +44 202 745520
 Telex: 41186 and 418123 TETRA G Cables: Tetradome Poole. Fax Group III (0202) 738299

Registered in England No 660457 Registered Office: Broom Road, Poole, BH12 4NN

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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 check status. These animals were killed within 24 hours of arrival.

The following macroscopic/microscopic changes were observed:

Two male rats with:	Skin: a scab (microscopic examination revealed one rat with a scab over healed skin and a second rat with fibrous thickening of dermis).
Third male rat with:	Cervical and deep cervical lymph nodes: enlarged (microscopic examination revealed cervical lymph nodes: plasmacytosis; deep cervical lymph nodes: no abnormality detected).
Fourth male rat with:	Cervical lymph nodes: enlarged (microscopic examination revealed cervical lymph nodes: lymphoid proliferation).
One female rat with:	Kidneys: increased pelvic dilatation (microscopic examination revealed kidneys: moderate renal pelvic dilatation).

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

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ADDENDUM 8

Foetal examinations - individual observations

Key:

Sternebral configuration

- U - unossified
- R - reduced ossification
- A/B - asymmetric or bipartite

Observations in [] were considered

- abnormalities too minimal to be observed and recorded consistently
- artefactual
- additional information not used for reporting purposes

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ADDENDUM 8 (continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus		Other observations								Foetus		Other observations	
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
1	1	3.11	♀						R		2	3.53	♂	[Minimal protrusion right median liver lobe]
	3	3.05	♀					U			4	3.41	♂	
	5	3.41	♂							[Subcutaneous haemorrhage right dorsal abdominal region - observed at autopsy - artefact]	6	3.50	♂	
	7	3.46	♂						R		8	3.28	♂	
	9	3.27	♂					U	U	Reduced ossification of sacro-caudal vertebral arches	10	2.83	♀	
	11	3.18	♂					U	U		12	3.22	♂	
	13	3.00	♀					U	U	Reduced ossification of sacro-caudal vertebral arches	14	2.73	♀	Minimal subdural haemorrhage left cerebral region (brain)
2	2	3.11	♀					U	R		1	2.64	♀	[Minimally abnormal lobation of liver]
	4	3.29	♂					U	R		3	3.12	♀	
	6	3.16	♀					U	U		5	3.20	♀	Abnormal lobation of liver
	8	2.90	♀					U	U		7	3.05	♀	Abnormal lobation of liver
	10	3.44	♂								9	3.42	♀	
											11	3.05	♂	[Minimally abnormal lobation of liver : damaged left forepaw]

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ADDENDUM 8 (continued)

Group 1 : Control														
Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Other observations										Foetus			Other observations
											No	wt(g)	sex	
3	1	3.35	♀								2	3.21	♀	(Minimal protrusion median liver lobe)
	3	3.15	♀								4	2.25	♀	(Small, no abnormality detected)
	5	3.28	♀								6	2.52	♀	
4	2	3.40	♀								1	3.64	♂	
	4	3.27	♀								3	2.86	♀	
	6	3.35	♂								5	3.50	♂	
	8	3.69	♂								7	3.50	♀	
	10	3.49	♀								9	3.67	♂	
											11	3.68	♂	

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APPENDUM 8 (continued)

Group 1 : Control															
Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
5	1	3.82	♂								2	3.74	♂		
	3	3.37	♀					U			4	3.60	♂		
	6	3.30	♀					U			5	3.65	♂	[Minimal subcutaneous haemorrhage right hindpaw]	
	7	3.28	♂								8	3.32	♀		
	9	3.79	♀								10	3.78	♀	[Minimally abnormal lobation of liver]	
	11	3.95	♂								12	3.74	♂		
6	2	3.28	♀					U			1	3.51	♀		
	4	3.72	♂								3	3.76	♂	[Minimally abnormal lobation of liver : minimal intra-abdominal haemorrhage]	
	6	3.48	♀					U	U		5	3.64	♀	Abnormal lobation of liver [Minimal intra-abdominal haemorrhage]	
	8	3.38	♀					U	R		7	3.68	♀		
	10	3.77	♀								9	3.65	♂		
	12	3.58	♀					U	R		11	3.56	♀		

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ADDENDUM 8
(continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination										Allocated to visceral examination		
	Foetus		Sternebral configuration							Other observations	Foetus		
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex
7	1	3.76	♂							[Minimal subcutaneous haemorrhage right hindpaw - observed at autopsy]	2	3.48	♀
	3	3.52	♀								4	3.71	♂
	5	3.50	♂								6	3.26	♀
	7	3.17	♀								8	3.34	♀
	9	2.99	♀						R	[Slightly reduced ossification of occipital]	10	3.07	♀
	11	3.42	♀								12	3.47	♀
	13	3.44	♂								14	3.28	♀
	15	3.05	♀				U						
8	2	3.40	♂								1	3.90	♂
	4	3.45	♂								3	3.56	♂
	6	3.60	♂								5	3.91	♀
	8	3.66	♂				R			Shortened 13th right rib	7	3.67	♂
	10	3.66	♂								9	3.65	♀
											11	3.38	♂

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ADDENDUM 8
(continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Fetus			Sternebral configuration							Fetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
9	1	3.83	♀								2	3.82	♂	(Minimal protrusion right median liver lobe)
	3	3.76	♂							Butterfly shaped 11th thoracic centrum	4	3.92	♂	
	5	3.57	♀								6	3.51	♂	
	7	3.63	♀					U		Right cervical rib (Slightly reduced ossification of interparietal)	8	3.54	♀	
	9	3.55	♀								10	3.59	♂	
	11	3.65	♀							Reduced ossification of sacro-caudal vertebral arches (Slightly reduced ossification of occipital)	12	3.40	♂	
	13	3.61	♀											

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ADDENDUM 8 (continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination												Allocated to visceral examination				
	Foetus			Sternebral configuration						Other observations			Foetus			Other observations	
	No	wt(g)	sex	1	2	3	4	5	6				No	wt(g)	sex		
10	2	3.84	♀					U					1	3.95	♀		
	4	3.58	♀					U	R				3	3.60	♂	Increased dilatation left renal pelvis	
	6	3.71	♂					R				Right lumbar rib	5	3.27	♂		
	8	3.27	♀					U	R			[Slightly reduced ossification of interparietal]	7	3.91	♂		
	10	3.58	♀					U					9	3.80	♂	[Moderate] intra-abdominal haemorrhage	
	12	3.83	♂					U					11	3.59	♀		
11	1	3.95	♂										2	3.81	♂		
	3	2.79	♀					U	U			Bipartite 4th, 11th and 13th, butterfly shaped 12th thoracic centra : reduced ossification of cervical vertebral arches	4	3.00	♀	[Minimal protrusion right median liver lobe]	
	5	3.61	♂										6	3.65	♂		
	7	3.13	♀					U	U				8	3.36	♀	Area of haemorrhage within left liver lobe	
	10	3.25	♂					U	U				9	3.83	♂	[Moderate] subcutaneous haemorrhage mandible region [Minimal protrusion right median liver lobe]	
	11	3.68	♂					U	U				12	3.22	♀		

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ADDENDUM 8 (continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination										Allocated to visceral examination					
	Foetus			Sternebral configuration						Other observations		Foetus			Other observations	
	No	wt(g)	sex	1	2	3	4	5	6	No	wt(g)	sex				
11(contd)	13	3.13	♀					U	R				14	3.59	♂	
	15	2.77	♀					U	U	Left cervical rib : bipartite 12th thoracic centrum; reduced ossification of left pubis						
12	2	3.78	♂						R				1	3.47	♀	
													3	4.12	♂	[Minimally abnormal lobation of liver : minimal intra-abdominal haemorrhage]
13	1	3.54	♂						U	Minimal distortions affecting 11th right rib			2	3.53	♂	[Minimally abnormal lobation of liver]
	3	3.40	♂					U	R				4	3.64	♂	
	5	3.23	♀					U	U	Minimal distortions affecting 11th and 12th left, 6th to 12th right ribs			6	3.54	♂	Abnormal lobation of liver
	7	3.35	♀					U		Minimal distortions affecting 11th right rib			8	3.46	♂	

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ADDENDUM 8
(continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Other observations										Foetus			
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	Other observations
14	2	3.82	♂								1	3.72	♀	
	4	3.58	♂							[Subcutaneous haemorrhage caudal region - observed at autopsy - artefact]	3	3.25	♀	
	6	3.71	♀					U			5	3.66	♂	
	8	3.76	♂								7	3.49	♀	[Minimally abnormal lobation of liver]
	10	3.96	♂								9	3.55	♀	
	12	3.54	♀								11	3.54	♂	
	14	3.48	♀								13	3.88	♂	
											15	3.52	♀	
15	1	3.76	♂						R		2	3.70	♀	
	3	3.73	♂							[Slightly reduced ossification of occipital]	4	3.67	♂	
	5	3.16	♂						U	Reduced ossification of sacro-caudal vertebral arches	6	3.43	♀	
	7	3.50	♀								8	3.76	♀	[Minimal haemorrhage within 3rd ventricle (brain)]
	9	3.61	♂								10	3.62	♂	[Minimal protrusion median liver lobe]

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ADDENDUM 8 (continued)

Group 1 : Control																
Litter No.	Allocated to skeletal examination											Allocated to visceral examination				
	Foetus			Sternebral configuration						Other observations	Foetus			Other observations		
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex			
15(con td)	11	3.36	♀					R					12	3.84	♂	[Minimal haemorrhage within 3rd ventricle (brain)]
	13	2.53	♀						U	[Slightly reduced ossification of interparietal]						
16	2	3.36	♀									1	3.51	♀		
	4	3.73	♀									3	2.93	♀		Increased dilatation left renal pelvis [Minimal intra-abdominal haemorrhage]
	6	3.71	♀									5	3.65	♂		
	8	2.96	♂							[Slightly misshapen 11th thoracic centrum]		7	3.33	♂		[Moderate] intra-abdominal haemorrhage [Small area of haemorrhage within right median liver lobe]
	10	3.29	♀					U	R			9	3.08	♀		Increased dilatation bilateral renal pelvis
	12	3.62	♀					R	U			11	3.46	♂		
	14	3.14	♀						R			13	3.32	♂		
												15	3.47	♂		Minimal subdural haemorrhage between hypothalamus and right cerebral hemisphere (brain)

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ADDENDUM 8 (continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration						Other observations		Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No	wt(g)	sex			
17	1	2.69	♀					U	U	Shortened 13th bilateral rib		2	3.22	♂	
	3	3.44	♂						R			4	3.58	♂	
	5	3.39	♂						R			6	3.35	♂	[Minimally abnormal lobation of liver]
	7	3.35	♀						U			8	3.52	♀	
	9	3.69	♀							Shortened 13th right rib		10	3.79	♂	Absent innominate artery: increased dilatation bilateral renal pelvis and ureter
	11	3.18	♂									12	3.29	♀	
18	2	3.52	♀									1	3.68	♀	
	4	4.04	♂									3	3.35	♀	
	6	3.89	♂									5	3.70	♂	
	8	3.74	♂									7	3.81	♀	
	10	3.58	♀									9	4.04	♂	
	12	3.37	♂									11	4.04	♂	
												13	3.31	♂	[Minimally abnormal lobation of liver]

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ADDENDUM 8
(continued)

Group 1 : Control																	
Litter No.	Allocated to skeletal examination											Allocated to visceral examination					
	Foetus			Sternebral configuration								Other observations		Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6			No	wt(g)	sex			
19	1	3.05	♀							U			2	3.31	♀	[Minimal protrusion right median liver lobe]	
	3	3.25	♂							U			4	3.03	♀		
	5	3.42	♂							U	Reduced ossification of interparietal and occipital		6	3.18	♀		
	7	3.41	♂										8	3.05	♀		
	9	3.70	♂										10	3.51	♀		
	11	3.66	♂							R			12	3.41	♀		
	13	3.27	♀							R							

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ADDENDUM 8 (continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Other observations										Foetus			
											No	wt(g)	sex	Other observations
20	2	3.78	♂								1	3.65	♂	[Minimal haemorrhage within 3rd ventricle (brain)]
	4	3.40	♂							[Slightly reduced ossification of occipital : connected sacro-caudal centrum to vertebral arch]	3	3.30	♀	
	6	3.53	♀							[Slightly reduced ossification of interparietal]	5	3.67	♂	[Minimal haemorrhage within 3rd ventricle (brain)]
	8	3.70	♂							[Damaged sternum]	7	3.64	♂	
	10	3.85	♂								9	3.58	♀	Thinning of diaphragm with protrusion of right median liver lobe
	12	3.66	♀								11	3.87	♂	[Minimally abnormal lobation of liver]
											13	3.13	♂	

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ADDENDUM 8
(continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus						Other observations				Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
21	1	3.40	♂								2	3.58	♂	
	3	3.59	♀						R		4	3.54	♀	
	5	3.54	♀								6	3.61	♂	[Damaged left forepaw]
	7	3.63	♀								8	1.61	♂	[Small with minimal intra-abdominal haemorrhage : minimal anterior displacement right testis]
	9	3.56	♀							[Slightly reduced ossification of interparietal]	10	3.57	♀	
	11	3.43	♂								12	3.45	♀	
	13	2.96	♀					U	R					

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ADDENDUM 8 (continued)

Group 1 : Control															
Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations		Foetus		Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No	wt(g)	sex			
23	1	3.70	♀							2	3.43	♀			
	3	3.82	♂							4	3.35	♀			
	5	3.11	♀						R	6	3.60	♂			
	7	3.63	♂							8	3.81	♀			
	9	3.61	♂							10	3.44	♀			
	11	3.60	♀							12	3.32	♀			
	13	3.32	♀												

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ADDENDUM 8
(continued)

Group 1 : Control																
Litter No.	Allocated to skeletal examination											Allocated to visceral examination				
	Other observations											Other observations				
	Foetus		Sternal configuration						Foetus			Foetus				
	No	wt(g)	sex	1	2	3	4	5	6	No	wt(g)	sex	No	wt(g)	sex	
24	2	3.47	♀					U	U			♀	1	3.73	♀	[Minimally abnormal lobation of liver]
	4	3.50	♀					U	R	Right lumbar rib		♀	3	3.59	♀	[Minimally abnormal lobation of liver]
	6	3.24	♀					U	U			♂	5	3.79	♂	Thinning of diaphragm with protrusion of median liver lobe
	8	3.51	♀					U		Reduced ossification of interparietal and right pubis [Slightly reduced ossification of occipital]		♂	7	3.66	♂	
	10	3.51	♂						R			♀	9	3.64	♀	
	12	3.42	♀					U	R			♂	11	3.91	♂	[Minimally abnormal lobation of liver]
	14	3.44	♀					U	U			♀	13	3.51	♀	
	16	3.15	♀					U	R			♀	15	3.41	♀	

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ADDENDUM 8 (continued)

Group 1 : Control

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus						Other observations				Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
25	1	3.39	♀								2	3.82	♂	
	3	3.62	♀						U		4	3.50	♂	Thinning of diaphragm with protrusion of median liver lobe
	5	3.53	♀					U			6	3.60	♀	
	7	3.58	♂								8	3.70	♂	
	9	3.50	♂						U	Bipartite 12th thoracic centrum	10	3.69	♂	[Damaged left forepaw]
	11	3.84	♀							Butterfly shaped 11th thoracic centrum	12	3.04	♀	
	13	3.60	♂						R	Reduced ossification of sacro-caudal vertebral arches	14	3.53	♀	

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ADDENDUM 8
(continued)

Group 2 : Toluene, 250 ppm

Group 2 : Iouene,250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration						Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
26	2	3.50	♂								1	3.61	♀	
	4	3.69	♂								3	3.40	♀	
	6	3.57	♀								5	3.61	♀	
	8	3.59	♂								7	3.33	♀	
	10	3.28	♀								9	3.41	♀	
	12	4.02	♂								11	3.30	♀	
											13	3.51	♀	
27	1	3.47	♂							R	2	3.28	♂	
	4	3.57	♂					R	R		3	3.23	♀	[Minimal subcutaneous haemorrhage right ventral cervical region]
	5	3.66	♂							R	6	3.45	♂	
	7	3.41	♂							R	8	3.22	♀	
	9	3.23	♀					U			10	3.44	♂	
	11	3.68	♂							R	12	3.40	♀	
	13	3.20	♂							R				

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ADDENDUM 8 (continued)

Group 2 : Toluene, 250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
29	1	3.51	♂								2	3.04	♂	[Minimally abnormal lobation of liver]	
	3	3.12	♂							R	4	3.13	♀		
	5	3.04	♀					U			6	3.03	♂		
	7	2.74	♂							R	8	3.06	♀	Minimal subdural haemorrhage olfactory lobes and cerebral hemispheres (brain) : increased dilatation right renal pelvis	
	9	3.19	♂							R	10	3.07	♀		
	11	3.09	♀							R	12	2.90	♀		
	13	2.93	♂							R					
30	2	2.61	♀					U	U		1	3.34	♀	[Minimally abnormal lobation of liver]	
	4	3.33	♀					U	R		3	3.24	♀		
	6	2.24	♀					U	U		5	3.38	♂		
	8	3.35	♂								7	3.23	♂		
	10	3.33	♀					U			9	3.45	♂	[Damaged right testis]	
	12	3.32	♀								11	3.13	♀		
											13	2.46	♀	[Small, no abnormality detected]	

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ADDENDUM 8
(continued)

Group 2 : Toluene, 250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
31	1	3.20	♀								2	3.21	♂	[Minimally abnormal lobation of liver]	
	3	2.95	♀					U	R		4	2.90	♀		
	5	2.88	♂						R	Bipartite 10th thoracic centrum	6	2.71	♀		
	7	3.03	♀						R		8	2.75	♀	[Minimally abnormal lobation of liver]	
	9	3.06	♀					U	R		10	3.28	♂		
	11	3.51	♂				U	U		Bipartite 11th thoracic centrum : reduced ossification of sacro-caudal vertebral arches and pubes. [Slightly reduced ossification of occipital]	12	3.02	♂	[Minimally abnormal lobation of liver]	
	13	2.93	♀					U	U	Reduced ossification of sacro-caudal vertebral arches	14	2.93	♂	Reduced left side of thyroid [Minimally abnormal lobation of liver]	
32	2	3.57	♂								1	2.93	♀		
	4	3.37	♂						R		3	3.01	♀		
	6	3.33	♂						U		5	3.47	♂		
	8	3.30	♂					U	R	[Slightly reduced ossification of occipital]	7	3.30	♂		
	10	3.16	♂						R		9	3.23	♂		
											11	3.32	♂		

Group 2 : Toluene, 250 ppm

group 2 : toluene, 250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Sternebral configuration										Other observations	Foetus			Other observations
	Foetus		Sternebral configuration												
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
33	1	3.83	♂					R	R		2	3.52	♂	[Minimal protrusion median liver lobe]	
	3	3.06	♀					U	U		4	3.55	♂	Abnormal lobation of liver	
	5	3.42	♀						U		6	3.82	♀		
	7	3.50	♀						U	Reduced ossification of cranial centres, cervical and sacro-caudal vertebral arches, right ischium	8	3.58	♂	Abnormal lobation of liver	
	9	3.56	♂						U	Butterfly shaped 12th thoracic centrum	10	3.76	♂		
	11	3.83	♀							[Connected sacro-caudal centrum to vertebral arch]	12	3.39	♀	[Minimally abnormal lobation of liver]	

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ADDENDUM 8
(continued)

Group 2 : Toluene, 250 ppm														
Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus						Other observations				Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
34	2	3.55	♂					U			1	3.42	♀	
	4	2.28	♀				R	U	U	Small with reduced ossification of sacro-caudal vertebral arches	3	3.58	♂	
	6	3.08	♂		R	R		U	U	Reduced ossification of occipital, sacro-caudal vertebral arches and left pubis [Slightly reduced ossification of Interparietal]	5	3.48	♂	Increased dilatation right renal pelvis
	8	2.85	♀					U	R		7	3.38	♀	
	10	2.77	♀					U	U	Bilateral lumbar rib	9	3.42	♂	
											11	2.86	♀	
35	1	3.75	♂								2	3.63	♂	
	3	3.66	♂								4	3.47	♀	
	5	3.64	♀								6	3.80	♂	
	7	3.57	♀					U			8	3.91	♂	(Minimally abnormal lobation of liver)
	9	3.78	♂								10	3.94	♂	
	11	3.53	♂						R		12	3.39	♂	

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ADDENDUM 8 (continued)

Group 2 : Toluene, 250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination		
	Foetus						Other observations				Foetus		Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex
36	2	3.27	♂							[Slightly reduced ossification of interparietal]	1	2.89	♀
	4	2.61	♀					U	U		3	2.89	♂
	7	2.59	♂		R			U	U	Reduced ossification of cranial centres and sacro-caudal vertebral arches [Reduced ossification of 2nd metacarpals and 5th metatarsals]	5	2.60	♂
	8	2.99	♀						R	[Slightly reduced ossification of interparietal]	6	2.72	♀
	10	2.60	♀					U	U	Bipartite 5th thoracic centrum	9	2.65	♀
	12	3.01	♂		R					Shortened 13th right rib	11	3.10	♂
	14	2.61	♀					U	U	Shortened 13th bilateral rib : reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal and occipital]	13	2.96	♂

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ADDENDUM 8 (continued)

Group 2 : Toluene, 250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination		
	Foetus		Sternebral configuration						Other observations		Foetus		
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex
37	1	3.98	♂							Shortened 13th bilateral rib : reduced ossification of cranial centres (minimal) and sacro-caudal vertebral arches [Minimally asymmetric pelvic girdle]	2	3.57	♀
	3	3.77	♀					R	U	[Slightly reduced ossification of interparietal]	4	3.71	♀
	5	3.89	♂						R		6	3.56	♀
	7	3.96	♂					U	R	Shortened 13th right rib : one less [18] thoraco-lumbar vertebra	8	4.07	♂
	9	3.88	♂						U	[Slightly reduced ossification of interparietal and occipital]	10	3.59	♀
	11	3.59	♀						R				

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ADDENDUM 8 (continued)

Group 2 : Toluene, 250 ppm													
Litter No.	Allocated to skeletal examination										Allocated to visceral examination		
	Foetus		Other observations								Foetus		Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex
39	1	3.79	♂		R	R	R			Distortions/ossification irregularities affecting 4th to 13th right and 6th to 12th left ribs. Also reduced ossification of cranial centres (marked), cervical, lumbar and sacro-caudal vertebral arches, pelvic girdle and metacarpals	2	3.47	♀
	3	3.48	♂						R	Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal and occipital]	4	3.36	♀
	5	3.19	♀				U		R	Ossification irregularity affecting 9th right rib. Also reduced ossification of cranial centres, sacro-caudal vertebral arches and pelvic girdle	6	3.38	♂
	7	2.95	♂						U	Reduced ossification of interparietal, occipital sacro-caudal vertebral arches and pubes.	8	3.68	♂
	9	3.47	♀					U		Reduced ossification of interparietal [Slightly reduced ossification of occipital]	10	3.29	♀
	11	3.50	♂					U			12	3.35	♂
	13	3.22	♀						R	Minimal distortions affecting 6th and 7th bilateral ribs : reduced ossification of cranial centres and sacro-caudal vertebral arches			

ADDENDUM 8

(continued)

Group 2 : Toluene, 250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Other observations										Foetus			
	No	wt(g)	sex	1	2	3	4	5	6	Other observations	No	wt(g)	sex	Other observations
40	2	3.34	♂					U	R		1	3.67	♂	
	4	3.45	♂					U	U		3	3.26	♀	
	6	3.33	♂					U	U		5	3.19	♀	Thinning of diaphragm with protrusion of right median liver lobe [Damaged right kidney]
	8	3.34	♂						U		7	2.55	♀	
	10	3.33	♀					U	R	Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of occipital]	9	3.37	♂	
	12	3.52	♂					U	U	Reduced ossification of right ischium [Slightly reduced ossification of interparietal]	11	3.42	♀	
	14	2.98	♀				R	U	U	Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal]	13	3.47	♀	Increased dilatation left ureter
											15	2.83	♀	
41	1	3.15	♀						R		2	3.36	♂	[Damaged right forepaw]
	3	3.43	♂								4	3.39	♂	[Damaged right testis]
	5	3.37	♂						R		6	3.29	♀	
	7	3.12	♀						U	Reduced ossification of interparietal	8	3.30	♂	
	9	3.38	♂								10	3.26	♂	
	11	3.52	♂								12	3.30	♂	

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ADDENDUM 8 (continued)

Group 2 : Toluene, 250 ppm

Group 2 : Torusae, 250 ppt																	
Litter No.	Allocated to skeletal examination										Allocated to visceral examination						
	Foetus			Sternebral configuration			Other observations				Foetus			Other observations			
	No	wt(g)	sex	1	2	3	4	5	6	No	wt(g)	sex					
42	2	3.87	♂					U	R				1	3.92	♂		
	4	3.82	♂							Bipartite 12th thoracic centrum				3	3.67	♂	Abnormal lobation of liver
	6	3.40	♂					U	R				5	3.60	♂		
	8	3.41	♀					U	R				7	3.58	♂		
	10	3.52	♀						R				9	3.10	♀	Ankyloglossia [tip of tongue connected to base of buccal cavity]	
	12	3.40	♀						R				11	3.58	♀	[Minimal protrusion of right median liver lobe]	
													13	3.19	♀		
43	1	2.90	♂					U	R				2	3.09	♀		
	3	2.92	♀					U	U	Butterfly shaped 11th thoracic centrum				4	3.31	♂	
	5	2.99	♀					U	U				6	3.15	♀		
	7	2.80	♀					U	U				8	3.29	♀		
	9	3.30	♀					U	R								

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ADDENDUM 8 (continued)

Group 2 : Toluene, 250 ppm

Group 2 : Rotenone, 250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration						Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
44	2	3.07	♀						U		1	3.43	♀	
	4	3.33	♂						R		3	2.93	♀	
	6	3.17	♂		R				U	Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal and occipital]	5	3.57	♀	(Minimally abnormal lobation of liver)
	8	3.49	♂								7	3.46	♂	Abnormal lobation of liver
	10	3.32	♀					R	R	Distortions/ossification irregularities affecting 8th to 11th right ribs. Also reduced ossification of cranial centres. [Reduced ossification of 2nd metacarpals]	9	3.43	♂	
	12	3.35	♀						R	Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal and occipital]	11	3.35	♂	
	14	3.10	♀						R		13	3.17	♂	
											15	2.87	♀	

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Group 2 : Female, 250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Fetus			Sternebral configuration						Other observations	Fetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
45	1	3.68	♂							R	2	3.45	♀	[Minimal intra-abdominal haemorrhage]
	3	3.50	♂								4	3.80	♂	
	5	3.28	♀								6	3.48	♂	
	7	3.37	♀								8	3.25	♂	
	9	3.64	♂								10	3.65	♀	
	11	3.90	♂								12	3.27	♂	
46	2	3.67	♀								1	3.78	♂	
	4	3.95	♀								3	3.41	♀	
	6	3.52	♀					U			5	3.71	♂	
	8	3.64	♀					U			7	3.71	♀	
	10	3.28	♂					U			9	3.43	♀	[Minimally abnormal lobation of liver]
	12	3.61	♀					U			11	3.16	♀	[Minimal protrusion of median liver lobe]
											13	3.82	♂	[Minimally abnormal lobation of liver]

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ADDENDUM 8
(continued)

Group 2 : Toluene, 250 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
48	2	2.99	♀				R	U	U		1	3.48	♀		
	4	2.53	♀					U	U		3	3.25	♀		
	6	2.98	♂						R		5	3.15	♀		
	8	3.28	♀								7	3.15	♀		
	10	3.45	♀						R		9	3.08	♂		
	12	3.50	♂					R			11	2.71	♀		
											13	2.56	♂	[Minimal subcutaneous haemorrhage nasal region]	
49	1	3.11	♀						U		2	3.46	♂		
	3	3.13	♂						R	[Slightly reduced ossification of interparietal]	4	3.47	♀	[Minimal protrusion right median liver lobe]	
	5	3.52	♂								6	3.70	♂	[Minimal protrusion right median liver lobe; minimally abnormal lobation of liver; damaged left forepaw]	

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ADDENDUM 8 (continued)

Group 2 : Toluene, 250 ppm

group 2 : toluene, 250 ppm															
Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
50	2	3.06	♀								1	3.04	♀	[Minimal protrusion right median liver lobe]	
	4	3.36	♀								3	3.02	♂	[Damaged left kidney]	
	6	3.47	♀								5	3.15	♀		
	8	3.00	♂						R		7	3.28	♂	[Minimally abnormal lobation of liver]	
	10	3.63	♀								9	3.32	♂		
	12	3.44	♂								11	3.32	♂		
	14	3.14	♀						R		13	3.25	♀		

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ADDENDUM 8
(continued)

Group 3 : Toluene, 750 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration						Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
51	1	3.83	♂								2	3.98	♂	
	3	3.47	♀								4	3.54	♀	
	5	3.63	♀								6	3.13	♀	
	7	3.72	♂					R			8	3.93	♂	
52	2	3.61	♀							Butterfly-shaped 11th thoracic centrum	1	3.58	♀	[Minimally abnormal lobation of liver]
	4	3.89	♂								3	3.50	♀	
	6	3.84	♂								5	3.49	♀	
	8	3.50	♀								7	3.56	♀	
	10	3.89	♂								9	3.68	♀	[Damaged left forelimb]
	12	3.74	♂								11	4.05	♂	[Minimally abnormal lobation of liver]
	14	3.71	♂						R	{Minimal subcutaneous haemorrhage left hindpaw - observed at autopsy - artefact}	13	3.60	♂	[Minimally abnormal lobation of liver]
											15	3.75	♂	

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ADDENDUM 8 (continued)

Group 3 : Toluene, 750 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration						Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
54	2	4.00	♀							[Slightly reduced ossification of interparietal]	1	3.73	♂	[Minimally abnormal lobation of liver]
	4	3.96	♀								3	3.72	♀	
	6	3.75	♀			R				Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal and occipital]	5	3.95	♀	[Minimal intra-abdominal haemorrhage]
											7	3.96	♀	
55	1	3.31	♀								2	3.48	♂	
	3	3.25	♀						R		4	3.23	♀	
	5	3.38	♂								6	3.32	♂	
	7	3.16	♀								8	3.21	♀	[Minimally abnormal lobation of liver]
	9	3.31	♂					U			10	2.95	♂	
	11	3.58	♂								12	3.09	♂	[Damaged right forepaw]

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ADDENDUM 8

(continued)

Group 3 : Toluene, 750 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration						Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
56	2	3.48	♀					U	R		1	3.81	♂	
	4	3.14	♀					U		Shortened 13th right rib	3	3.72	♂	
	6	3.59	♀								5	3.42	♀	[Minimal subcutaneous haemorrhage left pinna]
	8	3.62	♂					U	R		7	3.61	♀	
	10	3.53	♂					R			9	3.28	♀	
	12	3.11	♀					U			11	3.48	♀	
											13	3.23	♀	[Minimal subcutaneous haemorrhage cranium : minimally abnormal lobation of liver]

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ADDENDUM 8 (continued)

Group 3 : Toluene, 750 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination						
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations		
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex				
57	1	3.56	♂							U			2	3.24	♀		
	3	3.01	♀					U	U		Reduced ossification of cervical and sacro-caudal vertebral arches		4	3.55	♂		
	5	3.51	♂						U		Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal]		6	3.20	♀		
	7	3.10	♀					U	U		Reduced ossification of sacro-caudal vertebral arches		8	3.36	♂		
	9	3.23	♀					U	R				10	3.36	♀	[Minimally abnormal lobation of liver]	
	11	3.19	♂						U		Right lumbar rib		12	3.44	♂		
	13	3.41	♀					R					14	2.69	♀		
	15	3.17	♀		R			U	U		Reduced ossification of cranial centres, sacro-caudal vertebral arches and pelvic girdle						

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ADDENDUM 8

(continued)

Group 3 : Toluene, 750 ppm

Allocated to skeletal examination															Allocated to visceral examination		
Litter No.	Foetus			Sternebral configuration						Other observations	Foetus			Other observations			
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex				
58	2	3.37	♀							1	3.20	♀					
	4	3.05	♀					R		3	3.26	♀					
	6	2.89	♀						R	5	3.16	♀					
	8	3.12	♂						R	7	2.76	♀					
	10	3.23	♂				A	U	R	9	3.31	♀					
	12	3.15	♀						R	11	3.15	♀					
										13	3.18	♂					
59	1	3.78	♀					U		2	3.72	♂		[Minimally abnormal lobation of liver]			
	3	3.90	♂							4	3.49	♀					
	5	3.61	♀					U		6	3.56	♂					
	7	3.93	♂							8	3.68	♀		[Moderate] intra-abdominal haemorrhage			
	9	3.28	♀					U		10	3.64	♀		[Minimal intra-abdominal haemorrhage]			
	11	3.82	♂							12	3.57	♂		[Minimally abnormal lobation of liver]			

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ADDENDUM 8 (continued)

Group 3 : Toluene, 750 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration						Other observations		Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex		
60	2	3.23	♂						U	[Slightly reduced ossification of interparietal]	1	3.08	♀		
	4	3.10	♂						U	Butterfly shaped 12th thoracic centrum : reduced ossification of interparietal and sacro-caudal vertebral arches [Slightly reduced ossification of occipital]	3	3.07	♀		[Minimally abnormal lobation of liver]
	6	2.90	♀						R	[Damaged vertebral column]	5	3.36	♂		
	8	3.20	♂						U		7	3.20	♂		[Minimally abnormal lobation of liver : damaged right testis and left forelimb]
	10	2.90	♂					U	U		9	3.30	♂		
											11	3.19	♂		
61	1	3.50	♀					U	R	[Slightly misshapen 11th thoracic centrum]	2	4.21	♂		[Minimal intra-abdominal haemorrhage]
	3	3.80	♀								4	4.06	♂		
	5	3.85	♀					R		Bipartite 11th thoracic centrum [Damaged mandibles]	6	3.68	♀		Area of haemorrhage within left liver lobe

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ADDENDUM 8

(continued)

Group 3 : Toluene, 750 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination							
	Foetus			Sternebral configuration						Other observations		Foetus			Other observations			
	No	wt(g)	sex	1	2	3	4	5	6	No	wt(g)	sex						
63	1	3.58	♀							Reduced ossification of sacro-caudal vertebral arches					2	3.63	♂	
	3	3.52	♂							Bipartite 10th thoracic centrum					4	3.70	♀	
	5	3.34	♀					U	U	Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of right parietal and interparietal]					6	3.70	♂	(Minimal protrusion right median liver lobe)
	7	3.41	♀												8	3.71	♂	
	9	3.58	♂															
65	1	3.55	♂												2	3.44	♂	
	3	3.35	♂					U							4	3.50	♂	
	5	3.34	♂					U	R						6	2.98	♀	
	7	3.40	♂						R	Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal]					8	3.41	♂	
	9	3.35	♀					U	U						10	3.54	♀	
	11	3.37	♂						R						12	3.50	♂	
	13	2.85	♂					U	U									

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ADDENDUM 8

(continued)

Group 3 : Toluene, 750 ppm														
Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus		Other observations								Foetus		Other observations	
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
66	2	3.18	♂					U	R	[Subcutaneous haemorrhagic ring mid-caudal region - observed at autopsy]	1	3.37	♂	
	4	3.20	♂							Reduced ossification of occipital and sacro-caudal vertebral arches [Slightly reduced ossification of interparietal]	3	3.21	♀	
	6	3.60	♂								5	3.37	♀	
	8	3.37	♂								7	3.46	♂	Increased dilatation left renal pelvis
	10	2.76	♀					U	U	Reduced ossification of cranial centres and sacro-caudal vertebral arches	9	3.19	♂	[Minimally abnormal lobation of liver]
	12	3.30	♀					R	R		11	3.22	♂	
	14	3.48	♂						R	[Slightly reduced ossification of interparietal and occipital]	13	3.35	♀	
											15	3.03	♀	

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ADDENDUM 8
(continued)

Group 3 : Toluene, 750 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination		
	Foetus					Sternebral configuration					Foetus		
	No	wt(g)	sex	1	2	3	4	5	6	Other observations	No	wt(g)	sex
67	1	3.29	♂					R	B		2	3.28	♀
	3	3.15	♀					U	R		4	3.25	♀
	5	3.27	♂					U		Shortened 13th bilateral rib	6	3.27	♀
	7	3.18	♀					U			8	2.62	♀
	9	3.05	♀					U			10	3.46	♂
	11	3.54	♂							Absent 13th right, shortened 13th left rib	12	3.10	♀
	13	3.19	♀					U		Absent 13th right, shortened 13th left rib : one less [18] thoraco-lumbar vertebra	14	3.32	♀
	15	3.23	♂					U					
68	2	3.48	♂								1	3.39	♂
	4	3.19	♀					U			3	3.37	♂
	6	3.14	♂								5	3.47	♂
	8	3.15	♂					U	R		7	3.05	♂
	10	3.31	♀								9	3.37	♀

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ADDENDUM 8 (continued)

Group 3 : Toluene, 750 ppm

Group 3 : 10mg/kg, 750 ppm															
Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
69	1	3.08	♀								[Slightly misshapen 10th thoracic centrum]	2	2.82	♀	
	3	1.71	♂	U	U	U	U	U	U		Small with asymmetric alignment of costal cartilage elements : bipartite 11th thoracic centrum : reduced ossification of occipital, sacro-caudal vertebral arches and pubes : unossified presphenoid and 5th metacarpals.	4	3.13	♀	
	5	3.11	♀					U				6	2.85	♀	Increased dilatation right renal pelvis
	7	3.21	♂									8	3.33	♀	

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ADDENDUM 8 (continued)

Group 3 : Toluene, 750 ppm														
Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Other observations							Foetus			Other observations
	No	wt(g)	sex	Sternebral configuration						No	wt(g)	sex		
				1	2	3	4	5	6					
70	2	3.52	♂					U	R	1	3.57	♀	[Minimal intra-abdominal haemorrhage]	
	4	3.46	♀					U	R	3	3.31	♀		
	6	3.56	♀					U		5	2.86	♀	[Minimally abnormal lobation of liver]	
	8	3.56	♂					U	R	7	3.29	♀	[Minimally abnormal lobation of liver]	
	10	3.55	♀					U		9	3.23	♀		
	12	3.16	♀					U		11	2.99	♀		
	14	3.60	♂							13	3.67	♂	[Moderate] subdural haemorrhage right cerebral region and between hypothalamus and right cerebral hemisphere (brain) [Minimal intra-abdominal haemorrhage]	
	16	3.13	♀							15	3.29	♀		
	18	3.21	♀							17	3.27	♂		

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ADDENDUM 8

(continued)

Group 3 : Toluene, 750 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination		
	Other observations										Other observations		
	Sternebral configuration										Foetus		
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex
72	2	3.48	♀								1	3.79	♂
	4	3.77	♀								3	3.36	♀
	6	3.64	♀						R	Shortened 13th right rib	5	4.02	♂
	8	3.59	♀					R	R		7	3.36	♀
	10	3.53	♂								9	3.47	♂
	12	3.78	♂								11	3.67	♀
	14	3.38	♀								13	3.82	♀
73	1	3.84	♂								2	3.58	♂
	3	3.89	♀							[Slightly reduced ossification of interparietal]	4	3.74	♂
	5	3.50	♂							Reduced ossification of interparietal	6	3.40	♀
	7	3.52	♀						U		8	3.34	♂
	9	3.49	♀								10	3.41	♀
	11	3.40	♂						U	[Slightly reduced ossification of interparietal]	12	3.26	♀
													[Minimal subcutaneous haemorrhage cranium]

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ADDENDUM 8

(continued)

Group 3 : Toluene, 750 ppm

group 3 : Iomene, 750 ppm															
Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration						Other observations	Foetus			Other observations	
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex		
75	1	3.45	♂							R		2	3.73	♂	
	3	3.97	♀									4	4.05	♀	[Damaged right kidney]
	5	3.90	♀									6	3.30	♀	
	7	3.73	♂					R			Shortened 13th left rib [Minimally asymmetric pelvic girdle]				

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ADDENDUM 8 (continued)

Group 4 : Toluene, 1500 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration							Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
76	2	3.43	♂							Misshapen 13th thoracic centrum	1	3.77	♀	
	4	3.83	♂								3	3.47	♂	
	6	3.37	♀								5	3.28	♀	
	8	3.71	♂								7	3.36	♀	
	10	3.43	♀							Left lumbar rib	9	3.90	♂	
	12	3.38	♀					U	R		11	3.25	♀	(Minimally abnormal lobation of liver)
											13	3.35	♂	
77	1	3.46	♀					U			2	3.74	♂	Small interventricular septal defect
	3	3.18	♂					U	R		4	3.64	♂	
	5	3.28	♀					U		[Slightly reduced ossification of interparietal]	6	3.10	♀	
	7	3.34	♂					U			8	3.10	♀	
	9	3.53	♀					B	R		10	3.67	♂	[Minimal haemorrhage within 3rd ventricle (brain) and abdominal cavity]
	11	3.21	♀					R	R	Butterfly shaped 11th thoracic centrum	12	3.37	♀	[Minimal haemorrhage within 3rd ventricle (brain) and abdominal cavity]
	13	3.44	♀					U			14	4.00	♂	
	15	3.37	♀					U	U	[Slightly misshapen 11th thoracic centrum]				

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ADDENDUM 8 (continued)

Group 4 : Toluene, 1500 ppm

Litter No.	Allocated to skeletal examination											Allocated to visceral examination		
	Foetus			Other observations								Foetus		
	No	wt(g)	sex	1	2	3	4	5	6	Sternebral configuration		No	wt(g)	sex
78	2	3.56	♂						U			1	3.56	♀
	4	3.69	♂						R			3	3.47	♀
	6	3.30	♀					B	U			5	3.63	♀
	8	3.49	♀					U	U			7	3.22	♀
	10	3.52	♀									9	3.52	♂
	12	3.49	♀									11	3.41	♀
	14	3.21	♀					R	U			13	3.69	♂
80	2	3.28	♀					U				1	3.25	♂
	4	3.37	♀					R				3	3.60	♂
	6	3.43	♀									5	3.36	♀
	8	3.33	♀					U	U			7	3.34	♂
	10	3.23	♀					R	R			9	3.36	♀
	12	2.97	♂					U	U			11	3.11	♀

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ADDENDUM 8
(continued)

Group 4 : Toluene, 1500 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination							
	Focius			Sternebral configuration			Other observations				Focius			Other observations				
	No	wt(g)	sex	1	2	3	4	5	6					No	wt(g)	sex		
81	1	2.54	♂				A	U	U	Misshapen 13th thoracic centrum				2	3.12	♂	[Minimal anterior displacement right testis]	
	3	2.80	♀					U	U					4	1.98	♀	Small with minimal subdural haemorrhage left cerebral region (brain) [Diffuse subcutaneous haemorrhage cranium]	
	5	3.30	♀						U					6	3.37	♂		
	7	2.86	♀					U	U					8	2.88	♀		
	9	2.74	♀					U	U					10	2.91	♂		
	11	2.87	♂						U					12	2.70	♀		
	13	3.08	♀					U						14	2.96	♂	[Damaged left forelimb]	
	15	3.11	♀					U	U					16	2.61	♂		
82	2	3.33	♀					R	R	Bipartite 12th thoracic centrum [Subcutaneous haemorrhagic ring mid-caudal region - observed at autopsy - artefact]				1	3.36	♀		
	4	3.20	♂							Butterfly shaped 12th thoracic centrum				3	3.35	♀	[Minimal protrusion median liver lobe]	
	5	3.18	♀							[Skin damaged ventral cervical region - observed at autopsy - artefact]				6	3.22	♂	Abnormal lobation of liver	
	8	2.44	♀					R	U	U	[Small with damaged right pubis - artefact]				7	3.23	♀	Thinning of diaphragm with protrusion of median liver lobe

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ADDENDUM 8

(continued)

Group 4 : Toluene, 1500 ppm													
Litter No.	Allocated to skeletal examination							Allocated to visceral examination					
	Foetus			Sternebral configuration				Other observations			Foetus		
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex
83	1	3.34	♂								2	1.73	♀
	3	2.94	♀								4	2.91	♂
	5	3.27	♂								6	2.53	♂
	7	2.80	♂								8	2.99	♂
	9	2.85	♀								10	2.84	♀
	11	3.04	♀								12	2.96	♀
	13	3.02	♀								14	2.38	♀

Incomplete aortic arch [carotids and right subclavian arise from ascending aorta, left subclavian from descending aorta]; partially fused ascending aorta and pulmonary trunk. Also absent innominate artery [Minimally abnormal lobation of liver]

[Small, no abnormality detected]

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ADDENDUM 8 (continued)

Group 4 : Toluene, 1500 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration							Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
85	1	2.74	♂					R	U		2	3.27	♂	
	3	3.62	♂						R		4	3.49	♂	
	5	3.14	♀						R		6	3.57	♀	Increased dilatation right renal pelvis
	7	3.02	♂		R			U	U		8	2.91	♀	Increased dilatation left renal pelvis and ureter
	9	3.33	♂					U	R		10	3.58	♀	
	11	2.88	♀					U	U		12	2.84	♀	
	13	2.21	♀		R		R	U	U	Small with reduced ossification of cervical vertebral arches : unossified 5th metatarsals				
86	2	3.51	♂								1	3.48	♂	Medial displacement right testis
	4	3.27	♀						R	Right lumbar rib	3	3.49	♂	
	6	2.77	♂				R	U	U	Fused occipital condyle to 1st right cervical vertebral arch : additional thoracic vertebral arch/rib between 11th and 12th right (Additional rib fused to 11th, branched 11th left rib. Also bipartite 11th thoracic centrum [Reduced ossification of 11th right and additional thoracic vertebral arches])	5	3.54	♀	
	8	3.51	♂							[Medial displacement left testis - observed during processing for skeletal examination]	7	3.37	♂	

ADDENDUM 8 (continued)

Group 4 : Toluene, 1500 ppm

Litter No.	Allocated to skeletal examination											Allocated to visceral examination			
	Foetus		Sternebral configuration						Other observations	Foetus			Other observations		
	No	wt(g)	sex	1	2	3	4	5		6	No	wt(g)		sex	
89	1	3.46	♂						R		2	3.36	♂	[Minimally abnormal lobation of liver : damaged right testis]	
	3	3.40	♂						U	Shortened 13th left rib	4	3.38	♂	[Subcutaneous haemorrhage right hindpaw - observed at autopsy - artefact]	
	5	3.12	♂						U	R	6	3.13	♀		
	7	3.35	♂								8	3.34	♀	[Minimally abnormal lobation of liver]	
	9	3.22	♀						U	R	10	3.32	♀		
	11	3.45	♂							Reduced ossification of cranial centres and sacro-caudal vertebral arches					
90	2	3.81	♂								1	2.97	♂	[Minimally abnormal lobation of liver]	
	4	3.43	♂								3	3.49	♀		
	6	2.72	♀						U	[Slightly misshapen 12th thoracic centrum]	5	3.69	♂		
	8	3.25	♀								7	3.13	♂	Anury [anus patent]	
	10	3.49	♂								9	3.33	♂	[Minimal protrusion median liver lobe]	
	12	3.42	♂								11	3.63	♂		
	14	3.03	♂								13	3.36	♀		

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ADDENDUM 8 (continued)

Group 4 : Toluene, 1500 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus					Other observations					Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
91	1	3.56	♀								2	3.05	♀	(Minimally abnormal lobation of liver)
	3	3.11	♀					U			4	3.42	♂	
	5	2.99	♀					U	R		6	3.35	♂	
	7	2.05	♀	R	U	U	U	U	U	Small with termination of normal vertebral column at 3rd cervical vertebra : bilateral forelimb flexure : malrotated hindlimbs : umbilical hernia. Also reduced ossification of occipital and pelvic girdle [Slightly distended abdomen : anus patent : slightly reduced ossification of interparietal : reduced ossification of 5th metatarsals]	8	3.41	♂	
	9	2.51	♀					U	U	[Slightly reduced ossification of interparietal and occipital]	10	3.50	♀	
	11	3.04	♀					U	U	Minimal distortions affecting 7th and 10th right, 11th bilateral ribs : reduced ossification of cranial centres and pubes [Reduced ossification of 2nd metacarpals]	12	3.50	♂	(Minimally abnormal lobation of liver)
	13	3.35	♂					U			14	3.12	♀	
	15	3.20	♂							[Slightly reduced ossification of interparietal]				

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ADDENDUM 8 (continued)

Group 4 : Toluene, 1500 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
92	2	2.43	♀		R		R	U	U	Small with reduced ossification of cervical vertebral arches : unossified 5th metatarsals	1	3.82	♂		
	4	3.23	♂					U	R		3	3.39	♀		
	6	3.53	♂						R		5	3.32	♀		
	8	3.28	♂					U	R		7	3.28	♀		
											9	3.26	♀		
93	1	3.00	♀						R		2	2.97	♀		
	3	3.33	♂								4	2.80	♀		
	5	3.11	♂						R		6	2.99	♀		
	7	3.11	♀					U			8	3.27	♀		
	9	3.16	♀					U	R		10	2.94	♂		

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ADDENDUM 8 (continued)

Group 4 : Toluene, 1500 ppm

Group 4 : Louene, 1500 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration						Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
94	2	3.05	♂						R	Reduced ossification of sacro-caudal vertebral arches	1	3.22	♀	
	4	3.00	♀					U	U	Reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal]	3	3.20	♂	Anterior displacement right testis
	6	2.97	♂					R	R		5	3.52	♂	Medial displacement left testis
	8	3.40	♂					R	R		7	3.08	♀	
	10	3.40	♂						R		9	3.23	♀	
	12	2.83	♀					R	R		11	3.36	♂	
	14	3.02	♂						U		13	3.06	♀	
95	1	2.95	♀			R		U	U	Shortened 13th right rib	2	3.12	♂	
	3	3.13	♀					U	U		4	3.41	♂	[Minimally abnormal lobation of liver]
	5	3.17	♀					U	R		6	3.15	♀	
	7	3.03	♂					U	U	Shortened 13th right rib	8	3.23	♀	
	9	3.22	♀					U	R	Reduced ossification of left pubis	10	3.01	♀	[Subcutaneous haemorrhage terminal caudal region - observed at autopsy - artefact]
	11	3.20	♀					U			12	3.01	♀	[Damaged right forepaw]

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ADDENDUM 8
(continued)

Group 4 : Toluene, 1500 ppm

Litter No.	Allocated to skeletal examination												Allocated to visceral examination				
	Foetus			Sternebral configuration						Other observations			Foetus			Other observations	
	No	wt(g)	sex	1	2	3	4	5	6				No	wt(g)	sex		
96	2	2.85	♀					U	U	[Minimally asymmetric pelvic girdle]			1	3.20	♀		
	4	3.45	♂						U				3	2.98	♀		
	6	3.41	♀					R					5	2.93	♀		
	8	3.42	♂					U					7	3.09	♀		
	10	2.78	♀					U	U	[Reduced ossification of 5th metatarsals]			9	3.36	♀	[Minimally abnormal lobation of liver]	
	12	3.26	♂					U	R	Bipartite 11th thoracic centrum [Minimally asymmetric pelvic girdle]			11	3.17	♂		
	14	3.54	♂							Butterfly shaped 12th thoracic centrum			13	3.57	♂	Duplicated inferior vena cava [Damaged left testis]	
													15	3.43	♂	[Damaged bladder]	
97	1	3.44	♂										2	3.26	♂		
	3	2.91	♀					U	R				4	3.46	♂		
	5	3.04	♂										6	3.23	♂		
	7	2.97	♂					R	U				8	3.33	♀	[Minimal protrusion median liver lobe]	
	9	3.25	♂										10	3.21	♀		
	11	2.69	♂						U	U				12	3.08	♀	[Damaged uro-genital system]

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ADDENDUM 8 (continued)

Group 4 : 10luene, 1300 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination								
	Foetus			Sternebral configuration						Other observations	Foetus			Other observations					
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex						
100	3	3.05	♀						U	[Slightly misshapen 12th thoracic centrum]						1	2.97	♀	
	4	2.88	♀						R							2	2.73	♀	[Minimal subcutaneous haemorrhage ventral cervical region]
	6	2.93	♀													5	2.90	♂	
	8	2.94	♂						R							7	2.93	♀	Increased dilatation right renal pelvis
	10	3.07	♀						R	Butterfly shaped 11th thoracic centrum						9	2.41	♀	
																11	3.28	♂	

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ADDENDUM 8

(continued)

Group 5 : Toluene, 3000 ppm														
Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus					Other observations					Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
101	1	3.34	♂								2	3.25	♂	
	3	3.24	♀						R	Bipartite 11th thoracic centrum	4	3.22	♀	[Minimal protrusion median liver lobe]
	5	2.69	♀						U	[Subcutaneous haemorrhagic ring terminal caudal region - observed at autopsy - damaged caudal vertebrae at skeletal examination - artefact]	6	3.12	♀	
	7	3.30	♂						U		8	3.44	♂	[Damaged caudal region]
	9	3.19	♂						U		10	3.29	♀	Moderate haemorrhage anterior chamber right eye
	11	2.96	♀						U		12	2.97	♂	
	13	2.99	♂						U					
102	2	3.47	♂						R		1	3.50	♂	[Minimal intra-abdominal haemorrhage]
	4	3.18	♂						B		3	3.62	♂	[Minimally abnormal lobation of liver]
	6	3.16	♀						U		5	3.54	♂	[Minimal protrusion right median liver lobe]
	8	3.56	♂								7	3.32	♂	
	10	3.47	♀								9	3.28	♀	
											11	3.27	♀	

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ADDENDUM 8

(continued)

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination		
	Foetus		Sternebral configuration						Other observations		Foetus		
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex
103	1	2.97	♀								2	3.60	♂
	3	3.24	♂								4	3.07	♀
	5	3.39	♂								6	3.43	♀
	7	3.12	♀								8	3.21	♂
	9	3.07	♂							Left cervical rib	10	3.39	♂
	11	3.48	♀								12	3.27	♀
	13	3.20	♂						R		14	3.40	♂

[Minimally abnormal lobation of liver]

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ADDENDUM 8 (continued)

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration							Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
104	2	3.37	♀								1	3.39	♀	
	4	3.51	♂							[Connected sacro-caudal centrum to vertebral arch]	3	3.19	♂	
	6	3.37	♂							[Minimal subcutaneous haemorrhage terminal caudal region - observed during processing for skeletal examination - caudal vertebrae within normal limits]	5	3.60	♂	
	8	3.58	♂								7	3.25	♀	
	10	3.41	♀								9	3.65	♂	
	12	3.43	♀							[Minimal subcutaneous haemorrhage right hindpaw - observed during processing for skeletal examination - skeleton within normal limits]	11	3.62	♂	
	14	3.55	♂								13	3.45	♀	

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Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Fetus			Sternebral configuration						Other observations	Fetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
105	1	2.80	♂					U	R		2	2.65	♂	
	3	3.21	♀					R			4	3.28	♂	
	5	2.93	♀					U	R		6	3.20	♀	
	7	2.81	♀					U	U		8	3.25	♂	Marked intra-abdominal haemorrhage
	9	3.25	♀					U	R		10	2.93	♀	(Pale at autopsy - no abnormality detected)
	11	3.01	♂					R	U		12	2.99	♀	
	13	3.48	♀					R			14	3.15	♀	
	15	3.02	♂					U	R					

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ADDENDUM 8 (continued)

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination											Allocated to visceral examination				
	Foetus			Sternebral configuration								Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No	wt(g)		sex			
106	2	3.00	♀					U				1	3.54	♂		
	4	3.46	♂					U				3	2.62	♂		
	6	3.35	♂					U				5	2.79	♀		
	8	3.17	♀					U				7	3.54	♀		
	9	2.47	♀	U				U				10	3.42	♂		
	12	3.34	♂							R		11	3.67	♂		
108	2	3.42	♀									1	3.68	♂		
	4	3.54	♂									3	3.10	♀		
	6	2.93	♀					U	R			5	3.61	♂		
	8	3.79	♂									7	3.56	♀		
	10	3.24	♂									9	3.27	♀		
	12	3.32	♀					U				11	3.33	♂		
												13	3.29	♀		

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
109	1	2.86	♂							U	2	3.18	♂		
	3	3.13	♀					R	R		4	2.96	♂		
	5	2.77	♀					U	R		6	2.89	♀		
	7	2.98	♂					R	R		8	2.87	♀		
	9	2.81	♀					U	U		10	2.96	♀		
	11	2.87	♀					U	U						
110	2	3.41	♂								1	3.64	♂		
	4	3.12	♀					R	R		3	3.36	♂		[Minimal subcutaneous haemorrhagic ring caudal region - observed at autopsy - not apparent at detailed examination : damaged right testis]
	6	3.20	♀					U	R		5	3.52	♂		

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ADDENDUM 8 (continued)

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus		Sternebral configuration						Other observations		Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No	wt(g)	sex		
111	1	2.62	♀					U	U	Butterfly shaped 9th thoracic centrum	2	2.62	♀	
	3	2.85	♀					R			4	2.70	♀	Ankyloglossia [tip of tongue connected to base of buccal cavity : subcutaneous haemorrhage right forepaw - observed at autopsy - artefact]
	5	3.32	♀								6	2.89	♀	
	7	2.96	♀					U	U		8	3.01	♂	
	9	2.86	♀					U	U		10	2.90	♂	
	11	2.92	♀					R			12	3.09	♀	
	13	3.01	♀					U			14	2.66	♀	
	15	2.29	♀					U	U	Small with left lumbar rib [Damaged vertebral column and ribcage]				

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ADDENDUM 8

(continued)

Group 5 : Toluene, 3000 ppm														
Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration			Other observations				Foetus		Other observations	
	No	wt(g)	sex	1	2	3					4	5		
112	2	2.95	♀					U	R		1	2.71	♀	
	4	2.91	♀					U			3	2.86	♂	
	6	2.85	♀					U	R		5	3.05	♀	
	8	2.97	♂								7	2.71	♀	
	10	2.78	♂			R		U	U		9	2.66	♂	
	11	2.54	♀					U	U	Bipartite 12th thoracic centrum : reduced ossification of sacro-caudal vertebral arches [Slightly reduced ossification of interparietal: reduced ossification of 5th metatarsals]	12	1.70	♀	Small with small left eye [Minimal subcutaneous haemorrhage cranium]
	14	2.55	♀					U	U	[Slightly reduced ossification of occipital]	13	2.72	♀	
											15	2.97	♂	

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ADDENDUM 8

(continued)

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus						Other observations				Foetus			Other observations
	No	wt(g)	sex	Sternebral configuration							No	wt(g)	sex	
113	1	2.48	♀								2	2.76	♂	
	3	2.77	♂								4	2.84	♂	[Minimal subcutaneous haemorrhage cranium - observed at autopsy - artefact]
	5	2.38	♀	R	R						6	2.58	♂	[Moderate] subcutaneous haemorrhage caudal region [Minimally abnormal lobation of liver]
	7	2.90	♂								8	2.46	♀	[Small, no abnormality detected]
	9	2.76	♂								10	2.63	♀	[Minimally abnormal lobation of liver : damaged right uterine horn, kidney and ureter]
	11	2.69	♀								12	2.79	♀	
	13	2.77	♀								14	2.52	♀	[Minimal subcutaneous haemorrhage cranium : minimal area of haemorrhage within right anterior liver lobe]

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ADDENDUM 8 (continued)

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration							Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
115	1	2.93	♀					U	U		2	3.11	♂	
	3	2.94	♂					U	R		4	2.72	♀	Increased dilatation right renal pelvis
	5	3.05	♂					U			6	2.95	♂	
	7	3.08	♂					U			8	2.98	♂	[Minimal anterior displacement right testis]
	9	2.91	♀					U	U		10	2.82	♀	
	11	2.88	♂					R	R		12	3.11	♂	
	13	3.08	♂						R	Right lumbar rib	14	3.07	♀	
	15	3.33	♂								16	3.17	♂	
116	2	3.08	♀					U	R		1	3.20	♂	
	4	2.66	♀					U	U		3	3.11	♀	
	6	3.38	♂					R			5	3.02	♂	
	9	3.20	♂							[Minimal subcutaneous haemorrhage terminal caudal region - observed at autopsy - skeleton within normal limits]	7	3.30	♀	
	10	2.77	♀					U	U		8	2.72	♀	
	12	3.24	♂						R	Right lumbar rib	11	3.06	♀	
											13	2.91	♂	

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ADDENDUM 8 (continued)

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Sternebral configuration							Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
117	1	3.08	♀					U	U	[Slightly reduced ossification of interparietal and occipital]	2	3.09	♀	
	3	2.93	♂						U	[Slightly reduced ossification of interparietal]	4	3.02	♀	
	5	3.29	♂								6	3.06	♀	Increased dilatation left renal pelvis
	7	3.13	♀								8	3.20	♂	
	9	2.95	♀						R		10	3.10	♂	
	11	2.77	♀						U	Unossified 5th metatarsals	12	3.17	♂	
	13	2.94	♀						R		14	2.68	♀	Minimal haemorrhage within 4th ventricle (brain)
118	2	2.60	♀					U	R		1	2.59	♀	
	4	2.46	♀					U	R	[Small, skeleton within normal limits]	3	2.61	♀	
	6	2.81	♂					U	R		5	2.81	♀	
	8	2.56	♂					U	R		7	2.88	♂	
	10	2.85	♀							Bipartite 12th thoracic centrum [Damaged sternum]	9	2.80	♂	
	12	2.74	♂					U	U		11	2.73	♀	

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ADDENDUM 8 (continued)

Group 5 : Toluene, 3000 ppm

Group 5 : Toluene, 3000 ppm															
Litter No.	Allocated to skeletal examination										Allocated to visceral examination				
	Foetus			Sternebral configuration							Other observations	Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6	No		wt(g)	sex		
119	1	3.17	♂								2	3.46	♂		
	3	3.10	♂					U	R		4	3.05	♂	[Minimal internal] hydrocephaly [affecting lateral ventricles]	
	5	3.32	♂								6	3.01	♀		
	7	3.29	♀					U			8	3.27	♂		
	9	3.07	♂						R		10	2.87	♀		
	11	3.12	♂						R		12	3.10	♂	Minimal subdural haemorrhage right cerebral region (brain)	
121	1	3.14	♀					U			2	3.16	♂	[Damaged right testis]	
	3	2.78	♀					U	R		4	3.13	♀		
	5	3.25	♂					U	R		6	3.28	♀		
	7	3.20	♀					U	R		8	3.52	♂		
	9	3.32	♂					U			10	3.13	♀	[Damaged left kidney]	
	11	3.21	♀					U	R		12	2.86	♀	Thinning of diaphragm with protrusion of median liver lobe	

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ADDENDUM 8
(continued)

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Fetus						Other observations				Fetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
122	2	2.52	♂					U	R		1	2.80	♂	[Minimal variation contra-lateral eye size]
	4	2.43	♀		R			U	R	[Small with slightly reduced ossification of occipital]	3	2.59	♀	[Minimal protrusion median liver lobe]
	6	2.58	♀					U	U		5	2.18	♀	Small with retroesophageal right subclavian artery; interventricular septal defect. [Minimal variation contra-lateral eye size]
	8	2.66	♂					U			7	2.66	♂	
	10	2.25	♀				R	U	U	Small with reduced ossification of occipital and sacro-caudal vertebral arches : unossified 5th metatarsals [Connected sacro-caudal centrum to vertebral arch : damaged cranium]	9	2.54	♂	
123	1	3.35	♂						R	Butterfly shaped 12th thoracic centrum	2	3.27	♂	
	3	3.48	♂								4	3.16	♂	
	5	2.75	♀					U	U	Bipartite 11th thoracic centrum [Slightly misshapen 12th thoracic centrum]	6	3.16	♀	
	7	3.12	♀						R	[Slightly misshapen 12th thoracic centrum]	8	3.26	♂	[Damaged left testis]
	9	2.89	♂					U	U		10	2.98	♀	
	11	2.76	♀					U	U	Butterfly shaped 13th thoracic centrum				

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ADDENDUM 8 (continued)

Group 5 : Toluene, 3000 ppm

Litter No.	Allocated to skeletal examination										Allocated to visceral examination			
	Foetus			Other observations							Foetus			Other observations
	No	wt(g)	sex	1	2	3	4	5	6		No	wt(g)	sex	
124	2	2.62	♀					U	U	Left lumbar rib [Slightly misshapen 10th thoracic centrum]	1	2.96	♂	
	4	3.07	♂								3	2.85	♀	
	6	2.58	♂					U	U		5	3.17	♂	
	8	2.92	♂					U	R		7	2.89	♀	
	10	3.05	♀					R			9	2.71	♀	
	12	2.90	♂					U	R		11	3.15	♂	
125	1	2.15	♀		R	R	U	U	U	Small with unossified 5th metatarsals [Slightly misshapen 12th thoracic centrum]	2	2.15	♀	[Small with minimal variation in contra-lateral eye size]
	3	2.26	♀				R	U	U	Small with bipartite 11th thoracic centrum [Slightly misshapen 10th thoracic centrum: reduced ossification of 5th metatarsals : minimal subcutaneous haemorrhage right pinna region - observed at autopsy - artefact]	4	2.04	♀	Small with absent innominate artery [Lens adhered to cornea - assumed artefact]
	5	2.34	♂					U	U	Small with misshapen 11th thoracic centrum [Reduced ossification of 5th metatarsals]	6	2.37	♂	[Small, no abnormality detected]
	7	2.12	♀		R		R	U	U	Small with misshapen 13th thoracic centrum: unossified 5th metatarsals [Damaged cervical vertebrae and right hindpaw]	8	2.13	♀	[Small with minimally abnormal lobation of liver]
	9	2.17	♀				R	U	U	Small with unossified 5th metatarsals	10	2.57	♂	Diaphragmatic hernia
	11	2.38	♂					U	U	[Small with reduced ossification of 5th metatarsals]	12	2.06	♀	Small with abnormal lobation of liver

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ADDENDUM 9

Historical control data for foetal changes - study incidences

A. Malformations

Study#:	1	2	3	4	5	6	7	8	9
Number examined:	238 (22)	314 (27)	282 (25)	214 (20)	227 (20)	250 (23)	336 (30)	226 (20)	261 (22)
Number with malformations: foetuses (litters)									
Description									
CRANIAL	2 (2)	1 (1)	-	-	-	-	-	-	-
Microphthalmia/anophthalmia	-	-	-	-	-	-	-	-	-
Rhinencephaly	-	-	-	-	-	-	-	-	-
Agnathia	-	-	-	-	-	-	-	-	-
Hydrocephaly	-	-	-	-	-	-	-	-	-
Cyclopia	-	-	-	-	-	-	-	-	-
Ablepharia	-	-	-	-	-	-	-	-	-
Ankyloglossia	-	-	-	-	-	-	-	-	-
CERVICAL	-	-	-	-	-	-	-	-	-
Multiple vertebral irregularities	-	-	-	-	-	-	-	-	-
Termination at 3rd vertebra	-	-	-	-	-	-	-	-	-
Fused vertebral elements	-	-	-	-	-	-	-	-	-
THORACIC	-	2 (2)	-	-	1 (1)	1 (1)	1 (1)	-	-
Malformed cervico-thoracic arteries	-	2 (2)	-	-	2 (2)	-	1 (1)	1 (1)	-
Interventricular septal defect	1 (1)	1 (1)	-	-	-	1 (1)	-	-	-
Multiple vertebral irregularities	-	1 (1)	-	-	-	-	-	-	-
Distortions/ossification irregularities ribs	-	1 (1)	-	-	-	-	-	-	-
LUMBAR/ABDOMINAL	-	-	-	-	-	-	-	-	-
Duplicated inferior vena cava	1 (1)	-	-	-	1 (1)	-	-	1 (1)	1 (1)
Diaphragmatic hernia	-	-	-	-	-	-	-	-	-
Umbilical hernia	-	-	-	-	-	-	-	-	-
SACRO-CAUDAL	-	-	-	-	-	1 (1)	-	-	-
No tail apparent	-	-	-	-	-	-	-	-	-
APPENDICULAR	-	-	-	-	-	-	-	-	-
Forelimb flexures/malrotated hind limbs	-	-	1 (1)	-	-	-	-	-	-

From 9 studies performed 1990 - 91, using time-mated animals of the same strain and source, identified chronologically

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APPENDUM 9

(continued)

B. Visceral anomalies

Study#:	1	2	3	4	5	6	7	8	9
Number examined:	116 (22)	153 (27)	141 (25)	107 (22)	111 (20)	125 (23)	167 (30)	112 (20)	127 (22)
Number with anomaly: foetuses (litters)									
Description									
Subcutaneous haemorrhage:	-	-	1 (1)	-	-	-	-	-	-
cranium	-	-	-	-	-	-	-	4 (3)	-
trunk, tail, limbs									
CRANIUM									
Haemorrhages affecting:	1 (1)	1 (1)	3 (3)	2 (1)	1 (1)	4 (4)	5 (5)	5 (5)	3 (3)
brain/spinal cord	-	1 (1)	-	1 (1)	-	1 (1)	2 (2)	-	2 (2)
eyes/surrounding tissue	1 (1)	1 (1)	-	-	-	-	1 (1)	-	-
Small eye									
CERVICAL									
Reduced thyroid	-	-	1 (1)	-	-	-	-	1 (1)	-
THORACIC									
Anomalous cervico-thoracic arteries	1 (1)	1 (1)	2 (2)	-	1 (1)	-	1 (1)	-	2 (2)
Interventricular septal defect (small)	1 (1)	-	-	-	-	1 (1)	1 (1)	-	1 (1)
ABDOMINAL									
Thinning of diaphragm/protrusion liver	-	-	-	-	-	2 (2)	-	-	-
Liver - abnormal lobation	-	1 (1)	2 (2)	3 (3)	-	4 (3)	7 (7)	3 (3)	4 (4)
haemorrhage within lobe	-	-	1 (1)	-	-	1 (1)	-	-	-
Intra-abdominal haemorrhage	-	-	1 (1)	-	-	-	-	-	-
Increased dilatation renal pelvis/ureter	2 (2)	3 (3)	8 (6)	1 (1)	1 (1)	4 (3)	3 (3)	1 (1)	4 (4)
Displaced testis(es)	-	2 (2)	1 (1)	-	1 (1)	2 (2)	3 (3)	-	2 (1)

From 9 studies performed 1990 - 91, using time-mated animals of the same strain and source, identified chronologically
Malformed foetuses are excluded

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ADDENDUM 9
(continued)

C. Skeletal anomalies

Study#:	1	2	3	4	5	6	7	8	9
Number examined:	115 (22)	155 (27)	140 (25)	107 (20)	113 (20)	122 (23)	168 (30)	112 (20)	131 (22)
Number with anomaly: foetuses (litters)									
Description									
Reduced ossification of: one or more cranial centres	12 (7)	6 (4)	5 (3)	-	6 (5)	6 (5)	6 (6)	6 (5)	10 (8)
cervical vertebral arches	2 (1)	5 (3)	-	-	2 (2)	2 (2)	2 (2)	-	-
sacro-caudal vertebral arches	16 (9)	20 (9)	20 (10)	14 (9)	13 (6)	21 (10)	24 (13)	14 (8)	14 (6)
one or more centres pelvic girdle	6 (3)	6 (4)	5 (4)	1 (1)	3 (2)	8 (7)	3 (3)	5 (4)	5 (3)
digital centres	4 (1)	2 (2)	7 (4)	-	2 (2)	2 (2)	4 (3)	3 (3)	-
CERVICAL									
Cervical rib(s)	1 (1)	-	1 (1)	-	-	3 (3)	3 (3)	1 (1)	-
THORACIC									
Irregular ossification vertebral									
centra	4 (4)	2 (2)	8 (7)	-	1 (1)	5 (5)	1 (1)	4 (3)	6 (4)
Minimal distortions ribs	-	-	-	-	1 (1)	1 (1)	1 (1)	-	-
Shortened/absent 13th rib(s)	12 (7)	5 (3)	2 (1)	1 (1)	4 (3)	1 (1)	3 (1)	-	2 (2)
Asymmetric costal cartilage elements	-	-	-	-	-	-	-	-	-
LUMBAR									
Lumbar rib(s)	2 (1)	3 (3)	1 (1)	1 (1)	4 (3)	-	6 (4)	-	1 (1)
One less thoraco-lumbar vertebra	4 (2)	3 (1)	-	-	2 (1)	-	-	-	-

From 9 studies performed 1990 - 91, using time-mated animals of the same strain and source, identified chronologically
Malformed foetuses are excluded

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ADDENDUM 10

Summary of results from preliminary study

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 reduction in water and 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 reduction in water and food consumption.
- reductions in bodyweight gains.

2000 ppm

- slight signs indicative of a gradual narcosis confined to the period of direct exposure.
- an initial reduction in food and water intake.
- retardations in bodyweight gains.

1000 ppm

- slight initial retardation in bodyweight gain.

500 ppm

- slight initial retardation in bodyweight gain.

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ADDENDUM 10

(continued)

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 embryofetal 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 embryofetal toxicity study should not exceed 3500 ppm.

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