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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**



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QUALITY ASSURANCE/GLP COMPLIANCE STATEMENT

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

Testing Facility: Huntingdon Research Centre Ltd.

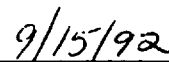
Testing Facility Number: APT 1/91309 : APT 2/91279

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
7/30/90	Prel. Atmosphere Gen. Data Review	7/30/90
7/31/90	Prel. Protocol Review	7/31/90
9/19-20/90	Audit Preliminary Data	10/11/90
10/14-15/90	Site Visit, In-life and Necropsy Inspection	10/17/90
5/7-10/91	Draft Report Audit	6/5/91
2/20/92	2cd Draft Report Audit	2/20/92
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


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."

APT 2/91279

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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10.9.92

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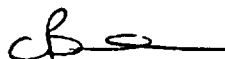
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We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.



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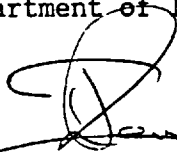
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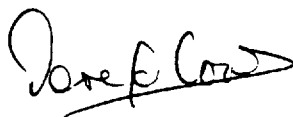
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QUALITY ASSURANCE STATEMENT

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.

References

- | | | |
|---|-------------------|--|
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Statistical references

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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.

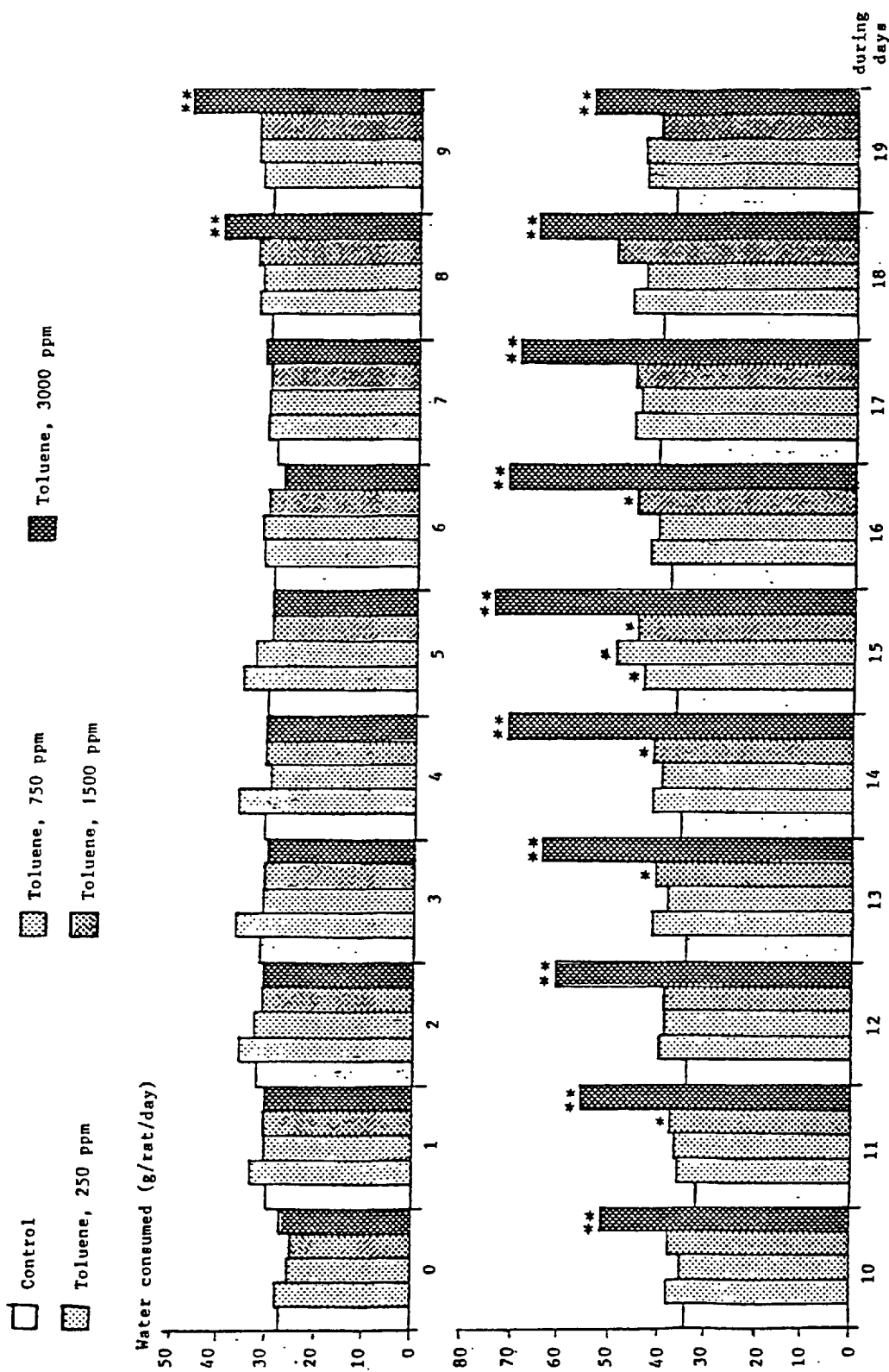
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The number of fetuses showing visceral or skeletal anomalies was similar among the groups (intergroup comparison with the control did not attain statistical significance).

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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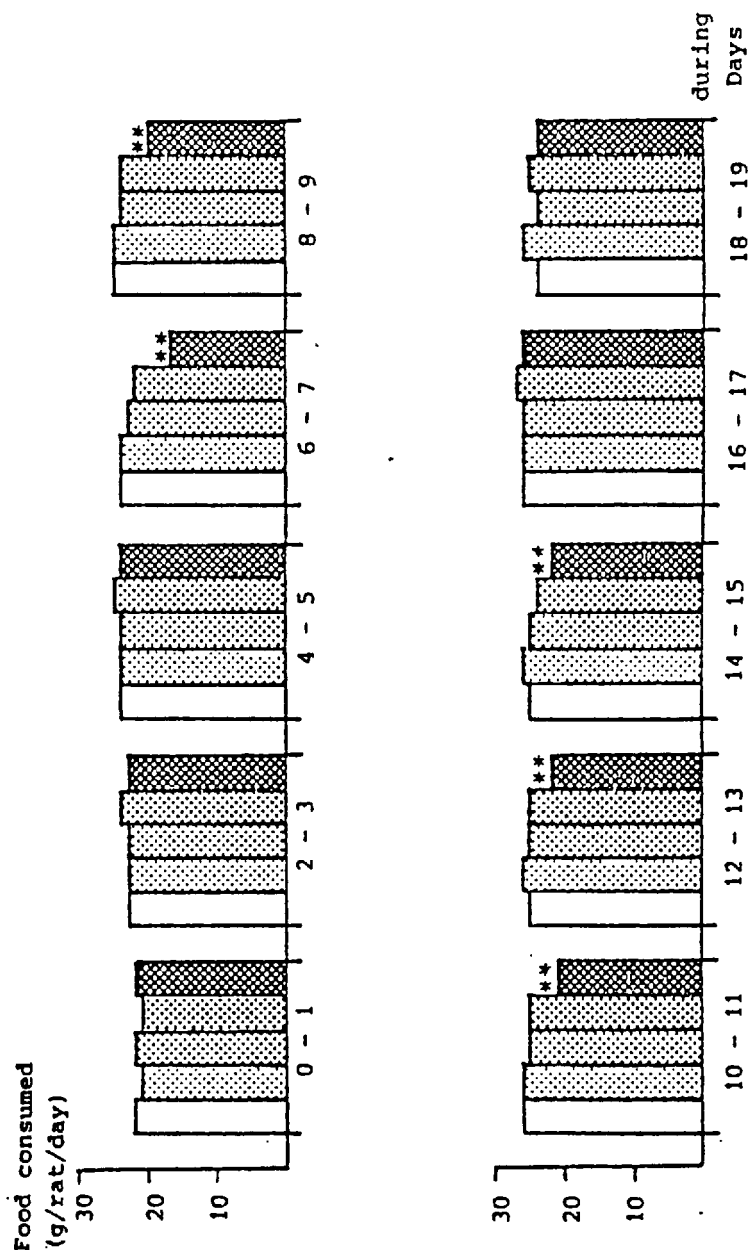
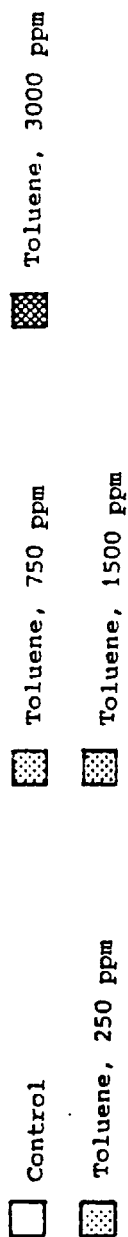
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FIGURE 2

Food consumption - animals with live young - group mean values



Treatment period Days 6 to 15 of pregnancy inclusive

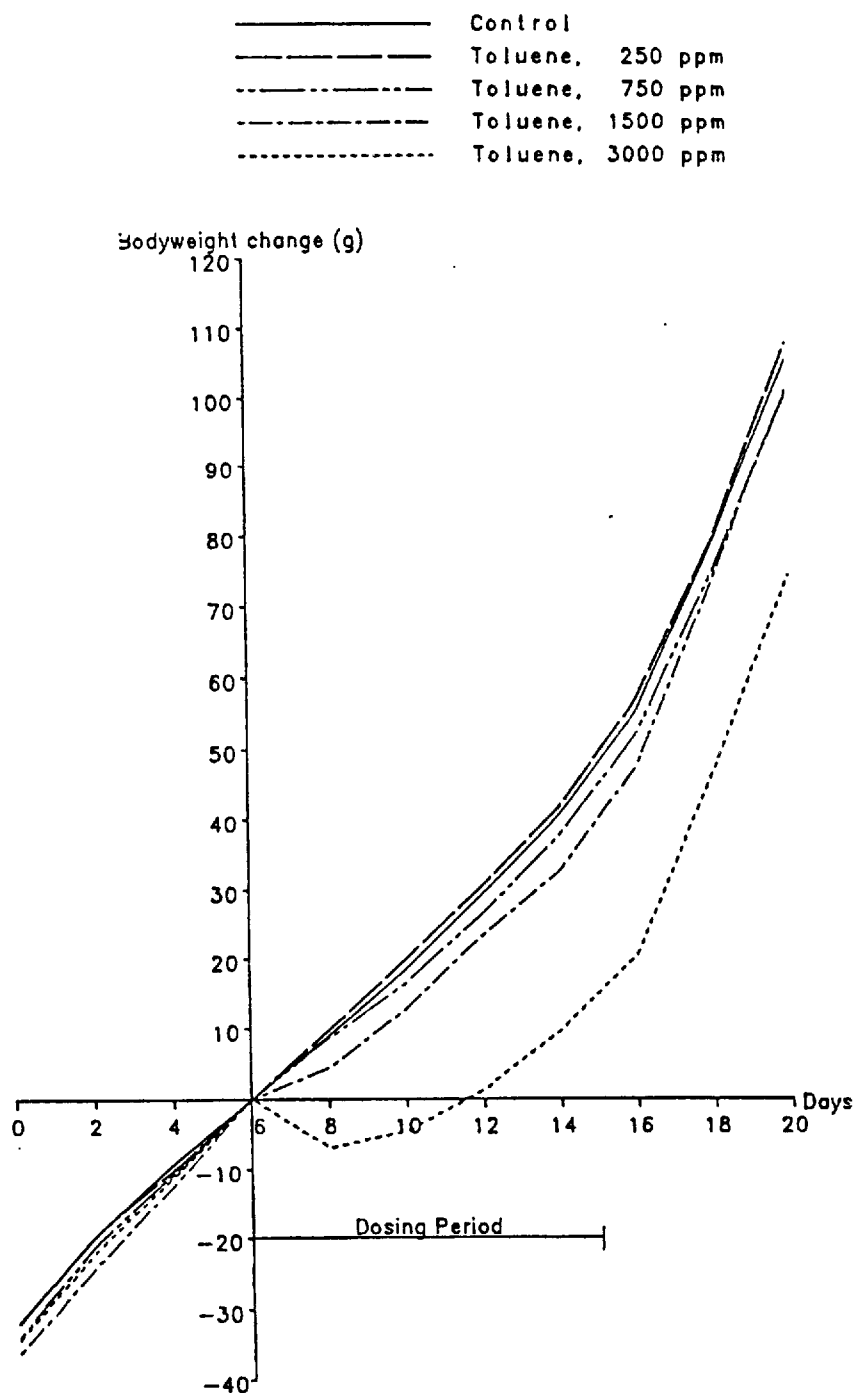
Statistical significance

** 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
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 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

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			Toluene								
Exposure (ppm):	-	250	750	1500	3000							
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	Examined
Number examined	146	131	116	116	138	24	22	20	19	22	
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

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

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