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Health and Environmental
Sciences Department

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

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

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

AUTHORS' SIGNATURE PAGE

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



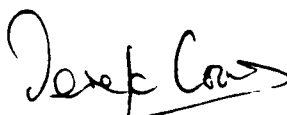
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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

HRC Report No. APT 1/91309

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

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

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

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

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3-9-92

Date

DEPARTMENT OF QUALITY ASSURANCE

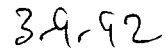
REPORT AUDIT STATEMENT

HRC REPORT No. APT 1/91309

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

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

16.10.91



Peter H.C.V. Richold, B.Sc.,
Senior Systems Compliance Auditor,
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DEPARTMENT OF QUALITY ASSURANCE

DATES OF STUDY INSPECTIONS

HRC REPORT No. APT 1/91309

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

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

 3.9.92

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

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

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

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SUMMARY

1. In this assessment of the effect of Toluene, a solvent, on the pregnancy and in utero development of the rat, dosages of 0, 500, 1000, 2000, 3500 and 5000 ppm were administered by inhalation for a period of 6 hours a day from Day 6 to 15 of pregnancy inclusive. On Day 20, surviving females were sacrificed, subjected to post mortem examination with liver weights being recorded, litter values were determined and foetuses were examined externally.

2. Exposure to Toluene was associated with the following maternal effects which were generally dosage-related in degree:

5000 ppm

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

3500 ppm

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

2000 ppm

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

1000 ppm

- slight initial retardation in bodyweight gain.

500 ppm

- slight initial retardation in bodyweight gain.

3. Examination of the litter data revealed:

6 animals at 5000 ppm with total resorptions.

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

Conclusion

Within the confines of the study, exposure to Toluene was associated with clear signs of maternal toxicity at 2000 ppm and above. Signs of 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.

INTRODUCTION

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

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

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

Key dates of the study were as follows:

Protocol approval by:

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

Arrival of animals:	19 July 1990
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Mating

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

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

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

PROCEDURE

Animal management and accommodation

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

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

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

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

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

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

Experimental design

The experimental design was as follows:

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

Exposure of rats to the test substanceDosing regimen

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

$$T_{90} = \frac{V}{Q} \times \frac{\log_{10} \frac{100}{100-90}}$$

where V = chamber volume (litres)

Q = chamber air flow rate (litres/min)

Observation

The following observations were made during the study:

1. Parent animals

(a) Signs

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

(b) Mortalities

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

(c) Food and water consumption

Food consumption was measured from weighday to weighday.

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

(d) Bodyweights

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

2. Litter data and foetal examinations

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

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

Embryofoetal deaths were classified as:

Early: only placenta visible at termination.

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

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

Assessment of results

Individual litter values

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

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

Post implantation loss was similarly calculated from the formula:

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

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

Group values

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

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

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

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

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

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

Location of study records

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

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

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

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

References

- a Salewski, E. 1964 Farbemethode zum makroskopischen
Nachweis von Implantationsstellen am
Uterus der Ratte. Naunyn-Schmiedebergs
Arch. exp. Pathol. Pharmacol. 247:367

RESULTS

1. PARENT ANIMALS

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

Treatment period: Days 6 to 15 of pregnancy inclusive.

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

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

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

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

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

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

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

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

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

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

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

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

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

(e) Terminal post mortem examination (Appendix 1)

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

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

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

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

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

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

FIGURE 1

Water consumption of pregnant females - group mean values

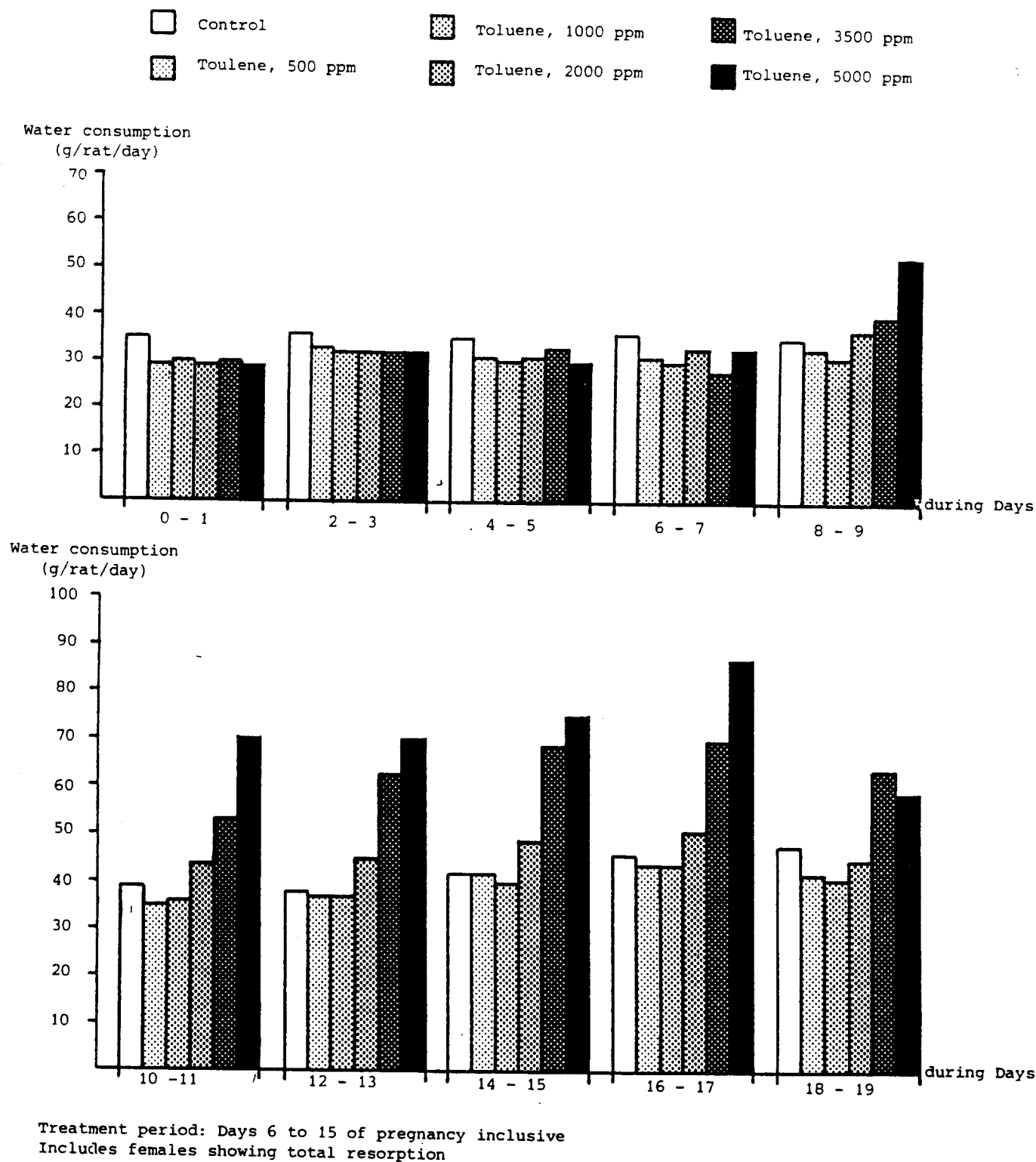
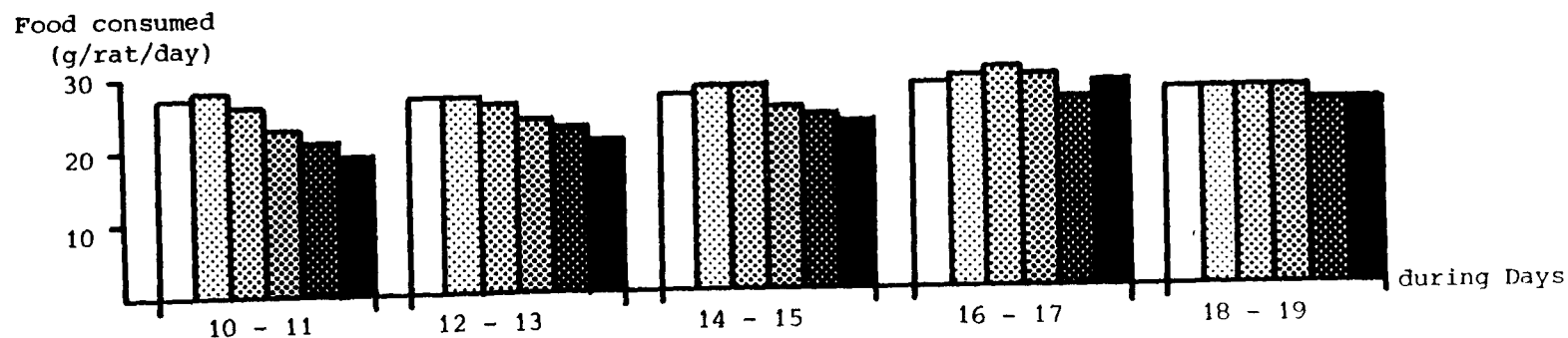
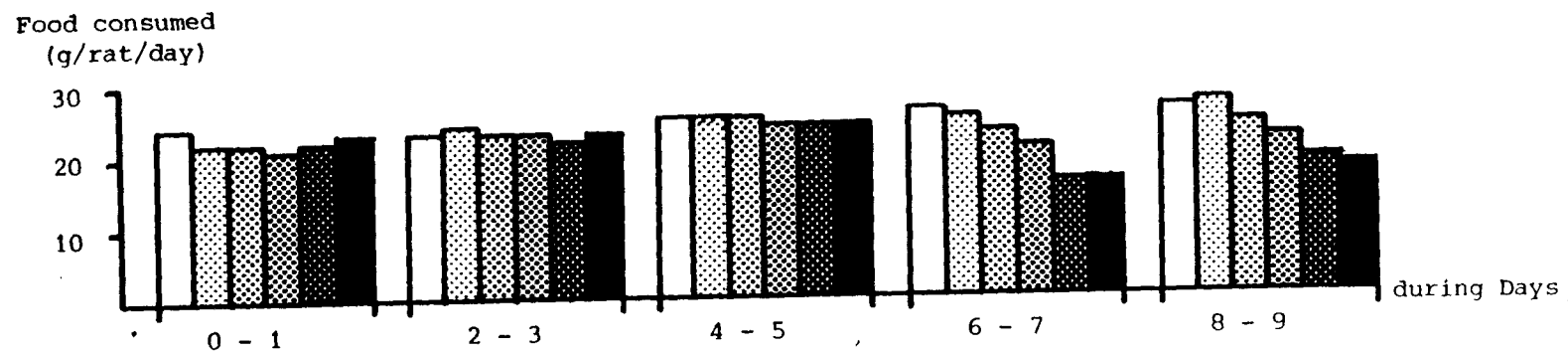
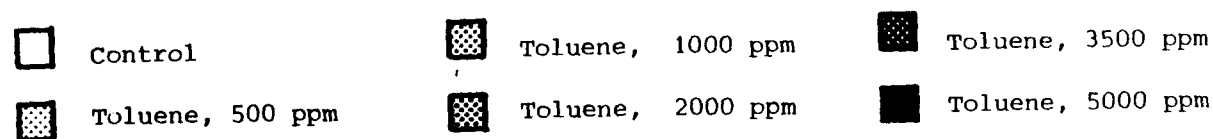


FIGURE 2

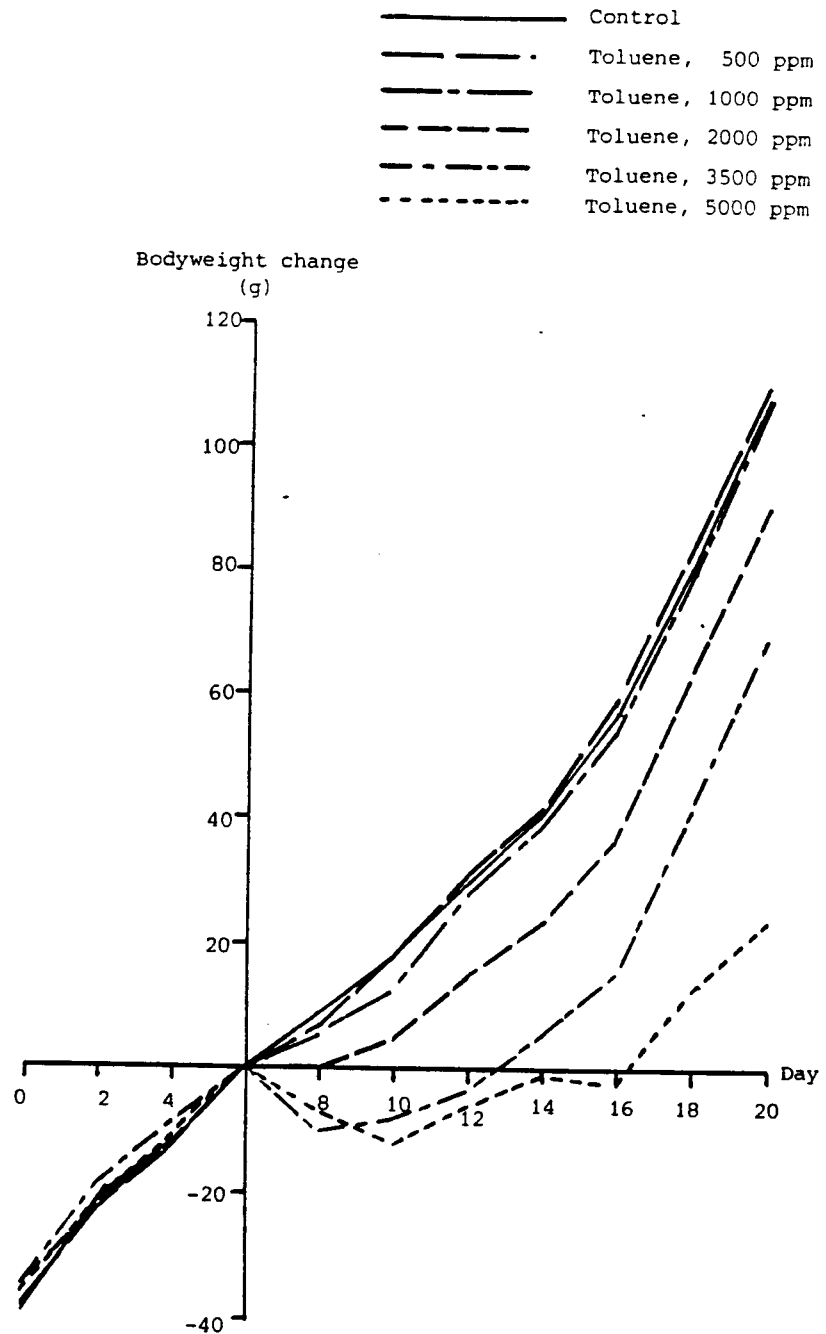
Food consumption of pregnant females - group mean values



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

FIGURE 3

Bodyweight change of pregnant females - group mean values



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

TABLE 1

Adult performance - summary

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

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

TABLE 2

Water consumption - animals with live young - group mean values

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

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

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

TABLE 3

Food consumption - animals with live young - group mean values

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

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

Treatment period Days 6 to 15 of pregnancy inclusive

TABLE 4

Bodyweights - animals with live young - group mean values

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

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

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

TABLE 5

Liver weights - group mean values

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

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

TABLE 6

Litter data - group mean values

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

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

Superscript indicates number of values excluded

TABLE 7

Sex ratios - group mean values

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

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

^a Excludes one pup, sex not recorded in error

APPENDICES